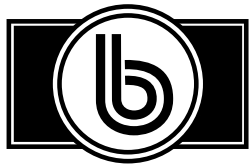

Hrvatsko biološko društvo 1885
SOCIETAS BIOLOGORUM CROATICA 1885
Croatian Biological Society

# 8. HRVATSKI BIOLOŠKI KONGRES
s međunarodnim sudjelovanjem
27. rujna – 2. listopada 2003.
Zagreb, Hrvatska

# 8th CROATIAN BIOLOGICAL CONGRESS
with International Participation
27th September- 2nd October 2003
Zagreb, Croatia

ZBORNIK SAŽETAKA

PROCEEDING OF ABSTRACTS

Zagreb, 2003.

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Croatian Biological Society

Zagreb, 2003.

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Tradicionalni Hrvatski biološki kongres u organizaciji Hrvatskog biološkog društva 1885 osmišljen je te po prvi puta organiziran još 1981. godine u Poreču u Istri. Kongres je organizirao tadašnji predsjednik Društva profesor Oskar Springer. Tijekom proteklih dvadesetak godina Kongres je postao središnji skup hrvatskih biologa na kojemu su se predstavljala dostignuća hrvatske biologije, ali i uspoređivalo njeno stanje s europskom te svjetskom biologijom.

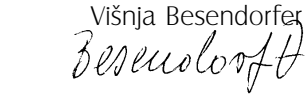
Godine 1984. u Zadru organiziran je Drugi kongres kada je predsjednik Društva bio profesor Hrvoje Gomerčić sa Veterinarskog fakulteta Sveučilišta u Zagrebu. Uslijedio je Treći kongres 1987. godine u Malome Lošinju za predsjedanja profesora Ivana Bašića. Zbog ratnih nedaća otkazan je Četvrti kongres koji se trebao održati 1991. godine u Dubrovniku tijekom mandata profesora Draška Šermana sa Medicinskog Fakulteta Sveučilišta u Zagrebu. Peti kongres je održan 1994. godine u Puli, kada je predsjednik Društva bio profesor Nikola Ljubešić sa Instituta "Ruđer Bošković" u Zagrebu, dok je Šesti kongres održan 1997. godine u Opatiji kada je Društvom predsjedavao profesor Ivica Valpotić sa Veterinarskog fakulteta Sveučilišta u Zagrebu. Konačno, u Hvaru na Hvaru 2000. godine održan je Sedmi kongres pod predsjedanjem profesorice Jasne Franekić Čolić sa Prehrambeno-biotehnološkog fakulteta Sveučilišta u Zagrebu.

Sadašnje vodstvo Društva, želeći maksimalno smanjiti troškove organizacije Kongresa, umjesto privlačnih jadranskih krajolika, prelijepih otočkih ili priobalnih hrvatskih gradova i gradića, te smirujućeg šuma mora izabralo je Zagreb, užurbanu i poslovnu hrvatsku metropolu kao mjesto zbivanja Osmoga hrvatskoga biološkog kongresa smatrajući da će na taj način Kongres biti dostupan najširoj biološkoj zajednici, a pogotovo mlađim istraživačima(icama).

Osmi hrvatski biološki kongres nastupa u vrijeme kada svjetska biologija, možda više nego ikada preispituje vlastita ranija stajališta te traži nove paradigme i eksperimentalne pristupe za njihovu provjeru. Ovo je također vrijeme velikih svjetskih znanstvenih dostignuća, poput sekvenciranja ljudskog genoma, ali i vrijeme trezvenijeg suočavanja sa sve većom prijetnjom specifičnog ljudskog djelovanja na prirodu. Konačno, ovo je i vrijeme kada bazična biologija sa svojim fundamentalnim dostignućima, nepovratno, pomoću nevjerojatnih tehničkih rješenja i primjena, ali i novih paradigmi, mijenja pristupe i razmišljanja ne samo u modernom liječenju ili, na primjer u supernaprednoj poljoprivredi te proizvodnji lijekova, već i u filozofskim razmatranjima mjesta čovjeka u cjelokupnoj zbilji.

Hrvatska biologija u nekima od tih područja ravnopravno slijedi svjetske trendove dok u drugima vidljivo zaostaje. Osmi hrvatski biološki kongres, u tradiciji ciljeva Prvoga kongresa, iskreni je pokušaj da se te razlike prezentiraju te pokušaju prevladati. Štoviše, ovaj Kongres, zadržavajući naslov središnji hrvatski biološki skup, najširi je sajam cijelokupne palete hrvatskih bioloških istraživanja te razmišljanja i ideja hrvatskih biologa.

Predsjednica Hrvatskog biološkog društva 1885
Višnja Besendorfer



The now traditional Croatian Biological Congress was first held in 1981 in the city of Poreč in Istria. The first Congress was organized by Prof. Oskar Springer then president of the Croatian Biological Society.

During the last twenty years this Congress has become the premiere event in which the full spectrum of Croatian biological research is highlighted and presented with reference to current trends in European and world biological research.

The current leadership of the Croatian Biological Society has chosen Croatia's dynamic capital city of Zagreb as this year's venue. In this way we hope to broaden our access to the Congress to the widest possible audience, especially to young scientists and students.

The eighth Croatian Biological Congress is taking place at a time when biology is undergoing a renaissance and reflecting on past knowledge in search of new paradigms and new experimental approaches to unresolved questions. Not only is this a time of great achievements (the sequencing of the human genome to name but one), it is also a time of serious contemplation of man's impact on the biological world. Fundamental biological research has transcended previously held boundaries to fundamentally change the fields of medicine, agriculture, pharmaceuticals and even philosophy and the definitions of man's place in the world.

In some areas Croatia's biological research community is in full step with the wider international community, while in others we lag behind. As was true for the first Congress, so too today, the Congress hopes to bridge the gaps and strengthen our ties to the international research community.

President of the Croatian Biological Society
Višnja Besendorfer

Previous Congresses:

1981, Poreč: President, Prof. Oskar Springer, Faculty of Science, University of Zagreb
1984, Zadar: President, Prof. Hrvoje Gomerčić, Faculty of Veterinary Medicine, University of Zagreb
1987, Mali Lošinj: President, Prof. Ivan Bašić, Faculty of Science, University of Zagreb
1991, Dubrovnik: President, Prof. Draško Šerman, Medical School, University of Zagreb (canceled due to the war situation in Croatia)
1994, Pula: President, Nikola Ljubešić, Institute "Ruđer Bošković", Zagreb
1997, Opatija: President, Ivica Valpotić, Faculty of Veterinary Medicine, University of Zagreb
2000, City of Hvar: President: Jasna Franekić Čolić, Faculty of Food Technology and Biotechnology, University of Zagreb

Hrvatski biološki kongres u organizaciji Hrvatskoga biološkog društva postao je tradicionalan, dobro poznati skup hrvatskih biologa. U radu Kongresa sudjeluju i znanstvenici iz susjednih zemalja, Europe i svijeta. Idejni začetnik i glavni organizator prvoga Kongresa 1981. godine u Poreču bio je tadašnji predsjednik Hrvatskoga biološkoga društva, profesor dr. sc. Oskar Springer. Hrvatski biološki kongres bio je tada zamišljen, prvenstveno, kao smotra hrvatske biologije te prikaz naših dostignuća i mogućnosti u danome trenutku. Neobično nam je drago što je ova znanstvena koncepcija kongresa i ove godine ostala sačuvana. Posebna pozornost posvećena je mladim istraživačima koji po prvi puta dobivaju priliku izložiti rezultate svojih istraživanja te sudjelovati u znanstvenim raspravama. Nastavnici biologije također sudjeluju u radu Kongresa čime im se otvara mogućnost da se upoznaju s novim istraživanjima i dostignućima u polju biologije, što će se zasigurno pozitivno odraziti i u nastavnom procesu.

Ovaj se Kongres održava u vrijeme izuzetnih otkrića u polju biologije; od onih na molekularnoj i staničnoj razini, pa do novih spoznaja o raznolikosti i međuovisnosti živoga svijeta i njegova okoliša. Kongres omogućuje cjelovit pogled u modernu biologiju, daje poticaj za postavljanje jasnih znanstvenih pitanja s ciljem potpunijeg razumijevanja živoga svijeta. Zastupljena su sva područja moderne biologije: od genetike i genomike do evolucije, od stanične i molekularne biologije do proteomike, bioinformatike i genomskih tehnologija, od botanike do kulture biljnoga tkiva i genetički promijenjenih biljaka, od zoologije i biologije mora do molekularne sistematike i ponašanja životinja, od ekologije i konzervacijske biologije do zaštite prirode.

Međunarodno sudjelovanje omogućuje izravan susret širokog kruga naših biologa sa sudionicima iz svijeta, upoznavanje s njima, razgovore i razmjenu iskustava, a možda i pronalaženje zajedničkih interesa te međusobno povezivanje u budućem radu. Nadamo se da će strani sudionici s ovog skupa ponijeti sliku o znanstvenom radu hrvatskih biologa, a također će nam prenijeti svoje najnovije spoznaje i poglede na pojedina područja suvremene biologije.

U okviru Kongresa održat će se okrugli stol o pitanjima unaprjeđenja nastavnih planova i programa u visokoškolskoj nastavi iz biologije i prirodoslovlja pri čemu valja slijediti smjernice Bolonjskog procesa, ali u skladu s vlastitim osobitostima. Važan događaj za članove Hrvatskoga društva za biljnu fiziologiju bit će Simpozij iz biljne fiziologije, koji se održava u okviru Hrvatskog biološkog kongresa. Poseban dan posvećen je pitanjima kopnenih voda, a predviđene su i stručne ekskurzije.

Osmi hrvatski biološki kongres s međunarodnim sudjelovanjem daje pregled sadašnjih znanstvenih i stručnih bioloških istraživanja u Hrvatskoj, a zahvaljujući sudjelovanju stranih znanstvenika ostvarit će se i izravan dodir s uzbudljivim i najnovijim dostignućima u svijetu. Nadamo se kako će i ovoga puta prikaz onoga što se u Hrvatskoj događa u području biologije biti sveobuhvatan da će pomoći upoznavanju stučnjaka i znanstvenika koji se bave različitim problematikama te time stvoriti preduvjete što širem ispreplitanju i povezivanju različitih područja.

U ime Znanstvenog odbora
Marijana Krsnik-Rasol

Miroslav Plohl

The Croatian Biological Congress as organized by the Croatian Biological Society has become a traditional event well known to Croatian biologists. Congress participants include scientists from neighboring countries, Europe and the world. In 1981, Prof. Dr. Oskar Springer first conceived and organized the initial Croatian Biological Congress. From the outset the Congress was envisioned as an event where a wide range of biological science representing Croatia's scientific achievements could be showcased. We are pleased that over the years this primary goal of the Congress has been maintained. We especially draw your attention to the many young scientists for whom this Congress represents their first official appearance on the scientific stage. Biology teachers are also an integral component of the congress where they can see the newest trends in biological research and discuss ways to communicate the wonder and excitement of this field to future students.

This Congress is taking place at a time of breathtaking discoveries in biology from the molecular to the cellular, through to novel aspects of biological diversity and the inter-relationship of the living world with its environment. The Congress reflects the breadth of modern biology and will stimulate the generation of novel ideas and new questions to be answered in our search for an understanding of the living world.

All areas of modern biology are represented at the Congress: from genetics and genomics to evolution, from cell and molecular biology to proteomics, bioinformatics and genome technologies, from botany to plant tissue culture to genetically engineered plants, from zoology to the biology of the sea and molecular systematics, from the biology of animals, to ecology, biological conservation and environmental protection.

International participation in the Congress allows for direct contact of a wide Croatian audience with international scientists where we envision stimulating interactions which may even lead to scientific collaboration. We hope that our international participants will leave with a wider recognition of biological research in Croatia as well as imparting their latest results and scientific views to the Croatian audience.

In the Congress programme we will also have a round table discussion on questions of how to improve the teaching of university level biology in the context of the Bologna declaration, but also considering local specificities. Another important sub-event within the Congress is a Symposium dedicated to the subject of Plant Physiology. In addition, a single day of the Congress has been dedicated to fresh water issues. Expert excursions are foreseen as well.

We hope that the presentations of the 8th Croatian Biological Congress will generate widespread interest in the diverse fields of biological research and continue to foster the evolution of inter-disciplinary research among members of Croatia's research community.

On Behalf of the Scientific Committee

Marijana Krsnik-Rasol

Miroslav Plohl

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VIROLOGIJA, MIKROBIOLOGIJA I MIKOLOGIJA
*Biologia virorum et microborum, Biologia fungorum*
VIROLOGY, MICROBIOLOGY AND MYCOLOGY

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*Biologia animalium*
ANIMAL BIOLOGY

Animalna fiziologija, imunologija, histologija, embriologija
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# UVODNO PREDAVANJE
# OPENING LECTURE

PL-1

# FROM MACOREVOLUTION TO MICROEVOLUTION - TRACING THE EVOLUTION OF DEVELOPMENTAL PROCESSES AND IDENTIFYING GENES INVOLVED IN ADAPTATIONS

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Studying the evolution of developmental processes promises to yield insight at the macroevolutionary level, i.e. the major evolutionary transitions that have shaped the organismic diversity. It is now clear that all animals use a conserved set of genes to set up the body axes and to pattern their body. Changes in the regulation of these genes or changes in their interactions can yield evolutionary novelties. In my talk I will take the comparison between segmentation in arthropods and somitogenesis in vertebrates as an example of how to trace conserved patterns as a basis for understanding the changes that can bring about evolutionary innovations. There is, however, also another level of evolutionary research that deals with the microevolutionary processes that lead to environmental adaptations and the formation of species. Little is so far known about the genes that are involved in these processes. I will present approaches of how such genes can be identified. It can be anticipated that studying the genetics of adaptive traits will be a major theme of future evolutionary biology.

# ZAVRŠNO PREDAVANJE
# CLOSING LECTURE

PL-10

## THE WARFARE BETWEEN PARASITES AND HOSTS

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The Red Queen paradigm posits that parasites and hosts are engaged in a genetic arms race creating a reciprocal selective pressure pushing their respective evolution towards high paces. Unilateral slowing down can be lethal. Hyper-mutation of immunoglobulin genes and of viruses are *par excellence* examples of selection for high mutation rates - in immunoglobulin genes to generate efficient antibodies and in viruses to escape such antibodies. In this arms race, parasites and hosts often use similar molecular strategies (recombination and point mutation) and even the same weapons, such as cytosine deaminases. Extensive cytosine deamination is an awesome modifyer of genetic information, and the repair of uracil in single-stranded DNA becomes a destroyer of genetic information (akin to restriction of phage genomes by bacterial hosts) effectively used as an innate immunity against retroviruses such s the HIV. To survive such restriction by cytosine deamination HIV has evolved the Vif (viral infectivity factor) protein – a potent inhibitor of cellular cytosine deaminase. We are allowed to speculate that such awesome mutator system was readily adopted by the immune cells to hypermutate their own immunoglobulin genes: cytosine deaminase AID is required for immunoglobulin class-switch recombination, hyper-conversion and hyper-mutation processes. Bacteria have evolved at least three strategies for tuning the level of genetic variation in their populations: (i) mutagenesis localized to the genes under frequent selective pressure (contingency genes), (ii) second-order selection for general constitutive mutators arising by the loss of genetic fidelity functions, such as mismatch repair, and (iii) turning on and off cellular mutagenic activities, such as the SOS polymerases. The paradigm of biological evolution by vertical and horizontal variation applies adequately to human cultural evolution. Ideas, or memes, evolve similarly by small changes (mutations) and by recombination of different ideas, but the horizontal (or lateral) transfer akin to infectious processes seems to be maximized in the evolution of ideas. Can we consider the human brain as an ecosystem for competing selfish and parasitic ideas?

# PLENARNA PREDAVANJA
# PLENARY LECTURES

PL-2

## ISLANDS, BEETLES AND MOLECULAR PHYLOGENIES

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Islands, and in particular oceanic archipelagos, evoke images of remote idealised worlds, often utopical. In the mind of biologists, this sometimes transforms in an opportunity of understanding the modes and intricacies of evolution in isolation after unlikely arrival. Darwin himself, after returning from his expedition around the world, formally established the close association between islands and evolution. Back in London, the diversity of sampled Galapagos finches triggered his idea of evolutionary change. Beetles comprise one of the most successful organism assemblages. They show a diversity that exceeds that of any other animal or plant group on Earth. As Dr. Crowson put it "*...they provide excellent illustrations and test cases for almost every evolutionary principle...*" Phylogenetic information, together with biogeographical and ecological data contributes in understanding the processes that have led to this species richness. In the last decades, advances in both obtaining and analysing DNA sequence data, provide a phylogenetic framework from which reconstruct the evolutionary history of beetles and the DNA sequences they harbour. Molecular phylogenies allow deducing colonisation and diversification patterns in oceanic islands. The Canary archipelago, which Darwin sadly missed on the *Beagle* voyage because of quarantine regulations, is part of Macaronesia (Canary Is., Madeira, the Azores, the Salvages and Cape Verde Is.). The Canaries are the closest to the Northwest African coast. The archipelago was formed sequentially by volcanic activity over the last 20 my and occur in one of the "hot spots" of worldwide biodiversity. Of the enormous existing diversity on the islands, around 1200 of the 7500 known species of terrestrial invertebrates are endemic Coleoptera. As it is the norm for island biota, many beetles on the Canaries are the product of rapid speciation and extensive radiations. Traditionally, many groups were considered as relicts from the Tertiary, but recent phylogenetic studies based on mitochondrial DNA sequences have changed this picture. The work of many research teams, including ours on beetles, suggests more recent speciation than once assumed, even during the Pleistocene. The accumulation of phylogeographic data allows generalising, but also shows that many factors can complicate the interpretation of particular patterns. Islands usually are fragile ecosystems. Isolated insects usually show limited dispersal ability, small populations and/or major adaptive shifts. These factors relate to an increment of extinction risks. Phylogenies, seen from both a geographical and a historic perspective, can contribute to conservation efforts, identifying particularly endangered groups.

PL-3

# INITIATION OF MEIOTIC RECOMBINATION BY SPO11: NEWS FROM A CENTRAL MEIOTIC PATHWAY

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Meiosis is a fascinating process, during which chromosomes are sorted into four complete sets, to allow generation of haploid gametes to close the cycle of sexual reproduction. The repair of programmed DNA-double strand breaks (DSBs) is exploited to achieve temporal linkage between corresponding chromosomes (chiasmata), which in turn serve to align homologous chromosomes on the spindle and thus enable a reductional division. Spo11, an evolutionarily conserved homolog of the archaebacterial Topoisomerase VI A-subunit, has been identified as the catalytic activity responsible for those breaks. DNA cleavage by Spo11 is highly controlled and in *S. cerevisiae* at least eleven genes are essential for its activity. We have set out to investigate physical interaction between Spo11 and recombination hotspots using chromatin immuno precipitation (ChIP). Using a Myc-tagged version of Spo11, we determined three essential factors for Spo11 – hotspot association. Moreover, we could determine which genes are required for full levels of this interaction, for its local precision or for correct dissociation. Most importantly a set of observations leads us to reconsider some aspects of the current working model for Spo11 cleavage. Briefly, by analogy to topo2 cleavage reactions, Spo11 is currently thought to act as a dimer, attacking the phosphodiester backbone of the DNA by transferring the ester bond to the enzyme's conserved tyrosine. As a result, the "cleavage complex", a covalent Spo11-5'DNA intermediate is predicted which is still able to reseal the break. In an effort to understand how irreversible breaks arise, it is assumed that the covalent attachment of Spo11 to the DNA ends first become irreversible and are later removed in the course of repair. Because *rad50S*, *mre11S* and *com1?* mutants accumulate breaks with Spo11 attached to the ends, it was thought that these genes were involved in repairing DNA ends blocked by Spo11. Several observations lead us to think differently. First of all, we are unable to detect the predicted covalent, irreversible intermediate between DNA and Spo11 by ChIP. Second, we observed novel phenotypes for *rad50S* and *com1?* mutants. We noticed a delay for about an hour in formation of stable breaks and a corresponding accumulation of a novel intermediate, a form of Spo11, tightly bound to uncleaved hotspot. We propose that this form corresponds to the reversible cleavage complex. If true, the primary failure in *rad50S* and *com1?* mutants lies in the termination of the reversible stage and not in the repair of blocked DNA ends. Thus the role of Rad50, Mre11 and Com1 could be to give directionality to the Spo11 cleavage reaction, by helping Spo11 to cleave itself off the broken DNA. We propose that no stable covalent Spo11-DNA intermediate occurs in wild type cells, and that Rad50 and Mre11 are not capable to repair stable Spo11-DNA prod-

ucts, once they have formed. Formation of these products in *rad50S* and *com1?* mutant cells may in fact be a secondary consequence of the inability of these cells to exit continuous cleavage and resealing cycles.

PL-4

## THE FUNCTION OF GENOMES IN BIOENERGETIC ORGANELLES

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Chloroplasts and mitochondria originated as bacterial symbionts. The larger, host cells acquired genetic information from their prokaryotic guests by lateral gene transfer. The prokaryotically-derived genes of the eukaryotic cell nucleus now function to encode the great majority of chloroplast and mitochondrial proteins, as well as many proteins of the nucleus and cytosol. Genes are copied and moved between cellular compartments with relative ease, and there is no known obstacle to successful import of any protein precursor from the cytosol, so there ought to be a selective advantage for chloroplasts and mitochondria abdicating all genes and gene expression to the nucleus and to cytosolic translation. What might chloroplast- and mitochondrially-encoded proteins have in common that confers a selective advantage on the cytoplasmic location of their genes? The proposal advanced here is that co-location of chloroplast and mitochondrial genes with their gene products is required for rapid and direct regulatory coupling. Redox control of gene expression is suggested as the common feature of those chloroplast and mitochondrial proteins which are encoded *in situ*. The hypothesis is abbreviated *CORR*, standing for *co-location for redox regulation*. Recent evidence is consistent with this hypothesis, and its underlying assumptions and predictions are described.

PL-5

## PROGRAM OF CELL DEATH: THE PLANT WAY

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Programmed cell death (PCD) is indispensable for the development of most multicellular organisms. Plants employ PCD mechanisms for the expression of tissue and organ functions, for efficient nutrition and reproduction, and for disease resistance. Plant PCD occurs in senescent leaves and petals, in the tissues of reproductive organs, in the xylem of vascular bundles, in root caps, and in cortex that is

forming aerenchyma. The cells of these tissues or organs display characteristic features as their PCD progresses. The activation of cell-death-associated hydrolytic enzymes, protein degradation, and breakage of nuclear DNA strands are observed frequently. The mechanism of PCD in different categories of plant cells appears to be different, suggesting that more than one pathway of PCD is likely to operate in plants in comparison to animals, where there seems to be only one program. Several spatially and temporally distinctive programs of cell death, evidenced by loss of nuclei and all sub-cellular membranous organizations, have been detected in developing caryopses of maize. The TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP-fluorescein nick end labeling) stain that is diagnostic of apoptotic-like PCD, identified TUNEL-positive nuclei in the nucellus during the early stages of kernel development. 14 – 20 days after pollination the positive TUNEL reaction was also detected in the scutellum, coleoptile, root cap and principally in the suspensor. In placento-chalazal (PC) cell layers of maternal pedicel tissue two distinctive sub-domains of PCD are present. The early wave of PCD was TUNEL-negative and specific to only the fertilized caryopses, indicating that the signaling for enucleation in these maternal cells originated in the zygotic tissues. The distinctive feature of the cell walls of enucleated cells was prominent callose deposit. The later wave of PCD was restricted to the files underneath the already enucleated cells, and was TUNEL-positive and callose -negative. Based on collective evidence we infer that the early PCD in PC layer may have osmolytic etiology and may lead to activation of the post-vascular transport function of the PC layer. On the other hand, the second wave of PCD may be senescent-related, and involved in the formation of an abscission layer associated with seed maturity.

PL-6

## MOLEKULARNA KONTROLA PRIJELAZA OD JAJNE STANICE DO ZAMETKA

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Reprogramiranje genoma dviju diferenciranih stanica- jajne stanice i spermija- se normalno desava u citoplazmi jajne stanice i omogucuje kreiranje totipotentnih stanica ranog zametka. Isto tako je moguce u toj sredini reprogramirati jezgre diferenciranih somatskih stanica . Jajna stanica sadrzava sve molecule neophodne za reprogramiranje i aktivaciju genoma zametka. Cilj naseg istrazivanja je potpuno razumijevanje te jedinstvene molekularne sredine. U tu svrhu odredjujemo koji su geni znacajno eksprimirani u tom periodu i pokusavamo ih grupirati na bazi kompjuterske analize funkcionalnih motiva. Istovremeno eliminiramo ciljanim mutacijama aktivnost specificnih gena u jajnoj stanici s ciljem utvrdjivanja njihove uloge u tom periodu.

## MOLECULAR CONTROL OF THE OOCYTE TO EMBRYO TRANSITION – THE REPROGRAMMING ENVIRONMENT

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Normal reprogramming of the genomes from two differentiated cell types, the sperm and egg, occurs in the oocyte cytoplasm resulting in the totipotent stem cells of the cleavage stage embryo. Nuclei from differentiated somatic cells can also be reprogrammed to totipotency in the oocyte milieu during the oocyte to embryo transition. The full-grown oocyte contains all of the molecules sufficient for reprogramming and activation of the embryonic genome. To understand this unique molecular environment we are analyzing the abundant transcripts present by annotating ESTs from the oocyte and 2-cell stage embryo cDNA libraries using the Gene Ontology vocabulary. Perturbation of specific molecules by oocyte-targeted mutagenesis, coupled with a search of the transcriptome for members of specific molecular pathways indicates the networks expressed in the newly formed embryo.

PL-7

## THE EMERGENCE OF CONSERVATION BIOLOGY: AN EMERGENCY DISCIPLINE WITH INSOLUBLE TASKS?

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Life on Earth has undergone several drastic mass exctinction events. They were caused either by intrinsic (atmospheric changes) or by extrinsic (asteorid collision) factors. From its origin onwards Man (that can merely be seen as a new intrinsic cause of species collapse) has altered ecosystems drastically, initiating long ago the presently ongoing mass extinction. The new extinction process has also dramatically accelerated over the past decades due to an unprecedented, rapidly burgeoning human population. At present *Homo sapiens* uses and/or redirect about 40% of Earth's net primary production (NPP) for its own needs. A corresponding amount of energy and resources is simply no longer available to other organisms coexisting with us on this planet. This NPP extraction rate by human beings is going to grow up further given that 1) human population is expected to reach 9 billions before the end of this century (vs 6 billions today); 2) amelioration of human life standards is going to increase further dramatically, across all nations. As a scientific discipline, Conservation biology is principally concerned with increasing the probability that living organisms (from local geographic races to entire ecosystems) survive in the

long term. For that purpose it has to provide the necessary scientific knowledge for the development of sound strategic tools for biodiversity management. However, given the presently prevailing environmental conditions on this planet, where should Conservation Biology acts in priority? There is no simple and definite answer to that question. Indeed strategic responses and solutions to that major issue are probably as numerous as the community of people working in Conservation Biology (from fundamental researchers to activists involved in practice) is diverse. As an example I will present the strategy followed by our research group at the Zoological Institute, Bern University, in collaboration with the Swiss Ornithological Institute.

PL-8

## SPERMATOGENESIS IN FISH: A MODEL SYSTEM FOR THE ANALYSIS OF BIOLOGICALLY ACTIVE COMPOUNDS

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The process of spermatogenesis is initiated when the primordial germ cells in the gonadal anlage of the embryo obtain the signal to differentiate into spermatogonia (as opposed to oogonia). A series of mitotic divisions is then initiated followed by meiosis and, finally, the process of spermiogenesis takes place which involves the cytological differentiation of the post-meiotic cells into functional spermatozoa. We have studied all of these processes in fish (medaka, tilapia) with the aim in mind to quantify the respective developmental processes such that the experimental interference with specific substances of interest can be analysed. As model compounds we chose selected flavonoids which are common in food (e.g. genistein and quercetin) since the biological activities of these natural compounds are of considerable medical interest. The process of sex determination during early juvenile development has been studied by exposing monosex populations of the medaka (*Oryzias latipes*) to ethynylestradiol or the isoflavone genistein. Genetic males exposed to low concentrations of these estrogens led to complete sex reversal and the development of fertile females. The expression of several genes involved in the early sex-specific differentiation of the gonads was studied by RT-PCR and *in situ* hybridisation. While the genetic control of sex determination can only be studied *in vivo*, the later differentiation processes proceed *in vitro* under suitable conditions, in particular the mitotic proliferation of spermatogonia and the differentiation of spermatocytes into spermatozoa. We have prepared and characterised primary cultures of Medaka testis cells. Quantification of the differentiation processes has been achieved by measuring the incorporation of BrdU into proliferating cells and by means of flow cytometry using the fluorescent dye carboxyfluorescein. The differentiation of spermatocytes into spermatozoa *in vitro* can be blocked efficiently by the

isoflavone genistein. We have also paid attention to physiological properties of spermatozoa which are relevant for fertility and the effects of flavonoids on parameters like cell motility, the level of free $Ca^{++}$, the production of reactive oxygen species and the mitochondrial membrane potential. The flavonol quercetin was shown to affect some of these parameters. In summary, the obtained results show that efficient test systems have been developed which are suitable to analyse the process of spermatogenesis *in vitro* and to study the effects of test compounds both on the cellular and the molecular level.

PL-9

## CONSERVATION BIOLOGY: SCIENCE FOR THE 21ST CENTURY

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Conservation biology has been a branch of applied science for many decades, but was formally developed as a discipline in the United States in the 1980's. As ecologists became more aware of declining species, loss of biodiversity, and habitat fragmentation, we saw a need for sound science in policy-making. We also recognized that conservation requires more than basic biological information – human needs and the values of society must also be considered. The Society for Conservation Biology and the Union of Conservation Scientists are composed of biologists, ecologists, social scientists, economists, and educators. We play a critical role in policy-making by advising governments on sustainable resource use, land use planning, and pollution mitigation. We work with industry and communities to reduce the effects of human activities on the environment. Most importantly, conservation biologists provide a critical link between academic science and the transfer of our knowledge to society. Our quality of life is closely tied to the health of the environment and the maintenance of ecosystems. As human population growth continues to pressure these systems, it will fall to conservation scientists to identify the critical components and processes needed for all species on our planet, including *Homo sapiens*.

# POSEBNA PREDAVANJA
# SPECIAL LECTURES

Predavanje povodom dodjele medalje "Spiridon Brusina"
Hrvatskog prirodoslovnog društva

PL-11

## LIJEČENJE ZLOČUDNIH BOLESTI PRESAĐIVANJEM STANICA: POSTIGNUĆA I IZAZOVI

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Najčešći oblici liječenja zločudnih bolesti presađivanjem stanica uključuju presađivanje koštane srži (ili krvotvornih matičnih stanica, KMS) i staničnu imunoterapiju. KMS se presađuju obično sa svrhom obnove krvotvornog sustava uništenog visokim dozama kemoterapije i ionizantnim ozračivanjem (namjerno kao pri liječenju leukemija ili kao popratne pojave liječenja drugih zločudnih bolesti). Pri staničnoj se pak imunoterapiji presađuju diferencirane imunosne stanice poput dendritskih stanica, T stanica i/ili NK stanica. Te se stanice presađuju sa svrhom poticanja obnove imunosti nakon presađivanja KMS i/ili za poticanje citotoksične imunosti specifične za tumor. U zadnje doba ta dva pristupa konvergiraju, posebno pri manipulaciji učinka presadka protiv leukemije i pri presađivanju KMS za liječenje čvrstih tumora. Da se poveća dostupnost KMS za presađivanje, te se stanice sada izdvajaju i iz periferne krvi i iz pupčane vrpce. Razmjerna je novina i presađivanje haploidentičnih KMS što je znatan korak prema univerzalnoj transplantabilnosti i, možda, presađivanju KMS potpuno nekompatibilnih transplantacijskih antigena. Velik se trud ulaže i u smanjivanje otrovnosti postupka pripreme bolesnika (tj. razaranja krvotvornog i imunosnog sustava) za prihvat presadka. U okviru tog truda vrijedno je navesti uspostavljanje krvotvornog kimerizma upotrebom "minipresadaka" nakon djelomične imunosupresije. Znatan se napredak postiže i u presađivanju diferenciranih imunosnih stanica. Primjerice, nedavno razvijeni presadci alogenskih T stanica kojima je uklonjena imuna reaktivnost prema primaocu se sada klinički ispituju u svrhu potiskivanja posttransplantacijskih infekcija (*Aspergillus*, citomegalovirus i dr.) i povećanog preživljenja. Novootkriveni mehanizmi regulacije aktivnosti NK stanica se istražuju kao dodatno sredstvo uklanjanja zločudnih stanica. Općenito, terapijska vakcinacija dendritskim stanicama i adoptivna imunoterapija imunosnim efektorskim stanicama specifičnim za tumor su se pokazale terapijski djelatnima u mnogim kliničkim istraživanjima. No, učinci su još uvijek nedovoljni i nepredvidljivi. Zato se trenutni napori u području stanične terapije tumora usmjeruju prema istraživanju činilaca koji stoje na putu boljem kliničkom učinku.

"Spiridon Brusina" Lecture

## CELL THERAPY OF CANCER: THE BENEFITS AND THE CHALLENGES

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The most common modes of cell therapy of cancer include bone marrow (or hematopoietic stem cell, HSC) transplantation and cellular immunotherapy. HSC transplantation has been used mostly to reconstitute patients' hematopoietic systems destroyed by high–dose chemotherapy and radiation therapy either intentionally as is the case of leukemia or as the side effect as in the case of other malignant diseases. Cellular immunotherapy has employed differentiated immune cells such as dendritic cells, T cells and/or NK cells to enhance establishment of immunity after HSC transplantation and/or for targeted immune ablation of malignancy. Recently, the two approaches have been merging, particularly through the manipulation of graft-versus-leukemia effect following allogeneic HSC transplantation and the use of HSC transplantation in the treatment of solid tumors. To increase the availability of HSC for transplantation, additional sources of HSC such as peripheral blood and umbilical cord blood have been introduced. Another new frontier has been opened by successful transplantation of haploidentical HSC, a further step towards universal access to transplantation and, possibly, to transplantation of fully HLA–mismatched HSC. Major efforts are underway to reduce toxicity due to conditioning (i.e., destruction of the hematopoietic system and immunity) for transplantation. Notable among these efforts is the establishment of hematopoietic chimerism by "minitransplants" following nonlethal conditioning. The use of differentiated immune cells in reconstitution of immunity following HSC transplantation and tumor eradication has made major advances as well. For example, the lately developed allogeneic immune T cell grafts devoid of host-specific immune reactivity are now clinically tested for suppression of post-transplant infections (due to Aspergillus, cytomegalovirus, etc.) and improvement of survival rates. The newly discovered mechanisms of regulation of NK-cell activity are now investigated as an additional tool in the eradication of malignancy. Dendritic-cell mediated therapeutic vaccination against cancer and adoptive immunotherapy by tumor-specific immune effector cells have been shown to be therapeutically feasible in numerous clinical trials. However, immune reconstitution and tumor remission rates are still low and unpredictable. Current efforts in cellular immunotherapy are focused onto the understanding of factors standing in the way of higher rates of clinical response.

Predavanje pod pokroviteljstvom Predsjednice Hrvatskog biološkog društva 1885

PL-12

## ANTROPOGENETIČKA ANALIZA (MIKRO)EVOLUCIJE JADRANSKIH IZOLATA – PROBLEMI I PERSPEKTIVE

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Bit će prikazani rezultati antropogenetičkih istraživanja koja su provedena tijekom trideset godina na stanovnicima istočnog Jadrana. Modeli proučavanja (mikro)evolucije primjenom holističkog analitičkog pristupa, bit će razmotreni prikazom različitih povijesnih, demografskih i lingvističkih populacijskih osobitosti te mono i poligeno determiniranih bioloških svojstava stanovništva (eritrocitnih antigena, serumskih proteina, izoenzima, DNA biljega: VNTR, HLA klasa I i klasa II, STR, mtDNA i kromosoma Y: STR i bialelnih polimorfizama, pa do niza kompleksnih svojstava). Mogućnost primjene antropogenetičkih istraživanja u stjecanju novih znanstvenih spoznaja bit će razmotrena s ciljem njihove praktične primjene u biologiji i biomedicini.

Lecture Under the Auspice of the President of the Craotian Biological Society

## ANTHROPOGENETIC (MICRO)EVOLUTION OF ADRIATIC ISOLATES – PROBLEMS AND PERSPECTIVES

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The results of anthropogenetic studies performed on Eastern Adriatic populations during the last thirty years will be presented. (Micro)evolution models based on holistic analytic approach will be considered using different historical, demographic and linguistic characteristics of these populations as well as mono and polygenetically determined biological variables (erythrocyte antigens, serum proteins, isoenzymes, DNA markers: VNTR, HLA class I and class II, STR, mtDNA and chromosome Y: STR and biallelic polymorphisms, and a number of complex traits). Potential practical application of anthropogenetic research in biology and biomedicine will be also discussed.

GENETIKA, MOLEKULARNA GENETIKA, EVOLUCIJA
*BIOLOGIA GENORUM ET EVOLUTIO*
GENETICS, MOLECULAR GENETICS, EVOLUTION

Uvodna tematska predavanja
Introductory Lectures

IL-1

## JESU LI NEKODIRAJUĆE CENTROMERNE SEKVENCE DNA ODGOVORNE ZA NASTANAK VRSTA?

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Heterokromatinski kromosomski odjeljci razlikuju se od eukromatinskih prema brojnim neobičnim molekularnim svojstvima, kao što su visoka razina kondenzacije kromatina tijekom cijelog staničnog ciklusa, kasna replikacija DNA i mali broj gena. Usprkos tomu, vitalne kromosomske funkcije, centromerne i telomerne, sadržane su u heterokromatinu. Glavni oblik sekvence DNA centromernog heterokromatina su nekodirajuće, uzastopno ponovljene sekvence, ili satelitne DNA. Dok je centromerna uloga sačuvana tijekom evolucije, elementi DNA nisu, te se sastav centromernih satelitnih DNA najčešće razlikuje već i među blisko srodnim vrstama. Nedavnim je pokusima dokazano kako srodne vrste dijele zbirku, ili biblioteku, satelitnih sekvenci koje istovremeno postoje u centromernom heterokromatinu, ali se značajno razlikuju prema broju kopija. Vrsti svojstven profil satelitnih DNA posljedica je umnažanja pojedinih satelita i nastanka glavne satelitne DNA koja je u određenoj vrsti zastupljena u velikom broju kopija, dok ostale satelitne sekvence ostaju prisutne u malom broju kopija. Nukleotidne sekvence satelitnih DNA mijenjaju se različitim brzinama te i time doprinose nastanku satelitnih profila svojstvenih za vrstu. Nadalje, reorganizacija i umnožavanje od ranije postojećih sekvenci dovodi do nastanka novih satelitnih jedinica ponavljanja. Razlike među glavnim satelitnim DNA centromernog heterokromatina mogu kod hibrida onemogućiti pravilno sparivanje i razdvajanje kromosoma. Promjene u glavnoj satelitnoj DNA praćene su također i promjenama u odgovarajućim centromernim histonskim proteinima, kao što su pokazali Henikoff i suradnici. Obje promjene mogu se odraziti na plodnost u križanjima jedinki koje ih nose, postavljajući time prepreku razmnožavanju, koja na koncu može predstavljati okidač u procesu nastanka vrsta.

## ARE NONCODING CENTROMERIC DNA SEQUENCES RESPONSIBLE FOR SPECIATION?

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Heterochromatic chromosomal segments differ from their euchromatic counterparts by a number of peculiar molecular characteristics, such as high level of

chromatin condensation throughout the cell cycle, late DNA replication, and paucity of genes. However, functions vital for chromosomes, those of centromeres and telomeres, are embedded within heterochromatin. Major DNA components of centromeric heterochromatin are non-coding tandemly repeated sequences, or satellite DNAs. While centromeric function is conserved in evolution, DNA components are not, and composition of centromeric satellites differs often even between closely related organisms. Recent experiments have provided evidence that related species share a collection, or a library, of satellite sequences that coexist in centromeric heterochromatin, but differ significantly in a copy number. Species–specific satellite DNA profiles result from amplification of a particular satellite into a major, highly abundant satellite DNA, while the others are still present as minor repeats. Nucleotide changes of satellite sequences occur within a broad variety of rates, thus contributing to the genesis of species–specific satellite profiles. In addition, rearrangements and amplifications of preexisting sequences lead to generation of novel satellite repeating units. Differences among major satellite components of centromeric heterochromatin could disable regular chromosome pairing and disjunction in hybrids. Changes in major satellite DNAs are followed by changes in corresponding centromeric histones, as demonstrated by Henikoff and coworkers. Both effects could reflect on the fecundity in crosses between individuals, thus raising a reproductive barrier that would ultimately act as a trigger in the speciation process.

IL-2

## GEN *recA* IZ BAKTERIJE *Streptomyces rimosus*: EKSPRESIJA I FUNKCIONALNA ANALIZA MUTIRANIH *recA*

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Proteini RecA kod streptomiceta su duži od većine bakterijskih RecA (376 ak kod bakterije *S. rimosus*), zato što posjeduju produženi C-kraj bogat lizinima i alaninima, koji predvidivo čini pozitivno nabijenu uzvojnicu. Svi donedavni pokušaji konstrukcije *recA* nul mutanta u streptomicetima su bili bezuspješni, pa se vjerovalo da je protein RecA neophodan za preživljavanje ovih bakterija, zbog uloge u očuvanju kromosomskih krajeva prilikom replikacije linearnog genoma. Mi smo prvi uspješno konstruirali RecA deficijentni soj bakterije *S. rimosus*. Ovaj *recA* mutant može sintetizirati samo vrlo kratki i nefunkcionalni protein $RecA_{1-151}$, kojem nedostaje 225 ak na C-kraju, pa je soj izrazito osjetljiv na UV-svjetlo. Unošenjem divljeg tipa gena *recA* u mutirani soj putem plazmida, otpornost na UV-svjetlo se vraća na normalu. No međutim, na isti način unešeni geni *recA* koji kodiraju RecA proteine s delecijom od 21 ak, odnosno 51 ak na C-kraju, ne mogu u cijelosti povratiti otpornost na UV-svjetlo. Koristeći metodu produženja klice uspjeli smo identificirati dva početka transkripcije gena *recA* u bakteriji *S. rimosus*,

smještene 134, odnosno 36 pb uzvodno od inicijacijskog kodona AUG. Duži transkript je poslijedica aktivnosti SEP (*Streptomyces-E.coli* like) promotora, koji sadrži i mjesto vezanja za protein LexA (Cheo-box). Zanimljivo, ovaj je promotor slabo aktivan, jer glavnina transkripta započinje s G na poziciji –36. Glavni promotor gena *recA* je UV-inducibilan i jedinstven zbog razmaka od samo 11 pb između položaja –35 (TTGTCA) i –10 (TAGCGT). Gotovo identičan promotor je odgovoran i za glavninu transkipta gena *recA* kod bakterije *Mycobacterium tuberculosis*, a pretragom genoma bakterije *S. coelicolor* našli smo ga uzvodno od mnogih (potencijalno) UV-inducibilnih gena. Kako je djelomično sačuvana i primarna struktura u 11 pb dugoj razmaknici između pozicija –35 i –10, konsensus za ovaj novi tip promotora kod streptomiceta glasi: TTGTCAGTGGC-N6-TAGggT.

## THE *recA* GENE FROM *Streptomyces rimosus*: EXPRESSION AND FUNCTIONAL ANALYSIS OF *recA* MUTANTS

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RecA proteins in *Streptomyces* are larger than most bacterial RecAs (376 aa in *S. rimosus*) and they contain a unique C-terminal extension rich in lysines and alanines, which predictably forms an alpha helix. As several attempts to construct *recA* null mutants in streptomycetes had been unsuccessful, it was believed that the requirement for RecA is connected with their linear chromosome and the specific role of RecA in preventing the loss of chromosomal ends. However, we recently successfully constructed the first viable RecA-deficient strain of *S. rimosus*. This *recA* strain can synthesize only very short, inactive $RecA_{1-151}$ protein lacking last 225 aa. Consequently, the strain is highly sensitive to the UV-light. Introduction of a plasmid carrying the wild type *S. rimosus recA* gene completely restored the UV resistance of the *recA* mutant to wild type levels. On the other hand, plasmids that carried mutant *recA* genes encoding proteins with 21 or 51 aa deletions at the C-terminus could not fully rescue UV sensitivity of the *recA* strain. Using primer-extension analysis we identified two transcription start sites for the *recA* gene in *S. rimosus*. A longer, weak transcript is initiated from the distal *Streptomyces-E. coli*-like promoter (SEP) that contains a Cheo-box like sequence, a putative LexA binding site: GAAC-N4-ATTC. The major start site of transcription is a G at position –36 and this shorter transcript significantly increases in response to DNA damage by the UV-light. The –35 box (TTGTCA) and –10 box (TAGCGT) of the strong promoter are only 11 bp apart and this promoter is almost identical to the major *recA* promoter of *Mycobacterium tuberculosis*. Moreover, the same type of promoter is present in upstream regions of many genes/ORFs in *S. coelicolor* genome, including numerous (potentially) UV-inducible genes. As DNA sequence between the predicted –35 and –10 boxes is also partially conserved, the consensus sequence for this new, *recA* type promoter in *Streptomyces* is: TTGTCAGTGGC-N6-TAGggT.

IL-3

## MEĐUDJELOVANJE REKOMBINACIJSKIH PUTOVA RecBCD I RecFOR U BAKTERIJI *Escherichia coli*

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Tri aktivnosti RecBCD enzima (helikaza, 5´→3´ egzonukleaza i nanošenje RecA proteina na jednolančanu DNA) su esencijalne za rekombinaciju u bakteriji *Escherichia coli*. Specifičan mutant u nukleaznom katalitičkom centru RecB-a (*recB1080*) producira enzim koji nema nukleaznu aktivnost i sposobnost nanošenja RecA proteina na jednolančanu DNA, a zadržava samo helikaznu aktivnost. Postoje kontradiktorni podaci o njegovoj rekombinacijskoj sposobnosti. U *recF*- genetičkom zaleđu, *recB1080* mutant je rekombinacijski deficijentan, dok je u *recF*+ genetičkom zaleđu rekombinacijski proficijentan. Objašnjenje za ove kontrastne fenotipove bi moglo biti da RecFOR promovira nastajanje filamenta RecA-jednolančana DNA i na taj način komplementira nedostatak RecB1080CD enzima. Mi smo testirali tu hipotezu koristeći tri eksperimentalna pristupa *in vivo*. Uspoređivali smo rekombinacijsku sposobnost jednostrukih mutanata *recB1080*, *recO*, *recR* i *recF* sa dvostrukim mutantima *recB1080 recO*, *recB1080 recR* i *recB1080 recF*. Pokazali smo da RecFOR funkcija komplementira deficijenciju u rekombinaciji i popravku DNA kod *recB1080* mutanta i da je nanošenje RecA proteina na jednolančanu DNA neovisno o RecFOR u *recB1080 recD* dvostrukom mutantu gdje je taj proces ovisan o RecB1080C(D-) enzimu. Prema našim rezultatima i podacima drugih autora, tri esencijalne aktivnosti za inicijaciju rekombinacije u *recB1080* mutantu su rezultat djelovanja različitih proteina: helikaza dolazi od RecB1080CD enzima, 5´→3´ egzonukleaza od RecJ proteina, a nanošenje RecA proteina na jednolančanu DNA je rezultat djelovanja RecFOR sistema.

## RecFOR FUNCTION IS REQUIRED FOR DNA REPAIR AND RECOMBINATION IN A RecA LOADING-DEFICIENT R*ecB* MUTANT OF *Escherichia coli*

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The RecA loading activity of the RecBCD enzyme, together with its helicase and 5´→3´ exonuclease activities, is essential for recombination in *Escherichia coli*. One particular mutant in the nuclease catalytic center of RecB, *i. e.*, *recB1080*, produces an enzyme that does not have nuclease activity and is unable to load RecA protein onto single stranded DNA. There are, however, previously published contradictory data on

the recombination proficiency of this mutant. In a *recF*- background the *recB1080* mutant is recombination deficient, whereas in a *recF*+ genetic background it is recombination proficient. A possible explanation for these contrasting phenotypes may be that the RecFOR system promotes RecA-single-strand DNA filament formation and replaces the RecA loading defect of the RecB1080CD enzyme. We tested this hypothesis by using three *in vivo* assays. We compared the recombination proficiencies of *recB1080*, *recO*, *recR*, and *recF* single mutants and *recB1080 recO*, *recB1080 recR* and *recB1080 recF* double mutants. We show that RecFOR functions rescue the repair and recombination deficiency of the *recB1080* mutant and that RecA loading is independent of RecFOR in the *recB1080 recD* double mutant where this activity is provided by the RecB1080C(D-) enzyme. According to our results as well as previous data, three essential activities for the initiation of recombination in the *recB1080* mutant are provided by different proteins, *i. e.*, helicase activity by RecB1080CD, 5´→3´ exonuclease by RecJ and RecA-single-strand DNA filament formation by RecFOR.

OP-1

## AN EVOLUTIONARY ANALYSIS OF ORPHAN GENES IN *Drosophila*

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Orphan genes are protein coding regions that have no recognizable homologue in distantly related species. A substantial fraction of coding regions in any genome sequenced so far consists of such orphan genes, but their evolutionary and functional significance is not understood. We present a re-analysis of the *Drosophila melanogaster* proteome that shows that there are still between 26 - 29% of all proteins without a significant match with non-insect sequences and that these orphans are under-represented in genetic screens. To analyse the characteristics of orphan genes in *Drosophila*, we have used sequence comparisons between cDNAs retrieved from two *D. yakuba* libraries (embryo and adult) and their corresponding *D. melanogaster* orthologues. We find that the cDNA library from adults yields double as many orphan genes than the one from embryos, suggesting a higher constraint on the use of conserved genes in embryos. The orphan genes evolve on average more than three times faster than non-orphan genes, although the width of the evolutionary rate distribution is similar for both classes. In particular, some orphan genes show very low substitution rates, which are comparable to otherwise highly, conserved genes. We propose a general model for orphan gene evolution that takes these large rate differences into account and suggests that they are caused by episodic phases of fast and slow divergence. The general characteristics of orphan genes in *Drosophila* suggest that they may be involved in the evolution of adaptive traits and that slow evolving orphan genes may be particularly interesting candidate genes for identifying lineage specific adaptations.

OP-2

## SATELITNE DNA RODA *Tribolium* (Coleoptera)

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Satelitne DNA (stDNA) su visoko zastupljeni, uzastopno ponovljeni odsječci DNA, smješteni poglavito u (peri)centromernim/telomernim regijama eukariotskih genoma. Premda nije utvrđena sveobuhvatno prihvaćena funkcija ovih nekodirajućih odsječaka, smatra se da bi mogli imati važnu ulogu u organizaciji kromosoma te u aktivnosti centromera. Važni skladišni štetnici iz roda *Tribolium* pokazali su se, s obzirom na visoki udio satelitnih odsječaka u genomu, kao vrlo prikladni eksperimentalni organizmi za proučavanje stDNA. Uz prethodno opisane

stDNA u genomima vrsta *T. freemani*, *T. confusum*, *T. madens* i *T. castaneum*, okarakterizirane su četiri nove stDNA u genomima vrsta *T. brevicornis*, *T. anaphe*, *T. destructor* i *T. audax*. Unatoč drastičnim razlikama u nukleotidnom slijedu, novoopisani sateliti dijele nekoliko zajedničkih obilježja. Najintrigantnija među njima složena je građa ponavljajućih jedinica, koja ukazuje na mogućnost tvorbe stabilnih struktura ukosnice, zahvaljujući učestaloj prisutnosti direktno i obrnuto ponovljenih motiva u nukleotidnom slijedu. U kontekstu evolucijske dinamike, stDNA vrsta *T. audax* i *T. madens* nedvosmisleno pripadaju satelitnoj porodici istog porijekla, bez obzira na određene promjene u nukleotidnom slijedu te u organizaciji ponavljajuće jedinice. Satelitne DNA preostalih šest vrsta roda *Tribolium* vrsno su specifične i ne pokazuju međusobnu sličnost. S obzirom na tako oprečne evolucijske obrasce, sateliti roda *Tribolium* pogodan su model za proučavanje evolucijske dinamike kongeneričnih vrsta, kao i za objedinjavanje često kontroverznih objašnjenja evolucije stDNA eukariotskih organizama općenito.

## SATELLITE DNAs OF THE GENUS *Tribolium* (Coleoptera)

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Satellite DNAs (stDNAs) are highly abundant, tandemly repeated DNA sequences located mainly at the (peri)centromeric/telomeric regions of the eukaryotic genomes. Albeit no universal and unambiguous function has been assigned to these noncoding sequences, it has been assumed that they play a role in chromosomal organization and centromere activity. Important stored product pests from the genus *Tribolium* have appeared to be suitable experimental organisms for stDNA analyses owing to high percentage of satellite sequences in their genomes. In addition to the satellites previously described in the *T. freemani*, *T. confusum*, *T. madens,* and *T. castaneum* genomes, four novel stDNAs have been characterized in the genomes of the *T. brevicornis*, *T. anaphe*, *T. destructor*, and *T. audax*. Although these four stDNAs differ in nucleotide sequences drastically, they share several features common to all *Tribolium* satellites among which the most provocative peculiarity is the complex structure of monomeric units, reflecting the possibility to form stable dyad formations due to frequent presence of direct and inverted repeats. Concerning evolutionary dynamics, stDNAs of *T. audax* and *T. madens* belong undoubtedly to the same ancestral satellite family, in spite of certain variations in nucleotide sequence and its organization. Contrarily, the other six *Tribolium* species possess species-specific stDNAs, which show no mutual similarity. In view of such opposite patterns observed, the *Tribolium* satellites represent an appropriate model for gaining deeper insight into the evolutionary dynamics among congeneric species, as well as for getting a clue to the controversial interpretations of stDNA evolution among eukaryotic organisms in general.

OP-3

## RAZNOLIKOST VARIJANTI SATELITSKIH DNA U GENOMU VRSTE *Donax trunculus*

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Satelitske DNA su nekodirajuće uzastopno ponovljene sekvence DNA koje u genomu sačinjavaju dugačke nizove i koje su obično smještene u heterokromatinskim dijelovima kromosoma. U morskih beskralješnjaka satelitske DNA su dosad bile proučavane samo kod nekoliko vrsta uključujući i mekušca *Donax trunculus*. U vrsti *D. trunculus* već je opisano pet različitih satelitskih porodica. Sve one se nalaze u malom broju kopija (manje od 1% od ukupne genomske DNA) i razlikuju se po sekvenci monomera iako neki od satelita dijele oligonukleotidne motive. Otkrili smo tri nove satelitske DNA unutar genoma vrste *D. trunculus*. Iako svi imaju jednaku duljinu monomera, samo se dva satelita mogu smatrati srodnim, a razlikuju se jedino po dijagnostičkim točkastim mutacijama, dok je treći različit i po nukleotidnom slijedu, GC sastavu i udjelu u genomu. Ovi sateliti su posebno intrigantni zbog očigledne razlike u razini homogenosti sekvence između kloniranog podskupa i cijelog genomskog skupa svake od tih satelitskih DNA. Ovakav suživot visoko homogeniziranih ali i međusobno divergentnih satelitskih podgrupa može ukazivati na djelovanje dinamičnih procesa koji dovode do raznolikosti satelitskih DNA unutar genoma.

## DIVERSIFICATION OF SATELLITE DNA VARIANTS IN THE *Donax trunculus* GENOME

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Satellite DNAs are non-coding, tandemly repeated DNA sequences that comprise long arrays in the genome and are usually located within heterochromatic regions of chromosomes. Among marine invertebrates, satellite DNAs have so far been studied in only a couple of species including the mollusc *Donax trunculus*. In *D. trunculus*, five distinct satellite families have been described previously. They are all present in low number of copies (less than 1% of total genomic DNA) and are distinct in their monomer sequence although some families share oligonucletide motifs. We have discovered three new satellites resident within the *D. trunculus* genome. Even though they all share the same monomer length, only two satellites can be considered related, differing only by diagnostic point mutations, whereas the third one is dissimilar in its

nucleotide sequence, GC content, and genomic abundance. These satellites are especially intriguing because of the apparent discrepancy in the level of their sequence homogeneity between the cloned subset and the entire genomic set of these satellite families. Such coexistence of highly homogenized but divergent satellite subgroups might indicate the presence of dynamic processes of satellite diversification within the genome.

OP-4

## DISTRIBUCIJA I ORGANIZACIJA rDNA KROMATINA U KROMOSOMIMA I JEZGRI NEKIH VRSTA RODA *Quercus* I AKTIVNOST 18S-5.8S-26S rDNA LOKUSA

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U identičnim kariotipovima vrsta *Q. petraea* i *Q. robur* dva su 18S-5.8S-26S (18S) rDNA lokusa – glavni (NOR-1) i mali (NOR-2) rDNA lokus - smještena na dva različita kromosoma komplementa. Prema intenzitetu hibridizacijskog signala nakon fluorescentne hibridizacije *in situ* (FISH) utvrđeno je da glavni 18S rDNA lokus sadži puno više kopija rRNA gena od malog lokusa. Upotrebom FISH tehnike s 18S rDNA sondom na metafazne/interfazne kromosome (svjetlosna mikroskopija) te hibridizacije *in situ* na sekcije tkiva korjenčića (elektronska mikroskopija) studirana je distribucija i organizacija rDNA kromatina unutar glavnog 18S rDNA lokusa, odnosno jezgrice. rDNA kromatin unutar ovog lokusa ograniziran je kao vrlo kondenzirani odnosno slabije kondenzirani, disperzni kromatin. Dva tipa rDNA kromatina mogu se razlikovati nakon FISH-a na metafaznim/interfaznim kromosomima u vidu lokaliziranog FISH signala odnosno točkastog, disperznog FISH signala. Lokalizirani signal, koji odgovara vrlo kondenziranom rDNA kromatinu, nalazi se u proksimalnom i/ili distalnom djelu lokusa, dok je disperzni signal, koji odgovara slabije kondenziranom kromatinu, prisutan u obliku točkica oko jezgrice. Željelo se utvrditi kako su dva tipa rDNA kromatina organizirana unutar jezgrice. U tu je svrhu primijenjena *in situ* hibridizacija s 18S rDNA sondom na ultra tanke sekcije meristematskog tkiva. Vrlo kondenzirani kromatin utvrđen je izvan jezgrice te asociran s jezgricom. Međutim, fokusi kondenziranog kromatina identificirani su i unutar jezgrice zajedno s mjestima difuznog signala za koji se pretpostavlja da odgovara potpuno dekondenziranoj rDNA. Dekondenzacija je u uskoj vezi s transkripcijskom

aktivnosti rRNA gena. Prema tome, u jezgrici ovih vrsta rDNA segmenti koji su sačinjeni od transkripcijski aktivnih gena izmjenjuju se sa rDNA segmentima u kojima su geni transkripcijski neaktivni i heterokromatinizirani. Ovaj rezultat ukazuje da se unutar jezgrice, usprkos uvriježenom mišljenju, može nalaziti i transkripcijski neaktivni heterokromatinizirani rDNA kromatin. FISH na pahitenskim nitima potvrdio je da jezgricu uvijek organizira samo glavni lokus kod obje vrste pa se vjeruje da je mali 18S rDNA lokus transkripcijski neaktivan.

## ACTIVITY OF 18S-5.8S-26S rDNA LOCI AND DISTRIBUTION OF rDNA CHROMATIN IN NUCLEI OF SOME *Quercus* SPECIES

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Distribution of rDNA chromatin within the individual locus was investigated in *Quercus michauxii, Q. petraea, Q. robur* and *Q. suber* using fluorescence *in situ* hybridization (FISH) to metaphases and interphases in light microscope (LM) and on nuclei in electron microscope (EM) level. The four species have two 18S-5.8S-26S (18S) rDNA loci; the major (NOR-1) and the minor one (NOR-2) positioned on two different chromosomes. Major 18S rDNA locus consists of two types of rDNA chromatin in these species – highly condensed and less condensed dispersed rDNA chromatin seen as localized and punctuate FISH signal, respectively. Localized FISH signal was present at the proximal and/or the distal end of the locus. Dispersed rDNA chromatin could have been seen as a large amount of pear-like dots spreading between the two ends of the locus and associated with the nucleolus/nucleoli. Distribution of the two types of rDNA chromatin in metaphases/interphases of *Q. petraea* and *Q. robur* was directly related with the location and organization of the rDNA chromatin in nucleolus of this species at EM level. The condensed rDNA chromatin, visible as localized signal positioned away from the nucleolus in LM, was detected in EM as large labeled extra-nucleolar and peri-nucleolar rDNA chromatin. However, labeled condensed rDNA chromatin of variable sizes was also detected inside the nucleolus (intra-nucleolar rDNA chromatin) together with decondensed rDNA chromatin visible as many diffuse labeled sites. The decondensed rDNA chromatin probably contained transcriptionally active rRNA genes. Thus, expressed rDNA segments were separated with unexpressed condensed rDNA segments inside nucleolus of the both species. Comparison of

*in situ* hybridization patterns with 18S rDNA probe in metaphases of *Quercus michauxii, Q. petraea, Q. robur* and *Q. suber* (LM) and *in situ* hybridization pattern in nucleolus of *Q. petraea* and *Q. robur* (EM) suggest that organization of rDNA chromatin could be similar in all four species. In addition, the activity of NOR-1 and NOR-2 loci was analysed in *Q. petraea* and *Q. robur* after FISH to pachyten spreads. The association of the major but not the minor 18S rDNA locus with nucleolus/nucleoli in mitosis and meiosis suggested that only the major rDNA locus was active, at least in these two species.

OP-5

## 16S rRNA DIVERSITY OF SULFATE-REDUCING BACTERIA BELONGING TO DELTA-PROTEOBACTERIA IN SALT MARSH SEDIMENTS

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Sulphate-reducing bacteria (SRB) dominate the mineralization of organic matter in salt marsh environments. In these environments, SRB are believed to be represented by two phylogenetically distinct groups, Gram-positive and delta-Proteobacteria. We assessed the 16S rRNA diversity of delta-Proteobacterial SRB in salt marsh sediments of Plum Island Sound, MA by PCR, cloning and sequencing. Samples taken over the course of a year were extracted to ensure representation of shifts in SRB community tied to seasonal growth cycles of the marsh grass Spartina. A clone library was constructed using 385F (selective for SRB delta-Proteobacteria) and 1492R (general bacteria) primers. Phylogenetic relationships were detected by the neighbour-joining method, whereas overall sampling efficiency and diversity were estimated using rarefaction analysis and the Chao-1 richness estimator. The estimated diversity was much higher than expected, indicating that even the extensive sampling of over 1,000 sequences did not account for the total diversity as extrapolated by rarefaction analysis. A large fraction of this diversity can be attributed to the ribotypes with the >99% identity and we have shown that this microheterogeneity is not due to technical or experimental errors. Most of the ribotypes belonging to delta-Proteobacterial group were associated with members of the Desulfobacteriaceae (*Desulfosarcina, Desulfobacterium* and *Desulfonema*) and Desulfobulbaceae (*Desulfobulbus, Desulfofustis* and *Desulfotalea*) group. Sequences from the Desulfomicrobiaceae and Desulfoarculaceae groups were also observed. Over two thirds of the delta-Proteobacterial ribotypes were most similar to *Desulfosarcina* and *Desulfobacterium*, suggesting that complete oxidizers dominate this environment.

OP-6

## MRE11 MUTATION CAUSE GENOME INSTABILITY, DEVELOPMENTAL DEFECTS AND STERILITY IN *Arabidopsis thaliana*, BUT THE TELOMERE LENGTH IS UNAFFECTED

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Mre11/Rad50/Xrs2 (MRX) complex has a central role in both homologous and non-homologous end-joining DNA repair pathways. This complex is important for DNA double strand breaks (DSB) repair and processing of DSBs during meiosis. Mutations in MRX complex in vertebrates are lethal. This, however, is not the case in plants, where viable mutants of the model plant *Arabidopsis thaliana* have been recently characterised. Interestingly, MRX complex is localised to human telomeres. Since Mre11p has nuclease activities, there is a possibility that Mre 11p is involved in resection of the telomeric C-strand, and consequently in telomere replication or T-loop formation. In order to test the role of Mre11p in telomere length maintenance, we characterised mutants with T-DNA insertion in MRE11 gene. Mutant plants exhibited developmental defects and were sterile. Cytogenetic analyses showed relatively high percentage of anaphase bridges in mitosis, indicative for chromosome/chromatid fusions. To investigate whether these defects were caused by telomere dysfunction, we analysed telomere structure using terminal restriction fragment analysis (TRF). Our results showed that telomere length of *mre11* mutants remained unchanged and similar to the wild type. To further study genetic interactions between MRE11 and other genes involved in the telomere maintenance (TERT-telomerase reverse transcriptase, KU70 non-homologous end-joining factor), we generated *mre11 ku70* and *mre11 tert* double mutants as well as *mre11 tert ku* triple mutant. These mutants are currently being examined for cytogenetic defects and telomere structure.

OP-7

## ODGOVOR NA SELEKCIJU I PROMJENE FREKVENCIJE ALELA U M3S POPULACIJI KUKURUZA POD REKURENTNOM SELEKCIJOM

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Populacija kukuruza Maksimir 3 sintetik (M3S) je stvorena međukrižanjem 12 inbred linija s dobrom kombinacijskom sposobnošću u odnosu na testere iz Reid Yellow Dent i Lancaster heterotične skupine. Nakon završena dva ciklusa rekurentne selekcije, na osnovi ocjene potomstava iz samooplodnje, na prinos zrna u M3S istražen je učinak selekcije na srednju vrijednost svojstava populacije i promjenu frekvencije alela. Odgovor na selekciju je procjenjen u S0 generaciji (populacija *per se*) i S1 generaciji (samooplođena populacija *per se*) na osnovi poljskih pokusa provedenih na tri lokacije. Da bi se procijenile promjene u frekvenciji alela, 96 jedinki iz početne (M3SC0) kao i poboljšane (M3SC2) populacije su genotipizirane na devet SSR lokusa. Prinos zrna nije se promijenio u S0 generaciji nakon dva ciklusa selekcije. Selekcija je bila učinkovitija u S1 generaciji, gdje je opaženo prosječno povećanje prinosa od 0.44 $Mgha^{-1}ciklus^{-1}$ ili 12.4% $ciklus^{-1}$. Inbriding depresija za prinos zrna je bila za 10% manja u M3SC2 u usporedbi s M3SC0 populacijom. Testom selekcijske neutralnosti je utvrđeno nekoliko ne-neutralnih SSR lokusa u populaciji čije promjene frekvencije alela se ne mogu objasniti samo učinkom genetskog drifta. Prosječna genska raznolikost se smanjila s 0.57 u M3SC0 na 0.45 u M3SC2 populaciji, rezultirajući s interpopulacijskim koeficijentom inbridinga od 0.15. Rezultati dobiveni u poljskim pokusima i SSR analizama upućuju na akumulaciju gena s dominantnim učinkom u M3S populaciji.

## RESPONSE TO SELECTION AND ALLELIC FREQUENCY CHANGES IN THE M3S MAIZE POPULATION UNDER RECURRENT SELECTION

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Maksimir 3 Synthetic (M3S) maize population was created by intercrossing 12 inbred lines possessing good combining ability with testers from both Reid Yellow Dent and Lancaster heterotic groups. Two cycles of selfed progeny recurrent selection for grain yield was completed in the M3S and the effects of breeding on mean performance of the population as well as on allele frequency changes were examined. Response to selection was estimated in the S0 generation (population *per se*) and S1 generation (population *per se* selfed) on the basis of field trials conducted at three locations. To

estimate changes in allele frequencies 96 individuals from the base (M3SC0) as well as improved (M3SC2) population were genotyped at nine simple sequence repeats (SSR) loci. Grain yield in the S0 generation was not affected after two cycles of selection. Selection was more effective in the S1 generation in which the average rate of response was 0.44 $Mgha^{-1}cycle^{-1}$ or 12.4% $cycle^{-1}$. Inbreeding depression for grain yield was 10% lower in the M3SC2 compared to M3SC0 population. A test of selective neutrality identified several non-neutral SSR loci in the population whose allele frequency changes cannot be explained by genetic drift alone. Mean gene diversity decreased from 0.57 in the M3SC0 to 0.45 in the M3SC2 population, resulting in an interpopulation inbreeding coefficient of 0.15. Results obtained from the field trials and molecular marker analysis suggested accumulation of genes with dominant action in the M3S population.

OP-8

## CROATIAN GENETIC LANDSCAPE – Y CHROMOSOMAL PERSPECTIVE

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Sixteen biallelic and eight microsatellite markers were typed in the sample of 457 Croatian men from the mainland and four Eastern Adriatic islands (Krk, Brač, Hvar, Korčula). Based on the analysis of biallelic markers nine different haplogroups were characterized. The most frequent was haplogroup I followed by R1a and R1b haplogroups. High frequency of haplogroup I in Croatian populations and the pattern of its microsatellite diversity make Adriatic coast a likely source of the recolonization of Europe following the Last Glacial Maximum. A higher frequency of haplogroup I in the southern island populations is contrasted with higher frequency of haplogroup R1a chromosomes in the northern island of Krk and in the mainland confirming different routes of settlement. R1a frequency could be a sign of the Slavic impact in the Balkan region. Haplogroups J, G, and E that are related to the spread of farming characterize the minor part (12.5%) of the Croatian Y chromosomal lineages. A relatively high frequency (14%) of lineages belonging to P*(xM173) cluster, unusual for European populations, was found in the southern island of Hvar indicating a connection with Central Asian populations, possibly the Avars. In sum, clear and meaningful in terms of ethnogenesis, gradients and diversity of Croatian paternal lineages testify to their largely Paleolithic European ancestry, a minor Neolithic influx from the Near East, as well as Slavic and Avar expansions.

OP-9

## CROATIAN GENETIC LANDSCAPE – MITOCHONDRIAL DNA PERSPECTIVE

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Mitochondrial DNA variation was analyzed in a sample of 721 Croatians from the mainland and four Eastern Adriatic islands (Krk, Brač, Hvar, Korčula). The hypervariable segment I (HVS-I) of the control region of mtDNA was sequenced between nps 16024 and 16383 and a set of twenty one RFLP sites diagnostic for main Eurasian haplogroups was analyzed in order to confirm the exact haplogroup affiliation of mtDNA HVS-I lineages. This analysis yielded 27 maternal haplogroups with the highest predominance of haplogroup H (41%). The phylogenetic analysis of haplogroups showed extremely high number of haplotypes that confirm their heterogeneity as well as their early age ancestry. A finding of larger contribution of Paleolithic than Neolithic mtDNA markers confirms Paleolithic legacy of the majority of the investigated Croatian gene pool. Distribution of mtDNA Paleolithic and Neolithic markers corresponds to findings in geographically proximate populations. When mainland and island populations were considered separately, a pronounced effects of evolutionary forces, especially founder effect and genetic drift, mediated a specific distribution of mtDNA haplogroups on the islands. The greatest haplogroup diversity was noted in the mainland population, whereas the lowest in the island of Korčula. The results of the Exact test showed a statistically significant difference in mtDNA haplogroup frequency among all investigated populations.

OP-10

## UČESTALOST ALELA TRI MIKROSATELITSKA LOKUSA (D6S265, D6S273, I MIB) KOD BOLESNIKA S INZULIN OVISNIM DIJABETESOM (IOD)

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Inzulin ovisan dijabetes (IOD) je autoimuna bolest uzrokovana razaranjem b stanica gušterače koje stvaraju inzulin. U nastanku bolesti sudjeluju genetski čimbenici, kao i čimbenici okoliša. Brojna istraživanja ukazala su na povezanost klasičnih gena HLA, kao i drugih gena unutar regije HLA, u razvoju

bolesti. Ovim istraživanjem obuhvaćeno je 100 bolesnika, podijeljenih u 4 skupine na osnovi tipizacije HLA (DR3/DR4, DR3, DR4, non DR3/DR4), te 150 zdravih, nesrodnih osoba. Geni HLA razreda II tipizirani su metodom PCR-SSP, dok su PCR produkti mikrosatelitskih lokusa (D6S265, D6S273, i MIB) analizirani elektroforezom u automatskom sekvenceru (ALFexpress). Analiza raspodjele učestalosti alela otkrila je razlike između bolesnika i zdravih ispitanika za lokuse D6S273 i MIB, dok za lokus D6S265 nije uočena nikakva razlika. Aleli D6S273-126bp (p=0,02565), D6S273-138bp (p=0,01967), D6S273-140bp (p=0,00035), MIB-348bp (p=0,01302), MIB-350bp (p=0,00677) i MIB-352bp (p=0,02289) bili su prisutni s većom učestalošću među bolesnicima, dok su aleli D6S273-130bp (p=0,02945) i MIB-334bp (p=0,00105) unutar bolesnika bili slabije zastupljeni. Usporedba bolesnika koji nisu bili pozitivni za antigene DR3 i DR4 s kontrolom ukazala je na postojanje razlike samo za alele D6S273-132bp (p=0,03986), MIB-332bp (p=0,04376) i MIB-350bp (p=0,01212) što ukazuje na njihovu neovisnu ulogu u razvoju bolesti. Usporedba DR3 i DR4 pozitivnih skupina pokazala je da su svi ostali mikrosatelitski aleli povezani s lokusom HLA-DR. Iz naših rezultata može se zaključiti da su lokusi D6S273 i MIB dva moguća nova biljega u podložnosti za IOD u Hrvatskoj.

## DISTRIBUTION OF ALLELES AT THREE MICROSATELLITE LOCI (D6S265, D6S273, AND MIB) AMONG PATIENTS SUFFERING FROM TYPE I DIABETES

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Type I diabetes is an autoimmune disease caused by destruction of the insulin producing b cells in pancreas. Both genetic and environmental factors participate in the development of disease. Recent studies have indicated the existence of non-HLA loci associated with type I diabetes in addition to previously known association with the HLA class II genes. One hundred patients selected on the basis of their HLA alleles, where classified in 4 groups (DR3/DR4, DR3, DR4, non DR3/DR4). Control group consisted of 150 healthy individuals. PCR-SSP method was performed for HLA class II typing, while 3 microsatellite loci (D6S265, D6S273, and MIB) were analysed using automated ALFexpress sequencer. Analysis of the allele distribution revealed differences between patients and controls for D6S273 and MIB loci while there was no difference observed for D6S265 locus. Alleles D6S273-126bp (p=0,02565), D6S273-138bp (p=0,01967), D6S273-140bp (p=0,00035), MIB-348bp (p=0,01302),

MIB-350bp (p=0,00677) and MIB-352bp (p=0,02289) were present with an increased frequency among patients, while alleles D6S273-130bp (p=0,02945) and MIB-334bp (p=0,00105) appeared with decreased frequency. We also examined the possibility that these alleles showed association with disease as result of their linkage with HLA class II genes, by excluding individuals positive for DR3 and/or DR4 antigens from further analysis. Difference was then observed only for alleles D6S273-132bp (p=0,03986), MIB-332bp (p=0,04376) and MIB-350bp (p=0,01212). Comparison of DR3 and DR4 positive groups showed that other alleles were in linkage disequilibrium with HLA class II genes. In conclusion, our results suggest two new possible susceptibility markers for type I diabetes: D6S273 and MIB loci.

OP-11

## MOLEKULARNA ANALIZA NAJUČESTALIJIH ALELA LOKUSA HLA-B U HRVATSKOJ

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Pet najčešćih antigena lokusa HLA-B u Hrvatskoj su HLA-B5, -B7,-B8, B-12, te - B35. Molekularne metode pokazale su da nije riječ o jednom entitetu, već o većem broju podtipova svake od navedenih specifičnosti, koje se ne mogu otkriti serološkim testovima. Cilj ovog rada bio je ispitati raznovrsnost podtipova navedenih antigena na uzorku od 156 zdravih, nesrodnih ispitanika s područja grada Zagreba. Istraživanje je bilo dio antropološke studije u sklopu 13-og međunarodnog radnog sastanka o histokompatibilnosti. Aleli su određivani lančanom reakcijom polimeraze i sondama specifičnim za ekson 2 i 3 gena HLA-B (metoda PCR-RLS). Za specifičnost HLA-B5 predominanat je bio alel B*5101, dok je između 16 različitih podtipova gena B*07 najučestaliji bio podtip B*0702 (9,9%). Analiza specifičnosti HLA B*35 pokazala je nazočnost 4 različita podtipa: B*3501 (5,6%), B*3502 (2,9%) i B*3503 (2,9%), a podtip B*3508 uočen je samo jedanput. U grupi alela skupine B*08 najzastupljeniji je bio podtip B*0801, dok su aleli B*0804 i B*0805 bili sporadičmo zastupljeni. Rezultati analize alela HLA-B44 pokazuju da se podtip B*4402 triput češće javlja nego B*4403, dok je alel B*4501 imalo samo dvoje ispitanika. Tri najčešća haplotipa bila su: A*0201- B*5101 (2,7%), A*0301- B*0702 (3,7%), te A*0101- B-0801 (3,5%). Analizom haplotipova A9-B44 uočili smo da je alel B*4403 uvijek dolazio u kombinaciji s aleleom A*2302, dok je alel B*4402 dolazio u kombinaciji s alelom A*2402. Rezultati ovog rada biti će od velike pomoći u budućim antropološkim studijama, te u programu transplantacije tkiva i organa, osobito kod nesrodnog davateljstva gdje je nužna podudarnost u podtipovima gena HLA

## MOLECULAR ANALYSIS OF THE MOST FREQUENT HLA-B GROUP ALLELES IN THE CROATIAN POPULATION

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The HLA-B5, -B7, -B8, -B12, and B35 are five of the most frequent antigens in Croatians. These alleles are very heterogenous HLA-B specificity with many subtypes, which cannot be distinguished by serology, and only few of them are revealed by biochemical methods. In order to define subtypes of these HLA-B antigens in Croatians, we studied 156 healthy, unrelated donors from wider area of the Croatian capital city, Zagreb. Genomic DNA was isolated by a standard salting-out method. All samples were amplified using PCR and tested with Reverse Line Strip method. Among B*51 alleles, B*5101 was predominant (11.9%) while B*5102 allele occurred twice (0.6%). Allele B*0702 (9.9%) was the only observed allele between 16 different B*07 subtypes. Four different B*35 alleles were observed (B*3501-5.6%, B*3503-2.9%, B*3502-2.9%, B*3520-1.3%, while B*3508 was present once (0.3%). In the group of B8 alleles, B*0801 was most frequent (2.2%), but we also observed B*0804 (1.3%) and B*0805 (0.6%). B*4402 (6.1%) occurred three times more than B*4403, and one individual had B*4407 (0.3%). Allele B*4501 was present twice (0.6%). Analysis of haplotypic associations showed that A*0201-B*5101 (2.7%), A*0301-B*0702 (3.7%), A*0201-B*3501 (2.9%), A*0101-B*0801 (3.5%) and A*0201-B*4402 (1.6%) were most frequent. When HLA-A9-B12 haplotypic associations were analysed, A*2402 associated with B*4402, while B*4403 was always associated with A*2302. This molecular data will be helpful for further antropology studies as well as for comparing findings for our population with results from other Caucasoid populations. It will also serve as reference for donor/recipient matching in organ and bone marrow transplantation program.

OP-12

## ZDRAVKO LORKOVIĆ (1900-1998): BIOGRAFIJA I ZNANSTVENI RAD

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Zdravko Lorković, lepidopterolog ekolog, taksonom, i genetičar, bio je jedan od najvećih hrvatskih biologa 20-tog stoljeća, kako kod nas tako i u svijetu. Bio je dugogodišnji profesor biologije na Medicinskom i Veterinarskom fakultetu. Godine 1965. postao je redoviti član Jugoslavenske Akademije znanosti i umjetnosti (poslije 90-ih, Hrvatske akademije znanosti i umjetnosti). Objavio

je 86 znanstvenih radova i više od 50 stručnih radova, kongresnih sažetaka i nekrologa. Svoje znanje sažeo je 1990. u knjizi iz edicije "The Butterflies of Europe" u poglavlju koje govori o kromosomima leptira, te njihovoj ulozi u sistematici i filogeniji leptira. Lorković je prihvatio i promijenio Mayr-ovu definiciju pojma semispecies, koja je i prihvaćena prema pravilima međunarodne zoološke literature. Opisao je i više novih vrsta leptira. 1986. donirao je svoju zbirku leptira od 40 000 primjeraka Hrvatskom prirodoslovnom muzeju u Zagrebu. Pola čini zbirku faune, dok drugu polovicu zbirke čine leptiri iz pokusa križanja, zajedno s pratećom dokumentacijom. Zbirka leptira zajedno s mikroskopskim preparatima, bibliotekom, pismima, fotografijama, dnevnicima, rukopisima i bilješkama čini posebnu cjelinu u Hrvatskom prirodoslovnom muzeju.

## ZDRAVKO LORKOVIĆ (1900-1998): BIOGRAPHY AND SCIENTIFIC WORK

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Zdravko Lorković was one of the greatest biologists in Croatia in the 20th century and an eminent in world terms. He was a lepidopterist, ecologist, taxonomist, geneticist and an expert in all these fields. He was for many years Professor of Biology in the Medical and Veterinary faculties. From 1965 he was a regular member of the Yugoslav Academy of Sciences and Arts (after 90s', the Croatian Academy of Sciences and Arts). He published 86 scientific papers and more than 50 professional papers, congress reports and obituaries. In 1990 his great knowledge of butterfly chromosomes resulted with writing a special chapter about chromosomes and their role in systematic and phylogeny in the book from the series of monographs "The Butterflies of Europe". Lorković accepted and modified Mayr's definition of the concept of semispecies, and urged that the semispecies category was accepted in international rules of zoological nomenclature. As a butterfly specialist he described several new butterfly species. In 1986 Professor Lorković donated his collection of about 40,000 butterflies to the Croatian Natural History Museum in Zagreb. Half of these form the faunistic collection. Other halves are specimens from his famous crossing experiments between different butterfly species. Together with documentation they are forming the crossing collection. The collection is kept as a special unit, with his microscope slides, library, letters, photographic documentation, diaries, manuscripts and notes in the Croatian Natural History Museum in Zagreb.

P-1

## THE ROLE OF A *recS* MUTATION ON RECOMBINATIONAL DNA REPAIR IN *Escherichia coli*

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Genetic recombination (recombinational DNA repair) is universal to all organisms and it is an important component of all the processes that contribute to DNA metabolism. In *Escherichia coli* there are three pathways of recombination: RecBCD, RecFOR, and RecE. A new *recS* genetic locus in *E. coli*, identified and characterized in our laboratory, might help us to gain a better insight into the interplay of these pathways of recombination, especially RecBCD and RecFOR. A mutation in this gene makes the cells extremely sensitive to UV light. Much of the radiosensitivity is lost when *E. coli* carries an additional mutation in the *recD* gene. We infer that the product of the RecS protein has an essential role(s) in the early steps of homologous recombination and recombinational repair in *E. coli*.

P-2

## ODREĐIVANJE PRIMARNE STRUKTURE MITOHONDRIJSKOG GENOMA MORSKE SPUŽVE *Suberites domuncula*

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Mitohondrijski genom predstavlja jedan od najznačajnijih modela u izučavanju evolucije genoma Metazoa. Čine ga visoko sačuvani geni i većinom se sastoji se od 2 gena za ribosomske RNA, 22 za tRNA, te 13 gena koji kodiraju za proteinske podjedinice uključene u oksidativnu fosforilaciju. Spužve (Porifera) su najstarije i najjednostavnije višestanične životinje. Usporedba rasporeda gena u mitohondrijskom genomu, kao i različite uporabe kodona, korisna je u utvrđivanju filogenetskih odnosa između glavnih skupina Metazoa. U ovome radu analizirani su dijelovi mitohondrijskog genoma morske spužve *Suberites domuncula* (Demospongiae). Do sada smo klonirali, te djelomično odredili primarnu strukturu gena za 16S RNA (oko 800 pb), velike dijelove gena koji kodiraju za citokrom b i citokrom c oksidazu (podjedinice I i II), kao i cjelovite gene koji kodiraju za ATPazu 8 i tRNA Lys. Preliminarni rezultati pokazuju da je uporaba kodona kod *S. domuncula* ista kao i kod filamentoznih alga, odnosno jedina razlika u odnosu na univerzalni genetički kod je što se stop kodon TGA (UGA) čita kao triptofan. Isto je nađeno i kod *Tetilla*

sp., također morske sužve, te kod mnogih koralja. AT sastav gena koji kodiraju za tri spomenuta proteina je visok (66%), što je i glavna karakteristika mitohondrijskih gena kod Metazoa.

## PARTIAL DNA SEQUENCES OF THE MITOCHONDRIAL GENOME FROM THE MARINE SPONGE *Suberites domuncula*

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The mitochondrial genome (mtDNA) represents a major model system in studies on evolutionary genomics in Metazoa. The gene content of metazoan mtDNA is highly conserved and typically consists of genes for 2 ribosomal subunit RNAs, for 22 tRNAs and for 13 protein subunits involved in oxidative phosphorylation.

Sponges (Porifera) are the most ancient and the simplest multicellular organisms. Comparisons of the mitochondrial gene arrangements, as well as genetic code variations, may be useful for evaluating phylogenetic relationships among major metazoan groups. We report here characterization of a portion of the mitochondrial genome from *Suberites domuncula* (Demospongiae). Up to now we cloned and sequenced a 800-bp long part of 16S rRNA gene, large portions of the genes encoding Cytochrome b and Cytochrome c oxidase subunit I (COI) and subunit II (COII), as well as complete gene encoding ATPase subunit 8 and tRNA Lys. Preliminary results already show that the usage of genetic code in *S. domuncula* is the same as in filamentous fungi, i.e. the only difference from the universal code is usage of the stop codon TGA (UGA) for tryptophan. The same was also found in *Tetilla* sp., another marine sponge, and in several corrals. The AT content of tree protein coding sequences is high (66%) what is a general characteristic of mtDNAs in Metazoa.

P-3

## KARAKTERIZACIJA HMGB PROTEINA IZ MORSKE SPUŽVE *Suberites domuncula*

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High mobility group (HMG) proteini su široko rasprostranjeni ne-histonski proteini, smješteni u jezgrima eukariota. Ime su dobili zbog velike elektroforetske pokretljivosti u poliakrilamidnom gelu. HMGB proteine, jednu

od tri skupine HMG proteina, karakterizira HMG-box, DNA-vezno mjesto koje uvodi čvrstu petlju u DNA i veže se s visokim afinitetom za iskrivljene DNA strukture. HMG proteini olakšavaju stvaranje nukleoproteinskih kompleksa, npr. posreduju pri udruživanju kompleksa uključenih u rekombinaciju i transkripciju. Također pomažu remodeliranje nukleosoma. Dva najznačajnija obilježja visoko homologih HMG1 i HMG2 proteina kralježnjaka su dvije slične, no ipak različite, ponovljene HMG-domene (A i B) i dugi kiseli C-terminalni «rep», sastavljen od oko 30 (HMG1), odnosno 20 (HMG2) kiselih (Asp i Glu) ostataka. Identificirali smo i analizirali cDNA koja kodira za HMGB protein iz morske spužve *Suberites domuncula*, te je nazvan HMGBSD. cDNA duga je 1115 pb i kodira za protein dug 183 aminokiseline, predviđene $M_r$ od 21.3 kDa. C-terminalni «rep» čini 13 aminokiselina i ovaj protein vjerojatno spada u skupinu HMG2, HMG proteina. U usporedbi s ostalim HMG2 proteinima, protein HMGBSD pruža najveću homologiju s onim iz *D. melanogaster* (DSP1), te iz sisavaca (svinja, čovjek, štakor, miš).

## CHARACTERISATION OF HMGB PROTEIN FROM MARINE SPONGE *Suberites domuncula*

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High mobility group (HMG) proteins are abundant non-histone proteins in the nuclei of eukaryocytes, so named because of their high electrophoretic mobilities in polyacrilamide gels. The HMGB proteins, one of the three classes of HMG chromosomal proteins are characterized by the HMG-box, a DNA-binding domain that both introduces a tight bend into DNA and binds preferentially to distorted DNA structures. HMGB proteins seem to act primarily as architectural facilitators in the manipulation of nucleoprotein complexes; for example, in the assembly of complexes involved in recombination and transcription. These proteins can also facilitate nucleosome remodelling. The two distinguishing features of the highly homologous vertebrate HMG1 and HMG2 proteins are two similar, but distinct, tandem HMG-box domains (A and B) and a long acidic C-terminal «tail», consisting of ~30(HMG1) or 20 (HMG2) acidic (Asp and Glu) residues. We have identified and analysed a cDNA encoding the HMGB from the marine sponge Suberites domuncula. Therefore the protein was named HMGBSD. Nucleotide sequence of sponge cDNA is 1115 bp long and predicts a protein of 183 amino acid residues with calculated $M_r$ of 21.3 kDa. C-terminal «tail» consists of 13 aa and this protein probably belongs to HMG2 group of HMGB proteins. The highest homology was found with HMG2 proteins from *D. melanogaster* (DSP1) and mammals (pig, human, rat, mouse).

P-4

## PRIMARNA STRUKTURA I FILOGENETSKA ANALIZA TIROZIN-KINAZA IZ MORSKE SPUŽVE *Suberites domuncula*

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Tirozin-kinaze (TK) čine mnogobrojnu porodicu enzima, koji u višestaničnih organizama imaju presudnu ulogu u međustaničnoj komunikaciji i prijenosu izvanstaničnih signala kroz staničnu membranu u unutrašnjost stanice. Dijele se u dvije skupine: receptorske TK (transmembranski proteini koji prenose signale kao rezultat vezanja izvanstaničnog liganda) i nereceptorske TK (sudjeluju u prijenosu signala kroz citoplazmu). U cilju istraživanja filogenetski sačuvanih gena/proteina koji su tipični za višestanične životinje, odlučili smo proučavati morske spužve (Porifera), najdrevnije i najjednostavnije višestanične životinje. Koristeći degenerativne klice, konstruirane na temelju visoko sačuvanih poddomena VIB-IX katalitičke domene TK, izvedena je lančana reakcija polimerazom (PCR) na cDNA biblioteci iz morske spužve *Suberites domuncula* (Demospongiae). Umnoženi fragmenti DNA su sekvencirani i kodiraju različite poddomene VIB-IX TK. Ovi su fragmenti korišteni kao hibridizacijske probe za pretraživanjem cDNA biblioteke iz *S. domuncula*. Detaljna analiza odabranih cDNA klonova pokazala je da svi sadrže cjelovite (ili gotovo cjelovite) cDNA inserte koji kodiraju različite TK. Tri analizirane TK iz *S. domuncula*, nazvane SRK1SD, SRK2SD i SRK3SD su nereceptorske TK iz porodice Src-kinaza, dok kinaza nazvana BTKSD pripada nereceptorskim TK iz porodici Tec. EPHBSD kinaza pripada porodici efrin receptorskih TK, a RTKSD pokazuje najveću sličnost s TK iz porodice receptora za faktor rasta fibroblasta (FGFR). Rezultati ovog rada ukazuju da je već rano u evoluciji mnogostaničja došlo do kreiranja visoko specijaliziranih, višedomenskih proteina kompleksne arhitekture, čije su strukture i organizacije domena sačuvane i kod najsavršenijih, evolucijski mladih predstavnika višestaničnih životinja.

## THE STRUCTURE AND PHYLOGENETIC ANALYSIS OF PROTEIN-TYROSINE KINASES FROM THE MARINE SPONGE *Suberites domuncula*

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Protein-tyrosine kinases (PTKs) represent a large family of enzymes, which play a major role in multicellular organisms in intercellular communication and the transduction of extracellular signals across the plasma membrane into the interior of the cells. They are generally divided into two classes, the class of receptor PTKs and the class of non-receptor PTKs. In the search of ancient versions of phylogenetically conserved genes/proteins, which are typical for multicellular animals, we have decided to analyse marine sponges (Porifera), the most ancient and the most primitive metazoan organisms. Using degenerate oligonucleotide primers designed for the highly conserved kinase catalytic domain we performed polymerase chain reactions (PCR) on the cDNA library from marine sponge *Suberites domuncula* (Demospongiae). Amplified PCR-fragments were sequenced and almost all encoded kinase domains of PTKs. Further screening of the *S. domuncula* cDNA library, using amplified PCR-fragments as hybridization probes, identified several cDNA clones encoding PTKs. Six of those cDNA clones were analysed in details. Three cDNAs encode proteins, named SRK1SD, SRK2SD and SRK3SD that belong to the non-receptor PTKs from the Src family. Protein named BTKSD shows best homology with the Tec family of non-receptor PTKs. The encoded protein EPHBSD displays high homology with the ephrin receptor family and RTKSD with receptor PTKs from the fibroblast growth factor receptor family. Our results suggest that the formation of highly specialised multidomain proteins with complex architecture, whose structure and domain organisation are preserved even in the most advanced and evolutionary new multicellular animals, happened early in the evolution of multicellularity.

P-5

## ANALIZA FILOGENETSKIH ODNOSA UNUTAR RODA *Orobanche* POMOĆU RAPD BILJEGA

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Raznolikost između 52 populacije unutar 20 taksona roda *Orobanche* (*O. alba, O. amethystea, O. arenaria, O. ballotae, O. cernua, O. clausonis, O. cumana, O. crenata, O. densiflora, O. foetida, O. foetida var. broteri, O. gracilis, O. haenseleri, O. hederae, O. latisquama, O. mutelii, O. nana, O. ramosa, O. rapum-genistae i O.*

*santolinae*.) analizirana je pomoću RAPD biljega. Korištenjem pet nasumičnih RAPD klica amplificirana su 202 polimorfna biljega. Matrica udaljenosti dobivena je Diceovim koeficijentom, a dendrogram je izrađen metodom neighbor-joining. Dobiveni obrazac međuvrsne raznolikosti uglavnom se podudara s rezultatima taksonomskih istraživanja temeljenih na morfološkim svojstvima, a razdvajanje na dvije sekcije (*Trionychon i Orobanche*) je uglavnom jasno vidljivo. No, vrsta *O. clausonis* ne prati tradicionalnu klasifikaciju, jer se na dendrogramu nalazi zajedno s članovima sekcije *Trionychon*. Unutar navedene sekcije, *O. arenaria* je relativno izolirana od drugih članova sekcije: *O. mutelii, O. nana i O. ramosa*. Unutar sekcije *Orobanche* sve populacije vrste *O. ramosa* imale su isti amplifikacijski obrazac, dok su unutar vrste *O. crenata* uočene razlike između populacija koje rastu na različitim domaćinima. Populacije vrsta *O. foetida i O. densiflora* smještene su u istu skupinu, što je razumljivo jer su navedene vrste morfološki i citološki vrlo slične, te rastu na istim domaćinima.

## PHYLOGENETIC RELATIONSHIPS WITHIN THE GENUS *Orobanche* AS REVEALED BY RAPD ANALYSIS

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RAPD markers were used to study variation among 52 populations belonging to 20 taxa in the genus *Orobanche: O. alba, O. amethystea, O. arenaria, O. ballotae, O. cernua, O. clausonis, O. cumana, O. crenata, O. densiflora, O. foetida, O. foetida var. broteri, O. gracilis, O. haenseleri, O. hederae, O. latisquama, O. mutelii, O. nana, O. ramosa, O. rapum-genistae and O. santolinae*. A total of 202 amplification products generated with five arbitrary RAPD primers was obtained and species-specific markers were identified. Distance matrices and dendrograms were generated using Dice coefficient and neighbor-joining cluster analysis. The pattern of interspecific variation obtained is in general agreement with previous taxonomic studies based on morphology, and the partition into two different sections (*Trionychon* and *Orobanche*) is generally clear. However, the position in the dendrogram of *O. clausonis* did not fit this classification since it clustered with members of section Trionychon. Within this section, *O. arenaria* was relatively isolated form the other members of the section: *O. mutelii, O. nana and O. ramosa*. Within section

*Orobanche*, all *O. ramosa* populations showed a similar amplification pattern, whereas differences among O. crenata populations growing on different hosts were found. *O. foetida* and *O. densiflora* clustered together, supporting the morphological and cytological similarities and the host preferences of these species.

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## STABILNOST BILJNIH RETROTRANSPOZONA U BAKTERIJI *Agrobacterium tumefaciens*

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LTR-retrotranspozoni su pokretni genetički elementi koji sa strukturnog i funkcionalnog gledišta nalikuju retrovirusima. Jednako kao i u retrovirusa, kodirajuća regija LTR-retrotranspozona omeđena je na krajevima dugim izravnim ponavljanjima (LTR-ima), po kojima su i dobili ime, a koja su ključna za transkripciju i transpoziciju. Iako su sastavni dio genoma svih biljaka i često obuhvaćaju većinu genoma (npr. više od 70% genoma kukuruza), do danas su utvrđena samo tri u cijelosti aktivna LTR-retrotranspozona – *Tnt1* i *Tto1* u duhanu, te *Tos17* u riži. Istraživanja aktivnosti retrotranspozona otežana su zbog velikog broja kopija u genomu, pa se u tu svrhu retrotranspozoni nastoje ugraditi u genom novog domaćina, najčešće u biljku *Arabidopsis thaliana*. Budući da se najzastupljenija metoda za ugradnju željenih gena u biljni genom temelji na primjeni bakterije *A. tumefaciens*, cilj ovog rada bio je istražiti stabilnost biljnih LTR-retrotranspozona u toj bakteriji. U istraživanje su bila uključena dva retrotranspozona (*Tto1* i *Tnt1*) za koje je dokazano da su aktivni u nekoliko različitih biljnih vrsta, te jedan neaktivni element (*Tpv2*). Utvrđeno je da je retroelement *Tto1* izrazito nestabilan u bakteriji *A. tumefaciens* jer već nakon dva mjeseca dolazi do izrezivanja kodirajuće regije uslijed homologne rekombinacije između LTR-ova. Za razliku od retrotranspozona *Tto1*, elementi *Tnt1* i *Tpv2* su bili stabilni tijekom cijelog perioda istraživanja koji je trajao šest mjeseci.

## STABILITY OF PLANT LTR-RETROTRANSPOSONS IN *Agrobacterium tumefaciens*

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LTR-retrotransposons are a family of mobile genetic elements, structurally and functionally related to retroviruses that transpose via an RNA intermediate. As in

retroviruses, the coding region of LTR-retrotransposons is flanked by long direct repeat sequences (LTRs) that are necessary for both transcription and transposition. Although they are ubiquitous constituents of plant genomes and represent a large fraction of some of them (e.g. more than 70% of maize genome), only *Tnt1* and *Tto1* of tobacco and *Tos17* of rice have been demonstrated to be transpositionally active. An impediment to investigation of retrotransposon activity in native hosts is the considerable background of copies. To circumvent this problem retrotransposons are transferred into heterologous hosts to investigate their life cycle. Since *Agrobacterium*-mediated transformation of plant genomes is the most widely used method to transfer transgenes into plant cells, the aim of our study was to investigate the stability of LTR-retrotransposons in *A. tumefaciens* prior to transfer into plant genomes. Two retrotransposons, previously shown to be active in plants (*Tnt1* and *Tto1*), and one inactive (*Tpv2)* element were used in this study. The tobacco element *Tto1* appeared to be highly unstable in *Agrobacterium*. The coding region of *Tto1* was deleted in *Agrobacterium* due to homologous recombination between LTRs within two months. In contrast, neither *Tnt1* nor *Tpv2* did show any instability even after six months in *Agrobacterium*.

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## AKTIVNOST RETROTRANSPOZONA Tpv2 U STANICAMA UROČNJAKA *Arabidopsis thaliana* L.

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LTR-retrotanspozoni su najčešći pokretni genetički elementi u eukariota i obuhvaćaju značajan dio genoma svih biljnih vrsta (npr. više od 70% genoma kukuruza). Unatoč tome, do danas je izolirano svega nekoliko aktivnih biljnih LTR-retrotranspozona. Istraživanja aktivnosti retrotranspozona otežana su, naime, zbog velikog broja kopija u genomu, pa se u tu svrhu retrotranspozoni nastoje ugraditi u genom novog domaćina, najčešće u biljku *A. thaliana*. Cilj ovog rada bio je transformirati stanice uročnjaka *A. thaliana* hibridnim LTR-retrotranspozonom Tpv2 graha u svrhu istraživanja njegove aktivnosti. Hibridni element Tpv2 sastavljen je iz dijelova dviju nefunkcionalnih kopija izoliranih iz genoma graha *Phaseolus vulgaris*. U svrhu olakšanog praćenja aktivnosti, napravljen je i retrotranspozon nazvan Tpv2i, u koji je ugrađen intron iz gena za glutamin sintetazu biljke *Lotus japonicus*. Stanice korjenja uročnjaka transformirane su retrotranspozonom Tpv2 ili Tpv2i primjenom bakterije *Agrobacterium tumefaciens*. U istraživanju su korištena dva

ekotipa uročnjaka, WS i Col-O, pa su proizvedena ukupno četiri tipa transgenih biljaka. Nakon izolacije ukupne biljne DNA, analiza aktivnosti retrotranspozona provedena je PCR-om (lančana reakcija polimerazom) i hibridizacijom po Southernu. Nije utvrđena aktivnost niti jednog oblika retrotranspozona Tpv2.

## ACTIVITY OF COMMON BEAN Tpv2 RETROTRANSPOSON IN *Arabidopsis thaliana* L.

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LTR-retrotransposons are the most common class of eukaryotic mobile elements and have been recently shown to occupy a large proportion of all plant genomes (e.g. more than 70% of maize genome). However, only few transpositionally active plant LTR-retrotransposons have been isolated so far. An impediment to investigation of retrotransposon activity in native host is the considerable background of copies. The aim of our study was to transform *A. thaliana* with hybrid common bean Tpv2 retrotransposon to investigate its activity. The hybrid Tpv2 element was made out of the fragments of two non-functional copies isolated from the genome of *Phaseolus vulgaris*. In addition, the intron of glutamine synthetase gene of *Lotus japonicus* has been inserted into one Tpv2 construct named Tpv2i. Roots of *A. thaliana* WS and Col-O ecotypes were transformed with either Tpv2 or Tpv2i element via *Agrobacterium*-mediated transformation. Four types of transgenic *Arabidopsis* plants were obtained this way. Upon the isolation of plant genomic DNA, the activity of retroelements was analysed by PCR, using primers flanking intron, and by Southern blotting. The activity of neither Tpv2 nor Tpv2i has been detected in any transgenic plant studied.

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## GENETIČKA ANALIZA MULTIPLICITETNE REAKTIVACIJE UV-OZRAČENOG FAGA ë

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Multiplicitetna reaktivacija (MR) je pojava da ozračeni fag preživljava znatno bolje u višestruko inficiranoj bakteriji nego u jednostruko inficiranoj bakteriji. MR

UV-ozračenog faga ë*red* u bakteriji *Escherichia coli* ovisi o aktivnosti bakterijskog proteina RecA. Proveli smo genetičku analizu MR faga ë*red* u nizu bakterijskih sojeva mutiranih u genima za rekombinaciju i/ili popravak DNA. Pronašli smo da je MR potpuno dokinuta u mutantima ruvC i ssb, smanjena u mutantima lexA, recD, recF, recG, recR, ruvA i ruvB, dok u mutantima recB, recBC sbcA, recBC sbcBC, recJ, recQ, mutH, mutL, mutS, umuC, uvrA, uvrB i uvrC nije promijenjena. Zaključujemo: (i) MR je fenomen temeljen na RecF putu rekombinacije i rekombinacijskog popravka DNA (budući da ovisi o genima recA i recF, a ne ovisi o genu recB); (ii) MR je lexA-ovisan proces (kao što su i neki drugi recF-ovisni fenomeni), što znači da MR zahtjeva blagu indukciju SOS odgovora koja se događa u neozračenim stanicama nakon infekcije ozračenim fagima; (iii) u recB mutantu MR nije osjetljiva na prisutnost mutacija sbcB,C,D koje inače suprimiraju rekombinacijski defekt recB,C mutanata; (iv) MR ne zahtjeva aktivnost mutH,L,S-ovisnog sustava za popravak krivo sparenih baza, aktivnost uvrA,B,C-ovisnog sustava za ekscizijski popravak UV-oštećenja, niti umuD'C-ovisnu translezijsku sintezu DNA.

## GENETIC ANALYSIS OF MULTIPLICITY REACTIVATION OF UV-IRRADIATED ë PHAGE

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Some phages survive irradiation much better upon multiple than upon single infection, a phenomenon known as multiplicity reactivation (MR). MR of UV-irradiated ë*red* phage in *Escherichia coli* cells was shown to depend on the activity of bacterial recA gene product. We have analyzed MR of UV-irradiated ë*red* in a number of bacterial mutants deficient in DNA recombination and/or repair. We found that MR is abolished in ruvC and ssb mutants, reduced in lexA, recD, recF, recG, recR, ruvA, and ruvB mutants, and not affected in recB, recBC sbcA, recBC sbcBC, recJ, recQ, mutH, mutL, mutS, umuC, uvrA, uvrB, and uvrC mutants. From these results several inferences are made: (i) because of its dependence on recA and recF genes, and independence on the recB gene, we classify MR as a phenomenon based on the RecF pathway of recombination and recombinational DNA repair; (ii) MR is a lexA-dependent process (as are some other recF-dependent phenomena) suggesting that MR requires a slight SOS induction that occurs in unirradiated cells upon infection with irradiated phage; (iii) MR in recB cells is insensitive to the sbcA or sbcB,C,D mutations that usually suppress recombination deficiency in recB,C mutants; (iv) MR does not require the activity of mutH,L,S-dependent system for the correction of mispaired bases, neither the activity of uvrA,B,C-dependent system for the excision repair of UV lesions, nor umuD'C-dependent trans-dimer DNA synthesis.

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## MEHANIZAM POPRAVKA DNA RADIOREZISTENTNE BAKTERIJE *Deinococcus radiodurans*

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Bakterija *Deinococcus radiodurans* poznata je po svojoj iznimnoj otpornosti na zračenje. Nakon izlaganja ekstremnim dozama ionizirajućeg zračenja, *D. radiodurans* može popraviti više od 100 dvolančanih lomova u DNA bez gubitka vijabilnosti i bez pojave mutacija. Osnovu radiorezistencije ove bakterije čine mehanizmi prevencije, tolerancije i popravka oštećenja, pri čemu najvažniju ulogu ima učinkovit rekombinacijski popravak DNA. Proučavali smo rekonstituciju genoma bakterije *D. radiodurans* koji je gama-zračenjem pocijepan u male (~25 kb) fragmente. Utvrdili smo postojanje RecA-neovisnog popravka DNA koji, međutim, nije dovoljan za rekonstituciju cjelovitog kromosoma. Pronašli smo da je popravak DNA praćen intenzivnom sintezom DNA koja je ovisna o DNA polimerazi I. Zaključujemo da se mehanizam popravka pocijepanog genoma bakterije *D. radiodurans* sastoji od RecA-neovisnog, PolI-ovisnog produljivanja i sparivanja jednolančanih regija DNA fragmenata i RecA-ovisnog sklapanja cjelovitog kromosoma.

## MECHANISM OF DNA REPAIR IN RADIORESISTANT BACTERIUM *Deinococcus radiodurans*

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*Deinococcus radiodurans* is the most radiation resistant organism known to date. Following exposure to extremely high doses of ionizing radiation, it can repair more than 100 double strand breaks per genome without any loss of viability or evidence of mutation. A combination of damage prevention, tolerance, repair and export seems to account for the extreme radiation

resistancy, of which the most important is a highly efficient recombinational DNA repair. This is a study of the mechanism of reconstitution of the *D. radiodurans* genome after an exposure to gamma irradiation breaking its genome down to about 25 kb fragments. Our results confirm the existence of a RecA-independent DNA repair, which, however, is not sufficient for the reconstruction of full-size chromosomes. We found that the assembly of DNA fragments is concomitant with an extensive DNA synthesis totally dependent on DNA polymerase I. We suggest that the efficient repair of highly fragmented *D. radiodurans* genome involves a RecA-independent inter-fragment priming of a massive PolI-catalysed DNA synthesis and a RecA-dependent maturation of intact chromosomes.

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## THE EFFECTS OF IONIZATING RADIATION ($^{60}$Co) ON DEVELOPMENT OF FABA BEAN

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In purpose to investigate the influence of ionizating radiation, when the seed is irradiated in germination phase, 90 h. *Vicia faba* var. *major* cultures are treated with different doses of g-rays, $^{60}$Co (40, 65 and 90 Gy, with doses rate 0,406 Gy/min). Changes registrated after the treatments are investigated during 3 vegetation period, in $M_1$, $M_2$ and $M_3$ generation respectively. There were changes analyzed immediately after the irradiation, changes in elongation phase, flowering phase and pod phase, when the morphological characteristics of root, steam, leaves, flowers, pods and seed have been analyzed, as well as cytological changes during mitotic and meiotic cycle trough detailed analyzes of chromosomes, mitotic index, and frequency of all noticed defects, tabulated for all phases. After the treatments, in the frame of primary reconstruction of the kariotype, many structural changes, as well as high % of micronuclei, fragmentation of the nuclei, polynuclear cells, cytomixis and polyploidy are noticed. There is stabile tetraploidy in all generation respectively, obtained by the treatment with 40 Gy g-rays, which results with significantly higher weight of all investigated morphological parameters - root, steam, leaves and pod. Hence, it is estimated that positive mutations after the treatment with g-rays can be obtained by this dose, which is optimal. There are normal conjugations and 12 bivalents, which confirms the balance and harmony in the polyploidization.

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## IZOLACIJA I UMNAŽANJE STARE DNA IZ MUZEJSKIH PREPARATA ZA GENETIČKA ISTRAŽIVANJA CRVENOKLJUNE GALICE (Aves: Corvidae: *Pyrrhocorax pyrrhocorax*)

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Muzejski preparati predstavljaju izuzetno vrijedan izvor podataka za populacijsko-genetička istraživanja s vremenskog stanovišta. Svrha ovog istraživanja jest ispitati upotrebljivost metode za izolaciju i umnažanje DNA iz kože muzejskih ptica za daljnje genetičke analize, posebice za sekvenciranje mtDNA. Istraživanjem su obuhvaćena 24 muzejska primjerka iz muzeja The Natural History Museum, Velika Britanija, od kojih su 22 primjerka bile jedinke vrste crvenokljuna galica (*Pyrrhocorax pyrrhocorax*), a dva primjerka su pripadala vrsti žutokljuna galica (*P. graculus*). Najstariji primjerak potječe iz 1874. godine, a najmlađi iz 1946. Laboratorijski dio istraživanja proveden je u laboratoriju namijenjenom isključivo za rad sa starom DNA kako bi se izbjegla moguća kontaminacija uzoraka današnjom DNA ili proizvodima reakcije PCR. DNA je izolirana iz komadića kože uzetog s ventralne strane nožnog prsta. Dio kontrolne regije mtDNA umnožen je reakcijom PCR u prisustvu specifičnih DNA klica. Proizvodi reakcije PCR razdvajani su elektroforezom u agaroznom gelu i bojani etidij-bromidom. Proizvodi reakcije dobiveni su iz 22 uzorka. Dva uzorka nisu dala proizvod, vjerojatno uslijed uznapredovale razgradnje DNA ili uslijed prisustva inhibitora reakcije PCR. Naknadnim sekvenciranjem proizvoda reakcije PCR utvrđeno je postojanje 6 haplotipova. Slijepa proba i negativna kontrola potvrdile su da niti u jednom slučaju nije došlo do kontaminacije uzoraka. DNA je uspješno izolirana iz uzoraka kože i umožena reakcijom PCR u gotovo 92 % uzoraka. Ovim istraživanjem pokazalo se da se uz primjerene metode može postići vrlo dobar uspjeh pri radu sa starom DNA. Kvaliteta ovako dobivene DNA dovoljno je visoka za primjenu u zahtjevnijim laboratorijskim postupcima kao što je sekvenciranje.

## EXTRACTION AND AMPLIFICATION OF ANCIENT DNA FROM MUSEUM SKINS FOR USE IN GENETIC STUDIES OF THE CHOUGH (Aves: Corvidae: *Pyrrhocorax pyrrhocorax*)

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Museum skins represent an invaluable source of data for temporal population genetic studies. Here we evaluate a DNA extraction and amplification protocol from avian museum skins for further use in genetic analysis, namely sequencing of mtDNA. Twenty-four museum specimens from the Natural History Museum, UK, were included in this study, 22 of which were choughs (*Pyrrhocorax pyrrhocorax*) and two were alpine choughs (*P. graculus*). The oldest specimen had been collected in 1874. and the most recent in 1946. All laboratory work was carried out in an ancient DNA lab following stringent standards to avoid contamination with recent DNA or PCR products. DNA was extracted from small pieces of footpad skin and mtDNA control region was amplified by means of PCR with a pair of specific primers. PCR products were resolved in agarose gel and stained with ethidium-bromide. Products were yielded from 22 samples. Two samples did not yield products, either due to DNA degradation or the presence of inhibitor substances. Subsequent sequencing of PCR products revealed six different haplotypes. The blank and the negative control confirmed that there was no contamination of the samples in either step of the process. In all, DNA was successfully extracted and amplified from almost 92 % of the samples. Our study shows that a high success rate in working with ancient DNA can be achieved by following adequate laboratory standards and procedures. The DNA obtained in this way can be of good enough a quality for use in more sensitive procedures such as sequencing.

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## MOLEKULARNI BILJEZI U POPULACIJSKO-GENETIČKIM ISTRAŽIVANJIMA CRVENOKLJUNE GALICE (Aves: Corvidae: *Pyrrhocorax pyrrhocorax*)

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Populacije crvenokljune galice (*Pyrrhocorax pyrrhocorax*) u svijetu su fragmentirane i u opadanju. Smanjenje britanske populacije bilo je vrlo snažno, te se ona danas procjenjuje na svega 340 parova. Poznavanje genetske raznolikosti populacija neophodno je prilikom odabira načina upravljanja populacijama u svrhu njihova očuvanja i očuvanja vrste. Cilj ovog istraživanja bio je ispitati primjenjivost dva tipa molekularnih biljega u populacijsko-genetičkim istraživanjima crvenokljune galice: kontrolnu regiju mtDNA i postojeće mikrosatelitske lokuse. DNA je izolirana

iz uzoraka 23 uginule jedinke crvenokljune galice s različitih lokacija u Walesu i iz 22 muzejska primjerka iz muzeja The Natural History Museum. Devet mikrosatelitskih lokusa umnoženo je reakcijom PCR pomoću DNA klica razvijenih u vrste marijanska vrana (*Corvus kubaryi*). Analiza proizvoda pokazala je da pet lokusa posjeduje samo jedan alel. Rezultati ukazuju da se ovi lokusi ne mogu koristiti u istraživanjima crvenokljune galice zbog svoje monomorfnosti. Za buduća populacijsko-genetička istraživanja ove vrste potrebno je izolirati nove mikrosatelite. Umnožen je i sekvenciran dio kontrolne regije mtDNA u duljini 365 parova baza uz upotrebu postojećih klica. U svih uginulih jedinki današnje velške populacije i u 17 muzejskih primjeraka pronađen je jedan haplotip, te još tri haplotipa u preostalih muzejskih primjeraka. Mitohondrijska kontrolna regija pokazala je vrlo malu raznolikost u crvenokljune galice. Ovakav nalaz najvjerojatnije je posljedica snažnog smanjenja populacije za vrijeme zadnjeg ledenog doba te naknadnih, od strane čovjeka izazvanih smanjenja populacije u prethodna dva stoljeća. Trebalo bi prikupiti i sekvencirati još uzoraka s različitih lokacija u Europi kako bi se ustanovilo postoji li još haplotipova te kako bi se utvrdila njihova geografska raspodjela.

## MOLECULAR MARKERS FOR POPULATION GENETICS OF THE CHOUGH (Aves: Corvidae: *Pyrrhocorax pyrrhocorax*)

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The chough (*Pyrrhocorax pyrrhocorax*) has a fragmented distribution with world populations declining in number. The decline has been very severe in Britain, where extant population is estimated at 340 pairs only. Knowledge of genetic diversity of the remaining chough populations is essential for successful conservation and management strategies. This study aimed at evaluating two types of genetic markers for population genetic research of the chough, namely mtDNA control region sequence and existing microsatellite loci. DNA was extracted from 23 dead specimens from Wales and 22 museum specimens from The Natural History Museum, UK. Microsatellite loci were amplified by means of PCR using primers developed previously in Mariana crow (*Corvus kubaryi*). Analysis of the products revealed that five loci were monomorphic. Results suggest that these microsatellite loci cannot be used in the chough. New microsatellites will have to be isolated for any future population-genetic studies of the chough that would involve this type of a genetic marker. A 365 bp segment of the mtDNA control region was amplified and sequenced using existing primers. One haplotype was found in all extant birds and 17 chough museum specimens and three more among the remaining museum specimens. Mitochondrial control region showed very little variation in the chough. This finding is

most likely due to a severe bottleneck during the last ice-age and subsequent human-induced bottlenecks in the last two centuries. Nevertheless, more samples from different localities in Europe should be sequenced in order to investigate the presence of other haplotypes and to determine their geographic distribution.

P-13

## GENETIČKI DIVERZITET PASMINE TUROPOLJSKA SVINJA

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Turopoljska svinja je autohtona hrvatska pasmina. Povijesni podaci ukazuju da je nastala u procesu domestifikacije, a kao pasmina uz udjel svinja nekih europskih pasmina. U ovom trenutku populacija Turopoljske svinje iznosi približno 250 jedinki, te je ona prema kriterijima FAO-a kritično ugrožena. Stoga se u Hrvatskoj provodi projekt zaštite i očuvanja Turopoljske svinje. U istraživanju su korištene metode genotipizacije pomoću mikrosatelita i sekvenciranja mitohondrijske D-petlje (hipervarijabilna kontrolna regija). Deset lokusa odabrano je sa liste mikrosatelitskih markera koje FAO preporuča za ovaj tip istraživanja na svinjama. Cilj istraživanja je odrediti stupanj genetičke raznolikosti kod Turopoljske svinje i usporediti je s ostalim pasminama svinja. Genomska DNA izolirana je iz 35 uzoraka krvi Turopoljske svinje i 10 uzoraka krvi križanaca Turopoljske svinje i komercijalne pasmine svinja. Mikrosatelitski lokusi umnoženi su lančanom reakcijom polimerazom uz korištenje specifičnih klica. Reverzna klica u svakom paru je fluorescentno označena sa Cy5. Duljina ovako dobivenih alela (DNA fragmenata) određena je na sekvenatoru DNA ALFexpress. Lančanom reakcijom polimerazom na svim uzorcima umnožen je i 557 parova baza dug dio mitohondrijske D-petlje kojem je određena primarna struktura DNA. Rezultati pokazuju nisku heterozigotnost na mikrosatelitskim lokusima, kao i dominaciju jednog mitohondrijskog haplotipa, što je u skladu s očekivanjima, budući da je Turopoljska svinja u nedavnoj prošlosti preživjela demografsko "usko grlo".

## GENETIC DIVERSITY IN THE TUROPOLJE PIG BREED

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Turopolje pig is an autochthonous Croatian breed. Historical evidence suggests that it originated through wild boar domestification and partly from other European

breeds. Presently, there are only around 250 surviving Turopolje pigs, and this breed is, according to FAO, critically endangered. As a part of an effort to preserve domestic animal genetic biodiversity, a conservation project for this breed is being carried out in Croatia. Microsatellite genotyping and mitochondrial D-loop (hypervariable control region) sequencing were the methods used in this research. Ten loci were chosen from the list of microsatellite markers recommended by FAO for this type of studies on pigs. The aim was to determine the degree of genetic diversity in Turopolje pig and to compare it with other pig breeds. Genomic DNA was isolated from 35 purebred Turopolje pigs and 10 hybrid pigs (Turopolje and commercial pig crosses). Microsatellite loci were amplified by PCR using specific primers. Reverse primer in each pair was fluorescently labeled with Cy5. Sizes of the resulting DNA fragments were determined using ALFexpress DNA sequencer. Part of the mitochondrial D-loop (557 base pairs) was also amplified by PCR and directly sequenced. The results for 45 analyzed samples show low heterozygosity on microsatellite loci as well as the domination of a single mitochondrial haplotype. This finding is in accordance with our expectations because Turoplje pig recently experienced a severe demographic bottleneck event.

P-14

## OBSERVATION AND DETERMINATION OF THE CHROMOSOME NUMBER OF SOME CULTIVARS OF GRAPEVINE

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In this paper were investigated the metaphase chromosomes at some cultivars of grapevine – cardinal, alphonse lavallée, italia, žilavka, vranec, cabernet sauvignon and chardonnay. The examined cultivars of grapevine are mainly characterized with 2n = 38 chromosomes. The analysis of the chromosomes was conducted during mitoses-cyclus. For analysis of somatic chromosomes were used meristems of small roots (5 - 10 mm) of the examined cultivars. Observation of grapevine chromosomes is difficult. They are small and do not scatter well through the cell surface during squashing, because of the density in the cytoplasm. The examined materials were treated with the method of Thio and Levan, and standard "squash" method of Battaglia. The main steps are: before-treatment with 8-hydrooxyquinoline (0,002 M), treatment – fixation in ethanol-acetic (3:1), treatment with 75% ethanol, staining by Schiff – reagent after hydrolysis in 1N HCL ($60C^0$) and squashing in a drop of 1% aceto – orcein. The method is suitable for accurate counting but not for investigation morphological differences between chromosomes. The aim of the researches was only determination of the chromosome number.

P-15

## INFERTILITET I MALFORMIRANO DIJETE KAO INDIKACIJA ZA PRENATALNU CITOGENETSKU ANALIZU

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Od 18555 prenatalnih citogenetskih analiza, 1798 trudnica imalo je indikaciju malformirano potomstvo ili više spontanih pobačaja. Kromosomske analize pokazale su 40 balansiranih kariotipa i 31 patološki kariotip fetusa, što je sveukupno iznosilo 71 (3,95%) kromosomsku abnormalnost. Nađeno je 16 trisomija (10 žena bile su starije od 35 godina, što povećava rizik), 9 numeričkih aberacija gonosoma, 1 slučaj s dvostrukom kromosomskom anomalijom inverzije i trisomije, 1 mozaični slučaj ring kromosoma, te 44 (62%) strukturnih aberacija. Među strukturnim abnormalnostima recipročnih translokacija bilo je 28 (63,6%), robertsonovih translokacija 11 (25%), te inverzija 5 (11,6%), a sve su većinom bile majčinog porijekla (63,6%). Balansirana kromosomska aberacija u jednom od roditelja može dovesti do produkcije gameta i embrija s nebalansiranim kromosomskim setom i tako uzrukovati spontane pobačaje ili rođenje malformirane djece. U 83 (4,6%) slučaja, od sveukupno njih 1798, jedan je od roditelja već bio poznat kao balansirani nositelj kromosomske aberacije. Od toga, 52 (62,6%) je imalo normalan fetalni kariotip, 29 (34,9%) balansirani i 3 (3,.6%) nebalansirani kariotip fetusa. Nađeno je 3 slučaja *de novo* kromosomskih aberacija, gdje postoji neznatno povećan rizik gubitka malog dijela kromosoma, što metodama klasične citogenetike ne možemo vidjeti. Mali postotak (0,56%) nositelja balansiranih kromosomskih aberacija koji su otkriveni prenatalnom dijagnostikom, ukazuje na to da je većina parova koji imaju malformiranu djecu ili spontane pobačaje upućena u genetsko savjetovanje i citogenetsko ispitivanje.

## INFERTILITY AND MALFORMED CHILD AS AN INDICATION FOR PRENATAL CYTOGENETIC EXAMINATIONS

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From 18555 prenatal cytogenetic diagnosis, 1798 were pregnant women who had previous abnormal offspring or repeated spontaneous abortions. Chromosome analysis showed 40 balanced and 31 pathological fetal karyotypes, bringing the total number of chromosomal abnormalities to 71 (3.95%). It is found 16 trisomies (10 women were over the age of 35, that increased the risk), 9 numerical aberrations of gonosomes, 1 case with double chromosome anomaly of in-

version and trisomy, 1 mosaic case with ring chromosome, and 44 (62%) structural rearrangements. Among structural abnormalities 28 (63.6%) were reciprocal translocations, 11 (25%) Robertsonian translocations and 5 (11.6%) were inversions. Also, they were mainly maternal origin (63.6%). A balanced chromosomal aberration in one of the parents could lead to the production of gametes and embryos with unbalanced chromosome sets, and may cause repeated abortions or giving birth of malformed children. In total of 1798 cases, there were 83 (4.6%) cases with one of the parents already known as a carrier balanced rearrangement. From those, 52 (62.6%) had normal karyotype, 29 (34.9%) balanced and 3 (3,6%) unbalanced fetal karyotype. It was found 3 cases of *de novo* chromosome aberrations and there is a slightly increased risk due to loss of minute portions of the gene. A small percentage (0.56%) of balanced carriers identified through prenatal diagnosis points that most of the couples with malformed child or spontaneous abortions were indicated to genetic counselling and cytogetetic examinations.

P-16

## PRVI SLUČAJ UNIPARENTALNE DISOMIJE, PRADER-WILLI SINDROMA U HRVATSKOJ

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Prader-Willi sindrom (PWS) i Angelman sindrom (AS) su prepoznatljivi sindromi s mentalnom retardacijom. Približno 70% navedenih sindroma uzrokuje mikrodelecija dugog kraka kromosoma 15 (15q11-13) očevog kromosoma (PWS) ili majčinog kromosoma (AS). Preostalih 28% slučajeva PWS-a i 3-5% AS-a nastaje kao posljedica uniparentalne disomije kromosoma 15 (jedan roditelj tijekom diobe stanice predaje obje kopije kromosoma). U Zavod za genetiku i bolesti metabolizma upućeno je dvoje dijece s kliničkom slikom koja odgovara Prader-Willi sindromu. U oba slučaja za potvrdu kliničke slike bila je potrebna molekularno-genetička analiza. DNK je izolirana iz uzoraka krvi bolesne djece i njihovih roditelja pomoću NucleoSpin Blood kita za izolaciju (Macherey-Nagel). Primjenom mikrosatelitskog lokusa HUMFES/FPS koji se nalazi na kromosomu 15 (15q25-qter) željeli smo utvrditi postojanje uniparentalne disomije kod bolesne djece. Analizom polimorfizma mikrosatelitskog lokusa utvrdili smo da su roditelji u oba slučaja informativni na ispitivanom lokusu, tj. da ne dijele zajednički alel. Analizom alela lokusa HUMFES/FPS kod obitelji 1. odredili smo da je dijete naslijedilo kromosom 15 od oba roditelja. Kod obitelji 2. otac je bio homozigot za alel 10 dok je majka imala 2 alela, alel l1 i alel 13. Analizom lokusa HUMFES/

FPS odredili smo da je dijete naslijedilo oba alela majke (alel 11 i 13). Na temelju toga smo zaključili da se radi o uniparentalnoj disomiji majke i time potvrdili kliničku sliku Prader Willi sindroma.

## FIRST CASE OF UNIPARENTAL MATERNAL DISOMY IN PRADER-WILLI SYNDROME IN CROATIA

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Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are complex and distinct mental retardation syndromes. Approximateely 70% of PWS and AS have microdeletions in a common region of 15q11-13 either on the paternal chromosome in PWS or the maternal chromosome in AS. Approximately 28% of PWS and 3-5% of AS cases are caused by uniparental disomy at chromosome 15 (inheriting two normal copies of chromosome 15 from only one parent). Two patients with a suspected clinical diagnosis of PWS were referred to the Division of Genetics and Metabolism. In both cases, molecular genetic analysis was requested to confirm the clinical diagnosis. DNA from blood samples of affected childs and their parents was extracted using Nucleospin blood kit (Machery Nagel). PCR-based analysis for polymorphism of microsatellite marker HUMFES/FPS, which is located between 15q25 and qter, was used to detect possible uniparental disomy. In both families probands and parents were informative for tested loci. In first family analysis of alleles at HUMFES/FPS suggest a normal biparental inheritance. In second family father was homozygous (allel 10) and mother heterozygous (alleles 11 i 13) for tested loci. The proband was disomic for maternal chromosomes 15 (alleles 11 and 13). This finding confirmed the clinical diagnosis of PWS.

P-17

## POLIMORFIZAM GENA RAZREDA I SUSTAVA HLA U BOLESNIKA S PSORIJATIČNIM ARTRITISOM U HRVATSKOJ

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Psorijatični artritis (PsA) je upalni artritis koji obično slijedi početak psorijaze i u većini slučajeva je negativan za reumatoidni faktor. Etiologija PsA je vrlo složena, a posljedica je kombinacije genetskih čimbenika i čimbenika okoliša. Cilj ovog istraživanja bio je istražiti učestalost alela HLA razreda I u skupini od 55 nesrodnih bolesnika s PsA-om i kontrolnoj skupini od 150 zdravih, nesrodnih ispitanika. Standardnom metodom isoljavanja izolirana je DNK iz leukocita periferne krvi. Svim ispitanicima bili su tipizirani aleli HLA razreda I i to lokusi HLA-A, -B, -Cw metodom PCR-SSP. Rezultati istraživanja su: 1.povećana učestalost alela HLA-B*13, -B*27,-B*39 I B*57 u bolesnika s PsA; 2.najveći relativni rizik pronađen je za alel B*39(RR=9.24); 3.statistički značajno smanjenje učestalosti alela HLA-B*38 u bolesnika s PsA; 4.slaba povezanost između bolesti i alela HLA-Cw*02; 5.nije pronađena povezanost između psorijatičnog artritisa i alela Cw*06. Zaključno, naši rezultati su potvrdili složenost genetske pozadine psorijatičnog artritisa. Oni pokazuju da je suspektni lokus smješten između HLA-B i Cw lokusa, ali daljnja istraživanja bi trebala također uključiti i lokuse izvan regije HLA koji su važni zbog određivanja gena-patogena.

## HLA CLASS I POLYMORPHISM IN CROATIAN PATIENTS WITH PSORIATIC ARTHRITIS

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Psoriatic arthritis (PsA) is an inflammatory arthritis that usually follows the onset of psoriasis and it is in the most cases negative for the rheumatoid factor. The aetiology of PsA is complex, caused by combination of genetic and environmental factors. The aim of this study was to investigate the distribution of HLA class I alleles in the group of 55 unrelated patients with PsA and 150 healthy unrelated controls. DNA was isolated from peripheral blood leukocytes by standard salting-out method. All individuals were typed for HLA alleles (HLA-A,-B, and –Cw) by PCR-SSP method. Findings from this study were:1.HLA-B*13, -B*27, -B*39 and -B*57 alleles were largely increased in PsA; 2.the strongest relative risk for PsA was observed for allele B*39 (RR=9.24); 3.HLA-B*38 allele was significantly decreased in patients; 4.HLA-Cw*02 allele showed a slight association with the disease; 5.no association between PsA and Cw*06 allele was found in our population. In conclusion, our results confirm the complexity of the genetic background of the PsA. Our results demonstrate that the susceptibility locus is clearly located between HLA-B and Cw loci, but further investigations that would also include non HLA loci are necessary to determine the actual gene in question.

P-18

## THE INSERTION/DELETION POLYMORPHISM ANGIOTENSIN – CONVERTING ENZYME GENE IN THE PATIENTS WITH CORONARY ARTERIES DISEASES IN THE BOSNIAN POPULATION

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In our study we have analysed correlation between insertion/deletion (I/D) polymorphism of gene for angiotensin converting enzyme (ACE) and CAD in Bosnian population. We have analysed 262 patients with CAD who were hospitalised in 1998 – 2002 in Cardiovascular Clinic Tuzla. Diagnosis of CAD was confirmed by coronary angiography. Polymerase chain reaction (PCR) was used to determine the genotypes for an insertion/deletion polymorphism in intron 16 of the ACE gene (Laboratory of molecular genetics, Clinical Centre Ljubljana). The allels frequency of the ID polymorphism angiotensine converting enzyme ((ACE) gene were also investigated. Control group has consisted from 185 healthy people. Whole participants included in our study were ages under 50 years. Our result shows statistically significant increase of ACE/DD (deletio/deletio) genotype (risk genotype) in CAD group compared with control group ($P < 0.05$). Higher prevalence of DD genotype in CAD Bosnian population (35.80 %) and ACE – D allele (0.590) confirmed some results from others studies from literature and suggest that the DD genotype of the ACE gene might be associated with positive history of CAD. These examinations are the first in our country and will be continued.

# STANIČNA I MOLEKULARNA BIOLOGIJA
*BIOLOGIA CELLULARIS ET BIOLOGIA MOLECULARIS SENSU LATO*
CELL AND MOLECULAR BIOLOGY

IL-4

## *Arabidopsis* SR DOMAIN-CONTAINING CYCLOPHILINS ARE INVOLVED IN PRE-mRNA SPLICING

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SR proteins are important splicing factors in both general and alternative splicing. By binding to specific sequences on pre-mRNA and interacting with other splicing factors they mediate different intraspliceosomal contacts, thereby helping in splice site selection and spliceosomal assembly. Spliceosome assembly is highly ordered and dynamic process involving many structural rearrangements. RNA helicases are believed to be responsible for those involving RNA-RNA base pairings, but how specific protein-protein interactions are formed and disrupted is not known. While characterizing new members of this SR protein family in *Arabidopsis,* we have identified two proteins, CypSR64 and CypSR92, consisting of an N-terminal cyclophilin domain and a C-terminal domain having many RS/SP dipeptides, which are separated by a region enriched in serines and basic amino acids (KRS domain). We show that CypSR64 and CypSR92 interact with *Arabidopsis* SR proteins and with U1 and U11 snRNP-specific proteins, U1-70K and U11-35K, in a yeast two-hybrid and *in vitro* pull-down assays. CypSR64 is a nuclear protein, localised in Cajal bodies and speckles. Moreover, CypSR64 was found to colocalize with its interacting partners in nuclear speckles. We also show that both RS/SP and KRS domains of CypSR64 are required for proper localization of the protein in Cajal bodies and for efficient binding to its interacting partners. Our results strongly suggest a role for CypSR64 in early steps of spliceosomal assembly and/or in assembly of snRNPs. In particular, its peptidyl/prolyl *cis/trans* isomerase activity could regulate interactions of SR proteins with U1-70K and U11-35K proteins directly or by influencing their phosphorylation status. The later can be supported by our observation that CypSR64 interacts with SR protein-specific kinase and is also a good substrate for it.

IL-5

## SERIL-tRNA SINTETAZE: STRUKTURA, FUNKCIJA I EVOLUCIJA U SVE TRI DOMENE ŽIVOTA

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Aminoacil-tRNA-sintetaze (aaRS) sudjeluju u biosintezi proteina katalizirajući specifičnu aminoacilaciju pripadne tRNA. Dostupnost brojnih genomskih i cDNA sljedova omogućila nam je kloniranje gena koji kodiraju seril-tRNA sintetaze (SerRS) iz različitih taksonomskih domena te istraživanje interakcije ovih enzima s njihovim pripadnim supstratima tijekom evolucije. Točnost aminoacilacijske reakcije vrlo je važna jer greška dovodi do ugradnje pogrešne aminokiseline u protein. Naša kinetička istraživanja otkrila su da se vjerna sinteza seril-tRNA u kvascu i biljkama postiže tRNA-potpomognutom optimizacijom vezanja aminokiseline u aktivno mjesto enzima. Predložen je mehanizam u kojem prolazni kompleks protein:RNA aktivira pripadnu aminokiselinu efikasnije i specifičnije nego sam apoenzim. Neke eukariotske aaRS stvaraju komplekse s drugim staničnim proteinima time pospješujući vjernost i efikasnost aminoacilacijskih reakcija. Koristeći sustav dvaju hibrida identificiran je peroksin Pex21p kao protein koji intereagira s kvaščevom SerRS. Kompleks SerRS:Pex21p je učinkovitiji u aminoacilacijskoj reakciji od same SerRS. Predlaže se da C-terminalni peptid SerRS intereagira s Pex21p, stabilizirajući konformaciju aktivnog mjesta u koje se veže tRNA. U biljaka, biosinteza proteina odvija se u citosolu, mitohondrijima i kloroplastima. Dosad su uvijek pronađene dvije, ne tri, izoforme određene AARS, od kojih jedna djeluje u dva odjeljka: citosol/mitohondrij ili kloroplast/mitohondrij. Izolirali smo dvije cDNA koje kodiraju seril-tRNA-sintetaze iz kukuruza. Jedna cDNA kodira protein sličniji eukariotskim citosolnim homolozima. Na temelju kinetičkih analiza, komplemetacije kvaščevog mutanta s disrupcijom gena u mitohodrijskoj SerRS, analize signalnog slijeda na N-kraju i filogenetske analize predloženo je da druga cDNA kodira sintetazu koja djeluje i u mitohondrijima i u kloroplastima. Neke metanogene arheje sadrže netipične SerRS koje pokazuju vrlo nisku homologiju s ostalim SerRS. U potrazi za identitetim elementima u arhealnim tRNA, osmišljeno je nekoliko hibridnih transkripata $tRNA^{Ser}$ u kojima su neke domene $tRNA^{Ser}$ iz metanogene arheje zamijenjene s odgovarajućima iz kvaščeve $tRNA^{Ser}$. Pošto su dostupni mnogobrojni proteinski sljedovi SerRS iz sve tri domene života odredili smo njihove filogenetske odnose te ih povezali s našim saznanjima o njihovoj strukturi i funkciji.

## SERYL-tRNA SYNTHETASES: STRUCTURE, FUNCTION AND EVOLUTION ACROSS ALL THREE DOMAINS OF LIFE

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Aminoacyl-tRNA synthetases (aaRS) are involved in protein biosynthesis by catalyzing specific aminoacylation of cognate tRNA. The availability of many genomic

and cDNA sequences enabled us to clone the genes encoding seryl-tRNA synthetases (SerRS) from different taxonomic domains and to investigate the interaction of these enzymes with their cognate substrates through evolution. Accuracy of the aminoacylation reaction is very important since mistakes cause incorporation of the incorrect amino acid into proteins. Our kinetic studies have revealed that accurate seryl-tRNA synthesis in yeast and plants is accomplished via tRNA-assisted optimization of amino acid binding to the enzyme active site. A mechanism is proposed by which transient protein:RNA complex activates the cognate amino acid more efficiently and more specifically than the apoenzyme alone. Some eukaryotic aaRS participate in protein complexes that improve fidelity and efficacy of aminoacylation reactions. Using two-hybrid assay we have identified peroxin Pex21p as a protein that interacts with yeast SerRS. SerRS:Pex21p complex is more efficient in the aminoacylation reaction than SerRS alone. It has been proposed that C-terminal peptide of SerRS interacts with Pex21p, thereby stabilizing conformation of the active site, where tRNA binds. In plants, protein biosynthesis takes place in the cytosol, mitochondria and chloroplasts. Thus far, there have always been found two, not three, isoforms of the respective AARS, one being dual-targeted: either to cytosol/mitochondria or plastids/mitochondria. We have isolated two maize cDNAs encoding SerRS enzymes. One cDNA encodes a protein more similar to eukaryotic cytosolic counterparts. Based on the kinetic analysis, complementation of yeast gene disruption mutant in mitochondrial SerRS, analysis of the N-terminal signal sequence and phylogenetic analysis it is proposed that the other cDNA encodes a synthetase dual targeted to mitochondria and chloroplasts. Some methanogenic archaea contain atypical SerRSs that share very low sequence homology with other SerRSs. In search for identity elements in archaeal tRNAs, several hybrid $tRNA^{Ser}$ transcripts have been designed where domains of $tRNA^{Ser}$ from methanogenic archaea have been replaced with the ones from yeast $tRNA^{Ser}$. Since many SerRS sequences from all three domains of life are available we have determined their phylogenetic relationships and correlated them with our findings on their structure and function.

IL-6

## INTERORGANELLAR COMMUNICATION. ALTERED NUCLEAR GENE EXPRESSION PROFILES IN A YEAST MITOCHONDRIAL DNA MUTANT

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Communication between mitochondria and the nucleus is important for a variety of cellular processes such as carbohydrate and nitrogen metabolism, mating and sporulation, and cell growth and morphogenesis. It has long been known

that the functional state of mitochondria can influence nuclear gene expression. For example, in yeast cells lacking the mitochondrial genome, the expression of several nuclear genes, such as CIT2 (citrate synthase), MRP13 (mitochondrial ribosomal protein), and DLD3 (d-lactate dehydrogenase) has been reported to be altered. Here we show by microarray analysis of the genome-wide transcription profile of Saccharomyces cerevisiae that yeast petite mutants lacking mitochondrial DNA induce genes coding for mitochondrial proteins, enzymes of the glycolytic pathway and of the citric acid cycle, cell wall components, membrane transporters, and genes normally induced by nutrient deprivation and a variety of stresses. Consistent with the observed induction of genes related to cell stress and those encoding membrane transporters, yeast petite cells showed increased resistance to severe heat shock and exhibited a pleiotropic drug resistance phenotype. The observed changes in nuclear gene expression in cells lacking mitochondrial DNA may have implications for the role of mitochondria in processes such as carcinogenesis and aging.

IL-7

## ULOGA SFINGOLIPIDA U APOPTOZI

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Apoptoza je oblik stanične smrti kao odgovor na različite normalne i patološke stimulanse. Brojni genski produkti su identificirani u kontroli apoptoze ali nije uvijek neophodna njihova *de novo* sinteza. Naša istraživanja pokazuju da *de novo* sinteza sfingolipida igra značajnu ulogu u apoptozi. Poznato je da *de novo* sinteza sfingolipida ide putem enzima serin palmitol transferaze (SPT) koja katalizira kondenzaciju palmitol CoA i serina u 3-ketosfinganin koji prelazi uz NADPH reduktazu u sfinganin. U ranijim radovima smo pokazali da fototerapija uz silikonski ftalocianin (PDT-Pc4) snažno inducira apoptozu. Povećana količina sfingolipida kao i keramida prati tako induciranu apoptozu. Opazili smo da inhibitori SPT-a kao što je myoricin, ISP-1, sprečavaju apoptozu induciranu PDT-Pc4. Do sad je pokazano da je za aktivnost SPT neophodna ekspresija dva gena LCB1 i LCB2. U predstavljenoj studiji pokušavamo rasvijetliti ulogu sfingolipida u apoptozi induciranoj oksidativnim stresom. Koristili smo eksperimetalne modele: Jurkat stanice, CHO stanice, stanice LY-B koje su mutanti SPT aktivnosti i LCB1 transfektirane LY-B stanice sa SPT cDNA. Genetski i biokemijski pokazatelji potvrđuju uključenost *de novo* sinteze sfingolipida u PDT induciranu apoptozu. Oštećenje mitohondrija i gubitak membranskog potencijala u direktnoj je ovisnosti o *de novo* sintezi sfilgolipida. Nije međutim dobivena direktna korelacija s produkcijom kisikovih reaktivnih radikala (ROS) iako je njihova produkcija nakon PDT implicirana kao osnovni

mehanizam indukcije apoptoze. Naši rezultati također nisu mogli pokazati da je PDT inducirana apoptoza praćena povećanom ekspresijom LCB1 i LCB2 gena. Naši rezultati ukazuju na sljedeće: 1. PDT inducirana produkcija ROS-a je prije *de novo* sinteze sfingolipida, 2. gubitak membranskog potencijala u sfingolipid-ovisnoj apoptozi mogao bi biti posljedica lokalizacije produkcije sfingolipida, 3. PDT inducirana apoptoza nije praćena povećanom ekspresijom gena odgovornih za SPT aktivnost, 4. sfingolipid inducirana apoptoza mogla bi biti ostvarena posttranskripcijskom regulacijom npr. konverzijom istih u keramide.

## SPHINGOLIPIDS INVALUMENT IN CELLULAR STRESS RESPONSES, INCLUDING APOPTOSIS

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Our recent studies have provide evidence for the role of the *de novo* sphingolipids in apoptosis. We have shown that apoptosis following the novel cancer treatment photodynamic therapy (PDT) with the silicon phthalocyanine Pc4 is supressed by ISP-I, an inhibitor of serine palmitoyltransferase (SPT), the rate-limiting enzyme in the sphingolipid biosynthetic pathway. *De novo* biosynthesis of sphingolipids gives rise to ceramide, which has been also associated with apoptosis. At least two genes, LCB1 and LCB2, are required for expression of SPT activity. In the present study, PDT was used as an oxidative stress inducer to assess the involvement of SPT-generated sphingolipids in apoptosis. The following cell model systems were employed: Jurkat cells, CHO and CHO-derived LY-B cells, which are deficient in SPT activity and *de novo* sphingolipids, as well as LY-B cell transfected with hamster LCB1 cDNA. The genetic and biochemical evidence supports the involvement of *de novo* sphingolipids in apoptosis through mitochondrial damage and the loss of membrane potential (Dym). Although single oxygen is considered the damaging reactive oxygen species (ROS) after PDD, our data suggest that ROS production is not regulated by *de novo* sphingolipids. Our results indicate that rapid apoptotic responses, initiating within 1-6 h post PDD was not associated with transcriptional upregulation of LCB1 or LCB2 genes responsible for SPT activity. Our results have several suggestion: 1. PDD-induced ROS production is upstream of the *de novo* sphingolipid biosynthesis, 2. loss of membrane potention in sphingolipid-mediated apoptosis after oxidative stress may depend on localization of sphingolipid generation, 3. the transcriptional upregulation of SPT subunits is not associated with rapid apoptosis after PDT, 4. sphinganine may induced apoptosis via its conversion to ceramide.

IL-8

# ULOGA TELOMERA U STANIČNOM STARENJU

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Većina normalnih staničnih linija sisavaca pokazuje slično ponašanje prilikom rasta u kulturi. Mlade kulture imaju inicijalni period intenzivnog rasta tokom kojega se većina stanica dijeli, ali kako se povečava broj dioba, potencijal rasta se sve više smanjuje uz istovremeno povečanje postotka starih stanica koje ne sudjeluju u diobama. Na kraju, sve stanice prestanu s daljnjim diobama što se naziva staničnо starenje. Nakon prestanka rasta, stare stanice ne umiru već ostanu viabilne pri ćemu pokazuju morfološke i biokemijske promjene kao npr. povećanje staničnog volumena, nemogućnost ugradnje $^{3}$H-timidina, bojanje sa endogenom b-galaktozidazom (endogenous senescence associated (SA)-b-gal) kod pH6 i promjene u ekspresiji gena. Predloženo je da su ove i druge promjene prilikom starenja stanica povezane sa promjenama na telomerama, repetitivnim sekvencama na krajevima eukariotskih kromosoma. Ti procesi uključuju skraćivanje i produžavanje telomera, konformacijske promjene na telomernoj DNA te promjene u interakciji telomera sa proteinima. Svaki predloženi model mora objasniti neke važne fenomene staničnog starenja i imortalizacije kao što su: - *Postepeno nagomilavanje starih stanica u kulturi:* Klasična analiza ugradnje $^{3}$H-timidina u staničnu DNA pokazala je da postepeno nagomilavanje starih stanica u kulturi rezultira ograničenim brojem dioba, a ne simultano starenje svih stanica na kraju rasta kulture. - *Stare stanice se pojavljuju naglo i nasumično u kulturi:* Činjenica da se stare stanice pojavljuju u kulturi naglo i nepredvidivo, fenomen nazvan Sindrom naglog starenja (Sudden Senescence Syndrome (SSS), je neopravdano zanemarivana. Smith i Whitney su pokazali da razlika u potencijalu rasta između dviju stanica proizišlih iz iste mitotske diobe može biti bilo gdje između 0 i >8 (sve do 35) dioba. Dakle, SSS uzrokuje da stanice stare unutar jedne diobe, daleko prije terminalne faze rasta čitave kulture. Također, stalna prisutnost SSS-a je demonstrirana i eksperimentom u kojemu su konsekutivno praćeni podklonovi u toku rasta kulture, tako da su individualni podklonovi kontinuirano generirali heterogenost u potencijalu rasta. - *Produženi stanični rast, kriza i imortalizacija:* Još jedan izazov za molekularne modele koji se zasnivaju na telomerama je da objasne fenomene produženog staničnog rasta i krize koje pokazuju razne ljudske stanice nakon ekspresije velikoga T antigena iz virusa SV40 (SV40 large T antigen (Tg). Pokazano je da SV40 Tg uzrokuje ovaj efekt kroz vezivanje (a time i inaktivaciju) na p53 i pRB, proteine važne za kontrolu staničnog ciklusa. Ovi podaci zorno potvrđuju sudjelovanje p53 i pRB u procesima normalnog staničnog starenja i karcinogeneze. Na kraju produženog rasta stanična kultura ulazi u krizu kada nastupa masovno ugibanje stanica zbog oštećenja kromosoma i nestabilnosti genoma. Iz ovog nestabilnog stanja izrastaju imortalne kolonije frekvencijom od ~$3x10^{-7}$. Da bi izbjegle krizu i stabilizirale imortalno stanje sve stanične linije moraju kontinuirano održavati telomere iznad određene minimalne dužine i to eksprimiranjem enzima telomeraze ili alternativnim produžavanjem telomera

(alternative lengthening of telomeres (ALT). Predloženo je nekoliko modela koji se zasnivaju na skraćivanju telomera , a nastoje objasniti fenomene staničnog starenja i imortalizacije. U ovom izlaganju biti će prezentirani eksperimentalni rezultati koji potvrđuju model naglog skraćivanja telomera kao mehanizma koji kontrolira opisane fenomene u usporedbi s ostalim predloženim modelima.

## TELOMERES AND STOCHASTIC NATURE OF CELLULAR AGING

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Most normal mammalian cell lines demonstrate a common growth pattern during proliferation in vitro. Young cultures initially have a period of rapid growth during which the majority of the cells divide vigorously. As the culture accumulates more divisions the growth potential declines due to the gradual increase in the fraction of nondividing, senescent cells. Eventually, the entire culture enters the terminally nondividing state referred to as replicative senescence. Upon growth cessation, senescent cells do not die but remain viable in culture, undergoing various morphological and biochemical changes, e. g. cell size enlargement, lack of $^{3}$H-thymidine incorporation, endogenous senescence associated (SA)-b-gal staining at pH6 and altered gene expression. It has been proposed that these and other phenomena have their origins in processes related to the maintenance of telomeres, which are repetitive sequences at the end of all eukaryotic chromosomes. Such processes include telomere shortening, telomere extension or conformational changes in the DNA in/et telomeres through changes in interaction of telomere associated proteins. Every model must explain some important features of cell senescence and cell immortalization such as:-*Gradual accumulation of senescent cells in culture:* Classic analyses of $^{3}$H-thymidine incorporation into cells have revealed that the limited population doubling (PD) potential of the culture is due to the gradually increasing fraction of nondividing cells, rather than all the cells that comprise a culture simultaneously entering senescence at the end of the in vitro lifespan. - *Senescent cells appear in the culture in sudden and stochastic fashion:* Fact that individual senescent cells appear in the culture unexpectedly, in a sudden and stochastic fashion due to Sudden Senescence Syndrome (SSS) has been unjustly overlooked. Smith and Whitney demonstrated that the degree of difference in doubling potentials between two cells arising from a single mitotic event can vary anywhere from between 0 and >8 (up to 35) PDs. Thus, SSS cause cells to senesce within one cell division, long before the terminal phase of the growth of the culture as a whole. Continuous presence of SSS in the proliferating culture has been demonstrated through consecutive subcloning experiments. These experiments have shown that individual subclones of the same culture constantly generate heterogeneity in proliferative potentials resulting in their distinct bimodal distribution. - *Extended lifespan, crisis and immortalization:* Another challenge for molecular models based on telomere biology is to explain the phenomenon of extended lifespan and crisis observed in various

OP-13

## NEOČEKIVANO PREPOZNAVANJE CISTEINA POMOĆU PROLIL-tRNA-SINTETAZA

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Aminoacil-tRNA sintetaze su ključni enzimi koji sudjeluju u procesima translacije proteina na ribosomu. Oni su odgovorni za specifično kovalentno vezanje aminokiselina na odgovarajuće tRNA. Točnost ovih procesa je obično vrlo visoka i često je još dodatno osigurana mehanizmima za popravak. Prolil-tRNA-sintetaze su aminoacil-tRNA sintetaze specifične za aminokiselinu prolin. Međutim, uz prepoznavanje prolina, prolil-tRNA-sintetaze prepoznaju značajno i neodgovarajuću aminokiselinu cistein, koju efikasno vežu na $tRNA^{Pro}$ *in vitro*. Učestalo netočno prepoznavanje aminokiselinskog supstrata primjećenog kod prolil-tRNA-sintetaza, nije dosad zabilježeno niti za jednu drugu obitelj aminoacil-tRNA sintetaza. Naši rezultati pokazuju da niti jedna od analiziranih prolil-tRNA sintetaza iz različitih arhebakterijskih, bakterijskih i eukariotskih organizama ne pokazuje sposobnost preciznog razlučivanja između prolina i cisteina, niti u inicijalnom odabiru, niti u mehanizmima popravka. Uz to, eksperimenti sa staničnim ekstraktima nisu pokazali aktivnost u hidrolizi krivo sparanog produkta Cys-$tRNA^{Pro}$, upućujući da cistein, jednom vezan na $tRNA^{Pro}$, ne može biti deaciliran *in vivo*. Prema tome, moguće je da krivo prepoznavanje cisteina pomoću prolil-tRNA-sintetaza ne može biti spriječeno uobičajenim mehanizmima na razini aminoacilacije. Ova činjenica otvara pitanja na koji način se onemogućava pogrešna ugradnja cisteina na mjesto prolina u proteine, te koliku razina pogrešne ugradnje može podnijeti živa stanica. U radu se nadalje razmatraju alternativni mehanizmi za onemogućavanje vezanja cisteina, te evolucijske i biotehnološke implikacije nespecifičnog prepoznavanja aminokiselina pomoću prolil-tRNA-sintetaza.

## UNUSUAL DISCRIMINATION OF CYSTEINE BY PROLYL-tRNA SYNTHETASES

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Aminoacyl-tRNA synthetases (AARSs) are the key enzymes in ensuring the fidelity of translation. They are responsible for correct attachment of the ap-

propriate amino acids to their specific tRNAs. Accuracy of this process is usually very high and is often additionally enhanced by editing activities of the same enzyme. Prolyl-tRNA synthetases (ProRSs) are AARSs specific for amino acid proline. Yet, in addition to proline, ProRSs exhibit a notable transfer of the non-cognate amino acid cysteine onto cognate tRNA *in vitro* that is to date unequalled among the AARSs. Here we show that none of the various ProRSs from archaeal, bacterial and eukaryotic origins tested were able to completely discriminate between proline and cysteine, either in initial selection, or by the post-transfer editing mechanism. In addition, experiments using whole-protein cellular extracts did not show significant Cys-tRNA$^{Pro}$ deacylation, suggesting that, once transferred onto tRNA$^{Pro}$, cysteine cannot be deacylated *in vivo*. Thus, mischarging of the cysteine by ProRSs might not be cleared out by typical mechanisms at the level of aminoacylation. This instigates the general question of how cysteine misincorporation into proteins is prevented, and how much mischarging could be tolerated by the living cell. Different scenarios to minimize cysteine binding, as well as possible evolutionary and biotechnological implications of cysteine mischarging are discussed.

OP-14

## ATIPIČNE ARHEALNE SERIL-tRNA SINTETAZE PREPOZNAJU DETERMINANTE tRNA$^{Ser}$ IZ SVA TRI ŽIVOTNA CARSTVA

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Metanogene arheje *Methanococcus jannaschii* i *Methanococcus maripaludis* sadrže atipične seril-tRNA sintetaze (SerRS), koje osim svojih homolognih supstrata tRNA$^{Ser}$ i tRNA$^{Sec}$ vrlo učinkovito prepoznaju eukariotske i eubakterijske tRNA$^{Ser}$. Relativna fleksibilnost u načinu prepoznavanja koju pokazuju SerRS metanogenih arheja pokazana je aminocilacijom i retardacijskom gel-elektroforezom. To ukazuje na konzerviranost pojedinih identitetnih elemenata u sve tri domene života, te dodatno potvrđuje postojanje funkcionalne veze između arhealnih, eubakterijskih i eukariotskih aminoacilacijskih sustava. S druge strane, strože prepoznavanje je zamijećeno kod kvaščeve SerRS, koja ne prepoznaje arhealne tRNA supstrate. Gel-filtracijskom kromatografijom izoliran je homologni kompleks dimerne SerRS iz *M. maripaludis* s jednom molekulom tRNA. Formiranje kompleksa veoma ovisi o konformaciji tRNA. Stoga su pomno istraženi uvjeti renaturacije za *in vitro* transkript izoakceptora tRNA$^{Ser}$GCT. Ova tRNA sklona je dimerizaciji, vjerojatno zbog nekoliko segmenata komplementarnih oligonukleotida sadržanih većinom unutar petlji. Dimerizaciju pospješuje porast koncentracije tRNA, a umanjuje ju brza renaturacija uz dodatak 5 mM magnezija.

## ATYPYCAL ARCHAEAL SERYL-tRNA SYNTHETASES RECOGNIZE tRNA$^{Ser}$ DETERMINANTS FROM ALL THREE DOMAINS OF LIFE

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Methane-producing archaea *Methanococcus jannaschii* and *Methanococcus maripaludis* comprise atypical seryl-tRNA synthetases (SerRSs), which recognize with high efficiency eukaryotic and bacterial tRNAs$^{Ser}$ in addition to their homologous tRNA$^{Ser}$ and tRNA$^{Sec}$ substrates. Relative flexibility in tRNA recognition pattern displayed by methanogenic SerRSs was shown by aminoacylation and gel mobility shift assays. This indicates the conservation of some serine determinants in all three domains and gives additional support to the existence of a functional connection between archaeal, bacterial and eukaryotic aminoacylation systems. On the other hand, more stringent recognition was observed by yeast SerRS, which does not recognize archaeal tRNA substrates. Homologous *M. maripaludis* complex of dimeric synthetase with presumably one tRNA$^{Ser}$ was isolated by gel-filtration chromatography. Complex formation strongly depends on the conformation of tRNA. Therefore, the renaturation conditions for *in vitro* transcribed tRNA$^{Ser}_{GCT}$ isoacceptor were carefully studied. This tRNA is prone to dimerization possibly due to several stretches of complementary oligonucleotides predominantly comprised within the loop regions. Dimerization is facilitated by increased tRNA concentration and can be diminished by fast renaturation in the presence of 5 mM magnesium.

OP-15

## POREMEĆAJ SPERMATOGENEZE UZROKOVAN TERATOGENOM 5-AZACITIDINOM: NASLJEĐIVANJE ABNORMALNOG METILACIJSKOG OBRASCA U POTOMSTVU TRETIRANIH ŠTAKORA

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5-azacitidin je poznati demetilirajući agens i koristi se u istraživanju procesa metilacije DNA. Koliko je proces metilacije važan za vrijeme razvitka govori podatak da čak i vrlo malo smanjenje metilacije DNA za vrijeme organogeneze uzrokuje ozbiljne abnormalnosti i smrt mišjeg embrija. U ovom radu su 13 dana stari štakori tretirani i.p. sa 5 mg/kg i 10 mg/kg 5-azacitidina tri puta svaki drugi dan. Iz svake grupe životinja odvojeni su svi mužjaci osim dva u dobi od dva mjeseca, žrvovani i njihovi testisi izolirani. Testisi su proučeni uz pomoć svjetlosnog i TEM mikroskopa. Dva mužjaka i sve ženke iz svake grupe životinja

stavljeni su na parenje, trudne ženke su odvojene i dobivene su dvije generacije potomaka. Njihovi testisi i epididimisi su također izolirani i proučeni. Prva generacija potomaka pokazala je statistički značajno slabije preživljenje, a druga je preživjela 100%. Testisi životinja tretiranih obim dozama 5-azacitidina bili su statistički značajno lakši i manji od kontrolnih, te su pokazivali poremećen obrazac spermatogeneze. Uz atrofiju sjemenskih kanalića bio je uočljiv gubitak stratifikacije germinativnog epitela. Pogođeni kanalići sadržavali su samo spermatogonije i spermatocite, bez prisustva spermatida i zrelih spermija. Prva generacija, potomci životinja tretiranih 5-azacitidinom, pokazivala je isti poremećeni obrazac spermatogeneze kao i njihovi tretirani roditelji. U sjemenskim kanalićima pronađene su samo spermatogonije i spermatocite. Ista slika sprematogeneze bila je prisutna i u drugoj generaciji, potomcima netretiranih životinja.

## DISTURBED SPERMATOGENESIS CAUSED BY TERATOGEN 5-AZACYTIDINE: INHERITANCE OF ABNORMAL METHYLATION PATTERN IN PROGENY OF TREATED RATS

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5-azacytidine is a well known demethylating agent and serves as a useful tool to study the process of DNA methylation. The importance of DNA methylation in development is shown when even the modest reduction in DNA methylation during organogenesis occurs, it causes severe abnormalities and death of the mouse embryo. In this work 13 day-old rats were treated i.p. with 5 mg/kg 5azaC and 10 mg/kg respectively, three times every other day. From each group all males but two were sacrificed at the age of two months and testicles were isolated. Testicle specimens were examined by light and TEM microscopy. Two males and all females from each group of animals were mated overnight, pregnant females were separated and two generations of offspring were obtained. Their testicles as well as epididymises were examined. The first generation showed statistically decreased survival. The next generation sired from these nontreated animals survived 100%. Testicles from animals treated with both doses of 5azaC were significantly smaller and lighter when compared to controls, and showed disturbed pattern of spermatogenesis. Besides the atrophy of seminiferous tubules, a loss of stratification within germinative epithelium was obvious. The affected tubules contained only spermatogonia and spermatocytes without spermatides and mature sperms. First generation of rats sired from treated animals showed almost the same disturbed picture of spermatogenesis as their treated parents. In seminiferous tubules only spermatogonia and spermatocytes were present. The same pattern was found in the testicles of the second generation of rats.

OP-16

## FUNCTIONAL AND STRUCTURAL ANALYSIS OF MYOSIN VIII USING BACULOVIRUS

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Complex proteins such as myosin depend on post-translational processing facilitated by eukaryotic systems. Baculovirus directed expression of muscle myosin II in insect cells has been shown feasible, thereby taking advantage of pathways involved in post-translational modification. In pursuit of the function and yet unknown structure of a class VIII type myosin we cloned myosin VIII, named Hamy1, from sunflower in order to produce biological active protein using baculovirus driven expression in Sf9 insect cells. Functional and biochemical analysis of recombinant myosin shows strong affinity to F-actin in ATP-sensible manner, whereas the Mg-ATPase activity of myosin VIII increases in the presence of F-actin. Electonmicrosope images of Hamy1 protein reveal a structure similar to myosin V, i.e. double headed molecules with a short rod-like tail region. Based on these data we assume, that Hamy1 protein is folded properly in insect cells, thereby showing for the first time the overall structure of a class VIII myosin.

OP-17

## ANTIOKSIDATIVNA AKTIVNOST RAZLIČITIH GLJIVA

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Različiti metabolički procesi u stanici, UV svijetlo i kemikalije mogu stvarati reaktivne kisikove radikale koji oštećuju strukturu i funkciju stanica. Oksidativno oštećenje staničnih organela javlja se kod mnogih kroničnih bolesti. Mnogi antioksidansi zaštićuju organizam od štetnog djelovanja slobodnih kisikovih radikala. Među njima su brojni plodovi voća i povrća značajni prirodni izvori antioksidansa. U našem radu ispitivali smo antioksidativna svojstva 47 vrsta gljiva skupljenih u šumama u okolini Zagreba. Od njih je 44 vrste bilo jestivo. Dobijeni uzorci su bili razdvojeni u klobuk i stabljiku sa korjenom te homogenizirani u fosfatnom puferu pH 7,5. Tamni homogenat je profiltriran i u čistom supernatansu su određeni različiti antioksidansi. Njihova koncentracija je bila dvostruko veća u klobuku prema ostalim djelovima ispitivanih uzoraka. Reducirani glutation je

najvažniji antioksidans u organizmu čovjeka. Njegova koncentracija je bila najviša u promjenjivoj puževici a najmanja u kovrčastoj kokici. Sličan odnos postoji i u koncentraciji polifenola koji su glavni biljni antioksdansi. Aktivnost antioksidativnih enzima superoksid dizmutaze i katalaze jedino nije ustanovljena kod vrste zavodnica. Između aktivnosti antioksidativnih enzima postoji polzitivna korelacija među različitim gljivama. Ukupni antioksidansi izraženi kao ukupni antioksidativni kapacitet imaju najveću vrijednost kod vrste rujnica. Ovo istraživanje pokazuje da gljive sadrže različitu koncentraciju prirodnih antioksidansa. Vjerojatno neke vrste kao prosenjak, grmačica i rujnica su izvor velike količine antioksidansa koje mogu biti korisne ljudima u zaštiti od štetnog djelovanja slobodnih radikala.

## ANTIOXIDANT ACTIVITY OF DIFFERENT MUSCHROOMS

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Endogenous metabolic processes in cell, UV light or different chemicals might produce reactive oxygen free radicals which damage structure and function of all cell macromolecules. Oxidative damage of cell organelles plays important role in development of chronical human diseases. Different antioxidants protect organism from harmful activity of free radicals. Many fruits from lemon, strawberry to different cruciferous vegetables are source high amount of antioxidants. In this report we investigated antioxidative properties 47 kinds of mushrooms collected in forests near Zagreb 44 kinds of mushrooms are edible. Obtained fungi were separated in cap with gills and stems. These compounds were homogenized (1 g/mL) in 0.1 M phosphate buffer pH 7.5. Dark homogenate were centrifuged and filtrated through Whatman No. 1 filter paper. In clear supernatant we have determined different antioxidative components. Their concentration was about twice higher in cap than stem. Main antioxidant in organism is threepeptide reduced glutathione. Its concentration is the highest in *Hygrophorus cossus* (56.2 μM/mg protein) and the least in *Sparassis crispa* (1.2 μM/mg protein). Similar difference existed in concentration mushroom polyphenols, which are principal antioxidants in vegetables. Only mushroom *Omphalatus oleanus* did not possess antioxidative enzymes superoxide dismutase and catalase. Between enzyme activity of different fungi existe positive correlation. Whole antioxidants in mushroom cap expressed as total antioxidative capacity have the highest concentration in *Lactarius deliciosus*. These results show that mushrooms contain different concentration of antioxidants and antioxidative enzymes. Probably, some of edible fungi as *Armillariella tabascus* and *Hydnum rependum* are source high amount of antioxidants, which might protect humans from damaging activity of free radicals.

OP-18

## STRUKTURNO SRODNI FLAVONOIDI S ANTIOKSIDATIVNIM SVOJSTVIMA RAZLIČITO UTJEČU NA PROLIFERACIJU I APOPTOZU STANICA HUMANE PROMIJELOCITNE LEUKEMIJE

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Flavonoidi su skupina fenolnih spojeva koji su prisutni u svim višim biljkama. Istražen je mogući odnos između između kemijske strukture 6 strukturno srodnih prirodnih flavonoida i njihovih učinaka na proliferaciju i apoptozu stanica humane promijelocitne leukemije (HL-60). Utvrđeno je da su kvercetin, miricetin, kempferol i izoramnetin snažni antiproliferativni agensi, dok su 3'-metilkvercetagetin i taxifolin neaktivni u tom smislu, indicirajući da dvostruka veza na položaju 2,3 i hidroksilna skupina na položaju 6 snažno moduliraju inhibitorne učinke flavonoida na rast stanica. Među testiranim flavonoidima kvercetin i miricetin pokazali su najsnažniji apoptotički učinak. Utvrđeno je da su iste minimalne strukturne značajke flavonoida nužne za aniproliferativni i apoptotički učinak flavonoida. Miricetin se pokazao najaktivnijim među testiranim flavonoidima u hvatanju 1,1'-difenil-2-pikrilhidrazil (DPPH) radikala, ali je bio gotovo potpuno neaktivan u redukciji menadionom induciranog oksidativnog stresa u HL-60 stanicama. Utvrđeno je da aktivnost flavonoida u hvatanju DPPH radikala ovisi o broju hidroksilnih grupa u prstenu B. Kempferol je bio najaktivniji u redukciji oksidativnog stresa uzrokovanog menadionom u HL-60 stanicama. Hidroksilna skupina na položaju 6 pokazala se kao nepovoljna strukturna značajka flavonoida s obzirom na njihovu učinkovitost u hvatanju DPPH radikala, kao i u redukciji oksidativnog stresa u stanicama.

## STRUCTURALLY RELATED FLAVONOIDS WITH ANTIOXIDATIVE PROPERTIES DIFFERENTIALLY AFFECT PROLIFERATION AND APOPTOSIS OF HUMAN PROMYELOID LEUKEMIA CELLS

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Flavonoids are a group of common phenolic compounds that are ubiquitous in all vascular plants. The possible relationship between the chemical structure of 6

structurally related naturally occurring flavonoids and their effects on proliferation and apoptosis of human promyeloid leukaemia (HL-60) cells were investigated. Quercetin, myricetin, kaempferol and isorhamnetin were found to be potent antiproliferative agents, whereas 3'-methylquercetagetin and taxifolin were ineffective, indicating that double bond in the 2,3 position and 6-hydroxyl group strongly modulate growth inhibitory effect of flavonoids. Among flavonoids tested quercetin and myricetin were the most potent apoptosis-inducing agents. The same minimal structural requirement could be postulated regarding to antiproliferative and apoptotic activities of flavonoids. Myricetin showed the strongest scavenging activity against 1,1'-dyphenyl-2-picrilhydrazyl (DPPH) radicals among the flavonoids tested, but it was almost ineffective in reducing menadion induced oxidative stress in HL-60 cells. DPPH radical scavenging activity of flavonoids was dependent on the number of hydroxyl group in the ring B. Kaempferol was the most effective compound in reducing menadion induced oxidative stress in HL-60 cells. The presence of the 6- hydroxyl group was shown to be unfavourable structural feature of flavonoids regarding to DPPH scavenging and antioxidative effects of flavonoids.

OP-19

## EFFECTS OF HUMAN INTERFERONS -a, -b AND -g ON GROWTH AND SURVIVAL OF CELLULAR MICROTUMORS.

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Interferons (IFNs) are proteins having an antiviral and antitumor activity, but their antiproliferative activity may differ according to the type of cells treated and a great heterogeneity in the sensitivity of different tumor cells has been reported in a number of *in vitro* and *in vivo* studies. In the present study we compared the direct effects of three human natural IFNs: á, â and  on the growth and survival of cellular microtumors of PLA (Adult pig kidney) cells. The organotypic culture method was used throughout the experiments. A continuous treatment lasting 10 days with 10, $10^2$, $10^3$ and $10^4$ I.U./ml of three IFNs led to growth inhibition and necrosis of PLA microtumors only in the presence of the two highest doses ($10^3$ and $10^4$ I.U./ml) of IFNs á and . In contrast, IFN â had very low growth inhibitory effect. Some of the PLA microtumors, especially at the lower dose levels (10 or $10^2$ I.U./ml) of IFNs â and  showed enhanced growth. IFN á treated microtumors show cytolysis starting with necrosis and vacuolisation. On the other hand the antitumor effect of IFNs á and  seems to result from the tumoristatic rather than the tumoricidal capacities of these interferons.

P-19

## PROTEINSKI KOFAKTOR KVAŠČEVE SERIL-tRNA SINTETAZE

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Peroksin Pex21p interagira *in vivo* i *in vitro* sa seril-tRNA sintetazom iz kvasca *Saccharomyces cerevisiae*. Pex21p ima osnovnu funkciju u biogenezi peroksisoma, ali se ponaša i kao specifični aktivator SerRS, povećavajući efikasnost aminoacilacije. Napravljeno je nekoliko delecijskih mutanata SerRS u svrhu pronalaska interagirajućih domena SerRS/Pex21p. Nakon delecije pozitivno nabijenog C-terminalnog peptida u SerRS, koji karakterizira sve eukariotske seril-tRNA sintetaze, interakcija peroksina i SerRS je gotovo izgubljena. Već smo pokazali da ovakvi krnji proteini *in vivo* djeluju toksično zbog promijenjenog načina u prepoznavanju supstrata. Sudeći po rezultatima, Pex21p je vjerojatno uključen u stabilizaciji konformacije aktivnog mjesta SerRS, te mu se predviđa važna uloga u točnosti serilacije.

## PROTEIN CO-FACTOR OF YEAST SERYL-tRNA SYNTHETASE

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Peroxin Pex21p was identified as seryl-tRNA synthetase-interacting protein in *Saccharomyces cerevisiae* both *in vivo* and *in vitro*. Besides its main function in peroxisome biogenesis, Pex21p acts as specific activator of SerRS by increasing the efficiency of aminoacylation. In order to map SerRS/Pex21p interactive domains, several deletion mutants of SerRS have been produced. The interaction with peroxin was almost entirely lost after deleting positively charged C-terminal peptide of SerRS, which characterizes all eukariotyc seryl-tRNA synthetases. We have previously shown that such truncation also leads to toxicity *in vivo,* due to altered substrate recognition. These results suggest the involvement of Pex21p in stabilizing the conformation of SerRS active site, which could be important for maintaining the accuracy of serylation.

P-20

## IMUNOFENOTIPIZACIJA LIMFOCITA PERIFERNE KRVI PACIJENATA UKLJUČENIH U POSTUPAK IZVANTJELESNE OPLODNE

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Cilj istraživanja bio je odrediti postotke subpopulacije T- i B- limfocita periferne krvi 30 neplodnih parova uključenih u postupak izvantjelesne oplodnje. Pacijenti su svrstani u četiri skupine: izostanak oplodnje u prethodnim postupcima (n=10), pozitivna anamneza ektopičnog zanošenja (n=5), prethodno neuspješna (više od 3) IVF/ET postupka (n=9), pozitivna anamneza spontanog poboačaja (n=6). Kontrolnu skupini činilo je 10 parova sa zdravom djecom. Odabrane subpopulacije limfocita analizirane su protočnom citometrijom. Postotak memorijskih $CD27^+$ B- limfocita u svim skupinama neplodnih pacijentica bio je značajno niži u poredbi sa zdravom kontrolom (medijan 30.4%, raspon 5.6, izražen kao postotak od ukupnih B- limfocita). Memorijski $CD27^+$ B- limfociti u skupini pacijentica s prethodnim izostankom oplodnje (medijan 22%, raspon 9.5) bio je značajno uvećan u poredbi sa skupinom pacijentica koje su prethodno imale ektopičnu trudnoću (medijan 15%, raspon 3.2). Razlike u postotku $CD27^+$ B- limfocita u skupini pacijentica s prethodno neuspješnim postupkom izvantjelesne oplodnje i skupinom koja je imala spontane pobačaje u poredbi sa skupinom pacijentica sa prethodnim izostankom oplodnje nije statistički značajna. Muški partneri skupine pacijentica s neuspješnom oplodnjom (medijan 21.9%, raspon 8.7) su imali značajno uvećan postotka $CD27^+$ B- limfocita u poredbi s partnerima skupine pacijentica s prethodnim ektopičnim trudnoćama (medijan 16.2%, raspon 5.9). Postotak T- limfocita, $CD8^+$ i $CD4^+$ T- limfocita, NK-stanica, te i apsolutnog broja $CD4^+$ T-limfocita u svim skupinama pacijentica nije bio značajno različit u poredbi s kontrolom. U dvije pacijentice iz skupine s pozitivnom anamnezom ektopične trudnoće ustanovljen je uvećan postotak NK-stanica u poredbi s kontrolom. Neplodni pacijenti imali su značajno smanjen postotak $CD27^+$ B- limfocita periferne krvi. Poremećaj u subpopulaciji memorijskih B- limfocita mogao bi biti pokazatelj imunološke patogeneze neplodnosti.

## IMMUNOPHENOTYPING OF PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS INCLUDED IN THE IVF/ET PROCEDURE

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The aim of this study was to determine the percentage of different T- and B-cell subsets in the peripheral blood of 30 infertile married couples included in the IVF/ET program. The patients were divided into four groups: with previous unsuccessful fertilisation (n=10), ectopic pregnancy (n=5), multiple (at least 3) failed IVF/ET (n=9)

and missed abortions (n=6). Control group consisted of 10 married couples with healthy children. Selected lymphocyte subpopulations were determined by flow cytometry. The percentage of memory $CD27^+$ B-cells in all groups of infertile patients was significantly decreased compared to healthy controls (median 30.4%, range 5.6, expressed as a percentage of total B-cells). Memory $CD27^+$ B-cells in women with previous unsuccessful fertilisation (median 22%, range 9.5) were significantly increased compared to women with previous ectopic pregnancy (median 15%, range 3.2). The difference in the percentage of $CD27^+$ B-cells in multiple failed IVF and missed abortion groups compared to women with unsuccessful fertilisation was not statistically significant. Male partners of women with previous unsuccessful fertilisation (median 21.9%, range 8.7) also had had significantly increased percentage of $CD27^+$ B-cells compared to the partners of women with history of ectopic pregnancy (median 16.2%, range 5.9). Percentage of T-cells, $CD8^+$ and $CD4^+$ T-cells, NK-cells and absolute count of $CD4^+$ T-cells in all groups of patients were not significantly different compared to the fertile control. Only two patients from the ectopic pregnancy group had increased percentage of NK-cells compared to the control. Infertile patients have significantly decreased percentage of $CD27^+$ B-cells in the peripheral blood. Abnormalities in the memory B-cell compartment may contribute to the pathogenesis of infertility.

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## EKSPRESIJA NUKLEARNOG ANTIGENA PROLIFERIRAJUĆIH STANICA TIJEKOM RAZVOJA ZAMETKA ŠTAKORA

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Visoki stupanj homologije gena za nuklearni antigen proliferirajućih stanica (PCNA) u životinjskom i biljnom svijetu ukazuje na njegov značaj u kontroli stanične proliferacije. Cilj ovog rada bio je istražiti endogeni proliferacijski potencijal različitih tkiva zametaka štakora tijekom embrionalnog razvoja pomoću proteinske ekspresije PCNA u pojedinoj stanici. Štakorski zameci soja Fischer starosti 12-15 dana izolirani su mikrokiruški pod binokularnom lupom, fiksirani u St. Marie fiksativu i uklopljeni u parafin. Rezovi (5 m) su obrađeni za imunohistokemijsko bojenje. Primarno monoklonalno mišje antitijelo na PCNA (DAKO) primjenjeno je u razrjeđenju 1:50, a kao negativna kontrola služio je standardni negativni kontrolni reagens - mišji IgG1 (DAKO). Za daljnju obradu primjenjen je DAKO LSAB® 2 Kit, Peroksidaza za štakorska tkiva sa DAB kromogenom i DAKO LSAB®+ Kit, Alkalna fosfataza sa fuksinskim

kromogenom prema uputama proizvođača. U svim stadijima razvoja ekspresija PCNA bila je prisutna u gotovo svim stanicama uz moždane komore, ali gotovo odsutna u ostalim bolje diferenciranim područjima mozga. U ganglijima je PCNA signal bio odsutan iz živčanih stanica, a prisutan u glija stanicama. U osnovi crijeva i u nezrelom epidermisu PCNA se eksprimirao u svim stanicama, a nije se eksprimirao u eritroblastima. U tkivu testisa PCNA bio je ubikvitaran u sjemenskom epitelu, ali bio je odsutan iz intersticija. U miotubama PCNA se nije eksprimirao, a eksprimirao se u mioblastima. Prema tome pronađena je odsutnost ekspresije PCNA u stanicama koje su krenule ka terminalnoj diferencijaciji, a ekspresija u stanicama koje još proliferiraju. Time je potvrđeno da je diferencijalna aktivnost PCNA jedan od ključnih regulatora razvoja sisavaca.

## PROLIFERATING CELL NUCLEAR ANTIGEN EXPRESSION IN THE DEVELOPING RAT EMBRYO

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High degree of homology between animal and plant genes for the proliferating cell nuclear antigen (PCNA) points to its importance for the control of the cell proliferation. The aim of this study was to investigate the endogenous proliferation potential of various fetal rat tissues during embryogenesis by PCNA expression in single cells. Fischer rat embryos 12-15-days-old were microsurgically isolated under the dissecting microscope. Fixation was done in St.Marie's fixative and embedding in paraffin. Embryos were cut (5 m) and processed for immunohistochemical staining. Primary monoclonal mouse antibody against PCNA (DAKO) was used (1:50). Mouse IgG1 (DAKO) was used for the standard negative control. DAKO LSAB® 2 Kit, Peroksidase with DAB chromogen and DAKO LSAB®+ Kit, Alkaline phosphatase (fuchsin chromogen) were used accordingly to manufacturer's instructions. In all stages of development PCNA expression was present in all cells surrounding the brain ventricles but absent from more differentiated regions of the brain. In ganglia PCNA was absent from neural cells but present in the glia. In the primitive gut and immature epidermis PCNA was expressed in all cells while it was absent from erythroblasts. In the testis PCNA was ubiquitous in sex cords but almost absent from the interstitial cells. In myotubes PCNA was not expressed in contrast to myoblasts where its expression was detected. In conclusion, the absence of PCNA expression was found in more differentiated cells in contrast to proliferating cells where PCNA was present. This confirmed the pivotal role of the PCNA differential expression for the regulation of mammalian development.

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## LIPID PEROXIDATION IN VARIOUS EXPERIMENTAL CONDITIONS IN RAT BRAIN HOMOGENATE IN MILDLY HYPERCHOLESTEROLEMIC RATS WITH DIFFERENT DIETARY SUPPLEMENT

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The cellular damage induced by fundamental role of free radicals, falls into three major categories: lipid peroxidation, DNA-modification and reaction with thiol-groups. The cerebral tissue is particularly vulnerable to oxidative insult. In recent years it has been established that oxidative stress is an important factor in the pathogenesis of several neurobiological disorders such as stroke, trauma and chronic neurodegenerative diseases. Cell membrane stability and protection against oxidative insult depend very much of the way in which the membrane is assembled from its lipid components. Structural organization requires that the "correct" ratios of phospholipids and their fatty acids are attached. This study has examined the degree of lipid peroxidation evaluated in brain homogenates, measuring both steady-state and stimulated concentrations of thiobarbituric acid reactive substances according to method described by Okhawa. Adult female Wistar strain rats, were randomized in 5 dietary groups: (1) intact female rats receiving no treatment (control group); (2) mildly hypercholesterolemic rats (receiving atherogenic diet for 180 days) with no supplementary treatment; (3) mildly hypercholesterolemic rats receiving high fat diet for 160 days, and after that put under intragastral supplementary diet (1.5 ml corn oil per day for period of 20 days); (4) mildly hypercholesterolemic rats with supplementary diet (1.5 ml soybean oil per day for 20 days) and (5) mildly hypercholesterolemic rats with supplementary diet (1.5 ml fish oil per day for 20 days). The obtained results indicate that the "in vivo" administration of high chronic doses of n-6 fatty acids (corn and soybean oil) increases the steady-state levels of lipid peroxidation products. Fish oil administration increases tissue resistance to lipid peroxidation triggered by metal-ascorbate system.

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## DETERMINACIJA EFG-u SLIČNOG ČIMBENIKA U TKIVNOM HOMOGENATU GUJAVICE

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G-90 je makromolekularna smjesa glikolipoproteinskih osobitosti izdvojena iz tkivnog homogenata gujavice *Eisenia foetida*. U *in vivo* testovima utvrđeno je da ne sadr?ava ni mutagene niti kancerogene. G-90 je makromolekularna smjesa (sastoji se od 17 proteinskih frakcija molekularne mase od 23 do 97 kDa) s dokazanim brojnim biolo?kim aktivnostima kao ?to su mitogenost, fibrinolitičnost, bakteriolitičnost, bakteriostatičnost, antioksidativnost antikoagulantnost. Vjerojatno je da je takva pleiotropna aktivnost G-90 povezana s molekulama sličnim inzulinu, serinskim peptidazama tripsinske natporodice i adhezinima imunoglobulinske natporodice izdvojenim iz pripravka. U prethodnim istra?ivanjima utvrđena je regeneracijska aktivnost G-90. Stoga smo u ovom radu metodama molekularne biologije ispitali prisutnost čimbenika rasta u smjesi G-90 ključnih u procesu regeneracije. Utvrdili smo da G-90 sadr?i i molekulu sličnu EGF-u. Ovi rezultati upućuju na potrebu daljnjih istra?ivanja u cilju kliničke primjene G-90 u humanoj i veterinarskoj medicini.

## EGF-LIKE FACTOR DETERMINED IN THE TISSUE HOMOGENATE (G-90) OF THE EARTHWORM

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The G-90 is a macromolecular mixture with glycolipoprotein characteristics obtained from tissue homogenate of the earthworm *Eisenia foetida*. No mutagens or cancerogens were recorded following *in vivo* tests. This macromolecular mixture (containing 17 protein fractions with molecular mass ranging from 23-97 kDa) exhibits *in vitro* and *in vivo* numerous biological activities such as mitogenicity, anticoagulation, fibrinolysis, bacteriolysis, bacteriostasis, and antioxidation. It is likely that such pleiotropic activity of G-90 could be associated with the insulin-like proteins, two tyrosine like serine peptidases and the immunoglobulin-like molecules isolated from the preparation. Previous studies have shown the regeneratory activity of G-90. Therefore, in this study the growth factors, essential for the regeneration process, were determined by the molecular biology methods in G-90. EGF-like factor was found in the G-90. Accordingly, it would be necessary to continue the evaluation of clinical potentials for practical use of G-90 in human and veterinary medicine.

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## MODULATORNO DJELOVANJE MET-ENKEFALINA NA ANTIOKSIDATIVNI STATUS U MOZGU MIŠEVA RAZLIČITE DOBI I SPOLA JE POSREDOVANO S IL-1

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Svrha ovog istraživanja bila je ispitati antioksidacijski status u mozgu miševa različite dobi i spola i učinak opioidnog peptida Menk-enkefalina (MENK) na tri glavna antioksidacijska enzima: superoksid dismutazu (SOD), katalazu (CAT) i glutation peroksidazu (Gpx) u homogenatu mozga ili supernatantu iz homogenata mozga u mužjaka i ženki CBA miševa u dobi od 1, 4, 10 i 18 mjeseci tretiranih ili netretiranih s MENKom (10 mg/kg ), te korelirati dobivene podatke s razinom IL-1 alfa u plazmi istih skupina miševa. Rezultati su pokazali da primjenom MENKa dodatno poraste već povišena razina lipidne peroksidacije (LPO) u mužjaka u dobi od 10 mjeseci dok u ženki nije zapažen nikakav učinak. MENK je različito modulirao učinak SOD u mužjaka (sniženje u dobi od 1 mjeseca) u odnosu na ženke (porast u dobi od 18 mjeseci). MENK je modulirao aktivnost CAT u veoma mladih mužjaka (dob od 1 mjeseca) i starih mužjaka (dob od 18 mjeseci), dok u ženki MENK nije imao učinka ni u jednoj starosnoj skupini. Nasuprot tome MENK je modulirao aktivnost Gpx u svim starosnim skupinama mužjaka, dok je u mozgu ženki aktivnost Gpx bila snižena u svim skupinama. Sažimajući podatke dobivamo rezultate odnosa SOD/CAT+Gpx u miševa tretiranih MENKom koji je snižen u mužjaka u dobi od 1 mjeseca i povišen u ženki u dobi od 18 mjeseci, i konačno u plazmi razina IL-1 mogla se korelirati sa sniženom aktivnošću SOD i odnosa SOD/CAT+Gpx u vrlo mladih mužjaka u dobi od mjesec dana tretiranih s MENKom.

## AGE AND GENDER RELATED MET-ENKEPHALIN MODULATED OF ANTIOXIDATIVE STATUS IN MOUSE BRAIN IS IL-1 MEDIATED

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The purpose of this study is to investigated the level of antioxidative status in mice brain of different age and sex and effect of opioide peptide Met-enkephalin (MENK) on three major antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx) in the brain homogenate or supernatant from brain homogenate of 1,4,10 and 18 months old male and female CBA mice treated or not treated with MENK (10mg/kg bw), as well as to correlate this

data to plasma level of IL-1 alpha in same groups of mice. Results showed that treatment with MENK increase already high lipid peroxidation (LPO) level in 10 months old male mice while in female mice have no effect. SOD activity was differently modulated by MENK in male (decrease in 1 month old) than in female mice (increase in 18 months old mice). CAT activity was modulated with menk in very young (1 month old male mice) and senescence (18 months old male mice), while in female mice MENK have no effect in all age groups. Contrary to this MENK modulate Gpx activity in all age groups of male mice, while in female mice brain Gpx activity was decreased in all age groups. Taken together this data lead to results of SOD/CAT+Gpx ratio in MENK treated mice which is decreased in 1 months male mice and increased in 18 months old female mice, and finally IL-1 level in plasma can be correlated with decreased SOD activity and SOD/CAT+Gpx ratio only in very young 1 month old male mice treated with MENK.

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## SINERGISTIČKI UČINAK ČIMBENIKA RASTA FGF-a I NGF-a NA REGULACIJU RASTA KULTIVIRANIH ZAMETAKA SISAVACA

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Fibroblastni faktor rasta (FGF) i neurotrofični faktor rasta živaca (NGF) sudjeluju u embriogenezi sisavaca. FGF je mitogen za različite stanice derivate mezoderma, uključujući 3T3 fibroblaste, endotelne stanice kapilara i endokarda, mioblaste, glija i astroglija stanice *in vitro*. FGF ima važne uloge u staničnoj proliferaciji i diferencijaciji tijekom embriogeneze, regeneraciji tkiva, razvoju CNS-a, zacjeljivanju rana i progresiji tumora. NGF je važan za preživljenje i razvoj simpatičkih neurona i derivata snsornih neurona neuralnog grebena. Oba proteina djeluju na gene preko specifičnih receptora. U kulturi *in vitro* NGF iskazuje mitotičku ili antimitotičku aktivnost ovisno o vrsti stanica na koje djeluje. Istraživali smo učinak signala za rast FGF-a i NGF-a na rast embrija štakora u kulturi organa *in vitro* i njihovo moguće sinergističko djelovanje. Modificirana metoda kulture organa razvijena u našem laboratoriju pogodna je za istraživanje rasta kultiviranih embrija, koji se razvijaju tijekom kultiviranja u teratome. Tretirali smo štakorske embrije u fazi gastrulacije sa 100 ng/ml i 200 ng/ml NGF-a ili FGF-a ili je upotrebljena kombinacija oba faktora u kemijski potpuno definiranom mediju (MEM) i mediju obogaćenom serumom. Procjena rasta vršena je određivanjem prosječne veličine eksplantata okularnim mikrometrom u određene dane kultiviranja tijekom 14-dnevne kulture. Vrijednosti su izražene kao artmetička sredina najmanjeg i najvećeg promjera eksplantata mjerenih okomito jedan na drugi. Statističke razlike su određene

Studentovim t-testom. Kontrolni zameci u mediju sa serumom maksimalnu veličinu postignu u prvom tjednu kulture, a onda se tijekom drugog tjedna rast postepeno smanji do veličine eksplantata u 5-om danu, dok u mediju bez seruma zameci udvostruče veličinu u prva dva dana kulture, a nakon toga do kraja kultiviranja nema promjene rasta. Obje upotrebljene koncentracije NGF-a značajno smanjuju rast (što je jasnije izraženo u mediju bez seruma), dok FGF stimulira rast, mada u pojedinim danima mjerenja razlika nije uvijek statistički značajna u usporedbi s kontrolnom grupom. Kada su NGF i FGF zajedno dodani u medij sa serumom rast je značajno povećan u usporedbi s kontrolom kao i eksperimentalnim grupama zametaka tretiranim NGF-om i FGF-om. Stoga izgleda da NGF i FGF djeluju sinergistički stimulirajući rast kultiviranih zametaka, dok u mediju bez seruma FGF smanjuje inhibicijski učinak NGF-a, ali ga ne može spriječiti i značajno povećati rast.

## GROWTH FACTORS NGF AND FGF ACT SYNERGISTICALLY IN REGULATION OF GROWTH IN CULTIVATED MAMMALIAN EMBRYOS

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Fibroblast growth factor (FGF) and neurotrophic nerve growth factor (NGF) takes a part in mammalian embriogenesis. FGF is a potent mitogenic agent for a wide variety of mesoderm-derived cells, including 3T3 fibroblasts, capillary and endocardial endothelial cells, myoblasts, mesothelial cells, glial and astroglial cells *in vitro*. FGF plays important roles *in vivo* in cell proliferation and differentiation in embryogenesis, tissue regeneration, CNS development, wound healing and tumour progression. NGF is physiologically significant for the survival and development of symphatetic and neural crest-derived sensory neurons. Both proteins affect genes via specific receptors. In *in vitro* culture NGF has mitogenic and antimitogenic activity (which depends on the type of cell involved). We investigated the growth of rat embryos *in vitro* in response to FGF and NGF signals and their possible synergistic action. The modified organ culture method devised in our laboratory is a very successful model for investigating growth of mammalian embryos developed in teratoma-like structures. We treated rat gastrulating embryos with 100 ng/ml and 200 ng/ml of NGF or FGF, and with both agents combined in serum-free (MEM) and serum-supplemented medium. Growth rate of explants *in vitro* was evaluated by measuring average size of explants using an eyepiece micrometer on a few particular days during 14 days of culture. The perpendicular major and minor diameters of each explant were measured and expressed as the arithmetic mean of those two values. Differences between respective groups were esti-

mated by Student's t-test. Control explants grown in serum supplemented medium reached the maximum in the first week and than gradually decreased in the second, whereas in serum-free medium embryos doubled their masses during the first two days and after that there was no more growth until the end of culture. Both concentrations of NGF impaired growth of explants (more obviously in serum-free medium), while FGF stimulated growth, although it was not always significantly different from control groups on a particular measure-day. When we applied NGF and FGF together in culture media the growth of explants was significantly improved in comparison to control and experimental NGF-treated or FGF-treated groups in serum-supplemented medium. It seems that FGF and NGF act synergistically, thus improving growth rate of cultivated embryos in serum-supplemented medium, while in serum-free medium FGF reduced inhibitory effect of NGF, but it could not prevent it and significantly increase growth.

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## INDUKCIJA DIFERENCIJACIJE NEUROBLASTA ČIMBENICIMA RASTA U EMBRIJIMA SISAVACA U KULTURI ORGANA *IN VITRO*

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Intracelularni signali faktorima rasta i neurotrofičnim faktorima imaju važnu ulogu u neurogenezi. Fibroblastni faktor rasta (FGF), koji je u početku istraživan kao induktor mezoderma, u poslijednje se vrijeme sve više istražuje kao induktor neurogeneze u kralješnjaka. Čimbenik rasta živaca (NGF) identificiran je zbog svoje trofičke uloge za različite neurone perifernog živčanog sustava. Pravilan obrazac razvoja živčanog sustava kralježnjaka započinje induktivnom diferencijacijom neuralne ploče dorzalne strane ektoderma na kraju gastrulacije. Pokušali smo istražiti više o ulogama FGF-a i NGF-a u kritičnom periodu embrionalnog razvitka sisavaca, gastrulaciji. U našem smo laboratoriju razvili jedinstveni sustav kulture organa postimplantacijskih zametaka glodavaca, koji osigurava optimalne uvjete za terminalnu diferencijaciju osnovnih tkiva tijekom dva tjedna kulture. Zameci štakora bez ekstraembrionalnih membrana kultivirani su na metalnoj mrežici sa lećnim papirićem, na granici plinovite i tekuće faze, u kemijski definiranom mediju bez seruma i proteina. Kao jedini signalni proteini u medij su dodavani FGF ili NGF, ili oba zajedno tijekom 9 dana kultiviranja. Tretirali smo embrije sa 100 ng/ml i 200 ng/ml FGF-a ili NGF-a ili smo upotrijebili kombinaciju oba agensa (100 ng/ml FGF-a + 100 ng/ml NGF-a). U kontrolnom

mediju bez seruma neuroblasti su bili prisutni vrlo rijetko ili uopće nisu. NGF nije poboljšao diferencijaciju neuroblasta. Nasuprot tome, učestalost diferencijacije neuroblasta bila je signifikantno povećana u embrijima tretiranim FGF-om (značajno bolje kada smo upotrijebili dozu od 200 ng/ml). Diferncijacija neuroblasta u embrijima tretiranim kombinacijom FGF/NGF, bila je otprilike jednaka kao kada smo primjenili 100 ng/ml FGF-a. Neuralna diferencijacija tijekom embriogeneze sisavaca je mnogostepeni proces, vjerojatno inicijalno reguliran FGF signalom, a ne NGF-om.

## GROWTH FACTORS-INDUCED NEUROBLASTS DIFFERENTIATION IN MAMMALIAN EMBRYO IN ORGAN CULTURE *IN VITRO*

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Intracellular signals provided by growth and neurotrophic factors play a critical role during neurogenesis. Fibroblast growth factor (FGF), previously studied as a mesoderm inducer has been more recently proposed to be a neural inducer in vertebrata. Nerve growth factor (NGF) was identified because of their trophic role for distinct populations of neurons in the peripheral nervous system. The development of the well-patterned vertebrate nervous system begins with the inductive differentiation of the neural plate from the dorsal side of flat ectoderm at the end of gastrulation. We tried to find out more about the role of FGF and NGF in critical period for mammalian development, gastrulation. In our laboratory we developed a unique organ culture system of rodent postimplantation embryo, which provide favourable conditions for the terminal differentiation of the main tissues for 2-week culture. Cultivation of gastrulating rat embryos without extraembryonic membranes, was performed on the metal grid supported lens paper, at the air-liquid interface in serum-free and protein-free medium. As a single signal-protein FGF or NGF were added, or both together in culture medium in time frame of 9 days. We treated embryos with 100 ng/ml and 200 ng/ml FGF or NGF or with both agents combined (100 ng/ml FGF + 100 ng/ml NGF). In control serum-free medium neuroblasts were very rare or absent. NGF did not improve differentiation of neuroblasts. On the contrary, incidence of differentiation of neuroblasts was significantly higher in FGF-treated embryos (more obviously when we used 200 ng/ml). Differentiation of neuroblasts in FGF/NGF-treated embryos was about the same as was when we applied 100 ng/ml of FGF. Neuronal differentiation in mammalian embryogenesis is multi-level process, probably initially regulated with FGF signal rather than NGF.

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## PUŠENJE CIGARETA I ANTIOKSIDATIVNO DJELOVANJE EKSTRAKTA BROKULE

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Pušenje cigareta je zdrastveni rizik koji utječe na razvoj kroničnih bolesti ljudi kao što su arterioskleroza i rak. Duhanski dim posjeduje slobodne kisikove radikale koji oštećuju funkciju organizma. U ovom ispitivanju mjerili smo antioksidativni status pušača (10-20 cigareta dnevno) koji su redovito uzimali ekstrakt cvijeta brokule. Ovaj ekstrakt je je registriran kao komercijani produkt Broccolin (proizvođač Pond, Zagreb). Preparat u obliku kapsula od 400 mg Broccolina dobijen je ekstrakcijom svježe brokule. Poznato je da brokula sadrži veliku količinu antioksidansa reduciranog glutationa i vitamina C. Trinaest dobrovoljaca pušača cigareta uzimalo je po tri kapsule Broccolina dnevno kroz mjesec dana. Od svih ispitanika uzeto je po 5 ml krvi na dane 0,15 i 30. Krv je centrifugirana i u plazmi ispitanika određeni su razni antioksidansi. Količina važnog oksidansa superoksidnog aniona $O_2^*$ smanjuje se u plazmi pušača nakon uzimanja Broccolina za 30 %. Količina C vitamina ostaje nepromjenjena dok se količina reduciranog glutationa povećava za 30%. Aktivnost antioksidativnih enzima superoksid dizmutaze i katalaze povećava se za 39 i 30% nakon tretmana sa Broccolinom. U ovom ispitivanju Broccolin ne mijenja koncentraciju holesterola i antioksidativni kapacitet u krvnoj plazmi ispitanih pušača cigareta. Ovi rezultati pokazuju da dijetetski preparat Broccolin povećava razinu antioksidansa i aktivnost antioksidativnih enzima u krvnoj plazmi ispitanika pušača cigareta. To pokazuje da Broccolin može pomoći organizmu u zaštiti od štetnog djelovanja slobodnih kisikovih radikala

## HUMAN SMOKING AND ANTIOXIDATIVE EFFECT OF BROCCOLI EXTRACT

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Cigarette smoking is strong risk factor for development cancer and different chronical diseases such as arteriosclerosis. Tobacco smoke possesses oxygen free radicals, which have harmful effect on cell function. In this investigation we measured antioxidative status in smokers (10-20 cigarette/day), which consumed broccoli extract. This extract was prepared as dietetic product commercially named Broccolin Pond, Zagreb). Every Broccolin capsule has 400 mg broccoli with quick extracted water from freshly gathered vegetable. Broccolin possesses high amount

of reduced glutathione (GSH) and vitamin C, which destroyed oxygen free radicals. 13 volunteers have ingested three Broccolin capsules every day through one month. All smokers gave 5.0 mL blood on days 0,15 and 30 after ingestion of Broccolin. Blood was centrifuged that separate plasma from erythrocytes and leucocytes. In plasma was determined different antioxidants and activity of antioxidative anzymes. Principal oxygen free radical superoxide anion $O_2^*$ decreased in smoker plasma for 30% after Broccolin treatment. Level of essential antioxidant reduced glutathione of oxidative stress decreased for 30% in smoker plasma after treatment. At the same time, activity of antioxidative enzymes superoxide dismutase and catalase increased for 39 and 30% after Broccolin ingestion. In this investigation Broccolin did not influence on cholesterol level and total antioxidative capacity in smokers. These results show that dietetic product Broccolin increased both level of antioxidants (GSH, vitamin C) and activity of antioxidative enzymes. This conclusion indicate on possibility that Broccolin might help in defence organism from damaging effect of oxygen free radicals in our environment.

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## KONFORMACIJSKE PROMJENE ALKALNE FOSFATAZE OPAŽENE POMOĆU OGRANIČENE PROTEOLIZE I MALDI-TOF MASENE SPEKTROMETRIJE

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Alkalna fosfataza (AP; E.C. 3.1.3.1.) iz bakterije *Escherichia coli* (AP) je homodimerni metalonezim koji sadrži dva vezna mjesta za $Zn^{2+}$ i jedno za $Mg^{2+}$ u svakom aktivnom mjestu. AP pokazuje odstupanja od Michaelis-Menten kinetike koja su tumačena negativnom kooperativnošću odnosno modelom dimernog enzima s nejednakim podjedinicama. Pretpostavlja se da su promjene konformacije izazvane vezanjem metalnih iona odgovorne za negativnu kooperativnost odnosno opaženu asimetriju enzima. U ovom radu ispitane su konformacijske promjene AP ovisne o metalnim ionima, za što je korištena ograničena proteoliza pomoću tripsina i MALDI-TOF masena spektrometrija. Opažano je značajno smanjenje podložnosti prema proteolitičkoj razgradnji nakon dodatka $Zn^{2+}$ apo-AP. Dodatak $Mg^{2+}$ zajedno s $Zn^{2+}$ dodatno povećava otpornost na triptičku razgradnju, dok sam $Mg^{2+}$ nema utjecaja. Analiza triptičkih fragmenata MALDI-TOF masenom spektrometrijom ukazala je na različitu proteolitičku osjetljivost peptidne veze iza Arg-293. Ovi rezultati upućuju na zaključak da vezanje metalnih iona izaziva konformacijske promjene koje se protežu daleko od mjesta njihovog vezanja na enzim.

## ALKALINE PHOSPHATASE CONFORMATIONAL CHANGES ASSESED BY LIMITED PROTEOLYSIS AND MALDI-TOF MASS SPECTROMETRY

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Alkaline phosphatase (AP; E.C. 3.1.3.1.) from *Escherichia coli* is a homodimeric metalloenzyme containing two $Zn^{2+}$ and one $Mg^{2+}$ binding site in each active centre. AP displays deviations from Michaelis-Menten kinetics that have been interpreted in terms of negative cooperativity or described by the model of a dimeric enzyme with unequal subunits. It has been postulated that conformational changes induced by binding of metal ions might be responsible for the expression of negative cooperativity or the observed enzyme asymmetry. In this study, metal-ion induced conformational changes of AP were examined by limited proteolysis and MALDI-TOF mass spectrometry. A marked decrease in susceptibility toward proteolytic degradation was observed upon addition of $Zn^{2+}$ to apo-AP. The addition of $Mg^{2+}$ together with $Zn^{2+}$ further enhanced the resistance to tryptic digestion, while $Mg^{2+}$alone had no effect. Analysis of AP tryptic fragments by MALDI-TOF mass spectrometry revealed differences in proteolytic susceptibility at the Arg-293 cleavage site. The results suggest that the binding of metal ions evokes conformational changes that extend far from the metal binding site.

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## DINAMIKA TELOMERA LJUDSKOG KROMOSOMA BROJ 1 U OKRUŽJU MIŠJIH STANICA A9

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Telomere su uređene strukture na krajevima eukariotskih kromosoma, a uloga im je zaštita i stabilizacija genoma. Sačinjava ih repetitivna DNA sekvenca (TTAGGG) na koju se veže niz proteina koji formiraju tzv. T-petlju. Stabilnost telomera je od izuzetnog značenja za stanicu jer izostanak njihove funkcije rezultira kromosomskim aberacijama, brojnim delecijama i na kraju apoptozom. Stoga je održavanje telomera esencijalno za sve imortalne stanične linije. U ovom radu smo koristeći PNA-FISH metodu, kvantitativnom analizom pratili dinamiku telomera u mišjoj staničnoj liniji A9. Pokazalo se da su telomere ovih stanica izuzetno nestabilne tako da ih je 28% neobilježenih što je popračeno i permanentno

promjenjivim kariotipom. U A9 stanice je uveden i ljudski kromosom broj 1 sa svrhom praćenja njegovih telomera u mišjem staničnom okružju. Iznenađujuće, uvođenje ljudskog kromosoma 1 je uzrokovalo skraćenje ukupne i prosječne dužine telomera što pokazuje Southern blot analiza, uz porast neobilježenih individualnih telomera na čak 66.5%. Pri tome nije bilo razlike u ponašanju telomera ljudskog kromosoma u odnosu na ostale telomere mišjih kromosoma. Uzrok ovakvom ponašanju telomera vjerojatno je ekspresija nekih ljudskih proteina koji interferiraju s mišjim proteinima važnima za održavanje telomera.

## HUMAN CHROMOSOME 1 TELOMERE DYNAMICS IN MOUSE A9 CELL LINE BACKGROUND

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Telomeres are specialized structures at the ends of all eukaryotic chromosomes and they protect and stabilize genome. They consist of repetitive DNA sequences (TTAGGG) which, bound to several proteins, form T-loop. It is important for every normal or immortal cell line to keep telomeres stable because their loss results in chromosomal aberrations, numerous deletions and ultimately apoptosis. In this work we used quantitative PNA-FISH analysis to follow telomere dynamics in A9 mouse cell line. As it has been shown, these cells have highly unstable telomeres among which 28% were not labeled in addition to permanently changed karyotype. Human chromosome 1 has been introduced into A9 cell line in order to follow its telomere dynamics in mouse cell background. Unexpectedly, introduction of human chromosome 1 resulted in total and average telomere shortening as it was shown by Southern blot, followed by increase of unlabeled telomeres up to 66.5%. Telomeres of human chromosome 1 behave same as telomeres of mouse chromosomes in the cell. We predict that such effect of human chromosome 1 is due to expression of some human proteins that interfere with mouse proteins on telomeres.

P-30

## EFFECTS OF DIAZEPAM ON MITOSIS OF HUMAN AND PLANT CELLS

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The goal of our work was to determine effects of diazepam (benzodiazepine anxiolytic) on the mitotic process: kinetics, separation, structure and organisation

of chromosomes in all division phases of human lymphocytes and *Allium cepa* root-tip cells. Human peripheral blood lymphocyte cultures and roots of *Allium cepa* were exposed to different concentrations (5, 10 and 20 mg/ml) of diazepam for 24 h. The results indicated a wide range of irregularities that included mitotoxic, chromo- and cytotoxic effects. Diazepam inhibited mitosis and caused mitotic arrest at pro- and metaphase. Among mitotic irregularities caused by spindle failure there were found irregular (disturbed) mitotic phases, multipolarity, laggards, vagrant chromosomes and C-mitosis. The chromatin abnormalities – chromosome stickiness and sticky bridges in ana- and telophase were observed. Based on established irregularities, it can be concluded that diazepam induces spindle aberrations and outstanding aneugenic activity. Also, this drug has mitodepressive action, caused stickiness of chromosomes and fragmentation of nucleus and therefore shows citotoxic activity. The identical findings obtained with *Allium* test and human peripheral blood lymphocyte *in vitro* test indices the possibility of extrapolation of results from one test system to another.

# GENOMIKA, FARMAKOGENETIKA, PROTEOMIKA, BIOINFORMATIKA I MOLEKULARNA DIJAGNOSTIKA *BIOLOGA MOLECULARIS SENSU STRICTO*
# GENOMICS, PHARMACOGENETICS, PROTEOMICS, BIOINFORMATICS AND MOLECULAR DIAGNOSTICS

IL-9

## TRANSKRIPTOMIKA I RAK

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Donedavno je istraživanje raka bilo temeljeno na vrlo dugotrajnim pokusima kojima je bilo moguće analizirati vrlo malen broj gena. Nove tehnologije, kao i Projekt humanog genoma, dovele su do znatnog pomaka u istraživanjima i temeljito promijenile naše razmišljanje o raku. Tako, na primjer, trankriptomika omogućuje otkrivanje mnogih procesa koji se u stanici dešavaju u određenoj jedinici vremena. Na žalost, cijene transkriptomskih analiza još su uvijek vrlo visoke, a analiza bi se, zbog osiguravanja reproducibilnosti rezultata, trebala na istom uzorku i istom metodom napraviti barem dva puta. Upravo zbog visoke cijene ove tehnologije rezultati se najčešće provjeravaju standardnim metodama. Ovaj problem dijelom je riješen "mikročipom" (Affymetrix, Inc) na koji je nanešeno 20 oligonukleotida po genu, 25-mera, koji su u 50% slučajeva istovjetni nukeleotidnom slijedu gena, dok u 50% slučajeva postoji nesuglasje ("mismatch") čime su znatno unaprijeđene osjetljivost i specifičnost metode. Na žalost, čak i u onim slučajevima u kojima su problemi vezani uz tehnologiju svedeni na najmanju moguću razinu, u području genetike raka neophodno je uspostaviti kriterije vrednovanja dobivenih rezultata, uspostaviti blisku suradnju između liječnika na klinici i znanstvenika u laboratoriju, te, najvažnije, osigurati kontrolu kvalitete rezultata. Navedene osobitosti transkriptomike bit će raspravljene u sklopu naših rezultata dobivenih "microarray" analizom zloćudnih tumora grkljana u ljudi.

## THE TRANSCRIPTOMICS OF CANCER

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Until recently, cancer research relied on a laborous and limited one-gene-at-a-time approach. Recent technological advances, coupled with the draft human genome sequence, have accelerated this research and fundamentally changed how we think about cancer. Transcriptomics is not only allowing us to see the whole cell at once, rather than bits of the cellular machinery in isolation, but also provides extremely detailed information and a global framework for understanding complex patterns in cells. The cost of arrays constrains the application of multiple replicates, and the validation of marked changes in individual genes must be checked with conventional approaches. This might be partially overcome by oligonucleotide arrays manufactured with twenty 25mers representing each gene on the array (10 perfectly matched sequences and 10 mismatched at the middle position) (Affymetrix, Inc). This provides some measure of

replication as target cRNA binds each independently, allowing an assesment of sensitivity and specificity. But even in those cases when the problems connected with the use of current technology are minimalized, the power of functional genomics in this particular field will require implementation of high throughput validation techniques, close collaboration between laboratory scientists and clinicians, data sharing formats and quality control measures. All these issues will be discussed in relation to our experience with human laryngeal carcinoma samples.

IL-10

## MECHANISMS OF APOPTOSIS IN CELLS EXPRESSING THYMIDINE KINASE OF THE *Herpes simplex* VIRUS TYPE 1 OR OF THE *Varicella zoster* VIRUS UPON EXPOSURE TO PURINE AND PYRIMIDINE NUCLEOSIDE ANALOGS

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Antiviral nucleoside analogs, as the purine analog ganciclovir (GCV) or acyclovir (ACV), have not only been used in the antiviral therapy of herpesvirus infections but also in the so-called suicide gene therapy of malignant tumors (e.g. *Glioblastoma multiforme*). The therapy is based on the transfer of a suicide gene (e.g. HSV*tk*) into target cells, followed by administration of an antiviral drug. To examine the response of target (potential tumor) cells to the suicide gene therapy, stable transfectants of Chinese hamster ovary (CHO) cells expressing thymidine kinase (TK) of either the Herpes simplex virus type 1 (HSV-1) or the Varicella zoster virus (VZV) were generated, and expression of the viral TKs determined by immunoblotting using specific antisera against HSV-1-TK or VZV-TK. The transfected clones were used as a sensitive model for studying cytotoxic (apoptotic) and genotoxic (clastogenic and recombinogenic) effects upon exposure to GCV and to the pyrimidine analog (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), respectively. Compared to the isogenic CHO-*neo* counterpart, CHO-HSV*tk* cells were hypersensitive towards GCV and CHO-VZV*tk* cells towards BVDU, indicating efficient conversion of both prodrugs to the corresponding monophosphates (*via* viral TK; rate-limiting) and to the active triphosphates (by cellular nucleoside kinases), which are incorporated into genomic DNA. In spite of similar viral TK-mediated drug conversion, different apoptotic pathways were induced. GCV triggered severe DNA damage and mitochondria-amplified apoptosis due to caspase-9/3-mediated cleavage of Bcl-2. Although efficiently incorporated, BVDU elicited only marginal DNA damage and triggered death receptor (Fas) dependent pathway involving AP-1-mediated induction of the Fas ligand and caspase-8 activation.

OP-20

## PROUČAVANJE IZRAŽAJA I ULOGE TRI MIŠJA GENA PREINAČENA POSTUPKOM GENSKE ZAMKE

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Postupkom genske zamke se mišji geni na slučajan način molekularno obilježeni, čime je omogućeno proučavanje njihove uloge i uzorka izražaja. Genetska preinaka učinjena je nehomolognim DNA umetkom putem embrionalnih matičnih stanica. Umetak se sastoji od primača prekrajanja iza kojeg slijede geni lacZ i neoR bez vlastitog promotora, te polyA signal na kraju. Ako je umetnut u gen koji se prepisuje, primač prekrajanja postaje aktivan i lacZ i neoR geni bivaju uključeni kao završni egzoni gena uhvaćenog na ovaj način u gensku zamku. NeoR daje otpornost na neomicin, što omogućava prepoznavanje preinačenih embrionalnih matičnih stanica, dok lacZ kodira bjelančevinu beta-galaktozidazu, čija prisutnost se može pratiti u tkivu jednostavnim histokemijskim bojanjem. Tri preinake genskom zamkom i odgovarajuće mišje linije nosioci ovih preinaka izdvojene su za daljnje istraživanje, te nazvane H15, lobel i kosenic. H15 mutacija odgovara preinaci genskom zamkom gena koji kodira bjelančevinu jezgrice, *Nol1* (*nucleolar protein 1*). Za ovu bjelančevinu smatra se da sudjeluje u umnažanju stanica. Prisutna je u svim organima i tkivima tijekom embrionalnog razvoja, a najviše je se nalazi u jezgrici stanice. Homozigotni zameci odumiru oko stadija blastociste. Lobel je mutacija gena uključenog u prijenos unutarstaničnih signala, *Stam2* (*signal transducing adapter molecule 2*). Odgovarajuća bjelančevina sudjeluje u toku signala preko endosoma, a prisutna je tijekom razvitka i u odraslom mozgu i srcu. Homozigotne životinje žive i pare se. Kosenic je doskora bio smatram novim genom, ali novi podaci dobiveni sekvencioniranjem mišjeg genoma pokazali su da je to mišji ortolog ljudskog gena *Klf8* (*Krüppel-like factor 8*). To je transkripcijski čimbenik nepoznate uloge. Smješten je na X kromosomu, a izražen je u skupini stanica osnove srca, te u embrionalnom i odraslom mozgu. Hemizigotni mužjaci, kojima nedostaje normalni alel ovog gena žive i pare se, premda se kongenični soj nije uspio uspostaviti, jer se na visoko srođenoj C57Bl/6 podlozi ove životinje prestaju pariti nakon nekoliko generacija.

## ANALYSIS OF EXPRESSION AND FUNCTION OF THREE MOUSE GENES MODIFIED BY GENE TRAP METHOD

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Gene trap method is a random approach, which enables molecular tagging of mouse genes, with their subsequent expression pattern and functional analysis. Genetic modification was done with a nonhomologous DNA construct via embryonic stem cells. Construct consisted of splice acceptor followed with promoterless lacZ and neoR genes, and the polyA signal at the end. If the construct was introduced within a transcribed gene, splice acceptor was active and lacZ and neoR acted as the last exons in the "trapped" gene. NeoR gave resistance to neomycine and enabled selection of the gene trap events in the embryonic stem cells, while lacZ coded for beta-galactosidase, which could be easily monitored in tissues by simple histochemical staining. Three gene trap modifications and corresponding mouse lines carrying these modifications were selected from the gene trap screen. They were called H15, lobel and kosenic. H15 mutation corresponds to an insertion of the gene trap vector within the gene coding for nucleolar protein 1 (Nol1), a gene implicated to be important in cell proliferation. Gene is expressed in all organs and tissues during embryo development, and it is strongly present in the cell nucleolus. Homozygous embryos die during embryo development around blastocyst stage. Lobel is a mutation within Signal transducing adapter molecule 2 (Stam2), which is involved in endosome mediated cell signalling pathway. It is expressed in the developing and adult brain and heart. Homozygous mice are alive and fertile. Kosenic was until recently thought to be a novel mouse gene, but the new data obtained from the sequencing of the mouse genome revealed it as a mouse homologue of the human Krüppel-like factor 8 (Klf8). This is a transcription factor with unknown function. It is located on X chromosome and expressed in the subset of cells in the developing heart, and in the developing and adult brain. Hemizygous males, which lack a non-mutated copy of the gene are alive and fertile, although congenic strain on C57Bl/6 inbred background was not achieved, as the mice failed to breed after several generations.

OP-21

## KOLIKO VISOK SISTEMSKI INDEKS JE POTREBAN ZA POUZDANO UTVRĐIVANJE IDENTITETA ŽRTAVA RATA ANALIZOM DNA?

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Analiza kratkih uzastupno ponavljajućih sljedova DNA (STR) je danas metoda izbora u identifikaciji ljudi, no značajnost sukladnosti među genotipovima se često pogrešno tumači. U sklopu procesa identifikacije žrtava Domovinskog rata uočili

smo neočekivano velik broj sukladnosti u 14 i 15 STR lokusa između ljudi koji nisu u međusobnom srodstvu i pretpostavili da bi to mogla biti posljedica međusobnog križanja bliskih rođaka u lokalnim populacijama. Potporu toj pretpostavci našli smo u nedostatku korelacije između vjerojatnosti pojedinog genotipa i broja sukladnih genotipova (potencijalnih roditelja i djece) u našoj bazi podataka. Razvili smo novi način usporedbe genotipova na osnovi kombinacije mini-haplotipova i pomoću njega ispitali skupinu od 295 osoba koje nisu u međusobnom srodstvu. Unatoč činjenici da je koeficijent srodnosti (inbreeding coefficient) bio nizak, neki od mini-haplotipova bili su i 90% učestaliji od očekivanog. Srodnost genotipova u kombinaciji s velikim brojem usporedbi koji se provode pri pretraživanju baza genotipova potencijalne rodbine može bitno smanjiti značajnost sukladnosti dvaju genotipova pa je potreban velik oprez pri tumačenju značajnosti laboratorijskih rezultata. Gdje god je to moguće identifikacije se trebaju temeljiti na kombinaciji analize većeg broja rođaka i ostalih "klasičnih" forenzičkih dokaza.

## HOW HIGH PATERNITY INDEX IS NEEDED FOR RELIABLE IDENTIFICATION OF WAR VICTIMS BY DNA TYPING?

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Typing of short tandem repeat (STR) DNA is the "golden standard" of human identification, but evidential value of a genetic match can be easily misinterpreted. While working in the process of identification of war victims in Croatia we observed unexpectedly high number of 14 and 15 STR loci matches between unrelated people and hypothesized that this might be a consequence of local inbreeding. Initial support for this hypothesis came from the lack of correlation between the probability of a genotype and the number of matching genotypes in the database. Novel mini-haplotype analysis that compares matches in all three-loci combinations of alleles was developed and used to study inbreeding in the group of 295 unrelated individuals. Despite the fact that inbreeding coefficient in the population was low, up to 90% overrepresentation of some mini-haplotypes was observed, indicating the existence of local inbreeding effects. The existence of local inbreeding effects, and the effect of large numbers (when genotypes in the database are randomly matched) can significantly decrease evidential value of a genetic match and careful use of statistical methods is essential for the proper evaluation of laboratory results. Whenever possible, multiple relatives should be analyzed, and other evidence based on the information about time, place and other conditions of disappearance, as well as anthropological and other "classical" forensic data should always be put together and compared before any final decision about the identity is made.

OP-22

## ANALIZA KRATKIH UZASTOPNIH PONAVLJANJA DNA U PRAĆENJU KIMERIZMA NAKON ALOGENE TRANSPLANTACIJE KOŠTANE SRŽI

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Miješani kimerizam (MC) je stanje u kojem su nazočne i primateljeve i davateljeve hematopoetske stanice, a opaža se nakon transplantacije koštane srži (TKS). U prijašnjim istraživanjima koristile su se standardne citogenetske tehhnike, krvne grupe ili metoda RFLP. Međutim, u brojnim slučajevima niti jedna od nabrojenih metoda nije informativna, te se analize kratkih uzastopnih ponavljanja DNK (Short Tandem Repeat –STR) može primjeniti i u praćenju kimerizma nakon TKS. Ovim istraživanjem pratili smo 15 bolesnika nakon TKS, dvanaest bolesnika bilo je podvrgnuto klasičnoj TKS, dok su preostala 3 bolesnika bila u programu minitransplantacije. Uzorci DNK bili su umnoženi lančanom reakcijom polimerazom za 5 STR lokusa (TH01, VWA31, FES/FPS, F13A01, i SE33) i jedan VNTR lokus (D1S80). U slučajevima minitransplantacije testirana su još 4 dodatna lokusa (D1S1656, D12S391, D18S535, te D22S683). PCR produkti su zatim analizirani elektroforezom u automatskom sekvenceru (ALFexpress). Kod svih 15 parova primatelja i davatelja uočene su razlike na barem dva STR lokusa. U većini slučajeva razlika je opažena na lokusu SE33. MC je pronađen kod sva 3 bolesnika u programu minitransplantacije, kod dva primatelja koji su transplantat KS primili od nesrodnog davatelja, te kod jednog bolesnika koji je imao davatelja KS unutar obitelji. Jedini slučaj u kojem je MC imao prognostičku vrijednost bio je kod bolesnika koji je pokazivao potpuni kimerizam do 160-og dana nakon transplantacije kad je uočeno 40% stanica primatelja. Istraživanje je pokazalo da su analiza lokusa STR zbog visoke informativnosti, relativne jednostavnosti i kratkotrajne analize, metoda izbora u praćenju kimerizma nakon TKS.

## SHORT TANDEM REPEAT LOCI – USEFUL TOOL FOR FOLLOWING CHIMERISM AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Mixed chimerism (MC) is a state in which there is a presence of both recipient and donor hemopoietic cells and is frequently observed after bone marrow transplantation (BMT). Previous studies have used cytogenetic techniques, changes in erytrocyte phenotype or RFLP analysis for following chimerism. In cases when none

of these markers is useful, analysis of short tandem repeat (STR) loci can be potential diagnostic tool. In this study we monitored 15 patients suffering from various hematological disorders. Twelve of them underwent classical conditioning regimen while the remaining 3 patients were subjected to nonmyeloablative conditioning (minitransplantation). DNA samples were amplified for 5 STR loci (TH01, VWA31, FES/FPS, F13A01, and SE33) and one VNTR locus (D1S80). In the cases of minitransplantation additional 4 loci (D1S1656, D12S391, D18S535, and D22S683) were also tested. Samples were analysed by electrophoresis in automated ALFexpress sequencer. In all 15 donor recipient-pairs we found differences for at least two STR loci. In most cases the difference was observed for SE33 locus. MC was detected in all 3 cases of minitransplantation, 2 cases with unrelated donor and one case with related donor. The only case in which MC was predictive of relapse was the case of a patient who showed full donor chimerism until day 160$^{th}$ post BMT when 40% of recipient cells were detected. In conclusion, this study showed that high informativeness, relative simplicity and short performing time of STR loci analysis makes this method a valuable tool for monitoring chimerism in BMT program.

OP-23

## UČINAK VRLO AKTIVNOG ANTIRETROVIRUSNOG LIJEČENJA NA FENOTIP T-LIMFOCITA OSOBA ZARAŽENIH VIRUSOM HUMANE IMUNODEFICIJENCIJE TIPA 1

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Cilj istraživanja bio je utvrditi učinak vrlo aktivnog antiretrovirusnog liječenja (HAART) na fenotip T-limfocita 27 terapijski-naivnih simptomatskih HIV-bolesnika s mjerljivom viremijom (medijan 5.6 log kopija HIV-1 RNA/ml plazme) i značajno smanjenim brojem $CD4^+$ T-limfocita prije liječenja (medijan 88 stanica/ml periferne krvi). Bolesnici su liječeni kombinacijom zidovudina, lamivudina i indinavira u preporučenim dozama. Ekspresija odabranih površinskih biljega $CD4^+$ i $CD8^+$ T-limfocita (CD45RA, CD45RO, CD62L, CD38, CD28) analizirana je protočnim citometrom (Coulter Epics XL) prije i nakon 1, 3, 6 i 12 mjeseci liječenja. Broj veznih mjesta za anti-CD38 antitijela određen je kvantitativnom protočnom citometrijom

(CELLQUANT, Biocytex). Viremija u plazmi određena je RT-PCR-om (Amplicor HIV-1 Monitor, Roche). Smanjenje viremije u plazmi na nemjerljivu razinu otkriveno je kod 90% bolesnika nakon 12 mjeseci primjene HAART-a. Porast subpopulacije $CD4^+$ T-limfocita opažen je kod 26 od 27 bolesnika s nemjerljivom viremijom. Značajan porast memorijskih $CD45RO^+CD4^+$ T-limfocita opažen je nakon prvog mjeseca liječenja. Značajan porast istinski naivnih $CD62L^+CD45RA^+CD4^+$ T-limfocita dokazan je tek nakon 12. mjeseci liječenja. Liječenjem potaknuto smanjenje viremije bilo je paralelno sa smanjenjem postotka aktiviranih $CD38^+CD8^+$ T-limfocita. Međutim, povećan broj veznih mjesta za anti-CD38 antitijela u bolesnika koji su imali nemjerljivu viremiju tijekom najmanje 9 mjeseci ukazuje na rezidualnu virusnu aktivnost. Postotci $CD28^+CD8^+$ T-limfocita porasli su do intervala referentnih vrijednosti nakon 12 mjeseci liječenja (za razliku od svih drugih imunoloških parametara). Jednogodišnja primjena HAART-a omogućava barem djelomičnu korekciju HIV-om potaknutih promjena fenotipa T-limfocita čak i u uznapredovaloj fazi HIV-bolesti.

## THE EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT (HAART) ON THE PHENOTYPE OF T-CELLS OF PERSONS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

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Our goal was to determine the effect of highly active antiretroviral treatment (HAART) on the phenotype of T-cells of 27 treatment-naive symptomatic HIV-patients with high pre-treatment viremia (median 5.6 log of HIV-1 RNA copies/ml) and reduced numbers of $CD4^+$ T-cells (median 88 cells/ml). HAART regimen consisted of zidovudine, lamivudine and indinavir at standard doses. The expression of selected markers (CD45RA, CD45RO, CD62L, CD38, CD28) on $CD4^+$ and $CD8^+$ T-cells was analysed at baseline and after 1,3,6 and 12 months of HAART by flow cytometry (Coulter Epics XL). The enumeration of anti-CD38 antibody binding sites was performed by quantitative flow cytometry (CELLQUANT, Biocytex). Viral load was determined by RT-PCR (Amplicor HIV-1 Monitor, Roche). HAART successfully decreased plasma viremia to undetectable levels in 90% of patients after 12 months.

An increase in $CD4^+$ T-cells was observed in 26 out of 27 patients with undetectable viremia. Increased percentages of memory $CD45RO^+CD4^+$ T-lymphocytes were observed after the first month of treatment. Significant increases in truly naive $CD62L^+CD45RA^+CD4^+$ T-lymphocytes were observed after 12 months of treatment. Therapy-induced decrease in plasma viremia paralleled a decrease in the percentages of $CD38^+CD8^+$ T-lymphocytes. However, increased numbers of anti-CD38 antibody binding sites in HIV-patients who had undetectable plasma viremia for at least 9 months, suggested residual viral activity. The percentages of $CD28^+CD8^+$ T-cells returned to reference values within one year of HAART (unlike other immune parameters). One year of HAART allows at least partial correction of HIV-induced changes in the phenotype of T-cells, even in advanced stages of HIV-disease.

OP-24

## PRIJENOS DNA IZ GENETIČKI PROMIJENJENIH BILJAKA U BAKTERIJU IZ TLA

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Obično se smatra da su vrste međusobno reproduktivno izolirane, međutim, za horizontalni prijenos gena pokazano je da se događa kod bakterija, a vjerojatno i kod viših organizama. Za oko 40 vrsta bakterija iz tla zna se da su prirodno sposobne apsorbirati ("uzeti") DNA iz svog okoliša. Ipak, pokušaji izravnog promatranja prijenosa DNA iz biljaka u bakterije često su davali negativne i upitne rezultate. U našim eksperimentima pokazan je prijenos DNA iz šest donorskih biljnih vrsta u bakteriju iz tla, *Acinetobacter* spp. BD413, korištenjem gena za neomicin-fosfotransferazu (*nptII*) kao markera za homolognu rekombinaciju. Neomicin-fosfotransferaza je enzim koji daje otpornost na antibiotike neomicin i kanamicin. Prijenos je dokazan već iz 0,1 g smrvljenog lišća duhana koji je imao *nptII* gen ugrađen u plastidnu DNA, te iz slične količine korijenja uljane repice s istim genom ugrađenim u jezgru. Prijenos iz smrvljenog lišća odvijao se u sterilnoj zemlji i vodi, bez dodatka hranjivih tvari. Bio je ovisan o masi biljnog tkiva tj. količini donorske DNA, osjetljiv na DNazu, a mutacije u *nptII* genu iz biljaka bile su popravljene u receptorskim bakterijama. S većom varijabilnošću detektiran je i prijenos DNA iz cjelovitih korjenčića uljane repice i ladoleža, te iz biljaka duhana i uročnjaka (*Arabidopsis thaliana* (L.)Heynh.) *in vitro*. Prijenos je varirao ovisno o veličini biljnog genoma i broju kopija gena markera u biljci donoru. Ovi rezultati ukazuju na mogućnost prijenosa DNA iz genetički promijenjenih biljaka u bakterije i u prirodi.

## DNA TRANSFER FROM GENETICALLY MODIFIED PLANTS TO A SOIL BACTERIUM

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Species are usually considered to be sexually isolated, but horizontal gene transfer is easily demonstrated in bacteria and likely occurs in higher organisms. Approximately 40 species of soil bacteria are known to acquire DNA from their environment, yet attempts to directly observe transfer of DNA from plants to bacteria have often produced negative or equivocal results. In our experiments, DNA transfer was demonstrated from six species of donor plants to the soil bacterium, *Acinetobacter* spp. BD413, using neomycin phosphotransferase (*nptII*) gene as a marker for homologous recombination. Neomycin phosphotransferase is an enzyme that gives resistance to antibiotics neomycin and kanamycin. In tobacco carrying a plastid insertion of *nptII*, transfer was detected with 0.1 g of disrupted leaves and in oilseed rape carrying a nuclear insertion with a similar quantity of roots. Transfer from disrupted leaves occurred in sterile soil and water, without the addition of nutrients. It was dose-dependent and sensitive to DNase, and mutations in the plant *nptII* were recovered in receptor bacteria. DNA transfer using intact oilseed and morning glory roots, and tobacco and *Arabidopsis* plants *in vitro* was also detected, but with greater variability. Transfer varied with plant genome size and the number of repeats of the marker DNA in the donor plant. These results imply the possibility that DNA transfer from genetically modified plants to bacteria could occur in nature.

OP-25

## STOHASTIČKO SIMULIRANJE STANIČNIH REAKCIJA

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Cilj rada je istražiti mogućnosti primjene kemijsko inženjerskih načela zakona sačuvanja tvari i energije za analizu dinamičkih pojava i regulacije intracelularnih reakcija u jednoj stanici. Osnovno koncept se zasniva na pretpostavci da se pojedina

živa stanica može matematički modelirati kao višefazni katalitički reaktor uz stalno povećanje volumena kao posljedica rasta stanice. Model regulacija metabolizma zamišljen je kao hijerarhijski organiziran i prostorno raspodijeljen upravljački sustav. Robusni pristup modeliranju dinamičkih pojava u bioreaktoru zasniva se na determinističkom opisu kinetike reakcija u uvjetima idealnog miješanja. Za razliku od reaktora, modeliranje pojedine stanice mora uzeti u obzir da se neke ključne molekule nalaze u stanici u vrlo malom broju zbog čega dolazi do značajnih slučajnih pojava. Model vremenski kontinuiranih promjena stanja prestaje i dolazi do diskretnih promjena stanja (broja molekula u stanici). U radu je primijenjen Gillespie algoritam egzaktne simulacije stohastičkih reakcijskih sustava. Stohastički modeli su izvedeni iz determinističkih modela i kinetičkih parametara za proračun vjerojatnosti pojedinih molekularnih interakcija. Primjenom ovog algoritma simulacijom stohastičkih reakcijskih sustava postiže se asimptotska konvergencija s determinističkim modelom kada broj molekula postaje dovoljno velik. Ovo svojstvo omogućuje usporedbu rezultata stohastičke simulacije s eksperimentalnim zapažanjima kada je broj stanica (pojedinih molekula) u promatranom uzorku (volumenu) dovoljno velik. Za numerički postupak simulacije primijenjen je programski sustav *W.R. Mathematica*. Analizirane su stohastičke pojave kod monosupstratne enzimske reakcije, koenzimske reakcije, reakcijskog niza (puta) s pozitivnim povratnim djelovanjem kada nastaju uvjeti graničnih ciklusa i oscilirajućih stanja. Primijenjen je deterministički model i parametri Lee i Bailey za istraživanje stohastičkog utjecaja na stabilnost regulacije operona laktoze (*lac*) u *E. coli*.

## STOHASTIC SIMULATION OF CELLUAR REACTIONS

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Aim of the work is to apply chemical engineering principles of mass and energy balances for analysis of dynamics and control of intracellular reaction systems. The basic concept is developed based on assumption that a living cell can be mathematically modelled as a multiphase catalytic reactor with expanding volume due to cell growth, and with hierarchical organised and spatially distributed internal control systems. The robust approach to modelling bioreactors is based on standard chemical engineering concept of a well mixed cup with deterministic kinetics. However, when a single cell is considered, small number of individual molecules present in a cell leads to important stochastic effects in molecular interactions. In time continuous change of state breaks down and discrete reaction occurrences must be accounted. In this work Gillespie algorithm for exact simulation of stochastic chemical reaction systems is applied. Stochastic models are formulated by translation of deterministic mechanisms and kinetic parameters into evaluation of probabilities of individual molecular interactions. Such stochastic modelling provides deterministic solutions as an asymptotic case of a stochastic

model. The asymptotic behaviour enables verification of stochastic predictions by experimental data determined in observations of cell populations. W.R. *Mathematica* software language and numerical procedure are applied. Analysed are effects of stochastic effects on a single enzyme reaction, coenzyme reaction, and a series of enzyme reactions with positive feedback, which leads to appearance of a limit cycle and sustained oscillations. Based on deterministic kinetic data by Lee and Bailey stochastic effects on stability of a regulation of the lactose (*lac*) operon in *Escherichia coli* are investigated.

OP-26

## MASENA SPEKTROMETRIJA U ANALIZI NESTABILNIH POSTTRANSLACIJSKIH MODIFIKACIJA: PRIMJENA U ANALIZI O-FUKOZILACIJE PROTEINA

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Iako se masena spektrometrija danas rutinski koristi u strukturnoj analizi proteina i proteoma, njena primjena u analizi određenih posttranslacijskih modifikacija, kao što su fosforilacija i O-glikozilacija proteina, još uvijek predstavlja izazov. Uslijed jednostavnog neutralnog gubitka tih modifikacija s proteinskog/peptidnog lanca u plinskoj fazi masenog spektrometra, rezultirajući spektri obično ne sadrže informacije o njihovoj strukturi i položaju. Mi smo nedavno optimirali uvjete za sudarom-uzrokovanu disocijaciju (eng. collision induced dissociation, CID), O-glikopeptida u nano-elektrosprej kvadrilpol-vrijeme-leta (eng. nano-electrospray quadrupole time-of-flight, nano-ESI Q-TOF) masenom spektrometru, pri kojima se samo jednim CID MS/MS mjerenjem mogu prikupiti informacije o glikozilacijskom statusu i glikozilacijskom mjestu analiziranog glikopeptida, kao i strukturama glikana i peptidnog lanca. Te smo uvjete primijenili u strukturnoj analizi posttranslacijske modifikacije uočene na ponavljajućim trombospondinskim sljedovima tipa 1 (eng. thrombospondin-type1 repeats, TSRs), strukturnim motivima očuvanim u mnogim proteinskim obiteljima. Nano-ESI Q-TOF CID masenom spektrometrijom i kromatografijom glikana na anionskom izmjenjivaču pri visokom pH, pokazano je da je dotična modifikacija disaharid koji se sastoji od fukoze i glukoze i koji je vezan putem fukoze na specifičnom glikozilacijskom mjestu unutar analiziranih sljedova. Ta modifikacija predstavlja novi tip O-fukozilacije proteina, prvi otkriven izvan domena sličnih epidermalnom faktoru rasta (eng. EGF). Štoviše, ta se modifikacija nalazi u veznim regijama TSR, za koje se pretpostavlja da posreduju u mnogim biološkim funkcijama proteina. Strukturni i funkcionalni aspekti ove nove posttranslacijske modifikacije proteina bit će dodatno raspravljeni.

# MASS SPECTROMETRY IN ANALYSIS OF LABILE POSTTRANSLATIONAL MODIFICATIONS: APPLICATION TO PROTEIN O-FUCOSYLATION

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Mass spectrometry (MS) is widely and routinely used in structural analysis of proteins and proteomes, yet its application to analysis of certain posttranslational modifications, such as protein phosphorylation or O-glycosylation still remains a challenge. Due to an easy neutral loss of these modifications from the protein/peptide backbone in the gas phase of the mass spectrometer, resulting spectra are usually devoid of useful information on their structure and location. We have recently optimized conditions for collision induced dissociation (CID) of O-glycopeptides in a nano-electrospray quadrupole time-of-flight (nano-ESI Q-TOF) mass spectrometer. In a single CID MS/MS measurement, we were able to obtain information on glycosylation status, glycan structure and glycosylation site of the analyzed glycopeptide. These conditions were applied to structural characterization of a posttranslational modification situated in thrombospondin-type1 repeats (TSRs), a structural motif conserved in many protein families. Using nano-ESI Q-TOF CID MS/MS and high-pH anion exchange chromatography (HPAEC), it was demonstrated that the modification is a disaccharide consisting of glucose and fucose, bound to the protein backbone through the fucose residue within specific glycosylation sites. This modification represents a new type of protein O-fucosylation, the first to be discovered beyond the epidermal growth factor (EGF) domains. Moreover, it is situated within the binding regions of TSRs, which are proposed to mediate many biological functions of TSR-containing proteins. Structural and functional implications of this novel protein modification will be discussed.

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## MIJENJANJE SPEKTROSKOPSKIH OSOBINA ZELENOG FLUORESCENTNOG PROTEINA Gfp+ POSTUPKOM *IN VITRO* MUTAGENEZE

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Gen *gfp* koji kodira zeleni fluorescentni protein (Gfp) često Se koriSTI kao genski biljeg i glasnik genske ekspresije. Do sada su razvijene brojne varijante Gfp sa svojstvima veće sjajnosti, uspješnijeg smatanja pri 37°C i boljih spektroskopskih osobina. Najboljom od njih smatra se protein Gfp+, koji je prilagođen ekspresiji u bakteriji *E. coli*. Cilj našega rada bio je postupkom istovremene višestruke ciljane mutageneze gena *gfp+* dobiti (*i*) cijan-plavi (Cfp+) i (*ii*) žuti (Yfp+) fluorescentni protein. Na temelju sekvenci gena *ECFP* i *EYFP* eksprimiranih u humanim stanicama odabrane su aminokiseline za koje se pretpostavilo da značajno pridonose stvaranju spektroskopskih svojstava. Za dobivanje proteina cijan-plave fluorescencije uvedeno je 6 mutacija koje mijenjaju 5 aminokiselina: K26R, Y66W, N146I, N164H i N212K, a za dobivanje proteina žute fluorescencije uvedeno je 7 mutacija koje mijenjaju 4 aminokiseline: T65G, V68L, S72A i T203Y. Potencijalnim transformantima *cfp+* i *yfp+* izmjerene su optičke gustoće i jačine fluorescencijskih signala. Za izračunavanje njihovih relativnih fluorescencijskih signala pomno je osmišljena matematička forma, pomoću koje su signali transformiranih uspoređeni sa signalima netransformiranih stanica *E. coli*. Analiziranim je mutantima potvrđeno svojstvo cijan-plavog odnosno žutog fluoresciranja. Klonovima s najboljim rezultatima određene su sekvence mutiranih gena *gfp+* te je utvrđena iznimna uspješnost mutageneze. Uz to, zaključeno je da zamjena aminokiseline u tripeptidnom aktivnom mjestu nužna, dok bi preostale zamjene mogle imati aditivan utjecaj na poboljšanje topljivosti i sjajnosti proteina. Pristup opisan ovim radom predstavlja važan početni korak za sve buduće postupke unapređivanja karakteristika grupe zelenih fluorescentnih proteina.

## THE CHANGE OF SPECTROSCOPIC PROPERTIES OF THE GREEN FLUORESCENT PROTEIN Gfp+ BY *in vitro* MUTAGENESIS

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The gfp gene that encodes green fluorescent protein (Gfp), is frequently used as a gene marker as well as a reporter gene. Numerous forms of Gfp that show greater brightness, better folding at 37°C and enhanced fluorescence properties have been developed until now. The aim of this research was to produce (i) cyan (Cfp+) and (ii) yellow (Yfp+) fluorescent protein using the method of multiple site-directed mutagenesis. Aminoacids that could be significant for the spectroscopic properties of the proteins have been chosen on the basis of sequences of the ECFP and EYFP genes expressed in human cells. In order to gain cyan fluorescent protein, 6 mutations that change 5 aminoacids have been introduced: K26R, Y66W, N146I, N164H and N212K. For the purpose of producing yellow fluorescent protein, 7 mutations that change 4 aminoacids have been introduced: T65G, V68L, S72A and T203Y. Optical density and fluorescence signals of potential cfp+ and yfp+ transformants were measured. Relative fluorescence signals of these clones were calculated using carefully conceived mathematical form and the signals were compared with signals of untransformed cells of E. coli. It was confirmed that analyzed mutants possess cyan and yellow fluorescing properties. Mutated gfp+ genes of the clones that showed the best results were sequenced and exceptional efficiency of mutagenesis was found. Further more, sequencing analysis confirmed the necessity of the mutations in the tripeptide active site while the other mutations might have cumulative effect on solubility and brightness of proteins. The approach described above represents a good starting point for all subsequent experiments of improving green fluorescent proteins.

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## ANALIZA HAPLOTIPOVA NA TRI STR LOKUSA KAO POKAZATELJ SRODNOSTI GENOTIPOVA U POPULACIJI

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Danas je općeprihvaćeno da analiza kratkih uzastopno ponavljajućih sljedova DNA (engl. short tandem repeat, STR) može dati dostatnu informaciju za jednoznačno utvrđivanje identiteta ili očinstva u većini forenzičkih slučajeva. Razvijene su sofisticirane statističke metode koje procjenjuju značajnost sukladnosti genotipova, no brojevi dobiveni na taj način nisu nužno točni u svim situacijama i potrebno im je pristupiti s oprezom. U sklopu procesa identifikacije žrtava Domovinskog rata proveli smo stotine tisuća nasumičnih usporedba

genotipova i uočili statistički neočekivano velik broj sukladnosti genotipova između ljudi koji nisu u srodstvu. Pretpostavili smo da bi to mogla biti posljedica povećane učestalosti određenih kombinacija STR alela zbog križanja u lokalnim zatvorenim populacijama. Da bismo ispitali tu pretpostavku razvili smo novu metodu koja ispituje učestalost svih mogućih kombinacija haplotipova od po 3 lokusa (mini-haplotip). Izračunavši učestalost svih mini-haplotipova potvrdili smo pretpostavku da je učestalost pojedinih mini-haplotipova bitno veća od očekivane. Utvrdili smo da su različiti mini-haplotipovi pretjerano zastupljeni u Hrvatskoj i Mađarskoj što potvrđuje pretpostavku da odstupanja su u učestalosti specifična za pojedine populacije. Vjerojatnost kompletnih (9 lokusa) genotipova izračunata kombinacijom vjerojatnosti mini-haplotipova može bitno odstupati od vjerojatnosti tog istog genotipa izračunate kombinacijom vjerojatnosti pojedinačnih alela, što upućuje na činjenicu da statističke metode koje se sada koriste mogu dati pogrešnu procjenu vjerojatnosti genotipa.

## ANALYSIS OF THREE-LOCUS STR HAPLOTYPES MIGHT PROVIDE INSIGHT INTO INBREEDING EFFECTS IN A POPULATION

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Today it is generally accepted that short tandem repeat (STR) sequences can provide sufficient information to determine identity or paternity in most forensic cases. Elaborate statistical methods have been developed to quantify the significance of genetic matches, but the calculated numbers may not be valid in all situations and have to be used with caution. As a part of the process of identification of war victims in Croatia we have randomly matched hundreds of thousands of genotypes and observed unexpectedly high number of statistically improbable matches between unrelated people. We speculated that this might be a consequence of higher than expected frequency of specific combinations of STR alleles because of local inbreeding. To assess this hypothesis we have developed a novel method that analyses incidence of all possible three-locus haplotype combinations (mini-haplotypes) in a population. After calculating frequencies for all possible mini-haplotypes we confirmed our hypothesis that some combinations are much more frequent than expected. Different combinations were found to be most frequent in Croatian and Hungarian populations supporting the hypothesis that these aberrations from expected values might be population-specific. Probabilities of complete 9-loci haplotypes calculated by combining frequencies of three-locus haplotypes can be significantly different from probabilities calculated using individual alleles, suggesting that currently used statistical methods might not be accurate in genotypes that contain these over-abundant mini-haplotypes.

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## PRIMJENA NOVOSINTETZIRANIH $MAN_9$ GLIKOPROBA ZA ANALIZU AKTIVNOSTI LEKTINA KOJI VEŽE MANOZU

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S ciljem unaprjeđivanja metoda za analizu lektina nedavno smo razvili novu skupinu složenih neoglikokonjugata koje smo nazvali Glikoprobe (Glycobiology 10:357, 2000). Glikoprobe se sastoje od oligosaharidnog liganda, fotoreaktivnog umreživača, i digoksinskog biljega, a omogućavaju izravnu analizu aktivnosti lektina u složenim biološkim uzorcima. Lektin koji veže manozu lectin (MBL) je C-tip lektin tipične topljive kolektinske strukture. Prepoznaje ponavljajuće šećerne strukture koje se javljaju na površini brojnih mikroorganizama. Zbog svoje sposobnosti da aktivira komplement ima značajnu ulogu u prvoj liniji obrane od patogenih mikroorganizama, i u nekim drugim patološkim procesima. Kako bismo omogućili analizu aktivnosti MBL u serumu čovjeka sintetizirali smo specifičnu glikoprobu koja kao ligand sadrži oligomanozni glikan ($Man_9$). Primjenom $Man_9$-glikoprobe ispitali smo aktivnost MBL u serumima 30 pacijenata oboljelih od reumatoidnog artritisa i 25 kontrolnih seruma. Ovdje je važno napomenuti da je ovo prvi primjer gdje je ispitivana aktivnost, a ne sama nazočnost MBL kao proteina. Uočena je vrlo visoka biološka varijabilnost aktivnosti MBL u obje ispitane skupine, dok nije uočeno statistički značajno odstupanje u skupini oboljelih. Ovaj rezultat ne podupire hipotezu da vezanje MBL na degalaktozilirane oligosaharide na IgG koji se javljaju u reumatoidnom artritisu doprinosi patologiji bolesti.

## APPLICATION OF THE NEWLY SYNTHESIZED $MAN_9$ GLYCOPROBE TO ANALYZE ACTIVITY OF MANNAN BINDING LECTIN IN HUMAN SERA

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Aiming to improve tools for the analysis of lectins, we have recently developed a new class of complex neoglycoconjugates named Glycoprobes (Glycobiology 10:357, 2000). Glycoprobes consists of an oligosaccharide ligand, photoreactive crosslinker and a digoxin tag, and enable direct analysis of lectin activity in complex biological samples. Mannan-binding lectin (MBL) is a C-type lectin with a typical soluble collectin structure. It functions as a pattern recognition molecule that binds repeating sugar arrays on many microbial surfaces. Its ability to activate complement response makes it an important player

in the first line of defense, as well as in some pathological processes. To be able to analyze MBL activity in human serum we synthesized a specific glycoprobe containing Mannose$_9$-N-Acetylganactosamine$_2$ (Man$_9$) glycan as a ligand. Using Man$_9$-glycoprobe we assayed MBL activity in 30 sera of patients with rheumatoid arthritis and 25 sera of matching healthy controls. It is important to note that this was the first example where activity, and not the simple presence of MBL protein was assayed. Very large biological variability of MBL activity was found to exist in both studied groups, while there appeared to be no significant difference between the groups. This result does not support the hypothesis that binding of MBL to degalactosylated olgosaccharides on IgG contributes to the pathology of rheumatoid arthritis.

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## DOKAZIVANJE REZISTENCIJE NA ANTIRETROVIRUSNU TERAPIJU

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U Klinici za infektivne bolesti u Zagrebu HIV-1 bolesnici liječeni su vrlo djelotvornom antiretrovirusnom terapijom (HAART-od engl. Highly active antiretroviral therapy). Ova terapija predstavlja kombinaciju inhibitora reverzne transkriptaze (nukleozidni i nenukleozidni analozi) i inhibitora proteaze. Iako HAART uspijeva sniziti broj kopija HIV-1 RNA po ml plazme na nemjerljivu, njena učinkovitost smanjuje se zbog pojave rezistencije na antiretrovirusne lijekove uzrokovane mutacijama u virusnom genomu. Cilj istraživanja bio je pokazati rezultate dokazivanja rezistencije na antiretrovirusnu terapiju kod HIV-1 bolesnika koji su usprkos liječenju imali više od 1500 kopija HIV-1 RNA u ml plazme (pokazatelj neuspjeha terapije). Primijenjena je metoda INNO-LIPA HIV-1 Test (Innogenetics) koja se temelji na principu reverzne hibridizacije. Ovim testom analiziraju se najčešće mutacije u kodonima gena za proteazu i reverznu transkriptazu koje su povezane s razvojem rezistencije. Od šest analiziranih HIV-1 bolesnika četiri su imala mutacije koje su povezane s rezistencijom na neki od antiretrovirusnih lijekova. Kod ostalih su svi analizirani kodoni predstavljali divlji tip (nije bilo mutacija). Kod dva bolesnika primijetili smo promjenu kod ponovljenog testiranja rezistencije nakon šest mjeseci koja je točno odražavala primjenu određenog lijeka. Rezultati određivanja rezistencije na pojedine antiretrovirusne lijekove trebali bi se uzimati u obzir prilikom prilagođavanja individualne terapije HIV-1 bolesnika.

## HIV-1 DRUG RESISTANCE TESTING BY GENOTYPING

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HIV infected patients in Croatia are treated with highly active antiretroviral therapy (HAART), which is, based on combination of reverse transcriptase inhibitors-RTI (nucleoside analoge-NA and non-nucleoside analoge-NNRTI) and protease inhibitors (PI). Although HAART regiments are able to suppress HIV-1 viral load to undetectable level and improves patients condition, its efficiency is limited by the development of drug resistance. HIV-1 drug resistance appears as a result of mutations within wild type viral genome. Our objective was to demonstrate the results of antiretroviral resistance in Croatian population of HIV-1 patients treated with HAART. Line probe assay INNO-LIPA HIV-1 based on reverse DNA hybridisation principle was used. This is a test for genotyping of codons in protease and reverse transcriptase gene of the HIV-1 virus. Codons chosen in this test are associated with most frequent known mutations. Four of six patients analyzed were mutant in one or more codons, which are associated with resistance, and two of them were wild type in all codons analyzed. We have also noticed a change in resistance in two patients during six month period corresponding to the usage of specific antiretroviral drugs. Data concerning drug resistance should be considered in order to optimise individual antiretroviral treatment.

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## EKSPRESIJA GENA ZA GALECTIN-3 I CD44V6 U PUNKTATIMA PROMJENJENOG TKIVA ŠTITNJAČE

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Mnogi bolesnici s promjenama štitnjače operiraju se u dijagnostičke svrhe, jer je često teško postaviti točnu preoperativnu dijagnozu. U novije vrijeme pokazano je da bi Galectin-3 i CD44v6 mogli poslužiti kao molekulski biljezi koji mogu povećati točnost preoperativne dijagnoze promjena štitnjače. U ovom istraživanju željeli smo provjeriti mogu li Galectin-3 i CD44v6 pomoći u

razlikovanju malignih i benignih promjena štitnjače u punktatima dobivenim aspiracijskom biopsijom tankom iglom. Ekspresija gena za Galectin-3 i CD44v6 određivana je tehnikom RT-PCR u punktatima dobivenim aspiracijskom biopsijom tankom iglom. Punktati su uzimani prije operacije od bolesnika s malignim i benignim promjenama štitnjače. Rezultati su uspoređivani s obzirom na citološku dijagnozu. Ekspresija gena za Galectin-3 nađena je u: 5 od 6 (83%) uzoraka papilarnog karcinoma, 3 od 15 (21%) uzoraka folikularnih promjena, 1 od 5 (20%) uzoraka Hashimotovog tiroiditisa i u 4 od 21 (19%) uzoraka nodularne strume. Ekspresija gena za CD44v6 je nađena u: 5 od 6 (83%) uzoraka papilarnog karcinoma, 3 od 15 (21%) uzoraka folikularnih promjena, 1 od 5 (20%) uzorka Hashimotovog tiroiditisa i u 2 od 21 (10%) uzoraka nodularne strume. Pokazali smo da su i Galectin-3 i CD44v6 biljezi koji mogu, uz određena ograničenja, povećati točnost preoperativne dijagnoze u punktatima dobivenim aspiracijskom biopsijom tankom iglom, u smislu razlikovanja malignih i benignih promjena štitnjače.

## GALECTIN-3 AND CD44V6 EXPRESSION IN FINE-NEEDLE ASPIRATES OF THYROID LESIONS

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Many patients with thyroid lesions are being surgically treated only for diagnostic purposes, because it is often difficult to make decisive preoperative diagnosis. Recently, Galectin-3 and CD44v6 have been proposed as tumour markers that could improve accuracy of preoperative diagnosis of thyroid lesions. In this study we wanted to assess weather Galectin-3 and CD44v6 could be used as potential markers to discriminate between benign and malignant thyroid lesions on fine-needle aspirates. RT-PCR analysis of Galectin-3 and CD44v6 expression was performed on RNA isolated from 50 fine-needle aspirates obtained preoperatively from patients with benign and malignant thyroid lesions. Results were evaluated against the fine-needle aspiration cytology diagnosis. Galectin-3 expression was found in: 5 of 6 (83%) papillary carcinoma samples, 3 of 15 (21%) follicular lesion samples, 1 of 5 (20%) Hashimoto thyroiditis samples and in 4 of 21 (19%) nodular goiter samples. CD44v6 expression was found in: 5 of 6 (83%) papillary carcinoma samples, 3 of 15 (21%) follicular lesion samples, 1 of 5 (20%) Hashimoto thyroiditis samples and in 2 of 21 (10%) nodular goiter samples. Our results suggest that, with some limitations, RT-PCR analysis of both Galectin-3 and CD44v6 expression in fine-needle aspirates could be used to improve preoperative diagnostic accuracy in discriminating between malignant and benign thyroid lesions.

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## POLIMORFIZAM C314T GENA ZA HISTAMIN N-METILTRANSFERAZU NIJE POVEZAN SA SKLONOŠĆU NASTANKA ALZHEIMEROVE BOLESTI

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Histamin je neurotransmiter središnjeg živčanog sustava čiji je metabolizam upleten u patofiziologiju više neuroloških poremećaja, uključujući i Alzheimerovu bolest (AD). Djelovanje histamina inhibira enzim histamin N-metiltransferazom (HNMT). Nedavno opisana mutacija C314T u genu za HNMT smanjuje njegov enzimski učinak, te usporava razgradnju histamina. Budući da postoje dokazi o upletenosti histamina u nastanak Alzheimerove bolesti, te da je HNMT ključni regulator histaminskog djelovanja, logično je pretpostaviti da bi abnormalnosti u HNMT mogle utjecati na nastanak AD. Testiranje moguće povezanosti Alzheimerove bolesti s mutacijom C314T u genu za HNMT provedeno je case-control studijom. Analizirano je 30 pacijenata (15 muškaraca i 15 žena) i 30 zdravih, po spolu i dobi odgovarajućih ispitanika. Alzheimerova bolest je dijagnosticirana prema Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), a kontrolni ispitanici odabrani su na temelju rezultata Mini-Mental State Examination of Folstein testa. Analiza molekule DNA i genotipizacija ispitanika temeljila se je na PCR-RFLP tehnologiji, a dobiveni rezlutati su statistički analizirani. Među pacijentima je pronađeno pet heterozigota za mutaciju C314T, te isto toliko među kontrolnim ispitanicima. Naši rezultati ukazuju da najvjerovatnije ne postoji povezanost ($p>0,05$) između Alzheimerove bolesti i smanjene razgradnje histamina (zbog mutacije C314T). Zbog ograničene veličine uzorka bilo bi potrebno provesti dodatna istraživanja.

## LACK OF ASSOCIATION OF ALZHEIMER DISEASE AND HISTAMINE N-METHYLTRANSFERASE POLYMORPHISM C314T

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Histamine is central nervous system neurotransmitter whose metabolism has been implicated in pathophysiology of several neurological disorders, including Alzheimer disease (AD). Histamine action is terminated via enzymatic action of histamine N-methyltrasferase (HNMT). Recently described mutation C314T, of HNMT impairs its enzymatic activity, thus slowering degradation of histamine.

Because of the evidence for possible role of histamine in the pathophysiology of Alzheimer disease and because of the critical role of HNMT in histamine metabolism, we hypothesized that abnormalities of HNMT could influence development of AD. Objective of our study was to test possible association of AD and C314T mutation in HNMT gene. In order to test our hypothesis we have conducted case-control study. Thirty patients with AD (15 men / 15 women) and 30 healthy, sex and age matched control subjects were included in our study. Alzheimer disease was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), and control subjects were selected using Mini-Mental State Examination of Folstein test. DNA analysis involved PCR-RFLP based genotyping of the individuals and obtained results were compared. We have found 5 heterozygous individuals with C314T mutation among AD patients and control individuals respectively. Our results suggest that inherited variation of HNMT activity (C314T mutation) is unlikely to be associated ($p>0.05$) with increased risk for Alzheimer disease. Because of limited sample size further studies are needed.

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## T- I B-STANIČNA KLONALNOST I UČESTALOST HUMANOG HERPESVIRUSA-6 I HUMANOG HERPESVIRUSA-8 U ANGIOIMUNOBLASTIČNOM T-STANIČNOM LIMFOMU

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Angioimunoblastični T-stanični limfom (AIL) je sistemska limfoproliferativna bolest nepoznate etiologije karakterizirana generaliziranom limfadenopatijom, hepatosplenomegalijom, povišenom temperaturom, kožnim osipom i hematološkim poremećajima. Postoje teorije o virusnoj infekciji kao mogućem etiološkom čimbeniku u nastanku AILa. Zbog niske učestalosti bolesti napravili smo retrospektivnu analizu T- i B-staničnih populacija na 22 arhivska slučaja AILa. DNA je izolirana iz biopsija tkiva uklopljenih u parafin. Koristeći PCR metodu željeli smo utvrditi: 1) pojavnost klonalnih/oligoklonalnih TCR i IgH preuredbi; i 2) moguću povezanost humanog herpesvirusa-6 (HHV-6) i humanog herpesvirusa-8 (HHV-8) s ovom bolesti. Klonalnu proliferaciju T-stanica našli smo u 11 (50%) slučajeva, dok je utvrđena jedna oligoklonalna i 2 monoklonalne proliferacije B-stanica (13,6%). HHV-6 sekvenca utvrđena je u 4 (18%) slučaja dok HHV-8 sekvenca nije utvrđena ni u jednom uzorku. Povezanost T-staničnе klonalnosti i HHV-6 infekcije je statistički značajna ($p=0,02$). Utvrđena veza HHV-6 infekcije i AILa zaslužuje daljnja istraživanja u cilju razjašnjenja patogenetskih mehanizama nastanka ove bolesti.

## T- AND B-CELL CLONALITY AND FREQUENCY OF HUMAN HERPES VIRUS-6 AND HUMAN HERPESVIRUS-8 IN ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Angioimmunoblastic T-cell lymphoma (AIL) is a systemic lymphoproliferative disorder of unknown etiology characterised by generalized lymphadenopathy, hepatosplenomegaly, fever, skin rash and haematological abnormalities. Much speculation has been centered on the possibility that a viral infection could trigger this disorder. Given the rarity of this disorder, we have undertaken a retrospective study of T and B cell populations in 22 archival cases of AIL (from 1978 to 2001). We used PCR amplification of DNA isolated from paraffin embedded tissue biopsies to assess: 1) the occurrence of clonal/oligoclonal TCR and IgH rearrangements; and 2) possible association of human herpesvirus 6 (HHV-6) and human herpesvirus 8 (HHV-8) with this disease. TCR clonal products were identified in 11 (50%) cases while IgH oligoclonal products were found in 1 case and clonal products in 2 cases (13.6%). HHV-6 DNA sequence was detected in 4 (18%) cases and HHV-8 DNA sequence was not detected by PCR. Statistically significant association between T-cell clonality and HHV-6 infection has been found (p=0.02). The relationship between AIL and HHV-6, demonstrated here, deserves further investigation in order to clarify its biological meaning in the pathogenesis of this condition.

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## USPOREDBA DVIJU PCR METODA ZA UTVRĐIVANJE PREUREDBE GENA TEŠKOG LANCA IMUNOGLOBULINA I OCJENU KLONALNOSTI B-LIMFOCITNIH POPULACIJA

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Mnoge B-limfoproliferativne bolesti nemaju specifični kromosomski ili molekularni biljeg, no populacija neoplastičnih limfocita ima istovjetno preuređen gen za teški lanac imunoglobulina. Ova se činjenica može koristiti

za utvrđivanje klonalnosti populacija limfatičnih stanica u svrhu razlikovanja reaktivnih (dobroćudnih) od neoplastičnih (zloćudnih) stanja. Geni teškog lanca imunoglobulina najviše se razlikuju u sekvenci oko V-D i D-J spojeva. Jedna od 3 hipervarijablne regije je i CDR3 koja se najčešće koristi u izradi molekularnog testa kojim se procjenjuje pojava ili stupanj klonalnosti ispitivane B-stanične populacije. Opisano je više metoda koje umnažaju segmente CDR3 regije. U ovoj studiji usporedili smo dvije (1,2) od kojih za jednu (1) njeni autori posebno ističu povišenu osjetljivost za razlučivanje manjeg broja B-klonalnih limfocita u miješanim populacijama. Za model studije izabrali smo uzorke koštane srži u kojima su patolozi opisali nakupine B-limfocita (nodularni infiltrati) za koje nije bilo drugim metodama moguće utvrditi njihovu klonalnost. Rezultati su pokazali da je metoda u kojoj se koristi ponovljena lančana reakcija polimerazom s internom početnicom osjetljivija (1) i da je njome moguće dokazati rearanžmane u uzorcima u kojima nisu utvrđeni drugom metodom.

## COMPARISON OF TWO PCR STRATEGIES FOR DETECTION OF IMMUNOGLOBULIN HEAVY CHAIN GENE REARRANGEMENT

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Many B lymphoproliferative diseases do not have specific chromosomal or molecular marker but population of neoplastic lymphocytes have the same unique immunoglobulin heavy chain gene rearrangement. This fact can be utilized for determinating lymphocyte population clonality and therefore differentiating between reactive (benign) and neoplastic (malignant) conditions. Immunoglobulin heavy chain genes diverge mostly in sequence around V-D and D–J junctions. One of tree hypervariable regions is CDR3 which is most frequently used for creation of molecular assay for determinating existence or clonality grade for given B cell population. There are tree described methods for amplification of CDR3 segments. In this study we compared two of them (1,2). For one (1) its authors point out higher sensitivity for detecting smaller number of B clonal lymphocytes in mixed population. For our model we selected bone marrow samples in which pathologists have found B lymphocytes clusters (nodular infiltrates). There was no other method for determinating monoclonality in those samples. Results have proven higher sensitivity of the seminested design of method 1 which may be relevant in some lymphoid pathologies.

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## POLIMORFIZAM GENA ZA RECEPTOR VITAMINA D JEST FAKTOR RIZIKA U NASTANAKU TIP 1 ŠEĆERNE BOLESTI

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Do sada je nekoliko studija pokazalo povezanost između polimorfizma gena za receptor vitamina D (VDR) i razvoja šećerne bolesti tip 1 (engl. *type 1 diabets mellitus*, T1DM). Kako bismo istražili vezu između VDR polimorfizma i prijemljivosti za T1DM u populaciji Dalmacije analizirali smo 134 oboljelih od T1DM i 132 kontrolne osobe. Polimorfizam VDR gena analiziran je koristeći lančanu reakciju polimeraze (PCR) i *BsmI, ApaI, TaqI* and *FokI* restrikcijske enzime. Rezultati su obrađeni $c^2$ testom. Raspodjela genotipova *TaqI* and *FokI* polimorfizama između oboljelih od T1DM i kontrolnih ispitanika značajno se razlikovala (*TaqI*: $c^2$=8,04, P=0.018; *FokI*: $c^2$=23,53, P=0,0001), s tt i ff genotipom više zastupljenim među oboljelima od T1DM. BBAAtt kombinacija genotipova pokazala se kao prijemljiva za T1DM (p=0,002). Također je BBAAttFf kombinacija alela pokazala prijemljivost a T1DM jer je bila tri puta zastupljenija među oboljelima od šećerne bolesti (p=0,05). S druge strane, kombinacija BbAaTtFf izgleda da ima zaštitnu ulogu jer je bila tri puta zastupljenija među kontrolnim ispitanicima (p=0,0033). Zanimljivo je istaknuti to da je BAt haplotip pronađen kao faktor rizika za T1DM u Njemačkoj populaciji, jedinoj testiranoj europskoj populaciji. Naši rezultati ukazuju da je VDR polimorfizam povezan s povećanim rizikom oboljevanja od šećerne bolesti.

## VITAMIN D RECEPTOR POLYMORPHISM IS RISK FACTOR FOR DEVELOPMENT OF TYPE 1 DIABETES

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Several studies have found a relationship between polymorphisms of the vitamin D receptor gene (VDR) and development of type 1 diabetes mellitus (T1DM). To examine the association of VDR polymorphisms and susceptibility to T1DM in the Dalmatian population we studied 134 individuals with type 1 diabetes and 132 control subjects. VDR genotyping was performed using poly-

merase chain reaction (PCR) and *Bsm*I, *Apa*I, *Taq*I and *Fok*I restriction enzymes. Data were analysed using the $c^2$ test. The distribution of genotype frequencies of the *TaqI* and *FokI* polymorphisms among T1DM patients and control subjects differed significantly (*TaqI*: $c^2$=8.04, P=0.018; *FokI*: $c^2$=23.53, P=0.0001), with the tt and ff genotype occurring more frequently in the T1DM individuals. The genotype combination, which conferred strongest susceptibility to T1DM, was BBAAtt (p=0.002). The BBAAttFf genotype conferred a higher risk for type 1 diabetes, being three times more frequent in T1DM patients (p=0.05). On the other side BbAaTtFf genotype seems to be protective since it was two times more frequent in controls (p=0.0033). Interestingly, the BAt haplotype was found to be a risk factor in a German population, the only European population tested thus far. Our results indicate that VDR polymorphisms are associated with increased risk of T1DM and warrant further studies.

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## ISTRAŽIVANJE RAZNOVRSNOSTI 4 MIKROSATELITSKA LOKUSA KOD PASMINE HRVATSKOG OVČARA

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Cilj ovog rada bio je po prvi puta analizirati raznovrsnost četiri mikrosatelitska lokusa (LEI07, LEI08, LEI22, LEI32) kod pasmine hrvatskog ovčara. Lokusi su odabrani na temelju literaturnih podataka. Uzorak se sastojao od 45 nesrodnih pasa s područja Slavonskog Broda, Županje i Đakova. Svi psi su zadovoljavali osnovne fenotipske osobine hrvatskog ovčara. Nakon izolacije DNA, 4 mikrosatelitska lokusa umnažali smo PCR metodom 4 odvojene reakcije, na različitim programima. Nakon umnažanja, dužina pojedinog fragmenta DNK određivana je elektroforezom na 6%-tnom poliakrilamidnom gelu u ALFexpress sekvenceru (Pharmacia-Biotech, Uppsala, Švedska). Rezultate elektroforeze analizirali smo uz pomoć kompjuterskog programa "AlleleLocator" (Pharmacia-Biotech, Uppsala, Švedska). Na 4 testirana mikrosatelitska lokusa broj heterozigota iznosio je od 54,3% za LEI32 do 77,8% za LEI08. Devet različitih alela uočeno je na lokusu LEI07, osam alela pronađeno je lokusu LEI08, dok je na lokusima LEI22 i LEI32 otkriveno po 6 različitih alela. Dobivene rezultate uspoređivali smo s rezultatima ostalih hrvatskih pasmina pasa za koje postoje podaci u literaturi (istarski gonič i dalmatinski pas). Usporedba je pokazala da pojedini aleli, visoko zastupljeni kod hrvatskog ovčara, nisu uočeni kod druge dvije navedene pasmine i obrnuto. Ovim radom započeto je stvaranje baze podataka o genetskom ustroju mikrosatelitskih lokusa hrvatskog ovčara.

# POLYMORPHISM AT 4 MICROSATELLITE LOCI IN THE CANINE POPULATION OF CROATIAN SHEPHERD

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The aim of the present study was to investigate 4 Short Tandem Repeat (STR) loci (LEI07, LEI08, LEI22, and LEI32) in the population of the Croatian shepherd. The loci were selected on the basis of literature data. The sample was comprised of 45 unrelated dogs from Slavonski Brod, Županja and Đakovo. All dogs had phenotypic characteristics of Croatian shepherd. After DNA isolation, samples were amplified in 4 separate PCR reaction and run on a 6% polyacrilamide gel in an ALF express sequencer (Pharmacia-Biotech, Uppsala, Sweden). The results were analysed using AlleleLocator program (Pharmacia-Biotech, Uppsala, Sweden). At 4 tested STR loci, the number of heterozygotes was in range of 54.3% (LEI32) and 77.8% (LEI08). Nine different alleles were observed at LEI07 locus, 8 alleles were found at LEI08 locus, while 6 alleles were observed at the LEI22 and LEI32 loci each. We compared our results with the data from literature for other Croatian canine species (Istrian hound, Dalmatian dog). Comparison demonstrated that some alleles specific for Croatian shepherd were not observed in the other two species and vice versa. This study is the beginning of the creation of a database containing information about the polymorphism of the STR loci in the population of the Croatian shepherd.

VIROLOGIJA, MIKROBIOLOGIJA I MIKOLOGIJA
*BIOLOGIA VIRORUM ET MICROBORUM,*
*BIOLOGIA FUNGORUM*
VIROLOGY, MICROBIOLOGY AND MYCOLOGY

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## FITOPLAZME – NEOBIČNE FITOPATOGENE BAKTERIJE

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Fitoplazme morfološki nalikuju mikoplazmama, odavna poznatim animalnim patogenima, te su isprva i nazivane *mikoplazmama slični organizmi*. Za razliku od uobičajenih bakterija, fitoplazme nemaju staničnu stijenku pa su promjenljiva oblika. U usporedbi s ostalim bakterijama znatno ih je teže istraživati jer se ne mogu kultivirati na umjetim hranjivim podlogama. Fitoplazme su do sada otkrivene u preko 600 biljnih vrsta koje pripadaju različitim porodicama. Na zaraženim biljkama fitoplazme najčešće uzrokuju žućenje listova, ozelenjavanje latica (virescencija) i sterilnost cvjetova. Čest simptom fitoplazmoza je sindrom vještičine metle nazvan tako zbog metličastih tvorbi koje nastaju izrazito jakom proliferacijom bočnih pupova. Fitoplazme u prirodi prenose neki kukci, a moguće ih je prenijeti i cijepljenjem. Zaražavaju mnoge poljoprivredne kulture, šumsko drveće i ukrasne biljke. Ekonomski najznačajnijim fitoplazmatskim bolestima pripadaju žutice vinove loze. Otkrivene su i u hrvatskim vinogradima, a na nekim lokalitetima poprimaju zabrinjavajuće razmjere. Laboratorij za biljnu virologiju Prirodoslovno-matematičkog fakulteta u Zagrebu doveo je moderne molekularno-biološke metode do rutinske primjene u detekciji i identifikaciji fitoplazmi. Istraživanja fitoplazmi u Hrvatskoj trebala bi rezultirati novim metodama koje bi unaprijedile detekciju fitoplazmi i omogućile istraživanja gena za koje se pretpostavlja da su bitni za interakciju biljke i patogena.

## PHYTOPLASMAS – THE UNUSUAL PHYTOPATHOGENIC BACTERIA

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Phytoplasmas have been known for decades to be important plant pathogens. Due to their morphological similarity with mycoplasmas, they were formerly called mycoplasmalike organisms. They differ from a vast majority of bacteria by the absence of a cell wall which enables them to shift from spherical to filamentous shape. The cultivation of these pleomorphic microorganisms on artificial media is not possible, which makes their investigation extremely difficult. So far, phytoplasmas have been isolated from more than 600 plant species belonging to a number of different families. The most frequently encountered symptoms of phytoplasmoses are leaf yellowing, virescence

(greening of floral organs) and flower sterility, as well as witches' broom caused by strong proliferation of axillary buds. Phytoplasmas are readily transmitted by vectors and grafting. They are causal agents of severe diseases in crops, horticultural species and forest trees. Phytoplasmoses of great economic importance are grapevine yellows identified also in Croatia as a factor of significant losses in viticulture. Laboratory for Plant Virology of the Department of Biology, University of Zagreb, introduced modern molecular biology methods for a routine detection and identification of these phytopathogens. The development of new molecular approaches will improve detection and enable in depth-investigation of phytoplasma genes presumed to be important for the plant-pathogen interactions.

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## EPIDEMIOLOGIJA ŽUTICA VINOVE LOZE U HRVATSKOJ

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Uzročnici žutica vinove loze (GY) uglavnom su fitoplazme, prokariotski patogeni. Simptomi tipični za GY uočeni su 1994. u zagrebačkoj regiji i od tada se prati širenje bolesti po cijeloj zemlji. Do danas su simptomi žutice vinove loze zabilježeni u svim dijelovima zemlje osim na otocima. U sjeverozapadnoj Hrvatskoj širenje fitoplazmoze bilo je jako, dok je u istočnom dijelu zaraza imala epidemijski karakter i uzrokovala velike ekonomske štete. U vinogradima Iloka, Baranje i Brodskog Stupnika bilo je zaraženo čak do 50% trsova. Najosjetljiviji kultivar je Chardonnay, a slijede Pinot Gris, Pinot Noir, Pinot Blanc, Ugni Blanc i Cabernet Sauvignon. Zaraza je također utvrđena i na autohtonim kultivarima Debitu, Plavini, Maraštini i Istarskoj Malvaziji. Detekcija se zasniva na ugnježđenoj lančanoj reakciji polimerazom uz primjenu univerzalnih i specifičnih početnica za amplifikaciju fitoplazmatskog 16S rRNA gena. Preciznu klasifikaciju patogena omogućuje primjena polimorfizma duljine restrikcijskih fragmenata. Fitoplazme iz svih uzoraka loze , te iz dvije korovne vrste *Taraxacum officinale* Web. i *Polygonum lapathifolium* L. klasificirane su u istu ribosomalnu podskupinu 16SrXII-A (Stolbur, Bois Noir), dok fitoplazma nađena u vrsti *Chenopodium album* L. pripada podskupini 16SrIII (X-disease). Prisutnost fitoplazmi u korovima koji su mogući izvor zaraze, kao i zabilježena prisutnost poznatog vektora (*Hyalestes obsoletus* Sign.) u zemlji, ukazuju na potrebu daljnjih istraživanja epidemiologije fitoplazmoza.

## EPIDEMIOLOGY OF GRAPEVINE YELLOWS DISEASE IN CROATIA

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Grapevine yellows diseases (GY) are mainly caused by plant pathogenic prokaryotes – phytoplasmas. Since 1994, when symptoms typical for GY were observed in Zagreb region, spreading of the phytoplasmoses was monitored throughout the country. Up to date GY symptoms were observed in all parts of the country except on the islands. In north-western Croatia the spread of the phytoplasmoses was wide, while in the eastern part it even had an epidemic character, causing significant economic losses.

In Ilok, Baranja and Brodski Stupnik up to 50% of the vines were infected. The most susceptible cultivar is Chardonnay, followed by Pinot Gris, Pinot Noir, Pinot Blanc, Ugni Blanc and Cabernet Sauvignon. Infection was also confirmed in indigenous cultivars: Debit, Plavina, Maraština and Istarska Malvazija. The detection was based on nested PCR (polymerase chain reaction) that employed phytoplasma generic and/or specific primers amplifying 16S rRNA gene. The RFLP (restriction fragment length polymorphism) experiments enabled precise classification of the pathogens. Phytoplasmas from all grapevine samples and from two weed species *Taraxacum officinale* Web. and *Polygonum lapathifolium* L. were classified in the same ribosomal subgroup 16SrXII-A (Stolbur, Bois Noir), while phytoplasma belonging to the 16SrIII subgroup (X-disease) was only found in *Chenopodium album* L. The presence of phytoplasmas in vineyard weeds, potential sources of the disease, as well as the recorded presence of a known vector (*Hyalestes obsoletus* Sign.) in the country, prompts us to further research on the epidemiology of phytoplasmoses.

OP-28

## MOLEKULARNI PROFILI *Citrus tristeza* VIRUSA I *Citrus exocortis* VIROIDA IZOLIRANIH IZ HRVATSKIH AGRUMA

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*Citrus tristeza Closterovirus* (CTV) i *Citrus exocortis viroid* (CEVd) najznačajniji su RNA-patogeni citrusa. Osim izazivanja sindroma koji nanose velike ekonomske štete u nasadima agruma diljem svijeta, ova dva patogena ujedno predstavljaju zanimljiv laboratorijski model za istraživanje specifičnih interakcija domaćin-patogen na molekularnoj razini. U istraživanju je obrađeno 30-ak uzoraka agruma s područja srednje Dalmacije od kojih velik broj uzorkovanih stabala nije pokazivao nikakve simptome CTV- ili CEVd-infekcije. Izolacija nukleinskih kiselina provedena je bez prethodnog umnožavanja patogena u odgovarajućim bioindikatorima. Poliakrilamidnom gel elektroforezom uspoređeni su profili replikativne forme (dsRNA) *Citrus trisreza* virusa te je ista dsRNA poslužila kao kalup u RT-PCR reakciji. Upotrebom specifičnog para početnica umnožena je regija kapsidnog proteina (CP) CTV-a. CTV-amplikoni obrađeni su s više restrikcijskih enzima te su uspoređeni njihovi RFLP-profili. S ciljem amplifikacije čitavih viroidnih molekula, izolirana je viroidna RNA te je proveden RT-PCR upotrebom dva specifična para početnica; jedan za centralnu konzerviranu, te jedan za lijevu terminalnu regiju viroidnog genoma. CTV i CEVd cDNA dodatno su podvrgnute SSCP (single-strand conformation polymorphism) te HMA (heteroduplex mobility assay) analizama. Molekularna karakterizacija navedenih izolata ukazuje na prisutnost više različitih molekularnih profila navedenih patogena u analiziranim uzorcima. Određivanje primarne strukture nukleinskih kiselina trebalo bi potvrditi rezultate obavljenih analiza te u konačnici pomoći objašnjenju složenih mehanizama interakcije domaćin-patogen.

## MOLECULAR PROFILES OF *Citrus tristeza VIRUS* AND *Citrus exocortis* VIROID INFECTING CROATIAN CITRUS

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*Citrus tristeza Closterovirus* (CTV) and *Citrus exocortis viroid* (CEVd) are RNA-pathogens widespread in major citrus-growing regions of the world causing economically important diseases. Both pathogens represent interesting models for investigation of the some specific host-pathogen molecular interactions. About 30 citrus samples (Satsuma mandarins, oranges, lemons) were taken from different orchards in the Croatian coastal region (Dalmatia) including some more isolated islands. Many of the sampled trees where the pathogen presence was indicated were without symptoms of tristeza, exocortis, or both. The procedures for the nucleic acid extractions were performed directly from the field trees. Double-stranded RNA patterns (dsRNA) of CTV were compared in the polyacrylamide gels. The same nucleic acids extracts were used as templates in RT-PCR experiments employing primer pair specific for CTV coat protein gene. CTV amplicons were singly digested with various restriction enzymes and their RFLP-patterns were compared. In order to obtain a full-length viroid sequence in the future, viroid RNA was isolated, used as a template for cDNA synthesis by RT-PCR employing two primer pairs, one covering the central conserved region and the other covering the left terminal region of viroid. CTV and viroid cDNAs were additionally subjected to the SSCP (single-strand conformation polymorphism) and HMA (heteroduplex mobility assay) analyses, as well. Molecular characterization of CTV and CEVd isolates indicate the existence of different molecular pathogen profiles in our samples. The differences should be confirmed by sequencing before exploring more complex host-pathogen interactions.

OP-29

## MOGUĆI OBRAZAC RASPADANJA ČESTICA VIRUSA MOZAIKA KRASTAVCA TEMELJEN NA REZULTATIMA IMUNODIFUZIJE U GELU

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Kukumovirus mozaika krastavca (CMV) pripada skupini malih izometričnih RNA virusa. Kapsida ovog virusa sastavljena je od 180 identičnih proteinskih podjedinica koje su organizirane unutar 12 pentamernih i 20 heksamernih

morfoloških jedinica. Svojstvo izuzetne nestabilnosti čestica virusa CMV može se povezati s prisustvom većih šupljina unutar kapside. Osim toga, za stabilnost čestica CMV naročitu važnost imaju RNA-protein elektrostatske interakcije. Naša istraživanja temeljila su se na serološkoj karakterizaciji pripravka virusa CMV upotrebom metode dvostruke radijalne imunodifuzije (DRID). U tu svrhu bilo je najprije potrebno prirediti reaktante seroloških reakcija. Nakon inokulacije i razmnožavanja virusa CMV u pokusnim biljkama, virusni pripravci su priređeni postupkom djelomične purifikacije virusnih čestica. Slobodne proteinske podjedinice kapside smo priredili tretiranjem virusnih pripravaka SDS-om. Poliklonski antiserum protiv virusa CMV priređen je imuniziranjem kunića djelomično purificiranim i formaldehidom fiksiranim virusnim pripravcima. Pokusi DRID pokazali su obrazac višestrukih precipitacijskih linija koje su nastale u reakciji između djelomično purificiranog pripravka virusa CMV i imunoglobulina priređenih protiv virusa CMV. Pošto se takav obrazac sastojao od pet precipitacijskih linija bilo je potrebno utvrditi njihovo podrijetlo. Dokazali smo da dvije precipitacijske linije potječu od cijelih virusnih čestica i slobodnih proteinskih podjedinica kapside. Dodatne dvije linije potječu od domaćinskih biljnih proteina. Obzirom na položaj i kvalitetu preostale precipitacijske linije, predložili smo njeno podrijetlo koje bi moglo potjecati od međuprodukta raspadanja čestica virusa CMV. Taj međuprodukt raspadanja najvjerojatnije predstavlja nukleoproteinski kompleks relativno definirane veličine.

## POSSIBLE DISINTEGRATION PATHWAY OF CUCUMBER MOSAIC VIRUS PARTICLES REVEALED BY THE GEL-IMMUNODIFFUSION ASSAY

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Cucumber mosaic virus (CMV), the type member of genus *Cucumovirus*, belongs to the group of small isometric RNA viruses. Its capsid is composed of 180 identical coat protein subunits organized into the 12 pentameric and 20 hexameric morphological units. CMV particles are considered to be extremely unstable and it could be correlated to the presence of extensive holes in the capsid as well as to the importance of RNA-protein electrostatic interactions. In our study we have focused on serological characterization of CMV samples employing double radial immunodiffusion (DRID) method. In order to prepare all necessary reactants for serological tests, CMV was first inoculated and propagated in the plants. Viral preparations were obtained by the partial purification of CMV particles. The isolated coat protein subunits were obtained by SDS treatment. Polyclonal antiserum to CMV was produced in rabbit with partially purified and formaldehyde-fixed CMV preparations. Our results in DRID tests re-

veled the formation of multiple precipitin line pattern in the reactions between partially purified CMV preparations and anti-CMV immunoglobulins. Since we have observed that the pattern was composed of five precipitin lines it was necessary to determine the origin of each one. We demonstrated that two of them originated from intact virions and free CMV capsid protein subunits. Furthermore we showed that other two lines originated from the host plant proteins. According to the position and quality of remaining precipitin line we suggested that it could originate from possible intermediate state of CMV particles degradation which involves nucleoprotein complex of relatively defined size.

OP-30

## 5-FLUOROURACIL (5-FU) AS POSSIBLE ANTIVIRAL THERAPY: CHARACTERIZATION OF HEPATITIS C VIRUS QUASISPECIES (HCV) IN PATIENTS UNDERGOING 5-FU ADJUVANT TREATMENT FOR COLORECTAL CANCER

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Hepatitis C virus (HCV) is small, enveloped RNA virus whose genome is a single-stranded positive RNA of about 9.6kb. Nowadays interferon and ribavirin combination therapy for hepatitis C is effective in almost 50% of patients infected with HCV genotype 1b. Studies in other viral models showed that 5-FU is capable to lower viral infectivity *in vitro* and therefore drive viral population into so-called error catastrophe, and finally to the extinction. To evaluate *in vivo* effect of 5-FU on HCV, we screened 250 patients with colorectal cancer attended at our Hospital and only 3 patients were infected with HCV. Serum samples from 3 patients were collected a month before and a day before 5-FU treatment, at fifth day of first week and last day ($4^{th}$ month) of treatment. Quasispecies structure was characterized in three HCV genome regions: 5'noncoding region (5'NC), E2 region (including hypervariable region HVR-1) and nonstructural NS5A region (including PKR binding domain (PKRbd)). The three viral regions were amplified by RT and nested PCR, using region specific sets of primers and proof reading DNA polymerase. Amplified fragment were cloned and an average of 26 clones per sample were sequenced. After alignment of obtained sequences complexity, diversity, mutation frequency and type of mutational change were calculated for each quasispecies and phylogenetic studies were performed. Our data indicate that 5-FU in concentration used does not drive HCV viral population into error catastrophe *in vivo*. Therefore, 5-FU alone does not seem to be an alternative therapy for chronic hepatitis C.

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## PROJEKT "INVENTARIZACIJA I KARTIRANJE GLJIVA HRVATSKE" - ČETIRI GODINE ISTRAŽIVANJA

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Gljive su daleko najslabije istražena velika taksonomska skupina u Hrvatskoj. Od oko 20000 vrsta gljiva za koje se pretpostavlja da žive u Hrvatskoj, do danas je zabilježeno tek oko 4000 vrsta. Tako izrazito slaba istraženost prvenstveno je rezultat vrlo malog broja mikologa koji su istraživali gljive ovog područja. Kako bi se što uspješnije popunila ova velika praznina u poznavanju biološke raznolikosti Hrvatske, Hrvatsko mikološko društvo je 1999. godine pokrenulo dugoročni znanstvenoistraživački projekt "Inventarizacija i kartiranje gljiva Hrvatske". Cilj projekta je ustanoviti koje vrste gljiva žive na području Republike Hrvatske, u kakvoj su vezi sa svojim okolišem i kako su rasprostranjene. Rezultati ovog projekta čine temelj za horološka, biogeografska, ekološka i taksonomska istraživanja gljiva u Hrvatskoj, kao i za zaštitu gljiva i njihovih staništa. Osim mikologa, u projekt su uključeni i brojni volonteri koji daju izuzetno velik doprinos u pronalaženju i uzorkovanju nalaza. Tijekom četiri godine trajanja ovoga projekta pronađene su 322 vrste i 55 rodova novih za mikofloru Hrvatske. Jedna pronađena vrsta objavljena je kao nova za znanost. Zbog malog broja hrvatskih mikologa, velikog broja vrsta gljiva, njihove izuzetno slabe istraženosti, te velikog broja različitih staništa, ovaj je projekt tek na svojem početku.

## PROJECT "RECORDING AND MAPPING OF FUNGI IN CROATIA" - FOUR YEARS OF RESEARCH

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Fungi are by far the least explored large taxonomic group in Croatia. Out of 20.000 species that is estimated to exist in Croatia, only about 4.000 species were recorded so far. Such a poor level of exploration is primarily a result of a small number of mycologists that have researched fungi of this area. In order to successfully overcome this great gap in the knowledge of Croatian biodiversity, Croatian Mycological Society started a long-term scientific project "Recording and Mapping of Fungi in Croatia" in the year 1999. The aim of the project is to find out which fungal species exist in Croatia, to explore interactions between

fungi and their environment, and to find out how they are distributed. The results of this project form the basis for chorological, biogeographical, ecological, and taxonomic research of fungi in Croatia, as well as for conservation of fungi and their habitats. Along with mycologists, there are numerous volunteers involved in the project that make a huge contribution in finding and collecting of fungi. During the four years of this project 322 species and 55 genera have been discovered as new to Croatian mycoflora. One species has been published as new to science. Because of the small number of Croatian mycologists, large number of fungal species, their poor level of exploration, and great diversity of habitats, this project is just at the beginning.

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## THIRTY YEAR CO-OPERATION OF CROATIAN AND HUNGARIAN RESEARCHERS IN PLANT VIRUS INVESTIGATION

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Similar soil and climate conditions exist in a fairly big part of territories of Hungary and Croatia. Because of similar indigenous flora and cultivated plant growing program similar epidemiological situation concerning plant virus diseases appears in those regions. Consequently, plant virologists in both countries have met with similar problems and tasks in the quoted aspect what led to an overlapping interest in some other aspects of plant virus and plant virus disease research as well. Following this a basis was found for mutual co-operation in that research field between Institute for Plant Protection (Budapest) and University of Veszprëm (Keszthely) and Faculty of Science (Zagreb). Investigations included identification of viruses unknown or less investigated in either or both countries, study of their epidemiological characteristics, biological and physico-chemical properties, etc. The common research work resulted in publishing 58 papers (40 in Current Contents journals) and in an adequate number of scientific meeting presentations. In either or both countries viruses from the following virus genera were found: *Caulimovirus* (CaMV), C*omovirus* (RaMV), *Cucumovirus* (CMV), *Nepovirus* (CLRV), *Nucleorhabdovirus* (PVYV), *Potyvirus* (CeMV, MVCV, TuMV, WMV), *Sobemovirus* (SoMV), *Tobamovirus* (RMV, TMV, ToMV), *Tymovirus* (TYMV, BelMV) and some other. The mentioned tobamoviruses were found in Hungarian rivers and lakes too. Other results included a number of new natural and experimental virus hosts, new virus strains (ToMV-H and $PVY^{NTN}$ in potato), serological, inclusion body (RMV, TMV, ToMV,TYMV) and adsorbtion-to-soil (TMV, TYMV) properties etc. Serious damages and losses were found in various cultivated plants.

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## ANTIMIKROBNO DJELOVANJE SJEMENKI GREJPA - *Citrus paradisi* MACFAYDEN

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Brojna su znanstvena izvješća o antimikrobnom djelovanju velikog broja prirodnih aktivnih tvari. Ekstrakt češnjaka, crvenog luka, eterična ulja mnogih biljaka te brojni proteini sjemnki različitih žitarica imaju inhibitorno djelovanje

na rast mikroorganizama. Posljednjih godina uveliko se istražuje antimikrobno djelovanje sjemenki grejpa (GSE) (*Citrus paradisi*). Međutim, objavljenja su i neka istraživanja koja taj učinak osporavaju i pripisuju ga konzervansima u pripravku. Zbog oprećnih mišljenja znanstvenika o djelotvornosti ekstrakta sjemenki grejpa, cilj rada je bio utvrditi antimikrobni učinak preparata tvorničkog imena "CITROSEPT®" i vlastitog pripravaka. Suhi ekstrakt usitnjene libovine (pulpa) i sjemenki grejpa otopljen u etanolu (250 mg/ mL) testiran je na 10 Gram-pozitivnih, 10 Gram-negativnih bakterija i 10 kvasnica. Postupkom difuzije dokazano je antimikrobno djelovanje "CITROSEPT®" pripravka sa promjerima zona inhibicija maks. 30mm. Ekstrakt grejpa pripremljen u laboratoruju nije pokazao antimikrobno djelovanje na Gram-negativne bakterije. Inhibicija rasta s promjerima zona inhibicija do 20 mm utvrđena je u grupi Gram-pozitvinih bakterija i kvasnica. Tankoslojnom-kromatografijom GSE dokazano je prisutstvo flavonoida naringina i hesperidina

## ASPECTS OF THE ANTIMICROBIAL EFFICACY OF GRAPEFRUIT SEED EXTRACT – *Citrus paradisi* MACFAYDEN

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A number of research studies reports on the antimicrobial activities of a large number of naturally compounds such as garlic and onion, essential oils as well as some proteins from plant seed which are potent inhibitors of microorganisms growth. In recent years, particular attention has been paid to the grapefruit seed extract (GSE), which product are on the market propagated as non-toxic natural product with a nearly universal healing power against a variety of ills and diseases. Reports of some authors showed that some preservative agents are present in commercially available GSE, and that antimicrobial effect of GSE may be related to these substances. The aim of the present work was to test the antimicrobial efficacy of one commercially GSE trade-mark «Citrosept® « which is available on our market in relation to self-made GSE which was prepared by hot extraction of seeds and juiceless pulp. Dry extract was dissolved in ethanol to 250 mg/mL and tested on ten Gram-positive, ten Gram-negative bacteria as well as ten yeasts. The results of the diffusion method show that all of tested bacteria and yeasts are inhibited with max. 30 mm of inhibition zones by «Citrosept®» Generally, self made GSE did not show any growth inhibiting potency against Gram-negative bacteria. All test strains of Gram-positive bacteria and yeasts showed bactericidal and fungicidal efficacy, but smaller inhibition zones up to 20 mm in diameter were observed. Thin-layer chromatography of GSE confirm existence of flavonoids naringin and hesperidin.

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## UTJECAJ DODATKA SULFATA NA RAD AEROBNOG SUSTAVA ZA OBRADU OTPADNE VODE

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U ovom radu ispitana je mogućnost pročišćavanja otpadnih voda u aerobnom sustavu pomoću aktivnog mulja bioaugmentiranog s fosfat-akumulirajućom bakterijom *Acinetobacter calcoaceticus*. Nakon utvrđivanja rada sustava s konvencionalnim i bioaugmentiranim aktivnim muljem, provedeni su pokusi s dodatkom sulfata u obliku otopine amonijeva sulfata (20 mg / L). Bioaugmentacijom konvencionalnog aktivnog mulja postignuto je bolje uklanjanje fosfora i bolji prirast aktivnog mulja. Dodatak sulfata u sustav s bioaugmentiranim muljem rezultirao je kratkim inhibitornim učinkom od 4 h na uklanjanje ukupnog fosfora i ortofosfata. Dodatak sulfata nije utjecao na koncentraciju aktivnog mulja, broj stanica *A. calcoaceticus*, pad kemijske potrošnje kisika i koncentraciju kisika u sustavu, ali je doveo do nepovratnog pada pH vrijednosti medija. Dobiveni rezultati važni su s praktičnog aspekta primjene u radu aerobnih sustava s aktivnim muljem za obradu otpadne vode, gdje dotok otpadne vode s povećanom koncentracijom sulfata često dovodi do narušavanja djelotvornosti sustava.

## INFLUENCE OF SULPHATE ADDITION ON THE PERFORMANCE OF AEROBIC WASTEWATER TREATMENT SYSTEM

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In this study, the possibility of the wastewater purification in an aerobic activated sludge system bioaugmented with a phosphate-accumulating bacteria *Acinetobacter calcoaceticus* was investigated. After establishing the system performance for the conventional and bioaugmented activated sludge, the experiments with the addition of sulphate in the form of ammonium sulphate solution (20 mg / L) were conducted. By the bioaugmentation of the conventional activated sludge better phosphorus removal and better activated sludge increment were achieved. Sulphate addition in the bioaugmented activated sludge system resulted in a short inhibitory effect of a 4 h on the removals of total phosphorus and orthophosphate. Sulphate addition did not influenced the mixed liquor suspended solids, bacterial numbers of *A. calcoaceticus*, removal of the chemical oxygen demand and the oxygen concentration in the system, but leaded to the irreversible decrease of the medium pH

value. The presented results are important from the practical aspect of the application in the performance of aerobic activated sludge wastewater treatment systems, where the inlet of the wastewater with the increased concentration of sulphate often leads to the disturbances of the system efficiency.

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## SASTAV MIKFOFLORE PLIJESNI OTVORENIH I ZATVORENIH PROSTORA DVIJU JAVNIH ZGRADA

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Svrha ovog istraživanja je praćenje mikroflore plijesni unutar i izvan dviju javnih zgrada u centru grada. Uzorkovanje je provedeno sezonski tijekom jedne, s početkom u jesen 2002. godine. Uzorci zraka unutar zgrada uzeti su na 3 lokacije (podrum, prizemlje i prvi kat). Uzorci zraka na otvorenom prostoru uzeti su na nasuprotnim stranama istraživanih zgrada. Uzorkovanje je provedeno aparatom za uzorkovanje zraka Millipore Air Tester. Za sakupljanje i identifikaciju plijesni korištene su komercijalne kazete s hranjivom podlogom (Saburaud dekstroza agar). Sito aparata kroz koje je zrak profiltriran sadrži oko 1000 mikroperforacija koje onemogućavaju preklapanje kolonija. Uzorkovani volumen zraka prilikom svakog uzorkovanja određen je na 100 litara. Kazete kroz koje je profiltriran zrak inkubirane su 7 dana na sobnoj temperaturi. Koncentracija je preračunata na broj kolonija plijesni u 1 $m^3$ zraka. Tijekom istraživanja mjerena je okolišna temperatura i relativna vlaga zraka. Izoliran je velik broj rodova plijesni među kojima prevladavaju *Aspergillus, Penicillium* i *Cladosporium*.

## AIRBORNE MICROBIAL MONITORING INSIDE AND OUTSIDE OF TWO PUBLIC BUILDINGS

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The aim of this study is airborne microbial monitoring inside and outside of two public buildings in the town center. Monitoring is performed seasonally during one year, started in autumn last year. Indoor air samples are taken at three localities (basement, ground and first floor). Outside air samples are taken at two localities at opposite sides of these buildings. Samples are taken by the Millipore Air Tester. It uses agar cassettes (Sabouraud dextrose) for collection and identification of fungi.

The sieve of the tester contains approximately 1000 micro perforations that minimize colony overlapping. Sampling volume is adjusted at 100 L of air. The exposed cassettes were incubated for seven days at room temperature. The concentrations were calculated as colony forming units in $m^3$ of air. During the study air temperature and relative humidity were measured. A large variety of mold genera were isolated among them *Aspergillus, Penicillium* and *Cladosporium* predominate. Indoor and outdoors airborne fungi observed until now, should be statistically evaluated to show eventual difference in the degree of number and species.

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## FACTORS INFLUENCING THE INHIBITION OF AFLATOXIN PRODUCTION IN SOY-BEANS BY *Trichothecium roseum*

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*Trichothecium roseum*, a mold commonly associated with *Aspergillus flavus* in damaged soy beans, interferes with production of aflatoxins when grown with *A. flavus* on autoclaved soy-beans. The pH of soy-beans meal disks was adjusted using NaOH-HCl, citric acid-sodium citrate, or water extracts of *T. roseum* fermented soybeans. Aflatoxin formation was completely inhibited below pH 2.5-3.5, irrespective of the system used for pH adjustment. When grown in association with *A. flavus* ATCC 26949 on autoclaved soy-beans, *T. roseum* lowered substrate pH sufficiently to suppress aflatoxin $B_1$ and $G_1$ production. The biodegradation of aflatoxin $B_1$ and $G_1$ were eliminated as potential mechanisms by which *T. roseum* reduces aflatoxin contamination. A water extract of soy-beans fermented with *T. roseum* caused an additional inhibition of aflatoxin formation apart from the effects of pH.

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## DEVELOPMENT OF THE CULTIVATABLE HIGHER *BASIDIOMYCETES* SPECIES' AND MUSHROOM HYBRIDS' GENE BANK

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Recently, the protection of living creatures' genome gets more and more significance. The increasing environmental harm reduces the chances for some populations to survive, that is why it is necessary to declare the endangered mushroom

species protected and to protect their habitat. In case of higher *Basidiomycetes*, on the basis of their fruiting body we can deduce, that the population is present, but the lack of the fruiting body doesn't mean in every case that the population has died out. Besides the protection of mushroom species and their habitats the most effective solution is the preservation of the species in the form of laboratory cultures. Our laboratory has undertaken to establish the stock breeding of first of all edible mushrooms which are mainly exposed to collection. In Hungary we are the only ones who have besides numerous wild stocks the collection of hybrids and basic material used for breeding from 20th century-breeders, like for example the world famous oyster mushroom hybrids (HK-35, HK-44, H-7, etc.) of Pál Gyurkó. The importance of these, as our national values is huge, furthermore they supply excellent basic material for breeding for the breeders of today as well. In order to keep the stability of our genetic cultures we applied cryopreservation techniques in the case of some mushroom species, stocks and hybrids. We determined the cryoprotective materials, the method of freezing and melting and besides exact data registration the place of certain cultures in the store. The refilling of the crypreservator is continuous.

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## METHODS FOR BREEDING THE OYSTER MUSHROOMS (*Pleurotus spp.*)

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In Hungary from a growing aspect the second most significant mushrooms species is the oyster mushroom (*Pleurotus ostreatus*). The first propagation material was collected and produced from the following oyster mushroom species: *P. ostreatus, P. pulmonarius* and *P. cornu-copiae*. Partly the development of extensive, but first of all that of intensive growing made continuous breeding work necessary. There is an increasing need worldwide to the new oyster mushroom hybrids. The hybrids of Hungarian breeders are spread and popular around the whole world. In Hungary formerly the University for Forestry and Wood Industry in Sopron, the Duna Agricultural Cooperation and the Agricultural Cooperation in Borota was engaged in oyster mushroom breeding and basic material production. Today the Vegetable Research Institute in Kecskemét, Szent István University and Korona Strain Research Laboratory is engaged in it now. Besides numerous Hungarian breeding specialists mention must be made of Pál Gyurkó, István Szili and István Szabó, who produced more strains which were qualified by the state as well. Another important result relating to Hungary was the HTTV (I. Heltay - L. Tóth

- E. Tóth - E. Véssey) patented procedure for oyster mushroom substrate which laid down the basics for large scale intensive growing. We inherited numerous basic materials for breeding and hybrids from these excellent specialists, which extended continuously with our breeding work means that we have at present on of the most significant gene banks of Central Europe. Our laboratory has besides hundreds of hybrids numerous other oyster mushroom species (*P. ostreatus, P. pulmonarius, P. columbinus, P. florida, P. cornu-copiae var. citrinopileatus, P. eryngii, P. flabellatus, P. sajor-caju,* etc.).

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## PCR BASED SEPARATING EXAMINATION OF WHITE BUTTON MUSHROOM (*Agaricus bisporus*)

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Since last year at the Strain Research and Molecular Biological Laboratory of National Korona Mushroom Union PCR-based molecular biological works are carried out in order to increase competitiveness, productivity and the separation of species furthermore for breeding. We would like to standardize the circumstances of the RAPD-PCR method, which is used during work, furthermore make a difference with random primers between some cultivated *Agaricus bisporus* hybrids according to their fingerprints. We plan individual primers with the isolating, cloning and sequention of the differentiating bands. Our further aim is to increase the effectiveness of the traditional breeding methods (selecting and separating the rare homocarion monospore cultures from the heterocarions, etc.) with the use of molecular markers. Our work till now was getting acquainted with and getting the mastery of the new techniques. We acquired numerous methods and protocols, which is used in Hungary for the first time within the frames of mushroom industry. Every step of our initial attempt became pioneer for the researches relating to Hungarian mushroom industry. We collected the wild species, which we would like to use as basic material for breeding. We placed the wild species in our stock collection into test tubes and Petri dishes, the testing of cryopreservation technique is in progress. During the education of the Agricultural Biotechnology Centre in Gödöll we acquired those basic techniques, which are indispensable for the mushroom breeding work with molecular markers. We made the application of devices and materials, in course of a long time we found the most optimal adjustments of the PCR device, the proper proportion of the ingredients, the most suitable values of some parameters (denaturation, anellation, extension, RAMP, etc.). We started the separation of our species according to the markings of their gel. These gel markings will allow us to achieve our aims.

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## UPDATING THE PROPAGATION MATERIAL (SPAWN) PRODUCTION PROCESS OF CULTIVATED MUSHROOM SPECIES

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Numerous higher *Basidiomycetes*, *Ascomycetes,* saprobiont and xilophag mushrooms have the possibility for intensive cultivation. We grow very few species from the above mentioned ones, first of all white button mushroom (*Agaricus bisporus*), oyster mushrooms (*Pleurotus ostreatus*) and shiitake (*Lentinus edodes*) hybrids. Our laboratory has hundreds of species, commercial hybrids, furthermore numerous basic materials for breeding. Within Hungary we have the largest gene bank in respect of the cultivatable mushrooms. The first step of the involvement into growing is the working out of keeping up the stocks, optimizing the ingredients of the culture medium, the determination of the process for mother culture and mushroom spawn production. Certain species grow optimally on different substrates. Our aim is to work out a substrate with relative universal ingredients for the production of propagation material for the above mentioned species, which is cheap, can be available for commercial sale and generally used for the optimal growth of different species. In the case of saprobionts we used organic materials, waste materials of plants (like grains, corn cobs) while in the case of xilophages we used waste wood, sawdust, spiles, etc. We made the mother culture experimentations at the Strain Research and Molecular Biological Laboratory of National Korona Mushroom Union and the spawn production experimentations at the Korona Spawn Plant.

P-50

## REARING CADDIS LARVAE (Trichoptera) IN AN ARTIFICIAL STREAM

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Defining *Trichoptera* larvae is very difficult, a preliminary study of more years is necessary, in order to be able to determine them. Hungarian scientific literature can give only referential information about the morphology of larvae, even

international scientific literature doesn't contain the description of each and every species' larva. With a light trap the flight periods of the imagoes can be registered, it is relatively easier to define the caught imagoes. During our work we collected *Trichoptera* larvae from the Szalajka- and Eger-brook in the Bükk hills in North Hungary, which we defined with binocular stereo microscope, put them into an aquarium with water stream where we brought them up. We fed the larvae with leaves and detritus and lead a diary about their improvement. We observed the time when they flew out as well. The bringing up of more species (*Sericostoma personatum*, *Odontocerum albicorne*, *Silo pallipes*) was successful. In our present paper we defined the larva on the basis of bringing up *Potamophylax rotundipennis*.

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## DISTRIBUTION OF PROTEOLITIC, AMYLOLITIC AND LYPOLYTIC BACTERIA IN LAKE OHRID

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The aim of this work was to confirm spatial and temporal distribution of proteolytic, amylolitic and lypolitic bacteria in the littoral (at 11 stations) and pelagic water (vertical profile) of Lake Ohrid, on a monthly basis from 2000-2002. Proteolytic bacteria are identified on the MPA (meat peptone agar) media with 1.5% gelatine, amylolitic on the MPA with 1.5 % starch and lipolytic bacteria on the tributyrrine agar. According to the results from this research, there is close relation between water pollution and bacterial number. Their quantity is usually higher in the litoral water, than in the pelagic water. The highest number of studied bacterial groups was recorded from littoral water at Grasnica, in the area where the majority of municipal and industrial effluents are received and spread in the surface layer. Locality of Grasnica is a very specific, shallow, with poor circulation. Major part of municipial wastewaters from the town of Ohrid are discharged there. Density of these bacterial groups decreased with the distance from this area. Seasonal oscillations showed summer maximum and winter minimum. In the pelagial, vertical distribution showed that their density increased with depth. Proteolytic bacteria occurred in greatest number, followed by amylolitic bacteria while lipolytic bacteria numbers were lowest. All this led us to conclude that increased proportion of complex organic compounds (proteins, carbohydrates, lypids) was favourable for the development of bacteria which decompose these compounds. Increased proportion of individual physiological bacterial groups may be a good indicator of pollution by defined organic compounds.

BILJNA BIOLOGIJA
*BIOLOGIA PLANTARUM*
PLANT BIOLOGY

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## FITOSOCIOLOGIJA VRSTA IZ RODA *Festuca* L. NA TRAVNJACIMA ISTRE (HRVATSKA)

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Vrste roda *Festuca* važni su članovi suhih travnjaka u krškom području Hrvatske gdje pripada i Istra. Cilj ovog istraživanja bio je ustanoviti kako su pojedine vrste roda *Festuca* povezane s određenim tipovima travnjačkih zajednica. U tu svrhu izrađena je 31 vegetacijska snimka standardnom srednjoeuropskom metodom. Snimke su analizirane fitocenološki i statistički pomoću klasterske analize i kanoničke analize korespodencije (CCA). Najrasprostranjenija vrsta roda *Festuca* na travnjacima Istre je *F. rupicola*. Rasprostranjena je i u eumediteranskim i u submediteranskim travnjačkim zajednicama, no centar njene rasprostranjenosti predstavljaju suhi, bazifilni travnjaci reda *Scorzoneretalia villosae*. Travnjaci tog reda predstavljaju i centar rasprostranjenosti vrste *F. valesiaca* s time da je ona na njih uglavnom i ograničena. Vrsta *F. lapidosa* vezana je isključivo za eumediteranske travnjake razreda *Thero-Brachypodietea* i to prije svega na pašnjačke zajednice razvijene iznad jako degradiranih, kamenjarskih tala koje pripadaju svezi *Cymbopogo-Brachypodion ramosi*. Vrste *F. filiformis* i *F. nigrescens* vezane su prije svega na relativno vlažnije i kisele travnjake s obronaka Učke koji pripadaju asocijaciji *Scorzonero-Hypochoeretum maculatae*, odnosno redu *Nardetalia*. Pored toga vrsta *F. nigrescens* pronađena je i u prijelaznoj zajednici između reda *Scorzoneretalia villosae* i razreda *Molinio-Arrhenatheretea*. Vrsta *F. trichophylla* pronađena je samo na vlažnim, podvirnim, kalcijevim-karbonatom bogatim livadama u podnožju Učke na rubu Ćepić polja. To je prvo i do sada jedino nalazište ove vrste u Hrvatskoj. Vrste *F. pratensis* i *F. arundinacea* vezane su na zajednice razreda *Molinio-Arrhenatheretea*, te nisu pravi članovi vegetacije krša.

## PHYTOSOCIOLOGY OF THE SPECIES FROM THE GENUS *Festuca* L. ON THE GRASSLANDS OF ISTRIA (CROATIA)

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Species of the genus *Festuca* are important members of grassland vegetation in the karstic area of Croatia where Istria also belongs. The aim of this study was to investigate how the particular species of genus *Festuca* are connected with specific grassland communities. To do this 31 vegetational records were made trough the Istria using standard middle-European method. The records were analysed

phytosociologically, and statistically using cluster analysis and canonical correspondence analysis (CCA). The most widespread species from the genus *Festuca* on the grasslands of Istria is *F. rupicola*. It is the member of eumediterranean as well as submediterranean grasslands, but the center of its distribution is still submediterranean, dry and basiphilous grasslands from the order *Scorzoneretalia villosae*. The grasslands of this order are also the centre of distribution of *F. valesiaca* and are predominantly restricted on this type of habitat. The species *F. lapidosa* is strongly restricted to eumediterranean grasslands of the class *Thero-Brachypodietea*, and within them predominantly on the pastures developed on degraded and stony soil, belonging to the alliance *Cymbopogo-Brachypodion ramosi*. The species *F. filiformis* and *F. nigrescens* are predominantly restricted to relative wet and acidophilous grasslands distributed on the hills of the Učka mountain, belonging to the association *Scorzonero-Hypochoeretum maculatae* and to the order *Nardetalia*. *F. nigrescens* was also found in transitive plant communities between the order *Scorzoneretalia villosae* and the class *Molinio-Arrhenatherethea*. The species *F. trichophylla* was found only on wet and calcareous meadows on the border of the Čepić polje near the foothills of the Učka mountain. The species *F. pratensis* and *F. arundinacea* are associated with communities of the class *Molinio-Arrhenatherethea* and are not real members of karstic grasslands.

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## FITOKEMIJSKA KARAKTERIZACIJA NEKIH VRSTA RODA *Plantago* L. (Plantaginaceae) IZ ZAPADNE HRVATSKE

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Rod *Plantago* L. polimorfan je rod porodice *Plantaginaceae*, zastupljen u flori Hrvatske s 22 vrste i nekoliko podvrsta. Srodstveni odnosi pojedinih svojti, razine specijacije, te dubine subspecifične diferencijacije nisu dovoljno poznati. U svrhu rasvjetljavanja spomenutih nejasnoća, provedena su fitokemijska istraživanja svojti: *P. altissima* L., *P. argentea* Chaix, *P. coronopus* L., *P. holosteum* Scop. (subsp. *depauperata*, subsp. *holosteum* i subsp. *scopulorum*), *P. lagopus* L. i *P. maritima* L. iz zapadnog dijela Hrvatske. Ispitivanju su podvrgnuti na zraku osušeni listovi navedenih biljnih taksona. Metodom micelarne elektrokinetičke kromatografije (MEKC) provedena je karakterizacija iridoidnih sastavnica. Utvrđeno je da vodeni

ekstrakti listova sadrže do 0,27% aukubina i do 1,81% katalpola (*P. argentea*). Uporabom metode reverzno-fazne tekućinske kromatografije visoke djelotvornosti (RP-HPLC) uspješno su karakterizirani flavonoidi i fenolne kiseline u metanolno-vodenim ekstraktima listova. Maseni udjeli analiziranih biološki aktivnih sastavnica znatno su varirali među taksonima. Sadržaj rutina iznosio je 0,005-0,024%, hiperozida 0,007-0,020%, kvercitrina 0,001-0,013%, kvercetina 0,006-0,028%, dok su maseni udjeli fenolnih kiselina bili u granicama od 0,002 do 0,115% za klorogensku, te od 0,001 do 0,046% za kavenu kiselinu. Eksperimentalni fitokemijski rezultati analize iridoida, flavonoida i fenolnih kiselina obrađeni su metodom multivarijatne statistike ("cluster" analiza), kao doprinos kemotaksonomiji roda *Plantago*. Utvrđeno je da analizirane niže svojte vrste *P. holosteum* ne pripadaju istoj kemotaksonomskoj skupini, jer se sadržajem bioaktivnih sastavnica podvrsta *holosteum* znatno razlikuje od druga dva niža taksona iste biljne vrste. Vrsta *P. argentea* se najviše fitokemijski razlikuje od ostalih istraženih biljaka, te posjeduje najveći fitoterapijski potencijal, kako u odnosu na sve ispitivane zapadnohrvatske taksone, tako i u odnosu na terapijski dokazano vrijednu vrstu *P. lanceolata*.

## PHYTOCHEMICAL CHARACTERIZATION OF SOME *Plantago* SPECIES (Plantaginaceae) FROM THE WESTERN PART OF CROATIA

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*Plantago* L. is a polymorfic genus of *Plantaginaceae* family, with 22 species and a few subspecies in Croatian flora. Taxonomic relationships, including levels of speciation and subspecific differentiation, are still not completely investigated. So, air-dried leaves of some *Plantago* species growing in the western part of Croatia were phytochemically investigated: *P. altissima* L., *P. argentea* Chaix, *P. coronopus* L., *P. holosteum* Scop. (subsp. *depauperata*, subsp. *holosteum*, and subsp. *scopulorum*), *P. lagopus* L., and *P. maritima* L. Micellar electrokinetic chromatography (MEKC) was used for the characterization of iridoid substances. The yield of aucubin and catalpol was up to 0.27% and 1.81% (*P. argentea*) of the dry mass of the leaves, respectively. Characterizations of flavonoids and phenolic acids were successfully performed by reversed phase HPLC. Yields of analysed substances varied among examined species: rutin (0.005-0.024%), hyperosid (0.007-0.020%),

quercitrin (0.001-0.013%), quercetin (0.006-0.028%), chlorogenic acid (0.002-0.115%) and caffeic acid (0.001-0.046%). Experimental phytochemical data were evaluated by multivariate statistic method (cluster analysis), as a contribution to the chemotaxonomy of genus *Plantago*. It was observed that *P. holosteum* subsp. *holosteum* was significantly different in contents and distribution of iridoids, flavonoids and phenolic acids compared to the other analysed lower taxa of the same species, so they formed different chemotaxonomic groups. The results of present study showed that *P. argentea* had the highest phytotherapeutic potential of all examined *Plantago* species and therefore it could be a very good resource of the investigated biologically active compounds.

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## ESTIMATION OF THE ISOENZYME GENETIC VARIABILITY OF THE SILVER FIR (Abies alba MILL.) FROM THE AREA OF GORSKI KOTAR (CROATIA)

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Variability of nine isoenzyme systems, composed of 16 locus and 33 alels, were investigated on 188 analysed Silver Fir trees from four populations in area of Gorski Kotar (Fužine, Skrad, Gerovo, Vrbovsko). Average number of the alels varied between 2,0626 in the Gerovo population and 2,3125 in the Fužine population. The level of heterozigosity (He) ranged from 0,1390 in the Fužine population to 0,1869 in population and multylocus difference (vgen) from 21,13 to 42,39 in the Skrad population. Statistically significant differences have been shown between investigated populations. High frequency of the alel 6Pgd-A2 in the populations indicates its connection with selection pressure on the locus caused by pollutants. The level of heterozigosity in the investigated populations, compared with Western European populations, shows small loss of original genetical potential as they are not too far from ice age refugies and also are in hybridisation area of Apenine and Balkan populations. The method used presents a very good picture of genetic structure of researched populations.

OP-35

## FIZIČKO KARTIRANJE JEZGRINIH GENA ZA RIBOSOMSKU RNA (rRNA) I KARAKTERIZACIJA 5S rDNA I ITS SEKVENCI U VRSTE *Abies alba* MILL.

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Ribosomski geni (18S-5.8S-26S rDNA i 5S rDNA) i njihove međugenske razmaknice (ITS i IGS) predstavljaju pogodne biljege za utvrđivanje filogenetskih odnosa između vrsta. U ovom radu istraživan je broj i položaj ribosomskih gena u kariotipu vrste *Abies alba* Mill. te slijed nukleotida unutarnjih transkribirajućih razmaknica (ITS) i 5S rDNA. Kariotip obične jele sastoji se od 24 meta- i submetacentrična kromosoma. Bojanjem s kromomicinom A3 (CMA) dobiveno je 10 CMA-pozitivnih signala smještenih interkalarno na 10 metacentričnih kromosoma. Nakon bojanja sa srebrom utvrđeno je najviše 10 jezgrica što se podudara s brojem CMA-pozitivnih signala. Osim toga, primjenom fluorescencijske hibridizacije *in situ* (FISH) utvrđeno je da se na tim mjestima nalaze geni za 18S-5.8S-26S rRNA. ITS1 i ITS2 razmaknice zajedno s 5.8S rRNA genom umnožene su pomoću lančane reakcije polimerazom (PCR). U vrste *A. alba*, kao i u većine četinjača PCR-om umnožena ITS1 razmaknica duža je u odnosu na ITS2. Duljina ITS1 razmaknice iznosi oko 1450 pb, dok je ITS2 zajedno s 5.8S rRNA genom duga oko 490 pb. Duljina PCR-om umnožene 5S rDNA iznosi oko 560 pb. U svrhu istraživanja filogenetskog odnosa vrste *A. alba* i drugih četinjača, slijedovi nukleotida ITS1 i ITS2 razmaknica, te 5S rDNA jele bit će uspoređivani s postojećim podacima za druge četinjače.

## PHYSICAL MAPING OF NUCLEAR RIBOSOMAL RNA (rRNA) GENES AND CHARACTERIZATION OF 5S rDNA AND ITS SEQUENCES IN *Abies alba* Mill.

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Ribosomal genes (18S-5.8S-26S and 5S rDNA) and their intergenic spacers (ITS, IGS) present useful markers for identification of phylogenetic relationship among species. The number and position of rRNA genes in chromosomes as well as characterization of internal transcribed spacer (ITS) and 5S rDNA sequences were investigated in *Abies alba* Mill. Karyotype of this species consists of 24 meta- and submetacentric chromosomes. After staining with chromomycine A3 (CMA), ten CMA-positive signals were detected at intercalary position on five metacentric chromosome pairs. The maximum number of ten nucleoli revealed by silver staining was in correlation with the number of CMA-positive signals. In addition, fluorescent *in situ* hybridization (FISH) revealed ten 18S-5.8S-26S rRNA loci on ten metacentric chromosomes. ITS1 and ITS2 together

with 5.8S rRNA gene were amplified by PCR. The length of amplified fragment is approximately 1450 bp for ITS1 and 490 bp for ITS2. The size of 5S rDNA fragment amplified by PCR is 560 bp. Amplified ITS1, ITS2 and 5S rDNA fragments should be sequenced and compared with known sequences in order to investigate phylogenetic relationships of *A. alba* and other conifer species.

OP-36

## DIFERENCIJACIJA HRVATSKIH POPULACIJA TAKSONA *Quercus pubescens sensu lato* (Fagaceae) UZ POMOĆ RAPD TEHNIKE

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Zbog velike varijabilnosti i intenzivnog protoka gena među srodnim vrstama, taksonomija roda *Quercus* L. je vrlo kompliciranа i često puta kontraverzna. Svrha ovoga istraživanja bila je determinirati odnose i taksonomski status hrvatskih populacija vrste *Q. pubescens* s.l. koristeći RAPD-PCR tehniku. Rezultati su analizirani uz pomoć multivarijatnih statističkih metoda. Uz pomoć RAPD tehnike nije moguće na području Hrvatske diferencirati različite taksone unutar kompleksa *Q. pubescens* s.l. Umjesto toga mogu se razlikovati sjeverna i južna skupina kao i jedna prijelazna populacija. Ovi rezultati uspoređeni su s rezultatima sličnih istraživanja te je zaključeno da se sjeverne populacije razlikuju od južnih zbog vjerojatne introgresije vrste *Q. petrea* u genom istraživanih populacija u sjevernom dijelu Hrvatske.

## RAPD DIFFERENTIATION OF THE CROATIAN POPULATIONS OF *Quercus pubescens sensu lato* (Fagaceae)

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Taxonomy of the genus *Quercus* L. is very complicated and often controversial because of its great variability and intense gene flow among related species. The purpose of this research was to determine the relationships and taxonomic status of Croatian populations of the *Q. pubescens* s.l. using RAPD-PCR technique. The results have been analyzed by means of the multivariate statistic methods. Different taxa within *Q. pubescens* s.l. in Croatia could not be differentiated by use of RAPD tech-

nique. Instead of that two groups of populations were noticed: the northern and the southern and a transitional population between them. The obtained results were compared to the already available studies on these species. The conclusion is that northern Croatian populations differentiate from the southern ones because of possible introgression of the *Q. petrea* into a genome of those populations.

OP-37

## FILOGENETSKI ODNOSI IZMEĐU ŠEST VRSTA RODA *Hypericum* USPOREDBOM KLOROPLASTNIH DNA BILJEGA

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Četiri autohtone vrste roda *Hypericum (Hypericaceae)*: *H. hirsutum* L., *H. maculatum* Crantz, *H. montanum* L. i *H. perforatum* L. kao i dvije vrste iz uzgoja: *H. androsaemum* L. i *H. calycinum,* uspoređene su na temelju restrikcijskih profila amplificiranih fragmenata kloroplastne DNK radi utvrđivanja njihovih filogenetskih odnosa. Analizirane su četiri regije kloroplastne DNK dobivene amplifikacijom u lančanoj reakciji polimerazom uz specifične početnice. Nakon cijepanja s dvije restrikcijske endonukleaze osam dobivenih restrikcijskih profila su identični između 10 do 12 jedinki iste vrste, dok je između vrsta ustanovljena znatna razlika. Odnosi između vrsta uspoređeni su *cluster* analizom i predstavljeni dendrogramom koji prikazuje visok stupanj specifičnosti strukture kloroplastne DNK pojedine vrste. Stupanj različitosti između vrsta u skaldu je s evolucijskim diagramom roda *Hypericum* temeljenom na morfološkim, ekološkim i citogenetičkim kriterijima.

## ESTIMATION OF PHYLOGENETIC RELATION AMONG SIX *Hypericum* SPECIES USING cpDNA MARKERS

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Six species of genus *Hypericum (Hypericaceae)*: *H. hirsutum* L., *H. maculatum* Crantz, *H. montanum* L. and *H. perforatum* L. native in Croatia and *H. androsaemum* L. and *H. calycinum* L. from cultivation were compared on the basis of restriction patterns of amplified cpDNA fragments to elucidate their phylogenetic relationships. Each species were represented with 10 to 12 individuals. Eight restriction pattern obtained by 4 amplified regions of cpDNA after digestion with two restric-

tion enzymes were analyzed. The investigated regions were amplified by PCR using the universal cpDNA primer pairs AS, CD, CS and FV. The restriction pattern after digestion with *Taq*I and *Hin*fI restriction endonucleases was identical among individuals of the same species, while considerable degree of interspecific polymorphism was detected. Species differs in fragments number and size. The dendrogram obtained from cluster data analysis demonstrates high species specificity of the cpDNA structure. Linkage distances among these species are in accordance with evolutionary diagram of genus *Hypericum* based on morphological, ecological and citogenetical criterions.

OP-38

## UPOTREBA HEMISFERNIH FOTOGRAFIJA U EKOLOŠKIM ISTRAŽIVANJIMA ŠUMSKE ZAJEDNICE *Omphalodo-Fagetum* U HRVATSKOJ

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Upotreba hemisfernih fotografija u raznovrsnim ekološkim istraživanjima, modeliranju i praćenju sve je raširenija. U svrhu usvajanja i razvoja metode, tijekom sezone 2002. snimljene su hemisferne fotografije sklopa krošnje u dinarskom dijelu areala bukovo-jelovih šuma na 151 lokaciji. Fotografije su digitalizirane, klasificirane prema kvaliteti njihove kontrastnosti i analizirane specijalističkim programom GLA (Gap Light Analyzer). Za izračunate osobine krošnji (otvorenost, indeks površine lista – LAI) ispitana je koreliranost s kvalitativnim i kvantitativnim podacima o flornom sastavu promatranih ploha u dva površinska razreda (cca. 79 i 491 $m^2$), te s drugim ekološkim parametrima (npr. nadmorska visina, klimatski parametri) i indeksom NDVI (Normalized Difference Vegetative Index). U ovisnosti o kvaliteti kontrasta fotografija ustanovljena je statistički značajna pozitivna korelacija indeksa LAI s indeksom NDVI i negativna s brojem vrsta. Dobiveni rezultati potvrdili su prikladnost upotrebe hemisfernih fotografija u ekološkim istraživanjima te ukazali na neke metodološke prilagodbe koje će povećati njihovu upotrebljivost.

## APPLICABILITY OF HEMISPHERE PHOTOGRAPHY IN ECOLOGICAL SURVEYS – A CASE STUDY FROM *Omphalodo-Fagetum* FORESTS IN CROATIA

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Today, hemisphere photography becomes more easily to apply and spread to various environmental monitoring, modelling and research. To adopt the methodology, hemisphere photography of forest canopy were taken at 151 plots in dinaric

part of the silver fir – beech forest areal during the 2002 season. Photographs were scanned, classified based on the quality of their contrast and analysed with GLA (Gap Light Analyzer) specialist software. For percentage of canopy openness and LAI (Leaf Area Index) index, existence of correlation with qualitative and quantitative data of floristic assemblage was examined in two areal classes (cca. 79 i 491 $m^2$), as well as with other ecological parameters (e.g. altitude, climatic parameters) and NDVI (Normalized Difference Vegetative Index) index. In dependence of the quality of contrast of the photographs, statistically significant positive correlation was established between LAI index and NDVI index, and negative between LAI index and number of plant species. Obtained results justified usage of hemisphere photography in ecological surveys and point at the methodological steps that have to be adjusted in order to increase their applicability.

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## UTVRĐIVANJE TIPA VRSTE *Limonium vestitum* (C. E. SALMON) C. E. SALMON (Plumbaginaceae)

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Prilikom opsežnijih taksonomskih istraživanja svojti roda *Limonium*, rasprostranjenih duž hrvatskog dijela Jadranske obale, istraživali smo i njihov tipski materijal. U potrazi za tipskim materijalom vrste *Limonium vestitum* C. E. Salmon (C. E. Salmon) (Salmon, 1924), ustanovili smo da je cijeli materijal u herbariju WU (Beč). Komparativna analiza tog materijala i literaturnih podataka pokazala je da nijedan primjerak nije izdvojen kao holotip ili izotip. Analiza Salmon-ovog prvog opisa, ilustracija i crteža vrste *L. vestitum*, tada opisane pod imenom *Statice vestita* C. E. Salmon (Salmon, 1923) i herbarijskog materijala, ukazala je na postojanje triju sintipova ove vrste: prvi s Jabuke (Pomo) (sabrali Ginzberger i Teyber 1911. pod imenom *S. cancellata* Bornh., revidirao Salmon 1923., kao *S. vestita*), drugi s Kamika (također sabrali Ginzberger i Teyber 1911. pod imenom *S. cancellata*, te revidirao Salmon 1923. kao *S. vestita*) i treći s Kamika (sabrao Ginzberger 1914., bez imena, a revidirao Salmon 1923 kao *S. vestita*). Prema Međunarodnom botaničkom nomenklaturnom kodeksu ICBN (Greuter et al., 2000), među tim sintipovima, izabrali smo lektotip vrste *L. vestitum*: *Limonium vestitum* C. E. Salmon (C. E. Salmon) in Salmon 62: 336, 1924. = *Statice vestita* C. E. Salmon in Salmon 61: 97-99, plate 567, fig. 1a., 1923. - Lectotype: specimen collected by Ginzberger A and Teyber A., s.n., 1911-06-03/09 (WU – Generale 27177). Greuter, W., McNeil, J., Barrie, F. R., Burdet, H. M., Demoulin, V., Filgueiras, T. S., Nicolson, D. H., Silva, P. C., Skog, J. E., Trehane, P., Turland, N. J. & Hawksworth, D. L. (eds.). 2000. *International Code of Botanical Nomenclature (Saint Louis Code) adopted by Sixteenth International Botanical Congress, St. Louis, Missouri, July-August 1999*. Koeltz Scientific Books, Königstein. [*Regnum Veg.* 138.]. Salmon, C. E. 1923. Notes on *Statice*. XV. *Statice vestita*, sp. nov. (Plate 567). *J. Bot.* 61: 96—99. Salmon, C. E. 1924. Short notes. *Statice* v. *Limonium*. *J. Bot.* 62: 335—336.

## TYPIFICATION OF THE *Limonium vestitum* (C. E. SALMON) C. E. SALMON SPECIES (Plumbaginaceae)

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Within larger taxonomical investigation of some interesting *Limonium* taxa distributed across the Croatian part of the Adriatic coast, we explored their type material. In quest of the original type material of the *Limonium vestitum* C. E. Salmon (C. E. Salmon) species (Salmon, 1924), we found out that the whole material is in the WU herbaria (Vienna). Comparative research of this material and literature data showed up that no one specimen was selected as holotype or isotype. Analyses of Salmon's description, illustrations and drawings of *L. vestitum*, under the name *Statice vestita* C. E. Salmon (Salmon, 1923) and herbarium material, pointed to the existence of three syntypes of this species: the first from Pomo (Jabuka) (collected by Ginzberger and Teyber in 1911 and named as *S. cancellata* Bornh., than revised as *S. vestita* by Salmon in 1923), the second sheet from Kamik (also collected by Ginzberger and Teyber in 1911 and also named *S. cancellata*, and than also revised as *S. vestita* by Salmon in 1923) and the third from Kamik (collected by Ginzberger in 1914, without name and than revised as *S. vestita* by Salmon in 1923). According to ICBN (Greuter et al., 2000), between those syntypes we selected the lectotype of *L. vestitum*: *Limonium vestitum* C. E. Salmon (C. E. Salmon) in Salmon 62: 336, 1924. = *Satice vestita* C. E. Salmon in Salmon 61: 97-99, plate 567, fig. 1a., 1923. - Lectotype: specimen collected by Ginzberger A and Teyber A., s.n., 1911-06-03/09 (WU – Generale 27177). Greuter, W., McNeil, J., Barrie, F. R., Burdet, H. M., Demoulin, V., Filgueiras, T. S., Nicolson, D. H., Silva, P. C., Skog, J. E., Trehane, P., Turland, N. J. & Hawksworth, D. L. (eds.). 2000. *International Code of Botanical Nomenclature (Saint Louis Code) adopted by Sixteenth International Botanical Congress, St. Louis, Missouri, July-August 1999*. Koeltz Scientific Books, Königstein. [*Regnum Veg.* 138.] Salmon, C. E. 1923. Notes on *Statice*. XV. *Statice vestita*, sp. nov. (Plate 567). *J. Bot.* 61: 96—99. Salmon, C. E. 1924. Short notes. *Statice* v. *Limonium*. *J. Bot.* 62: 335—336.

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## RAZLIKE U SADRŽAJU FLAVONOIDA HRVATSKIH ENDEMIČNIH VRSTA *Iris illyrica* Tomm. i *I. pseudopallida* Trinajstić (Iridaceae)

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Unatoč brojnim recentnim studijama koje podupiru mišljenje o zasebnom taksonomskom statusu vrsta *I. illyrica* Tomm i *I. pseudopallida* Trinajstić, navedena se dva taksona ipak najčešće smatraju jednom vrstom i to *I. illyrica* ili se ubrajaju u različite niže taksonomske kategorije unutar roda *Iris*. Budući da su razlike u sadržaju flavonoida uspješno korištene u rješavanje brojnih taksonomskih problema, svrha

je ove studije istražiti taksonomski odnos između vrsta *I. illyrica* i *I. pseudopallida* temeljen na sadržaju flavonoida. Metanolni ekstrakt listova dvadeset biljaka iz svake populacije podvrgnut je TLC i HPLC analizama. Utvrđene su significantne kvantitativne razlike u sadržaju flavonoida između listova istraživanih vrsta. Ovi rezultati podupiru mišljenje o odvojenom taksonomskom statusu vrsta *I. illyrica* i *I. pseudopallida*.

## FLAVONOID DIFFERENTIATION OF CROATIAN ENDEMIC SPECIES *Iris illyrica* TOMM. AND *I. pseudopallida* TRINAJSTIĆ (Iridaceae)

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Although there are several recent studies that support opinion about a separated taxonomical status of the species *Iris illyrica* Tomm. and *I. pseudopallida* Trinajstić, these two taxa have been mostly considered as only one species, *I. illyrica*, or as different lower taxonomic categories within different species of the genus *Iris*. Since the characteristics of the flavonoids have been successfully used for the solution of the many taxonomic problems, the aim of this study was to determine the taxonomical relationships between *I. illyrica* and *I. pseudopallida* using their flavonoid patterns. Methanolic extracts of the leaves of 20 plants for each population were submitted to TLC and reversed phase HPLC analyses. Significant quantitative differences in the leaf flavonoid patterns between *I. illyrica* and *I. pseudopallida* were obtained. This result supports opinion about separate taxonomical status of *I. illyrica* and *I. pseudopallida*.

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## VARIJABILNOST I RASPROSTRANJENOST VRSTE A*nthyllis vulneraria* L. U HRVATSKOJ

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Vrsta *Anthyllis vulneraria* L. rasprostranjena je duž cijele Europe (od Skandinavije do Mediterana), na sjeveru Afrike te u jugozapadnoj Aziji. To je polimorfna vrsta koja obuhvaća oko 36 različitih podvrsta. Pojedine podvrste međusobno su dobro povezane intermedijarnim populacijama, zbog čega se čini shodnim smatrati svojtu *A. vulneraria* L. jednom jedinom vrstom s velikom varijabilnošću. Podvrste su grupirane u 2 prirodna agregata (*vulneraria* i *alpestris*),

no i među njima postoje intermedijari. Prema literaturnim i herbarskim podacima u Hrvatskoj uz osnovnu vrstu dolazi i 6 podvrsta vrste *A. vulneraria* L.: *A. vulneraria* L. subsp. *alpestris* (Kit. ex Schult) Asch. et Graebn. i *A. vulneraria* L. subsp. *carpatica* (Pant.) Nyman iz agregata "*alpestris*" te podvrste *A. vulneraria* L. subsp. *polyphylla* (DC.) Nyman, *A. vulneraria* L. subsp. *pulchella* (Vis.) Bornm., *A. vulneraria* L. subsp. *rubriflora* (DC.) Arcang. i *A. vulneraria* L. subsp. *weldeniana* (Rchb.) Cullen iz agregata "*vulneraria*". Potonje dvije podvrste su endemi. Na osnovu postojećih literaturnih i herbarskih podataka te terenskih opažanja napravljene su karte rasprostranjenosti podvrsta u Hrvatskoj te su uočene neke nelogičnosti (npr. nalaz visokoplaninske podvrste *A. vulneraria* L. subsp. *alpestris* oko Zagreba). Morfometrijskom analizom izabranih varijabli različitih populacija u Hrvatskoj pokušat ću utvrditi stvarno stanje ovog problematičnog taksona, za kojeg pretpostavljam da obuhvaća više od šest podvrsta na području Hrvatske. Morfometrijsku analizu namjeravam potkrijepiti odgovarajućim analizama DNA.

## THE VARIABILITY AND DISTRIBUTION OF SPECIES A*nthyllis vulneraria* L. IN CROATIA

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The species *Anthyllis vulneraria* L. is distributed throughout whole Europe (from Scandinavia to Mediterranean), in North Africa and in southwest Asia. It is a polymorphic taxon that contains ca 36 different subspecies. The occurrence of a large number of intermediates between the subspecies indicates that *Anthyllis vulneraria* L. is one single species with high variability. Subspecies are grouped in 2 natural aggregates (*vulneraria* and *alpestris*), although there are intermediates between them, too. According to the literature and herbaria data along with common species there are 6 subspecies of *Anthyllis vulneraria* L. living in Croatia: *A. vulneraria* L. subsp. *alpestris* (Kit. ex Schult) Asch. et Graebn. and *A. vulneraria* L. subsp. *carpatica* (Pant.) Nyman from aggregate "*alpestris*" and subspecies *A. vulneraria* L. subsp. *polyphylla* (DC.) Nyman, *A. vulneraria* L. subsp. *pulchella* (Vis.) Bornm., *A. vulneraria* L. subsp. *rubriflora* (DC.) Arcang. and *A. vulneraria* L. subsp. *weldeniana* (Rchb.) Cullen from aggregate "*vulneraria*". The latter two taxa are endemic. Distribution maps of the subspecies in Croatia were made based on literature, herbaria data and filed observation. Some non-logic facts were discovered, i.e. the recording of high-mountain subspecies of *A. vulneraria* L. subsp. *alpestris* around Zagreb. Morphometric analysis of selected variables in different populations from Croatia will give the reins to new perception of diversity in group, and it can be assumed that more than 6 subspecies of *A. vulneraria* L. exist in Croatia. Morphometric analysis is going to be accompanied with responsive DNA analysis.

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## SISTEMATSKI ODNOSI SVOJTI RODA *Lilium* U HRVATSKOJ - PRELIMINARNI IZVJEŠTAJ NA TEMELJU MOLEKULARNIH PODATAKA

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Rod *Lilium* rasprostranjen je na sjevernoj zemljinoj polutci, a u Europi je zastupljen s deset vrsta. U hrvatskoj flori rasprostranjeno je pet taksona. Među njima nalazi se i nekoliko dvojbenih stenoendemičnih taksona o čijim točnim sistematskim položajima postoje mnoga različita mišljenja s obzirom da su na ovom području diferencirani u veći broj različitih svojti. *Lilium martagon* L., najrasprostranjeniji od svih ljiljana, na području svog areala pokazuje veliku varijabilnost. Prema nekim autorima vrsta se u Dalmaciji diferencira u dvojbeni takson *L. cattaniae* Vis. Na području Dinarida pojavljuje se još jedna dvojbena svojta *L. bosniacum* (Beck) Beck ex Fritsch. Ona je usko srodna i taksonomski povezana s *L. carniolicum* Bernh. ex Koch i obje pripadaju carniolicum skupini europskih ljiljana. Svrha ovog rada je utvrđivanje točnog taksonomskog položaja ovih svojti. Zbog potpunijeg razumijevanja međusobnih srodstvenih odnosa uključene su i ostale dvije hrvatske vrste, *L. bulbiferum* L. i *L. candidum* L., kao i vrste *L. pyrenaicum* Gouan. i *L. pumilum* DC. Jedinke iz populacija rasprostranjenih duž areala ovih vrsta analizirane su s pomoću molekularnih i morfoloških karakteristika. Metode korištene za utvrđivanje genetičke raznolikosti unutar i između populacija su polimorfizam dužine restrikcijskih ulomaka (RFLP) kloroplastne DNK i slučajno umnožena polimorfna DNK (RAPD). Analiza fragmenata kloroplastne DNK odvojila je *L. bulbiferum*, *L. canididum* kao i cijelu carniolicum grupu od ostalih vrsta, a uočeno je i moguće odvajanje *L. cattaniae* od *L. martagon*. Preliminarno RAPD istraživanje koje je uključilo četiri populacije svojti carniolicum grupe pokazalo je zamjetnu unutar- i između- populacijsku varijabilnost.

## SYSTEMATIC RELATIONSHIPS AMONG TAXA OF GENUS *Lilium* IN CROATIA - PRELIMINARY RESULTS BASED ON MOLECULAR DATA

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Ten species of *Lilium*, a Northern Hemisphere genus, are present in Europe. In Croatian flora, five taxa are distributed. Among them dubious stenoendemic taxa appears and causes much taxonomic confusion due to their differentiation into series of vicarious taxa in this region. The most widespread species, *Lilium martagon*

L., shows a significant variability throughout the entire range. In Dalmatian part of areal, according to some authors, it differentiates into doubtful *L. cattaniae* Vis. Another taxonomically questionable taxon found in Dinaric karst area is *L. bosniacum* (Beck) Beck ex Fritsch. It is related and taxonomically close to *L. carniolicum* Bernh. ex Koch and they both belong to carniolicum group of European lilies. The objective of this work is to determine the definitive status of these taxa. In addition, to infer their position in relation to other lily species, two other species found in Croatia, *L. bulbiferum* L. and *L. candidum* L., as well as species *L. pyrenaicum* Gouan. and *L. pumilum* DC. are included in the study. Individual plants from populations across the range of these taxa are analysed using molecular data and morphological characteristics. Restriction-site variation of PCR-amplified cpDNA fragments and random amplified polymorphic DNA are applied to determine the degree of genetic variability within and between them. The analysis of cpDNA fragments discriminated *L. bulbiferum*, *L. canididum* and species belonging to the carniolicum group from other species, and also provided a possible distinction between *L. martagon* and *L. cattaniae*. The preliminary RAPD study which included four populations belonging to the carniolicum group showed significant intra- and inter- population variability.

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## MOLEKULARNO SISTEMATSKA ISTRAŽIVANJA NEKIH SVOJTI RODA *Iris* (Iridaceae) SA JUŽNOEUROPSKOG PODRUČJA

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Rod *Iris* L. (*Iridaceae*) je kozmopolitski rod sa više od 250 vrsta rasprostranjenih u sjevernom umjerenom pojasu. Prema Flora Europaea rod *Iris* je u Europi zastupljen sa 30 vrsta. U Hrvatskoj dolazi 16 svojti koje su podijeljene u 6 serija: *Elatae* Lawr., *Pallidae* (A.Kern.) Trinajstić, *Pumilae* Lawr., *Sibiricae* (Diels) Lawr., *Laevigatae* (Diels) Lawr. i *Spuriae* (Diels) Lawr. Serija *Elatae* je najbrojnija u Hrvatskoj i sadrži 5 vrsta: *Iris croatica* I. Horvat et Horvat M., *I. florentina* Dykes, *I. germanica* L., *I. variegata* L. and *I. x rotschildii* Degen. Serija *Pallidae* je predstavljena sa *Iris illyrica* Tomm., *I. pallida* Lam. and *I. pseudopallida* Trinajstić. Unutar serije *Pumilae* dolaze 2 vrste: *I. adriatica* Trinajstić ex Mitić and *I. reichenbachii* Heuff. Predstavnik serije *Sibiricae* je *I. sibirica* L i njezina podvrsta *I. sibirica* L. *ssp. erirrhiza* (Pospischal) Wraber. Taksonomski položaj i odnosi među ovim svojtama su još uvijek nepoznati. Štoviše postojanje nekih vrsta je upitno. Glavni interes ovog istraživanja bio je istražiti odnose među svojtama hrvatskih irisa koristeći molekularno sistematske metode. U ovom istraživanju korištene su metode polimorfizam dužine restrikcijskih ulomaka (RFLP) kloroplastne DNA i slučajno amplificirana polimorfna DNA (RAPD). Dobiveni rezultati pokazali su odvajanje ser. *Sibiricae* od ostalih serija. Između

četiri populacije *I. sibirica* L i njezine upitne svojte *I. sibirica* L. *ssp. erirrhiza* (Pospischal) Wraber,razlike nisu utvrđene.

## MOLECULAR SYSTEMATIC RESEARCH OF SOME *Iris* TAXA (Iridaceae) FROM SOUTH EUROPE REGION

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The genus *Iris* L. (*Iridaceae*) is a cosmopolitan genus and consists of more than 250 species in northern, temperate region. According to the Flora Europaea the genus *Iris* in Europe is represented with 30 species. In Croatian flora 16 taxa are present and they are divided into six series: *Elatae* Lawr., *Pallidae* (A.Kern.) Trinajstić, *Pumilae* Lawr., *Sibiricae* (Diels) Lawr., *Laevigatae* (Diels) Lawr. and *Spuriae* (Diels) Lawr. The series *Elatae* is the most numerous in Croatia, represented with 5 species: *Iris croatica* I. Horvat et Horvat M., *I. florentina* Dykes, *I. germanica* L., *I. variegata* L. and *I. x rotschildii* Degen. The series *Pallidae* is represented with *Iris illyrica* Tomm., *I. pallida* Lam. and *I. pseudopallida* Trinajstić. The series *Pumilae* is represanted with 2 species: *I. adriatica* Trinajstić ex Mitić and *I. reichenbachii* Heuff. The representative of the series *Sibiricae* is *I. sibirica* L. and its subspecies *I. sibirica* L. *ssp. erirrhiza* (Pospischal) Wraber. Taxonomical position and relationships among these taxa are still unknown. Moreover the existence of some species is still questionable. The main interest of this study was to study the relationships between Croatian irises using molecular systematic methods. Restriction-site variation of PCR-amplified cpDNA fragments and RAPD techniques were used. The obtained results separated ser. *Sibiricae* from the other series. No differences between four populations of *I. sibirica* L. and its dubious taxon *I. sibirica* L. *ssp. erirrhiza* (Pospischal) Wraber were detected.

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## HAPLOTIPOVI HRASTOVA SEKCIJE *Quercus* U JUGOISTOČNOJ EUROPI – POSTGLACIJALNA KOLONIZACIJA I IDENTIFIKACIJA PRIBJEŽIŠTA

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Kloroplastna DNA listopadnih hrastova sekcije *Quercus* analizirana je tehnikom AFLP. Amplificirana su četiri segmenta kloriplastne DNA. Analizom

su obuhvaćene populacije od kontinentskih nizina hrvatske do visoravni Makedonije uključujući Jadransko priobalje od Istre do Albanije. Pojava relativno velikog broja haplotipova i njihova prostorna rasprostranjenost na istraživanom prostoru ukazuju na kompleksnost njegove kolonizacije. Po srodstvenim odnosima nađeni haplotipovi pripadaju trima grupama i potječu iz najmanje dva pribježišta. Na krajnjem zapadu od Gorskog Kotara do gornje Podravine nađen je haplotip porijeklom s jugozapada. Istra, Lika i dio središnje Hrvatske koloniziran je iz Apeninskog pribježišta, a jedan od ovih haplotipova nađen je u okolici Podgorice. Istočno od linije Repaš-Okućani-dolina Zrmanje nalazimo veliku zastupljenost haplotipova porijeklom iz Balkanskog pribježišta. Unutar ovog prostora možemo izdvojiti dvije grupe populacija sa specifičnim haplotipovima: 1)poluotok Pelješac s dolinom Neretve i 2) dolinu Bosne s unutrašnjošću Crne Gore. Ovi rezultati ukazuju i na mogućnost postojanja pribježišta uz tadašnju obalu srednjeg Jadrana, vjerojatno južno od današnjeg otoka Visa (dolina Neretve).

## HAPLOTYPES OF OAKS OF SECTION *Quercus* IN SOUTH-EAST EUROPE – POSTGLACIAL COLONISATION AND IDENTIFICATION OF REFUGIA

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Chloroplast DNA of oaks of section *Quercus* was analysed by AFLP technique. Four chloroplast sequences were amplified. Research covers populations from Croatia to Macedonia, including Adriatic from Istria in Croatia to Albania. Appearance of relatively big number of haplotypes and their geographic distribution point out to complexity of colonisation of this region. Haplotypes found belong to three fylogenetic groups and originate from at least two refugia. On very west from Gorski Kotar region to upper Podravina was found haplotype originating from southwest, Istria, Lika and part of central Croatia was colonised from appeninic refugia, and one of this haplotypes was even found down to suburb of Podgorica. Eastward from line Repaš-Okućani-Zrmanja valley haplotypes from Balkan refugia are very frequent. Within this space we can separate two geographically defined groups of populations with characteristic haplotypes: 1) half island Pelješac with Neretva valley and 2) Bosnia valley with inner part of Montenegro. This results point out at possibility of existence of refugia along than central Adriatic cost, perhaps south of today's island of Vis (Neretva valley).

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## NEW DATA ON THE LICHEN FLORA IN THE REPUBLIC OF MACEDONIA

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In our examination of lichen flora different localities, new or once again, were studied. The outcome was determination of extensive number of new lichens species in the Republic of Macedonia. The lichen flora material was collected in the period from 1997 to 2002, when varied localities in Macedonia were visited, as following: Shar mountain (altitude: 1400-1750), Galicica mountain (altitude: 750-1600), Karadjica mountain (altitude: 750-1600), Suva Gora mountain (altitude: 700-1520) and Skopska Crna Gora mountain (altitude: 550-1650). A large number of Fagus, Prunus and Salix lichens, as well as, silicate rocks, moss and soil was collected. A particular attention was paid that the lichens would be well developed, have productive body and fastening organs. For the first time from the investigated areas, a total of sixty (60) lichen species were found. Out of them, there were four (4) species: *Pertusaria flavida*, *Placynhielle oligotrrropha*, *Ramalina intermedia* and *Bucelia insignis*, new to the flora on the Balkan Peninsula. In their determination, the keys and manuals of more authors were used: Murati (1992, 1993), Moberg (1992), Wirth (1980, 1987, 1995 ), Clauzade (1985) and others.

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## MEDICAL, AROMATIC AND SPICING PLANTS IN THE FLORA OF MACEDONIA, USED IN FOOD AND PHARMACEUTICAL INDUSTRY

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The flora in Macedonia consists around 3500 taxons of high plants. In Macedonia exist around 1000 taxons of aromatic, spicing and medical plants and some of them are endemic only for the flora of Macedonia and neighbourhood. Plants of the families Lammiaceae, Brassicaceae, Apiaceae, Papaveraceae, Hypericaceae, Asteraceae etc., could be of great importance for the food and pharmaceutical industry. In Macedonia, which is hilly-mountainous area, these plants exist and give good harvest and quality of active substances. Autochtonous plants are adapted on drought and coolness more than introduced species. Therefore, performing of selection and crossing for this purpose is of great importance. For food and pharma-

ceutical industry could be used the following species: *Mentha piperita, Mentha aquatica, Melissa oficinalis, Salvia officinalis, Tymus serpillium, Lavandula vera, Origanum vulgare, Osmium basilicum, Sinapis alba, Carum carvi, Pimpinella anison, Anethum graveolans, Papaver somniferum, Hiperricum perforatum, Hiperricum olimpicus, Urtica dioica, Urtica urens, Syderitis scardica, Rosmarinus officinalis* etc.

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## FENOFAZA CVJETANJA NA CRETU TRSTENIK (GORSKI KOTAR)

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Cretovi u Hrvatskoj predstavljaju vrlo zanimljiva i iznimno ugrožena staništa. Trstenik po svojim obilježjima zauzima posebno mjesto među njima. Fitocenološki se razlikuju dva dijela, središnji – ostatak nadignutog creta, i periferni – prijelazni cret iz sveze *Caricion davallianae* Klika 1934. Tijekom vegetacijske sezone 2002. godine fenološki je istražena fenofaza cvjetanja (gdje je bilo moguće i fenofaza ploda) za 118 biljnih vrsta na ovom lokalitetu. Utvrđeni su početak i trajanje fenofaze, procijenjena je pokrovnost i izračunat je indeks fenološke aktivnosti (PAI) cvjetanja po mjesecima. Podaci su uspoređeni međusobno i analizirani s obzirom na Ellenbergove ekološke pokazatelje, spektar životnih oblika i duljinu trajanja dnevne svjetlosti pri početku cvjetanja. Spektar životnih oblika ukazuje na vegetacijsku sukcesiju koja se na ovom staništu odvija prema šumi smreke, uz međufazu travnjaka beskoljenke (*Molinia coerulea*). Biljne vrste ulazile su u fenofazu cvjetanja od ožujka (*Crocus vernus* (L.) Hill subsp. *albiflorus* (Kit.) Asch. Et Graebn.) do listopada (*Calluna vulgaris* (L.) Hull), većina tijekom lipnja (29 vrsta) i srpnja (30 vrsta). Najkraće je cvjetala *Gentiana clusii* Perr. et Song. (12 dana), a najduže *Trollius europaeus* L. (135 dana). Najveću fenološku aktivnost PAI je pokazao za kolovoz.

## FLOWERING PHENOPHAZE ON THE MOOR TRSTENIK (GORSKI KOTAR)

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Croatian moors are very interesting and extremely endangered habitats. The moor Trstenik consists of two different parts, the central area – remains of

the only high moor in Croatia, and the surrounding area – transitional fen belonging to the alliance *Caricion davallianae* Klika 1934. During the vegetation period 2002, the coverage of 118 plant species on the locality of Trstenik have been recorded and the phenophase of flowering was observed throughout the vegetational season. The beginning and duration of flowering period have been registered (where possible, the same has been done for fruiting season.) The start of flowering phenophase has been compared with the length of day. For each month a Phenological Activity Index for flowering has been assessed. Analysis of life forms and of ecological characteristics according to Ellenberg show progressed succession of the moor Trstenik towards the spruce forest through the interphase of *Molinia coerulea* grassland. The flowering season started in March (*Crocus vernus* (L.) Hill subsp. *albiflorus* (Kit.) Asch. Et Graebn), although the last species began to bloom in October (*Calluna vulgaris* (L.) Hull). The greatest number of plant species entered the flowering phenophase during June (29 species) and July (30 species). In the year 2002 species *Gentiana clusii* Perr. et Song. had the shortest flowerig season of only 12 days, whereas *Trollius europaeus* L. remained in this phenophase during 135 days. The highest flowering intensity (PAI) was computed for August.

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## FLORA OTOČIĆA UNUTAR PARKA PRIRODE TELAŠĆICA

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Uvala Telašćica je smještena na južnom dijelu Dugog otoka. 1988. godine je izdvojena iz Nacionalnog parka Kornati i proglašena parkom prirode. Osim uvale Telašćica, Park obuhvaća i najviše strmce hrvatske obale, slano jezero Mir i mnoge otoke. Botanička sekcija Udruge studenata biologije ("BIUS") je tijekom ljetnog kampa "Telašćica 2000" započela floristička istraživanja na području Parka. Istraživanja su nastavljena s mr. sc. Marijom Pandžom u proljeće 2001. i 2002. god. Zasebni floristički podaci za samo područje Parka ne postoje. Posljednja floristička istraživanja Dugog otoka, koja su obuhvatila i područje Parka, izvršio je 1991. godine prof. I. Trinajstić, a prije toga davne 1925. i 1926. god. provedena su prirodoslovna istraživanja sjevernodalmatinskih otoka (obradio I. Pevalek). Unutar granica Parka nalazi se 22 otočića i stijena od kojih smo većinu posjetili (Farfarikulac, Donji Školj, Burnji Školj, Katina, Korotan, Hrid Galijola, Garmenjak Veli, Garmenjak Mali, Sestrica Vela, Sestrica Mala, Abica, Gornja Aba, Buč Veli, Buč Mali) i zabilježili njihovu floru. Dosadašnji popis flore Parka prirode Telašćica, bez literaturnih podataka sadrži više od 400 taksona vaskularnog bilja. Ovdje iznosimo popis flore otočića unutar Parka prirode Telašćica.

## FLORA OF ISLETS OF TELAŠĆICA NATURE PARK

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Telašćica bay is situated in the southern part of the island Dugi otok. In 1988 Telašćica was separated from Kornati National Park and proclaimed Nature Park. Besides Telašćica bay, Park includes highest Croatian cliffs, salty lake Mir and many islets. Botanical group of Biology Students Association ("BIUS") carried out floristic research of the Park during summer camp "Telašćica 2000". The research was continued with mr.sc. Marija Pandža in the spring of years 2001 and 2002. Untill now there are no floristic data given separately for the Park. Last researches, that included Park area, were made for Dugi Otok Island in 1991 by Prof. I. Trinajstić and before that only in 1925 and 1926 by the Yugoslav Academy of Sciences (I. Pevalek). Within the boundries of the Park there are 22 islets and rocks. We visited most of them (Farfarikulac, Donji Školj, Burnji Školj, Katina, Korotan, Hrid Galijola, Garmenjak Veli, Garmenjak Mali, Sestrica Vela, Sestrica Mala, Abica, Gornja Aba, Buč Veli, Buč Mali) and noted the species growing there. Co-ordinates of each island were measured with GPS method. On the whole, without literature data, our list of flora of Telašćica Nature Park now consists of more than 400 vascular plant taxa.

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## KOROVNA FLORA ORANICA ŠIREG PODRUČJA JASTREBARSKOG (SZ HRVATSKA)

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Korovi su kompetitori usjevima za vodu, hranjive tvari i svjetlo i zato su zanimljivi objekt istraživanja. Korovna flora oranica istraživana je tokom dvije godine, na deset različitih lokaliteta šireg područja Jastrebarskog (sjeverozapadna Hrvatska). Istraživane su oranice strnih žita (pšenica), te oranice pod kukuruzom. Ove dvije kulture istraživane su budući da je na ovom području učestala primjena dvopoljnog sustava gospodarenja. Zabilježeno je oko 100 korovnih vrsta na oranicama šireg područja Jastrebarskog. Utvrđen je i određen broj zajedničkih korovnih vrsta za obje kulture, kao na primjer *Cirsium arvense* (L.)Scop., *Chenopodium album* L., *Echinochloa crus-galli* (L.) PB., *Panicum capillare* L., *Sorghum halepense* (L.) Pers. što se može objasniti upravo dvopoljnim sustavom gospodarenja. Ove vrste na oranicama prisutne su s velikom učestalošću i pokrovnošću. Ruderalna vrsta *Ambrosia artemisiifolia* L. koja se proširila sa ruderalnih staništa zabilježena je i na oranicama s pšenicom i s kukuruzom,

zajedno s vrstom *Abutilon theophrasti* Med. koja je zabilježena na oranicama s kukuruzom predstavlja veliki problem za obradu i cjelokupnu proizvodnju. Analizom životnih oblika oraničnih korova šireg područja Jastrebarskog utvrđeno je da prevladavaju terofiti. Rezultati će se prikazati Cluster-analizom.

## FIELD WEED FLORA ON THE JASTREBARSKO AREA (NW CROATIA)

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The weeds are crop competitors for water, nutritive matters and light and an interesting object for research. Field weed flora was researched on the wider Jastrebarsko area (north-west part of Croatia). This research has been realised during two years, at ten different localities.The purpose of the research was to explore weed flora in wheat and maize (corn) fields, because at this area is usual two cropping system: wheat – maize. More than hundred weed species were noted at fields on the Jastrebarsko area. It is interesting to point out the findings of *Cirsium arvense* (L.)Scop., *Chenopodium album* L., *Echinochloa crus-galli* (L.) PB., *Panicum capillare* L., *Sorghum halepense* (L.) Pers. They are common for both crops and present with the great frequency and cover. Ruderal weed species *Ambrosia artemisiifolia* L. was expanded from ruderal habitats to wheat and maize fields. Weed species *Abutilon theophrasti* Med. was noted at maize fields. These two species are special problem for complete tillage and caused weed control problems. The spectrum of life forms of weeds in wheat and maize fields of Jastrebarsko area shows the predomination of therophytes. The results will be represented with Cluster analysis.

P-63

## SEASONAL SUCCESSION OF PHYTOPLANKTON IN LAKE OHRID

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Lake Ohrid is a unique lake ecosystem and it is one of the oldest lakes in the world. In Lake Ohrid exists characteristic succession of dominant phytoplankton species during the year, with certain speciality on different depths. Phytoplankton population structure and dynamics was studied in the period from February 1999 to February 2000. Samples were collected by "Ruttner" water sampler from the pelagic zone from surface to 100 m depth. Population density of particular phytoplankton groups were determined using inverted micro-

scope. Identified taxa of Lake Ohrid belong to the following groups of algae: Cyanophyta, Bacillariophyta, Chlorophyta, Chrysophyta and Pyrrophyta. In quantitative sense Bacillariophyta is the most represent group, which was represented by two endemic species *Cyclotella fottii* and *Cyclotella hustedti*. From the surface to 40 m depth during the winter and early spring, and on larger depths beginning of 50 m depth all the year round, diatoms were dominant. The low nutrient concentration, typical for oligotrophic Lake Ohrid, restricts the development of green algae and representatives of Cyanophyta. The population of the Chrysophyta group were dominant mainly during the spring and autumn and mainly from the surface to 10 m depth. The main representative of this algal group was *Dinobryon bavaricum*. In this period total phytoplankton density was the highest. During the summer period total phytoplankton density began to decrease, especially on the surface and 10 m depth. At that time, on these depths, algal biomass was mainly represented by *Ceartium hirundinella* representative of Pyrrophyta.

P-64

## ALERGOGENE KOROVNE VRSTE U KONTINENTALNOM DIJELU HRVATSKE

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Kao odgovor na različite stresne situacije korovi su razvili višestruke mehanizme /fiziološke, mehaničke i kemijske/ samozaštite, što im omogućuje nastavak vrste i širenje. Mnogi korovi imaju brojne i neugledne cvjetove, ali s velikom produkcijom polena, koji je sposoban lebdjeti u zraku. Taj polen sadržava specifične tvari na koje sa različitim stupnjem bolesnih stanja reagira dio /10-30 %/ ljudske populacije. Mnoge korovne vrste imaju posebne dlake ili sadrže mnogo eteričnih ulja te druge sastojke na koje u dodiru s tim vrstama osjetljivi ljudi reagiraju sa dermatozama. Cilj ovog rada je dati informaciju o kvantitativnom prisutvu takvih korova u flori kontinentalnog dijela Hrvatske. Na osnovi vlastitih istraživanja korovne flore i općepoznatih literaturnih podataka o aeroalergogenom djelovanju korova napravljena je lista od 36 segetalnih i ruderalnih vrsta korova, koji pripadaju u 23 roda i 9 porodica. Od korovnih vrsta koje pri dodiru uzrokuju dermatitis navesti će se kao primjer vrste: *Achillea millefolium*, *Ambrosia artemisiifolia*, *Clematis vitalba*, *Conyza canadensis*, *Datura stramonium*, *Euphrasia spp.*, *Euphorbia spp.*, *Heracleum sphondylium*, *Primula vulgaris*, *Urtica dioica* i *Urtica urens*. Zaključno, potrebno je više pažnje posvetiti štetnom djelovanju korova na ljudsko zdravlje sa alergološkog stanovišta.Takva saznanja pomoći će u prevenciji.

# ALLERGOGENIC WEED SPECIES IN THE CONTINENTAL PART OF CROATIA

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As response on different stress situations weeds were developed manifold mechanisms / physiological, mechanical and chemical/ of the selfprotection, which make them possible for the species continuation and spreading. A lot of weeds have numerous and not attractive flowers but with high pollen production and by ability to share the representation of fluing pollen in the air. This pollen might comprise the specific substances on which the human population /10-30 %/ react with the different degree of illness states. Many weed species have special hairs or comprise a lot of eteric oils and other substances in which contact the susceptible peoples react with dermatoses. The aim of this contribution is to give the information about the quantitative presence of those weeds in the continental part of Croatia. On the basis of our investigations and literature data about the commonly known weeds with aeroallergizing effects it has been made a list of 36 plant species included in 23 genera and 9 families. Among weeds which could cause dermatoses are par examples: *Achillea millefolium*, *Ambrosia artemisiifolia*, *Clematis vitalba*, *Conyza canadensis*, *Datura stramonium*, *Euphrasia spp.*, *Euphorbia spp.*, *Heracleum sphondylium*, *Primula vulgaris*, *Urtica dioica* i *Urtica urens*. Conclusively, it is necessary to pay more intensive attention to harmful effects of weeds from this allergological point of view on human's health. That sort of the knowledge will help in prevention.

# BILJNA BIOLOGIJA
# 2. SIMPOZIJ HRVATSKOG DRUŠTVA ZA BILJNU FIZIOLOGIJU

# PLANT BIOLOGY
# 2ND SYMPOSIUM OF THE CROATIAN SOCIETY OF PLANT PHYSIOLOGY

IL-12

## REGULATORNI ELEMENTI OKSIGENE FOTOSINTEZE

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Tilakoidne kloroplastne membrane kataliziraju fundamentalni proces fotosintetske pretvorbe energije. Različiti mehanizmi reguliraju raspodjelu pobudne energije između dva fotosustava, a drugi prevode suvišnu pobudnu energiju u toplinu. Smatra se da je velik broj pomoćnih enzima uključen u ove fiziološke procese. Oni, na primjer, uključuju proteinske kinaze i fosfataze, molekularne pomagače te velik broj proteaza koje provode reguliranu degradaciju proteina. Brojni su drugi pomoćni proteini potrebni tijekom biogeneze fotosintetskih proteinskih kompleksa. Poslijednjih smo godina karakterizirali nekoliko pomoćnih tilakoidnih proteina koji su uključeni u regulaciju fotosinteze. TLP40 (*t*hylakoid *l*umen *P*Plase of 40 kDa) složen je ciklofilin s C-terminalnom katalitičkom domenom I N-terminalnim područjem koji sadrži nekoliko modula za vezanje drugih proteina. Pokazali smo da TLP40 interagira s tilakoidnim membranama i da regulira aktivnost proteinske fosfataze specifične za fotosustav II. TCP34 (*t*etratricopeptide-containing *c*hloroplast *p*rotein of 34 kDa) vjerojatno je uključen u ekspresiju plastidnih gena. TCP34 objedinjuje središnje postavljen tetratrikopeptidni ponavljajući slijed (TPR) s N-terminalnom domenom sličnom prokariotskim regulatorima odgovora. Rekombinantni TCP34 može specifično vezati kloroplastnu DNA (ptDNA), a nativni se TCP34 nalazi u sklopu transkripcijski aktivnog DNA-proteinskog kompleksa. Pretpostavljamo da dosad nekarakterizirani signalni put, s pomoću fosforilacije unutar regulatorne domene, može regulirati sposobnost TPC34 da veže ptDNA. Nedavno identificirani protein TROL (*t*hylakoid *r*h*o*danese (thiosulfate sulfurtransferase)- *l*ike) objedinjuje domenu sličnu fosfatazama s dvostrukom specifičnošću s ponavljajućim slijedom koji bi mogao biti uključen u vezanje feredoksin:NADP$^+$ oksidoreduktaze (FNR). TROL je lokaliziran u blizini citokrom $b_6f$ kompleksa i vjerojatno je uključen u ciklički transport elektrona ili u redoks reguliranu kaskadu prijenosa signala.

## REGULATORY ELEMENTS OF OXYGENIC PHOTOSYNTHESIS

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Thylakoid membranes in chloroplasts catalyse the fundamental process of photosynthetic energy conversion. Various mechanisms can regulate the distribution of excitation energy between the two photosystems, and other can convert ex-

cess excitation energy into thermal energy. A significant number of auxiliary enzymes are believed to be involved in these physiological processes. These include, for instance, protein kinases and phosphatases, chaperones, a substantial number of protease that catalyse regulated protein degradation, and various other protein components that are required during biogenesis of the photosynthetic multisubunit complexes. In recent years, we have characterized several thylakoid auxiliary components which are involved in regulation of photosynthesis. TLP40 (*t*hylakoid *l*umen *P*Plase of 40 kDa), is a complex cyclophilin with a C-terminal PPIase catalytic domain and an N-terminal domain with a number of protein binding modules. TLP40 has been shown to interact with the thylakoid membrane and regulate the activity of the photosystem II-specific protein phosphatase. TCP34 (*t*etratricopeptide-containing *c*hloroplast *p*rotein of 34 kDa) is probably involved in plastid gene expression. TCP34 combines a centrally positioned tetratricopeptide repeat (TPR) array with an N-terminal response regulator-like domain. Recombinant TCP34 exhibits specific binding to chloroplast DNA and native TCP34 was found in association with a transcriptionally active protein/DNA complex. An as yet unidentified phosphorelay signal may modulate its capacity for ptDNA binding through the phosphorylation state of the putative response regulator-like domain. Recently identified *t*hylakoid *rh*odanese (thiosulfate sulfurtransferase) -*l*ike protein TROL combines a domain similar to a dual specificity phosphatases with a repetitive module possibly involved in the binding of ferredoxin:$NADP^+$ oxidoreductase (FNR). TROL is located at the proximity of cytochrome $b_6f$ complex and might be involved in a cyclic electron transport, or a redox-regulated signal transduction cascade.

IL-13

## RAZVOJNO SPECIFIČNA EKSPRESIJA PROTEINA U KULTURI BILJNOGA TKIVA

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Nakon nekoliko desetljeća intenzivnih istraživanja i važnih otkrića o strukturi i funkciji gena, suočeni smo danas sa sve većim zanimanjem za proteom – funkcionalni komplement genoma. Eksperimantalni pristup obično uključuje dvodimenzionalnu elektroforezu, nakon koje može slijediti identifikacija proteina masenom spektrometrijom. U ovome radu istraživalo se biljni razvitak i morfogenezu u kulturi *in vitro* i to na razini uzorka izraženih proteina. Istraživalo se na modelu triju biljnih vrsta: šećerne repe (*Beta vulgaris* L.), hrena (*Armoracia lapathifolia* Gilib.) i kaktusa (*Mamillaria gracillis* Pfeiff.). Razvojni procesi potaknuti su unutarnjim staničnim čimbenicima, okolišnim uvjetima ili transformacijom stanica plazmidom Ti (divlji tip B6S3), a bez djelovanja vanjskih regulatora rasta. Otkrivene su promjene u programima aktivacije gena na razini staničnih i

izvanstaničnih proteina i izoenzima (peroksidaze, esteraze i invertaze). Proteini su analizirani jednodimenzionalnom i dvodimenzionalnom elektroforezom, obojeni srebrom ili su karakterizirani lektinima (Con A, UEA, RCA120). Razvojno specifični uzorci proteinskih mrlja dobiveni dvodimenzionalnom elektroforezom omogućit će identifikaciju nekih tkivno-specifičnih proteina masenom spektrometrijom.

## DEVELOPMENTALLY SPECIFIC PROTEIN EXPRESSION IN PLANT TISSUE CULTURE

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After many decades of intensive genome research and impressive revelations on gene structure and function we are facing the growing interest on a proteom - a functional genome complement. The experimental approach usually includes two-dimensional gel electrophoresis, which can be combined with protein identification by mass spectrometry. In the present work plant development and morphogenesis in *in vitro* culture were studied at the level of protein expression pattern. Three model species: sugar beet (*Beta vulgaris* L.), horseradish (*Armoracia lapathifolia* Gilib.) and cactus (*Mamillaria gracillis* Pfeiff.) have been studied. Developmental processes were triggered by intrinsic cellular factors, environmental condition and by Ti-plasmid (a wild type B6S3) transformation, without the action of any exogenous growth regulators. Changes in gene activation programs were detected at the level of cellular and extracellular proteins and isoenzymes (peroxidases, esterases, invertases). Proteins were analysed by means of one-dimensional and two-dimensional electrophoresis, silver-stained or characterised by lectins (Con A, UEA, RCA120). Developmentally specific protein patterns obtained by means of 2-D electrophoresis should enable us to identify some of tissue specific proteins by means of mass spectrometry.

IL-14

## CYTOKININS IN BIOTECHNOLOGY AND MEDICINE

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New series of cytokinins derived from 6-benzylaminopurine (BAP) have been developed for agricultural and biotechnological applications. These compounds exhibit very high biological activities in different cytokinin bioassays. Preferentially, the methoxy and fluoro derivatives had 2-10 times higher activity then *trans*-zeatin

and expressed very positive antisenescent and morphogenetic properties in different *in vitro* cultures of some horticulture species. These compounds have been recently identified as new natural cytokinins. Our research focused on the primary mechanism of action of plant hormones cytokinins in cell division cycle has showed that natural plant cytokinins are rather non-specific inhibitors of various protein kinases. Surprisingly, among aromatic cytokinin derivatives, we have discovered a compound, 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, named "olomoucine", which specifically inhibits some cyclin-dependent kinases (cdks) at micromolar concentration. One of the inhibited kinases, the $p34^{cdc2}$/cyclin B kinase, assumed to be a key mitotic factor, which is highly conserved and strongly implicated in cell cycle transitions in all eukaryotic cells. The design and inhibitory activity of OC was further improved by modifications at positions 2, 6,and 9, i.e., the positions that control binding to cdk1. This has recently led to discovery of novel specific cdk inhibitors named roscovitine, olomoucine II and purvalanol A, which display an enhanced inhibitory activity toward cdk1, a higher selectivity for some cdks, an increased antimitotic activity at the G1/S and G2/M points of the cell cycle, and stronger and more selective antitumour effects. The compounds are also effective *in vivo* and two of them are already in clinical trials. The purine analogue bohemine and roscovitine were also used, to study the role on CDKs in cell cycle progression and microtubule organisation in *Vicia faba* root tip cells. These compounds also induce apoptosis of different plant cells *in vivo*.

OP-39

## SINTEZA RUŽMARINSKE KISELINE U KOSMATOM KORIJENJU UKRASNE KOPRIVE *Coleus blumei* Benth.

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Prirodni antioksidans – ružmarinsku kiselinu (RA) nalazimo u vrsta iz porodice Lamiaceae te kod nekoliko vrsta porodice Boraginaceae. Dobro je poznato svojstvo ukrasne koprive da sintetizira značajnu količinu RA. Infekcijom lisnih eksplantata s bakterijom *Agrobacterium rhizogenes* A4 potaknut je rast kosmatog korijenja. Prisutnost T-DNA u kosmatom korijenju potvrđena je lančanom reakcijom polimeraze (PCR). Tijekom kultivacije u tekućoj hranidbenoj podlozi MS i CB praćeni su rast tkiva i biosinteza RA. Rast transgenog korijenja i biosinteza RA bili su bolji u hranidbenoj podlozi MS. Maksimalni indeks rasta u toj podlozi iznosio je 16,5 (nakon 42 dana), dok je maksimalni rast korijenja u podlozi CB postignut 49. dan, kada je indeks rasta bio 11,37. Kosmato korijenje raslo u podlozi MS sintetiziralo je 2,95 mg RA po tikvici (1,34% RA u suhoj tvari), u podlozi CB maksimalna sinteza RA po tikvici iznosila je 1,96 mg RA (1,15% RA u suhoj tvari). Ovaj rad prvi puta objavljuje sposobnost sinteze RA u kulturi kosmatog korijenja ukrasne koprive.

## ROSMARINIC ACID SYNTHESIS IN HAIRY ROOTS OF C*oleus blumei* Benth.

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Rosmarinic acid (RA) is a natural antioxidant found especially in plants of Lamiaceae and several Boraginaceae species. Decorative nettle *Coleus blumei* (Lamiaceae) is well known to produce significant amount of RA. *Coleus blumei* hairy roots were induced by infection with wild strain *Agrobacterium rhizogenes* A4. The presence of T-DNA genes in transformed tissue was confirmed by polymerase chain reaction (PCR). The growth of *Coleus blumei* transformed roots and biosynthesis of RA were analyzed during its cultivation in MS and CB liquid medium. Roots grew faster and better in MS medium and achieved maximum growth index of 16.5, after 42 days. In CB medium maximum growth index of 11.37 was after 49 days. In liquid MS medium hairy roots accumulated maximum 2.95 mg RA per tube, that is about 1.34% RA in dry tissue mass. In CB medium hairy roots accumulated 1.96 mg RA per tube that corresponds to 1.15% RA in dry tissue mass. This is the first report about feasibility of *Coleus blumei* hairy roots to synthesize RA.

OP-40

## IDENTIFIKACIJA I KARAKTERIZACIJA ESTERAZA VEZANIH UZ MORFOGENEZU U KULTURI TKIVA KAKTUSA *Mammillaria gracillis*

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Prilikom razmnožavanja biljaka kaktusa *Mammillaria gracillis* Pfeiff. (*Cactaceae*) spontano dolazi do prijelaza s organiziranog na neorganizirani način rasta i stvaranja obilnog kalusa. Bez dodatka regulatora rasta kalus regenerira izdanake s karakterističnim fenotipom ove biljne vrste, ali i hiperhidrirane izdanke. Tumorsko tkivo TW i TR, inducirano bakterijom *A. tumefaciens* (soj B6S3 i GV3101), nema organogeni potencijal. Tkiva kaktusa (izdanak, kalus, hiperhidrirani regenerant, tumori TW i TR) uspoređena su s obzirom na aktivnost esteraze (arylesteraze EC 3.1.1.2 i karboksilesteraze EC 3.1.1.1) i uzorak izoesteraza. Mjerenja su provedena svakih 7 dana tijekom trajanja jedne supkulture (28 dana) i to s dva supstrata, 1- i 2-naftilacetatom. Najviša aktivnost esteraza za oba supstrata izmjerena je u izdancima i tumorima 21. dan, dok je u kalusu i hiperhidriranim izdancima bila najviša sedmi dan. Esteraze su razdvojene elektroforezom u gelu s koncentracijskim gradijentom (8-18%) poliakrilamida pod nativnim uvjetima. Uočeno je ukupno 12 izoesteraza koje reagiraju s oba supstrata. Nije bilo razlika u uzorku i broju izoenzima s obzirom na starost tkiva kaktusa. Za bolju karakterizaciju esteraza korišten je selektivni inhibitor karboksilesteraza bis-p-nitrofenil fosfat (BNPP, 100 mM). Viši stupanj inhibicije s BNPP-om utvrđen je za sve uzorke uz 1-naftilacetat (oko 50%) nego uz 2-naftilacetat kao supstrat (oko 30%). Na gelu inkubiranom s 1-naftilacetatom i BNPP-om nedostajala je izoesteraza E10, dok je na gelu inkubiranom s 2-naftilacetatom i BNPP-om nedostajala izoesteraza E5.

## IDENTIFICATION AND CHARACTERIZATION OF ESTERASES RELATED TO MORPHOGENESIS IN *Mammillaria gracillis* TISSUE CULTURE

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Cactus *Mammillaria gracillis* Pfeiff. (*Cactaceae*), cultivated *in vitro*, spontaneously switch from an organised to unorganised way of growth, producing a ha-

bituated organogenic callus which regenerates normal and hyperhydric shoots without the addition of growth regulators. Tumour tissues TW and TR, induced by *A. tumefaciens* strain B6S3 and GV3101, do not express any organogenic potential. The esterase (arylesterase EC 3.1.1.2 and carboxyl esterase EC 3.1.1.1) activity and isoenzyme pattern of cactus tissues (shoot, callus, hyperhydric regenerant, tumours TW and TR) were tested every 7 days during the period of one subculture (28 days). Two esterase substrates, 1- and 2-naphthylacetate, were used. In shoots and tumours, the highest esterase activity for both substrates was measured on the 21st day, while in the callus and hyperhydric regenerants it had the highest value on the 7th day. Esterases were separated electrophoretically in polyacrylamide gradient gels under native conditions. In total, 12 isoesterases, reacting with both substrates, were resolved. No differences in isoenzyme profile were noticed in correlation to the age of tissues. A selective inhibitor for carboxyl esterase, bis-p-nitrophenyl phosphate (BNPP, 100 mM) was used to better distinguish the esterases. The higher degree of inhibition (50%) was obtained in all samples when 1-naphthylacetate was used as a substrate than when 2-naphthylacetate was used (30%). No colour reaction was found on the gel for the isoesterase E10 with BNPP and 1-naphthylacetate or for isoesterase E5 with BNPP and 2-naphthylacetate.

OP-41

## CITOKININI U BIJELOM KUKURIJEKU (*Helleborus niger* L.)

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U oplođenim cvjetovima bijelog kukurijeka (*Helleborus niger* L.), lapovi (za vrijeme cvatnje bijeli) razvijaju funkcionalne kloroplaste te ostaju dok ne dozriju sjemenke. U neoplođenim cvjetovima, taj proces ozelenjavanja može se pokrenuti primjenom *egzogenih* citokinina. Biološki aktivni *endogeni* citokinini i njihovi reverzibilni konjugati u cvjetovima s plodovima u razvoju analizirani su slijedom kromatografskih metoda i masenom spektrometrijom. Ponađeni su *trans*- i *cis*-zeatin, dihidrozeatin, $N^6$-(2-izopentenil)adenin te pripadni *O*-glukozidi, ribozidi, *O*-glukozidi ribozida i ribotidi, uz manje količine aromatskih citokinina (npr. $N^6$-benziladenin). U plodovima, ukupna razina aktivnih citokinina se u početnoj fazi razvoja sjemenki povećava za nekoliko redova veličine, a ostaje visoka do ~2 tjedna prije zriobe (zadnji dosad analizirani razvojni stadij), i to vjerojatno zato što razvoj embrija nije završen nego se nastavlja kad sjemenke ispadnu iz ploda. U lapovima, jedino su koncentracije ribozida i ribotida *cis*-zeatina slične ili veće nego u plodovima iz istih cvjetova, a jasno se povećavaju za vrijeme ozelenjavanja, dok razine drugih citokinina ostaju niske te u toku razvojnog ciklusa ne pokazuju očite promjene.

## CYTOKININS IN THE CHRISTMAS ROSE (*Helleborus niger* L.)

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In fertilized flowers of the Christmas rose (*Helleborus niger* L.), the sepals (initially white) develop functional chloroplasts and persist until the seeds are ripe. In unfertilized flowers, the greening process may be initiated by treatment with *exogenous* cytokinins. The biologically active *endogenous* cytokinins and their reversible conjugates in fruit-bearing flowers were analyzed by a combination of chromatographic methods and mass spectrometry. *Trans*- and *cis*-zeatin, dihydrozeatin, $N^6$-(2- isopentenyl)adenine and the corresponding *O*-glucosides, ribosides, riboside *O*-glucosides, and ribotides were detected, in addition to minor amounts of aromatic cytokinins (e. g. $N^6$-benzyladenine). In the fruit, the overall level of active cytokinins increased enormously, during early seed growth, and remained high until ~2 weeks before ripening (most advanced stage checked so far), possibly because embryo development is not complete and continues after the seeds are shed. In the sepals, only the concentrations of *cis*-zeatin riboside and ribotide were about the same, or higher than, in the fruit from the same flowers and increased consistently during the greening process, while the levels of the other cytokinins examined remained low and showed no obvious systematic changes.

OP-42

## DJELOVANJE NATRIJEVA KLORIDA I MANITOLA NA DUBROVAČKU ZEČINU (*Centaurea ragusina L.*)

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*Centaurea ragusina* L. je hrvatska endemična vrsta dobro prilagođena za život na stijenama okrenutim prema moru. Relativan prirast biljaka, aktivnost peroksidaza te broj izoenzima analiziran je u *in vitro* uzgojenim biljkama izloženim solnom (NaCl) ili osmotskom (manitol) stresu. Zakorijenjene biljčice koje su rasle na MS ¨ hranjivoj podlozi uz dodatak 300 mM manitola ili 150 mM natrijeva klorida uzimane su u pokus nakon 5, 10 ili 15 dana. Relativan prirast biljaka bio je značajno povećan u uvjetima solnog stresa, a smanjen u

uvjetima osmotskog stresa. Sadržaj proteina u izdancima i korijenju kontrolnih biljaka i biljaka izloženih stresu bio je vrlo sličan s iznimkom znatnog povišenja proteina na stresu izazvanom manitolom nakon 10 dana. Peroksidazna aktivnost bila je osjetno povećana kod biljaka izloženih manitolu (naročito nakon 10 dana stresnih uvjeta), dok je kod biljaka izloženim solnom stresu ta aktivnost bila slična onoj u kontrolnih biljaka ili čak i niža. U izdancima kontrolnih biljaka primijećeno je ukupno šest, a u korijenju do osam peroksidaznih izoenzima. U izdancima i korijenju biljaka izloženim solnom stresu neke vrpce nisu bile prisutne, dok se kod biljaka izloženih manitolu pojavila jedna nova vrpca, a neke vrpce su bile puno izraženije. Prikazani rezultati potvrđuju halofitnu prirodu ove vrste te toleranciju prema salinitetu. Na osnovu različite peroksidazne aktivnosti kod biljaka izloženih manitolu i onih izloženih solnom stresu, može se pretpostaviti da su u adaptaciju na solni i osmotski stres uzrokovan manitolom uključeni različiti mehanizmi.

## THE EFFECT OF NaCl AND MANITOL STRESS ON *Centaurea ragusina L.*

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*Centaurea ragusina* L. is an endemic Croatian species adapted to arid and salt-affected environments. Relative plant growth rate, soluble peroxidase (POD) activity and isoperoxidase pattern of *in vitro* cultured *C. ragusina* plants exposed to saline (NaCl) and osmotic (mannitol) stress were measured. Rooted plantlets grown on MS ¨ nutrient media supplemented with 300 mM mannitol or 150 mM NaCl were harvested after 5, 10 or 15 days. Relative plant growth was markedly promoted under NaCl and suppressed under mannitol stress. Protein content in shoots and roots of treated and non-treated plants was almost the same, except after 10 days when it was elevated under mannitol stress. Peroxidase activity was significantly increased in both roots and shoots of mannitol-stressed plants (especially after 10 days under stress conditions) while in salt-stressed plants it was either similar as in non-stressed plants or even decreased. Six isoforms in total were present in non-stressed shoots while in roots up to eight isoforms were noticed. Some of the bands, in both shoots and roots, were missing under NaCl stress while prominent accumulation of some isoforms as well as appearance of a new one was observed under mannitol stress. Presented results confirm halophylic nature of the species and its tolerance to osmotic stress caused by salinity. Differences in peroxidase activity of saline and osmotic stress suggest different mechanisms might be involved in adaptation to osmotic stress caused by NaCl and mannitol.

OP-43

## PRIMJENA UREĐAJA ZA GENERIRANJE ELEKTROMAGNETSKOG POLJA U BIOLOŠKIM ISTRAŽIVANJIMA

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Prisutnost sve brojnijih i učestalijih elektromagnetskih (EM) valova posljedica je današnjeg načina života. Izvori EM valova niskih frekvencija (50 Hz) su svi električni kućanski aparati, transformatori električne energije i dalekovodi. Također, posljednjih nekoliko godina sve se više rabe pokretni telefoni koji rade na frekvencijama EM polja od 400 do 1900 MHz. Zbog toga se sve više istražuje i raspravlja o učincima EM valova na žive organizme. Uređaji za generiranje EM polja koji se mogu rabiti za istraživanje bioloških učinaka nastalih uslijed izlaganja različitim EM valovima u kontroliranim uvjetima razvijeni su na Fakultetu elektrotehnike i računarstva u Zagrebu. Za istraživanje učinaka EM polja niskih frekvencija, vodena leća (*Lemna minor*), koja se koristi kao modelna biljka u mnogim biološkim istraživanjima, izlagana je magnetskom polju u Helmholtzovom svitku te električnom polju unutar dviju paralelnih kružnih elektroda. Učinci frekvencija koje rabe pokretni telefoni promatrani su nakon izlaganja vodene leće EM polju u GTEM komori (komora s transverzalnim elektromagnetskim načinom širenja vala u GHz području). Izlaganje magnetskom i električnom polju od 50 Hz, snage 1 mT tijekom 24 sata prouzročilo je neznatno smanjenje rasta vodene leće. Frekvencije mobilnih telefona od 400, 900 i 1900 MHz s niskom snagom polja (<10 V/m) nisu značajno utjecale na rast dok ga je polje veće snage (23 V/m), frekvencije 400 i 900 MHz, značajno smanjilo. Dobiveni rezultati pokazuju da su razvijeni uređaji pogodni za procjenu bioloških učinaka EM valova pa će se u sljedećim istraživanjima rabiti za procjenu učinka polja različitih snaga, različitog trajanja izlaganja te modulacije EM polja.

## INSTRUMENTATION FOR ELECTROMAGNETIC FIELDS GENERATION IN BIOLOGICAL MEASUREMENTS

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Electromagnetic fields (EMFs) are part of everyday life in modern world. Extremely low-frequency EMFs (50 Hz) are produced by every electric home appli-

ance, electric power transmission and distribution lines. The last ten years mobile phones have been widely used all around the world. They operate on the EMF frequencies from 400 MHz to 1900 MHz. The effects of EMFs on living organisms have been the subject of debate and research for the last thirty years. The instrumentation for generation of EMFs have been designed at the Faculty of Electrical Engineering and Computing, Zagreb, and can be used for controlled exposure to different EMFs. To study the effect of extremely low-frequency EMF, duckweed (*Lemna minor*) - the model plant in biological measurement, was exposed to the magnetic field in Helmholtz coil and to the electric field between two parallel circle electrodes. For the effect of mobile phones frequencies duckweeds were exposed in Gigahertz Transversal Electromagnetic Mode (GTEM) cell. Exposure for 24 hours to magnetic and electric field of 50 Hz with strength 1 mT slightly decreased the growth of *Lemna minor*. Mobile phone frequencies of 400, 900 and 1900 MHz with low field strength (<10 V/m) did not change the growth of duckweed significantly, but 400 and 900 MHz at higher field strength (23 V/m) reduced it significantly. The research confirmed that instrumentation used in these experiments is suitable for evaluation of biological effects of EMFs. The effect of different field strengths, exposure times and modulation can be tested with these instrumentation in further experiments.

OP-44

## RAZLIČITA SNOŠLJIVOST NA SLANOST TRIJU ODLIKA JAGODE UZGAJANIH U UVJETIMA *IN VITRO*

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Ispitana je osjetljivost jagode *Fragaria ananassa* Duch (odlike Elsanta, Marmolada i Miranda) na slanost (NaCl: 0; 0.5; 1.0; 2.0; 3.0; 4.0 g $L^{-1}$) tijekom tri supkulture multiplikacijske faze. Svaka supkultura trajala je 60 dana. Testirani parametri bili su: rast, razvitak, stopa umnažanja izdanaka, prinos svježe mase i suhe tvari te postotak suhe tvari (%S.T.). Koncentracija 0.5 g $L^{-1}$ NaCl u mediju MS nije značajno utjecala na ispitane parametre. Više koncentracije soli (1.0-4.0 g $L^{-1}$) i produljeno djelovanje (2-3 supkulture) značajno su smanjili stopu umnažanja i rast izdanaka, prinos svježe mase i suhe tvari te povećali %S.T. Odlika Elsanta bila je najosjetljivija i prva je odgovorila na povišenu koncentraciju soli nekrozom listova. Elsanta je formirala najmanje korisnih izdanaka i u odnosu na kontrolu imala najmanji prinos svježe mase i suhe tvari te prosječno najveći %S.T. Od tri ispitane odlike najveću snošljivost na slanost imala je Miranda. Dobiveni rezultati osnova su za daljnje istraživanje mehanizma kojim se jagoda prilagođava na slanost.

## VARIABILITY IN SALT TOLERANCE OF THREE STRAWBERRY CULTIVARS CULTURED *I/V VITRO*

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Responses of *Fragaria ananassa* Duch (cvs. Elsanta, Marmolada and Miranda) to salinity (NaCl: 0; 0.5; 1.0; 2.0; 3.0; 4.0 g $L^{-1}$) were tested during three subcultures of multiplication stage. One subculture lasted 60 days. Growth, development, shoot multiplication rate, fresh and dry weight, and dry weight percentage (%D.W.) were examined parameters. The concentration of 0.5 g $L^{-1}$ NaCl in MS medium did not affect significantly any parameters. Higher concentrations (1.0-4.0 g $L^{-1}$) and prolonged cultivation (two or three subcultures) on salt decreased multiplication rate, shoot growing, average of fresh and dry weight but increased %D.W. Cv. Elsanta was the most susceptible cultivar and among three cvs. it first responded to increased salinity by leaf necroses. Elsanta also produced the smallest number of usable shoots and had the highest average decrease of fresh and dry weight, and the highest increase of %D.W. Miranda was the less sensitive to salt stress. This report is a base for further studies on the mechanism of salt acclimation in strawberry.

OP-45

## MIKRORAZMNOŽAVANJE BUHAČA (*Chrysanthemum cinerariaefolium* VIS.)

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Buhač ili dalmatinska krizantema autohtona je trajnica koja samoniklo raste u Dalmaciji. Biljka sintetizira insekticid širokog spektra t.z. "piretrin" koji je netoksičan za ljude i toplokrvne životinje i brzo razgradiv te je zbog navedenih karakteristika prihvatljiv za široku upotrebu. Odabrane biljke koje imaju bujan prinos cvjetova i visok sadržaj piretrina mogu se vegetativno razmnožiti na više načina, no mikrorazmnožavanje je najbrža i ekonomski prihvatljiva metoda. Utvrđen je protokol za brzo vegetativno razmnožavanje buhača u uvjetima in vitro. Regeneracija biljaka postignuta je uvođenjem eksplantata vegetativnih vršaka biljaka raslih u prirodnom okolišu otoka Šolte. Od više ispitanih hranidbenih podloga modificirani Woody Plant Medium (WPM) s dodatkom BA i NAA bio je najbolji za rast i razvitak višestrukih

izdanaka (stopa umnažanja bila je 5 za 4 tjedna). Zakorijenjivanje pojedinačnih izdanaka postignuto je na podlozi mod. MS s dodatkom IAA. Biljke su preživljavale aklimatizaciju 100%. Sredinom travnja biljke, veličine 12-15 cm, presađene su u polje i tijekom svibnja formirale su cvjetne glavice.

## MICROPROPAGATION OF PYRETHRUM (*Chrysanthemum cinerariaefolium* VIS.)

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Pyrethrum or Dalmatian chrysanthemum is an autochthonous and wild growing perennial plant in Dalmatia. Plant is the source of the broad-spectrum "pyrethrum" insecticide. Its very low toxicity for humans and warm-blooded animals combined with its non-persistence in the environment has made pyrethrum the insecticide of choice in an increasingly ecology-conscious world. A vegetative propagation of selected plants with high flower yield and a high pyrethrins content can be achieved in several ways, but a micropropagation system is the fastest and economically feasible method of propagation. A protocol for rapid in vitro multiplication of pyrethrum has been standardized. Plant regeneration was successfully achieved from the apex explants of vegetative plants grown on the island Šolta. Among the different compositions of media investigated, the mod. Woody Plant Medium (WPM) supplemented with BA and NAA was suitable for growth and development of multiple shoots (multiplication rate was 5 every 4 weeks). For rooting, individual shoots were planted on mod. MS medium with IAA, and rooted plantlets were transplanted to fertilized substratum. With 100% survival the plants (12-15 cm high) were outplanted to field in the mid of April. During May plants formed the capitula.

OP-46

## RAST I PRIRAST PINIJE (*Pinus pinea*)

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Istraživanje rasta i prirasta provedeno je na stablu pinije starosti 95 godina. Nakon olujnog vjetra 2001. godine koji je pogodio područje nacionalnog parka

Brijuni, izvaljeno je nekoliko stabala bora. Jedno stablo kojem nije bilo oštećeno deblo, iskorišteno je za analizu rasta i prirasta. Cilj istraživanja je bio proučiti zakonitosti rasta i prirašćivanja stabla pinije koje je raslo na otoku Veliki Brijun kao soliterno stablo. Uobičajenom metodom ispiljeno je 10 kolutova na svaka dva metra od panja, te su izbrojani i izmjereni godovi na njima. Temeljem dobivenih podataka utvrđen je debljinski, plošni, visinski i volumni rast i prirast tog stabla. Načinjen je i uzdužni presjek debla. Stablo je bilo visoko 22 m, prsnog promjera 47 cm. Iz rezultata je vidljivo da krivulja rasta u visinu još uvijek nema S-oidan oblik, djelomično je konkavna (prvi dio nalikuje pravcu). Stablo pinije doživljava kulminaciju visinskog prirasta između 10. i 15. godine života i to od 80 cm. Nakon 45. godine visinski prirast je oko 5-6 cm i konstantan je. Krivulja rasta u debljinu pokazuje koliko toliko S-oidan oblik, ali dominira konveksnost. Plošni prirast kreće se oko 60 $cm^2$. Prosječni godišnji volumni prirast se između 25. i 90. godine života kreće oko 0,06 $m^3$ (0,04-0,075). S obzirom na utvrđenu starost stabla, oblik i dimenzije krošnje, stablo pinije je najvjerojatnije posađeno oko 1910. godine.

## GROWTH AND INCREMENT OF STONE PINE (*Pinus pinea*)

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Research of growth and increment has been carried out on a stone pine tree 95 years old. Several pine trees were thrown out by a gale-force wind in 2001, when a storm hit the area of national park Brijuni. One tree whose trunk was left undamaged was used for growth and increment analysis. The research aim was to study growth and increment pattern for stone pine tree growing as a solitary tree on an island Veliki Brijun. Ten rings were sawed on each two meters from the stump using the usual method, and annual rings on them were counted and measured. Diameter, basal area, height and volume growth and increment were determined on the basis of obtained data for this tree. Longitudinal section of the trunk was also made. The tree was 22 meters high and its d.b.h. was 47 cm. The results show that the height growth curve still has not acquired an S shape, it is partly concave (the first segment resembles a straight line). Stone pine tree has a height growth culmination between 10$^{th}$ and 15 year and it is 80 cm. Height increment after 45$^{th}$ year is around 5-6 cm and is constant. Diameter growth curve remotely resembles an S shape, but convexity prevails. Basal area increment is around 60 $cm^2$. Average annual volume increment between 25$^{th}$ and 90$^{th}$ year is about 0,06 $m^3$ (0.04-0.075). The stone pine tree was probably planted about 1910, judging by determined age, crown shape and dimension.

OP-47

## RESPONSE OF ROOTS OF DIFFERENT PLANT SPECIES TO NATURAL $CO_2$ ENRICHMENT

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Plant performance under elevated $CO_2$ has been intensively studied in last years. As a rule, research has been much more focused to shoot processes (photosynthesis, stomatal response) when compared to below ground ones. This holds true also for the studies performed at natural carbon dioxide sources ($CO_2$ springs, mofettes), which can efficiently serve as an alternative to $CO_2$ fumigation experiments. Soil gas measurements at the Stavešinci mofette (NE Slovenia) have showed that the effects of modified environmental conditions on root functioning and rhizosphere processes are to be expected in plants growing close to $CO_2$ springs. Beside direct and/or indirect action of very high $CO_2$ concentration, possible effects of low soil oxygen partial pressure, which is accompanying $CO_2$ increase, should not be neglected. In our study root respiration of different plant species growing at the Stavešinci $CO_2$ spring was measured by using Clark-type oxygen electrodes. In addition, the response of mycorrhizal symbiosis under elevated $CO_2$ was evaluated by using root screening methods and analysis of soil glycoprotein glomalin. In general, relatively high tolerance of root respiration and arbuscular mycorrhiza to extreme gaseous conditions has been found.

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## GVAJACILNE PEROKSIDAZE U KORIJENU MRKVE (*Daucus carota* L.)

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Mrkva (*Daucus carota* L.) se ubraja u poljoprivredne vrste koje se redovito koriste u prehrani. Cilj rada bio je istražiti distribuciju gvajacilnih peroksidaza i odrediti njihovu aktivnost u pojedinim dijelovima korijena: centralnom cilindru, korteksu i epidermalnom području. Riječ je o preliminarnom istraživanju koje će poslužiti kao temelj za buduća istraživanja utjecaja stresa na staničnu diferencijaciju u korijenu mrkve. Lokalizacija je izvršena na svježim prerezima, uranjanjem tkiva u reakcijsku smjesu koja je sadržavala gvajakol kao supstrat. U istu svrhu je korištena i tehnika otiska tkiva (tissue printing). Fotografije su snimljene uz upotrebu lupe. Aktivnost enzima je određena spektrofotometrijski, a izražena je kao specifična aktivnost ($ÄA_{470}min^{-1}mg^{-1}_{PROTEINA}$). Peroksidazna aktivnost utvrđena u centralnom cilindru i korteksu, lokalizirana je u provodnim elementima. Pojavljivanje smeđe boje bilo je najintenzivnije u epidermalnom području. Najniža specifična aktivnost peroksidaza izmjerena je u centralnom cilindru, nešto viša u korteksu (bez epidermalnog područja), a najviša u uzorku koji je sadržavao i korteks i epidermalno područje. Visoka aktivnost povezana s epidermom upućuje na moguću zaštitnu funkciju peroksidaza prilikom bilo kojeg mehaničkog ili kemijskog utjecaja. Uzorci su međusobno uspoređeni i s obzirom na sastav izoenzima.

## GUAIACOL PEROXIDASES IN CARROT (*Daucus carota* L.) ROOT

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Carrot (*Daucus carota* L.) belongs to the group of common edible vegetables. The aim of the present study was to establish a distribution of guaiacol peroxidases as well as to determine the enzymes activity in particular regions of the carrot root: central cylinder, cortex and epidermis. It has been a preliminary study for further investigations of stress influence on the cell differentiation in carrot root. For enzyme localisation hand-made transversal sections of the fresh root tissue were incubating in the reaction mixture containing the guaiacol as a substrate. The tissue printing technique was used for the same purpose. Enzyme activity was determined

spectrophotometrically and expressed as a specific activity ($ÄA_{470}min^{-1}mg^{-1}_{PROTEINS}$). The appearance of peroxidase activity in central cylinder and cortex was localised in the conducting elements. The most intensive brown colouration was noticed in the epidermal region. The lowest specific peroxidase activity was measured in the extracts of central cylinder. The activity was slightly higher in the cortex area (without epidermal region), being the highest in the samples containing tissues from both the cortex and epidermal region. Such high peroxidase activity could be the part of a defensive mechanism provoked by any mechanical or chemical attack. Samples were compared with regard to isoperoxidase patterns.

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## TKIVNO SPECIFIČNI STANIČNI I IZVANSTANIČNI PROFIL GLIKOPROTEINA U STANIČNIM LINIJAMA ŠEĆERNE REPE

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Glikoproteini su velika grupa proteina koji imaju šećernu komponentu na N ili C kraju. Uključeni su u važne biološke procese kao što su razvoj, rast i preživljavanje organizma. Tkivne linije šećerne repe: N (normalna, ovisna o hormonima), HO (prilagođena, organogena) i HNO (prilagođena, neorganogena) već su morfološki i biokemijski opisane. Utvrđeno je da se nalaze na različitim stupnjevima razvitka. Međutim, detaljna glikoproteinska slika nije analizirana. U eksperiment smo dodatno uvrstili tri transformirane linije: T, Tr i Tw. U radu smo koristili lektine da bi karakterizirali šećernu komponentu staničnih i izvanstaničnih glikoproteina staničnih linija šećerne repe. Proteine razdvojene SDS-PAGE-om te prenesene na nitrocelulozu membranu, tretirali smo s lektinima SNA, MAA, RCA, UEA i ConA. Stanični glikoproteini su locirani između 49 i 55 kDa te između 22 i 33 kDa. Potonji nisu reagirali s ConA. Glikoproteini od 22 do 30 kDa detektirani pomoću UEA, bili su vidljivi samo u HO, Tr i Tw linijama. Sijalinska kiselina, glikanska komponenta koja dosad nije otkrivena u biljkama, detektirana je u svim staničnim linijama lektinima SNA i MAA. Vrpca od 60 kDa nije otkrivena U T liniji dok su vrpce od 24 – 29 kDa nedostajale u HNO i Tr liniji. Izvanstanični glikoproteini od 32 i 70 kDa bili su zajednički u svim linijama osim linije HNO. Neoplastična i dediferencirana linija HNO izlučivala je više proteina od drugih linija te su specifični glikoproteini otkriveni na 67, 42 i 40 kDa. Glikoproteini su povezani s fenotipom i s promjenama na razvojnoj razini. Glikoproteinska slika izvanstaničnih proteina pokazala je više razlika među tkivima nego slika staničnih. Determinacija specifičnih glikoproteina će se nastaviti.

## TISSUE SPECIFIC CELLULAR AND EXTRACELLULAR GLYCOPROTEIN PATTERNS IN SUGARBEET CALLUS LINES

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Glycoproteins are large group of proteins that have sugar component attached on the N or C terminal domain. They are involved in crucial biological events as development, growth, function and survival of an organism. Sugarbeet callus lines: N (normal, hormone dependent), HO (habituated organogenic) and HNO (habituated non - organogenic) were already morphologically and biochemicaly characterised, and found to be in different stages of differentiation. However, detailed glycoprotein patterns of these lines were not established. Three transformed lines: T, Tr and Tw were also included in the experiment. Lectins were used to characterise a sugar component of cellular and extracellular glycoproteins. Proteins separated by SDS-PAGE and immobilized on nitrocellulose membrane were probed with SNA, MAA, RCA, UEA and ConA lectins. For cellular glycoproteins, common bands were resolved in the range between 49-55 kDa and between 22-33 kDa. The latter bands did not react with ConA. Glycoproteins of 22-30 kDa detected with UEA were expressed in HO, Tr and Tw lines only. Sialic acid, a glycan component so far not reported in plants was detected in all tissue lines with SNA and MAA. The 60 kDa band was missing in T and 24 –29 kDa bands were missing in HNO and Tr line. The extracellular 32 and 70 kDa glycoproteins were detected in all lines except in HNO. The HNO, neoplastic-dedifferentiated cell type excreted more proteins than other tissues and it had specific glycoproteins detected at 67, 42, 40 kDa. Glycoproteins are related with the phenotype and to changes in differentiation state. Extracellular glycoprotein patterns showed more differences between tissues than the cellular ones. Determination of specific glycoproteins will be the further step.

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## KVANTITATIVNA ANALIZA FLAVONOIDA PROPOLISA IZ SJEVERNE HRVATSKE

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Propolis je smolasta tvar što ju pčele medarice (*Apis mellifera* L.) sakupljaju s raznog drveća i nose u košnicu kako bi ju zaštitile od propuha, nametnika i sl.

Propolis se dugo upotrebljava u narodnoj medicini, a danas je dokazano da djeluje antimikrobno, protuupalno i antioksidativno. Najznačajnije skupine bioaktivnih spojeva u propolisu su flavonoidni aglikoni, fenilpropenske kiseline i esteri te eterično ulje. Sastav i koncentracija tih sastavnica ovisi o vrsti bilja s kojeg pčele sakupljaju propolis, godišnjem dobu i temperaturi. Stoga smo u ovom radu spektrometrijski odredili udjele flavonoida u 20 uzoraka sirovog propolisa iz Sjeverne Hrvatske (od Varaždina do Osijeka) koji su prikupili pčelari tijekom 2002. godine. Flavoni i flavonoli određeni su prema kvercetinu primjenom metode s aluminijkloridom, a flavanoni prema naringeninu korištenjem metode s 2,4-difenilhidrazinom. Utvrđeno je da ispitani uzorci propolisa sadrže 1.25-2.34% flavona i flavonola, dok su koncentracije flavanona određene u širokom rasponu od 3.91% do 22.19%. Rezultati su pokazali da je sadržaj ukupnih flavonoida propolisa podrijetlom iz sjevernih hrvatskih područja u granicama između 5.16% i 25.94% te da se primjenom dviju komplementarnih spektrofotometrijskih metoda brzo i jednostavno može odrediti koncentracija flavonoidnih aglikona kao pokazatelja kvalitete propolisa.

## QUANTITATIVE ANALYSIS OF FLAVONOIDS IN PROPOLIS SAMPLES FROM north CROATIA

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Propolis is resinous substance collected by honeybees (*Apis mellifica* L.) from various plants, which protects hives against draught and invaders. Propels has been used in folk medicine for many years, and there is evidence indicating that propolis has antimicrobial, anti-inflammatory and antioxidant properties. The most bioactive substances in propolis are flavonoid-aglycones, phenylpropen acids and esters and essential oils. Composition and concentration of these compounds depend on plant sources from which bees collect propolis, season, and temperature. Because of that we have quantitative analyzed percentage of flavonoids in 20 samples of raw propolis, collected by beekeepers from north Croatia during 2002 (from Varaždin to Osijek). Flavones and flavonols were determinated using method with quercetin and aluminium chloride, and flavanones with naringenin method and 2,4-dinitrophenylhydrazine). It is estimated that investigated propolis samples contains flavones and flavonols concentration from 1.25 to 2.34%, and flavanones from 3.91 to 22.19%. Result showed that total concentration of flavonoids in raw propolis from north Croatia ranged from 5.16 to 25.94%. Application of two complementray quantitative spectrofotometric methods is fast and simply method for estimation of flavonoids as key compounds for evaluating quality of raw propolis.

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## PROMJENE NA ULJNIM ŽLIJEZDAMA I OŠTEĆENJA PLODOVA MANDARINE UNSHIU OD NISKE TEMPERATURE TIJEKOM ČUVANJA OVISNO O TOPLINSKOM PREDTRETMANU

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Mandarina unshiu (*Citrus unshiu* Marc.) je glavna vrsta agruma u Hrvatskoj s godišnjom proizvodnjom od oko 40.000 tona. Slaba sposobnost skladištenja i pojava ozljeda od niskih temperatura tijekom čuvanja plodova je glavni ograničavajući čimbenik duže potrošnje plodova. Potapanje plodova u toplu vodu u trajanju tri minute može poboljšati sposobnost skladištenja. Mehanizam postanka makroskopski vidljivih ozljeda je nejasan s anatomskog gledišta, a oštećenja se u pojedinim godinama i ne moraju javiti bez obzira na postojanje povoljnih uvjeta za njihov nastanak. Smatra se da bi anatomske promjene uljnih žlijezda mogle prethoditi postanku vidljivih simptoma ozljeda. Istraživanje je obuhvatilo potapanje plodova (cv. 'Saigon') tri minute u vodu (HWD) temperature 48 i 52ș C i kontrolu (bez predtretmana) nakon čega je uslijedilo čuvanje 4 mjeseca na 1ș C, te držanje na sobnoj temperaturi u trajanju od 7 dana. Mikroskopski pregled zdravog i oštećenog staničja kore pokazao je promijenjeni oblik uljnih žlijezda na oštećenim mjestima. Niti jedna pregledana uljna žlijezda u oštećenom staničju nije imala eterično ulje, za razliku od onih u zdravom. Na preparatima se također mogao uočiti različit stupanj oštećenja tkiva, s time da je degradacija staničja bila najjača kod predtretmana HWD 52ș C i kontrole, a manja kod HWD 48ș C.

## CHANGES IN OIL GLANDS AND CHILLING INJURIES DURING STORAGE OF UNSHIU MANDARIN FRUITS IN RESPECT OF HOT WATER DIP PRE-TREATMENT

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Unshiu mandarin (*Citrus unshiu* Marc.) is the major citrus crop in Croatia with the production of approx. 40,000 tons per year. Low storage performance and appearance of chilling injuries during storage is the key limiting factor for longer period of fruit consumption. Storage performance can be improved by dipping the fruit into hot water. The mechanism of origin of the microscopically visible injuries is not clear from anatomic view. Namely, during certain seasons these injuries may not appear in spite of the conditions facilitating their development. It is believed that the anatomic changes of oily glands may precede the appearance of visible injury symptoms. The investigation involved dipping fruits (cv. 'Saigon') into hot water (HWD) at the temperatures of 48 and 52ș C, and control (untreated fruits). In continuation the fruits were stored for 4 months at the temperature of 1ș C, and for 7 days at 20ș C (shelf life). Microscopic examination of the healthy and the injured rind tissue showed changes in the shape of the oily glands on the injured or damaged spots. None of the examined oil glands contained etheric oil in the injured tissue, which was not the case with the healthy tissue. The preparations also showed different degrees of tissue injuries: the decay being higher with HWD pre-treatment at 52ș C and the control, but lower at 48ș C.

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## UČINAK TEMPERATURE NA STOPU FOTOSINTEZE I DISANJA U ZELENE ALGE *Chlorella kessleri*

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Temperatura je važan abiotički čimbenik koji u velikoj mjeri utječe na metaboličke reakcije stimulirajući ih pri temperaturnom optimumu i njemu bliskim vrijednostima. S udaljavanjem vrijednosti od optimalnih prema višim ili nižim temperaturama dolazi do inhibicije staničnih procesa. U ovom smo radu pratili učinak temperature na stopu fotosinteze i disanja mjerenjem oslobođenog, odnosno utrošenog kisika pri temperaturama 25, 30, 35, 40 i 45 °C te pri intenzitetima svjetlosti 25, 50, 75, 100, 150 i 200 μmol $m^{-2}$ $s^{-1}$ za svaku primijenjenu temperaturu. Mjerenja su provedena pomoću uređaja Chlorolab 2 (Hansatech, Velika Britanija) koji mjeri kisik pomoću elektrode Clark-ovog tipa. Kao testni organizam smo koristili jednostaničnu zelenu algu *Chlorella kessleri* Fott et Novák čiji se broj stanica kretao između $5,06 \times 10^6$ i $6,73 \times 10^6$ u mililitru suspenzije. Pri temperaturi 30 i 35 °C te intenzitetima svjetlosti od 50, 75, 100 i 150 μmol $m^{-2}$ $s^{-1}$ stopa fotosinteze je premašivala stopu disanja, dok je pri 40 °C oslobađanje kisika u većoj mjeri u odnosu na potrošnju zabilježeno pri intenzitetima svjetlosti 75, 100 i 150 μmol $m^{-}$

$^{2}$ $s^{-1}$. Pri primijenjenim intenzitetima svjetlosti produkcija kisika fotosintezom bila je veća od potrošnje disanjem u rasponu temperatura od ~ 33 °C do ~ 40 °C, a najviša je bila pri 35 °C pa se može zaključiti da je ta temperatura optimalna za fotosintezu zelene alge *Chlorella kessleri*. Pri optimalnoj temperaturi povećanje intenziteta svjetlosti iznad 75 μmol $m^{-2}$ $s^{-1}$ nije uzrokovalo daljnje povećanje stope fotosinteze.

## EFFECT OF TEMPERATURE ON PHOTOSYNTHETIC AND RESPIRATION RATE IN GREEN ALGA *Chlorella kessleri*

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Temperature is an important abiotic factor that has a great effect on metabolic reactions. It stimulates these reactions at optimal or optimum close values, but it inhibits cell reactions when distant from the optimum. The effect of temperature on photosynthetic and respiration rate was studied by measuring the amount of released and spent oxygen. The measurements were carried out at temperatures 25, 30, 35, 40 and 45 °C, and light intensities 25, 50, 75, 100, 150 and 200 μmol $m^{-2}$ $s^{-1}$ for every temperature using instrument Chlorolab 2 (Hansatech, UK) based on the Clark-type electrode. Unicellular green alga *Chlorella kessleri* Fott et Novák was used as a test organism. The number of cells was between $5.06 \times 10^{6}$ and $6.73 \times 10^{6}$ per mililitre of suspension. At temperatures 30 °C and 35 °C and light intensities 50, 75, 100 and 150 μmol $m^{-2}$ $s^{-1}$ photosynthetic rate was higher than respiration rate. Temperature of 40 °C caused higher oxygen release than its consumption at light intensities 75, 100 and 150 μmol $m^{-2}$ $s^{-1}$. The amount of oxygen spent by respiration was lower than the one released by photosynthesis in a temperature range from ~ 33 °C to ~ 40 °C at measured light intensities. The optimal temperature for photosynthesis in green alga *Chlorella kessleri* was at 35 °C because it caused the highest rate of photosynthesis. Light intensity above 75 μmol $m^{-2}$ $s^{-1}$ at optimal temperature did not cause further increase of the photosynthesis rate.

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## FITOKEMIJSKE VARIJACIJE U SASTAVU ETERIČNIH ULJA VRSTA *Satureja montana* L. I *S. cuneifolia* TEN. IZ DALMACIJE

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Rod *Satureja* (vrisak) koji raste na područja Hrvatske je zastupljen s devet vrsta, pet podvrsta i sedam varijeteta. Istraživali smo sastav eteričnog ulja dviju

vrsta *Satureja montana* L. (brdski vrisak) i *Satureja cuneifolia* Ten. (klinastolisni vrisak). Biljni materijal sakupiljen ja na tri različita lokaliteta u Dalmaciji (Biokovo, Brač, Kozjak), u različitim vegetacijskim periodima: prije, za vrijeme i nakon cvatnje. Analiza ulja vršena je GC/MS metodom. Poznato je da na sastav eteričnog ulja biljaka djeluju uvjeti okoliša i stadiji u razvoju biljke. Kvantitativni sastavi eteričnog ulja vrsta *S. montana* ukupno gledajući u svim istraživanim fazama kreće se od 1,1 - 2,75 %, a za *S. cuneifolia* 0,35 – 1,4 %. Kod vrste *S. montana* najzastupljenije satavnice eteričnog ulja su karvakrol (16,1 – 52,4%) i timol (1,9 - 20,6%). Obje ove glavne komponente su fenolni monoterpeni koje nastaju na istom biogenetskom putu s *p*-cimenom i γ-terpinenom. Vrsta S. *cuneifolia* s Brača bogata je α-pinenom (10,9 – 20,7 %), s Kozjaka linalolom (17,2 – 18.2 %), a ulje s Biokova â-kubrbenom (8,6 – 11,1 %). Istraživane biljke proizvode sekundarne metabolite kao normalan odgovor na rast , utjecaj patogene i stres.

## PHYTOCHEMICAL VARIATION OF *Satureja montana* L. AND *S. cuneifolia* TEN. ESSENTIAL OILS FROM DALMATIA

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The genus *Satureja* is represented in Croatia by nine species, five subspecies and seven varieties. In our study we investigated composition of essential oils from two species *Satureja montana* L. and *Satureja cuneifolia* Ten. The plant materials were collected from three different localities in Dalmatia (Biokovo, Brac, Kozjak) in three different stages of development: before, during and after flowering. It is well known that environmental conditions and stage of development can affect the relative amounts of essential oil components. The essential oils of both plants were subjected to a detailed GC-MS analysis. The yield of the essential oil of *S. montana* ranged from 1.1 to 2.75 %, and *S. cuneifolia* 0.35 to 1.4 %. *Satureja montana* shows large variations in the relative concentration of its major components, viz. carvacrol (16.1 – 52.4 %), thymol (1.9 – 20.6 %). Both of this oxygen-containing phenolic monoterpes were found to be the main constituents in all the samples of essential oils, the next most abundant being monoterpenic hydrocarbons, *p*-cymene and γ-terpinene. S. *cuneifolia* from Brac was rich in α-pinene (10.9 - 20.7 %), from Kozjak was rich in linalool (17.2 – 18.2 %) and from Biokovo oil was rich in â-cubebene (8.6 – 11.1 %). Investigated plants produce secondary metabolites as normal program of grow and development or in response to pathogen attack or stress.

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## SASTAV ETERIČNOG ULJA VRSTE *Thymus longicaulis* C. PRESL (Lamiaceae)

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*Thymus longicaulis* C. Presl, tankolisna majčina dušica, malena je aromatična trajnica iz porodice *Lamiaceae*. Vrlo je polimorfna vrsta s mnogo opisanih varijacija i formi, a svrstava se u skupinu ilirsko-mediteranskih biljaka. U ovom je radu istražen sastav eteričnog ulja izoliranog hidrodestilacijom iz osušenih nadzemnih dijelova navedene biljne vrste. Primjenom plinske kromatografije odijeljena je 51 sastavnica ulja, od kojih su 33 identificirane (97,3% ulja). Utvrđeno je da osnovu ulja čine monoterpenski spojevi (95,7%), praćeni malim udjelom seskviterpena (1,6%). Među monoterpenima prevladavali su fenoli (41,6%), ugljikovodici (39,3%) i alkoholi (12,7%). Određene su glavne sastavnice ulja: timol (40,1%), limonen + 1,8-cineol (16,0%), *p*-cimen (15,1%) i a-terpineol (8,4%). Udjeli a-pinena, sabinena, mircena, *o*-cimena, borneola, nerola, karvakrola i a-terpinilacetata bili su u granicama od 1,1% do 2,2%, dok su ostale sastavnice ulja bile pojedinačno zastupljene u količini manjoj od 1,0%. Rezultati su pokazali da vrsta *T. longicaulis* sadrži 1,2% (V/m) eteričnog ulja bogatog timolom koji ima snažna antimikrobna svojstva.

## COMPOSITION OF THE ESSENTIAL OIL OF *Thymus longicaulis* C. PRESL (Lamiaceae)

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*Thymus longicaulis* C. Presl is a small aromatic perennial herb of *Lamiaceae* family. It is a typical representative of the Illyric-Mediterranean flora. A great num-

ber of variations and forms were described within this polymorphic species. In the present work, the composition of the essential oil of *T. longicaulis* was investigated by capillary gas chromatography (GC). The oil was obtained by hydrodistillation from dried aerial parts of the plant sample. GC analysis indicated the presence of 51 components, out of which 33 were identified, representing 97.3% of the total oil. Essential oil was characterized by a small sesquiterpene fraction (1.6%) and a very high content of monoterpenes (95.7%): phenols (41.6%), hydrocarbons (39.3%) and alcohols (12.7%). Thymol (40.1%), limonene + 1,8-cineole (16.0%), *p*-cymene (15.1%) and a-terpineol (8.4%) were identified as the major components. Amounts of a-pinene, sabinene, myrcene, *o*-cymene, borneol, nerol, carvacrol and a-terpinyl acetate ranged from 1.1% to 2.2%. The other components were present in lower concentrations. The results showed that *T. longicaulis* contained 1.2% (V/m) of the essential oil, which was rich in thymol, a strong antimicrobial agent.

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## ETERIČNA ULJA NEKIH VRSTA RODA *Artemisia* L. U HRVATSKOJ

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Eterična ulja krajnji su proizvodi biljnog metabolizma i imaju značajnu ulogu u životu i razvoju biljke, osobito u prilagođavanju uvjetima okoliša. Mnoga ulja posljednjih se nekoliko desetljeća sve više proizvode u različite svrhe: za aromaterapiju, industriju hrane i pića, kozmetiku i drugo. U botanici, sastavnice eteričnog ulja mogu poslužiti kao kemotaksonomski markeri. U ovome radu obrađeno je 5 vrsta roda *Artemisia* L. s obzirom na sadržaj i sastav eteričnog ulja, uspoređujući ulja dobivena od biljaka sakupljenih na različitim nalazištima i u raznim stadijima razvoja same biljke. Četiri vrste su autohtone ili naturalizirane u Hrvatskoj: *A. absinthium* L., *A. alba* Turra, *A. verlotorum* Lamotte i *A. vulgaris* L., dok je jedna prisutna samo u uzgoju: *A. dracunculus* L. ssp. *redowskii* Ledeb. Eterična ulja su dobivena postupkom hidrodestilacije suhog biljnog materijala, u trajanju od 4 sata u aparaturi prema Clevenger-u. Ulja su potom analizirana sustavima GC i GC-MS. Glavne sastavnice eteričnih ulja prvih četiriju vrsta, u različitim omjerima, su tujoni, kamfor, artemisia keton i gremakren-D, dok se posljednje znatno razlikuje i u njega predominiraju sabinen, metil-eugenol i seskviterpeni.

## ESSENTIAL OILS OF SOME *Artemisia* L. SPECIES GROWING IN CROATIA

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Essential oils are final products of plant metabolism and have various purposes in plant life and development, especially for adapting to the environmental conditions. Many of them are widely produced for various purposes in past few decades, including aromatherapy, food and drinks industry, cosmetics etc. In botany, essential oils composition can be used as chemotaxonomic marker. In this study, 5 species of the genus Artemisia L. were analysed for their essential oil content and composition, comparing the results gained from the plants collected at various sites and different stages of development. Four species are natural or naturalised in Croatia: *A. absinthium* L., *A. alba* Turra, *A. verlotorum* Lamotte and *A. vulgaris* L., whilst one is only found in the culture: *A. dracunculus* L. ssp. *redowskii* Ledeb. Essential oils were obtained by 4 hour hidrodistilation in Clevenger apparatus, of dried plant material. They were analysed by GC and GC-MS. Main oil components for the first 4 species, in various percentages, were found to be thujones, camphor, artemisia ketone and germacrene-D, while the last one has significantly different oil composition with predominance of sabinene, methyl-eugenol and sesquiterpene.

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## OSOBITOSTI ANATOMSKE GRAĐE I SASTAV ETERIČNOG ULJA VRSTE *Hypericum perforatum* L.

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Kantarion, gospina trava ili trava Sv. Ivana (*Hypericum perforatum* L. subsp. *veronense* (Schrank) Fröhlich) je višegodišnja biljka koja raste na neobrađenim poljima, pašnjacima, livadama. U fitoterapiji i narodnoj medicini jedna je od najdragocjenijih biljaka danas. Biljni materijal sakupljen je u Dalmaciji (iznad Omiša) u dva različita vegetacijska perioda: prije i za vrijeme cvatnje. Izvršena su mikroskopska istraživanja anatomije vegetativnih organa lista, stabljike i korijena. GC-MS metodom su izolirana eterična ulja i određen sastav hlapljivih komponenti. Okoliš i stadiji u razvoju biljke su uvjetovali promjenu u sastavu i broju komponenata eteričnog ulja istraživane vrste.

Stabljika i listovi istraživane vrste su prekriveni kutikulom. Kutikula i eterična ulja smještena u sekretornim šupljinama blizu površine stabljike, te u žljezdama u mezofilu lista imaju ulogu u smanjenju transpiracije. Korijen je dobro razvijen, a anatomski dominira sekundarna građa, kao i prevladavanje provodnih elemenata ksilema. Postotak dobivenog eteričnog ulja veći je u razdoblju prije cvatnje. U oba vegetacijska perioda glavna komponenta ulja je biciklički spoj formule biciklo [4, 4, 0] dec –1-en.

## ANATOMY SPECIFICS AND ESSENTIAL OIL COMPOSITION OF *Hypericum perforatum* L.

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Saint John´s Wort or *Hyperici herb* (*Hypericum perforatum* L. subsp. *veronense* (Schrank) Fröhlich) is perennial plant growing in neglected fields, grazing lands and meadows. It is one of the most appreciated plants today in fitotherapy and folk medicine. The plant material was collected in Dalmatia (the mountain near Omiš) in two different stages of development: before and during flowering. We investigated anatomy of vegetative organs such as leaf, stem and root by light microscopic method. Essential oil was isolated and the composition of volatile components was established by means of GC-MS. Environmental conditions and stages of development affected the content of the researched plant essential oil and the number of its components. All the steam and the leaves parts are covered in cuticle. Both cuticle and essential oils located in secretory cavities near stem surface and in the mesophyl leaf glands have an important role in lowering the plant transpiration. The plant has a well developed root system in which the secondary built is dominant as well as the xylem vascular system. The content of essential oil was higher in the stage before flowering. In bouthvegetation stages the main oil component was a bicyclic compound with the chemical formula bicyclo [4, 4, 0] dec–1-en.

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## INFFLUENCE OF THE CULTIVAR AND ECOLOGICAL FACTORS ON THE ACHIEVMENT OF FULL RIPENESS AT SOME SORTS OF GRAPEVINES

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In Skopje vineyard during the period of 1999-2001 some phenologic observations have been performed on ten vine cultivars (Chardone, Semion, Muscat Otonel, Traminec, Rajnski Riesling, Italian Riesling, Rkaciteli, Zilavka, Zupljanka and

Smederevka). The aim of these phenologic observations was to establish the number of active days in the period of the vegetation, the needed active and effective temperature sums from the beginning of the shoot elongation until the full maturity at the experimented vine cultivars which are source of raw material for the white wines. In Skopje vineyard in the examined period the average annual air temperature was 13.3°C, and the average vegetative temperature had a value of 19.7°C, the number of active days had a value of 203, the active temperature sum had a value of 4131°C and the effective temperature sum was 2101°C. Besides the cultivar, as the main factor, the meteorological conditions in the years of examination had also great influence on the phenologic development and the needed temperature sums. The examined cultivars need active temperature sum of 3047°C (Traminec) to 3677°C (Smederevka). The necessity of effective temperature sums is 1650°C for Chardone to 1947°C for Smederevka.

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## PRILAGODBA LISTIĆA MAHOVINE *Polytrichum commune* L. NA NISKE ZIMSKE TEMPERATURE

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Stanice zimskih listića mahovine (*Polytrichum commune* L.) sadrže brojne malene vakuole raspoređene oko centralno smještene jezgre. Kloroplasti su veliki s obilnom stromom, a tilakoidni sustav sadrži grana od svega nekoliko tilakoida. U stromi ima više malenih škrobnih zrnaca i nakupina malenih plastoglobula. Mitohondriji su dobro razvijeni. U citoplazmi ima i pojedinačnih membrana endoplazmatskog retikuluma, diktiosoma i velikih lipidnih globula smještenih uz staničnu stijenku. U biljkama koje su bile trajno (oko mjesec dana) na temperaturi ispod ništice uočljive promjene vidljive su u prvom redu u citoplazmi stanica listića. Količina citosola je smanjena, a ribosomi često agregirani i stiješnjeni između vakuola. Diktiosomi i endoplazmatski retikulum nisu uočljivi. Istodobno s tim promjenama stanice poprimaju konkavne konture. Te osobine ukazuju da tijekom smrzavanja dolazi do dehidracije stanica, što rezultira smanjenjem njihovog volumena. Ako se prethodno smrznute biljke drže neko vrijeme (2 do 3 tjedna) na temperaturi iznad ništice oblik stanica postaje konveksan, citoplazma ponovno dobiva rahlu strukturu, a diktiosomi i endoplazmatski retikulum mogu se uočiti. Istodobno tilakoidi kloroplasta su dilatirani. Opisane ultrastrukturne promjene tijekom smrzavanja i odmrzavanja zimskih listića mahovine slične su onima nađenim u zimskim listovima višeg bilja /Ljubešić i sur. Acta Bot. Croat. 58 (2003)1-10/. Naša istraživanja pokazuju da mahovine imaju veću mogućnost prilagodbe na niske temperature od višeg bilja.

## ADAPTATION TO LOW WINTER TEMPERATURES OF THE MOSS-LEAFLETS OF *Polytrichum commune* L.

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Characteristic for winter moss-leaflets *Polytrichum commune* L., was the type of vacuolization of the cells. There were numerous small vacuoles in the cytoplasm surrounding the nucleus. The chloroplasts were large with an abundant stroma containing grana of several thylakoids, starch grains, and groups of small plastoglobules. The mitochondria were well developed. The cytoplasm also contained elements of the endoplasmic reticulum, some dictyosomes, and large lipid globules in the vicinity of the cell wall. When the plants were kept for prolonged periods (about a month) at temperatures below zero, the following changes in the cytoplasm were observed. The volume of the cytosol was reduced and the ribosomes were aggregated between the vacuoles. The dictyosomes and the endoplasmic reticulum were no longer visible. At the same time, the cell contours became concave. Both characteristics indicated that dehydration of the cells and a decrease of the volume occurred during freezing. When the frozen plants were held for some time (2 to 3 weeks) at temperatures above zero the cells became convex, the cytoplasm loose and the dictyosomes and the endoplasmic reticulum detectable. In the chloroplasts some grana thylakoids were dilated. The ultrastructural changes observed in frozen and defrozen winter moss-leaflet were similar to those described for winter leaves of some higher plants /Ljubešić et al. Acta Bot. Croat. 58 (2003)1-10/.Our observations indicate that moss plants endure much lower winter temperatures than higher plants.

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## PRETVORBE PLASTIDA TIJEKOM ZRIOBE I OZELENJAVANJA PLODA KRASTAVCA

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Osobitost plastida je njihova izuzetna sposobnost transformacije. U ovom radu istražili smo ultrastrukturne karakteristike naknadnog ozelenjavanja ploda krastavca (*Cucumis sativus* L.). Nezreli zeleni plod sadržavao je tipične kloroplaste sa dobro razvijenim tilakoidnim sustavom i malim plastoglobulima. Tijekom zriobe, plod je postupno mijenjao boju od zelene do žute. Kromoplasti

u žutom plodu bili su ovalni, relativno mali (duljine 2-3 μm) te bogati ribosomima. U tim plastidima, od tilakoidnog sustava preostale su samo pojedinačne, često jako dilatirane membrane. Kao mogući nosioci karotenoidnih pigmenata nađeni su veliki plastoglobuli (promjera 0,2-0,5 μm). U određenim uvjetima, dolazilo je do ozelenjavanja zrelog žutog ploda. Početak ovog procesa pratilo je povećanje broja vezikularnih struktura, posebno u rubnim dijelovima plastida. Istovremeno, u središnjem dijelu nekih plastida pojavila se struktura slična vakuoli. Ultrastrukturna istraživanja pokazala su da bi membrana ovih "vakuola" mogla biti mjesto stvaranja tilakoidnih membrana. Na kraju procesa ozelenjavanja, plastidi su sadržavali lećaste kloroplaste (dužine 5-8 μm) s normalno razvijenim tilakoidnim sustavom te stromom bogatom ribosomima. Jedino je prisustvo velikih plastoglobula ukazivalo na njihovo porijeklo od kromoplasta. Ova istraživanja na plodu krastavca potvrđuju mogućnost naknadnog ozelenjavanja kromoplasta, za koje se obično smatra da predstavljaju zadnji stadij u razvoju plastida. Prikazani rezultati još su jedan dokaz velike prilagodljivosti plastida čak i u jako odvedenim stadijima biljnog razvitka.

## PLASTID TRANSFORMATIONS DURING RIPENING AND REGREENING OF CUCUMBER FRUIT

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Plastids are characterized by a great ability of transformation. In this work, we investigate the regreening potential of the fruit of cucumber (*Cucumis sativus* L.) on the ultrastructural level. Unripe, green fruit contained typical chloroplasts with a well-developed thylakoid system and small plastoglobules. As the ripening process proceeded, the fruit gradually changed its colour from green to yellow. The chromoplasts in such yellow fruit were roundish, relatively small (2-3 μm in length), and rich in ribosomes. In these plastids, the only remnants of the thylakoid system were single membranes, mostly strongly dilated. The carotenoid-bearing structures were large plastoglobules (0,2-0,5 μm in diameter), which usually appeared in clusters. Under certain conditions, mature fruit regreened. The beginning of this process was characterized by an increasing number of vesicular structures, especially in the peripheral regions of the plastids. Simultaneously, in some plastids, large central vacuole-like structure could be observed. Ultrastructural investigations indicated that the membranes of these "vacuoles" could be the sites of formation of thylakoids. In the fruit, at the end of the regreening process, the plastids contained typical lens-shaped chloroplasts (5-8 μm in length) with a normally developed thylakoid

system and a stroma rich in ribosomes. The only indication of their origin from chromoplasts was the presence of large plastoglobules. The presented ultrastructural studies on cucumber fruit demonstrate the regreening potential of chromoplasts, a plastid type that has usually been considered the last stage in plastid development. These investigations confirm the great flexibility of plastids, even in highly differentiated stages of plant development.

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## NEKE MORFOLOŠKE I FIZIOLOŠKE ZNAČAJKE SJEMENA BUKVE (*Fagus sylvatica* L.)

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Obična bukva je najzastupljenija vrsta drveća u šumama Hrvatske koju nalazimo u sva tri područja (Panonskom, Dinarskom i Mediteranskom) na površini od 921.882 ha ili 44,72 % ukupne obrasle šumske površine. Bukva dolazi pretežno u mješovitim – rjeđe čistim sastojinama bjelogorice i crnogorice tvoreći 18 ekološko-gospodarskih tipova regularnih šuma na površini od 507.035 ha (55%) i 33 ekološko-gospodarska tipa prebornih šuma na površini od 414.847 ha (45%). Druga polovica 20. stoljeća prepoznatljiva je u šumskim ekosustavima po globalnim promjenama s negativnim posljedicama, a najnepoželjnije su one koje su dovele u pitanje prirodnu obnovu i vitalitet ove gospodarski značajne vrste drveća. Potreba pomoći prirodnoj obnovi gospodarskih vrsta nazočna je osobito kod obnove lužnjaka, kitnjaka, poljskog jasena, jele kao i bukve, te je nužnost namicanja kvalitetnog sjemena navedenih vrsta drveća postala svakodnevnica koju je bilo moguće podmiriti iz priznatih sjemenskih sastojina. Tijekom proteklih pola stoljeća istraživana je kakvoća sjemena gospodarski značajnih vrsta drveća u laboratoriju Šumarskog instituta, Jastrebarsko pa tako i za običnu bukvu. Na temelju tih laboratorijskih nalaza i istraživanja vezanih za morfološke i fiziološke osobine sjemena dobiveni su prosječni parametri za neke morfološke i fiziološke osobine sjemena obične bukve. Prosječna masa sjemena je 258 g (100-331), prosječan broj sjemenski u 1000 g iznosi 4.098 komada (6.062-10.000 komada), a on je obartno proporcionalan s masom sjemena; prosječna hektolitaraska težina sjemena iznosi 45 kg (33-58 kg); prosječna dužina sjemenke je 14,89 mm (11,31-16,20 mm), a prosječna širina sjemenke je 9,53 mm (7,63-10,34 mm). Veličina sjemenke je u dobroj direktnoj klorelaciji s masom sjemena; prosječan vitalitet sjemena iznosi 69 % (10-90%), a prosječno učešće šturog sjemena 19 % (0-50%). Zdravstveno stanje sjemena je posljednjih godina bolje nego na početku promatranog razdoblja, jer se učestalije koriste određena preventivna i kurativna sredstva zaštite sastojina i sjemena protiv biljnih bolesti, gospodarskih i karantenskih štetnika.

# SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF BEECH SEED (*Fagus sylvatica* L.)

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Beech is the most distributed tree species in the forests of Croatia and can be found in all three regions (Pannonian, Dinaric and Mediterranean) on an area of 921.882 ha, i.e. 44.72 % of the total forest stocked area. Beech grows predominantly in mixed - and more rarely - pure deciduous and coniferous stands, forming 18 ecological-management types of regular forests on an area of 507.035 ha (55 %), and 33 ecological-management types of selection forests on 414.847 ha (45 %). The second half of the 20th century was characterised by global changes with their negative effects on the forest ecosystems. The least desirable are those that threaten natural reforestation and vitality of this important tree species from the aspect of management. The need for help in natural reforestation of management types is evident in the reforestation of peduncled oak, sessile-flowered oak, narrow-leafed ash, fir and also beech. Consequently, the need to provide high-quality seed of the aforementioned species has become a daily problem, which has been possible to satisfy from acknowledged seed stands. In the course of the last 50 years, an investigation has been carried out of the seed quality of management important tree species, including beech, in the laboratory of the Forest Research Institute, Jastrebarsko. On the basis of laboratory analyses and research of morphological and physiological seed properties, average parameters were obtained for some morphological and physiological properties of the beech seed: average 1000-seed weight is 258 g (100-331 g); average number of seeds in 1000 g. amounts to 4.098 pieces (6.062-10.100 pieces), and is inversely proportional with 1000-seed weight; average hectoliter seed weight amounts to 45 kg (33-58 kg); average seed length is 14.89 mm (11.31-16.20 mm); average seed width is 9.53 mm (7.63-10.34 mm); size of the seed is in good direct correlation with absolute weight; average viability of the seed amounts to 69 % (10-90%); average number of empty seed amounts to 19 % (0-50 %). Health state of the seed, during the last few years, is better than at the beginning of the observed period, because more frequent use has been made of certain preventive and curative interventions in the protection of stands and seed against plant diseases, management and quarantine pests.

BIOLOGIJA ŽIVOTINJA
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ANIMAL BIOLOGY

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## STRES I IMUNOKOMPETENCIJA U DOMAĆIH ŽIVOTINJA

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Zanimanje za stres u veterinarskoj medicini, posebice u farmskih životinja, raste u posljednjih nekoliko desetljeća. Domestifikacija životinja tijekom prošlih 8000 godina predstavlja jedinstveni i najznačajniji izazov njihovu slobodnom i neovisnom načinu života. Međutim, domaće, kao i divlje životinje, uspijevaju preživjeti, unatoč neprekidnom zbivanju izvanjskih ili unutarnjih "podražaja", održavanjem izrazito složene biološke ravnoteže ili homeostaze. Zaista, ta je vitalna harmonija stalno ugrožena, pa su životinje razvile procese, posebice zaštitne imunosne odgovore, radi nadvladavanja iznenadnih promjena u vanjskom ili unutarnjem okolišu. Ovisno o načinu držanja, vrsti i pasmini životinje, njezinoj genetskoj osnovi, dobi i fiziološkom stanju, te lokalnim klimatskim uvjetima, njezin će se odgovor na stres iz okoliša razlikovati kvalitativno i kvantitativno. Općenito, ishod tog odgovora može biti dvojak. Ili urođeni i dinamički zaštitni procesi, uključujući i imunosni odgovor, uspijevaju uspostaviti homeostazu, ili su pak "napušteni" i preoblikovani u stresni odgovor pri kojem je ishod istih procesa nepovoljan po jedinku, a očituje se promjenama u fiziologiji i vladanju, te trajnom imunosupresijom. Međutim, endokrini i neuropeptidni činitelji koji se izlučuju tijekom odgovora na stres mogu utjecati na imunosni sustav tako da izazovu i imunostimulaciju ili pak nemaju izravnog učinka. Osim toga, međudjelovanje neuroendokrinog i imunosnog sustava je dvosmjerno. Naime, aktivirane imunosne stanice proizvode neuroendokrine činitelje i citokine i njima utječu na aktivnosti središnjeg živčanog sustava, koje se očituju povratnim slijedom izlučivanja neuropeptida i neurohormona iz mozga. U ovom kontekstu, namjera je ovog predavanja prikazati spoznaje o postuliranoj povezanosti između stresa i imunosnog sustava, te razmotriti vlastite i tuđe podatke o njihovom međudjelovanju pri tvorbi zaštitnih ili štetnih učinaka na mnoge biološke funkcije u domaćih životinja.

## STRESS AND IMMUNOCOMPETENCE IN DOMESTIC ANIMALS

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Interest in the importance of stress for veterinary medicine, particularly in farm animals, has grown in the past few decades. The domestication of animals by man throughout the past 8000 years represents the single most significant challenge to their free and independent existence. However, through an extremely

complicated biological equilibrium called homeostasis, domestic as well as wild animals maintain their survival despite of the continuous both externally and internally generated "stimuli". Indeed, this apparent harmony is constantly challenged, and thus, they have developed processes, particularly protective immune responses, to overcome immediate perturbations in both the internal and external environment. Depending on the nature of management and the animal species and breed, its genetic background, age, physiological status as well as the local climatic conditions, the host response to the environmental stress will vary both qualitatively and quantitatively. Generally, it can be said that two possible outcomes of such response might be distinguished. Either, the innate and dynamic protective processes, including also the immune response, are capable to restore homeostasis or they are "left on", and turned into a stress response whereby the same processes exhibit negative outcomes in physiology and behaviour of individuum, and may result in prolonged immunosuppression. However, the endocrine and neuropeptide factors elicited in response to varying stimuli as well as signalling molecules secreted by immune cells, may ultimately impact on the immune system to produce either immunoenhancement or have no effect. Besides, the interaction between neuroendocrine and immune systems is bidirectional. Namely, the activated immune cells release neuroendocrine factors and cytokines which influence central nervous system (CNS) activities exhibited as the feedback cascade of neuropeptides and neurohormones produced by the CNS. In this context, the objective of this lecture is to present already existed evidences for a postulated connection between stress and the immune system, and to discuss our data and those of other authors on their interactions in producing the protective and harmful effects on different biological functions in domestic animal species.

OP-48

## MORFOMETRIJSKE OSOBITOSTI IMUNOSNIH STANICA JEJUNUMA PRASADI OBRAĐENE LEVAMISOLOM I IZAZIVAČKI INFICIRANE ENTEROTOKSIGENIM SOJEM BAKTERIJE *Escherichia coli*

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Bakterija *Escherichia coli* nalazi se u normalnoj crijevnoj mikroflori životinja i čovjeka gdje sudjeluje u proizvodnji manje količine celuloze, vitamina K i B12, riboflavina i tiamina. Neke serološke varijante bakterije *E.coli* mogu uzrokovati bolesti, posebice kod djece i mladunčadi životinja.Među bolestima životinja kolienterotoksemija sisajuće i odbijene prasadi uzrokovana enterotoksigenim sojevima bakterije *E.coli*(ETEC) najčešća je infekcija u prasadi. Vrednovali smo imunostimulacijski učinak imunomodulatora, levamisola kojim je prasad bila obrađena, a potom inficirana sa ETEC sojem. Na uzorcima jejunuma, nakon imunohistokemijske obrade, kvalitativnom i kvantitativnom analizom vrednovali smo aktivaciju imunosnih stanica u sluznici crijeva. Brojnost limfoidnih i mijelodnih stanica u limfatičkim tkivima probavnog sustava (LTPS), kao što su lamina propria, podsluznica i Peyerove ploče jejunuma znatno je povećana u prasadi koja je prije infekcije bila obrađena levamisolom, u usporedbi s neobrađenom, pokusno inficiranom prasadi. Specifična reaktivnost monoklonskih protutijela (mPt) s membranskim molekulama imunosnih stanica, poput CD3, CD21, CD45RA, CD45RC, SWC3, IgA omogućila nam je jasan uvid u raspodjelu i udjel T i B limfocita, makrofaga, dendritičkih i plazma stanica u pretraživanim odjeljcima LTPS. Također smo rabili softverski program Lucia G za digitalnu analizu slike (DAS) i kvantifikaciju subpopulacija imunosnih stanica fenotipiziranih s pomoću protu-svinjskih CD/SWC mPt. Numerički izraženi podaci za te subpopulacije stanica pokazuju razlike u imunosnim odgovorima između levamisolom obrađene i neobrađene prasadi koja su potom bila inficirana ETEC sojem. Uvođenje DAS u naš istraživački model kolibaciloze odbijene prasadi može biti relevantan za vrednovanje kvantitativnih promjena među imunosnim stanicama u sluznici jejunuma odbijene prasadi nespecifično imunizirane levamisolom i izazivački inficirane ETEC sojem.

## MORPHOMETRIC CHARACTERISTICS OF JEJUNAL IMMUNE CELLS FROM PIGS TREATED WITH LEVAMISOLE AND CHALLENGED WITH ENTEROTOXIGENIC *Escherichia coli* STRAIN

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Bacteria *Escherichia coli* can be found in normal intestinal microflora of animals and humans where it participate in production of small amounts of cellulose, vitamin K, $B_{12}$, riboflavin and thiamine. Some serogroups of *E.coli* can cause diseases, particularly in children and young animals. Among animal diseases colienterotoxemia caused by enterotoxigenic *E. coli* (ETEC) strains is the most prevalent porcine infection, commonly occurring in suckling and weaned pigs. We evaluated non-specific immunostimulating effect of levamisole, an immunomodulator, on pigs that were challenged with ETEC strains. Following immunohistochemical analyses of jejunal sections they were qualitatively and quantitatively evaluated for the immune cell activation within mucosal sites. The numbers of lymphoid and myeloid cells in the gut-associated lymphoid tissues (GALT), such as jejunal lamina propria, submucosa and Peyer's patches were considerably increased in pigs treated with levamisole prior to the infection as compared to nontreated, infected pigs. Specific reactivity of monoclonal antibodies (mAbs) with surface molecules on immune cells, such as CD3, CD21, CD45RA, CD45RC, SWC3, and IgA enabled clear insight into distribution and proportion of T and B lymphocytes, macrophages, dendritic and plasma cells within the GALT compartments examined. Also, we have used software program Lucia G for digital image analyses (DIA) and quantification of anti-swine CD/SWC mAbs-phenotyped immune cell subpopulations. Numerically expressed data for these cell subsets demonstrated difference of immune responses between levamisole-treated and nontreated ETEC-infected pigs, respectively. Introduction of the DIA into our research model of post weaning colibacillosis may be of relevance for the validation of quantitative changes among immune cells residing jejunal mucosa of weaned pigs non-specifically immunized with levamisole, and challenged with ETEC strain.

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## STANIČNI IMUNOSNI ODGOVOR U MEZENTERIJSKIM LIMFNIM ČVOROVIMA ODBIJENE PRASADI IMUNIZIRANE F4ac$^+$ NE-ENTEROTOKSIGENIM SOJEM BAKTERIJE *Escherichia coli*

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Kolibaciloza je glavni uzrok oboljevanja i mortaliteta odbijene prasadi. Najčešće je posljedica enterotoksigenih sojeva bakterije *E. coli* (ETEC) koji proizvode enterotoksine što oštećuju stijenku crijeva, potiču lučenje sluzi i elektrolita i tako uzrokuju dijareju. Vakcinacija protiv kolibaciloze odbijene prasadi nije u široj uporabi kao profilaktička mjera. Naime, pokušaji da se potakne specifični imunosni odgovor protiv ETEC sojeva na mukoznim površinama crijeva nakon oralne imunizacije s fimbrijskim vakcinama bili su neučinkoviti. Zaštita od mikrobnih antigena, kao što su ETEC sojevi, na mukoznim površinama mogla bi se postići indukcijom i regulacijom specifičnih obrambenih mehanizama posredovanim imunosnim stanicama u limfatičkom tkivu probavnog sustava (LTPS). Vrednovali smo imunogenost F4ac$^+$ ne-ETEC soja kao izvora replicirajućeg antigena u specifičnoj imunizaciji odbijene prasadi temeljem kvalitativnih i kvantitativnih pokazatelja aktivacije njihovih imunosnih stanica u mezenterijskom limfnom čvoru (MLČ) uporabom protočne citometrije i imunohistologije, kao i *in vitro* određivanjem fagocitnih funkcija peritonejskih makrofaga. Sedam dana nakon intragastričke imunizacije sa $10^{10}$ CFU/ml F4ac$^+$ ne-ETEC soja, analizirali smo CD$^+$/SWC$^+$ imunofenotipove stanica iz MLČ uporabom monoklonskih protutijela specifičnih za CD3, CD8, CD4, CD21, CD14, SWC3 i MHC II površinske stanične molekule. U MLČ imunizirane prasadi utvrdili smo porast udjela CD4$^+$CD8$^+$ memorijskih stanica, aktiviranih CD25 T pomoćničkih i T citotoksičnih limfocita, B limfocita i stanica koje prikazuju antigen. Peritonejski makrofagi imunizirane prasadi imali su veću sposobnost ingestije čestica. Izrazitija razlika u brojnosti aktiviranih CD25$^+$ T-limfocita vidljiva je u MLČ imunizirane u odnosu na kontrolnu prasad. Prema ovim rezultatima, pokusni vakcinalni F4ac$^+$ ne-ETEC soj uporabljen za poticanje specifične imunosti odbijene prasadi protiv kolibaciloze izazvao je odgovore mijeloidnih i limfoidnih stanica u odjeljku LTPS tj. u MLČ, i unutar peritonejske šupljine odbijene prasadi.

## CELLULAR IMMUNE RESPONSE IN MESENTERIC LYMPH NODES OF WEANED PIGS IMMUNIZED WITH F4ac$^+$ NON-ENTEROTOXIGENIC *Escherichia coli* STRAIN

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Colibacillosis is a major cause of illness and mortality in recently weaned pigs. Usually it is a consequence of enterotoxigenic *E. coli* (ETEC) strains producing enterotoxins that disrupt the intestinal mucosal barrier, stimulate hyper secretion of mucus

and electrolytes and thus cause diarrhoea. Vaccination against postweaning colibacillosis has not been widely used as a mean of prophylaxis. Attempts to induce a specific immune protection against ETEC strains at the mucosal surfaces of intestine after oral immunization with fimbriae-based vaccines were ineffective. Protection against microbial antigens such as ETEC strains at the mucosal surfaces of intestine may be achieved by induction and regulation of specific defense mechanisms mediated by immune cells from the gut-associated lymphoid tissues (GALT). We have evaluated immunogenicity of $F4ac^+$ non-ETEC strain as a source of the replicating antigen in the specific immunization of weaned pigs based on qualitative and quantitative parameters of their immune cell activation in the mesenteric lymph node (MLN) using flow cytometry and immunohistological methods as well as *in vitro* phagocytic functions of peritoneal macrophages. Seven days after intragastric immunization with $10^{10}$ CFU/ml of $F4ac^+$ non-ETEC strain, immunophenotypes of porcine $CD^+$/$SWC^+$ cells from MLN were analyzed using monoclonal antibodies specific for CD3, CD8, CD4, CD21, CD14, SWC3, and MHC II cell surface molecules. In the MLN of immunized pigs we found increased proportions of $CD4^+CD8^+$ memory cells, activated CD25 T helper and T cytotoxic lymphocytes, B lymphocytes and antigen presenting cells. Peritoneal macrophages of immunized pigs had higher capability of particle ingestion. More pronounced numbers of $CD25^+$ T lymphocytes were visible in MLN of immunized than in control pigs. According to the results, a vaccine candidate $F4ac^+$ non-ETEC strain used in the specific immunization of weaned pigs against colibacillosis induced lymphoid and myeloid cells responses in the compartment of GALT, *i.e.* MLN, and within peritoneal cavity of weaned pigs.

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## PČELINJI PROIZVODI KAO TUMOR –PREVENTIVNE I/ILI - TERAPEUTSKE TVARI

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Pčelinji proizvodi se odavnina rabe kao zdrava hrana i u narodnoj medicini. Istražili smo učinak pčelinjeg otrova (PO), meda, matične mliječi (MM), vodene otopine propolisa (VOP) i njenih polifenolnih sastavnica (kafeinska kiselina-CA, fenetil kafeat-CAPE, kvercetin-QU) na tumorski rast i metastatsku sposobnost. Rabili smo transplantabilni karcinom mliječne žlijezde miša CBA. Plućne metastaze prouzročili smo intravenskim (iv) uštrcavanjem $2x10^5$ vijabilnih tumorskih stanica. Sve istraživane tvari su citotoksične za stanice HeLa, V79 i MCa *in vitro* osim VOP. Lokalna nazočnost PO, CA i CAPE u tkivu značajno odgađa pojavnost tumora. Uštrcavanjem $1x10^5$ tumorskih

stanica *iv* neposredno nakon uštrcavanja pčelinjeg otrova, broj tumorskih čvorića u plućima je značajno manji (p<0,001) od kontrolne skupine ili miševa obrađenih s pčelinjim otrovom subkutano (*sc*). Ovi nalazi jasno ukazuju da antitumorski i antimetastatski učinak PO je visoko ovisan o načinu unosa PO te o bliskom doticaju sastavnica PO s stanicama tumora. MM data intraperitonealno, *sc* ili *iv* 7 i 14 dan prije ili 2 i 9 dan nakon uštrcavanja tumorskih stanica nema učinak na stvaranje metastaza dok istovremena primjena MM i tumorskih stanica značajno (p<0,05) inhibira nastanak metastaza. Med, nadalje ima antimetastatski učinak (p<0,05) kada je primjenjen (per os 2 g/kg za miševe ili 1 g/kg za štakore, jedan put dnevno u nizu od 10 dana) prije unosa tumorskih stanica, dok obrada životinja VOP i/ili njenim polifenolnim sastavnicama pokazuje značajan antimetastatski učinak (p<0,01; p<0.001) prije i poslije unosa tumorskih stanica. VOP ima veći antimetastatski učinak od polučenog s CA ili CAPE. Apoptoza i nekroza su dokazane protočnim citometrom nakon obilježavanja stanica aneksinom i propidijskim jodidom te TUNEL metodom u stanicama mliječne žlijezde miša poslije obrade s PO ili CAPE. Druge istraživane komponente prouzročile su uglavnom apoptozu. Povećanje makrofagne citotoksičnosti na tumorske stanice te odnosa CD4/CD8 stanica T vjerovatno je uzrok supresije tumorskog rasta u životinja obrađenih istraživanim tvarima. Podaci sugeriraju da pčelinji proizvodi mogu imati važnu ulogu u stimulaciji specifične i nespecifične imunosti i utjecati izravno na rast tumora.

## HONEY-BEE PRODUCTS AS CANCER-PREVENTIVE AND/OR - THERAPAPEUTIC AGENTS

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Honey-bee products have been widely used as a healthy food and folk medicine. We investigated the effect of bee venom (BV), honey, royal jelly (RJ), water-soluble derivative of propolis (WSDP) and related polyphenolic compounds (caffeic acid-CA, caffeic acid phenethyl ester-CAPE, quercetin-QU) on tumor growth and metastasing ability. Tumor was a transplantable mammary carcinoma (MCa) of CBA mouse. Metastases in the lung were generated by 2 x $10^5$ viable tumor cells given intravenously (*iv*). When tested compounds were applied to HeLa, V79, and MCa tumor cells *in vitro* all but WSDP exhibited a cytotoxic effect; the growth of tumor cells were clearly inhibited. Local prence of BV, CA, and CAPE in the tissue caused significant delay of tumor formation. When mice were given 1 x $10^5$ tumor cells *iv* immemately after BV injection, the number of tumor nodules in the lung was significantly lower ($p < 0.001$) than in untreated mice or mice treated with BV subcutaneously (*sc*). These findings clearly

demonstrated that antitumor and antimetastatic effects of BV were highly dependent on route of injection and on close contact between components of the BV and tumor cells. RJ did not affect metastases formation when given intraperitoneally, *sc* or *iv* 7 and 14 days before or 2 and 9 days after tumor cell inoculation while in the case of synchronous application of tumor cells and RJ a significant ($p < 0.05$) inhibition of metastases formation was observed. On the other hand, honey exerted pronounced antimetastatic effect ($p < 0.05$) when applied before tumor cell inoculation (per os 2 gr/kg for mice or 1 gr/kg for rats, once a day for 10 consecutive days) while WSDP and related polyphenolic compounds had significant antimetastatic effect ($p < 0.01$; $p < 0.001$) given either before or after tumor cell inoculation. The antimetastatic effect of WSDP was of higher degree than that achieved by either CA or CAPE. Apoptosis and massive necrosis were identified by flow cytometry using annexin and propidium iodide or in situ by TUNEL method in MCa cells after treatment with BV or CAPE. Other tested compounds induced mostly apoptosis. In addition to suppression of tumor growth by tested compounds, there was an increase in ratio CD4/CD8 T cells as well as in macrophage cytotoxicity for tumor cells in mice treated with tested compounds. These data suggest that honey-bee products may have important role in stimulation of specific and non-specific immunity and influence tumor growth by direct action.

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## AKTIVNOST UGLJIKOVE ANHIDRAZE U TKIVIMA ŠKRŽNE ŠUPLJINE HLAPA *Homarus gammarus* UVJETOVANA PROMJENOM SALINITETA

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Aktivnost enzima ugljikove anhidraze (UA) istraživana je u tkivima škržne šupljine hlapova *Homarus gammarus* prilagođenih u morskoj (MV, S 38; 1140 mOsmol/l) i u razrijeđenoj morskoj vodi (RMV, S 20; 578 mOsmol/l). Hlap se može uspješno prilagoditi u RMV (do saliniteta 20), gdje ima sposobnost hiperosmotske regulacije. UA uz $Na^+$, $K^+$-ATPazu sudjeluje u regulaciji osmotski važnih iona koji služe u održavanju gradijenta između stanica i morske vode. UA je višenamjenski enzim, koji osim uloge u disanju, regulira i pH u stanici. Enzim opskrbljuje stanicu $H^+$ i $HCO_3^-$ ionima nužnim za protusmjerni i istosmjerni prijenos osmoregulacijski važnih iona $Na^+$ i $Cl^-$. Cilj istraživanja su nove spoznaje o prilagodbama ionsko-regulacijskih mehanizama morskih rakova u održavanju homeostaze s obzirom na promjene saliniteta MV tijekom njihovih migracija u okolišu. Aktivnost enzima UA određena je modificiranom metodom uspoređivanjem brzine nekatalitičke i katalitičke reakcije u puferskom homogenatu tkiva (uz dodatak Tritona-100). Diferencijalnim centrifugiranjem tkiva dobivene su podstanične frakcije u kojima je detaljno ispitana raspodjela enzima, te određen odnos i raspodjela otopljene citosolne UA i membranski vezane UA. U hlapova prilagođenih u RMV od ukupne

aktivnosti UA u tkivima škržne šupljine, oko 70% nalazi se u epipoditu i po 15% u branhiostegitu i škrgama. U podstaničnim frakcijama od ukupne aktivnosti UA u tkivu citosolna je u epipoditu 75,8%, a u branhiostegitu 61,0%, dok je membranski vezana u epipoditu 6,8%, a u branhiostegitu 16,2%. U homogenatu i membranskoj frakciji škrga povećanje aktivnosti UA je manje i nema značajne razlike između hlapova iz MV i RMV. Rezultati ukazuju na dominantni udio citosolne UA, pa pretpostavljamo da je uloga UA najvećim dijelom u prijenosu osmoregulacijski važnih iona i u procesu disanja. Rezultati po prvi puta dokazuju značaj epipodita i branhiostegita u indukciji UA u hlapova prilagođenih u RMV. Utvrđeni rezultati o najpovoljnijim životnim uvjetima hlapa doprinijet će novim spoznajama u ekofiziologiji morskih organizama, te praktičnoj primjeni u marikulturi i prvenstveno zaštiti prirodne populacije hlapa.

## ACTIVATION OF CARBONIC ANHYDRASE IN BRANCHIAL CAVITY TISSUES OF LOBSTER *Homarus gammarus* CONDITIONED BY SALINITY

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The activity of carbonic anhydrase (CA) and its distribution in the branchial cavity tissues had been studied in the lobster *Homarus gammarus* from ambient seawater (SW, salinity 38; 1140 mOsmol/l) and dilute seawater (DSW, salinity 20; 578 mOsmol/l). Lobsters were acclimated to DSW (salinity 20), where they have the ability of hyperosmotic regulation. In addition to $Na^+$, $K^+$-ATPase, CA represents a key enzyme involved in the ion osmoregulation where it maintains osmoconcentration gradients between the haemolymph and ambient seawater. CA is a multifunctional enzyme, with an additional role in respiration and acid-base regulation in the cells. CA supplies cells with ions $H^+$ and $HCO_3^-$, which then serve as counterions for the active uptake of important osmolyte ions $Na^+$ and $Cl^-$. The aim of our study was to provide more knowledge on the adaptive ion regulating mechanism in marine crustaceans, which maintain homeostasis when lobsters migrate to brackish water habitats with a fluctuating salinity. The activity of CA was measured using a modified method by comparison of uncatalysed and catalysed reaction times in tissue homogenates (with addition of Triton-100). Differential centrifugation of tissues homogenates was used for measurements of CA distribution in subcellular fractions, and to define the relation and distribution of cytosolic and membrane-bound CA. In DSW acclimated lobsters, almost 70% of the total CA in tissues of the branchial cavity was found in epipodites, and the rest was equally distributed between branhiostegites and gills. In subcellular fractions from DSW acclimated lobsters, the main proportion of 75.8% was in epipodites, 61.0% in branhiostegite of the total CA activity in these tissues remained in the cytosol portion. Partially purified membranes contained 6.8% in epipodites and

16.2% in the branhiostegite. A slight activation of CA in homogenates and in partially purified membranes of gills was not confirmed as a statistically significant difference between the SW and DSW groups. These results indicate the dominant proportion of the cytosolic CA. We suggest that CA is mainly involved in the transport of osmolyte ions and respiration. These results for the first time indicate the importance of epipodites and branchiostegite in the CA induction when lobsters are acclimated to DSW. Moreover, results provide knowledge to disclose problems applied in mariculture and protection of lobster's population in marine habitats.

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## GLUCOSE-6-PHOSPHATASE ACTIVITY DURING ACCLIMATION TO HIGH ENVIRONMENT TEMPERATURE IN FED AND FASTED RATS

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The aim of this experiment was to analyse the correlation of hepatic glucose-6-phosphatase activity and liver glycogen content and blood glucose level in fed and 4-days fasted rats, during the acclimation to hyperthermic environment. The experiments were performed on adult Wistar male rats. Fed animals were divided into 8 groups: 1, 4, 7, 14, 21, 30 and 60 days of acclimation respectively, and control group. Four days fasted animals were grouped in: 0+4, 3+4, 10+4, 17+4, 26+4 and 56+4 days of acclimation, and control group. Heat acclimation was obtained in a special heat-chamber with regulated temperature of 35±1°C and air humidity of 20-30%. Control animals were kept at a room temperature of 20±2°C. In fed rats glucose-6-phosphatase activity was significantly decreased, regardless of duration of acclimation period. The correlation coefficient of glucose-6-phosphatase activity, depending on duration to hyperthermic environment was $r = -0.823$, $p<0.050$. Liver glycogen content was significantly increased after $14^{th}$ day of acclimation till the end of acclimation period ($r = +0.927$, $p<0.050$ depending on time). High environmental temperature caused significant decreasing of blood glucose level during whole acclimation period ($r = -0.956$, $p<0.050$). There is negative correlation of changes between glucose-6-phosphatase activity and liver glycogen content ($r = -0.730$, $p<0.050$) and positive correlation with blood glucose level ($r = 895$, $p<0.050$). In 4-days fasted animals, there were decreased activity of glucose-6-phosphatase ($r = -0.886$, $p<0.050$), and decreased liver glycogen content ($r = -0.896$, $p<0.050$) during whole acclimation period. There is positive correlation between glucose-6-phosphatase activity and liver glycogen content ($r = 0.619$, $p<0.050$). No significant changes in blood glucose level were noticed in fasted groups during acclimation to high temperature.

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## UTJECAJ SADRŽAJA PROTEINA I LIPIDA U HRANI NA RAST EUROPSKOG SOMA (*Silurus glanis)*

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Cilj istraživanja je bio odrediti sadržaj proteina i lipida u paletama sirove riblje hrane, nužan za optimalni rast europskog soma *(Silurus glanis).* U svaki od ukupno 20 kaveza postavljenih na početku pokusa, smješteno je 30 mladoh somova. Ribe u svakom kavezu primale su drugačiju hrane tijekom 98 dana, pri povoljnim fizikalnim i kemijskim uvjetima vode. Količina proteina u hrani bila je od 37,5 do 45%, dok je količina lipida, dodanih u obliku sojinog ulja, bila od 3 do 11%. Ulje je sadržavalo odgovarajuću koncentraciju w-masnih kiselina, nužnu za rast riba. Najvažniji indikatori rasta određeni na kraju pokusa bili su: ukupni tjelesni prirast (total body gain, TBG), specifični prirast (specific growth rate, SGR) i stupanj konverzije hrane (feed conversion ratio, FCR). Za svaki parametar rasta pokazana je značajna statistička razlika između pokusnih grupa ($P<0,001$) na kraju pokusa. Oboje, i proteini i lipidi, utjecali su na faktore rasta, ali na različit način. Dodatne analize – izračunavanje postotka promjene u ovisnosti o omjeru lipida i proteina u hrani, kao i bivarijantna analiza površina – omogućili su točno prepoznavanje najekonomičnije kombinacije: 39,5% proteina + 9% lipida. 9% sojinog ulja dodanog ribljoj hrani smanjuje potrebnu količinu proteina u hrani za 5,5% uz identične efekte na rast soma, te tako smanjuje troškove uzgoja ribe.

## MUTUAL INFLUENCE OF PROTEIN AND LIPID FEED CONTENT ON EUROPEAN CATFISH (*Silurus glanis)* GROWTH

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We wished to determine protein and lipid content in pelleted raw fish food, necessary for optimal growth of European catfish *(Silurus glanis).* Experiments were set up in 20 cages, each holding 30 young catfish. Fishes in each cage received a different food combination over a 98-day period at favourable physi-

cal and chemical water conditions. Food protein content varied between 37.5 and 45%, while lipid content, added in the form of soybean oil, varied between 3 and 11%. The oil contained an adequate w - fatty acids concentration, necessary for fish growth. The main growth indicators determined at the end of experiment were total body gain (TBG), specific growth rate (SGR) and feed conversion ratio (FCR). There was a high statistical difference among the experimental groups receiving variable food combinations for each growth parameter (P<0,001). Both, protein and lipid food content affected growth parameters, but in a different manner. Further analysis – percentage of change depending on lipid to protein ratio and bivariate surface analysis – allowed us to recognize the most economical combination: 39.5% protein + 9% lipid content. The addition of 9% soybean oil to the fish food reduces the necessary protein concentration by 5.5%, with resulting identical catfish growth effects.

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## HISTOLOŠKE OSOBITOSTI GUŠTERAČE NEKIH VRSTA DUPINA

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Gušterača istraživanih vrsta dupina svojom morfologijom odgovara gušterači u sisavaca općenito. Dorzalnim dijelom povezana je naborom potrbušnice s uzlaznim kolonom i jetrom, a ventralnim dijelom leži u mezostrukturi dvanaesnika. Histološki je to miješana egzokrina i endokrina žlijezda. Egzokrini dio je složena acinusna žlijezda, a otočići endokrinog tkiva su manje nakupine stanica raspoređene između egzokrinih acinusa. Kako u nama dostupnoj literaturi nije opisana građa Langerhansovih otočića gušterače dupina i raspored pojedinih vrsta endokrinih stanica u njima, obavljeno je ovo istraživanje. Istraživanje je obavljeno na ukupno 7 životinja od čega su 4 dobri dupini *(Tursiops truncatus)* i 3 plavobijela dupina *(Stenella coeruleboalba)*. Uzorci tkiva obrađeni su standardnim histološkim tehnikama, bojenje hemalaunom i eozinom za prikaz osnovne građe te Azan bojenje i Anilin blue-orange G bojenje (modificirano bojenje po Malloryju) za prikaz endokrinih stanica gušterače. Osnovna histološka građa gušterače istraživanih dupina odgovara onoj u kopnenih sisavaca. Otočići egzokrinih stanica su sitniji, ali zato brojniji nego je to slučaj u kopnenih sisavaca. Unutar ovih otočića, primjenom Azan metode bojenja, mogu se razlikovati tri vrste

stanica, dok se modificiranom Malloryjevom metodom jasno razlikuju dvije vrste endokrinih stanica. Pri tome su stanice s crveno-narančasto obojenom citoplazmom (po Malloryju) smještene periferno i odgovarale bi A-stanicama endokrinog dijela gušterače. Stanice s plavo obojenom citoplazmom (po Malloryju) smještene su centralno unutar Langerhansovog otočića i odgovarale bi B-stanicama endokrinog dijela gušterače. Azan metodom bojenja dobiven je isti raspored spomenutih stanica.

## HISTOLOGICAL CHARACTERISTICS OF THE PANCREAS OF SOME DOLPHIN SPECIES

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Pancreas morphology of the investigated dolphins is generally similar to the pancreas of other mammalian specieses. Dorsal part of pancreas is connected to the ascending colon and liver with peritoneal fold. Ventral part lies in the mesoduodenum. Histologically, it is a mixed exocrine and endocrine gland. Exocrine part is complexed acinus gland, and islets of the endocrine tissue are disseminated between exocrine acinuses. Histological structure of the Langerhans islets and arrangement of different types of endocrine cells in dolphins pancreas wasn't described in accessible literature, so we made this investigation. Investigation was done at 7 animals: 4 bottlenosed dolphins *(Tursiops truncatus)* and 3 striped dolphins *(Stenella coeruleboalba)*. Tissue samples were processed by standard histological techniques, staining with hematoxilin and eosin for general morphology, and Azan staining and Anilin blue-orange G staining (variety of the Mallory staining) for showing of the pancreas endocrine cells. General histological structure of pancreas in the investigated dolphins is similar to those in the terestrial mammals but endocrine islets are smaller and mach numerous. With Azan staining can be different three type of cells inside of endocrine islets, and with modifficated Mallory staining can be different clearly two cells types. Cells with red-orange stained cytoplasm (Mallory) are peripherally placed and it can be A-cells of the endocrine part of pancreas. Cells with blue stained cytoplasm (Mallory) are placed centrally inside of Langerhans insula and that can be B-cells of the endocrine part of pancreas. The same cell ordering was produced with Azan staining.

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# UČINAK NOVOG NUKLEOZIDNOG ANALOGA TROXACITABINE (BCH-4556) I STI-571 PROTIV HUMANIH LEUKEMIJSKIH STANICA NA MODELU STRANOG PRESATKA

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Troxacitabine (b-L-dioxolane cytidine, BCH-4556, Troxatyl, BioChem Pharma Inc.) je novi nukleozidni analog koji u pokusima pokazuje snažnu antitumorsku aktivnost na leukemije i solidne tumore. Budući da je troxacitabin analog citidina nalik Ara-C (1-b-D-arbinofuranosylcytosine), koji se trenutno rabi u obradi akutne mijeloične leukemije, istražili smo učinkovitost BCH-4556 na CML stanice u uvjetima *in vitro* i *in vivo*. Istražili smo učinak BCH-4556 samog i združenog s Ara-C, NSC 680410 ili STI-571 na AML i CML stanične linije senzitivne i rezistentne na STI-571. Antiproliferativni učinak BCH-4556 je mjeren MTS metodom i metodom klonalnog rasta. Apoptoza je praćena flurometričkom analizom mjerenjem aktivnosti 3/7 kaspaza. U *in vivo* pokusima pratili smo antileukemijsku aktivnost BCH-4556 u odnosu na aktivnost BCH-4556 + STI-571 na modelu stranog presatka u ICR SCID miševima nositeljima humanih leukemijskih stanica. Antileukemijiska aktivnost oba lijeka procijenjena je mjerenjem njihovog učinka na postotak preživljavanja obrađenih miševa. U uvjetima *in vitro* broj vijabilnih AML ili CML stanica je reduciran s BCH-4556 ovisno o dozi. $IC_{50}$ u šest različitih staničnih linija kultiviranih s BCH-4556 tijekom tri dana je 0.5 do 1 mM. Učinak BCH-4556 s NSC-680410, STI-571 ili Ara-C ne pokazuje dosljedno dodatni učinak na smrtnost stanica. Istovremena obrada KBM-7 i ili KBM-7R stanica s BCH-4556 i STI-571 pokazuje povećan apoptotički odgovor u odnosu na učinak pojedinačnog lijeka. Međutim, ista kombinacija nema učinak na apoptozu u KBM-5 ili KBM-5R stanicama. Metodom klonalnog rasta nije utvrđena selektivnost BCH-4556 za CML progenitore u odnosu na kontrolu. U in vivo studiji BCH-4556 dat intraperitonealno u dozi 5, 10, 20 ili 25 mg/kg tijekom 5 dana bio je izrazito učinkovit na leukemijske stanice KBM-5 i KBM-5R (STI-571 rezistentne stanice) na modelu ICR SCID miševa kao nositelja leukemijskih stanica ljudi. Preživljavanje SCID miševa nositelja KBM-5 ili KBM-5 leukemijskih stanica obrađenih s BCH-4556 bilo je produženo u usporedbi s kontrolom i T/C vrijednosti bile su od 134% do 169% i 114% do 157%. Obrada SCID miševa nositelja KBM-5 leukemijskih stanica s BCH-4556 (10 ili 25 mg/kg) tijekom 5 dana i sa STI-571 (50 mg/kg) tijekom 10 dana rezultirala je dodatnim povećanjem postotka preživljavanja za 29 do 74% u odnosu na sam BCH-4556. Naši podaci ukazuju da BCH-4556 ima značajnu antileukemijsku aktivnost ne samo na AML, nego također i na CML leukemijske stanice koje su rezistentne na STI-571.

# EFFECT OF A NOVEL NUCLEOSIDE ANALOGUE TROXACITABINE (CH-4556) AND STI-571 AGAINST LEUKEMIC HUMAN TUMOR XENOGRAFTS

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Troxacitabine (b-L-dioxolane cytidine, BCH-4556, Troxatyl, BioChem Pharma Inc.) is a novel nucleoside analogue, which in experiments demonstrated potent antitumor activity both leukemias and solid tumors. Since troxacitabine is a cytidine analogue like Ara-C (1-b-D-arbinofuranosylcytosine), witch is currently used in treatment of acute myelogenous leukemia; we extended the efficacy validation of BCH-4556 to CML cells both in vitro and in vivo. We investigated the effect of BCH-4556 alone and combination with Ara-C, NSC-680410, or STI-571 on AML and CML cell lines sensitive and resistant to STI-571. The antiproliferative activity of BCH-4556 was evaluated using MTS and colony-forming unit assay. Apoptosis was assessed by fluorometric assay for caspase-3/7 activity. For in vivo studies, we compared antileukemic activity of BCH-456 with that of BCH-4556 + STI-571 in human leukemia xenograft models. The antileukemic activity of both drugs was evaluated by measurement of their effect on the percent increased life span. In vitro, the number of viable AML or CML cells was reduced at BCH-4556 in dose-dependent concentrations. The $IC_{50}$ in six different cell lines treated for 3 days was in the 0.5 to 1 mM concentration interval. The combined treatment with NSC-680410, STI-571, or Ara-C showed no consistent additional effect with regard to induced cell death. Concurrent treatment with the combination of BSC-4556 and STI-571 causes a greater apoptotic response than treatment with either agent alone in KBM-7 and KBM-7R cells, but did not in KBM-5 or KBM-5R cells. Clonogenic assay did not demonstrate selectivity of BCH-4556 for CML progenitors than that of control. In vivo studies, BCH-4556 was administrated i.p. to the animals at doses of 5, 10, 20, or 25 mg/kg on a daily x 5 regimen. BCH-4556 was very effective in human tumor xenograft models, including KBM-5 or KBM-5R (STI-571-resistant) cells. The survival time of SCID mice bearing KBM-5 or KBM-5R leukemia cells treated with BCH-4556 was extended as compared to control and T/C values ranged from 134% to 169% or 114 to 157, respectively. Sequential treatment of SCID mice bearing KBM-5 leukemia cells with BSC- 4556 (10 or 25 mg/kg) for 5 days and after with STI-571 (50 mg/kg) for 10 days resulted in additive outcome for 29 to 74% versus troxatyl alone. Our findings indicate that BSH-4556 has significant antileukemic activity not only to AML but also in CML which have developed resistance to STI-571.

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## UČINAK ADOFASTINA (NSC 680410) NA AKUTNU I KRONIČNU MIJELOIČNU LEUKEMIJU

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BCR/ABL, konstantno aktivirana tirozin kinaza, je onkogen koji uzrokuje Philadelphia –kromosom pozitivnu ($Ph^+$) leukemiju. STI-571, kompetitivni inhibitor za ATP-vezno mjesto BCR/ABL, pokazuje visoku aktivnost na ovaj tip leukemije. Ipak većina pacijenata s poodmaklom bolešću vraća se u prvobitno stanje usprkos obradi sa STI-571. U ovom istraživanju procijenili smo učinak NSC 680410 na proliferaciju apoptozu i aktivnost tirozin kinaze u Bcr/Abl pozitivnim staničnim linijama ($Ph^+$; $p^{210\ Bcr/Abl}$ KBM-5, $p^{210\ Bcr/Abl}$ KBM-7, $p^{210\ Bcr/Abl}$ K562), ili $Ph^-$ (AML-2, AML-3) staničnim linijama i procijenili selektivnost NSC 680410 na primarne CML ili AML stanice blasta. *In vitro* inhibitorni učinak NSC 680410 na rast mijeloičnih stanica utvrđen je MTS metodom i metodom klonalnog rasta.Uporabom Apo-One™ Homogeneous Caspase 3/7-assay kit mjerena je kaspazna aktivnost. Promjena ekspresije proteina Bcr/Abl i fosforilizacija istog utvrđena je western blotting analizom. NSC 680410 reducira rast i preživljavanje većine CML i AML leukemijskih staničnih linija u koncentraciji između 0.5 i 1 mM, osim u stanicama K562 ($IC_{50}$ ~13 mM). Nadalje, učinkovitost NSC 680410 u STI-571-senzitivnim i rezistentnim stanicama u sličnim koncentracijskim vrijednostima ukazuje na nedostatak križne rezistencije između dva lijeka. Istovremena ili obrada u nizu CML ili AML stanica s NSC 680410 i drugim kemoterapeutskim tvarima ima aditivni učinak na rast stanica. Metodom klonalnog rasta potvrđena je selektivnost NSC 680410 za CML i AML progenitore u odnosu na kontrolu. NSC 680410 pobuđuje apoptozu u $Ph^+$ i $Ph^-$ staničnim linijama ovisno o dozi i vrermenu. Isti učinak neovisan je o aktivnosti Bcr/Abl kinaza. Obrada stanica s NSC 680410 pokazuje postupno smanjenje fosforilizacije tirozina u Bcr/Abl i drugim polipeptidima ovisno o vremenu i dozi. NSC 680410 je obećavajući lijek za CML i AML pacijente, posebice za CML pacijente rezistentne na STI -571.

## EFFECTS OF ADAPHOSTIN (NSC 680410) ON CHRONIC AND ACUTE MYELOID LEUKEMIA

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*BCR/ABL*, a constitutively activated tyrosine kinase, is the oncogene that causes Philadelphia-chromosome-positive ($Ph^+$) leukemia. STI-571, a competitive inhibitor at the ATP-binding site of *BCR/ABL*, has been shown to have high activity in this type of leukemia. However, most patients with advanced disease relapse despite continued treatment with STI-571. In the present study, we evaluated in vitro effects of adaphostin (NSC 680410) on proliferation, apoptosis and tyrosine kinase activity in Bcr/Abl positive cell lines ($Ph^+$; $p^{210\ Bcr/Abl}$ KBM-5, $p^{210\ Bcr/Abl}$ KBM-7, $p^{210\ Bcr/Abl}$ K562), or $Ph^-$ (AML-2, AML-3) cell lines and assessed selectivity of NSC 680410 on primary CML or AML blast cells. *In vitro* growth inhibition effect of NSC on myeloid cells was determined by measuring MTS or with clonogenic assay. Caspase activity was measured with the Apo-One™ Homogeneous Caspase 3/7-assay kit. The modulation of Bcr/Abl protein expression and phosphorylation was performed by western blotting analysis. In our in vitro studies, NSC 680410 reduced both growth and survival of the most CML and AML leukemia cell lines at concentration between 0.5 and 1mM, except K562 ($IC_{50}$ ~13 1mM). Moreover, NSC 680410 was effective in both STI-571-sensitive and –resistant cells at the similar concentration range, suggesting lack of cross-resistance between the two drugs. Simultaneous or sequential treatment CML or AML cells with NSC 680410 and other chemotherapeutic agents resulted in additive effect on the cell growth. Clonogenic assay demonstrated selectivity of NSC for CML or AML progenitors, unlike that of the control. NSC 680410 induced apoptosis in $Ph^+$ and $Ph^-$ cell lines in dose- and time-dependent manner. This effect was independent of Bcr/Abl kinase activity. In brief, NSC 680410 treated cells showed gradual and progressive dose- and time-dependent decline in phosphorilation of Bcr/Abl and other tyrosine phosphorilated polypeptides. In conclusion, NSC 680410 appears to be very promising new drug for use in the treatment of CML and AML, and could potentially be explored as a useful approach in patients with CML whose disease is resistant to STI 571.

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## HEMOCITI VRSTE *Astacus astacus* IZ JEZERA U OKOLICI VELIKE GORICE

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Istražili smo svojstva i broj hemocita slatkovodnih rakova *Astacus astacus* iz Crnog jezera i Vukovine u okolici Velike Gorice ulovljenih u proljeće 2001. i predložili način bojanja i klasifikaciju. U rakova postoje tri osnovna oblika hemocita: hijalinociti koji sudjeluju u koagulaciju hemolimfe i otvrdnuću kutikule tijekom presvlačenja te granulociti i semigranulociti koji su uključeni u fagocitozu i enkapsulaciju stranih čestica

te aktivaciju profenoloksidaze (proPO sustav) važne u obrambenom mehanizmu u rakova. Ukupan broj hemocita (THC) utvrđen je brojanjem neobojenih stanica u hemolimfi razrijeđenoj u omjeru 1:1 s EDTA antikoagulansom u Neubauerovoj komorici. Za tri uzorka iz Crnog jezera THC se kreće između $2,35x10^5$ i $5,39x10^5$/ml hemolimfe, a za 24 uzorka iz Vukovine u rasponu od $1,08x10^5$ do $3,20x10^6$/ml. Diferencijalni broj hemocita (DHC) utvrđen je na razmazima hemolimfe fiksirane 10%-tnim formalinom u omjeru 3:1 i obojanim Wright-ovom bojom. Prebrojano je ukupno 200 stanica na jednom razmazu svakog uzorka hemolimfe. Udio hijalinocita kreće se za uzorke iz oba staništa između 70 i 80%, udio granulocita je između 10 i 17%, a semigranulocita između 7 i 12%. Razmazi su također obojani Giemsom i Crystal violet bojom, no Wrightova boja se pokazala specifičnom za granula granulocita i omogućila je pouzdanije razlikovanje dvije podskupine ovih hemocita. Na razmazima hemolimfe standardnim citokemijskim metodama dokazana je aktivnost nekih lizosomalnih enzima (kisela fosfataza i fenoloksidaza) u granulama granulocita.

## HEMOCYTES OF THE *Astacus astacus* SPECIES FROM THE LAKES NEAR VELIKA GORICA

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We investigated features and number of the hemocytes of the freshwater crayfish *Astacus astacus* from the lakes Crno and Vukovina near Velika Gorica and suggested method of their dyeing and classification. The research was conducted in the spring 2001. There are three basic types of hemocytes: hyalinocyte which contribute to hemolymph clotting and hardening of cuticle at molt and granulocytes and semigranulocytes involved in phagocytosis, encapsulation of foreign particles and activation proPO defense mechanism of the crayfish. Total hemocyte count (THC) was determinated in Neubauer chamber by counting unstained cells in hemolymph diluted with EDTA anticoagulant in 1:1 ratio. For three samples from the lake Crno THC were between $2,35x10^5$ and $5,39x10^5$ /ml hemolymph, and for 24 samples from the lake Vukovina THC were between $1,08x10^5$ and $3,20x10^6$/ml. Differential hemocyte count (DHC) was determinated on the hemolymph smears fixed with the 10% formaline (hemolymph-formaline ratio was 3:1, respectively) and dyed with the Wright stain. On one smear of each hemolymph sample we counted 200 cells in total. Hyalinocyte portion for the samples from the both lakes was between 70 and 80%, while the granulocyte portion was between 10 and 17% and semigranulocyte was between 7 and 12%. Smears were also dyed with the Giemsa and Crystal violet but the Wright stain makes better differentiation of the two subgroups of these hemocytes because it specificaly dyed granula of the granulocytes. We applied some standard cytochemical methods on the hemolymph smears and found positive activity of some lysosomal enzymes (acid phosphatase and phenoloxidase) in the granula of the granulocytes.

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## GRAĐA LIMFNIH ČVOROVA DOBROG DUPINA (*Tursiops truncatus*) I PLAVOBIJELOG DUPINA (*Stenella coeruleoalba*) IZ JADRANSKOG MORA

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Limfni čvorovi su inkapsulirani organi uklopljeni u tok limfnih žila. Vezivnotkivna čahura okružuje limfni čvor, a parenhim je podijeljen na vanjski sloj, koru i unutrašnji sloj, srž. Kora se sastoji od limfnih čvorića okruženih difuznim limfatičnim tkivom koje se u obliku sržnih tračaka proteže u srž limfnog čvora. Aferentne limfne žile prolaze kroz čahuru limfnog čvora i izlijevaju limfu u supkapsularni sinus, a potom, limfa kroz intermedijalni sinus utiče u medularni sinus. Eferentne limfne žile u hilusu limfnog čvora odvode limfu. Količina i raspored tkiva kore i srži limfnog čvora može znatno varirati između jedinki pojedine vrste životinja vezano za stadij aktivnosti i smještaj limfnog čvora. Građa limfnih čvorova može se razlikovati i između pojedinih vrsta životinja. Limfni čvorovi svinje su atipični s inverznim smještajem kore i srži i posljedično tome obrnutim tokom limfe. Kako su podaci o građi limfnih čvorova životinja iz reda kitova (Cetacea) nedostatni i vrlo neprecizni pa se čak navodi nejasna granica između kore i srži kao i raspored limfnih čvorića kroz cijeli limfni čvor odlučili smo histološki istražiti mezenterijalne i bifurkacione limfne čvorove u dobrog dupina i plavobijelog dupina iz Jadranskog mora. Dobiveni rezultati govore da građa većine istraženih limfnih čvorova odgovara uobičajenoj građi tog organa u kopnenih sisavaca, međutim bifurkacioni limfni čvor u plavobijelog dupina je potpuno jasan inverzni tip limfnog čvora.

## MORPHOLOGY OF THE LYMPH NODES OF THE BOTTLENOSE DOLPHIN (*Tursiops truncatus*) AND STRIPED DOLPHIN (*Stenella coeruleoalba*) FROM THE ADRIATIC SEA

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Lymph nodes are encapsulated organs occurring in series along the course of lymphatic vessels. The connective tissue capsule covers the lymph node and parenchyma is organised into an outer cortex and inner medulla. The cortex consists of lymphatic nodules surrounded by diffuse lymphatic tissue. Extensions of latter tissue into the medulla are called medullary cords. The afferent lymphatic vessels penetrate the capsule to join the subcapsular sinus. From there lymph flows through intermediate sinuses to medullary sinuses. Efferent lymphatic vessels at the hilus drain the node. The amount and arrangement of cortical and medullary tissue can vary within the same species depending on their state of activity and on location of lymph nodes in the body. The general organisation of lymph nodes can vary from species to species. The lymph nodes of the pig are atypical with the location of the cortical and medullary tissue, as well as the flow of the lymph being reversed. We decided to investigate general structure of the mesenteric and bifurcation lymph nodes of the bottlenosed dolphin and striped dolphin from the Adriatic Sea on account of insufficient and indistinct information about histological feature of the lymph nodes of cetaceans. It is even mentioned unclear boundary between cortical and medullary tissue and the arrangement of lymph nodules through the whole organ. The obtained results pointed that general organization most of the examined lymph nodes is analogous to the typical lymph nodes of terrestrial mammals but bifurcation lymph node of the striped dolphin is obvious reverse.

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## HISTOPATHOLOGICAL ANALYSIS OF LIVER IN FISH (*Barbus meridionalis petenyi* HECKEL) IN RESERVOIR "TREBENISTA"

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The *Barbus meridionalis petenyi* Heck. is a typical benthophagous fish, feeding on zoobentos and plant components and thanks to their capabilities for sensitivity to the changes in surrounding medium are ideal object that indicate the health of the aquatic ecosystems. The teleostei fish liver is susceptible to numerous, both toxic and metabolic disturbances. Liver pieces of 40 individuals collected from reservoir ,,Trebenista" in July 1999, were excised and processed for standard histopathological analysis. The obtained results revealed pathological changes in the liver tissue including parenchyma cell necrosis associated with hemmorhagia.

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## ACTIVITY OF HEPATIC PYRUVATE KINASE AND PHOSPHOENOLPYRUVAT CARBOXYKINASE IN RATS DURING THE ACCLIMATION TO HYPERTHERMIC ENVIRONMENT

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The effect of exposition to hyperthermic environment (35±1°C) on the activity of hepatic pyruvate kinase (PK) and phoshoenolpyruvate carboxykinase (PEPCK), liver glycogen content and blood glucose level in rats has been observed. Adult white Wistar female rats were used for this experiment. The animals were divided into 7 experimental groups (1, 4, 7, 14, 21, 30 and 60 days) respectively, depending on the time of the exposure to hyperthermic environment. One group was kept at room temperature (20±2°C) and it used as a control. The heat-acclimation was performed in special heated chamber with regulated temperature of 35±1°C and air humidity of 20-30%. In experimental rats, the activity of hepatic PEPCK and glycogen content are significantly increased regardless of duration of exposition to high environmental temperature (26.3% and 22.7%, $p<0.050$, respectively), while the PK activity and blood glucose level are significantly decreased (-16.8% and –23.2%, $p<0.050$, respectively). The elevating of PEPCK activity and glycogen content, and the declining of PK activity in liver, imply that in heat-acclimated rats processes of gluconeogenesis are increased and glycolysis is decreased.

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## SOCIO-ECONOMIC FACTORS INFLUENCE ON GROWTH AND DEVELOPMENT OF THE BOYS FROM THE REGION OF TUZLA (BOSNIA AND HERZEGOVINA)

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Growth as the most important phase of ontogenesis represents the mirror of a society state and that is why it is a central subject of anthropological re-

searches. Social factors, before all the economic ones, have a strong influence on the physical and neuropsychic development and working capability of children. this research aimed to investigate the socio-economic factors influence (parents' living standard, number of family members) on some anthropometric parameters like: body height, body mass, chest circumference, upper arm circumference, upper leg circumference, arm length, leg length, pelvis width, shoulders width. investigations were performed on the sample of 698 boys aged 11 to 16 years. Anthropometric measurements were done according to IBP. The results show that socio-economic status of the parents is in the positive correlation to the mean values of the investigated anthropometric properties, but in the negative correlation to the number of children in a family.

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## BIRTH ORDER INFLUENCE ON THE ANTHROPOMETRIC PROPERTIES OF THE BOYS FROM THE REGION OF TUZLA (BOSNIA AND HERZEGOVINA)

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Birth order and its effect on growth and development of children and youths have been rare subject of researches so far. The objective of this research was an analysis of the birth order effects on some anthropometric properties of the boys of 11-16 years old on the sample of 681 boys from the Tuzla region. As this sample involved very few boys born as the third, forth, or fifth child, we decided to analyze only differences in the mean values for some anthropometric properties between the boys of the first and the second birth order. Measurements were done according to IBP and the following parameters were investigated: body height, body mass, chest circumference, upper arm circumference, upper leg circumference, sitting height, arm length, leg length, pelvis width, shoulders width, length and width of head. We established that first-born boys in most generations have larger mean values for most anthropometric variables in comparison to the second born boys.

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# MOTHER'S AGE INFLUENCE ON SOME ANTHROPOMETRIC PROPERTIES OF THE BOYS FROM THE REGION OF TUZLA (BOSNIA AND HERZEGOVINA)

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The objective of this research was an analysis of mother's age influence on some anthropometric properties of their sons of 11-16 years old, on the sample of 726 boys. The sample was divided into two subsamples according to mothers' age: subsample that included mothers aged under 25 years (N=535 ); subsample that included mothers aged over 25 years (N=191). Measurements were performed according to IBP-u and the following parameters were investigated: body height, body mass, chest circumference, upper arm circumference, upper leg circumference, sitting height, arm length, leg length, pelvis width, shoulders width, length and width of head. We established that younger mothers' children had larger mean values for most parameters investigated in the period of prepuberty, than the older mothers' children. In the course of adolescent rise the situation was inverse, but in the age of 16 years, mean values for most parameters in both groups of boys were approximately equal, or insignificantly larger in the younger mothers' sons.

IL-16

# VAŽNOST I ZNAČAJKE IHTIOFAUNE SLATKOVODNIH RIBA HRVATSKE

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Slatkovodna ihtiofauna Hrvatske nedovoljno je istražena, premda smo po raznolikosti, odnosno brojem vrsta i endema jedno od ihtiološki najzanimljivijih područja Europe. Zoogeografska analiza naše ihtiofaune učinjena je tek djelomično. Veliki prostori nisu obuhvaćeni redovnim istraživanjima, tako da za mnoge vrste nisu utvrđeni areali rasprostranjenja. Manje poznate vrste i podvrste nisu do kraja opisane, a njihova biologija potpuno je nepoznata. Ukupan broj vrsta i podvrsta riba koje dolaze u našim slatkim vodama je oko 149. Oko 21 vrsta žive i u slatkoj i u morskoj vodi. Posljedica je to zemljopisnog položaja Hrvatske na dva riječna slijeva, jadranskom i crnomorskom. U zadnjih 10 godina opisano je oko 6 potpuno novih vrsta slatkovodnih riba, a taj broj zasigurno nije konačan. Premda je važnost ribe kao izvora proteina u ljudskoj prehrani ogromna i ekonomski vrlo realna, ostale funkcije ribljih zajednica daleko su važnije. Oko 40 vrsta dio su raznih tipova ribolovne privrede. Danas se smatra da je ekološka kvaliteta nekog vodenog ekosustava posljedica prostorno-vremenske integracije različitih fizikalnih, kemijskih i bioloških čimbenika. Uočivši ove značajke, Europska Zajednica je u cilju poboljšanja zaštite, dugotrajnog korištenja i odgovarajućeg monitoringa vodenih ekosustava uvela značajke riblje zajednice, kao komplementarne metode dosadašnjim metodama tradicionalne procjene kvalitete voda. Mnogi čimbenici utječu na ribe na različite načine. Najznačajniji su onečišćenja, melioracije i regulacije vodotoka, unos alohtonih vrsta, ogromne potrebe modernog čovjeka za tehničkom i pitkom vodom, prelov i turizam. Novoizgrađene brane na rijekama sprječavaju migracije na tisućljetna mrijestilišta. Povećanje količine nutrijenata i unos novih vrsta dovodi do smanjenja brojnosti riba, naročito rijetkih i osjetljivijih vrsta. Negativni utjecaji na ribe slatkih voda u zadnjih nekoliko desetljeća toliko su jaki da su mnoge vrste gotovo nestale ili su im se populacije jako smanjile. Oko 5 vrsta riba nestale su ili gotovo 40 godina o njima nema podataka, 32 vrste riba su jako ugrožene i ako u skoroj budućnosti ne dobiju zaštitu, nestat će iz naših krajeva. O statusu i biologiji 14 vrsta riba imamo nedovoljno podataka. Jedna od najozbiljnijih posljedica antropogenog utjecaja na ribe je unos stranih vrsta u neko područje. Ihtiofauna naše zemlje sadrži oko 15 vrsta unesenih vrsta riba koje su aklimatizirane na naše područje. Promjene koje su ove vrste izazvale u autohtonim zajednicama vrlo je teško kvantitativno procijeniti. Ako se održi u novoj sredini, novounesena vrsta može značajno promijeniti zajednicu riba, što često dovodi do nestajanja osjetljivih domaćih vrsta.

# IMPORTANCE AND CHARACTERISTICS OF CROATIAN FRESHWATER FISH

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Knowledge of freshwater fish of Croatia is insufficient despite that the biodiversity and number of endemic forms in the Dalmatian ichthyologic region represents one of the most diverse parts of Europe. Zoogeographical analysis of Croatian ichthyofauna was carried out only partially. Extensive parts of Croatian territory are not included in regular investigations and therefore areals of distribution for many species are still unknown. Some rare and data deficient species, subspecies and races are not described in detail and their ecology is completely unknown. The total number of fish species in the freshwaters of Croatia is 149. Twenty-one species live in freshwater as well as in saltwater habitats. This is a consequence of the geographical position of Croatia on two watersheds: Danubian and Adriatic. Over the last decade six new freshwater fish species have been described, and this number is not final. Even though freshwater fish are now an important source of proteins for human consumption as well as their economical values, other functions of fish communities are not less important. About 40 fish species are part of the fishing industry. The ecological quality of certain water ecosystems is a consequence of the temporal and spatial integration of different physical, chemical and biological factors. To improve conservation, long-term use and monitoring of water ecosystems, the European Community has added and implemented characteristics of fish communities as complementary methods to traditional water quality assessment. Fish are influenced by many environmental factors, the most important being pollution, river regulations, damming, introduction of alien species, the huge demand of modern society for technical and drinking water, overfishing and tourism. Dams prevent migrations from moving towards spawning grounds. Increased concentrations of nutrients and the introduction of alien species decrease fish diversity, especially of rare and vulnerable species. Due to the negative influences on freshwater fish, over the last decades many species have disappeared or their populations significantly diminished. About 5 fish species either disappeared from Croatian waters or there were no data on them for more than 40 years. Thirty-two species are endangered. In the near future they could also disappear if conservation measures are not implemented. The status and biology of 14 rare fish species are data deficient. One of the most dramatic consequences of human impact on fish is the introduction of alien species in some regions. Fifteen fish are invasive alien species adapted to our region. The changes which those fish made in native communities are unpredictable.

OP-53

## EKOLOŠKE ZNAČAJKE POPULACIJA MOČVARNOG OKAŠA *Coenonympha oedippus* FABRICIUS, 1787 (Lepidoptera, Satyridae) U HRVATSKOJ

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Močvarni okaš *Coenonympha oedippus* jedan je od najugroženijih danjih leptira Europe, gdje je zbog nestanka staništa izumro s velikog broja lokaliteta. U Hrvatskoj je vrsta rasprostranjena na osam vlažnih livada sjeverne Istre. Otkrivena je 1999. na tri, a kasnije na još pet lokacija. Populacijska istraživanja obavljena su tijekom dvije sezone u lipnju i srpnju 2001. i 2002. Korištena je standardna tehnika markiranja i ponovnog ulova leptira na dvije lokacije uz dolinu rijeke Mirne i dvije sjeverno od sela Šterna. Svih 133 jedinki 2001. i 264 jedinke 2002. individualno je markirano. Utvrđene veličine populacija ne razlikuju se bitno za obje godine. Isto tako nije utvrđeno značajnije miješanje populacija, što govori o njihovoj izoliranosti. Odnos spolova mijenjao se tijekom sezone leta: u početku su prevladavali mužjaci dok su kasnije dominantne ženke. Isto je tako opažano različito ponašanje spolova: većina ženki je mirovala, dok su mužjaci uglavnom letjeli. Preliminarna istraživanja gustoće populacija i utvrđeni uvjeti staništa (zarastanje livada) ukazuju na potrebu aktivne zaštite. Zbog sve veće izolacije staništa predložene mjere zaštite, kao što je redovita košnja, nužne su za opstanak vrste *Coenonympha oedippus*.

## ECOLOGY OF FALSE RINGLET *Coenonympha oedippus* FABRICIUS, 1787 (Lepidoptera, Satyridae) IN CROATIA

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The False Ringlet is one of the most threatened butterflies in Europe. It has disappeared from many locations due to habitat loss. In Croatia, the species lives only on eight wet meadows of Northern Istria. It was first discovered in 1999 on three localities and latter it was found on five more. The population study was conducted during June and July seasons of 2001 and 2002. The standard mark-release-recapture techniques were used on two populations in Mirna valley and two northern of village Šterna. All of 133 captured individuals in 2001 and 264 in 2002 were uniquely marked. The number of established population sizes was similar for both years. There was no significant movement between populations, which shows the isolation. The sex ratio changed with brood aging: early in flight season males were in majority while females where predominant at the end of flight.

Between sexes different behaviour was observed, most of the females were observed resting and males perching. Preliminary investigations of population density and registered changes in environment (primarily meadow overgrowing with shrubs) indicate the need for conservation actions. Evidently the localities are more isolated each year so key priority actions such as regular and well-timed mowing are essential for the survival of *Coenonympha oedippus*.

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## SITNA TERESTRIČKA FAUNA PRETPLANINSKE BUKOVE ŠUME NA ZAVIŽANU (VELEBIT, HRVATSKA)

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Prvi puta sustavno je prikupljana fauna sitnih terestričkih životinja u pretplaninskoj šumi bukve s urezicom (*Homogyno alpinae - Fagetum sylvaticae*) na lokalitetu Zavižan na sjevernom Velebitu. Uz podatke koji su uz pomoć obitelji Vukušić povremeno prikupljani uz dom na Vučjaku od 1974. godine do danas, godine 1995. i 1996. primijenjena je modificirana metoda lovnih posuda s pregradama/usmjerivačima. Na NE padini Velikog Zavižana na visini od 1650 m n/m, na plohi od 0,07 ha prikupljen je gotovo potpuni sastav sitne terestričke faune uz samu gornju granicu ove najviše šumske zajednice. Za prikupljanje više od 70% ukupnog broja vrsta sitnih sisavaca bila su potrebna tri lova, a tek tijekom druge godine lova kvantitativno su se jasno diferencirale dominantne vrste. Uočene su godišnje fluktuacije pojedinih vrsta koje traže vremenski dulja opažanja. Od kralježnjaka je dominantna šumska rovka *Sorex araneus*, a od beskralježnjaka trčak *Nebria dahlii velebitica*. Od oko 50 dosad identificiranih vrsta iz 7 skupina životinja, čak 30 je po prvi puta zabilježeno za ovu zajednicu u Hrvatskoj. Uz raspravu o utjecaju metode na rezultate, zabilježena fauna je preliminarno uspoređena s poznatom faunom nekih drugih gorskih i pretplaninskih šumskih staništa.

## SMALL TERRESTRIAL FAUNA OF THE SUBALPINE BEECH FOREST ON ZAVIŽAN (MT VELEBIT, CROATIA)

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In the subalpine forest of beech with alpine coltsfoot (*Homogyno alpinae – Fagetum sylvaticae*) on the peak of Zavižan, north Mt Velebit, small terrestrial fauna was systematically collected for the first time. In conjunction with

data collected randomly with the help of the Vukušić family near the mountain hut on Vučjak near Zavižan ever since 1974, in 1995 and 1996 we applied the modified method of a Y-shaped pitfall-drift fence array. Almost the complete small terrestrial fauna of the upper border of the highest forest association on the NE slope of Veliki Zavižan peak at 1650 m a.s.l. was registered on the plot of 0.07 ha. Over 70% of the entire small mammal species were registered in three catches, and in the second year the dominant species could be clearly quantitatively differentiated. Annual fluctuations of certain species were registered; these could take longer to study. The common shrew *Sorex araneus* was dominant among the vertebrates and the ground beetle *Nebria dahlii velebitica* among invertebrates. Of the about 50 species identified so far from 7 animal groups, as many as 30 were registered for the first time for this association in Croatia. The impact of the methodology on the results is discussed and the results are preliminary compared with those for the fauna of other mountain and subalpine forest habitats.

OP-55

## STRUKTURA IHTIOFAUNE RIJEKE NERETVE

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Tijekom ihtioloških istraživanja ušća rijeke Neretve i njenih pritoka u Hrvatskoj zabilježene su 34 vrste riba iz 15 porodica. Najbrojnija porodica bila je porodica Cyprinidae zastupljena s 14 vrsta, a slijede ju porodice Gobiidae s tri vrste te Salmonidae i Mugilidae sa po dvije vrste, dok su sve preostale porodice zastupljene samo sa po jednom vrstom. Na različitim staništima dominiraju različite vrste, ali u čitavom uzorku najčešće vrste su: *Anguilla anguilla, Scardinius erythropthalmus scardafa, Rutilus basak, Atherina boyeri, Gasterosteus aculeatus, Gambusia affinis, Cobitis taenia narentana, Phoxinellus adspersus, Blenius fluviatilis* i *Knipowitschia punctatissima*. Predatorne vrste su vrlo rijetke, ako isključimo jegulju i relativno rijetku pastrvu. Riblja zajednica ušća Neretve obuhvaća 12 endemskih ribljih vrsta istočno-jadranskog slivnog područja među kojima su i *Chondrostoma kneri, Leuciscus svallize, Leuciscus microlepis, Paraphoxinus adspersus, Rutilus basak, Knipowitschia punctatissima croatica* i *Salmothymus obtusirostris.* Strane unesene vrste su: *Gambusia affinis, Ictalurus nebulosus, Carassius auratus gibelio, Lepomis gibbosus* i *Cyprinus carpio.* Shanon-Wienerov indeks raznolikosti za riblju zajednicu iznosi 3.731 (log e) od mogućeg maksimuma od 5.044 s ujednačenošću od 74 %. Od 34 ekološki obrađene vrste njih 13 obuhvaćeno je Aneksom III Bernske konvencije.

## STRUCTURE OF ICHTHYOFAUNA OF THE RIVER NERETVA

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Icthyological investigation of the delta and tributary of the river Neretva gave list of 34 fish species belonging to 15 families. The most dominant family was Cyprinidae with 14 species, the family Gobiidae with three species and Salmonidae and Mugilidae with two species, and the remaining families represented by one species. Different habitats have different dominant species but in general the most common species are: *Anguilla anguilla, Scardinius erythropthalmus scardafa, Rutilus basak, Atherina boyeri, Gasterosteus aculeatus, Gambusia affinis, Cobitis taenia narentana, Phoxinellus adspersus, Blenius fluviatilis,* and *Knipowitschia punctatissima.* Predatory species are very rare if we exclude *Anguilla anguilla* and comparatively rare *Salmo trutta*. The fish community of Neretva watershed is comprised of 12 endemic fish from the east Adriatic watershed as *Chondrostoma kneri, Leuciscus svallize, Leuciscus microlepis, Paraphoxinus adspersus, Rutilus basak, Knipowitschia punctatissima croatica* and *Salmothymus obtusirostris.* Introduced alien species are: *Gambusia affinis, Ictalurus nebulosus, Carassius auratus gibelio, Lepomis gibbosus* and *Cyprinus carpio.* The Shanon-Wiener diversity index for the fish community is 3.731 (log e) from a maximum of 5.044 with an evenness of 0.740. Community of 34 species is ecologically analyzed and 13 are on the Bern Convention Annex III.

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## ŠPILJSKA FAUNA PARKA PRIRODE BIOKOVO

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Biospeleološka istraživanja Biokova počinju u 20. stoljeću, a intenziviraju se početkom 21. stoljeća suradnjom Parka prirode i Hrvatskog biospeleološkog društva. U radu su predstavljeni rezultati dosadašnjih istraživanja. Obrađeno je preko 100 speleoloških objekata, većinom jama. Definirana su i fotografirana podzemna staništa, mjereni njihovi mikroklimatski parametri, te sakupljana kavernikolna fauna i vršeno njeno makrofotografiranje. Na osnovi ekologije staništa, kao i strukture podzemne faune utvrđeno je pet biokovskih pojaseva. U podzemnim staništima preteže kopnena fauna, dok rijetki nalazi vodene špiljske faune potječu iz primorskih krških vrela. Utvrđeno je oko 120 taksona različitog stupnja prilagođenosti na špiljske uvjete, od kojih oko 40 % predstavlja endeme, dok čak 25 taksona živi samo u

podzemlju Biokova: *Oligochaeta (1), Amphipoda (1), Pseudoscorpiones (4), Aranea (1), Chilopoda (1), Diplopoda (4) i Coleoptera (13).* Prisutna je endemna obitelj - *Biokoviellidae* (*Diplopoda*), kao i sedam endemnih rodova: *Protoneobisium* (*Pseudoscorpiones*), *Biokoviella (Diplopoda*), *Zariquieyella*, *Hoffmanella, Roubaliella* i *Radziella* (*Coleoptera*, *Cholevidae, Leptoderinae*) i *Biokovoaphaenopsis* (*Coleoptera, Carabidae, Trechinae*). U sakupljenom biološkom materijalu utvrđeni su taksoni novi za znanost, a dio materijala još čeka specijalističku obradu. Prema dosadašnjim rezultatima Biokovo uz Velebit predstavlja centar endemizma podzemne špiljske faune u Hrvatskoj. Predstoji daljnji rad na utvrđivanju biocenološke strukture i svih ekoloških specifičnosti podzemnih staništa Biokova. Ovu jedinstvenu vrijednost, danas uključenu u Park prirode Biokovo, potrebno je valorizirati i zaštititi, te uključiti u edukativne i promotivne aktivnosti.

## CAVE-DWELLING FAUNA OF THE NATURAL PARK BIOKOVO

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Biospeleological research of Biokovo Mt. began in the 20th century and has become more intensive at the beginning of the 21st century in cooperation with the Natural Park and Croatian Biospeleological Society. This work shows results of the research conducted so far. More than 100 speleological objects, mostly pits, have been researched. Underground habitats have been defined and photographed, ecological features measured and cavernicoulos fauna collected and macrophotographed. Based on habitats' ecology, as well as the structure of cavernicoluos fauna, five Biokovo belts have been defined.

Underground habitats are dominated by terrestrial fauna while rare findings of the aquatic fauna originate from the seaside karstic springs. About 120 taxons with different degrees of adaptability to the cave life have been detected: 40 % of them are endemic while 25 taxa are exclusively endemic for Biokovo: *Oligochaeta (1), Amphipoda (1), Aranea (1), Pseudoscorpiones (4), Chilopoda (1), Diplopoda (4) and Coleoptera (13).* There is one endemic family - *Biokoviellidae* (*Diplopoda*), as well as 7 endemic genera: *Protoneobisium* (*Pseudoscorpiones*), *Biokoviella (Diplopoda*), *Zariquieyella*, *Hoffmanella, Roubaliella* and *Radziella* (*Coleoptera, Cholevidae, Leptoderinae*) and *Biokovoaphaenopsis* (*Coleoptera, Carabidae, Trechinae*). In the collected material there have been new scientific discoveries of taxa, but a portion of the material is yet to be analyzed by the specialists. Biokovo Mt., together with Velebit Mt., represents a centre of endemismus of cave-dwelling fauna in Croatia. Future exploration is to be continued on the estimation of the biocenological structure and specific ecoparameters of underground habitats. This unique value should be valorized, conserved and included in the educative and promotional activity.

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## NOVOSTI U FAUNI PODZEMNIH KORNJAŠA (Insecta: Coleoptera) HRVATSKE

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Proteklo je četvrt stoljeća od vremena kada je tiskan prvi dodatak monografiji o podzemnim kornjašima Hrvatske. U međuvremenu rezultat intenzivnih istraživanja su brojne nove endemične vrste i čak novi rodovi. Uz pregled otkrića koja su tek dijelom taksonomski obrađena i objavljena, raspravlja se o njihovu doprinosu ekologiji i zoogeografiji krškog podzemlja Dinarida, te uspoređuje biodiverzitet hrvatskog dijela krša sa susjednim područjima. Ekološki su osobito značajni nalazi novootkrivenih svojti koje nastanjuju dosad slabo poznata podzemna mikrostaništa.

## NEWS IN CROATIAN FAUNA OF SUBTERRANEAN COLEOPTERAN BEETLES (Insecta, Coleoptera)

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It has been 25 years since the publication of the first supplement of the subterranean coleopteran beetles monograph. In the meantime, intensive research resulted in findings of many new endemic subspecies and species, even new genera. With a review of discoveries which are only partially taxonomically investigated and published, their contribution to ecology and zoogeography of the Dinaric karst underground is discussed. Biodiversity of the Croatian part of the karst is compared with the neighbouring karst areas. Findings of new taxa in poorly known underground microhabitats are of significant interest for cave ecology.

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## TERMALNA OGRANIČENJA TJELESNE TEMPERATURE, UPOTREBE MIKROSTANIŠTA I HRANJENJA KOD PRIMORSKE GUŠTERICE (*Podarcis sicula*)

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Istraživali smo učinak termalnih ograničenja na različite aspekte biologije primorske gušterice (*Podarcis sicula*) na otoku Velika Palagruža. Mjerili smo tjelesnu temperaturu ($T_b$) aktivnih guštera te sakupili podatke o različim aspektima njihova termoregulatornog ponašanja. Pomoću "fizičkog modela" guštera mjerili smo operativnu temperaturu ($T_e$) različitih staništa te procijenili raspon i distribuciju dostupnih termalnih mikrostaništa. Njihovu termalnu prikladnost procijenili smo mjereći stupanj sličnosti između $T_e$ i raspona tjelesnih temperatura koje gušteri postižu u laboratorijskom termogradijentu. U istraživanoj populaciji *P. sicula* tijekom mjeseca rujna, termalni uvjeti omogućavali su gušterima održavajnje visoke i konstantne $T_b$ tijekom dana, međutim termalna ograničenja različitog intenziteta bila su cjelodnevno prisutna. Prvo, u različitim vremenima samo ‹5 do 30 % dostupne površine nudilo je prikladne termalne uvjete. Stoga su gušteri mogli postići visoku $T_b$ u samo jednom djelu dostupnih mikrostaništa. Drugo, relativna distribucija termalno prikladnih mjesta kroz klimatski različita mikrostaništa također se mijenjala s vremenom. Sklonost prema klimatskom mikrostaništu predviđena je na temelju konceptualnog modela koji podrazumjeva da gušteri ograničavaju svoje aktivnosti na termalno pogodna mjesta, te da upotrebljavaju ta mjesta proporcionalno njihovoj raspoloživosti. Općenito, promjene u opaženim sklonostima za različita klimatska mikrostaništa podudaraju se sa predviđenim sklonostima. Prema tome, termalne značajke različitih klimatskih mikrostaništa određuju njihovu upotrebu u različita doba dana i zbog toga ograničavaju prostornu aktivnost guštera. Također smo otkrili da je broj pokušaja lova bio najviši u satima kada je relativno veliki udio staništa bio termalno prikladan. Uvjeti okoliša određuju vrijeme uloženo u termoregulaciju, te indirektno ograničavaju mogućnosti hranjenja.

## THERMAL CONSTRAINTS ON BODY TEMPERATURES, MICROHABITAT USAGE AND FORAGING IN THE ITALIAN WALL LIZARD (*Podarcis sicula*)

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We studied the impact of thermal constraints on the biology of the lizard *Podarcis sicula* on Velika Palagruža Island during late summer (September). We

gathered data on the $T_b$s of active lizards and on aspects of their thermoregulatory behaviour. The range and distribution of thermal microhabitats was assessed using "physical models" that measure the operative temperatures ($T_e$) at different sites. We estimated the thermal suitability of microhabitats by quantifying the extent of similarity between the $T_e$s and the range of body temperatures that lizards maintain a laboratory thermogradient. Thermal conditions enabled the lizards to maintain high and constant $T_b$s throughout the day. Nevertheless, thermal constraints were present in variable intensities over the course of the day. First, in different hours only <5 to 30% of the available surface offered suitable thermal conditions. Hence, at all times of day, lizards could attain high $T_b$s in only a fraction of the available microhabitats. Second, the relative distribution of thermally suitable sites over different solar microclimates also changed with time. We predicted microclimate preferences based on a conceptual model that assumed that lizards restrict their activities to thermally suitable sites, and that they use these sites in proportion to their availability. Overall, the hourly variation in observed preferences for different microclimates coincided well with the predicted preferences. Thus, thermal characteristics of different microclimates determined their usage at different times of the day and therefore constrained the spatial activities by the lizards. An additional finding was that the number of foraging strikes was highest during hours when a relatively high proportion of the habitat was thermally suitable. Thus, environmental conditions determine the time invested in thermoregulation, and thereby indirectly constrain opportunities to forage.

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## DISTRIBUTION OF PARASITES IN FREE-LIVING PIGEONS IN LJUBLJANA

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For the last several years a successful sterilization program of the urban pigeon *Columba livia domestica* with Ornisteril® (maize treated with progesterone in 0.01% concentration) was being undertaken in the Slovenian capital Ljubljana. In the year 2001 an parasitological research on the health status of free-living pigeons was included. With regard to the most common ectoparasites and endoparasites, a total of 214 pigeons were captured. Oropharyngeal swabs as well as samples of faeces and feather were taken from captured birds. *Trichomonas gallinae* was microscopically confirmed using oropharyngeal swabs in 62.1% of examined birds. *Eimeria spp.* was identified in 67.8%,

*Capillaria sp.* in 13.1% and *Ascaridia columbae* in 4.7% of faecal samples examined. In faeces of twenty-two pigeons (10.3%) two different endoparasites were found. In faeces of three pigeons (1.4%) three different endoparasites were found. Among fifthy-nine pigeons (27.6%) eimeria, capillaria and ascaridia parasites were not found. Approximate 1 to 20 visible ectoparasites were detected on spread wings, neck and cloaca region of infestated pigeon. Two species of ectoparasites, *Columbicola columbae* in 72.0% and *Campanulotes bidentatus compar* in 2.8%, were confirmed from feather samples. In four pigeons (1.9%) both species of ectoparasites were found and in 58 pigeons (27.1%) they didn't find any of them.

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## DINAMIKA I RASPROSTANJENOST ELEONORINOG SOKOLA (*Falco eleonorae* GENE, 1839) NA HRVATSKOM DIJELU JADRANA

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Autori su 1998. godine započeli prvo sustavno istraživanje eleonorinog sokola u Hrvatskoj. Otada se svake godine vrši monitoring ekoloških parametara najveće hrvatske kolonije – prebrojavanje odraslih ptica, registracija aktivnih gnijezda, promatranje ponašanja i prstenovanje mladih ptica. Također, prvi cenzus poduzet je 2000. godine i otad se redovito provodi. Registrirano je šest kolonija, od kojih su dvije bile potpuno nepoznate. Hrvatska populacija eleonorinoih sokola smještena je na nekoliko srednjodalmatinskih otoka. Nalazi se na rubu područja rasprostranjenosti vrste i nije brojna, što indicira moguću nestabilnost i ugroženost u budućnosti. Procijenjuje se da u hrvatskom dijelu Jadrana gnijezdi 60 – 70 parova eleonorinog sokola.

## THE SPREAD AND DYNAMICS OF ELEONORA'S FALCON (*Falco eleonorae* GENE, 1839) IN THE CROATIAN PART OF ADRIATIC

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Authors began the first systematic research of Eleonora's Falcon in Croatia in 1998. Since then, ecological parameters of the biggest Croatian colony are monitored every year. Counting of birds, registration of active nests, observation of birds' behaviour and ringing of young birds were performed within this research. Also, the first census of total Croatian population was performed in 2000 and continued every year since then. Six colonies were registered, two of which were completely unknown. Croatian population of Eleonora's Falcon is situated on several Middle Dalmatian islands. It is on the very edge of species area and it is not numerous, which indicates its possible instability and vulnerability in future. The estimation of population numerousness in Croatian part of Adriatic is about 60 – 70 breeding pairs.

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## ISTRAŽIVANJE BIOLOGIJE KUKURUZNE ZLATICE (*Diabrotica virgifera virgifera* LE CONTE, Coleoptera: Chrysomelidae) NOVOG ČLANA ENTOMOFAUNE HRVATSKE

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Kukuruzna zlatica (*Diabrotica virgifera virgifera* LeConte) otkrivena je 1995.g. u istočnoj Hrvatskoj. Od tada, štetnik se širi Hrvatskom prosječnom brzinom od 30-50 km godišnje. Zbog velikih površina pod kukuruzom i šteta koje uzrokuje, kukuruzna zlatica predstavlja potencijalno najvažnijeg štetnika kukuruza. Danas je zaraženo područje od 19,000 $km^2$ na kojem se uzgaja 250,000 ha kukuruza. Temeljem literaturnih i klimatskih podataka prognozirani su rokovi prve pojave i pojave 50% ličinki, te prve pojave i pojave 50% kornjaša. Pregledom tla utvrđen je udio pojedinih razvojnih stadija ličinki u ukupnom broju ličinki. U entomološkim kavezima praćena je prva pojava kornjaša iz tla. Dobiveni rezultati su uspoređeni s prognozom. Temperaturni pragovi i termalne konstante koje su dali Levine i sur. (1992) za prvu pojavu i pojavu 50% ličinki odgovaraju podacima koji su dobiveni u polju uz neznatna odstupanja. Utvrđeno je da se prva pojava ličinki u uvjetima istočne Hrvatske može očekivati od 16. svibnja do 9. lipnja. Podaci o temperaturnim pragovima i termalnim konstantama mogu se uspješno koristiti za prognozu pojave prvih kornjaša u polju. Utvrđeno je da mužjaci izlaze iz tla između 15.lipnja i 11. srpnja, s prosjekom 30. lipnja. Izlazak ženki otpočinje 3-4 dana kasnije. Izlazak kornjaša iz tla trajao je 27-36 dana. Temeljem praćenja biologije štetnika izrađeni su fenogrami razvoja u Hrvatskoj. Rezultati istraživanja ukazuju da su odstupanja u biologiji kukuruzne zlatice u uvjetima Hrvatske u odnosu na podatke iz SAD neznatna.

## AN INVESTIGATION OF THE BIOLOGY OF THE WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* LE CONTE, Coleoptera: Chrysomelidae), A NEW MEMBER OF THE ENTOMOFAUNA OF CROATIA

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Western corn rootworm (WCR) was discovered in eastern Croatia in 1995. The pest is spread over a distance of 40 km per year. Because of the big corn production in Croatia, the damages caused by WCR on corn are of great importance. The pest has infested 19,000 km$^2$ in which area 250,000 ha of corn is sown. Based on the literature and climatic data the terms of the first emergence and the emergence of 50% of larvae and adults were calculated. First occurrence of larvae in the field was established by soil examination. In the emergency cages the emergence of adults was observed. Results were compared with the prognosis. The temperature thresholds and thermal constants for larval hatching given by Levine et al. (1992) correspond to the larval hatching in the field. First larval hatching in east Croatia could be expected between May 16$^{th}$ and June 9$^{th}$. The temperature thresholds and thermal constants for adult emergence could be used for prognosis of the first adult emergence in the field. Summarizing of the effective temperatures should start when egg hatching begins. The emergence of beetles starts between June 15 and July 4 with an average being June 30. The emergence of females began 3-4 days after the appearance of males. The beetles emerged period is 27-36 days. Phenograms of the development of WCR were made. Results of the investigation of the biology show that the differences between the biology in Croatia and in the USA are minor.

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## SEZONSKA DINAMIKA I EMERGENCIJA FAUNE TULARA (Trichoptera: Insecta) NA PLITVIČKIM JEZERIMA

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Na području Plitvičkih jezera, tijekom 2000. i 2001. godine, izvršeno je prikupljanje tulara (Trichoptera) metodom piramid-klopki. Klopke su bile postavljene na četiri lokaliteta tijekom 2000. godine (izvori Crne i Bijele rijeke, Crna rijeka-Plitvički Ljeskovac i barijera Labudovac), a u 2001. godini istraživanje je prošireno i na peti lokalitet (barijera Kozjak-Milanovac). Prve godine je prikupljeno je ukupno 779 primjeraka tulara, a druge godine 507 primjeraka. Analiza prikupljenih uzoraka pokazala je da su zabilježene 34 vrste u

2000. godini i 30 vrsta u 2001. godini. Vrsta *Micropterna lateralis* Steph. prvi je puta zabilježena na području NP "Plitvička jezera". Za svaku zabilježenu vrstu prikazan je period izljetanja imaga. Najveći broj vrsta prikupljen je 2000. godine u lipnju i srpnju te u lipnju i rujnu 2001. godine. Najveći stupanj raznolikosti faune tulara utvrđen je za obje godine (pomoću Shannon-Wienerovog, Simpsonovog, Brillouinovog indeksa bioraznolikosti i maksimalne bioraznolikosti) na barijeri Labudovac, a najmanji na izvoru Bijele rijeke. Najveći stupanj ujednačenosti faune utvrđen je na barijerama, u 2000. godini na postaji Labudovac, a u 2001. godini na postaji Kozjak-Milanovac. S¸rensenov indeks sličnosti ukazuje na najveći stupanj sličnosti faune tulara na različitim tipovima izvora, na limnokrenom izvoru Bijele rijeke i reokrenom izvoru Crne rijeke.

## SEASONAL DYNAMICS AND EMERGENCE OF CADDISFLIES (Trichoptera: Insecta) AT THE PLITVICE LAKES

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Adult Trichoptera were observed at the Plitvice Lakes over two years (2000 and 2001) using pyramid-type emergence traps. Traps were installed at four sampling sites in the year 2000 (Crna rijeka, Bijela rijeka, Crna rijeka-Plitvički Ljeskovac, Labudovac) and additional one in the year 2001 (Kozjak-Milanovac). A total of 779 specimens were collected during the 2000, belonging to 34 species, and 507 specimens during the 2001, belonging to 30 species. *Micropterna lateralis* Steph. was recorded at the Plitvice Lakes for the first time. The period of emergency is given for each species. During the 2000 most species emerged in June and July and in 2001 in June and September. Diversity (expressed as Shannon-Wiener, Simpson, Brillouin indices and maximal biodiversity) was highest at the travertine barrier Labudovac and lowest at the limnocrenic spring Bijela rijeka. Equitability was highest at the two travertine barriers, Labudovac in 2000 and Kozjak-Milanovac in 2001. We determined the highest similarities (expressed as S¸rensen index) at the two kind of springs – reocrenic spring Crna rijeka and limnocrenic spring Bijela rijeka.

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## UČESTALOST BAKTERIJA *Campylobacter jejuni, Salmonella* spp. i *Chlamydophila psittaci* U NEKIH VRSTA PTICA S PODRUČJA HRVATSKE

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Kampilobakterijske infekcije, salmoneloze i klamidioza ptica su bolesti koje ugrožavaju zdravlje ptica, a istovremeno i ljudi (zoonoze). Neke vrste divljih ptica imaju važnu ulogu kao prirodni rezervoari uzročnika navedenih bolesti. Istraživanja učestalosti prirodnih infekcija bakterijama *Campylobacter jejuni, Salmonella* spp. i *Chlamydophila psittaci* provedena su na 13 vrsta iz 11 rodova ptica s područja Hrvatske. *C. jejuni* izdvojena je iz 3 (2,3%), a *Salmonella* spp. iz 7 (5,4%) od ukupno 130 pretraženih izmeta divljih ptica. *S. typhimurium* izdvojena je iz 5 (3,8%) gradskih golubova (*Columba livia domestica*) i jednog riječnog galeba (*Larus ridibundus*), a *S. enteritidis* iz izmeta jedne šumske sove (*Strix aluco*). Imunološki odgovor na bakteriju *C. psittaci* nije dokazan niti u jedne od istraženih vrsta ptica. Posebna pozornost pridana je epidemiološkim stajalištima i mogućoj ulozi divljih ptica za infekcije čovjeka i drugih domaćih životinja.

## THE OCCURRENCE OF *Campylobacter jejuni, Salmonella* spp. and *Chlamydophila psittaci* IN SOME SPECIES OF WILD BIRDS ON CROATIAN TERRITORY

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Campylobacteriosis, salmonellosis and avian chlamydiosis are zoonotic diseases in which birds have been suggested to play an important role as a natural reservoir. We investigated the prevalence of *Campylobacter jejuni, Salmonella* spp. a *Chlamydophila psittaci* in 130 wild birds belonging to 13 species from 11 orders on Croatian territory with the aim of examining the nature of *C. jejuni, Salmonella* spp. and *C. psittaci* infection in this bird species. Cloacal swabs and fresh fecal specimens from wild birds were cultured for *C. jejuni* and *Salmonella* spp. The carrier rates detected were as follows: *C. jejuni*, 2.3% and 5.4% *Salmonella* spp. *S. typhimurium* was the most common serotype isolated from pigeons. Among birds inhabiting the city of Zagreb, the highest isolation rate for *S. typhimurium* was found in pigeons (*Columba livia domestica*) (3.8%) and one black-headed gulls

(*Larus ridibundus*). *S. enteritidis* was isolated from only one tawny owl (*Strix aluco*). Immunity reactiveness to the bacteria *C. psittaci* during the research was not found. Epidemiological aspects and the possible role of the examined birds as a source of infection for man and domestic animals are discussed.

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## LISIČJE JAZBINE NA NEKIM LOKALITETIMA SJEVEROZAPADNE HRVATSKE

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Crvena lisica (*Vulpes vulpes*) je pripadnik porodice pasa (*Canidae*) rasprostranjen diljem Republike Hrvatske. Lisičja jazbina je podzemna jama locirana uglavnom na suhim obroncima, nasipima napuštenih puteva, a nerijetko i u blizini cesta i magistrala. Lisica rijetko kopa jame sama, uglavnom se koristi prirodnim jamama ili onima koje iskopa jazavac. Lisica obično na teritoriju kopa jednu glavnu i nekoliko sporednih, pričuvnih jama. U sklopu ovog istraživanja pretraženo je pet (5) lokaliteta na području sjeverozapadne Hrvatske - Jakovlje, Stubičke toplice, Solarske šume, Starča i Hrastje. Od utvrđenih 36 aktivnih lisičjih jama, 32 su koristile isključivo lisice, a 4 jame su koristile zajedno lisice i jazavci. Prosječan broj ulaza-izlaza u aktivnim jamama kretao se oko 4,7. U neaktivnim jamama je zabilježen manji broj otvora. Najpovoljnija mjesta za smještaj jama su padine, a najnepovoljnija uvale.

## DISTRIBUTION OF FOX BURROWS AT THE NORTH-WEST AREA OF CROATIA

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Red fox (*Vulpes vulpes*) is a member of the *Canidae* family, with distribution range all over the Republic of Croatia. Fox burrows are underground hole located mostly on the dry hills, abandoned causeways, and they are not infrequently placed near the roads and highways. The burrow is rarely disinterred by foxes only, usually they use natural holes or those disinterred by badgers. On its territory foxes usually

forms one main and few accessory burrows. In this investigation few places in north-west region of Croatia have been surveyed – Jakovlje, Stubičke toplice, Solarske šume, Starča and Hrastje. From fortified 36 active burrows, 32 were used by foxes only, and 4 of them were used by foxes and badgers. The average number of entrance and exit per active burrow was 4.7. In the inactive burrows minor number of openings was registered. The most favourable places for burrows were hillsides, while the less suitable were ground depressions.

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## BRZINA RASTA I RITAM PRESVLAČENJA JADRANSKOG JASTOGA (*Palinurus elephas*)

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Tijekom perioda od dvije godine praćena je brzina rasta i ritam presvlačenja nedoraslih jastoga držanih u kontroliranim uvjetima. Sve jedinke (154 kom.) u pokusu iskazale su ritmičnost presvlačenja, na način da se većina istraživane skupine rakova (90,4 %) presvlačila dva puta godišnje – u proljeće i u jesen. Nije utvrđena razlika u brzini i ritmičnosti presvlačenja jedinki različitog spola prije pojave prve spolne zrelosti, no nakon spolnog sazrijevanja ženke su iskazale sporiji rast u odnosu na mužjake. Nedorasli jastozi prosječno su pri svakom presvlačenju narasli od 2 do 2.5 cm, što znači da je godišnji dužinski prosjek rasta iznosio 4 do 5 cm. Pošto je pojava prve spolne zrelosti u jadranskog jastoga zabilježena pri ukupnoj dužini tijela od 21 cm (minimalno), to znači da je pri zabilježenoj brzini rasta jastozima potrebno najmanje četiri godine da dostignu minimalnu veličinu prve spolne zrelosti. U istraživanih jastoga utvrdili smo fiziološku i funkcionalnu spolnu zrelost, s tim da se fiziološka spolna zrelost (proljetno presvlačenje) kod većine jedinki (87,5%) javlja u prosjeku pola godine ranije od funkcionalne (jesensko presvlačenje).

## GROWTH RATE AND MOLT CYCLE OF ADRIATIC SPINY LOBSTER (*Palinurus elephas*)

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During two years period growth rate and molt cycle of sub-adult Adriatic spiny lobsters maintained in controlled condition were recorded. All specimens (154) in

experiment showed rhythmic molt in a manner that vast majority of lobsters (90.4 %) have molt twice a year – in spring and autumn period. No significant difference in growth rate and molt rhythm between specimens of opposite sex was observed before first sexual maturity, but sexually matured females showed lower growth rate than sexually matured males. During each molt length growth of sub-adult lobsters was in average 2.0 to 2.5 cm, what imply that average yearly growth rate was between 4 and 5 cm. According to fact that first sexual maturity of spiny lobsters in Adriatic was recorded at total length (TL) of 21 cm (minimum) it is obvious that Adriatic spiny lobsters need at least four years to reach the length of first sexual maturity. All studied lobsters manifested two types of sexual maturity – physiological and functional, and in most specimens (87.5%) physiological sexual maturity (spring molt) has shown half a year earlier than functional sexual maturity (autumn molt).

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## NEFRITIS U KUNA ZLATICA I BJELICA

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Kuna zlatica (*Martes martes*) i kuna bjelica (*Martes foina*) naše su autohtone zvijeri iz porodice kuna (*Mustelidae*). Ove kune većinom se hrane sitnim glodavcima i pernatom divljači, ali i svim ostalim životinjama koje mogu svladati (zec, lane, bolesna srna), jajima ptica, medom i slatkim voćem. Smještaj na kraju hranidbenog lanca upućuje na mogućnosti kumuliranja raznovrsnih štetnih tvari, što se prvenstveno odražava na organima koji sudjeluju u detoksikaciji i ekskreciji. Obzirom na važnost mokraćnog sustava u ekskreciji, u ovom radu naglasak je stavljen na patohistološku pretragu bubrežnog tkiva. Tijekom 2002. i proljeća 2003. razuđeno je 5 kuna zlatica i 5 kuna bjelica, s područja Desinića, Pokupskog bazena, okolice Požege i Slavonskog Broda i patohistološki su im pretraženi bubrezi. Nefritis različitog karaktera popraćen nefrozom nađen je u devet (9) od deset (10) kuna.

## NEPHRITIS IN PINE AND STONE MARTENS

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Pine marten (*Martes martes*) and stone marten (*Martes foina*) are referred to as autochthonous carnivores from the family of *Mustelidae*. Primarily they feed on small rodents and feathered game, but also on all animals they can surmount (hares, roe deer fawn, feebly roe deer), as well as bird eggs, honey and fruits. Exactly the position on the top of the food chain points on possibilities of accumulation of different harmful materials, which has a reflection on excretion organs and organs for detoxification. As the urinary system is the most important excretion system in the body, in this paper we have put accent mainly on the pathohistological survey of kidney tissue. During the 2002 and the spring of 2003 we have performed a necropsy and pathohistological survey of kidneys, originating from 5 pine martens and 5 stone martens, collected from the areas of Desinići, Pokupski bazen, broad area of Požega and Slavonski Brod. A different characters of nephritis accompanied with nephrosis was found in nine (9) from ten (10) martens.

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## ANALIZA PREHRANE VUKA U HRVATSKOJ

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Istraživane su prehrambene navike vuka na području Gorskog kotara i Dalmatinske zagore analizom uzoraka izmeta (N=147) i želudaca (N=10) prikupljenih u razdoblju od 1999. do 2002. godine. Određena je učestalost pojavljivanja (%) pojedinih kategorija nalaza i vrsta životinja za svaku regiju posebno. Na području Dalmatinske zagore većinu vučje hrane čine domaće životinje (73,4%). Pronađen je i relativno učestali udio dlake kanida (32,6%), a koja je mogla većim dijelom potjecati od lizanja vlastitog tijela, te nije uračunata u udio hrane vuka. U Gorskom kotaru glavni plijen su divlji parnoprstaši (jelen, srna, divlja svinja), a koji su u prehrani zastupljeni 84,2%.

## ANALYSIS OF WOLF FOOD HABITS IN CROATIA

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Wolf food habits have been studied in the areas of Gorski kotar and Dalmatinska Zagora by analyses of scats (N=147) and stomachs (N=10) collected during 1999 to

2002. Frequency of occurrence (%) has been determined for each food category and studied region. In the Dalmatinska Zagora region domestic animals comprised the majority of wolf food (73.4%). The relatively high canid hair occurrence (32. 6%) may in big part the own licked hair of a wolf. In Gorski kotar the main food were wild ungulates (red deer, roe deer, wild boar) with the 84.2% frequency of occurrence.

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## EHOLOKACIJA, OBLIK KRILA I LET U DUGONOGOG ŠIŠMIŠA *Myotis capaccinii* (BONAPARTE, 1837)

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Dugonogi šišmiš (*Myotis capaccinii*) jedan je od sedam najugroženijih europskih vrsta šišmiša, a o biologiji vrste ima malo podataka. Multidisciplinarno istraživanje ekologije ove vrste provodi se od 1999. godine na najvećoj poznatoj porodiljskoj koloniji ove vrste u Europi (špilja Miljacka II, NP Krka). Temelj za ekološko istraživanje bilo koje vrste šišmiša je poznavanje akustične ekologije i morfologije leta. Snimanje eholokacije ove vrste provedeno je od svibnja do rujna 2000. Eholokacija je snimana prilikom izlaska šišmiša iz spilje u noćni lov, prilikom leta kroz gustu vegetaciju, prilikom hranjenja nad rijekom te prilikom ispuštanja iz ruke. Analizirane su četiri spektralne i tri temporalne karakteristike po zvuku. Mjere teoretske brzine leta izračunate su prema mjerama krila uzetih sa 160 ulovljenih šišmiša koji borave u špilji. U ovom radu 1) opisujemo i uspoređujemo eholokacijske zvukove šišmiša u različitim tipovima staništa i različitim ekološkim situacijama te ih uspoređujemo s literaturnim podacima za vrste slične ekologije, 2) opisujemo teoretski način leta ove vrste prema poznatoj ekologiji vrste, 3) raspravljamo značaj rezultata za zaštitu vrste.

## ECHOLOCATION, WING DESIGN AND FLIGHT PERFORMANCE IN THE LONGFINGERED BAT *Myotis capaccinii* (BONAPARTE, 1837)

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The Long-fingered bat (*Myotis capaccinii*) is one of the seven most endangered bat species in Europe and little is known about its biology. Since 1999 a

three-year multidisciplinary study (funded by The Whitley Laing Foundation) has investigated aspects of the ecology of this species at its largest nursery colony in Europe (Miljacka II Cave, Croatia). Fundamental to any study of the ecology of a microchiropteran bat, is an understanding of its acoustic ecology and flight morphology. Between May and September 2000, echolocation calls were recorded while bats were exiting the cave, flying through dense foliage, foraging over a river, and on release from the hand after capture. Four spectral and three temporal features of calls were analysed. Measures of theoretic flight performance were also calculated from wing tracings of 160 individuals inhabiting the cave. In this paper we 1) describe and compare the echolocation calls emitted in different habitat types and ecological situations, and compare these results with those published for species with similar ecologies, 2) describe the theoretical flight performance of this species in relation to what is known about its ecology, and 3) discuss our results in terms their relevance to the conservation of *M. capaccinii*.

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## POPULATION SIZE AND DISTRIBUTION OF BOTTLENOSE DOLPHINS (*Tursiops truncatus*) AROUND THE LOŠINJ AND CRES ARCHIPELAGO

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Bottlenose dolphins are the only regularly sighted cetaceans in Croatian waters. The Adriatic Dolphin Project (ADP) has monitored the resident bottlenose dolphin population of the Lošinj and Cres archipelago since 1987. Research has particularly focused on the socio-ecology of the population. Data was collected, using photo-identification of natural markings of individual dolphins, from May to October of the years 2001 and 2002. Population abundance was then estimated by using the software SOCPROG. The data was examined using analytical methods applied to minimise bias. It was found that a 'Mortality + Trend Model', was best fit to describe the population. The estimated number of dolphins with long-term markings accounted for 100 individuals (95% CI = 82.3 – 125.5, SE = 12.1). This is well below the IUCN's classification of 'critically endangered isolated populations' with less than 250 individuals. The combined mortality, mark change and emigration rate was estimated at 0.02 % per year with a positive trend of 0.04 % per year. A lagged identification model also showed that during the field season

individuals emigrated from, and re-immigrated back into the study area. These results also indicate that the study area is only part of the home range of the dolphins frequenting the area. It is essential to determine the population size and trend of animal species to establish and develop the implementation of management and conservation strategies for vulnerable populations. Further studies are necessary before an accurate status of the bottlenose dolphins in Croatian waters can be determined.

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## A PRELIMINARY STUDY OF CETACEAN PRESENCE AND ABUNDANCE in the ARCHIPELAGO OF THE KORNATI NATIONAL PARK (CROATIA)

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Little is known about the occurrence and distribution of cetaceans in the Central Adriatic Sea, with data available only from a long-term study of bottlenose dolphins in the Kvarnerić. The aim of this preliminary survey was to better understand the occurrence and distribution of cetaceans in the Kornati National Park and its adjacent waters, in order to identify the ideal area for conducting a long term study. A total of 17 surveys were conducted between July and August 2002, data was collected on a daily basis from a sailing boat, with identification of individuals based on standard identification procedures. Bottlenose dolphins were the only cetacean species encountered with all sightings occurring outside the boundaries of the National Park. In total they were observed six times in positive weather conditions for a total of 528.56 km surveyed. The encounter rate of 0.011 sightings/km was lower than that recorded in the Kvarnerić during the same year (0.016 sightings/km). From the photo-identification data collected 14 dolphins have been classified. Every dolphin classified was re-sighted two or more times: among these re-sighted dolphins two mothers and calves were identified. Comparisons with the 160-catalogued dolphins of the Kvarnerić area did not show any match. The long-term aim of the Kornati Dolphin Project is to identify the relevance of this geographic area to cetaceans, the potential benefits that the National Park can provide to these species and the benefits that the presence of cetaceans may provide to the National Park.

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## STRUCTURE OF SOIL ELATERIDES (Coleoptera: Elateridae) COMMUNITY IN BEECH ECOSYSTEM IN MAVROVO NATIONAL PARK COMPARED TO TURKEY AND ITALIAN OAK ECOSYSTEMS IN MACEDONIA

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Structural features of the soil Elaterides in the beech ecosystem in Mavrovo National Park, in comparison with the turkey and oak ecosystems in Galichica National Park and on Ramnishte locality near Veles were investigated. In Mavrovo, Galichica and Veles, five, four and three species of Elateridae family have been found, respectively. The highest average annual density of Elaterides in the three ecosystems investigated has been registered in the layer of 0-5 cm, and density plummets with the increase of depth and towards the forest floor. The fauna of Elateridae in Mavrovo has a Euro-Siberian form; in Veles it has south European form, while in Galicica it is of a mixed type – Euro-Siberian and south European. The zoocenological valuation of the structural characteristics of the Elaterides in these three ecosystems investigated has shown that the values of the structural indices (richness, diversity, homogeneity) are the highest in the oak ecosystems in Galicica, then follows the beech ecosystem in Mavrovo, whereas they are the lowest in the oak ecosystem in Veles. The communities of the soil Elaterides respond differently and with different characteristics, as a reflection of the concrete ecological conditions in the investigated ecosystems in Mavrovo, Galicica, and Veles.

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## PRIKAZ BIO-BIBLIOGRAFSKOG KAZALA IMENA ENTOMOLOGA U HRVATSKOJ

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U svakodnevnom radu s entomološkim zbirkama i literaturom, stručnjaci se susreću s brojnim imenima osoba - autorima, sabiračima i drugima iz razdoblja od 18. stoljeća nadalje. Često su im potrebni detaljniji podaci. U svijetu ima nekoliko relevantnih tiskanih publikacija, a u novije vrijeme i na internetu ("Entomologen der Welt" i druge). Za dio Hrvatske podatke o osobama obradio je G. Nonveiller 1989 i 1999. u knjizi o povijesti entomologije u Dalmaciji (592 imena). Nedostajali su relevantni podaci za cijelu Hrvatsku i današnje stanje. Na serveru Hrvatskoga entomološkog društva autor je od prosinca god. 2002. do svibnja 2003. postavio probne stranice hrvatskog Bio-bibliografskog kazala

s podacima za više od 1100 osoba. Kazalo je jedno od pomoćnih sredstava u istraživanju entomofaune Hrvatske. U prikazu se razmatraju kriteriji i problemi sastavljanja ovog Kazala. Analiziraju se raspoloživi izvori i mogućnosti primjene suvremene tehnologije u Kazalu. Uspoređene su relevantne strane publikacije i baze podataka. Kazalo "Index bio-bibliographicus notorum hominom Nonveilleriana" posvećeno je uspomeni na entomologa G. Nonveillera (1913-2002), istraživača entomofaune u Hrvatskoj i povijesti entomologije u Hrvatskoj.

## PRESENTATION OF THE BIO-BIBLIOGRAPHIC INDEX OF NAMES - ENTOMOLOGISTS IN CROATIA

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In everyday work with entomological collections and references, experts meet with many names of persons - authors, collectors and others from the period from the 18th c. onwards. They often need more detailed data. In the world, there have been several relevant publications printed, and lately also on the internet ("Entomologen der Welt" and others). For a part of Croatia, data on persons were processed by G. Nonveiller in 1989 and 1999 in the book on the history of entomology in Dalmatia (592 names). Missing were the relevant data for the entire Croatia and the present condition. On the server of the Croatian Entomological Society, the author has from December, 2002 to May 2003 set up the test pages of the Croatian Bio- bibliographic index with data for over 1,100 people. The index is one among the auxiliary means in researching the entomofauna of Croatia. The presentation considers criteria and problems encountered in the compilation of the said Index. Analysed are the available sources and possibilities of applying modern technology in the Index. Compared were the relevant foreign publications and databases. The index "Index bio-bibliographicus notorum hominom Nonveilleriana" has been dedicated to the memory of entomologist G. Nonveiller (1913-2002), researcher of entomofauna in Croatia and history of entomology in Croatia.

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## NOVI POGLEDI NA RASPROSTRANJENOST I EKOLOGIJU VRSTA RODA *Plecotus* (Chiroptera: Vespertilionidae) U HRVATSKOJ

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Pojedini autori opisivali su unutar roda *Plecotus* (Geoffroy, 1818) brojne vrste i podvrste na temelju zapaženih morfoloških osobitosti koje su kasnije tumačene kao dio geografske varijabilnosti jedine priznate vrste *P. auritus* (Linnaeus, 1758).

Nakon što je prije 43 godine potvrđena specifička osobitost *P. austriacus* Fisher, 1829, tek je poslije 1985. bio priznat i specifički status alopatričke svojte *P. austriacus teneriffae* (Barret-Hamilton, 1907). Molekularno-genetičkim istraživanjima mitohondrijalnih markera naknadno su otkrivene još tri nove vrste na području Evrope, od kojih dvije nalazimo i u Hrvatskoj. Te dvije vrste su dosad bile poznate kao *P. austriacus kolombatovići* Đulić, 1980 i *P. auritus macrobullaris* Kuzjakin, 1965. Morfološka usporedba muzejskog materijala pojedinih grupa jedinki sve četiri vrste čiji predstavnici tvore posebne haplotipove, omogućila je dobivanje novih podataka o rasprostranjenju vrsta ovog roda u pojedinim staništima u Hrvatskoj. Utvrđena je simpatrija pojednih vrsta, kao i slučajevi sintopije, te su tako dobiveni temeljni podaci potrebni za daljnja istraživanje ekologije novootkrivenih vrsta. Rezultati omogućuju i početak valorizacija ugroženosti, odnosno utvrđivanje mjera zaštite.

## NEW VIEW ON DISTRIBUTION AND ECOLOGY OF THE GENUS *Plecotus* (Chiroptera: Vespertilionidae) IN CROATIA

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Several authors have described numerous species and subspecies of the genus *Plecotus* on the basis of morphological characters which have later been considered as part of geographical variability of just one confirmed species *P. auritus* (Linnaeus, 1758). Before 43 years specific difference of *P. austriacus* Fisher, 1829 has been confirmed, and after 1985 the specific status of alopatric species *P. austriacus teneriffae* (Barret-Hamilton, 1907) has been admitted. Molecular-genetically investigations of mitochondrial markers revealed three new species in Europe, two of which are found in Croatia. They were formerly known as *P. austriacus kolombatovići* Đulić, 1980 and *P. auritus macrobullaris* Kuzjakin, 1965. Morphological comparison of museum material of groups of specimens of all four species whose representatives make different haplotypes, made it possible to collect new data on distribution of species from this genus in different habitats in Croatia. Founded are sympatric relations between some species and cases of syntopy, and in this way basic data was collected for further ecological investigations of new founded species. These results allowed start for status evaluation and preparation of conservation measures.

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## PORODILJSKE KOLONIJE MOČVARNOG PATULJASTOG ŠIŠMIŠA *Pipistrellus pygmaeus* (Chiroptera: Vespertilionidae) U DONJEM MIHOLJCU (HRVATSKA)

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Tijekom proljeća 2002. otkriveno je u potkrovljima zgrada u Donjem Miholjcu, Hrvatska, nekoliko većih kolonija patuljastih šišmiša (*Pipistrellus spp.*). U pronađenim kolonijama utvrđena je njihova veličina i sastav s obzirom na vrste te vremenski raspon i fenologija korištenja skloništa. Novootkrivene kolonije čine uglavnom ženke i (nešto kasnije) mladunci močvarnog patuljastog šišmiša *Pipistrellus pygmaeus*. Zabilježena opažanja prilog su poznavanju ekologije i ponašanja ove vrste čiji je taksonomski status tek nedavno potvrđen. Na primjercima *P. pygmaeus* u jednoj od pronađenih porodiljskih kolonija analizirane su i morfološke osobitosti koje su dosad navođene kao presudne za identifikaciju vrste, odnosno njezino razlikovanje od morfološki vrlo sličnog patuljastog šišmiša, *P. pipistrellus*. U rezultatima su također prikazani odnos spolova te starost jedinki.

## NURSERY COLLONIES OF SOPRANO PIPISTRELLE *Pipistrellus pygmaeus* (Chiroptera: Vespertilionidae) IN DONJI MIHOLJAC (CROATIA)

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During spring 2002. we have found in the attics of the buildings in Donji Miholjac, Croatia, several large colonies of pipistrelle bats (*Pipistrellus spp.*). Founded colonies size and composition regarding the species and time scale together with phenology of the use of shelters have been recorded. Newly discovered colonies consist mostly of females and (somewhat later) juveniles of soprano pipistrelle *Pipistrellus pygmaeus*. Recorded data therefore present important contribution to the knowledge of ecology and behaviour of this species whose taxonomic status has been just recently confirmed. On specimens of *P. pygmaeus* from one colony we have analyzed morphological characteristics which have up to now been regarded as most important for field identification of the species, i.e. its differentiation from the morphologically very similar common pipistrelle, *P. pipistrellus*. Also, among the results sex ratio and age of the animals are presented.

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## IHTIOFAUNA HIDROAKUMULACIJA SREDNJEG TOKA RIJEKE DRAVE U HRVATSKOJ OD 1983. DO 2002. GODINE

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Raznolikost, sastav vrsta i struktura riblje zajednice istraživani su u srednjem toku rijeke Drave u Hrvatskoj u razdoblju od 1983. do 2002. godine. U ovom radu opisan je utjecaj triju hidroelektrana i njihovih akumulacija na zajednicu riba. Tijekom razdoblja od dvadeset godina ulovljeno je 25,913 jedinki riba koje obuhvaćaju 56 različitih vrsta iz 16 porodica. Porodica Cyprinidae zastupljena je s 31 vrstom, porodica Percidae sa 7 vrsta, a porodica Salmonidae s 3 vrste, dok su sve ostale porodice zastupljene sa po jednom ili dvije vrste riba. U samim akumulacijama deverika je dominantna vrsta, dok su ostale značajne vrste za rijeku Dravu bodorka, klen, uklija i balavac.

## FISH FAUNA IN RESERVOIRS OF DRAVA RIVER IN CROATIA FROM 1983 TO 2002

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The diversity, species composition and structure of the fish community were studied in the middle part of Drava River in Croatia. Impacts of three hydroelectric power plants with their reservoirs on fish communities are described. Over a period of twenty years 25,913 fish specimens were collected, which includes 56 different fish species belonging to 16 families. The family Cyprinidae is represented by 31 species, the family Percidae by 7 species and the Salmonidae by 3 species, while the remaining families were represented by one to two species. Within the reservoirs, the Bream was the dominant fish species. The other important fish species on this part of Drava River are Roach, Chub, Bleak and Ruffe.

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## MORPHOMETRIC CHARACTERISTICS OF *Leuciscus souffia muticellus* IN CROATIA

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The distribution and morphometrics of *Leuciscus souffia* and its subspecies in France, Italy, Slovenia and Monte Negro have been described in many studies. However, all these studies have failed to include any data on the endemic population of *Leuciscus souffia muticellus* from the Cetina River in Croatia. Twenty morphometrical characteristics of this population were determined on 190 samples of *L. souffia muticellus* at five stations in the Cetina River. The meristic characteristics were measured as: D II (8), A III (9), C II (9)+III (8), P II (13), II (7); 40-41 vertebrae and 1.5-5.1. pharyngeal teeth. Comparisons with other *L. souffia* populations showed significant statistical ($p$<0.01) differences in 9 morphometric characteristics.

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## FISH OF RIVER BRKA

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In purpose to establish actual condition of fish community of the river Brka (right tributary of the river Sava) during May 2001 ichthyologic research was carried out. The research was performed in five characteristic localities (Rašljani, Maoča, Gornji Rahić, Palanka and Brka) on longitudinal profile of the upper course of the river Brka. The lower course of the river Brka was not investigated because the region is mined. Fish were collected by electrofishing unit, and fixed in 4% formaldehyde solution. Laboratory analysis was made in the laboratories at the Biology Department of Philosophical faculty. The sample consisted of 911 individuals of ten fish species belonging to two fish families: *Cyprinidae* and *Cobitidae*. The family of *Cyprinide* was represented by species: *Barbus meridionalis petenyi* Heck, *Gobio gobio* L., *Alburnoides bipunctatus* Bl., *Rhodeus sericeus amarus* Bl., *Phoxinus phoxinus*, and *Pseudorasbora parva* Temminck et Schlegel, that was registered in this river for the first time. The *Cobotidae* family was represented by species: *Cobitis taenia* L., *Cobitis aurata* Filippi and Nemachilus *Barbatulas* L. Kosoric et al. (1975) in the Brka reported presence of *Chondrostoma* sp., *Leuciscus idus*, *Barbus sp.*, *Rutilus sp.*, *Esox sp.*, *Abramis sp.*, and also some of undetermined species. Based on our observations, signifficant differences in the structure of fish community could be seen. Reasons of fish community changes are discussed.

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## VARIJABILNOST ŠKOLJKI VRSTE *Dreissena polymorpha* PALLAS U RIJECI DRAVI (HRVATSKA)

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Provedenim istraživanjem nastojala se utvrditi morfometrija školjki vrste *Dreissena polymorpha* skupljenih u sustavu hidroelektrane Dubrava, izgrađene na rijeci Dravi (Hrvatska). Odabrane postaje razlikovale su se po vrsti podloge, dubini i fizikalno-kemijskim značajkama. Za postaju 1 odabrana je obala rijeke, dok su postaje 2 i 3 bile smještene u akumulacijskom jezeru Dubrava na dubini od 3, odnosno 8 metara. Ukupno je skupljeno 1200 školjkaša. Mjerene su slijedeće morfološke varijable: dužina školjke (DŠ), širina školjke (ŠŠ), visina školjke (VŠ) i težina školjke (TŠ). Osim toga, definirana su tri omjera: ŠŠ/DŠ, VŠ/DŠ i ŠŠ/VŠ. Analiza varijance (ANOVA) i testovi višestruke usporedbe (Duncan test) primijenjeni su kako bi se testirale razlike u omjerima između tri postaje te odredilo koje se postaje razlikuju međusobno. Provedena analiza pokazala je da se omjeri ŠŠ/DŠ i ŠŠ/VŠ statistički značajno razlikuju između sve tri postaje ($p<0.0001$), dok se omjer VŠ/DŠ ne razlikuje između postaja. Nadalje, analiza glavnih komponenata primijenjena je na logaritamski transformirana obilježja skupa od ukupno 540 školjki. ANOVA i Duncan test primijenjeni su na rezultirajuće komponente kako bi se testirale razlike u vrijednostima komponenti između sve tri postaje. Primijenjena analiza razdvojila je riječnu i jezersku populaciju školjkaša u dvije morfološke grupe ukazujući da među njima postoji značajna fenotipska plastičnost.

## VARIABILITY OF THE ZEBRA MUSSEL, *Dreissena polymorpha* PALLAS IN THE DRAVA RIVER (CROATIA)

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Investigation was carried out to examine the shell morphometrics of the zebra mussel (*Dreissena polymorpha*) collected from the system of hydroelectric power plant Dubrava on the Drava River (Croatia). Chosen sites had different sediment types, depth, and physical and chemical conditions. Site 1 was the shallow of the Drava River, while sites 2 and 3 were located in the artificial lake Dubrava at depths of 3 and 8 m, respectively. A total of 1200 mussels were collected from all three sites. The following morphological variables were exam-

ined: shell length (SL), shell width (SW), shell height (SH) and shell mass (SM). In addition three different ratios were defined: SW/SL, SH/SL and SW/SH. Analysis of variance (ANOVA) and Duncan multiple comparison test were applied to test the differences of the ratios among the three sites and to determine which of them was significantly different from the others. The analysis showed that the ratios SW/SL and SW/SH differ significantly between all three sites ($p<0.0001$), while the ratio SH/SL did not differ between sites. The log-transformed variables were further analysed using principal component analysis on 540 shells. ANOVA and Duncan test were applied on the resulting principal components to test for the differences in component scores among the three sites. The analysis separated the riverine and the lake populations of mussels into two morphological groupings, indicating that considerable phenotypic plasticity exists among the populations of the zebra mussel.

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## KRUPNOZUBI DUPIN (*Ziphius cavirostris*) PRVI PUTA NAĐEN U HRVATSKOM JADRANU

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Godine 2001. i 2002. prvi puta je u hrvatskom Jadranu nađen krupnozubi dupin (*Ziphius cavirostris)* iz porodice Ziphiidae. Ova vrsta uobičajna je u Sredozemlju, no nikada nije nađena u Hrvatskoj, ali je nekoliko mrtvih primjeraka nađeno u Italiji. Prva životinja je boravila u ožujku i travnju godine 2001. uz mjesto Srebreno pokraj Dubrovnika, gdje je 37. dana uginula. Kako se radilo o mladoj ženskoj životinji nije se moglo sa sigurnošću utvrditi, radi li se o sjevernoj kljunastoj ulješuri (*Hyperoodon ampullatus*) ili o krupnozubom dupinu. Čak ni detaljnom obdukcijom nije bilo moguće 100%-tnom sigurnošću utvrditi vrstu. Druga životinja je izbačena u raspadajućem stanju na Korčulu veljače 2002., te je s ozirom na oblik zubiju zaključeno da je to jedna od sljedećih vrsta iz porodice Ziphiidae: krupnozubi dupin, sjeverna kljunasta ulješura, *Berardius bairdii, Berardius arnuxii, Hyperoodon planifrons, Mesoplodon europaeus, Mesoplodon mirus* ili *Mesoplodon pacificus*. U uzorcima obje životinje standardnim metodama

je izolirana ukupna genomska DNA, čiji je DNA fragment na 5' kraju kontrolne regije mtDNA dugačak 500 bp amplificiran lančanom reakcijom polimerazom i sekvenciran. Sekvence kontrolne regije mtDNA ove dvije životinje su uspoređene s referentnom bazom sekvenci koja sadrži sekvence za svu 21 vrstu porodice Ziphiidae. Sekvence uzoraka obje životinje su tvorile skupinu s referentnom sekvencom krupnozubog dupina, što je isključivalo sve ostale vrste. Prva životinja je bila ženka dužine 430 cm i mase 610 kg, a druga je bio mužjak dužine 500 cm, mase oko 1000 kg.

## FIRST RECORD OF THE CUVIER'S BEAKED WHALE (*Ziphius cavirsotris*) IN THE CROATIAN PART OF THE ADRIATIC SEA

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In 2001 and 2002 two Cuvier's beaked whales (*Ziphius cavirsotris)* were for the first time recorded in the Croatian part of the Adriatic Sea. It is a relatively common species in the Mediterranean Sea, but has never been found before in the Croatian part of the Adriatic Sea, although a few specimens stranded on the Italian coast. The first animal was observed live during March and April 2001 in the area of Srebreno near Dubrovnik where it died on 37$^{th}$ day of its appearance. As it was a young female, neither external morphology nor a detailed dissection could confirm the species. In February 2002 the second animal stranded died in an advanced state of decomposition on the island of Korčula. Based on teeth shape the animal was recognized as belonging to the beaked whale family (Ziphiidae), but exact species could not be identified. Muscle samples were collected from both animals. Total genomic DNA was isolated. A 500 base pair fragment of the 5' end of the mitochondrial DNA control region was amplified via the polymerase chain reaction and sequenced. The resulting sequences were compared to the beaked whale reference database. Sequences from both specimens grouped strongly with reference sequences from Cuvier's beaked whale, to the exclusion of all other beaked whale species in the database. The first animal was a female of total body length 430 cm and body mass 610 kg, and the second was a male of total body length 500 cm and body mass ca. 1000 kg.

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## PRISUTNOST I RASPROSTRANJENOST VRSTA IZ REDA KITOVA (Cetacea) U ZADARSKOM AKVATORIJU

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Od kolovoza 2000. do lipnja 2002. godine tijekom 121 sata i 16 minuta istraživana je prisutnost i rasprostranjenost vrsta iz porodice *Cetacea* u zadarskom akvatoriju izravnim promatranjem iz brodice dužine 5,5 m s motorom 55 KS opremljene GPS uređajem i fotoopremom. U početku istraživanja podaci su prikupljani nesustavno, a od srpnja 2001. do lipnja 2002. godine prema izrađenom protokolu svakih 15 minuta bilježeni su podaci o položaju i broju promatranih životinja, te vremenskim uvjetima. Jedina prisutna vrsta bila je dobri dupin (*Tursiops truncatus*). U istraživanom području utvrđena je velika uspješnost pronalaska dupina (100%), budući da je tijekom svakog dana istraživanja (N=24) promatrana barem jedna skupina dobrih dupina. Prosječan broj promatranih dobrih dupina u skupini bio je 4,7 (od 1 do 9), a najčešći 6. S obzirom na prijeđeni put u 15-minutnom razdoblju dobri dupini kretali su se prosječnom brzinom od 2,99 km/h, a najvećom od 9,06 km/h. Najveća individualna brzina bila je 13,5 km/h. Metodom fotoidentifikacije identificirano je 13 jedinki s obje strane tijela, 13 samo s lijeve, a 16 samo s desne, te je utvđeno da su četiri dobra dupina promatrana 2001. godine ponovno boravila u području istraživanja 2002. godine.

## ABUNDANCE AND DISTRIBUTION OF WHALE SPECIES (ORDER: Cetacea) IN THE AREA OF ZADAR

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Direct observations conducted from a 5.5 m inflatable boat with an 55 HP outboard engine and equipped with a GPS instrument and cameras were carried out from August 2000 to June 2002 to estimate the abundance and distribu-

tion of some whale species (Cetacea) in the Zadar aquatorium. A total of 121 hours and 16 minutes were spent at sea during the study. From July 2001 to June 2002, after the initial period of irregular data collection, observations of school size and position and weather conditions were recorded following a 15-minutes protocol. Bottlenose dolphin (*Tursiops truncatus)* was the only cetacean species observed. A high sighting frequency for bottlenose dolphins (100%) was recorded in the study area because every day of the study (N=24) at least one dolphin group was encountered. The average size of the bottlenose dolphin school was 4.7 (range from 1 to 9), most encountered 6. The average speed of bottlenose dolphin schools calculated in 15-minutes intervals was 2.99 km/h and the highest was 9.06 km/h. The highest individual speed was 13.5 km/h. Both body sides of 13 bottlenose dolphins were photoidentified, just the left side of 13 and just the right side of 16. Four bottlenose dolphins encountered in 2001 were resighted in 2002.

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## POJAVLJIVANJE CUVIEROVOG KLJUNASTOG KITA (*Ziphius cavirostris*) U VODAMA HRVATSKOG JADRANA

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Većina publikacija o fauni kitova (Cetacea) u Hrvatskom Jadranu ne navodi prisutnost Cuvierovog kljunastog kita (*Ziphius cavirostris*) u tim vodama; vjerojatno stoga što nisu postojali očigledni podaci kao dokaz. Pregled povijesnih podataka i prirodoslovnih zbirki u Hrvatskoj te nedavno prikupljeni i neobjavljeni vlastiti podaci otkrivaju tri zabilježena pojavljivanja *Z. cavirostris* uz hrvatsku obalu Jadrana. Prvi zabilježeni primjerak uhvaćen je 1939. u blizini Cavtata. Događaj je zabilježio Miroslav Hirtz koji ga netočno odredio kao *Hyperoodon ampullatus*. Ovim radom po prvi puta prezentiramo reviziju postojećih podataka i slika pomoću kojih možemo utvrditi da se u stvarnosti radilo o *Z. cavirostris*. Drugi primjerak prvo je opažen u blizini Cavtata u ožujku 2001. gdje je boravio tijekom više od mjesec dana nakon čega je uginuo. Ostaci posljednjeg zabilježenog primjerka pronađeni su u uznapredovanom stanju

raspadanja u veljači 2002 u Pupnatskoj luci na Korčuli. *Z. cavirostris* je vrsta skupine kitova zubana koja pravi duboke urone te je pronalazimo uglavnom na otvorenom moru gdje se hrani velikim vrstama glavonožaca. Ovako mali zabilježeni broj životinja upućuje na zaključak da Jadransko more ne predstavlja značajno stanište ove vrste, no može se očekivati da je pojavljivanje lutajućih jedinki u dubljem srednjem i južnom Jadranu učestalije nego što upućuju podaci o pronađenim uginulim primjercima.

## THE OCCURRENCE OF CUVIER'S BEAKED WHALE (*Ziphius cavirostris*) IN CROATIAN ADRIATIC WATERS

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The majority of publications regarding cetacean fauna in the Croatian Adriatic omit the presence of *Ziphius cavirostris* in these waters; this was due to the lack of clear data concerning its presence. A review of the historical data together with inspection of the natural history collections in Croatia and recent original unpublished data has revealed that there have been three recorded occurrences of *Z. cavirostris* along the Croatian coast. The first recorded specimen caught in 1939, near Cavtat and recorded by Miroslav Hirtz was misidentified as *Hyperoodon ampullatus*. This paper presents for the first time a revision of the available data and pictures that establishes that the specimen was in fact *Z. cavirostris*. The second specimen was initially sighted near Cavtat in March 2001, the animal remained in the area for approximately a month before being found dead. The last specimen was found in an advanced state of decomposed in Pupnatska luka on the island of Korčula in February 2002. *Z. cavirostris* is a deep diving Odontocete species found mainly in offshore waters feeding mainly on large cephalopod species. The small number of recorded animals implies that the Adriatic Sea does not represent a significant habitat for this species, however, the occurrence of vagrant individuals in the southern and central basins may be more frequent than the stranding data suggests due to the greater depths found there.

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## DISTRIBUTION OF THE GENUS *Zospeum* BOURGUIGNAT 1856 (Gastropoda, Ellobiidae) IN CROATIA

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Genus *Zospeum* Bourguignat 1856 (Ellobiidae, Archaeopulmonata, Gastropoda) includes land troglobiont snails. They inhabit subterranean habitats of the central Pyrenées, the southern Alps and the Dinaric karst from a few meters above sea level to an altitude of 2,000 meters. Specimens live in caves and fissures where they probably feed on the detritus that is found in loam and in cave sediment. In caves, they occur on walls, on the ground beside puddles, or on organic matter (rotten wood). First record of Zospeum findings in Croatia was published by famous malakologist Spiridion Brusina, based on material collected in middle of 19. Century. That was also first record of troglobitic snails in Croatia. The most systematically research of troglobitic *Zospeum* snails was performed by J. Bole and F. Velkovrh in second part of 20. Century. During recent research performed by authors and members of Croatian Biospeleological Society, new localities and taxa have been recorded. At the moment it is known that eight species of the genus *Zospeum* occupy karst in Croatia. Three of them are strictly endemic for Croatia. Sites of finds of individual taxa are cited and an areal map of the UTM net of Croatia is presented.

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## ŠPILJSKA FAUNA PARKA PRIRODE ŽUMBERAK-SAMOBORSKO GORJE

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Biospeleološka istraživanja Žumberka i Samoborskog gorja počinju u 20. stoljeću, a intenziviraju se početkom 21. stoljeća suradnjom Parka prirode i Hrvatskog biospeleološkog društva. U radu su predstavljeni rezultati dosadašnjih istraživanja. Obrađeno je preko 70 speleoloških objekata. Definirana su i fotografirana podzemna staništa, mjereni njihovi ekološki parametri, te sakupljana kavernikolna fauna i vršeno njeno makrofotografiranje. Na osnovi ekologije staništa, kao i strukture podzemne faune unutar Parka utvrđene su četiri mikroregije. U podzemnim staništima preteže kopnena, ali nađena je i bogata vodena fauna. Utvrđeno je preko 80 taksona prilagođenih na uvjete podzemnih staništa, od kojih

oko 40 % predstavlja endeme, dok je nekoliko taksona endemno za regiju. Utvrđena fauna pripada kompleksu tzv. Kranjske faune, karakteristične za područje sjevernih Dinarida. Ističe se stigobiontna fauna puževa (*Gastropoda*), virnjaka (*Tricladida*) i rakušaca (*Amphipoda*), te troglobiontna fauna striga (Chilopoda), lažištipavaca (*Pseudoscorpiones*) i najzastupljenijih kornjaša (*Coleoptera*) sa čak 21 vrstom iz 14 rodova, od kojih su karakteristični *Aphaobius, Leptodirus* (*Coleoptera, Cholevidae, Leptoderinae*), te *Anophthalmus* (*Coleoptera, Carabidae, Trechinae*). Neke vrste utvrđene su po prvi puta za faunu Hrvatske, a za neke je područje Parka njihov rubni areal. U sakupljenom biološkom materijalu utvrđeni su taksoni novi za znanost, a dio materijala još čeka specijalističku obradu. Predstoji daljnji rad na utvrđivanju biocenološke strukture i svih ekoloških specifičnosti podzemnih staništa. Ovu jedinstvenu vrijednost, danas uključenu u Park prirode potrebno je valorizirati i zaštititi, te uključiti u edukativne i promotivne aktivnosti.

## CAVE-DWELLING FAUNA OF THE NATURAL PARK ŽUMBERAK-SAMOBORSKO GORJE

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Biospeleological research of Žumberak and Samoborsko gorje began in the 20th century and has become more intensive at the beginning of the 21st century in cooperation with the Natural Park and Croatian Biospeleological Society. This work shows results of the research conducted so far.

More than 70 speleological objects have been researched. Underground habitats have been defined and photographed, ecological features measured and cavernicoulos fauna collected and macrophotographed. Based on habitats' ecology, as well as the structure of cavernicoluos fauna, four microregions have been defined. Underground habitats are dominated by terrestrial fauna, but also rich aquatic fauna have been found. More than 80 taxons with some adaptability to the cave life have been detected: about 40 % of them are endemic, few of them exclusively endemic for the region. Defined fauna belong to the Fauna of Kranj complex, characteristic for Nord Dinaric region. It should stress stigobitic snails (*Gastropoda*), flatworms (*Tricladida*), crustaceans amphipods (*Amphipoda*); and terrestrial centipedes (Chilopoda), pseudoscorpions (*Pseudoscorpiones*) and most dominated beetles (*Coleoptera*) with even 21 species from 14 genus, most characteristic *Aphaobius, Leptodirus* (*Coleoptera, Cholevidae, Leptoderinae*) and *Anophthalmus* (*Coleoptera, Carabidae, Trechinae*). Some species have been found first time for Croatian fauna and for some Park region is areal border. In the collected material there have been new scientific discoveries of taxa, but a portion of the material is yet to be analyzed by the specialists. This unique value should be valorized, conserved and included in the educative and promotional activity.

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## PRILOG POZNAVANJU FAUNE ŠIŠMIŠA PARKA PRIRODE "BIOKOVO"

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Cilj istraživanja provedenog u okviru projekta "Biokovo 2002" Udruge studenata biologije - "BIUS" bio je prikupljanje podataka o fauni šišmiša na području Parka prirode "Biokovo". Istraživanje je provedeno u sklopu inventarizacije koju provodi Javna ustanova Park prirode Biokovo. Frekvencije glasanja šišmiša bilježene su bat - detektorom. Šišmiši su lovljeni mrežama postavljanim uz lokve, koje im tijekom ljeta služe kao izvor pitke vode. U razdoblju od 15. do 23. lipnja 2002. godine na četiri lokacije s južne strane Biokova ukupno je ulovljeno, izmjereno i determinirano 55 jedinki. Utvrđen im je spol, izmjerena dužina podlaktice, tragusa i palčanog nokta. Utvrđeno je najmanje pet vrsta šišmiša prethodno nezabilježenih na ovom području: *Myotis blythii, M. emarginatus, M. mystacinus, M. nattereri* i *Plecotus spp*. Od sedam vrsta šišmiša poznatih iz ranijih istraživanja, ponovno su zabilježene četiri: *Rhinolophus ferrumequinum, Tadarida teniotis, Eptesicus serotinus* i *Hypsugo savii*. Uključujući ovaj nalaz, na području Parka prirode "Biokovo" zabilježeno je najumanje 12 vrsta šišmiša. Primjerci roda *Plecotus* nisu se sa sigurnošću mogli odrediti do vrste.

## CONTRIBUTION TO BAT FAUNA OF THE BIOKOVO NATURE PARK

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The aim of this study, conducted by the Biology student association –"BIUS", as a part of the "Biokovo 2002" Project, has been to collect data related to bat population in the Biokovo Nature Park. The research was conducted as part of inventarisation led by Public Institution "Nature Park Biokovo". The echolocation sound frequencies of flying bats were detected using the bat detector. Bats were collected using mist-nets set next to the ponds, the source of drinking water for bats in summer. Between June 15 and June 23, 2002, total of 55 bats were collected, measured and determined at four different locations on the southern side of Biokovo Mountain. Upon the capture of the animal its gender was determined and basic morphological characteristics were mea-

sured, including body mass, length of the forearm, tragus and thumb claw. Total of 10 bat species have been identified, and at least five of them were new species for this area: *Myotis blythii, M. emarginatus, M. mystacinus, M. nattereri* and *Plecotus* spp. Four out of seven previously identified species were also found: *Rhinolophus ferrumequinum, Tadarida teniotis, Eptesicus serotinus*, *Hypsugo savii*. Based on cumulative data from previous and current studies, total number of bat species identified in Biokovo Nature Park is at least 12. Members of the genus *Plecotus* have not been determined with certainty.

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## NEW DATA OF HOVERFLIES (Diptera: Syrphidae) IN MACEDONIA

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In the past 20 years investigations (1987-2003) were carried out of the Fauna of Hoverflies in the territory of the Republic of Macedonia. The results of our additional investigations included re-determination of the Glumac collection preserved in Macedonian Museum of Natural History and the Institute of Biology in Novi Sad where they were analyzed. This paper presents data on 27 species of 18 genera of Hoverflies which are new to the fauna of Macedonia. The list below presents general distribution of each species and distribution in Macedonia, their visit flowers and flight period. The species *Cheilosia* (*Nigrocheilosia*) *gagatea* Loew, 1857; *Cheilosia himantopa* (Panzer), 1798; *Cheilosia lenta* Becker, 1894; *Cheilosia ranunculi* Doczkal, 2000; *Dasysyrphus friuliensis* (van der Goot), 1960; *Dasysyrphus postclaviger* (Stys and Moucha), 1962; *Epistrophe grossulariae* (Meigen), 1822; *Eristalinus* (*Lathyrophtalmus*) *megacephalus* (Rossi), 1794; *Eristalis jugorum* Egger, 1858; *Eumerus argyropus* Loew, 1848; *Eumerus sulcitibus* Rondani, 1868; *Eupeodes lapponicus* (Zetterstedt), 1838; *Eupeodes latifasciatus* (Macquart), 1829; *Ferdinandea ruficornis* (Fabricius), 1775; *Meligramma cingulata* (Egerr), 1860; *Merodon aeneus* Megerle in Meigen, 1822; *Merodon crymensis* Paramanov, 1925; *Myolepta dubia* (Fabricius), 1805; *Myolepta obscura* Becher, 1882; *Neoascia meticulosa* (Scopoli), 1763; *Parasyrphus punctulatus* (Verrall), 1873; *Pipizella annulata* (Macquart), 1829; *Platycheirus fulviventris* (Macquart), 1829; *Platycheirus manicatus* (Meigen), 1822; *Scaeva dignota* (Rondani), 1857; *Sphegina clavata* (Scopoli), 1763 and *Syrphus nitidifrons* Becker, 1921, are registered for the first time. Systematic investigations of hoverflies continued with the project "Fauna of Macedonia", when investigations were carried out on the whole territory of Macedonia. Besides the mentioned systematic investigations, there are also data from other sources about particular species of Hoverflies.

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## EARLY DEVELOPMENT OF THE SUSPENSORIUM OF *Salmo letnica* (KARAMAN, 1924) (Teleostel: Salmonidae)

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In this study the ontogenetic development of the suspensorium of *Salmo letnica* (Karaman, 1924) was studied from hatching until the age of a 92 days posthatching. Samples at age of 200 days posthatching were also included in this study, as well as trouts one year old. Most of the samples were *in toto* trypsine cleared and stained, some specimens were used for making serial histological sections. The centre of ossification and the type of the bony tissues was established with the help of serial histological sections. A chronological evaluation of the formation of the suspensorium during early development of *Salmo letnica* was performed. Development of the elements of the suspensorium at this salmon fish was found to be similar to the other salmonid fishes. Most of the cartilaginous structures which build the suspensorium were present at the moment of hatching. The first ossified cartilage bones were *os symplecticum* and *os quadratum*, which appear at age of 14 days posthatching. At the same age the formation of the first dermal bone os entopterigoidem was noticed. Os autoplatainum was the last cartilage bone that ossified.

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## VELIČINA POLOGA, DIMENZIJE JAJA I STRATEGIJA GNIJEŽĐENJA VRSTE BRGLJEZ (*Sitta europaea)*

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Veličina pologa, obilježja jaja i strategija gniježđenja vrste brgljez istraživano je na području sjeverozapadne Hrvatske u razdoblju od 2000. do 2002. godine. Svi parovi gnijezdili su u škrinjicama (umjetnim dupljama). Svaka je imala pomični otvor kako bi se mogli uzimati oološki podaci. Ova su istraživanja dala odgovore na više pitanja. Primjerice, postoji li razlika oooloških podataka po godinama, jesu li značajne razlike između populacija različitih geografskih širina, je li redoslijed nesenja jaja utječe na dimenzije jaja i drugo. Usporedbom dimenzija jaja po godinama nisu zabilježene značajne razlike ($p>0,05$). Također nije zabilježena značajna korelacija između redoslijeda nesenja jaja i dimenzija jaja. Prema relativnoj veličini posljednjeg snesenog jajeta (%D) brgljez vjerojatno primjenjuje strategiju

"redukcije" legla. U ptica koje nesu relativno veliko posljednje jaje zastupljena je strategija "preživljavanja" legla, a u ptica koje nesu relativno malo posljednje jaje strategija "redukcije" legla. Ptice koje primjenjuju strategiju "redukcije" odnosno "smanjivanje" legla u slučaju nestašice hrane "žrtvuju" jednog ili više potomaka.

## CLUTCH SIZE, EGG DIMENSIONS AND BREEDING STRATEGY IN NUTHATCH *(Sitta europaea)*

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Egg-characteristics and breeding strategy in nuthatch were studied during 2000 -2002 in NW Croatia. Only cluthces from pairs that bred in nest boxes. All nest boxex had a sliding top in order to monitor nesting. This study has several tasks. First, to present the breeding characteristics of nuthatch and compare them with data obtained from other countries. Second, to investigate the influence of laying order on egg dimensions. There were no significant differences in egg during 2000 - 2002 ($p>0.05$). Generally, in this study there was no significant correlation between egg dimensions and the egg laying order. According to the relative size of the final egg laid (%D) nuthatch adopts the brood-reduction strategy. Birds which lay relatively big final eggs are adopting the brood- survival strategy (the last nestling is capable of rivalry with his older siblings), whereas birds which lay relatively small final egg are adjusting to the brood-reduction strategy (the last nestling will be sacrificed in the event of food-shortage).

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## TAXONOMICAL INVESTIGATIONS OF CHIRONOMID FAUNA (Diptera: Chironomidae) IN UPPER FLOW OF THE RIVER TRESKA R. MACEDONIA

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The main task of this scientific work was the investigation of chironomid fauna in the upper flow of the river Treska. Samples of the sediment were taken with Surber net from different colony. 13 species were determinated, among them, *Paratendipes albimanus* and *Cricotopus gr. sylvestris* were with the highest abundance. The most numerous population was established in May 2001 on the mud colony with 1620 ind/$m^2$ belonging to the species *Paratendipes albimanus*. Minimal population numberness was established for 7 species: *Paratanytarsus lauterborni*, *Paratanytarsus confusus*, *Eukeifferiela hospita*,

*Tanytharsus longipes*, *Prdiamesa olivacea*, *Cryptochironomus gr.defectus*, and *Polypedilum nubeculosum* that were not found at all in some of analysed samples.

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## VIŠEGODIŠNJA OPAŽANJA POPULACIJE UGROŽENOG LEPTIRA *Parnassius apollo* NA JEDNOM LOKALITETU U GORSKOM KOTARU

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Od 1979. godine prati se populacija ugroženog leptira *Parnassius apollo* L. (Rhopalocera) na jednom staništu u Gorskom Kotaru na 800 metara nadmorske visine i oko tri kilometra udaljenosti zračnom crtom od morske obale. Primjeirc koji lete na staništu fotografirani su s gornje i donje strane krila, tako da je do sada prikupljeno oko 1000 snimljenih jedinki (makro snimke s dva centimetra udaljenosti). Utvrđeno je da u rasporedu i veličini crnih i crvenih pjega leptiri pokazuju izrazitu individualnost - svi se primjerci međusobno razlikuju. Na terenu su bili označene sve uhvaćene jedinke da bi se moglo pratiti njihovo kretanje i procijeniti trajanje života. Sve faze u životu i patološke pojave apolona s ovog staništa dokumentirane su fotografski, VHS i S-VHS zapisima. Tijekom istraživanja značajno se smanjila površina područja na kojem žive leptiri i skratilo razdoblje u kojem oni lete. Površina areala populacije se smanjila skoro trostruko do 1996. godine. Razdoblje leta leptira skratilo se s oko 75 dana na oko 40 dana. Od 1996. godine do 2002. pratio se broj primjeraka koji lete u lipnju i srpnju, te je utvrđeno da se brojnost populacije također smanjila. Od 1998. prati se čitavživotni ciklus ove populacije, od izlaska gusjenica iz jajašaca do smrti leptira. Pri tom su uočene neke zanimljivosti u ponašanju leptira i gusjenica, od izlaska gusjenica iz jajašaca, načina svlačenja, priprema za svlačenje i kukuljenje, do ponašanja gusjenica i leptira kada su uznemireni. Uočene su i neke karakteristične refleksne reakcije gusjenica po kojima se može ocijeniti njihovo zdravstveno stanje, i ustanovljeno je da leptiri produciraju karakterističan i prepoznatljiv zvuk. Registrirane su dosta česte i raznolike patološke pojave kod gusjenica i leptira. Ova se populacija apolona smanjuje i vjerojatno će nestati ako joj se na neki način ne pomogne. Diskutira se o mogućem preseljenju dijela populacije na neka druga, pogodna staništa, koja bi se mogla održavati radom dobrovoljaca.

## SEVERAL YEAR OBSERVATIONS OF ENDANGERED BUTTERFLY *Parnassius apollo* POPULATION AT ONE LOCALITY IN GORSKI KOTAR

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Since 1979 one islolated habitat of the endangered butterfly *Parnassius apollo L.* on unnamed site in Gorski Kotar at 800 metres height above sea-level and about three

kilometres air distance from the coastline was investigated. All individual specimens from this location were photographed from the both wing side (close-up photographs from two centimetres) so that now we have about 1,000 photographed individuals. It is evident that in the pattern and size of their black and red spots, the butterflies express a marked individuality – of all the individual exemplars no two are alike. In addition, marking method for order to moving of individuals was used. All phases in their lives and pathological manifestations from this locality are well-documented photographically with VHS and S-VHS. In investigated period the distribution area of the butterflies decreased considerably and the period in which the butterflies fly became shortened. The area of population decreased nearly threefold till 1996. The period of the butterfly's flight became shortened from about 75 days to about 40 days. From 1996 to 2002 the denstiy in June and July are investigated, and has also decreased too. Some interesting features in behaviour of the butterfly and its caterpillar are noticed, like as their getting out of eggs, their way of sloughing, arrangements for such casting off and chrysalising, as far as the behaviour of caterpillars and butterflies when being upset. They are also noticed a few characteristic reflex reactions of caterpillars, by which their health condition can be estimated and found out that the butterflies generate a characteristic and recognisable sound. In their development rather frequent and various pathological manifestations at caterpillars and butterflies are registered. This population of apollo butterfly is diminishing and is likely to disappear, if it could not be helped somehow. Possible introducing the population to some other suitable biotopes, which would be kept up by work of volunteers, are discussed.

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## VISINSKA RASPODJELA DANJIH LEPTIRA (Rhopalocera) NA ŽUMBERKU

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Istraživana je fauna danjih leptira Žumberačke gore tijekom 2000. i 2001. godine. Odabrane su četiri postaje različitih nadmorskih visina: Pribić, 125 m/nm; kanjon Slapnice, 200 m/nm; Boljare, 900 m/nm; Sveta Gera, 1178 m/nm. Željelo se utvrditi faunističke i zoogeografske značajke, kao i visinsku razdiobu danjih leptira. Terenskim istraživanjima i pregledom entomoloških zbirki Hrvatskog prirodoslovnog muzeja u Zagrebu utvđeno je 70 vrsta danjih leptira. Stupanj istraženosti Žumberačke gore utvrđen je usporedbom faune Žumberka sa relativno dobro istraženim dijelovima kontinentalne Hrvatske i Slovenije (Podravina, Gorski Kotar, Turopolje, Prekmurje). Sličnosti obzirom na vrste danjih leptira izražene su Sörensenovim indeksom.

## ALTITUDE DISTRIBUTION OF BUTTERFLIES (Rhopalocera) ON Mt. ŽUMBERAK

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During 2000 and 2001 butterfly fauna of Žumberak Mt. was researched. Four localities on the different altitudes were selected (Pribić, 125 m/a.s.l.; Slapnica, 200 m/a.s.l.; Boljare, 900 m/a.s.l.; St. Gera, 1178 m/a.s.l.). The objective of research was to find out faunistical and zoogeografical characteristics, as well as butterflies' altitude distribution. 70 species of butterflies were find out by field and laboratory work and reviewing entomological collections in Croatian Natural History Museum in Zagreb. The fauna of Žumberak Mt. was compared with relativly well researched part of continental Croatia and Slovenia (Podravina, Gorski Kotar, Turopolje, Prekmurje). Sörensen index was used to examine the similarity of locations with regard to butterfly species.

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## NEKI ASPEKTI BIOLOGIJE SKUPINE NOTOSTRACA (Crustacea) U POVREMENIM STANIŠTIMA PARKA PRIRODE LONJSKO POLJE, HRVATSKA

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Istraživanje je provedeno od ožujka do srpnja 2001. i 2002. godine u cilju utvrđivanja prisutnosti i rasprostranjenosti, trajanja životnog ciklusa te utvrđivanja ekoloških obilježja staništa skupine Notostraca u Lonjskom polju. Istraživanje je provedeno na šest lokaliteta, koji obuhvaćaju 8 lokvi i 1 rukavac. Vrsta *Triops cancriformis* (Bosc, 1801) je prvi puta zabilježena na području Lonjskog polja, a za vrstu *Lepidurus apus* (L., 1758) su utvrđena nova nalazišta. Vrsta *T. cancriformis* utvrđena je na dva lokaliteta – Mužilovčica (1 lokva) i Lonja (1 lokva), a *L. apus* samo na lokalitetu Mužilovčica (2 lokve). Obje se vrste pojavljaju u proljeće, nakon punjenja lokvi obilnim kišama. Simpatrijske populacije su zabilježene samo tijekom sušnog proljetnog razdoblja 2001. godine u lokvi na lokalitetu Mužilovčica. Vrsta *T. cancriformis* nastanjuje povremene lokve koje nastaju zadržavanjem oborinske vode, dok vrsta *L. apus* nastanjuje lokve koje se uz oborinsku pune i podzemnom vodom. Utvrđene su značajne razlike u tipu sedimenta i zasjenjenosti pojedinih

lokvi. Obje vrste nastanjuju lokve poplavnih šuma, a lokve pašnjaka jedino vrsta *T. cancriformis*. Životni ciklus stenotermne vrste *L. apus* završava sredinom svibnja, iako je voda u lokvi još prisutna. Euritermnoj vrsti *T.cancriformis* životni ciklus završava sredinom lipnja, nakon potpunog isušivanja lokvi. Rezultati istraživanja upućuju na potrebu i mogućnosti zaštite skupine Notostraca na području Hrvatske.

## SOME ASPECTS OF NOTOSTRACA (Crustacea) BIOLOGY IN TEMPORARY HABITATS OF NATURE PARK LONJSKO POLJE, CROATIA

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Research was conducted from March until July 2001 and 2002. The goal was to determine the occurrences and distribution of Notostraca in Lonjsko polje. The research also focused on their life cycle and ecological characteristics of the ephemeral ponds they inhabit. The research took place at six localities, which included 8 ponds and an oxbow lake. *Triops cancriformis* (Bosc, 1801) was found in Lonjsko polje for the first time, and a new collection site of *Lepidurus apus* (L., 1758) was observed. *T. cancriformis* was determined in Mužilovčica and Lonja, in one pond at each locality. *L. apus* was determined in two ponds only in Mužilovčica. Both species appear in spring, after inundation of ephemeral ponds. Only during the dry spring of 2001, sympatric populations were observed in pond at Mužilovčica locality. *T. cancriformis* inhabits ephemeral ponds which are formed by retaining of rainwater, while *L. apus* inhabits ponds which are filled with rainwater and groundwater. We were determined differences in type of sediment and level of shadiness at each pond. Both species inhabit ponds in flooded woods, while *T. cancriformis* inhabits grassland ponds, too. The life cycle of the stenothermal species *L. apus* ends in mid-May, even though the ponds don't dry out. The life cycle of the eurithermal species *T. cancriformis* ends in mid-June, after the ponds dry out. The results of this research implicate possible requirement for legal protection and conservation of Notostracan group in Croatia.

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## SADAŠNJI STATUS I DISTRIBUCIJA PAKLARA (Cyclostomata: Pteromyzontidae) U HRVATSKOJ

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Porodica paklara (Petromyzontidae) iz razreda kružnoustih (Cyclostomata) na području Europe broji osam vrsta. Sve vrste osim morske paklare, *Petromyzon marinus* izrazito su slatkovodne. Ova skupina kralješnjaka s manjim ili većim intenzitetom predmet je istraživanja na području Hrvatske gotovo dvjestotinjak godina. Iako ne brojna, na prostoru Hrvatske nije još utvrđen točan faunistički sastav, morfološke značajke i rasprostranjenost zabilježenih vrsta. Zbog navedenih razloga počelo se je sa sistematskim prikupljanjem i istraživanjem ove skupine prije nekoliko godina. Osim terenskog i laboratorijskog rada, te analize literaturnih podataka izvršen je i djelomičan uvid u zoološke zbirke muzeja i instituta u Zagrebu, Rovinju, Rijeci, Splitu i Dubrovniku, a izvršeni su i kontakti sa kolegama iz Ljubljane, Sarajeva i Beča o primjercima pojedinih vrsta u njihovim kolekcijama. Na temelju provedenih istraživanja i uvida u literaturne podatke utvrđeno je da na području Hrvatske zasigurno dolaze tri vrste: *Petromyzon marinus*, *Eudontomyzon mariae* i *Lethenteron zanandrei*, a literaturni podaci o vrstama *Lampetra fluviatilis* i *Lampetra planeri* jako su diskutabilni i njihovo prisustvo u fauni Hrvatske tek treba utvrditi.

## CURRENT STATUS AND DISTRIBUTION OF LAMPREY (Cyclostomata: Pteromyzontidae) IN CROATIA

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Family lamprey (Pteromyzontidae) from the class cyclostomes (Cyclostomata) on the region of Europe includes eight species. All species except the marine lamprey, *Pteromyzon marinus* are strictly fresh-water. This group of vertebrates with lower or greater intensity has been a subject of research on the territory of Croatia for over two hundred years. Although not numerous, on the territory of Croatia, the exact number of species has not yet been determined, nor there morphological characteristics and the distribution of the recorded species. Because of the following reasons a gradual collecting and the research of this group has started a few years ago. Besides field and laboratory work and the analysis of the literature data there has been a partial insight in zoological collections of museums and institutes in Zagreb, Rovinj, Rijeka, Split and Dubrovnik as well as contacts with colleges from Ljubljana, Sarajevo and Vienna about the specimens in there collections. On the basis of the research and the

insight in literature data it has been established that three species are certainly present on the territory of Croatia: *Pteromyzon marinus*, *Eudontomyzon mariae* and *Lethenteron zanandrei*, but the literature data about species *Lampetra fluviatilis* and *Lampetra planeri* are debatable and there present in fauna of Croatia is yet to be determined.

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## LIČINKA VRSTE *Drusus ramae* MARINKOVIĆ, 1970 (Trichoptera: Limnephilidae)

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Osamnaest vrsta iz roda *Drusus* zabilježeno je na području Dinarida i zapadne Makedonije. Mnoge vrste su endemske. Najveći broj endemskih vrsta pronađen je u fauni Bosne i Hercegovine: *D. bosnicus* Klá., *D. klapaleki* Mar., *D. radovanovici* Mar., *D. vespertinus* Mar., *D. ramae* Mar. *D. medianus* Mar. and *D. schmidi* Boto. Postoji vrlo malo podataka o morfološkim značajkama endemskih, dinarskih vrsta iz roda *Drusus*. Radom prikazujemo nepoznte morfološke karakteristike ličinke vrste *D. ramae* Mar., i dajemo karatk osvrt na njene zoogeografske i ekološke značajke.

## THE LARVA OF *Drusus ramae* MARINKOVIĆ, 1970 (Trichoptera: Limnephilidae)

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Eighteen species of the genus *Drusus* were recorded from the Dinarides and western Macedonia. Many of them are endemic species. The highest number of endemic species were found in fauna of Bosnia and Herzegovina: *D. bosnicus* Klá., *D. klapaleki* Mar., *D. radovanovici* Mar., *D. vespertinus* Mar., *D. ramae* Mar. *D. medianus* Mar. and *D. schmidi* Boto. There is a general lack of data on the morphology of endemic Dinaric *Drusus* species. We presented morphological description of unknown larvan of *D. ramae* Mar. We have also presented some zoogeographical and ecological notes.

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## BIOLOGICAL DIVERSITY OF THE SPECIES OF THE GENERA: *Otiorynchus*, *Trachyphloeus* AND *Phyllobius* (Curculionidae: Otiorynchinae) IN THE FAUNA OF MACEDONIA

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In this work, the author presents her own results of the researches of many years about the biological diversity of the species of the genera: *Otiorynchus, Trachyphloeus and Phyllobius* in the fauna of Macedonia. Most of these species have been collected in the meadows in the high mountains throughout Macedonia, by means of the standard methods for colleting this group of insects. Based on the taxonomic-faunal analysis, presence has been established of 40 species; of which 26 belong to the genus *Otiorynchus,* 2 to the genus *Trachyphloeus,* and 12 to the genus *Phyllobius*. In the list of established species, the presence of new and endemic species is especially significant for the fauna of Macedonia. This has been the result of the specific geological, geographical and climate conditions that are dominant in the region. All these species are phytophagous and so are known as huge pests for certain plant species. Therefore these insect species have economic impact as well.

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## ODREĐIVANJE SPOLA U NEKIH VRSTA KITOVA (Cetacea) POMOĆU LANČANE REAKCIJE POLIMERAZOM

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Pri istraživanju biologije kitova znanstvenici često nailaze na probleme prilikom prikupljanja podataka na terenu. Ponekad nije moguće morfološki odrediti spol životinje čije je truplo pronađeno u uznapredovalom stanju truljenja. Također je teško ili čak nemoguće promatranjem u prirodi odrediti spol žive jedinke, budući da u većine vrsta kitova spolni dimorfizam nije izražen. Ovim istraživanjem pokušali smo uvesti pouzdanu molekularno-genetičku metodu kojom bi se određivao spol kitova na uzorcima tkiva

uzetih s lešina, kao i na biopsijama kože živih jedinki. Ukupna DNA je izolirana iz mišićnog tkiva 49 jedinki; 27 dobrih dupina (*Tursiops truncatus*), 9 plavobijelih dupina (*Stenella coeruleoalba*), 7 glavatih dupina (*Grampus griseus*), 2 krupnozuba dupina (*Ziphius cavirostris*), 2 velika sjeverna kita (*Balaenoptera physalus*) i 2 uzorka neidentificiranih vrsta. U jednom slučaju je DNA izolira na iz kože. Svi su uzorci uzeti s lešina različitih stupnjeva raspadanja. Lančanom reakcijom polimerazom uz tri oligonukleotidne početnice umnoženi su dijelovi ZFY i ZFX homolognih gena na Y- i X-kromosomima. Proizvodi reakcije, različite duljine i specifični za spol, razdvajani su elektroforezom u 1% agaroznom gelu. Uspješno je određen spol na 40 uzoraka. Pokazalo se da metoda nije primjenjiva u slučajevima raspadnutog tkiva, ali je pouzdana i učinkovita kad se primijeni na svježim ili dobro očuvanim uzorcima.

## SEX DETERMINATION IN SOME CETACEAN SPECIES BY THE POLYMERASE CHAIN REACTION

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Investigations of cetacean biology often encounter problems in gaining information from the field. It is not always possible to morphologically determine sex of an animal whose carcass was found in an advanced state of decomposition. Determining the gender of living cetaceans is also difficult or impossible, since in most species sexually dimorphic characters are poorly marked. The purpose of this study was to introduce a reliable method for molecular sexing of cetaceans that could be used on tissue samples from carcasses, as well as on skin biopsies from living animals. Total DNA was extracted from muscle tissue of 49 cetacean specimens; 27 bottlenose dolphins (*Tursiops truncatus*), 9 striped dolphins (*Stenella coeruleoalba*), 7 Risso's dolphins (*Grampus griseus*), 2 Cuvier's beaked whales (*Ziphius cavirostris*), 2 fin whales (*Balaenoptera physalus*) and 2 samples of unidentified species. In one case DNA was extracted from skin. All samples were taken from corpses in different state of decomposition. The introduced method uses polymerase chain reaction (PCR) to amplify regions on ZFY and ZFX, which are the homologous genes located on Y- and X-chromosomes, respectively. Three oligonucleotide primers were employed to produce fragments specific for the ZFY and ZFX sequences. Amplification products differed in length and therefore gave distinct, sex-specific bands on 1% agarose gel after electrophoresis. The sex of 40 cetacean specimens was success-

fully determined. Method failed in cases of strongly decomposed tissues, but showed reliable and efficient when applied on fresh or well preserved samples.

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## MEĐUODNOS DULJINE KUKOVLJA, DULJINE TIJELA I TJELESNE MASE DOBROG DUPINA (*Tursiops truncatus*) IZ JADRANSKOG MORA

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U 52 dobra dupina (*Tursiops truncatus*), koji su pronađeni mrtvi od listopada 1990. do ožujka 2003. godine u Jadranskom moru, istraživan je međuodnos duljine kukovlja s tjelesnom masom i duljinom tijela. Istraženo je 29 ženki, tjelesne mase od 23,65 do 261 kg, duljine tijela od 122 do 288 cm i 23 muške životinje tjelesne mase od 75 do 350 kg duljine tijela 163 do 312 cm. Prosječna duljina kukovlja bila je 7,95 cm (od 1,8 do 12,7 cm). Razlika između duljine lijevog i desnog kukovlja iste životinje u prosjeku je iznosila 0,37 cm, s najvećom razlikom od 1,5 cm. Velika korelacija (R=0,971) između duljine kukovlja i duljine životinje opažena je kod ženki dobrog dupina, dok kod mužjaka korelacija nije uočena. Kod ženki također postoji korelacija (R=0,941) između duljine kukovlja i tjelesne mase životinje dok je kod mužjaka nema. Koristeći ove korelacije kod ženki dobrog dupina na temelju prosječne duljine kukovlja možemo procjeniti duljinu tijela (duljina tijela u cm = 100,37Ln(duljina kukovlja u cm) + 49,787) i njezinu tjelesnu masu (tjelesna masa u kg = 26,147duljina kukovlja u cm - 29,419).

## CORRELATION BETWEEN PELVIC BONE LENGTH, BODY LENGTH AND BODY MASS OF BOTTLENOSE DOLPHINS (*Tursiops truncatus*) FROM THE ADRIATIC SEA

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The correlation of pelvic bone length, body length and body mass of 52 bottlenose dolphins (*Tursiops truncatus*) found dead from October 1990 till March 2003

in the Adriatic Sea was investigated. This study included 29 females of body mass in range from 23.65 to 261 kg, body length in range from 122 to 288 cm and 23 males of body mass in range from 75 to 350 kg and body length from 163 to 312 cm. The average pelvic bone length was 7.95 cm (in range from 1.8 to 12.7 cm). The average difference in length of the left and right pelvic bone of the same animal was 0.37 cm, and the highest difference was 1.5 cm. A high correlation (R=0.971) between pelvic bone length and body length was observed in females although the correlation was not found in males. Females also show a correlation (R=0.941) between pelvic bone length and body mass while males do not. Using this correlations in females based on the average pelvic bone length the body length (body length in cm = 100.37Ln (pelvic bone length in cm) + 49.787) and body mass (body mass in kg = 26.147pelvic bone length in cm – 29.419) can be calculated.

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## PROCJENA STAROSTI DOBROG DUPINA (*Tursiops truncatus*) IZ JADRANSKOG MORA PREMA BROJU ZONA PRIRASTA U ZUBNOM DENTINU I USPOREDBA S DULJINOM TIJELA I TJELESNOM MASOM ŽIVOTINJE

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U 51 dobrog dupina iz Jadranskog mora procijenjena je starost na temelju zona prirasta u zubnom dentinu. Na podužnim i poprečnim presjecima zubiju obojenim hemalaunom brojane su zone prirasta u dentinu. Najveća procijenjena starost dobrog dupina bila je 26 godina, a najmlađe životinje mlađe su od godinu dana. Prosječna starost istraženih dobrih dupina je 10,6 godina, a najčešća 12 godina. Histogram starosti istraživanih dobrih dupina ukazuje na mladu populaciju s povećanom smrtnošću muških jediniki u dobi od 9 do 12 godina. Starost dobrih dupina u boljoj je korelaciji s duljinom tijela (R=0,900) nego s tjelesnom masom (R=0,792). Poznavanjem duljine tijela dobrog dupina koristeći jednadžbu: starost u godinama = $0{,}091e^{0{,}0179\text{duljina tijela u cm}}$ može se procijeniti starost životinje, a za procjenu starosti životinje na temelju tjelesne mase može se koristiti jednadžba: starost u godinama = $1{,}4383e^{0{,}0097\text{tjelesna masa u kg}}$. Poznavanjem spola životinje preciznije se procjenjuje starost životinje na temelju duljine tijela pomoću jednadžbe za mužjake (starost u godinama = $0{,}0786e^{0{,}0189\text{duljina tijela u cm}}$ uz faktor korelacije R=0,918) i za ženke (starost u godinama = $0{,}0909e^{0{,}0174\text{duljina tijela u cm}}$ uz faktor korelacije R=0,907).

## AGE ESTIMATION IN THE BOTTLENOSE DOLPHIN (*Tursiops truncatus)* FROM THE ADRIATIC SEA BASED ON GROWTH LAYER GROUPS IN DENTINE AND CORRELATION OF AGE WITH BODY LENGHT AND BODY MASS

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The age of 51 bottlenose dolphins found in the Adriatic Sea based on growth layer groups in dentine has been estimated.. Growth layer groups were counted on longitudinal and transversal tooth sections stained with hematoxylin. The highest estimated age of a bottlenose dolphin was 26 years, and the youngest animals were less then one year old. The average age of studied bottlenose dolphins was 10.6 years and the most frequent 12. Age histogram of investigated bottlenose dolphins shows a young population with an increased mortality of males in the age of 12 years. The age of bottlenose dolphins is in higher correlation with body length (R=0.900) than with body mass (R=0.792). The following formula can be used for age determination if the body length of an animal is known: age in years = $0.091e^{0.0179\text{body length in cm}}$ and the second one if the body mass is known: age in years = $1.4383e^{0.0097\text{body mass in kg}}$. More precise age determination can be calculated if the sex of the animal is known by following formula for males: age in years = $0.0786e^{0.0189\text{body length in u kg}}$, correlation factor R=0.918), and for females: age in years = $0.0909e^{0.0174\text{body length in kg}}$ , correlation factor R=0.907).

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## RADIO-TELEMETRY STUDY OF THE WILDCAT (*Felis silvestris*) IN SOUTHERN SLOVENIA

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A marked decrease in the historical range of the wildcat has taken place over the last centuries in most of Europe. Many recent European populations

of the wildcat are relatively small, isolated and exposed to break-up, reduction and alteration of the habitat. Wildcats were captured for radio collaring in a study of their spatial distribution and social organisation. The study was carried out in the northwestern part of Dinaric Mountains. The centre of the study area is situated at 45°40' N, 14°41' E in Kocevje forests of southern Slovenia. The research focused on activity patterns, spatial distributions and social interactions of the wildcats. Habitat selection was studied in relation to some relief and vegetation parameters. Influences of some abiotic factors on wildcats' activity patterns were examined and compared between seasons. Nine wildcats, one lynx and one feral cat were caught as target species. General, time independent spatial distribution of the wildcats in the study area was non-random and depended primarily on the forest type. Except for one male with a circadian activity pattern, all animals had a crepuscular–nocturnal activity. Their travel activity increased at night, while spot activity showed several peaks during day and night. The influence of daylight cycle, day length, moon phase and temperature partly explained variability in wildcats' activity. Home range sizes ranged from 264 ha to 1876 ha and overlapping was found only between sexes. Despite overlapping, dynamic interactions showed strong avoidance between specimens. This indicates that they are strongly solitaire animals, with rare direct contacts and successful chemical communication.

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## CAPTURE AND CHEMICAL IMMOBILIZATION OF FREE LIVING WILDCATS (*Felis silvestris*) IN SOUTHERN SLOVENIA

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Wildcats were captured in the course of a radiotelemetric study of wildcats in southern Slovenia. One goal of the study was also to establish a safe, efficient procedure for capture and immobilization of free-living wildcats. Cats were captured using double-door box traps placed at trails they regularly used. Nine wildcats and one feral cat were captured. The cats were immobilized with a combination of medetomidine and ketamine. In the first two cats, the drugs were administered by a blowpipe. All subsequent captures were restrained with a restraining plate, and the drug injected using a hand-held syringe. The animal was monitored during the immobilization period. Readings of body temperature, heart rate and breathing frequency were taken at regular intervals. A physical examination of the animal was performed, biometric data measured and blood and urine samples taken. The animal was photographed and checked for ectoparasites. A small piece of skin tissue was taken for ge-

netic analyses, and a radio-collar was fitted. The cat was given atipamezole, a specific medetomidine antidote, to facilitate its recovery. The animals were put back in the box trap and released once they seemed completely recovered. Subsequently, a control group of eight domestic cats was immobilized using the same combination of drugs and the results compared with the results obtained from the wildcats. Combination of medetomidine and ketamine seemed to be an effective, safe method for immobilization of wildcats, and the effective doses did not differ significantly from the results obtained from the control group.

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## CAPTURE EFFICIENCY OF BOX TRAPS FOR WILDCATS (*Felis silvestris*), CAPTURE-RELATED INJURIES AND HEALTH STATUS OF CAPTURED ANIMALS

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Wildcats were captured for radio collaring as a part of a study of their spatial distribution and social organisation in southern Slovenia. This provided a chance to evaluate efficiency of box traps for capturing wildcats and enabled assessment of their health status. Double-door box traps have been used to capture individuals. Nine wildcats, one lynx and one feral cat were caught as target species and 19 badgers and one bear cub as non-targets. The cats were immobilised with a combination of medetomidine and ketamine. A thorough physical examination of the cats was preformed, and samples for haematology, blood chemistry, most common infectious diseases, urine analysis and genetics were taken. Samples were taken from a control group of healthy domestic cats and results compared with the results from the wildcats. The catching success was 1 wildcat/58 trap-days. Five of the cats had no injuries, and four had minor abrasions and lacerations. Values of glucose, BUN, Cl, albumin, MCV, basophil count and ALT were significantly higher than the values of the control group. All urine samples contained WBC and proteins, and seven of them contained RBC. All cats were checked for FeLV and FIV, seven were checked for corona viruses and five were checked for parvovirosis. All checked animals were negative. Box traps seem a fairly efficient method for trapping wildcats and do not cause many injuries to the trapped animal. The health status of the animals seemed good, but the sample size is too low to extrapolate conclusions to the whole population.

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## HOME-RANGE DYNAMIC OF WILDCATS (*Felis silvestris*) IN THE REGION OF THE DINARIC FORESTS (SLOVENIA)

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The wildcat is distributed through most of Eurasia and throughout the African continent. Despite this vast distribution, it is still poorly known. Unlike many other felids, which live in open habitats, wildcats prefer forests. Due to the limestone geological foundation, dinaric area is subject to karts phenomena. This results in a typical relief with mosaic-structured microclimate of the study area. The research has been carried out as a part of a radio-telemetry study in Kočevje forests. The analysis of the wildcat's home range and habitat use was carried out at two levels of sampling. We compared habitat use between seasons and between sexes. We used fixed 95% kernels as a utilization estimator and minimal convex polygons – MCP's as a range estimator of wildcats' home ranges. Spatial shifts and associated habitat use were examined using Jenrich-Turner centroids and wildcats' core areas. They showed preference for steep, SW exposed slopes and altitudes between 680 and 920 m. These preferences were stronger in the cooler seasons. The analysis of wildcats' habitat use in the cooler seasons 99/00 and 00/01 respectively revealed more frequent use of the open habitat in the season 00/01. No significant difference in home range sizes between the sexes was found, except for one female and the feral cat. The formation of home ranges was changing seasonally with spatial shifts between seasons. The shifts were most synchronized at the end of the summer 2000. It was typical only for the females that they returned to the same seasonal home ranges in subsequent years.

KONGRESNI DAN KOPNENIH VODA
THE CONGRESS FRESHWATER DAY

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## FITOPLANKTON VISOVAČKOG JEZERA

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U sklopu ekoloških istraživanja, u području Nacionalnog parka Krka, istraživana je vertikalna i sezonska distribucija mrežnog fitoplanktona te neki fizikalno-kemijski parametri, na profilu K1 u Visovačkom jezeru. Uzorkovanja su provođena jednom mjesečno u razdoblju od travnja 1995. do ožujka 1996. godine. Uzorci su dobiveni filtriranjem jezerske vode s dubina: 1, 2, 5, 10, 15 i 20 m. Apsolutna učestalost fitoplanktonskih vrsta (broj stanica/l) dobivena je prebrojavanjem stanica pojedinih vrsta u komorici s milimetarskom mrežicom. Maksimalna je brojnost fitoplanktona tijekom istraživanja utvrđena na dubini od 5 do 10 m, izuzev u hladnijim mjesecima. Sezonska distribucija gustoće fitoplanktona pokazuje dvije vršne vrijednosti i to tijekom lipnja te rujna odnosno listopada. Tijekom proljetnog, pa sve do sredine ljetnog, razdoblja u fitoplanktonskoj zajednici prevladavaju: *Asterionella formosa* Hass., *Cyclotella melosiroides* (Kirch.) Lemm i *Fragilaria crotonensis* Kitt. Dominantna je vrsta *Asterionella formosa* s maksimumom u kasnoproljetnom razdoblju (1,5-1,7x$10^6$ stanica/l). Vrste *Cyclotella melosiroides* i *Fragilaria crotonensis* maksimum brojnosti postižu tijekom ranog proljeća (1,9-7,7x$10^3$ stanica/l). Od sredine ljeta do rane jeseni u fitoplanktonskoj zajednici dominira *Ceratium hirundinella* (O.F. Müller) Dujardin. Tijekom kasne jeseni i početkom zime najveću gustoću stanica razvija vrsta *Fragilaria crotonensis*. Zimi je utvrđen i znatniji broj bentoskih vrsta iz skupine *Bacillariophyceae*.

## PHYTOPLANKTON OF LAKE VISOVAC

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During ecological research in National park Krka, vertical and seasonal distribution of net phytoplankton together with some physical and chemical parameters, on K1 profile of Lake Visovac was researched. Samples were taken once a month in period from April 1995 to March 1996. Water samples were taken from depths of 1, 2, 5, 10, 15 and 20 meters and filtrated. From number of counted cells on millimeter grid cell, relative abundance (number of cells per litre) of species was calculated. Highest number of phytoplankton was observed in depths from 5 to 10 meters, except in colder period of year. Seasonal distribution of phytoplankton density showed two peaks: during June and September/October. Most abundant species during spring and midsummer

were: *Asterionella formosa* Hass., *Cyclotella melosiroides* (Kirch.) Lemm. and *Fragilaria crotonensis* Kitt. Most abundant species was *Asterionella formosa* with peak in late spring period (1.5-1.7x$10^6$ cells/l). Species *Cyclotella melosiroides* and *Fragilaria crotonensis* had their peaks during early spring (1.9-7.7x$10^3$ cells/l). Species *Ceratium hirundinella* (O.F. Müller) Dujardin was dominant in phytoplankton community from midsummer until early fall. During late fall and beginning of winter highest phytoplankton density was noted for species *Fragilaria crotonensis*. In winter, considerable number of benthic Bacillariophyceae was also observed.

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## VERTIKALNA DISTRIBUCIJA PERIFITONA U UJEZERENOM DIJELU RIJEKE KRKE

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U razdoblju od srpnja do listopada 1995. godine, na vertikalnom profilu (epilimnij, metalimnij, hipolimnij) donjeg ujezerenog dijela rijeke Krke, eksponirane su pleksiglas ploče tijekom 30, 60 i 90 dana. Istraživanje je provedeno u svrhu utvrđivanja intenziteta obrastanja umjetnih podloga. Uz kvalitativni sastav i brojnost algi na okomito (direktno strujanje vode) i horizontalno (gornja i donja strana) postavljenim podlogama, praćeni su i neki fizikalno kemijski parametri. Provedenim je istraživanjima determinirano 90 vrsta. Unutar dominantne skupine *Bacillariophyceae* redovito su u svim uzorcima bile prisutne: *Cyclotella radiosa* (Grun.) Lemmer., *Achnanthes affinis* Grun., *Cocconeis placentula* Ehr. i *Navicula minima* Grun. Najveći potencijal obrastanja zabilježen je u epilimniju na gornjoj strani horizontalno postavljene plohe, nakon ekspozicije od 60 dana. Pri istoj dužini ekspozicije zabilježen je i najmanji potencijal obrastanja u hiplolimnionskom sloju na okomito postavljenoj podlozi. Rezultati ukazuju na znatnije razlike u gustoći, broju i sastavu vrsta između obraštajnih ploha i dubina.

## VERTICAL DISTRIBUTION OF PERIPHYTON IN LOTIC AREA OF RIVER KRKA

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The aim of this study was to determine rate of periphyton growing over artificial surfaces. Plexy slides were exposed for 30, 60, and 90 days on vertical profile

(epilimnion, metalimnion and hypolimnion) of lower, lotic area of River Krka from July to September 1995. Besides qualitative and quantitative composition and abundance of algae on perpendicular (direct water flow) and parallel (upper and lower side) surfaces, some physic-chemical parameters were also researched. During research time 90 different species were observed. The most dominant group, *Bacillariophiceae* was well represented with species. *Cyclotella radiosa* (Grun.) Lemmer., *Achnanthes affinis* Grun., *Cocconeis placentula* Ehr. and *Navicula minima* Grun. that were present in all samples. Highest growth potential was observed after 60 days of exposition on upper side of horizontally placed surface in epilimnion. During the same exposition time, the lowest growth potential was noted on perpendicular surface in hypolimnion. Results show significant differences in abundance, number and composition of algae between growth surfaces and depths.

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## IHTIOFAUNA RIJEKE SAVE U BLIZINI ZAGREBA

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Tijekom 2001. i 2002. godine istraživana je rijeka Sava u blizini Zagreba u svrhu procjene zajednice riba. Uzorkovanja su provedena tijekom proljeća i ljeta, pomoću elektroagregata (2,5 kW) na tri lokaliteta na rijeci. Tijekom istraživanja u rijeci Savi u okolici Zagreba utvrđene su 24 vrste riba. Pet vrsta riba su česte vrste, 10 ih je srednje često dok se 9 vrsta smatra rijetkima. Uz ribe koje često obitavaju u ovom dijelu rijeke Save utvrđeno je i 7 vrsta koje se javljaju tek sporadično. Unatoč tome što nije potvrđeno 6 vrsta karakterističnih za donje tokove rijeka uočava se određeno povećanje broja vrsta nizvodno. Sa sve intenzivnijim antropogenim utjecajima nakon 60-ih godina 20. stoljeća struktura zajednica riba počela se znatno mijenjati. Kao rezultat tih promjena došlo je i do nekoliko većih pomora riba tijekom 60-ih i 70-ih godina. Danas rijeka Sava pripada skupini srednje onečišćenih rijeka (III. kategorija) iako ponekad za niskog vodostaja ulazi i u IV. kategoriju. U zadnjih 15-ak godina nakon izgradnje Nuklearne Elektrane Krško došlo je do dodatnih ekoloških promjena u rijeci Savi prvenstveno zagrijavanjem vode rijeke za više od dva stupnja. Dakle, osnovni razlog nestajanja pojedinih vrsta riba su promjene staništa i kvalitete vode.

## ICHTHYOFAUNA OF SAVA RIVER IN VICINITY OF ZAGREB

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The river Sava, in close proximity of Zagreb, was investigated in 2001 and 2002 to evaluate fish communities. Sampling was performed by electric gear (2.5 kW) at three places on the river during the spring and autumn. Based on sampling, throughout our study 24 species have been detected inhabiting the river Sava in the proximity of Zagreb. Five species are common, ten intermediate, and nine rare. Seven species that only occasionally occur near Zagreb were included into the list together with species, which we assume to occur frequently in this part of the river Sava. Six species of typical lowland waterways fish communities have not yet been confirmed at the river Sava nearby Zagreb but a gradual increase in the number of species is noted in the downstream direction. The structure of fish communities has been changed since 1960 under the strong anthropogenic influence. As a result of that several fish mortalities have been detected during the 1960's and 1970's. Now days river Sava belongs to the category of semi-polluted rivers (IIIrd category), however it reaches IVth category at some places during the minimal discharges. For the last 15 years the Nuclear Power Plant Krsko has been changing the ecological conditions in Sava River by warming the river water by more than 2 degrees. Hence, habitat and water-quality changes influenced the disappearance of some fish species in the river.

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## MACROINVERTEBRATES IN WATER QUALITY ASSESSMENT OF THE RIVER BOSNA (BOSNIA AND HERZEGOVINA)

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Sampling of macroinvertebrates was carried out in waters of river Bosna at seven sites in period from July 1999 to June 2000 using kick-sampling method. Physical and chemical parameters (water depth, characteristics of sediment, conductivity, pH, concentration and saturation with oxygen, BPK5, nitrates and phosphates) were measured during the investigation. Localities in the river Bosna include mainstream 500 m down the spring, up to mouth of the Lašva river, near Zenica. Qualitative and quantitative composition of macroinvertebrates, their spatial and temporal variability was analysed. Index of diversity was counted according to Shannon-Weawer (1949). Spreaded biotic index (modification, Ghetti 1986) was used in estimation of the water quality. In the benthos samples from the river

Bosna 82 taxa were found. Results indicate high degree of pollution of the river Bosna after the mouth of the Miljacka river. There is a correlation between qualitative and quantitative composition of macroinvertebrates and physical and chemical parameters throughout year. Values of the diversity index vary from 1.14 (benthos samples before mouth of the river Ljubina) to 2.37 in the river Mala Bosna. Spreaded biotic index had lower value before mouth of the river Ljubina (3.12), and the highest in Mala Bosna (7.62). There is need for modification of used biotic index and its adaptation to character of investigated waterway. This research could help to get data on species that are most reliable for assessment of water quality in waterways of Bosnia and Herzegovina.

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## ODNOS VELIČINE ŽENKI VRSTE *Austropotamobium torrentium* I NJIHOVIH JAJA

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Rak kamenjar (*Austropotamobius torrentium*) je najmanja vrsta porodice Astacidae o čijoj se ekologiji, biologiji i životnom ciklusu ne zna dovoljno. Stoga je svrha istraživanja bilo sakupljanje podataka o reprodukcijskom ciklusu ove vrste. Važan čimbenik koji utječe na fekunditet ženki je veličina jaja. Zna se da se s povećanjem veličine jaja smanjuje fekunditet, no nije poznato kakav je odnos između veličine jaja i veličine ženki pa smo u ovom radu istražili taj odnos. Istraživanje je provedeno od listopada 1998. do travnja 2000. godine u potocima Medvednice. Ukupno je ulovljeno 767 ženki, od čega su 92 (11,99 %) imale jaja pričvršćena za pleopodalne noge. Vanjska jaja su na ženkama pronalažena od početka studenog do sredine lipnja slijedeće godine. Najmanja ulovljena ženka s pleopodalnim jajima je bila ukupno duga (od vrha rostruma do kraja telzona) 5,4 cm, a najveća 9,29 cm. Promjer pleopodalnih jaja kretao se od 0,24 do 0,36 cm (srednja vrijednost ± SD = 0,28 ± 0,02), a njihov broj po ženki od 1 do 104. Zabilježena je pozitivna korelacija između veličine ženki i broja jaja (r = 0,39), veličine ženki i promjera jaja (r = 0,30) te negativna između broja jaja i njihovog promjera (r = -0,04). Do sada su u procjeni fekunditeta, odnosno prirasta u populaciji, korišteni odnosi veličine ženki i broja jaja, a dobivene spoznaje o pozitivnoj korelaciji između veličine ženki i veličine, tj. promjera jaja, ukazuju da se i ovaj odnos može koristiti za preciznije procjenjivanje fekunditeta i prirasta.

## THE RELATIONSHIP BETWEEN FEMALE SIZE AND EGG SIZE IN FRESHWATER CRAYFISH *Austropotamobius torrentium*

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The stone crayfish (*Austropotamobius torrentium*) is the smallest species in Astacidae family and little is known about its ecology, biology and life cycle so the aim of our research was to gather information about it's reproduction cycle. In crayfish reproduction, one of the main factors affecting fecundity is egg size. It is known that an increase in egg size causes decrease in fecundity, but it is not known what is the relation between female size and egg size. The

research was conducted from October 1998 until April 2000 in streams on Medvednica Mountain. We caught 767 females, from which 92 (11,99 %) were berried. Berried females were found from the beginning of November till the mid June next year. The total length (from the top of the rostrum till the end of thelson) of the smallest female with pleopodal eggs was 5,4 cm, while the longest one was 9,29 cm. Diameter of pleopodal eggs varied from 0,24 mm to 0,36 mm (mean ± SD = 0,28 ± 0,02) and their number per females from 1 to 104. We found positive correlation between female size and number of eggs (r = 0,39) and between female size and egg diameter (r = 0,30), while negative correlation was recorded between number of eggs and their diameter (r = -0,04). In order to have an accurate fecundity estimate, which is used in the estimation of recruitment, in addition to the relation between egg number and female size, the relation between female size and egg size should also be considered.

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## VODENI MAKROFITA I KVALITETA VODE U KANALU ČONAKUT

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Uzorci vode i makrofita sakupljeni su na devet postaja duž lijeve obale kanala Čonakut u Parku prirode Kopački rit prilikom devet terenskih izlazaka tijekom vegetacijskog razdoblja: srpanj - listopad 2001. te travanj - srpanj 2002. Utvrđeno je ukupno 14 vodenih makrofita: *Nymphoides peltata*, *Ceratophyllum demersum*, *Potamogeton gramineus*, *P. lucens*, *Carex* sp., *Polygonum amphibium*, *Salvinia natans*, *Spirodella polyrhiza, Lemna minor, Phragmites communis, Myosotis scorpioides, Rorippa amphibia, Ranunculus trichophyllus,* and *Nuphar luteum.* Vodeni makrofita u sporotekućim i stagnantnim vodama pružaju fizički i kemijski kompleksno stanište. Postaje istraživanja razlikovale su se u vrsnom sastavu makrofita, ali su bile vrlo slične s obzirom na dubinu i abiotičke čimbenike. Dubina vode na postajama uzorkovanja gotovo je uvijek bila manja od dva metra. Izmjerena temperatura na površini vode iznosila je između 16 i 28 °C, koncentracija otopljenoga kisika bila je između 2,30 i 13,42 $mgO_2$/L, kemijska potrošnja kisika između 71 i 209 $mgO_2$/L, ukupni Kjeldahlov dušik između 0,32 i 3,48 mg/L, ukupni fosfor između 0,02 i 2,78 mg/L, dok je izmjerena elektroprovodljivost bila između 339 i 791 µS/cm (treća klasa kvalitete). Vrijednosti indeksa trofičkog stanja na istraživanim postajama bile su između 55,36 i 87,65 te su ukazale na eutrofne uvjete s tendencijom ka hipereutrofizaciji.

## AQUATIC MACROPHYTES AND WATER QUALITY IN THE ČONAKUT CHANNEL

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Water and macrophyte samples were collected at nine sites on the left bank of the Čonakut Channel in Kopački rit Nature Park. Fieldwork was done on nine occasions during vegetation season in the period: June - October 2001 and April-July 2002. A total of 14 aquatic macrophytes species were recorded: *Nymphoides peltata*, *Ceratophyllum demersum*, *Potamogeton gramineus*, *P. lucens*, *Carex* sp., *Polygonum amphibium*, *Salvinia natans*, *Spirodella polyrhiza, Lemna minor, Phragmites communis, Myosotis scorpioides, Rorippa amphibia, Ranunculus trichophyllus,* and *Nuphar luteum*. The aquatic macrophytes provides physical and chemical complex habitat in the slow and stagnant waters. The sites differed with respect to the species composition of macrophytes, but were similar in water depth and chemistry. The water depth at the sampling sites was almost less than two meters even. Water surface temperature was between 16 and 28 °C, dissolved oxygen concentration was between 2.30 and 13.42 mg$O_2$/L, chemical oxygen demand was between 71 and 209 mg$O_2$/L, total Kjeldahl nitrogen was between 0.32 and 3.48 mg/L, total phosphorus was between 0.02 and 2.78 mg/L, and conductivity was between 339 and 791 µS/cm (third class of quality). Values of trophic state index were between 55.36 and 87.65 at the investigated sites, pointed up to eutrophic conditions with tendency to hypereutrophic conditions.

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## FAUNA OBLIĆA SEDIMENATA LOTIČKIH I LENTIČKIH VODENIH EKOSUSTAVA ISTOČNE HRVATSKE

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Raznovrsnost i gustoća faune oblića lotičkih i lentičkih vodenih ekosustava u istočnom dijelu Hrvatske istraživani su u nekoliko proteklih godina. Postaje istraživanja podijeljene su u svije grupe na osnovu mjesta uzorkovanja – eulitoralne postaje (od 100 cm udaljenosti od kontaktne zone voda-obala do 0,5 m dubine ispod površine vode: rijeka Karašica, rijeka Drava, kanal Crni fok, obalno područje Sakadaškog jezera) i plitke litoralne postaje (od 0,5 m do 9 m dubine: akumulacija Borovik, središnji dio Sakadaškog jezera i ribnjaci Belje). Na sedam postaja ukupno je zabilježeno 88 nvrsta koje pripadaju u 27 porodica oblića i 45 rodova. Samo tri vrste oblića (*Tobrilus gracilis, Mononchus aquaticus* i *Eumonhystera filiformis vulgaris* grupa) bile su zajedničke za

plitke litoralne postaje kao i sve postaje istraživanja. Na eulitoralnim postajama zajedničke su bile četiri vrste oblića: *T. gracilis, M. aquaticus, E. filiformis vulgaris* grupa i *Diplogaster rivalis*. Najniža raznovrsnost (10 vrsta) pronađena je u sedimentu kanala Crni fok, dok je najveća raznovrsnost (50 vrsta) zabilježena na eulitoralnim postajama duž rijeke Drave. Statistička metoda PCA izdvojila je na temelju zabilježene gustoće oblića eulitoralne postaje rijeke Drave i Sakadaškoga jezera.

## SEDIMENT NEMATOFAUNA OF LOTIC AND LENTIC FRESHWATER ECOSYSTEMS IN THE EASTERN CROATIA

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Nematofauna diversity and abundance in the lotic and lentic water ecosystems in the eastern part of Croatia were investigated in a few last years. Investigated sites were divided in two groups, according to the sampling position - the eulittoral sites (from 100 cm in land to 0.5 m below the water surface: the Karašica river, the Drava river, the Crni fok Channel, Lake Sakadaš shoreline) and the shallow littoral sites (from 0.5 m to 9 m deep: Borovik reservoir, central part of Lake Sakadaš, and Belje fishponds). A total of 88 species, which belongs to the 27 nematode families and 45 genera, were recorded at seven sites. Only three nematode species (*Tobrilus gracilis, Mononchus aquaticus,* and *Eumonhystera filiformis vulgaris* group) were common at the shallow littoral sites as well as at all investigated sites. At the eulittoral sites, four species were common: *T. gracilis, M. aquaticus, E. filiformis vulgaris* group and *Diplogaster rivalis*. The lowest nematode diversity (10 species) were found in the sediment of the Crni fok Channel, while the greatest diversity (50 species) was recorded at the eulittoral sites along the Drava river. The principal component analysis (PCA) discriminated the eulittoral sites of the Drava river and Lake Sakadaš on the basis of recorded mean nematode abundances.

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## ZAJEDNICE OBLIĆA NA VODENIM MAKROFITIMA U KANALU ČONAKUT

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Kanal Čonakut je 3 km dugačka i 300 m široka vodena veza Sakadaškoga jezera s Hulovskim kanalom u Parku prirode Kopački rit. Tijekom vegetacijske

sezone od lipnja do listopada 2001. na osam postaja duž kanala Čonakut sakupljeni su uzorci makrofita prema standardnim hidrobiološkim metodama. Meiofauna je odvojena od vodenih makrofita i oblići su izdvojeni standardnim postupcima. 31 vrsta oblića nađena je u asocijaciji s ukupno osam vodenih makrofita: *Nymphoides peltata*, *Ceratophyllum demersum, Phragmites communis, Carex* sp., *Polygonum amphibium, Potamogeton gramineus, P. lucens* i *Salvinia natans*. Najveći broj vrsta oblića (19) zabilježen je u «čistoj» zajednici *P. gramineus*, dok je najniži broj (svega 6 vrsta) nađen u bitipskoj zajednici vodenih makrofita: *N. peltata* i *P. communis*. *Chromadorina* sp. (46 jed./0,25 $m^2$) i *Eutobrilus notus* (37 jed./0,25 $m^2$) bili su zastupljeni s većom gustoćom u odnosu na ostale zabilježene vrste oblića. Ove dvije vrste imale su najveću gustoću na vodenim makrofitima: *P. lucens, P. gramineus, Carex* sp. i *C. demersum* tijekom razdoblja istraživanja: *E. notus* sa 128 jed./0,25 $m^2$ i *Chromadorina* sp. sa 112 jed./0,25 $m^2$.

## NEMATODE ASSOCIATIONS ON THE AQUATIC MACROPHYTES IN THE ČONAKUT CHANNEL

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The Čonakut Channel is 3 km long and 300 m wide water connection between the Sakadaš Lake and the Hulovo Channel in the Kopački rit Nature Park. During the vegetation season, from June to October 2001, the samples of macrophytes were collected at eight sites along the Čonakut Channel, according to standard hydrobiological methods. Meiofauna was separated from the aquatic macrophytes, and nematodes were picked out following the standard procedure. Thirty-one nematode species are found associated with total of eight aquatic macrophytes: *Nymphoides peltata, Ceratophyllum demersum, Phragmites communis, Carex* sp., *Polygonum amphibium, Potamogeton gramineus, P. lucens* and *Salvinia natans*. The highest number of the nematodes, 19 species, was recorded in the fully-dominated stand of *P. gramineus*, while the lowest number, only 6 species, was recorded in the bitypic stand of the aquatic macrophytes *N. peltata* and *P. communis*. In relation to the other recorded nematode species, *Chromadorina* sp. (46 ind./ 0.25 $m^2$) and *Eutobrilus notus* (37 ind./0.25 $m^2$), were represented by the larger abundance. These species had the highest density in the investigated period: *Eutobrilus notus*, with 128 ind./0.25 $m^2$, and *Chromadorina* sp., with 112 ind./0.25 $m^2$, on the aquatic macrophytes: P. *lucens, P. gramineus, Carex* sp., and *Ceratophyllum demersum.*

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## WATER QUALITY OF BORACKO LAKE (BOSNIA AND HERZEGOVINA)

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In last decades evaluation of water quality of freshwater ecosystems is based in the first place on a qualitative-quantitative composition of living populations. Natural lakes are interesting objects for investigation, from a standpoint of the biodiversity and protection of this valuable ecosystems. Boracko Lake is glacial lake situated in foothills of Mountain Prenj in Hezegovina. It is the only natural lake in Bosnia & Herzegovina in the region of xerotherm broadleaved forests. In the early 20-century (1928, 1935) Protic investigate hydrobiology of this lake. From the September to December of 2002, analysis of zooplankton, zoobentos, phytoplankton and phytobentos was carried out. Samples were collected with plankton net and zoobentos with Ekmann grab. Previous and recent analysis had shown relatively low diversity of the plankton community what is most probably result of strong influence of Borački brook and rains, especially in autumn. Composition of zooplankton and phytoplankton shows oligo–betamesosaprobic water quality and benthos communities shows saprobity index of 1,93 and 1,73. Also, Boracko lake is characterised with rich population of european freshwater crayfish *Astacus astacus,* Fabricius 1875. There is antropogenic influence on the lake from the weekend houses on a coastline and therefore these investigations could help in a future as a tool in monitoring of ecosystem condition and nature protection.

BIOLOGIJA MORA
*BIOLOGIA MARIS*
MARINE BIOLOGY

IL-17

## ŠTETNE FITOPLANKTONSKE CVATNJE

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Fitoplanktoni su jednostanični biljni organizmi koji žive u moru i slatkoj vodi. Većina fitoplanktonskih organizma nije štetna i služe kao primarni proizvođači energije i organske tvari koja se preko hranidbenog lanca prenosi na više trofičke razine. Međutim, za vrijeme toplog i mirnog vremena kao i povećane koncentracije hranjivih soli određene fitoplanktonske vrste mogu vrlo brzo rasti odnosno "cvjetati" i stvarati guste, golim okom vidljive mrlje na površini vode. S obzirom da su učestelast i intenzitet štetnih fitoplanktonskih cvatnji znatno porasli u posljednjem desetljeću sve je veća zabrinutost i interes za istraživanje ovih pojava. Uobičajena i česta posljedica fitoplanktonskih cvatnji je djelomičan ili potpuni nedostatak kisika u pridnenim slojeviama što može izazvati pomor morskih organizama. Pojedini fitoplanktonski organizmi imaju teške celulozne oklope, različite bodlje i nastavke pa nakupljanje takvih organizama u škrgama riba uzrokuje iritaciju, prekomjernu proizvodnju sluzi, a može izazvati i smrt. Fenomen sluzavih cvatnji u Jadranu popraćen je cvatnjom pojedinih dijatomejskih vrsta: *Nitzschia closterium*, *Chaetoceros sp.*, *Leptocylindrus sp.* i *Skeletonema costatum*. Izvanstanično otpuštanje organske tvari normalan je fiziološki proces kod fitoplanktonskih organizama i usko je povezan s procesom fotosinteze. Nakupljanje želatinozne sluzi ispod vode kao i na površini uzrokuje velike probleme i štetu u turizmu i ribarstvu. Moguće je da promjenu u omjeru hranjivih soli dušika i fosfora igraju važnu ulogu u stvaranju sluzavih nakupina u Jadranu. Mali broj fitoplanktonskih vrsta proizvodi vrlo jake neurotoksine koji prenošenjem kroz hranidbeni lanac mogu usmrtiti organizme na višim trofičkim razinama kao što su ribe, ptice, morski sisavci, pa čak i ljude. Zanimljivo je da određene toksične fitoplanktonske vrste proizvode toksin na nekom području dok ga na drugom području te iste vrste ne proizvode. Faktori okoliša kao i omjer hranjivih soli dušika i fosfora odlučujući su parametri koji uvjetuju proizvodnju toksina.

## HARMFUL ALGAL BLOOMS

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Phytoplankton are microscopic, single-celled plants that live in the sea and fresh water. Most species of algae or phytoplankton are not harmful and serve

as the energy producers at the base of the food web, without which higher life on this planet would not exist. Under certain circumstances like nutrients increase, hot and calm weather the algae can grow very fast or "bloom" and accumulate into dense, visible patches near the surface of the water. Concerns about harmful algal blooms (HAB) have increased over the last decade largely because of the perceived increase in the number and duration of events. The most common consequence of phytoplankton bloom is anoxia in the bottom layer that can cause the marine biota mortality. Some phytoplankton species has spines with serrated edges, which can lodge in fish gill tissues, causing irritation, over production of mucous and eventual death. The phenomenon of mucilage formation in Adriatic Sea is associated with some species of diatoms: *Nitzschia closterium*, *Chaetoceros sp.*, *Leptocylindrus sp.* and *Skeletonema costatum*. The extracellular release of organic matter by marine phytoplankton is a normal physiological process which is closely related to the rate of photosynthesis. The accumulations of gelatinous masses on and below the water surface have led to problems and damage for both tourism and fishing industry. Imbalance of nitrogen to phosphorus N:P ratio seems to play important role of mucilage aggregates in the Adriatic Sea. Small number of phytoplankton species produces potent neurotoxins that can be transferred through the food web where they affect and even kill the higher forms of life such as fish, birds, marine mammals and even humans that feed either directly or indirectly on them. The intrigue notice is that the same toxic species produce toxin in one area while at the same time it is not produce toxin in another area. Environmental factors as well as nutrient ratio are seems to be determining factors that can cause a toxin production.

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## TOVILIŠTA TUNA I ODGOVORNO RIBARSTVO

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Prikazat će se rezultati istraživanja bentoskih biocenoza u područjima pod utjecajem tovilišta tuna uz otoke Drvenik, Kluda, Fulija i Kudica. Drastične negativne promjene uočene u naseljima morskih cvjetnica ukazuju na nedovoljnu kritičnost pri izboru područja za smještaj kaveza. Prelov gospodarski značajnih vrsta riba u Jadranu jedan je od argumenata za poticanje marikulture. Pokušat ćemo, međutim, ukazati da intezivna marikultura u unutrašnjem ribolovnom moru Republike Hrvatske može imati pogubni utjecaj na ekosustav mora. Odgovornim ponašanjem u ribarstvu, proglašavanjem posebnih staništa te zaštitom morskih cvjetnica i njihovih staništa mogla bi se u relativno kratkom razdoblju osigurati obnova ribljeg fonda i zaštita Jadranskog mora.

# TUNA FARMS AND RESPONSIBLE FISHERY

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The results of benthic biocoenoses survey in the area of Drvenik, Kluda, Fulija and Kudica islands, which are under the influence of tuna farms, will be presented. Drastic negative changes that have been noted among sea grass communities indicate that tuna cages have been inadequately placed. Overfishing of the economically important species has been one of the arguments for encouraging mariculture in the Adriatic Sea. We will try to point out that intensive mariculture, within the near-shore fishing area of the Republic of Croatia, can influences disastrously on the marine ecosystem. On the other hand, the renewal of fishing stock and the protection of the Adriatic Sea can be provided in the relative short period by responsible fishery, the proclamation of special marine reserves and by the protection of the sea grass communities.

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## TROPSKA ZELENA ALGA *Caulerpa taxifolia* (VAHL.) C.AG. U SJEVERNOM JADRANU (MALINSKA, OTOK KRK)

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U sjevernom Jadranu, u obalnom pojasu Malinske (otok Krk) alga *Caulerpa taxifolia* (Vahl.) C.Ag. (Caulerpales, Chlorophyta) prvi je put bila primijećena 1994. godine. Kartiranje naselja alge je provedeno u obalnom pojasu Malinske na četiri postaje: Haludovo, Lukobran, Luka i Kupalište, prema standardnim kartografskim postupcima jednokratno u 1998., 2000. i 2002. godini. Tijekom 1998. godine ukupna površina zahvaćena uniformnim naseljem alge *Caulerpa taxifolia* je iznosila 7 988 $m^2$, a 2000. godine se povećala na 13 252 $m^2$. Godine 2002. latentno stanje alge je zabilježeno sve do polovice mjeseca srpnja, kada se *Caulerpa* počela sporadično pojavljivati na istraživanim postajama. Na istraživanoj postaji Lukobran alga je prekrivala pjeskovito-muljevito dno na dubini od 4 do 15 m. Uzorci su sakupljani metodom kvadrata jednom mjesečno autonomnim ronjenjem od listopada 1997. do studenoga 1999. godine. U laboratoriju su provedene morfometrijske analize alge uz praćenje njene biomase. U jesensko-zimskom razdoblju utvrđene su najviše vrijednosti biomase od 295 g suhe težine alge na $m^2$. Najveća raznolikost epifita na steljkama alge je utvrđena u zimsko-proljetnom razdoblju. Unutar naselja alge *Caulerpa taxifolia* je zabilježeno 30 vrsta alga, 2 morske cvjetnice i 32 vrste raznih morskih zoobentoskih organizama. U sedimentu naselja alge *Caulerpa* je primijećena velika raznolikost unutar skupina Gastropoda, Bivalvia i Polychaeta, a sveukupno je iz sedimenta izdvojeno 115 vrsta makrofaune.

## TROPICAL GREEN ALGA *Caulerpa taxifolia* (VAHL.) C.AG IN THE NORTHERN ADRIATIC SEA (MALINSKA, KRK ISLAND)

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The alga *Caulerpa taxifolia* (Vahl.) C.Ag. (Caulerpales, Chlorophyta) was for the first time noted in the northern Adriatic Sea, in the coastal zone of Malinska (Krk Island), in 1994. The cartography of the *Caulerpa taxifolia* settlement in the littoral zone of Malinska was carried out at four stations: Haludovo, Lukobran, Luka and Kupalište. The standard cartographic procedure was applied once a year, in 1998, 2000 and 2002. During 1998 the total affected area, covered with a uniform settlement of *Caulerpa taxifolia*, was 7 988 $m^2$ and by the year 2000 had increased to 13 252 $m^2$. In 2002 the latent condition of *Caulerpa* was recorded up to the middle of July, at which time *Caulerpa* began sporadic growth. At the station Lukobran, *Caulerpa* settled on a silty sandy bottom (4–15 m depth). Algae were collected monthly by SCUBA divers, using a square method, from

October 1997 to November 1999. In the laboratory, the seaweed samples were subjected to morphometric analyses and biomass measurements. In the autumn-winter period the greatest standing crop of 295 g d.w./$m^2$ was noted. On the algal thalli in the winter-spring period a greater variety of epiphytes occurred. In *Caulerpa* settlements 30 seaweed, 2 seagrass and 32 different macrobenthic species were observed. In sediment inside the *Caulerpa* settlement a total of 115 invertebrate species was noted, with a higher diversity within the groups of Gastropoda, Bivalvia and Polychata.

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## POJAVA I ŠIRENJE "INVAZIVNOG VARIJETETA" TROPSKE ALGE *Caulerpa racemosa* JADRANSKIM MOREM

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"Invazivni varijetet" tropske zelene alge *Caulerpa racemosa* u Sredozemnom je moru zabilježen prvi puta 1991. godine u Siriji. Za područje Jadranskog mora prvi je nalaz zabilježen u jesen 2000. godine uz južnu stranu otočića Marinkovac (Pakleni otoci). Do danas još pronađena na 7 udaljenih područja: ispred grada Cavtata, tri nalza s južne strane poluotoka Pelješac (uvale Česminova, Luka i Prižežba), otok Mljet (pored Sobre), otok Hvar (uvala Vela Grška) i otok Biševo (uvala Mezuporat). Nalazišta alge većinom nisu mjesta uobičajena za sidrenje te se širenje alge na veće udaljenosti vjerojatno odvija prijenosom fragmenata alge i zigota gibanjem mora (struje i valovi). Alga raste na svim tipovima morskoga dna od površine do dublje od 50 m. Gusta naselja u potpunosti i u više slojeva isprepletenih kauloida prekrivaju dno i sesilne organizme. Maksimalo gusta naselja sadržavala su 2700 m/$m^2$ puzajućih kauloida s više od 26000 filoida. Godišnji vrhunac razvoja doseže u jesen, dok tijekom zime talusi značajno propadaju. U proljeće iz preostalih fragmenata započinje obnavljanje naselja.

## APPEARANCE AND SPREAD OF THE "INVASIVE VARIETY" OF TROPICAL ALGAE *Caulerpa racemosa* IN THE ADRIATIC SEA

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The first record of the «invasive variety» of the tropical green algae *Caulerpa racemosa* in the Mediterranean Sea was in 1991 in Syria. For the Adriatic Sea the first

report was in autumn 2000 on the south side of the Marinkovac Island (Archipelago of the Pakleni Islands). Until today it was found on additional 7 locations: in front of Cavtat Town, three records on the Pelješac Peninsula (coves of Česminova, Luka and Priježba), Mljet Island (near Sobra), Hvar Island (Vela Grška Coves) and Biševo Island (Mezuporat Cove). Most of the locations are not suitable for anchoring. Because of that it is supposed that the long distant dispersion of the algae is caused by the transportation of the algae fragments and zygotes by sea movement (currents and waves). The alga proliferates on all kinds of the substrate from the surface up to more then 50 m depth. A dense colony completely covers a substrate and sessile organisms by several layers of interlaced stolons. The extreme dense colony is made of 2700m/m2 of creeping stolons with more then 27000 fronds. The annual growing maximum reaches in autumn while during winter a thalus is significantly damaged. A regeneration of the colony started during spring.

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## UTJECAJ "INVAZIVNOG VARIJETETA" TROPSKE ALGE *Caulerpa racemosa* NA PRIRODNE BENTOSKE ORGANIZME

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Posljednja istraživanja "invazivnog varijeteta" tropske zelene alge *Caulerpa racemosa* potvrđuju kako njegovo širenje dovodi do drastičnog pada biološke raznolikosti. Tijekom ljeta alga raste brzinom od gotovo 2 cm na dan pri čemu se razvija gusta mreža puzajućih kauloida. Alga prerasta, zasjenjuje i zagušuje druge sesilne organizme, dovodi do pojačane sedimentacije i razvoja anoksičnih stanja. Naime, gusta mreža kauloida (2600 m kauloida $m^{-2}$) sakuplja organske i anorganske čestice koje se talože nad primarno dno. U blizini livade cvjetnice *Posidonia oceanica*, većina organskih čestica su dijelovi otpalih listova cvjetnice. Njihovim se taloženjem ispod spleta alge razvija 2-5 cm debeli detritusni sloj. Organskom razgradnjom detritusa troši se kisik pa u detritusnom sloju te ispod njega dolazi do hipoksičnih i anoksičnih stanja. Bakterijskom se razgradnjom u anoksičnim uvjetima razvija sumpro-vodik koji je otrovan za većinu bentoskih organizama. Između talusa vidljive su bijele prevlake bakterije *Beggiatoa* sp. Redoks potencijal u sedimentu ispod naselja alge iznimno je negativan (do -300 mV) što je potvrda razvoja sumporovodika. Analizom epifitskih algi na rizomima cvjetnice *Posidonia oceanica* zahvaćenih algom *C. racemosa*, utvrđeno je drastično smanjenje brojnosti (manje od 30% zabilježenih vrsta u odnosu na referentno područje) i pokrovnosti epifitskih makroalga (između 15 i 50 % pokrovnosti u odnosu na referentno područje). Vrlo je vjerojatno kako anoksični uvjeti u sedimentu imaju negativno djelovanje i na samu cvjetnicu *P. oceanica*. Zahvaćeni rizomi cvjetnice su crni, a listovi često nekrotizirani. Zbog brzog i nepovratnog djelovanja na sesilne bentoske organizme mišljenja smo kako širenje "invazivnog varijeteta" alge *C. racemosa* predstavlja opasnost biološkoj raznolikosti Jadranskog mora.

## IMPACT OF THE "INVASIVE VARIETY" OF THE TROPICAL ALGAE *Caulerpa racemosa* ON THE BENTHIC ORGANISMS

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A recent investigations of the "invasive variety" of the tropical green algae *Caulerpa racemosa* confirmed that its spread would lead to significant decrease of biodiversity. During summer, the algae grow 2 cm per day and develop a dense canopy of the creeping stolons. The algae overgrow, shade and smother other sessile organisms, increase sedimentation and cause anoxic conditions. Namely, a dense canopy (2600 $m/m^2$ of stolons) accumulated organic and inorganic particles on the bottom. Close to the *Posidonia oceanica* seagrass meadow, most of the organic particles are fragments of the seagrass leaves. By its accumulation under a canopy, a 2 –5 cm thick detritus layer is developed. Because of the oxygen consumption due to organic decomposition, a hypoxic and anoxic conditions occurred inside and under detritus layer. In anoxic sedimentary condition, bacterial sulphate reduction produces $H_2S$, which can be toxic to the most of the benthic organisms. Inside canopy it is possible to observe white mat of sulphur bacteria *Beggiatoa* sp. Redox potential in the sediment covered by the canopy is extremely negative (-300 mV) and also indicates on appearance of $H_2S$. The investigation of epiphytic macroalgae on the *Posidonia oceanica* rhizomes has demonstrated a significant decreasing of number of taxa (lower then 30% compared to the referent site) and species cover (15 – 50% comparing to referent site). It is highly possible that anoxic condition in the sediment has also negative impact on the seagrass *Posidonia oceanica* itself. Affected rhizomes are black and leaves usually necrotic. Due to fast and irretrievable impact on the sessile benthic organisms we believe that spread of the "invasive variety" of the *C. racemosa* represent a main threat for the biodiversity of the Adriatic Sea.

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## FLORA SMEĐIH ALGA (Phaeophyta) UZ ISTOČNU OBALU JADRANA

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Posljednji popis bentoskih algi Jadranskoga mora koji uključuje i smeđe alge (odjeljak Phaeophyta) dao je Giaccone (1978). Temeljna manjkavost toga rada je nedostatak podataka o bentoskoj flori smeđih algi u južnom Jadranu. Ta se manjkavost u ovom radu pokušala izbjeći. U radu se iznose podaci o smeđim algama uz istočnu obalu Jadranskoga mora s posebnim osvrtom na njegov sjeverni, srednji i južni dio.

Popis se temelji na literaturnim podacima koji su objavljeni 1950 godine i kasnije. Ta je godina uzeta za graničnu jer su na sličan način oblikovani popisi zelenih alga Sredozemlja (Gallardo et al.., 1993) i istočne obale Jadrana (Antolić et al., 2001), te smeđih alga Sredozemlja (Ribera et al., 1992). U radu je ukupno uključeno 172 svojte bentoskih smeđih alga koje su svrstane u razrede, redove, porodice, rodove, vrste, podvrste, odlike i oblike. Najveći broj svojti (159) zabilježen je u srednjem Jadranu, manji u sjevernom (124 svojte) a najmanji u njegovom južnom dijelu (79 svojti).

## FLORA OF THE BROWN MARINE ALGAE (Phaeophyta) ON THE EASTERN ADRIATIC COAST

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The recent and more complete survey of benthic algae of the Adriatic Sea that includes the brown algae (Phaeophyta) has been published by Giaccone (1978). The deficiency of that work was the lack of data regarding main brown marine algae of the South Adriatic. We tried to avoid that lack in the present work. This paper deals with data of benthic flora of the brown marine algae on the eastern Adriatic coast with particular consideration of the North, Middle and South Adriatic. The survey is based upon the data published in 1950 and later. The 1950 is taken as "border" year due to the similar way of listed Mediterranean green algae (Gallardo et al., 1993); the list of Mediterranean brown algae (Ribera et al., 1992); the list of green algae on the eastern Adriatic coast (Antolić et al., 2001). In this work there are a total of 172 taxa of benthic brown marine algae that are classified into classes, orders, families, genera, species, subspecies, varieties and forms. The greatest number of taxa has been registered in the middle Adriatic (159), lower number in the northern Adriatic (124) and the lowest number (79) in the southern Adriatic.

OP-66

## UTJECAJ SLATKE VODE IZ HIDROELEKTRANE PLAT NA BENTOSKE ORGANIZME U ŽUPSKOM ZALJEVU

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Hidroelektrana kod Plata (2,5 km sjeverno od Cavtata) u Župski zaljev donosi do 90 $m^3/s$ slatke vode. U sklopu studije utjecaja na okoliš proučeno je područje

oko tunela kojim se voda ulijeva u more. U lučici ispred tunela mjerena je slanost i strujanje, a metodom direktnog opažanja autonomnim ronjenjem promatran je bentos. Utvrđeno je ukupno 110 vrsta makrozoobentosa te 15 vrsta fitobentosa. Od ukupno 125 vrsta organizama unutar lučice, povećana gustoća u odnosu na uobičajenu, utvrđena je za 19 vrsta, a smanjena za 7 vrsta. Povećana gustoća utvrđena je za pasivne filtratore, i to na mjestima s pojačanim strujanjem, kao npr.: za spužve vrsta *Crambe crambe* i *Chondrilla nucula*, hidroide vrsta *Eudendrium racemosum* i *Plumularia* sp. te mahovnjaka vrste *Schizobrachiella sanguinea*. Nalaz ježinca vrste *Sphaerechinus granularis*, organizma bez osmoregulacije, također ukazuje na posebni režim strujanja u lučici. U moru ispred lučice, 200 m ispred tunela nije bio zamijećen utjecaj slatke vode na bentoske organizme. Iako rijekama Krkom i Zrmanjom u more dotječe otprilike ista količina vode kao i iz HE Plat, utjecaj slatke vode može se primijetiti na daleko većem području – području cijelog estuarija - nego u slučaju HE Plat, gdje se utjecaj može primijetiti na svega 2 ha. Glavni razlog ovoj pojavi je otvorenost akvatorija ispred ispusta elektrane prema dubokom moru Župskog zaljeva.

## EFFECT OF FRESHWATER INFLOW FROM PLAT HYDROELECTRIC POWER PLANT ON BENTHIC ORGANISMS IN ŽUPA BAY

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The hydroelectric power plant (HEPP) near Plat (2,5 km north from Cavtat) brings up to 90 $m^3$/s of freshwater into the Župa Bay. As a part of an environmental impact assessment study, the area around tunnel through which freshwater enters into the sea was surveyed. In an artificial port in front of the tunnel salinity and current measurements were performed. Also, SCUBA divers observed benthic organisms. About 110 macrozoobenthic and 15 fitobenthic species were noted. From total of 125 species inside the port, a higher density of organisms in comparison to usual was noted for 19 species, and lower for 7 species. Higher density was noted for passive filter feeders, especially on the locations with stronger current, such as for sponges *Crambe crambe* and *Chondrilla nucula*, hydroids *Eudendrium racemosum* and *Plumularia* sp., as well as for bryozoan *Schizobrachiella sanguinea*. Occurrence of sea urchin *Sphaerechinus granularis*, the organism without osmoregulation, so deep inside the port points towards the special current pattern in the port. In front of the port, some 200 m in front of the tunnel, there was no visible freshwater effect on benthic organisms. Although approximately the same amount of freshwater like for HEPP Plat enters into the sea through Krka or Zrmanja river, freshwater influence there could be registered on a much broader area –

whole estuary, while in the case of HEPP Plat influence is limited to the small port (approx. 2 ha). The main reason for that is wide connection between aquatorium in front of freshwater release and open sea.

OP-67

## DINAMIKA POPULACIJE CIPLA BALAVCA, *Liza* (*Liza*) *ramada* (RISSO, 1826) U PODRUČJU SREDNJEG JADRANA (ŠIBENSKI ZALJEV)

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Iznose se rezultati jednogodišnjih istraživanja dinamike populacije cipla balavca *Liza* (*Liza*) *ramada* (Risso, 1826) u Šibenskom zaljevu gdje je u lovinama od svih vrsta cipla ova vrsta bila najzastuljenija vrsta. Podaci su sakupljeni u razdoblju od siječnja do studenog 1984. Odrasli primjerci ove vrste su bili dostupni ribolovu u Šibenskom zaljevu za vrijeme mirovanja u spolnom ciklusu, što se podudara s reproduktivnom migracijom ove vrste. Prikazane su vrijednosti dužinske i starosne raspodjele, odnos dužine i težine, parametri rasta, koeficijenti prirodne, ribolovne i ukupne smrtnosti, razina iskorištavanja i stopa preživljavanja. Utvrđena je blaga negativna alometija u relativnu rastu ove vrste ($b$ = 2,931). Teoretska krivulja rasta je izračunata temeljem dviju metoda, Casie-a (1954) i Bhattachary-a (1967), unoseći u von Bertalanffy-jevu eksponencijalnu jednadžbu rasta vrijednosti $L_\infty$ =52,5 cm odnosno 52,7 cm totalne dužine; $K$ = 0,25 odnosno 0.3, $t_0$ = - 0.1. Rezultati ukazuju na relativno spori rast cipla balavca, koji je dosegao 95% od svoje ukupne dužine u dvanaestoj godini života. Procjene veličine populacije izložene ribolovu su izvedene temeljem triju metoda: kombinirane metode kohorta i VPA metode, VPA metode i metode globalnih modela. Sve upotrijebljene metode su dale vrlo slične vrijednosti procijenjene količine cipla balavca u Šibenskom zaljevu. Biološki maksimalno dozvoljena razina iskorištavanja iznosila je 110 odnosno 120 tona.

## POPULATION DYNAMICS OF GREY MULLET, *Liza* (*Liza*) *ramada* (RISSO, 1826) FROM THE MIDDLE ADRIATIC (ŠIBENIK BAY)

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A study of population dynamic characteristics of the grey mullet, Liza (*Liza*) *ramada* (Risso, 1826) was carried out over a period of one year in Šibenik Bay. Grey mullet was the most abundant species of the mullet catches in the area of the Šibenik Bay. The total length distribution, length-length and length-weight relationship, growth param-

eters, instantaneous rate of total, natural and fishing mortalities, rate of exploitation, rate of survival, including the observation of sexual cycle and migratory behaviour were carried out. Mild negative allometry was established ($b$ = 2,931) in length-weight relationship. Theoretical growth curve of this species was estimated according to Casie's (1954) and Bhattachary's (1967) methods carrying into von Bertalanffy equation the values of L8 =52,5 cm and 52,7 cm respectively; $K$ = 0.25 and 0.3 respectively as well as $t_0$ = - 0.1. Results point out the relatively slow growth of grey mullet. Stock assessment and maximum sustainable yield (MSY) were carried out by three quantitative approaches: combined VPA and cohort analysis, global production model and VPA. These quantitative approaches gave very similar amounts of grey mullet in the Šibenik Bay (110 and 120 tons respectively).

OP-68

## PRVA SPOLNA ZRELOST BRGLJUNA *Engraulis encrasicolus* (L.), U ISTOČNOM JADRANU

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U radu se iznose minimalne dužine prvog spolnog sazrijevanja brgljuna, *Engraulis encrasicolus* (L.), u istočnom Jadranu. Daju se dužine pri kojima je 50% i 100% populacije spolno zrelo. Analizirano je 2530 primjeraka brgljuna tijekom maksimalne spolne aktivnosti ove vrste – u srpnju i kolovozu 1990 te svibnju 2003. Dane su raspodjele totalnih dužina i podaci o stadijima zrelosti gonada (zreli, u mriješćenju, izmriješteni). Uzorci su uzimani iz lovina plivarice ostvarenih u estuariju rijeke Zrmanje koje je područje obitavalište i hranilište najvažnijih pelagičkih vrsta. Ukupne dužine primjeraka brgljuna korištenih u razmatranju minimalnih dužina prvog spolnog sazrijevanja su se kretale između 4,4 i 12,5 cm, a težine između 0,4 i 13,8 g. Minimalna dužina prvog spolnog sazrijevanja bila je 8,2 cm (mužjak), $L_{50}$ je iznosila 8,2 cm, a $L_{100}$ 11,5 cm ( $r^2$=0.9537; $SE$ = 0.0019).

## FIRST SEXUAL MATURITY OF ANCHOVY *Engraulis encrasicolus* (L.), IN THE EASTERN ADRIATIC

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The minimal length at first maturity, as well as $L_{50}$ and $L_{100}$ of anchovy, *Engraulis encrasicolus* (L.), were estimated. Length-frequency and gonad data (ripe, spawning, spent, with semi hydrated and hydrated oocytes) of 2 530 anchovy specimens

were analysed during the peak of anchovy spawning period - in July and in August 1990 and in May 2003. Anchovy specimens were caught by purse seine in the Zrmanja River estuary, which is a nursery ground of the small pelagic fish species. Total length of anchovy specimens used for obtaining the lowest size of its sexual maturation ranged from 4.4 to 12.5 cm and weight range from 0.4 to 13.8 g. The minimal length at first maturity of these fish species was 8.2 cm of total length (male), $L_{50}$ was 9.0 cm and $L_{100}$ 11.5 cm. Highly significant correlation was found ( $r^2$=0.9537; $SE$ = 0.0019).

OP-69

## ŠKOLJKAŠI KAO EPIBIONTI I PODLOGA ZA NASELJAVANJE DRUGIH VRSTA ORGANIZAMA

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Autori iznose višegodišnja zapažanja o nalazima školjkša na raznim čvrstim podlogama (alge, ljušture mekušaca, oklopi rakova, razne zaštitne tvorbe organizama, staklo, drvo, metal, PVC folije, amfore, podmorske konstrukcije i drugi predmeti). Mnogi školjkaši naseljavaju razne, dok drugi odabiru određenu podlogu, a zbog čvrstih ljuštura su često i sami predmetom za naseljavanje. Navode se najzanimljiviji primjeri školjkaša kao obraštaj ili podloga za naseljavanja. Zapaženo je da su najgušće naseljene obraštajem periske (*Pinna nobilis*), kunjke (*Arca noae*), kopita (*Spondylus gaederopus*), kamenice (*Ostrea edulis*), ali i neke druge vrste. Kao obraštajni organizmi školjkaši *Striarca lactea, Flexopecten glaber, Laevicardiun oblongum* naseljavaju razne alge, vogu i svilinu, *Hiatella arctica, Lima lima, Papillicardium papillosum* spužve, *Pteria hirundo* žarnjake, *Pododesmus patelliformis* oklope škampa, *Chlamys multistriata* mahovnjake i kožaste koralje, *Arca noae, Musculus subpictus* plaštenjake i predstavnike drugih skupina. Školjkaši dolaze na raznim predmetima odbačenim s brodova ili potonulim zajedno s njima. Tako su na amforama ili fragmentima amfora nađene *Chama gryphoides, Anomia ephipippum, Gastrochaena dubia* i drugi školjkaši. Na podmorskim betonskim konstrukcijama najčešći su *Gastrochaena dubia, Irus irus, Mytilus galloprovincialis*. Rezultati zapažanja ukazuju da su brojne vrste školjkaša važna komponenta u obraštajnim zajednicama na živim organizmima i raznim čvrstim podlogama.

## BIVALVES AS EPIBIONTS AND SUPPORT FOR SETTLEMENT OF OTHER ORGANISMS

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Authors present the observations, during long period of time, about bivalve finding place on hard supports (algae, molluscan shells, crab carapax, macrofaunal protected formation, glass, wood, metal, plastic, amphoras, undersea constructions and other objects). Many bivalve species inhabit various, the others select special support, and their hard shells are often favourable for larval settlement. The examples of the most interesting bivalve species as fouling or support for settlement are presented here. The high fouling density have been observed at fan shell (*Pinna nobilis*), Noah,s Arche (*Arca noae*), thorny oyster (*Spondylus gaederopus*), flat oyster (*Ostrea edulis*), and some other. Bivalve species as *Striarca lactea*, *Flexopecten glaber*, *Laevicardiun oblongum* have been observed on various algae and phanerogams, *Hiatella arctica, Lima lima, Papillicardium papillosum* on spongia, *Pteria hirundo* on cnidaria, *Pododesmus patelliformi,* on cephalothorax of Norvegian lobsters, *Chlamys multistriata* on bryozoa, *Arca noae, Musculus subpictus* on tunicata, and other macrofaunal species. Bivalves have been found on various objects thrown from ships or went down by ships to bottom. Thus, amphoras or their fragments had the following species *Chama gryphoides, Anomia ephipippum, Gastrochaena dubia* and others. The species *Gastrochaena dubia, Irus irus, Mytilus galloprovincialis* have been found in high densities at the undersea concrete constructions. The observations show that most of bivalve species are an important component in the fouling communities on living organisms and at various hard supports.

OP-70

## GODIŠNJA RASPODJELA GUSTOĆE I BIOMASE TINTININA U KAŠTELANSKOM ZALJEVU

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Sezonska je raspodjela lorikatnih trepetljikaša praćena u sjeveroistočnom dijelu Kaštelanskog zaljeva od svibnja 1998. do studenog 1999. godine. Uzorkovalo se u slojevima od 0, 5, 10 i 28 m, upotrebom Niskin crpca. Za mikroskopsku je analizu korišten obrnuti mikroskop IMT-2 pri povećanju od 100, 200 i 400 puta. Dobiveni su rezultati prvi za ovaj dio Kaštelanskog zaljeva, a podaci o biomasi među prvima za Jadransko more. Godišnja je raspodjela tintinina obilježena proljetnim i jesenskim porastom gustoće populacija s najvišom vrijednosti u listopadu 1999. godine od 433 jed.$l^{-1}$, na dubini od 5 m. U tom je razdoblju i njihova zastupljenost u skupini protozoa najviša (36%). S druge strane, najniže su gustoće populacija i najmanji udio u skupini proto-

zoa zabilježene tijekom toplijeg dijela godine. Kolebanje biomase tintinina na vremenskoj skali se preklapa s raspodjelom abundancije ovih organizama, što potvrđuje vrlo visoki koeficijent korelacije između spomenutih parametara. Vrijednosti biomase variraju u granicama od 0,061 do 3,277 µgC $l^{-1}$ uz srednjak od 0,637 ±0,675 µgC $l^{-1}$. Taksonomski sastav skupine ukazuje na smanjenu bioraznolikost u odnosu na ostale dijelove zaljeva. Od ukupno 32 determinirane vrste, čak su 19 vrste obalnog i estuarskog mora. Među njima su kvantitativno najzastupljenije vrste *Helicostomella subulata*, *Codonellopsis schabi*, *Salpingella rotundata* i *Tintinnopsis nana* s udjelom od 65% u ukupnom broju tintinina. Takva zastupljenost upućuje na važnost ovih vrsta u sekundarnoj proizvodnji Kaštelanskog zaljeva. Stoga bi tijekom sljedećih istraživanja osobitu pažnju trebalo usmjeriti na proučavanje hranidbenih odnosa između trepetljikaša i drugih komponenti "mikrobnog kruga".

## ANNUAL DISTRIBUTION OF TINTINNINE DENSITY AND BIOMASS IN KAŠTELA BAY

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Seasonal distribution of the loricate ciliates was studied in the northeastern part of the Kaštela Bay from May 1998 to November 1999. Samples were collected at 0, 5, 10 and 28 m depth, using Niskin bottles. The analysis was performed with inverted microscope IMT-2 at magnification of x100, x200 and x400. These results are the first data form investigated part of Kaštela Bay and one of very few for the whole Adriatic Sea. Annual tintinnine variability was characterised by spring and autumn increase in population density, with maximum of 433 ind.$l^{-1}$ in October 1999, at 5 m depth. In that period tintinnine contribution to the total protozoans number was the highest (36%). On the other hand, the lowest abundances and share in assemblages of protozoa were during the warmer season. Seasonal oscillations of tintinnine biomass were overlapped with fluctuations of their abundances what was also confirmed by statistically significant correlation. The biomass varied from 0.061 to 3.277 µgC $l^{-1}$, with mean of 0.637 ±0.675 µgC $l^{-1}$. The taxonomic structure of tintinnine community pointed at attenuate biodiversity in relation with other part of the Bay. From the total of 32 tintinnine species, even 19 species were neritic and estuarine ones. Among them, quantitatively dominant were *Helicostomella subulata*, *Codonellopsis schabi*, *Salpingella rotundata* and *Tintinnopsis nana*, which participated in total tintinnine number with 65%. Such data stress the importance of this species in secondary production of Kaštela Bay. Therefore, during further researches, exceptional attention should be aimed to study of trophic relationship between ciliates and other components of "microbial food web".

OP-71

## PRELIMINARNI REZULTATI O MORFOMETRIJI I REPRODUKTIVNIM ZNAČAJKAMA HRIDINASTOG JEŽINCA *Paracentrotus lividus* IZ UVALE BISTRINA, MALOSTONSKI ZALJEV

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Jedan od prioritetnih zadataka hrvatske marikulture je diverzifikacija proizvodnje novim vrstama morskih organizama. Skupina koja ima veliki tržišni potencijal su morski ježinci, posebice hridinasti ježinac, *Paracentrotus lividus*. U ovom radu su prikazani rezultati istraživanja morfometrijskih i reproduktivnih značajki populacije hridinastog ježinca iz uvale Bistrina u Malostonskom zaljevu. Ježinci su prikupljani s obale ili ronjenjem do dubine od tri metra, te obrađivani svježi. Ukupno je uzorkovano 840 jedinki, u razdoblju od kolovoza 2002. do travnja 2003. Srednja vrijednost ukupne tjelesne mase ježinaca iznosila je 23,2 ± 11,4 g s modalnom vrijednosti od 16,9 grama. Mjesečna srednja vrijednost ukupne mase kretala se u rasponu od 18,7 g do 27,1g. Najmanja jedinka je težila 0,5 g, a najveća 78,2 g. Srednja vrijednost promjera skeleta iznosi 37±7,4 mm s modalnom vrijednosti od 40 mm. Najmanja zabilježena vrijednost promjera je 9 mm, a najveća 65 mm. Srednja vrijednost mase gonada ukupnog uzorka iznosi 0,62±0,57 g s modalnom vrijednosti od 0,40 g. Mjesečna srednja vrijednost mase gonada kreće se u rasponu od 0,36 g u rujnu do 1,38 g u travnju. Najmanja zabilježena masa gonada iznosi 0,04 g, a najveća 5,30 g. Srednja vrijednost gonadosomatskog indeksa ukupnog uzorka iznosi 2,71± 2,48%. Prosječni mjesečni GSI počinje rasti od siječnja kada iznosi 2,4%, a najveći je u travnju 5,22%. Najmanji pojedinačni GSI zabilježen je u siječnju od 0,15%, a najveći u travnju, 42,87%. Tijekom travnja su uočene i prve jedinke koje su aktivno ispuštale gamete, što ukazuje na početak mriješćenja.

## PRELIMINARY RESULTS ON MORPHOMETRY AND REPRODUCTIVE CHARACTERISTICS OF PURPLE SEA URCHIN *Paracentrotus lividus* FROM BISTRINA MALI STON BAY

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One of the most urgent tasks of Croatian mariculture is diversification of production with new marine species. A group of such species with great market potential is sea urchins, especially purple sea urchin, *Paracentrotus lividus*. In this paper, preliminary results are displayed on morphometry and reproductive characteristics of purple sea urchin in the bay of Bistrina. Urchins were collected from the shore or by diving

technique and examined fresh. The sample in the whole was 840 specimens and research was conducted in the period from August 2002 until April 2003. Weight median for the whole population is 23,2±11,4 g with modal value of 16,9 grams. Monthly weight median ranges from 18,7 g to 27,1 g. Lightest sample weighs 0,5 g and heaviest 78,2 g. Median value of the test diameter is 37±7,4 mm with modal value of 40 mm. The smallest specimen recorded is 9mm and the largest is 65 mm in diameter. Gonadal weight median is 0,62 g with modal value of 0,40 g. Monthly median values range from 0,36 g in September to 1,38 g in April. The smallest specimen has gonades that weigh 0,04 g, and the largest weigh 5,30 g. Median value of the GSI (gonadal somatic index) is 2,71±2,48%. Monthly median starts to grow in January when it is 2,40%, and peaks in April with value of 5,22%. The smallest individual GSI is registered in January and it is 0,15%. The largest individual GSI was registered in April and it is 42,87%. In April, the first individul extraction of gametes was noted, indicating the beginning of hatching cycle.

OP-72

## UTJECAJ UZGAJALIŠTA TUNA NA BENTOSKE BIOCENOZE

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U obalnom moru Republike Hrvatske u plutajućim kavezima 2002. godine proizvedeno je oko 4000 t tuna vrste *Thunnus thynnus* (L.). Za njihovu ishranu utrošeno je preko 40000 t male plave ribe. Nakon hranjenja tuna, na dnu ispod kaveza taloži se dio njihovog fecesa te neiskorištena hrana. Ova «svježa» organska tvar značajno djeluje na bentoske biocenoze ispod kaveza. Metodom direktnog opažanja i ronilačkih transekata autonomni ronioci pratili su utjecaj tri uzgajališta (tovilišta) tuna u srednjoj Dalmaciji, u periodu od 1997. do 2003. godine. Pozicija uzgajališta i transekata je određena GPS uređajem. Dubina mora na mjestu uzgajališta je od 29 do 59 m, a dno je većinom sedimentno. Prije postavljanja uzgoja na dnu su bile razvijene cirkalitoralne biocenoze uobičajene za to područje: biocenoza obalnih terigenih muljeva, biocenoza obalnih detritusnih dna i mjestimice koraligenska biocenoza. Rezultati istraživanja prikazani su na geokodiranim kartama mjerila 1:25000. Transekti su prikazani grafički, a utjecaj kao tri kategorije «nema utjecaja», «primjetan utjecaj» i «značajan utjecaj». Utjecaj uzgajališta tuna na bentoske biocenoze mogao se primijetiti do oko 100 m od okomite projekcije kaveza na dno te se očitovao kao nestajanje većine organizama. Utjecaj je bio veći na plićim dijelovima dna ispod kaveza. Utjecaj je bio negativan za većinu makrobentoskih organizama. Naprotiv, za neke organizme kao npr.: *Beggiatoa* sp., *Ulva* sp., *Aplysia punctata, Pleurobranchia meckeli, Macropipus depurator, Psammechinus microtuberculatus, Holothuria tubulosa*, te neke vrste mnogočetinaša utjecaj je bio povoljan.

## IMPACT OF TUNA FARMS ON BENTHIC BIOCOENOSES

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In 2002, in Croatian coastal sea in semi-offshore cages approximately 4000 t of tuna *Thunnus thynnus* (L.) were produced. For their feeding more than 40000 t of small fish (mostly sardines and anchovies) were used. After the feeding, part of tuna faeces and uneaten food settle on the bottom beneath the cages. That "fresh" organic matter has distinctive impact on benthic biocoenosis beneath the cages. By direct observation and diving transects SCUBA divers have monitored impact of three tuna farms in middle Dalmatia, from 1997 to 2003. Position of farms and transects was determined with GPS device. Sea depths beneath cages are from 29 to 59 m and bottom is mostly sediment. Before tuna farms were established, bottom was inhabited by usual circalittoral biocoenoses: biocoenosis of coastal terrigenous mud, biocoenosis of coastal detritic bottom and somewhere coralligenous biocoenosis. Research results were shown on georeferenced maps in scale 1:25000. Transects were shown graphically, and the impact was assessed through three categories: «no influence», «notable influence» and «significant impact». The impact on benthic biocoenoses could have been noted up to 100 m from vertical projection of cages on the bottom and it was manifested as disappearance of majority of organisms. The impact was stronger on the shallower parts of bottom beneath the cages. For majority of benthic organisms the impact was negative. However, for some benthic organisms such as: *Beggiatoa* sp., *Ulva* sp., *Aplysia punctata, Pleurobranchia meckeli, Macropipus depurator, Psammechinus microtuberculatus, Holothuria tubulosa*, as well as some species of polychaetes the impact was positive.

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## UTJECAJ IZLOVA PRSTACA (*Lithophaga lithophaga* L.) NA STRUKTURNU RAZNOLIKOST HRIDINASTOG DNA

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Brojni morski organizmi koriste kamenito dno kao supstrat, oni žive na kamenu (epilithion), buše kamen (endolithion) ili žive u najmanjim pukotinama (mesolithion) i svi zajedno sačinjavaju vrlo značajne oblike hridinastih zajednica. Prstaci (*Lithophaga lithophaga* L., Bivalvia) su endolitni školjkaši, koji se razvijaju unutar galerija u površinskom sloju vertikalnih i nagnutih stijena, koje aktivno buše. Rasprostranjeni su na vapnenačkim podlogama duž čitave mediteranske i istočno atlantske obale. Usprkos zakonskih zabrana, prstaci se neprestano izlovljavaju rušenjem i razaranjem nagnutih

hridinastih površina s posljedicom cjelovitog odstranjenja pratećih epi-, endo- i mezolitijskih organizama i do promjena mikroreljefa supstrata. Duž 50 km zapadne obale Istre na području Rovinja (Sjeverni Jadran) pregledano je 41 postaja i procijenjeno je da do 10 m dubine bilo ogoljeno približno 28% od ukupne dužine nagnutih hridinastih površina. Zbog dubine donji slojevi hridinaste obale nisu intenzivno izlovljeni. Radi numeričkog prikazivanja promjena mikrostrukture nastalih uslijed rušenja hridinastih podloga, na nagnutim površinama na skali od 30 mm, izmjereno je pet mikrotopografskih metričkih i kutnih varijabla supstrata. Zatim je nakon analize svih pet pokazatelja strukturne kompleksnosti za pojedine postaje i analizom varijanse u odnosu na sve postaje, testirana je statistička značajnost razlike (PCA) između netaknutih i oštećenih podloga. Pokazalo se da je, osim promjene indeksa mikrostrukturne raznolikosti, došlo i do značajnih promjena srednje nagnutosti i posječenosti na oštećenim hridima. Rezultati dokazuju da je izlov prstaca vrlo agresivna djelatnost, koja dovodi do trajnog smanjenja strukturne raznolikosti hridinastog dna i do dugotrajnog osiromašenja i destabilizacije biološke raznolikosti izvorno bogatih biocenoza na hridinastoj obali.

## IMPACT OF DATE MUSSEL (*Lithophaga lithophaga* L.) HARVESTING ON THE ROCKY BOTTOM STRUCTURAL COMPLEXITY

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Numerous marine organisms live on the rocky surface (epilithion); some others bore into the limestone substrate (endolithion) or use the numerous small crevices (mesolithion) for their shelter. European date mussels (*Lithophaga lithophaga* L.) are active boring bivalves, distributed in calcareous limestone along the entire Mediterranean and east Atlantic coast. In spite of strong protection legislature, date shells are continuously harvested following a permanent destruction of the bottom structures and substrate microtopography, eradication of the accompanying epi-, endo- and mesolithion species and drastic degradation of the originally rich rocky bottom community. A survey of 41 stations alongside 50 km of coast adjacent to Rovinj (West istrian coast, North Adriatic, Croatia) showed that up to 10 m depth, nearly 28% of the total length of inclined surfaces has been bared. The lower horizonts are not critically harvested. The microtopography of the inclined surfaces was measured at a 30 mm scale and five indices of structural complexity, including metric and angular variables, were calculated. The mean values of the five indices from single stations were analysed by means of the analyse of variance and, following the principal component analysis (PCA) we were able to differentiate the intact from damaged profiles. Additionally, the mean angle of surfaces significantly changed suggesting that the shadowing effect and the general profile of the limestone substrate was modified. Our results prove that harvesting of date shells disturbance cause a permanent reduction structural complexity, following a long-term pauperation and destabilization of the originally rich biodiversity of the rocky bottom communities.

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## STANJE PSINA U ISTOČNOM JADRANU

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Porodica Lamnidae je u Jadranu predstavljena s 3 vrste morskih pasa: velikom bijelom psinom, *Carcharodon carcharias* (Linnaeus, 1758), psinom dugonosom, *Isurus oxyrinchus* Rafinesque, 1810, te sa atlanskom psinom, *Lamna nasus* (Bonnaterre, 1788). S obzirom da pripadaju skupini velikih morskih pasa koji su na vrhu hranidbenog lanca u morskom ekosustavu te se zbog toga smatraju tzv. K - vrstama, ovi morski psi su izrazito ranjivi i podložni štetnim utjecajima prekomjernog izlovljavanja kojima su izloženi kao ciljane vrste ili prilov. U Mediteranu njihov broj je drastično opao što je izazvalo veliku zabrinutost za njihov status. Trenutno se u Jadranu te vrste smatraju rijetkima ili veoma rijetkima, iako se nekad veliku bijelu i dugonosu psinu smatralo stalnim stanovnicima Jadrana. Ovaj rad donosi sve nalaze ovih vrsta u istočnom dijelu Jadrana od 19. stoljeća pa do današnjih dana, te razmatra moguće uzroke smanjivanja njihova broja kako u Mediteranu tako i u Jadranu.

## STATUS OF LAMNIDAE SPECIES IN THE EASTERN ADRIATIC

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Family Lamnidae have been presented with 3 species in the Adriatic: great white shark, *Carcharodon carcharias* (Linnaeus, 1758), shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, porbeagle shark, *Lamna nasus* (Bonnaterre, 1788). As large sharks, which are apex predator of marine ecosystem and therefore considered as K - species, those sharks are highly vulnerable to overfishing, either as target species or by catch. In the Mediterranean their decline has already been observed which raised a concern for their status. Currently, in the Adriatic these species have been considered as rare or very rare, although, previously, for great white and mako shark it was believed that they are constantly present in that area. This paper present their records in the Eastern Adriatic since 19th century, including newest record, and deals with possible causes of their decline in the Mediterranean, as well as in the Adriatic.

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## PRVI NALAZ PUŽA *Melibe fimbriata* U JADRANSKOM MORU

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Prirodna rasprostranjenost puža *Melibe fimbriata* Alder and Hancock, 1864 je indopacifičko područje (od južne i istočne Afrike do Australije, Filipini i Japan). Dosadašnji nalazi u Sredozemlju su iz Jonskog mora, južnog Egejskog mora, Korintskog zaljeva i Tunisa. Vrsta M. fimbriata je u Jadranskom moru prvi put zabilježena u listopadu 2001. godine. Brojne su jedinke zabilježene u Starogradskom zaljevu (43°11'N-16°35'E) na otoku Hvaru. Tijekom razdoblja istraživanja temperatura mora je iznosila 18°C. Većina jedinki dužine od 10 do 15 cm je puzala i hranila se u livadama cvjetnica Cymodocea nodosa i Posidonia oceanica na pjeskovitim i pjeskovito-muljevitim dnima na dubini od 2 do 15 m. Na površini od 100 m2 je bila prisutna otprilike jedna jedinka. Jedinke vrste M. fimbriata bile su mnogo aktivnije noću kada su se hranile. Tijekom dana su usporeno puzale i odmarale se u livadama morskih cvjetnica. Mriješćenje je također uočeno. Ovo je do sada najsjeverniji nalaz ove lesepske vrste na području Sredozemnog mora.

## FIRST RECORD OF GASTROPOD *Melibe fimbriata* IN THE ADRIATIC SEA

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The original distribution of gastropod *Melibe fimbriata* Alder and Hancock, 1864 is the Indo-Pacific (South and East Africa to Australia, the Philippines and Japan). Previous records in the Mediterranean Sea were from the Ionian Sea, the South Aegean Sea, the Gulf of Corinth and from Tunisia. We recorded M. fimbriata in the Adriatic Sea for the first time in October 2001. A large number of specimens were observed in Stari Grad Bay (43°11'N-16°35'E), Island of Hvar, Croatia. During the observation period the seawater temperature was 18°C. Most specimens, 10-15 cm in length, were creeping and feeding in Cymodocea nodosa and Posidonia oceanica beds on sandy and sandy-muddy bottoms at depths of 2 to 15 m. The density of the population was about 1 specimen per 100 m2. Melibe fimbriata specimens were significantly more active during the night when they captured prey. During the day they were creeping slowly and resting in sea-grass beds. Spawning was also observed. Presently, this is the northernmost record of this Lessepsian immigrant in the Mediterranean basin.

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## KARTIRANJE LIVADA POROSTA, *Posidonia oceanica* (L.) DELILE U HRVATSKOM DIJELU JADRANSKOG MORA

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"Livade" porosta, morske cvjetnice *Posidonia oceanica*, imaju s biološkog i gospodarskog stanovišta izuzetan značaj za ekosustav Sredozemnog, a time i Jadranskog mora. Brojne međutim, ljudske djelatnosti negativno utiču na razvitak pa i opstanak ove osjetljive životne zajednice. U posljednjih je četrdesetak godina povećano onečišćenje mora, intenzivno koćarenje i unos tropske alge *Caulerpa taxifolia* utjecalo na smanjenje površine ove biocenoze u Sredozemnom moru za preko 30%. Nizom međunarodnih konvencija (Barcelona 1976/1995, Bern 1979, Rio 1992 itd.) primorske se zemlje obvezuju na očuvanje i zaštitu morske vegetacije, a posebno zaštitu morskih cvjetnica od kojih je najugroženija vrsta sredozemni endem *P. oceanica*. Većina razvijenih sredozemnih zemalja već se niz godina bavi kartiranjem i izradom atlasa rasprostranjenosti ove vrste radi smislenog upravljanja obalnim područjem, odgovornog ribarenja i učinkovite zaštite prirodne baštine i bioraznolikosti. U ovim je istraživanjima predviđeno kartiranje naselja porosta u hrvatskom dijelu Jadranskog mora. Kartira se autonomnim ronjenjem i snimanjem in situ, a započima se snimanje i lateralnim sonarom s QTC VIEW 5 programom, najmodernijim sustavom za klasifikaciju morskog dna. Predviđena je izrada atlasa u mjerilu 1:25000. Ovom će se prilikom prikazati rezultati dosada obavljenih istraživanja na području oko otoka Silbe, Lastova i Dugog otoka te u okolici Šibenika.

## MAPPING OF THE *Posidonia oceanica* MEADOWS IN THE CROATIAN PART OF THE ADRIATIC SEA

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Biocoenosis of Posidonia meadows is the most valuable settlement in biological and economical sense for ecosystem of Mediterranean and also Adriatic Sea. Intense influence of human activities has negative impact on evolution and survival of this sensitive community. In last 40 years due to higher sea pollution, intense trawling and introduction of tropical alga *Caulerpa taxifolia*, areas covered by this biocoenosis have been reduced for

more than 30 %. By signing many international conventions (Barcelona 1976/ 1995, Bern 1979, Rio 1992, etc.) coastal countries took obligation to conserve and protect marine vegetation especially marine phanerogams from which the most endangered is *P. oceanica* - a Mediterranean endemic species. For many years the most developed countries have been mapping benthic communities and making distribution maps especially for marine phanerogams in order to achieve wise coastal zone management, responsible fishing and effective protection of natural resources and biodiversity. In this research Posidonia meadows mapping in Croatian part of Adriatic Sea is proposed. Mapping will be done by SCUBA diving and in situ recording and marine bottom will be surveyed by side scan sonar with high sophisticated QTC VIEW 5 program for seabed classification. Making an atlas in scale 1:25 000 is also proposed. Results of up to now undertaken research from the area around islands of Silba, Lastovo and Dugi otok and in the Šibenik area will be presented on this occasion.

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## NEKI ASPEKTI BIOLOGIJE LISTA OBIČNOG, *Solea solea* (LINNAEUS, 1758) (Teleostei, Soleidae) DUŽ SJEVERNOG DIJELA ZAPADNE OBALE ISTRE

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List obični je jedna od najbrojnijih vrsta porodice Soleidae u istočnom dijelu Jadranskog mora, naročito na pjeskovitim dnima zapadne obale Istre. U lovinama mreža listarica, ribolovnom alatu kojim se najviše lovi, čini u tijeku glavnog ribolovnog razdoblja (listopad - veljača) od 22,5 do 83,1% (prosječno 70,9%) broja i od 21,0 do 77,8% (prosječno 70,4%) mase. Njegova je biologija izučavana u razdoblju od prosinca 2002. do veljače 2003. Analizirano je ukupno 2798 primjeraka. Dužinski i maseni raspon je bio: Lt = 20,0 - 39,8 cm (Lt ± SD = 29,4±2,493) i W = 62-592 g (W ± SD = 228,9±76,92). Starost je bila od 2 do 7 godina (2o = 25,3 cm; 3o = 29,2; 4o = 34,0; 5o = 37,3; 6o = 38,6; 7o = 39,5 cm). Izračunate vrijednosti za odnos dužine i mase tijela za oba spola su bile: a = 0,0016, b = 3,510; r2 = 0,867, što karakterizira pozitivni alometrijski rast. Parametri rasta izračunati pomoću von Bertalanffy-jeve jednadžbe rasta u STATGRAPH 3.0 statističkom programu su za oba spola bili: L?.= 43,55; K = 0,327; to = -0,603. Izračunata teoretska vrijednost L? je nešto niža od utvđenih vrijednosti najvećih dužina za ovu vrstu (50 cm za Jadran; 70 cm za Sredozemno more). Dobivena ukupna i prirodna smrtnost je bila Z = 1.40 god. -1 i M = 0.895 god. -1. Stopa iskorištavanja, E = 0.505, ukazuje na intenzivno iskorištavanu populaciju ove vrste na istraživanom području.

## SOME ASPECTS OF THE BIOLOGY OF COMMON SOLE, *Solea solea* (LINNAEUS, 1758) (Teleostei, Soleidae) ALONG THE NORTHERN PART OF THE WEST ISTRIAN COAST

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Common sole is one of the most numerous species of Soleidae family in eastern part of the Adriatic Sea, particularly on the sandy bottoms of the west Istrian coast. In the trammel bottom set catches, called "listarice", specialised fishing gear for its catches, S.solea participated in number from 22.5 to 83.1% (average 70.9%) and in weight from 21.0 to 77.8% (average 70.4%) during the main fishing season (from October to February). Its biology was investigated from December 2002 to February 2003. Total of 2798 specimens were analysed. Total length and weight of individuals ranged as follows: Lt = 20.0 - 39.8 cm (Lt ± SD = 29.4±2.493) and W = 62 - 592 g (W ± SD = 228.9±76.92). Age of analysed specimens ranged from 2 to 7 years (2o = 25.3 cm; 3o = 29.2; 4o = 34.0; 5o = 37.3; 6o = 38.6; 7o = 39.5 cm). Calculated values of the length - weight relationship for both sexes were: a = 0,0016, b = 3,510; r2 = 0,867, which indicate positively allometric growth. Growth parameters calculated used von Bertalanffy growth equation in STATGRAPH 3.0 statistical programme were for both sexes as follows: L?.= 43,55; K = 0,327; to = - 0,603. Calculated theoretical values L? is smaller than the maximum recorded total length for this species (50 cm for the Adriatic Sea; 70 cm for the Mediterranean). Total and natural mortality were found to be Z = 1.40 years -1 and M = 0.895 years -1. The exploitation ratio, E = 0.505 revealed intensively fished stock conditions in the studied area.

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## ANHIHALINE JAME OTOKA BRAČA (CROATIA)

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Postanak otoka Brača vezan je uz proces podizanja razine mora nakon zadnjeg glacijala. Speleološki oblici nastali potapljanjem , a smješteni do 1 km od ruba otoka svrstani su u kategoriju anhihalinih jama. Na otoku Braču istražene su ukupno tri anhihaline jame , a rezultati obuhvaćaju podatke o fizikalno-kemijskim mjerenjima i pregled faune makroavertebrata.

## ANCHIHALINE CAVES OF THE ISLAND OF BRAČ (CROATIA)

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The formation of the island of Brač was determined by the raising of the sea level after the last glacial. The speleological objects situated about 1 km far from the island's coast fill with brackish water are called anchihaline caves. There are three anchihaline caves on the island, which have been explored. The exploration included physical and chemical parameters as well as the faunal research of macroinvertebrate species.

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## PLANKTONSKI KOPEPODI PRIOBALNOG DIJELA JUŽNOG JADRANA

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Kopepodi su najbrojniji herbivorni organizmi u pelagijalu mora. Istraživanja su obavljena tijekom 44 izlaska od svibnja 1996 do svibnja 1997, na postaji kod Dubrovnika, dubokoj 100 m (42038'N, 18003'E). Kopepodi su sakupljeni Nansenovom mrežom na zatvaranje s promjerom pora 200 µm, promjerom obruča 54 cm, u slojevima: 0-25 m, 25-50 m, 50-90 m. Prosječna abundancija kopepoda se kreće između 290 jed.m-3 (sredinom rujna) i 2172 jed.m-3 (početak lipnja). Veliki broj jedinki zabilježen je i polovinom prosinca, 1973 jed.m-3, te krajem siječnja, 1898 jed.m-3. Dominirali su kopepoditi roda Paracalanus i Clausocalanus s udjelom od 22%. Slijede kopepoditi roda Oithona (8%), te vrste Paracalanus parvus, Ctenocalanus vanus, Oihona similis i Oithona plumifera (7%). Mann-Whitneyevim U-testom utvrđene su značajne razlike ($p<0.001$-$p=0.05$) dnevne vertikalne raspodjele brojnosti stalno prisutnih i čestih vrsta. Više vrijednosti u sloju iznad 25 m dubine odnose se na kopepodita, te vrsta Paracalanus parvus, Mecynocera clausi, Clausocalanus furcatus, Centropages typicus, Temora stylifera, Acartia clausi. U sloju od 25-50 m, dominiraju Paracalanus denudatus, Ctenocalanus vanus, Calocalanus contractus, Caloacalanus styliremis, Clausocalanus arcuicornis, Farranula rostrata, a Clausocalanus paululus, Lucicutia flavicornis a Oithona setigera ispod 50 m dubine. Vrsta Calocalanus pavo je brojna unutar dva gornja sloja, a Calanus helgolandicus, Mesocalanus tenuicornis, Nannocalanus minor, Calocalanus plumulosus, Clausocalanus jobei, Clausocalanus pergens, Candacia giesbrechti, Oithona plumifera, Oncaea mediterranea od 25 m do dna. Značajne razlike vertikalne raspodjele brojnosti nisu utvrđene za kopepode Oithona similis i Monothula subtilis, vrste roda Corycaeus i Oncaea (< 1 mm).

## PLANKTONIC COPEPODS IN THE OFFSHORE SOUTHERN ADRIATIC

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Copepods play a key role in the flow of energy and material in marine planktonic ecosystems, owing partly to the fact that they often are the most numerous herbivores present. Material for this study derives from plankton collected at an offshore station (100-m depth) in the Southern Adriatic (442038'N, 18003'E) during 44 daytime cruises from May 1996 to May 1997. Copepods were sampled with 200-µm Nansen nets, 54 cm in diameter, equipped with a closing system at 0-25 m, 25-50 m, and 50-90 m. Average abundance ranged from 290 ind. m-3 in mid September to 2172 ind. m-3 at the beginning of June. A high number of individuals were recorded in the middle of December (1973 ind. m-3) and at the end of January (1898 ind. m-3). Paracalanus and Clausocalanus were dominant, contributing about 22% of total abundance, followed by Paracalanus parvus, Ctenocalanus vanus, Oihona similes, and Oithona plumifera (7%). The Mann-Whitney U-test ($p<0.001$-$p=0.05$) was used to compare the difference in vertical distribution of the most commonly encountered species. Copepodites of the following species dominated above 25 m: Paracalanus parvus, Mecynocera clausi, Clausocalanus furcatus, Centropages typicus, Temora stylifera and Acartia clausi. In the 25 - 50 m layer, Paracalanus denudatus, Ctenocalanus vanus, Calocalanus contractus, Caloacalanus styliremis, Clausocalanus arcuicornis, and Farranula rostrata dominated; Clausocalanus paululus, Lucicutia flavicornis, and Oithona setigera were most abundant below 50 m. Calocalanus pavo was numerous from the surface to 50 m, and Calanus helgolandicus, Mesocalanus tenuicornis, Nannocalanus minor, Calocalanus plumulosus, Clausocalanus jobei, Clausocalanus pergens, Candacia giesbrechti, Oithona plumifera, and Oncaea mediterranea from 25 m to the bottom. Significant differences in vertical distribution were not found nor in Oithona similis and Monothula subtilis, neither in species of Corycaeus and Oncaea (<1 mm).

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## NAJČEŠĆE VRSTE ALGI U OBRAŠTAJU U JADRANU

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Pojam "obraštaj" koristi se za zajednicu životinja i alga koja raste na umjetnim strukturama uronjenim u vodu i ne odnosi se na zajednice koje rastu na kamenju ili drugim prirodnim objektima. Najčešće je upotreba tog pojma ograničena na zajednice čiji rast ima ekonomski štetne posljedice. Postoji znatna razlika između obraštajnih

zajednica koje se javljaju na različitim strukturama (brodovi, plutače, kavezi za uzgoj ribe, test-ploče...). Razlike između navedenih obraštajnih zajednica ovise više o okolnostima pod kojima su uronjene i stupnju do kojeg je dopušteno da struktura obrasta (dužina izlaganja), a manje o tipu uronjene strukture. Neposredan efekt obraštanja je povećanje otpora broda, čime se smanjuje brzina broda, a time su povećani troškovi goriva, te se javlja gubitak vremena i novca zbog neophodnih dokovanja. Obraštanjem kaveza povećaju se vertikalne sile koje djeluju na strukturu kaveza, a ujedno se smanuje veličina oka mrežnog tega. Time se smanjuje protok vode kroz kavez i pogoršavaju se uvjeti uzgoja. Obraštaj može povećati težinu kaveza i izazvati potapanje kaveza. Cilj ovog rada je dati pregled najčešćih alga u obraštaju u brodogradilištu u Vela Luci na poliesterskim i epoksidnim test-pločama tijekom jedne godine, te u ribogojilištima u Rogoznici (uvala Peleš) i na Šolti (uvala Stračinska) na polietilenskom mrežnom tegu izrađenom tijekom ljeta i jeseni. Pronađene su vrste koje su karakteristične za obraštaj u Jadranu i koje su tipične za čista područja niskog stupnja trofije.

## THE MOST FREQUENT SPECIES OF ALGAE FOUND IN THE ADRIATIC SEA

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The term fouling is used for communities of algae and animals which grow on artificial structures immersed in water and it does not refer to plants and animals communities which grow on rocks or on the other natural objects. Fouling implies negative economic effect of growing communities. There are significant differences between fouling communities, which grow on different structures (ships, cages for fish rearing, test-panels...). The differences between fouling communities depend more on the conditions of immersion and duration of immersion and less on the type of immersed structure. The fouling of the ship increases the resistance of the ship, which reduces ship speed and increases fuel consumption costs as well as money and time lost due to ship docking. The fouling represents a serous problem in fish rearing in cages because used cage materials are ideal surfaces for the attachment of fouling organisms. The fouling of the cage increases vertical forces on cage structure and at the same time reduces mesh size of net which reduces water flow trough the cage and makes rearing conditions worse. Accumulated fouling can increase the weight of the cage and cause cage sinking. The main purpose of the work is to give the review of the most frequent algae species found in fouling community of the Adriatic Sea based on two researches, the first - on polyester and epoxy test panels immersed in shipyard "Greben" in the Vela Luka Bay during one year, the second - on polyethylene cage nets in fish farms in the Peles Bay (Rogoznica) and Stracinska Bay (island of Solta) during the summer and the autumn. The results we get correspond to those from the literature. The algae we found are typical for the Adriatic Sea fouling communities and for clean waters of low bioproductivity.

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## HIPERBENTOSKI GLAVOČI (Pisces, Periformes, Gobiidae) U KVARNERSKOM PODRUČJU, JADRANSKO MORE

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Od 1993. do 2002. godine izvršeno je 179 ihtioloških istraživanja na 77 postaja u području Kvarnera. Većina nađenih glavoča bile su epibentoske ili kriptobentoske vrste. Četiri vrste zabilježene su kao hiperbentoske tj. kao slobodno plivajuće u prostoru do 1 m iznad dna: *Aphia minuta mediterranea* De Buen, 1931; *Pomatoschistus knerii* (Steindachner, 1861; *Pomatoschistus quagga* (Heckel, 1840); *Pseudaphya ferreri* (De Buen & Fage, 1908). Sakupljeni su podaci o ekologiji ovih vrsta (brojnost, sezonsko pojavljivanje, stanište, biocenoza, te zajednica riba). Hiperbentoske vrste zabilježene su 29 puta na 14 postaja. Ovo istraživanje pokazalo je da su *P. quagga* i *P. knerii* infralitoralne hiperbentoske vrste okupljene u plovama. P. quagga nalažen je u raznovrsnim uvjetima sastava i dubine dna, u odnosu na *P. knerii* koji bira plića dna, sa slabo razvijenom vegetacijom i pod utjecajem slatke vode. Pri postojećoj razini spoznaje o ovim dvjema vrstama nema dokaza da one provode dio života na drugim dubinama (cirkalitoral) ili u drugom odnosu prema dnu (bentopelagično ili pelagično) od sada opisanog. *A. minuta* i *P. ferreri* povremeno se pojavljuju blizu infralitoralnog dna u području Kvarnera.

## HYPERBENTHIC GOBIES (Pisces, Perciformes, Gobiidae) IN THE KVARNER AREA, ADRIATIC SEA

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The 179 ichthyological researches using SCUBA diving were performed on 77 locations in the Kvarner area (Adriatic Sea) in the period from 1993 to 2002. The majority of the found gobies were epibenthic or cryptobenthic fishes. However, four identified species were observed as hyperbenthic (meaning within one meter above the sea bottom): Transparent goby, *Aphia minuta mediterranea* De Buen, 1931; Kner's goby, *Pomatoschistus knerii* (Steindachner, 1861); Quagga goby, *Pomatoschistus quagga* (Heckel, 1840); Ferrer's goby, *Pseudaphya ferreri* (De Buen & Fage, 1908). The species ecology (abundance, seasonal occurrence, habitat, biocenosis, fish assemblage) of these four species is described. A total of 29 findings of hyperbenthic gobies were recorded. They were collected on the fourteen locations. The presented research shows that *P. quagga* and *P. knerii* are infralittoral hyperbenthic schooling species. *P. quagga* occurred at more variable bottom conditions and depths, com-

pared to *P. knerii*, which preferred shallow water, bare bottoms and freshwater influence. At the present state of knowledge there is no evidence that these two species spend part of their lives at different depths (circalittoral) or in different relation to the bottom (benthopelagic, pelagic) from the presently described. *A. minuta* and *P. ferreri* occasionally occurred closely above the infralittoral bottom in the Kvarner area.

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## UČINAK UV ZRAČENJA I OZONA NA PREŽIVLJAVANJE CISTA I NAUPLIJA RAČIĆA Artemia (Crustacea, Branchipoda) - MOGUĆNOST PRIMJENE ZA OBRADU BRODSKIH BALASTNIH VODA

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Brodska balastna voda jedan je od najznačajnijih načina unosa nedomicilnih vrsta u moru. Posljedice unosa vrsta balastnom vodom mogu biti ekološke i gospodarske, te je stoga potrebito osigurati mehanizme kontrole prijenosa i unosa vrsta. Cilj ovog istraživanja bio je odrediti učinkovitost UVC zračenja (254 nm) i ozona, zasebno i kombinirano, za usmrćivanje organizama u balastnoj vodi, koristeći ciste i nauplije planktonskog račića Artemia kao test organizme. Ciste račića Artemia izlagane su UV zračenju tijekom 10, 30, 60, 180, 300 i 420 min, ozonu (0,124 g/dm3h) tijekom 30 i 180 min, te UV zračenju i ozonu tijekom 10, 30 i 180 min. Naupliji su izlagani UV zračenju i ozonu (0,124 g/dm3h) tijekom različitog vremena (1, 3, 5, 8 min). Rezultati istraživanja pokazuju da se naupliji račića Artemia mogu usmrtiti primjenom UV zračenja i ozona, zasebno i kombinirano, za razliku od cista čija potpuna inaktivacija nije ostvarena tijekom ovog istraživanja. Najučinkovitija inaktivacija cista i nauplija ostvarena je primjenom ozona i UV zračenja kombinirano.

## INACTIVATION OF Artemia CYSTS AND NAUPLII INDUCED BY UVC RADIATION AND OZONE - A POSSIBLE METHOD FOR BALLAST WATER TREATMENT

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Ship's ballast water is one of the most important vectors of species introduction into new marine environment. Consequences of such introduction can be ecological and economical, and it is therefore necessary to provide effective control mechanisms of species transport and introduction. The objective of this research was to examine the potential of UVC irradiation (254 nm) and ozone, individually and in combination, to kill organisms in ballast water, using cysts and nauplii of brine shrimp Artemia as test organisms. Artemia cysts were exposed to UV irradiation for 10, 30, 60, 180, 300 and 420 min and to ozone (0,124 g/dm3h) for 30 and 180 min, and to UV irradiaton and ozone for 10, 30 and 180 min. Brine shrimp nauplii were exposed to UV irradiation and to ozone (0,124 g/dm3h) for various periods (1, 3, 5, 8 min). Results of this study show that brine shrimp nauplii can be killed using UV irradiation and ozone, individually and in combination. Artemia cysts were not inactivated completely in this research. The most successful cysts inactivation was achieved using combination of UV irradiation and ozone.

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## NEKE BIOLOŠKE OSOBITOSTI PAPALINE, *Sprattus sprattus* (LINNAEUS, 1758) IZ ESTUARIJA RIJEKE ZRMANJE (NOVIGRADSKO MORE)

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Ukupno je analizirano 270 primjeraka papaline, *S. sprattus* iz komercijalnih lovina plivarice ostvarenih u području Novigradskog mora. Uzorci potječu iz rujna 2000. i 2002. godine. Daje se prikaz analiziranih merističkih i morfomerijskih osobitosti papaline. Totalna dužina analiziranih jedinki se u cjelini kretala između 7,7 i 15,0 cm, sa srednjom vrijednošću od 9,85±1,133 cm i modalnom vrijednošću od 9,5 cm (44,81%). Masa analiziranih jedinki je bila u rasponu od 4,03 do 23,05 g, sa srednjom vrijednošću 7,59±3,452 g. Parametri dužinsko-masenog odnosa su: a=0,0168 i b=2,6535 (r2=0,8109). Vrijednost eksponenta b se statistički značajno razlikuje od 3,0 i ukazuje na negativni alometrijski rast papaline u ovom području. Najveće vrijednosti koeficijenta kondicije su zabilježene kod manjih primjeraka, u dužinskim razredima od 7,5 do 8,5 cm. Nakon tih vrijednosti je uslijedio pad te ponovni porast pri dužinama iznad 12,5 cm.

## SOME BIOLOGICAL ASPECTS OF SPRAT, *Sprattus sprattus* (LINNAEUS, 1758) FROM THE ZRMANJA RIVER ESTUARY (NOVIGRAD SEA)

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A total of 270 specimens of sprat *S. sprattus* were analysed. Samples were taken from purse seine commercial catches during September 2000 and 2002. Some meristic and morphometric characters were analysed. Total lengths of analysed specimens ranged from 7.7 to 15.0 cm, with mean values 9.85±1.133 cm and modal length of 9.5 cm (44.81%). Weight of analysed specimens ranged from 4.03 to 23.05 g, with mean value of 7.59±3.452 g. The parameters of the length-weight relationship are: a=0.0168 and b=2.6535 (r2=0.8109). The exponent b value differs considerably from 3.0 and denotes negative allometric growth of the sprat from this region. The highest condition coefficient was established in specimens of length groups from 7.5 to 8.5 cm and in groups of 12.5 and 13.0 cm. Sprat specimens of intermediate lengths showed the lowest condition coefficient.

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## *Hippolyte prideauxiana* LEACH, 1817: NOVA VRSTA KOZICE ZA JADRANSKO MORE

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Zadnjih desetak godina u Jadranu je zabilježeno nekoliko novih vrsta desetonožnih rakova. Tako velik broj nalaza dekapoda u kratkom vremenskom roku možemo zahvaliti većoj angažiranosti znanstvenika karcinologa istraživanju podmorja metodom autonomnog ronjenja. Terenskim radom Prirodoslovnog muzeja Rijeka 2001. godine pronađena je nova vrsta kozice za Jadran. Vrsta *Hippolyte prideauxiana* Leach, 1817 sakupljena je na lokaciji Stare vode u Kostreni pored Rijeke. Ovaj nalaz je proširio dosad poznatu geografsku rasprostranjenost te vrste.

## *Hippolyte prideauxiana* LEACH, 1817: THE NEW SHRIMP SPECIES FOR THE ADRIATIC SEA

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In the last ten years several decapod species have been recorded for the first time in the Adriatic sea. Such a rapid increase in the number of recorded species

of decapod crustaceans in the recent years is the result of the increased number of carcinologists engaged in SCUBA diving. In 2001 during the ordinary Natural History Museum Rijeka SCUBA field work a new caridean species of decapod crustaceans for the Adriatic sea was recorded. The species *Hippolyte prideauxiana* Leach, 1817 was collected at the locality of Kostrena near city of Rijeka in the north Adriatic. The new record widen the so far recognized geographical distribution of the investigated decapod.

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## ISHRANA ŠNJURA (*Trachurus trachurus* L.) I ŠNJURA PUČINARA (*Trachurus mediterraneus* STEIND.) U ISTOČNOM DIJELU JADRANSKOG MORA

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U Jadranskom moru šnjur (*Trachurus trachurus* L.) i šnjur pučinar (*Trachurus mediterraneus* Steind.) imaju značajnu ulogu u koćarskom i pelagičkom ulovu. Iako su vrste vrlo rasprostranjene u Jadranskom moru malo se zna o njihovoj ishrani. Cilj rada je istraživanje ishrane u odnosu na dužinu i sezonske promjene u sadržaju želuca. Prema našim podacima šnjur i šnjur pučinar su karnivorne vrste. Glavna hrana su im planktonski eufauzidni račići *Nyctiphanes couchii* i *Euphausia krohni*. Skupina plijena koja obuhvaća više od 50% od ukupnog IRI (indeks relativnog značaja) može se smatrati glavnom hranom. Ribe kao što su *Gadiculus argenteus*, *Maurolicus muelleri* i *Engraulis encrasicolus* imaju manju važnost u ishrani osim u dužinskim razredima preko 28 cm TL, pa se smatraju sekundarnom hranom. Ostale skupine plijena kao što su dekapodni i mizidni rakovi, mnogočetinjaši i glavonožci imaju malu važnost i predstavljaju slučajnu hranu. Sastav hrane značajno se mijenja porastom ribe. Kod obje vrste značajno se poveća udjel riba, a smanji se udio eufauzidnih račića. Male su sezonske promjene u sastavu hrane. Eufauzidi su dominantni tijekom svih sezona posebno u razdoblju od ljeta do zime.

## FEEDING OF HORSE MACKEREL (*Trachurus trachurus* L.) AND MEDITERRANEAN HORSE MACKEREL (*Trachurus mediterraneus* STEIND.) IN THE EASTERN ADRIATIC SEA

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In the Adriatic Sea horse mackerel (*Trachurus trachurus* L.) and Mediterranean horse mackerel (*Trachurus mediterraneus* Steind) are of major importance in the pelagic and demersal fisheries. However, despite its abundance, very little is known about the trophic ecology. The purpose was to examine the feeding habits, including the influence of predator size and seasonal variations in the stomach contents. According to our data, main prey items of these two species were plankton euphausiids *Nyctiphanes couchii* and *Euphausia krohni*. The prey group which represents 50% or more of total IRI (index of relative importance) can be classified as main food. Teleosts, such as *Gadiculus argenteus*, *Maurolicus muelleri* and *Engraulis encrasicolus* and some others were less important in the diet, except in the large size classes exceeding 28 cm TL, and can therefore be considered as secondary food. Other prey groups i.e. mysids, decapods, cephalopods and polychaeta, were of minor importance and thereby considered as occasional food. Diet composition considerably change as fishes grows. In the both species the proportion of fish prey increases and that of euphausiids decreases. There was little seasonal variation in food composition. Euphausiids were dominant prey during all seasons, and were especially abundant from summer to winter.

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## SASTAV I SEZONSKE PROMJENE MAHOVINASTIH NASLAGA U PODMORJU RTA OŠTRO (RIJEČKI ZALJEV, SJEVERNI JADRAN)

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Sastav i sezonske promjene mahovinastih naslaga makroalgi istraživane su u podmorju rta Oštro (Riječki zaljev, sjeverni Jadran, Hrvatska) od veljače do prosinca 1997. g. Slučajnim odabirom s površine od 1/16 m2 sakupljeno je u sezoni ukupno 36 uzoraka od morske površine do 20 m dubine. Svi tipovi morskog dna (kamenito, pjeskovito, muljevito) bili su pokriveni mahovinastom naslagom. Ukupno su utvrđene 33 vrste algi od kojih su 20 Rhodophyceae (60,6%), 6 Fucophyceae (18,2%) i 7 Chlorophyceae (21,2%). Najzastupljenije su crvene alge Rhodomelaceae (45,5%) i zelene alge Cladophoraceae (18,2%). Postotak uspravnih algi relativno je nizak (12,1%). Prosječni broj vrsta u istraživanom periodu u osnovi je konstantan. Glavne vrijednosti indeksa raznolikosi po Shannon-u i R/P indeksa, od 1.1 i 3.8, ukazuju na nisku vrijednost složenosti sastava algi i sub-tropski tip flore istraživanog područja Za vrijeme ovih istraživanja crvena tropska alga visoke invazivnosti *Womersleyella setacea* (Hollenberg) R. E. Norris po prvi puta je zabilježena u Riječkom zaljevu.

Ova je vrsta danas široko rasprostranjena u Mediteranu (Verlaque, 1989; Airoldi et al., 1995; Rindi et al., 1999) i sve do sjevernog Jadrana (Sartoni & Rossi, 1998). Postotak zastupljenosti alge *W. setacea* mjenja se sezonski sljedeći uzorke na različitim dubinama. Za vrijeme istraživanja kod ove vrste nisu ustanovljeni reproduktivni organi. Vrlo nisko bogatstvo i raznolikost vrsta algi istraživanog područja ukazuje da mahovinaste nakupine algi, uglavnom s prevladavajućim dijelom alge *W. setacea* mogu negativno utjecati na razvoj uspravnih algi. Dosadašnjim istraživanjima teško je donijeti zaključke o mogućim utjecajima ove invazivne mahovinaste alge za akvatorij Riječkog zaljeva.

## STRUCTURE AND SEASONAL VARIATIONS OF A SUBTIDAL TURF-DOMINATED ASSEMBLAGE OF THE OŠTRO CAPE (RIJEKA BAY, NORTHERN ADRIATIC SEA)

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The structure and the seasonal variations of a subtidal turf-dominated macroalgal assemblage were studied at Oštro cape (Rijeka Bay, Northern Adriatic Sea, Croatia) from February to December 1997. A total of 36 samples were seasonally collected from randomly selected surfaces of 1/16 m2 from sea level to the depth of 20 m. All types of substrates (rocky, sandy, muddy) were overgrown by turf. A total of 33 species, among which 20 Rhodophyceae (60.6 %), 6 Fucophyceae (18.2 %) and 7 Chlorophyceae (21.2 %), were found. The most abundant were the red algae Rhodomelaceae (45.5 %) and the green algae Cladophoraceae (18.2 %). The percentage of the erect algae, was relatively low (12.1 %). The average species number was basically constant in time. The mean values of the Shannon diversity index (H') and of the R/P index, respectively 1.1 and 3.8, indicate a low complexity of the assemblage and a sub-tropical type of the flora. During this study the high invasive tropical red alga *Womersleyella setacea* (Hollenberg) R. E. Norris, was recorded for the first time in Rijeka Bay. This species, has recently become widespread in the Mediterranean (Verlaque, 1989; Airoldi et al., 1995; Rindi et al., 1999) till Northern Adriatic Sea (Sartoni & Rossi, 1998). The percentage cover of *W. setacea* varied seasonally following the same pattern at different depths. During the investigation no reproductive organs were found for this species. In conclusion, we can say that the low specific richness and diversity, of the studied assemblage, suggest that the turf-forming algae, mainly composed by *W. setacea*, might negatively affect the development of erect algae. But in the present state of investigations it is difficult to formulate relevant conclusions on the impact of this turf-forming macroalgae in the region of Rijeka Bay.

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## MEIOFAUNA PRIOBALNOG PODRUČJA OKOLICE ROVINJA

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Meiofauna sedimenata Jadranskog mora u plitkom priobalnom području je vrlo slabo istražena, stoga se ovim istraživanjem željelo pridonijeti njenom boljem poznavanju. Istraživanje je obavljeno na deset postaja u priobalnom području grada Rovinja na pjeskovitom i muljevitom sedimentu. Uzorkovalo se mjesečno tijekom jednogodišnjeg razdoblja metodom autonomnog ronjenja uz upotrebu ručnih plastičnih korera. Na svakoj postaji uzeta su po tri poduzorka sedimenta za analizu kvalitativnog i kvantitativnog sastava meiofaune. Uzorci su obrađeni standardnom laboratorijskom procedurom koja uključuje fiksiranje, elutraciju, sijanje (50 µm sito), te brojanje i određivanje glavnih skupina organizama koji ulaze u sastav meiofaune. Ukupno su utvrđene 23 skupine koji ulaze u sastav meiofaune. Prosječna godišnja brojnost meiofaune na istraživanom području kretala se od 745 do 2277 jedinki/10 cm2. Dominantna skupina bili su Nematoda (68 - 85%) izuzev dvije postaje. Skupina Copepoda je bila druga po brojnosti, osim na dvije postaje gdje su dominirali (57 - 60%). Ostale skupine meiofaune bile su zastupljene s udjelima 2 - 16%. Meiofauna priobalnog područja okolice Rovinja odlikuje se relativno visokom brojnošću i raznolikošću skupina. Sezonska dinamika meiofaune na istraživanom području nije bila jasno izražena.

## SHALLOW WATER MEIOFAUNA OF ROVINJ AREA

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Meiofauna of sediments in the shallow water of Adriatic Sea is very poorly investigated, so this research presents a contribution to better knowledge of this problematics. Samples were collected monthly during the period of one year at ten stations in the shallow water of Rovinj area, at sandy and muddy bottom. All samples were collected by SCUBA divers, using plastic hand cores. At each station three cores for qualitative and quantitative description of meiofauna were taken. The samples were treated using standard laboratory procedure including fixation, elutriation and sieving (50 µm mesh size). Later the samples were counted and determined to main meiofaunal taxa. Entirely 23 meiofaunal taxa were found. The mean yearly density of meiofauna on the investigated area ranged from 745 to 2277 ind./10 cm2. Free-living Nematodes were the most abundant organisms (68 - 85%) at all but two stations. Harpacticoid copepods were second in overall abundance and most abundant organisms at two stations (57 - 60%). The abundance of the other meiofaunal taxa ranged from 2 to 16%.

Meiofauna of shallow water near Rovinj showed relatively high diversity and abundance. Dinamics of meiofauna on investigated area was without clear seasonal trend.

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## GENETIČKA ANALIZA IVAZIVNOG VARIJETETA ALGE *Caulerpa racemosa* UPOTREBOM ITS PODRUČJA rRNA GENA KAO MOLEKULARNOG BILJEGA

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Početkom 1990-tih godina alga *C. racemosa* naglo se i agresivno raširila cijelim Sredozemnim morem. Agresivno širenje ove alge podrazumijeva stvaranje livada ispod kojih, zbog građe njezinog talusa, dolazi do anoksije i ugibanja svih živih organizama. Pojava uniformnih livada alge *C. racemosa* u podmorju Sredozemnog mora moglo bi imati dalekosežne posljedice po biološku raznolikost. Postoje indikacije da ova vrsta alge odgovara novom morfološkom i genetičkom varijetetu prozvanom invazivni. Posljednje dvije godine ovaj je varijetet pronađen i morfološki opisan i na dva nalazišta u Jadranskom moru (uvala Marćuleti na poluotoku Pelješac i Pakleni otoci kraj Hvara). Da bi genetički potvrdili postojanje morfološki opisanog invazivnog varijeteta alge *C. racemosa* u Jadranskom moru analizirano je ITS (ITS1-5.8S-ITS2) područje rRNA gena u genomu tri jedinke iz populacije Pelješac. U sve tri jedinke utvrđen je polimorfizam ITS-ova koji ukazuje na recentno hibridno porijeklo invazivnog varijeteta. Štoviše, sve tri jedinke imale su isti uzorak polimorfizma ITS-ova što bi moglo ukazivati da su one genetički klonovi nastali vegetativnim razmnožavanjem jedne ''roditeljske'' alge koja je donešena, bilo morskim strujama bilo antropogeno, na područje uvale Marćuleti. Analiza varijabilnosti polimorfizma ITS-ova znatno većeg broja jedinki iz ove populacije mogla bi otkriti genetičku strukturu populacije.

## GENETIC ANALYSIS OF *Caulerpa racemosa* "INVASIVE VARIETY" USING ITS REGION OF rRNA GENES AS THE MOLECULAR MARKER

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In the early 1990's *Caulerpa racemosa* has spread rapidly and aggressively throughout the Mediterranean Sea. This alga has characteristic organisation of

talus so that it creates vast undersea meadows under which no life can be sustained due to anoxic conditions. Such Caulerpa meadows have an enormous effect on the biodiversity of the Mediterranean Sea. There are indications that aggressive *C. racemosa* represents a new morphologically and genetically distinct variety called ''invasive''. Since 2000, the "invasive variety" has been found and morphologically described on two localities in the Adriatic Sea (peninsula Pelješac and islands Pakleni near the island of Hvar). ITS1-5.8S-ITS2 region of rRNA genes was analysed from three samples of *C. racemosa*, population Marćuleti, peninsula Pelješac, in order to determine genetically if the algae from this population correspond to "invasive variety". All three samples showed ITS polymorphism, which is considered to indicate recent hybrid origin of the "invasive variety". Moreover, the three samples showed the same pattern of ITS polymorphism, which could indicate their clone origin e.i. the population propagated vegetativelly from a "paternal'' plant which has been probably introduced here unintentionally. More individuals should be analysed to reveal the genetic origin of the population.

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## RIJETKI NALAZ PELAGIČKE HOBOTNICE *Tremoctopus violaceus violaceus* DELLE CHIAJE, 1829 U JUGOISTOČNOM JADRANU

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Cirkumtropska pelagička hobotnica *Tremoctopus violaceus violaceus* Delle Chiaje, 1830 je već prije zabilježena za jadranske vode (Verany, 1851; Kolombatović, 1889), ali usprkos povremenom ulovu u ribarske mreže ovo je prvi nalaz živog primjerka ove vrste od 1936. godine (Lane, 1974). Glavni cilj ovog rada je dodatak nalazima ove rijetke vrste u Jadranu. Dane su i osnovne morfološke karakteristike ulovljene jedinke, ženke koja je nađena u uvali Šunj na otoku Lopud (42° 41,5"N and 17° 57,5" E) 27. travnja 2000. godine. Šunj je plitka pješčana uvala južno-jugoistočne ekspozicije koja je otvorena prema dubokim vodama južnog Jadrana. Dorzalna duljina plašta je 33 mm, a totalna masa ulovljene jedinke je 19,32 g.

## RARE FINDING OF PALMATE OCTOPUS (*Tremoctopus violaceus violaceus* DELLE CHIAJE, 1829) IN SOUTHEASTERN ADRIATIC WATERS

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Circumtropical pelagic octopod *Tremoctopus violaceus violaceus* Delle Chiaje, 1830 has been reported for the Adriatic (Verany, 1851; Kolombatović,

1889), but though it can be found in fisherman's nets on occasion, this is first reported finding of live specimen of Tremoctopus violaceus violaceus since 1936 (Lane, 1974). The main purpose of this report is to make an update on findings of this rare cephalopod in the Adriatic area. As on addition, given are the morphological characteristics of captured specimen, the female that differs largely from male of the same species, e.g. has marked sexual dimorphism. One specimen, (female) was collected in Šunj bay on island of Lopud (42° 41,5"N and 17° 57,5" E) on April 27th, 2000 on south-southeastern exposure sandy beach looking to the deep open waters of southeastern Adriatic. Dorsal mantle length (DML) was 33 mm and total mass 19,32 g.

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## SASTAV POPULACIJA PLOSNATICA (Pisces, Pleuronectiformes) U PLITKIM UVALAMA JUŽNOG JADRANA

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U razdoblju od svibnja 1998. do prosinca 2002. u plitkim vodama četiri uvale na području južnog Jadrana (Gornji Molunat, Donji Molunat, Gornje Čelo i Lopud) istraživan je sastav i ukupna brojnost, sezonska kolebljivost te duljinsko-težinski odnos vrsta plosnatica. Uzorci su sakupljani priobalnom mrežom potegačom 25 metara duljine i visine 500 cm na središnjem dijelu s centralnom vrećom. Promjer oka je 8 mm na vanjskim krilima i 4 mm na središnjem dijelu mreže. Tijekom istraživanja ulovljeno je ukupno 325 jedinki plosnatica, pripadnika 2 porodice i 4 vrste. Brojnošću prevladava razok (*Bothus podas*, Delaroche 1809) s 66,15% ukupnog ulova, nakon kojeg slijedi plosnatica bljedica (*Arnoglossus laterna*, Walbaum 1792) s 32,61%, plosnatica tamnuša (*Arnoglossus kessleri*, Schmidt 1915) s 0,92% i list (*Solea vulgaris*, Quensel 1806) s 0,30%. U usporedbi s drugim grupama riba, brojnost plosnatica je niska i predstavlja 1,46% ukupnog ulova. Zabilježena je sezonska kolebljivost u brojnosti ulova pojedinih jedinki i vrsta s najvišim vrijednostima početkom i krajem ljeta (svibanj i rujan 2000; N=29) i najnižim u jesen (listopad 1998; N=2). Također su izražene i razlike u ulovu individualnih vrsta među uvalama, s većim vrijednostima u onima s pjeskovitim dnom. Krivulja duljinsko-težinskog rasta za obje najbrojnije vrste pokazuje negativni alometrijski rast (*Bothus podas* b=2,28; *Arnoglossus laterna* b=2,55). Prevladavanje stadija mlađi u ukupnom ulovu ukazuje na ulogu istraživanjih uvala u razvojnom ciklusu promatranih vrsta kao hraništa i rastilišta.

## SPECIES COMPOSITION OF THE FLATFISHES (Pisces, Pleuronectiformes) IN SHALLOW BAYS OF SOUTH ADRIATIC

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Structure and seasonal variation, total number and length-weight relationship in catch with small beach seine are reported for a flatfish population at four shallow bays (Gornji Molunat, Donji Molunat, Gornje Čelo and Lopud), southeastern Adriatic from May 1998 to December 2002. Samples were collected with modified beach seine 25 m long, and 500 cm high in central part with the central sac. Mesh size was 8 mm on outer wings and 4 mm in central part of the net. A total of 325 individuals representing 2 families and 4 species were collected. A wide-eyed flounder (*Bothus podas*, Delaroche 1809) was the most numerous making up 66.15% of the total catch, followed by scaldfish (*Arnoglossus laterna*, Walbaum 1792) with 32.67%, Kessler's scaldfish (*Arnoglossus kessleri*, Schmidt 1915) with 0.92%) and common sole (*Solea vulgaris*, Quensel 1806) with 0.30%. In comparison with other fish groups, flatfishes abundance was low and these four species comprised 1.46% of the total catch. There was seasonal variation in the abundance of individuals, with catches peaking in early and late summer (May and September 2000; N=29), and the lowest in autumn (November 1998; N=2). There was also difference in the catches of individual species between bays, occurring most frequently in soft bottoms bays, rather than in hard bottom substrate bays. The slope of the total length-weight relationship for both species indicates negative allometric growth (*Bothus podas* b=2.28; *Arnoglossus laterna* b=2.55). The dominance of juveniles in the catches indicates that these areas serve as nursery and feeding grounds for juvenile flatfishes.

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## PORODICE Scyliorhinidae I Triakidae (Squaliformes) U RIBARSKOM ULOVU NA PODRUČJU HRVATSKOG DIJELA JADRANSKOG MORA

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U razdoblju od 1997. do 2002. godine bilježen je ulov jedinki iz porodice Scyliorhinidae, *Scyliorhinus canicula* (Linnaeus, 1758) i *Scyliorhinus stellaris* (Linnaeus, 1758) i porodice Triakidae, *Galeorhinus galeus* (Linnaeus, 1758), *Mustelus asterias* (Cloquet, 1821), *Mustelus mustelus* (Linnaeus, 1758) and *Mustelus punctulatus* (Risso, 1826) na području hrvatskog dijela Jadranskog mora. Sve ulovljene jedinke morfološki su obrađene, a upisani su i podaci poput mjesta, datuma, dubine i metode ulova. U istraživanju su osim vlastitih podataka korišteni i podaci dobiveni u suradnji s lokalnim

ribarima. Za istraživane vrste napravljene su karte s ucrtanim mjestima ulova. Ukupno je ulovljeno 1076 jedinki, od toga 626 jedinki vrste *Scyliorhinus canicula* (Linnaeus, 1758), 119 jedinki vrste *Scyliorhinus stellaris* (Linnaeus, 1758), 9 jedinki vrste *Galeorhinus galeus* (Linnaeus, 1758), 123 jedinki vrste *Mustelus asterias* (Cloquet, 1821), 160 jedinki vrste *Mustelus mustelus* (Linnaeus, 1758) i 39 jedinki vrste *Mustelus punctulatus* (Risso, 1826). Sve istraživane jedinke imaju gospodarstveni značaj u ribarstvu Hrvatske. Kako za istočni dio Jadranskog mora ne postoje sustavni podaci o ulovu morskih pasa, teško je odrediti stupanj ugroženosti za svaku od istraživanih vrsta.

## FAMILIES Scyliorhinidae AND Triakidae (Squaliformes) IN FISHING CATCH IN THE CROATIAN PART OF THE ADRIATIC SEA

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Specimens of family Scyliorhinidae, *Scyliorhinus canicula* (Linnaeus, 1758) and *Scyliorhinus stellaris* (Linnaeus, 1758) and of family Triakidae, *Galeorhinus galeus* (Linnaeus, 1758), *Mustelus asterias* (Cloquet, 1821), *Mustelus mustelus* (Linnaeus, 1758) and *Mustelus punctulatus* (Risso, 1826) were caught from 1997 to 2002 in the Croatian part of the Adriatic Sea. All the specimens were morphologically analysed and the other data concerning place, date, depth and method of the catch were recorded as well. Besides personal observations, in this research data recorded by local fishermen were also taken in account. The map of Adriatic Sea with positions where the specimens were caught is given for all investigated species. A total of 1076 specimens were caught, of which 626 belonged to *Scyliorhinus canicula* (Linnaeus, 1758), 119 to *Scyliorhinus stellaris* (Linnaeus, 1758), 9 to *Galeorhinus galeus* (Linnaeus, 1758), 123 to *Mustelus asterias* (Cloquet, 1821), 160 to *Mustelus mustelus* (Linnaeus, 1758) and 39 to *Mustelus punctulatus* (Risso, 1826). All this investigated species have commercial importance in Croatian fisheries. Since there have been no systematic data about shark fishery for the eastern Adriatic sea, it is not possible to determine precise level of threat for each of this species in this area.

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## SMANJENJE GUSTOĆE ZOOKSANTELA KOD KORALJA *Cladocora caespitosa* (L., 1767) U LABORATORIJSKIM UVJETIMA

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*Cladocora caespitosa* je česti kolonijalni kameni koralj u Jadranskom moru. Živi u simbiozi sa mnogobrojnim endosimbiontskim, fotosintetskim zooksantelama iz roda

*Symbiodinium*. U nepovoljnim uvjetima koralji mogu izbaciti, a ponekad čak i probaviti zooksantele. Ovaj rad istražuje broj zooksantela u polipima koralja *C. caespitosa* kod kolonija u različitim eksperimentalnim uvjetima. Dvije kolonije držane su u odvojenim akvarijima, u filtriranoj morskoj vodi i na temperaturi od 15 - 20 °C. Jedna kolonija hranjena je s tek izlegnutim račićima vrste *Artemia salina* Linnaeus, 1758 u vremenskim razmacima od 2-3 dana, a druga kolonija nije hranjena. Oba akvarija bila su za vrijeme čitavog istraživanja u uvjetima potpunog mraka. Svaka tri dana zooksantele iz polipa koralja brojene su pomoću hemocitometra. Za svaki uzorak brojanje je ponavljano desetak puta, a iz dobivenih rezultata izračunata je srednja vrijednost i standardna devijacija. U prvom mjesecu istraživanja broj zooksantela kod kolonije koja nije hranjena varirao je od 7,17 x 106 to 7,09 x 106 stanica po polipu (SV = 7,12 x 106; SD = 8,76 x 105; n = 122), a kod kolonije koja je hranjena od 6,93 x 106 to 6,86 x 106 stanica po polipu (SV = 6,9 x 106; SD = 7,58 x 105; n = 138). Prvi značajan pad u broju zooksantela zabilježen je 28. dan od početka istraživanja i to kod obje kolonije. Zooksantele nisu pronađene u polipima koralja 61. dan nakon početka istraživanja kod kolonije koja nije hranjena, a 85. dan kod hranjene kolonije. Mali broj zooksantela pronađen je i u morskoj vodi u oba akvarija.

## DECREASING OF ZOOXANTHELLAE DENSITY IN CORAL *Cladocora caespitosa* (L., 1767) IN LABORATORY CONDITIONS

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*Cladocora caespitosa* is a common colonial scleractinian coral in the Adriatic Sea. It occurs naturally with numerous endosymbiotic photosynthetic zooxanthellae from genus *Symbiodinium*. In extreme conditions corals can expel zooxanthellae or may even digest them. This study investigates number of zooxanthellae in polyps of *C. caespitosa* in colonies under different experimental conditions. Two colonies were held in separate glass aquaria in filtered sea water at temperature of 15 - 20 °C. One colony was fed with freshly hatched brine shrimp (*Artemia salina* Linnaeus, 1758) at intervals of 2-3 days and the other colony was starving. Both aquaria were kept in dark conditions during experiment. Every three days zooxanthellae from polyps were enumerated using haemocytometer. Ten replicates were counted from each sample and the mean and standard deviation were calculated. In the first month of the experiment the zooxanthellae density ranged from 7.17 x 106 to 7.09 x 106 cells per polyp (mean = 7.12 x 106; SD = 8.76 x 105; n = 122) in colony that was not fed and from 6.93 x 106 to 6.86 x 106 cells per polyp (mean = 6.9 x 106; SD = 7.58 x 105; n = 138) in colony that was fed. The first significant decrease in zooxanthellae density was noted on 28th day of the experiment in both colonies. The zooxanthellae were not detectable in polyps at 61st day experiment in colony that was not fed and at 85th day in fed colony. A small number of zooxanthelae was also found in sea water during the experiment in both aquaria.

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## ISHRANA NEDORASLOG I ODRASLOG ŠPARA, *Diplodus annularis* (LINAEUS, 1758) (Teleostei, Sparidae) NA ISTOČNOJ OBALI JADRANSKOG MORA

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Iako je špar vrlo uobičajna priobalna vrsta porodice Sparidae, znanja o njegovoj biologiji i ekologiji, pa tako i ishrani su oskudna. Za analizu su uzeta probavila 489 (od čega 100 nedoraslih) jedinki špara sakupljene na području Novigradskog mora i Kaštelanskog zaljeva. U ishrani je nedoraslih špara utvrđena i biljna i životinjska komponenta hrane. Neophodna i glavna hrana nedoraslih su špara makrobentoske zelene alge (Chlorophyta: MFI=4154,47; Q=6201,15; IRI=4755,60), a dodatna hrana školjkaši (Bivalvia: MFI=32,72; Q=55,38; IRI=48,12), dok sve ostale zabilježene skupine plijena predstavljaju slučajnu hranu. Dobiveni je koeficijent punoće probavila (%Jr) nedoraslog špara relativno nizak i iznosi 1,54, dok je nasuprot tome dobiven relativno visok koeficijent praznoće probavila (%V) od 33,00. U analiziranim su probavilima odraslih špara pronađeni ostaci biljne (5 skupina) i životinjske (14 skupina) komponente bentoskog podrijetla što ukazuje na izraženu neizbirljivost odraslog špara u ishrani. Neophodni i glavni plijen u njegovoj ishrani su puževi (Gastropoda: MFI=331,81; Q=265,64; IRI=839,17), desetonožni raci (Decapoda: MFI=1132,50; Q=96,79; IRI=2239,99), školjkaši (Bivalvia: MFI=174,04; Q=86,85; IRI=517,45), zelene alge (Chlorophyta: MFI=110,49; Q=119,73; IRI=898,22) i nedefinirana jaja različitih životinja (uglavnom rakova i riba) (MFI=87,62; Q=47,45; IRI=309,32), dok morske cvjetnice predstavljuju dodatnu hranu (Spermatophyta: MFI=39,01; Q=29,89; IRI=426,47). Ostale su skupine prisutne u manje značajnim postocima promatrajući ukupni analizirani uzorak probavila. Utvrđene su kvalitativne i kvantitativne sezonske razlike u ishrani špara. Iz dobivenih rezultata proizlazi da su špari omnivori s tim da nedorasli pokazuju preferentnost za biljnom, a odrasli za životinjskom hranom.

## FEEDING OF ANNULAR SEABREAM, *Diplodus annularis* (LINAEUS, 1758) (Telostei, Sparidae) IN THE EASTERN PART OF THE ADRIATIC SEA

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Although annular seabream is a very common coastal species from a family Sparidae, little is known about its biology, ecology and feeding. In this study 489 annular seabream specimens (out of which 100 were immature) collected in the

Novigrad Sea and Kaštela bay were analysed for stomach contents. Feeding of immature specimens consisted of plant and animal components. Essential and main food items of immature seabream were macrobenthic green algae (Chlorophyta: MFI=4154.47, Q=6201.15, IRI=4755.60), secondary food items were bivalves (Bivalvia: MFI=32.72, Q=55.38, IRI=48.12), while other prey groups constituted occasional food. Obtained coefficient of stomach fullness (%Jr) was relatively low in immature seabream (1.54), while obtained value for coefficient of stomach emptiness (%V) was rather high (33.0). In analysed stomachs of adult seabream 5 groups of plant and 14 groups of animal components of benthic origin were found, indicating pronounced lack of food selectivity in adult seabream. Essential and main food items of adult specimens were gastropods (Gastropoda: MFI=331.81, Q=265.64, IRI=839.17), decapod crustaceans (Decapoda: MFI=1132.50, Q=96.79, IRI=2239.99), bivalves (Bivalvia: MFI=174.04, Q=86.85, IRI=517.45), green algae (Chlorophyta: MFI=110.49, Q=119.73; IRI=898.22) and undetermined eggs of different animal species (mostly crustaceans and fish) (MFI=87.62, Q=47.45, IRI=309.32), while sea grasses constituted secondary food (Spermatophyta: MFI=39.01, Q=29.89, IRI=426.47). Other groups were present in smaller percentages. Qualitative and quantitative seasonal differences were determined. Obtained data show that seabream is omnivorous species whose immature individuals show preference for plant components, while adults prefer animal component.

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## SPUŽVE U ČETIRI MORSKE ŠPILJE NA JUGOZAPADNOJ STRANI OTOKA PREMUDE, DUGOG OTOKA I GARMENJAKA VELOG

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Spužve su značajna skupina organizama u biocenozi polutamnih špilja, koja naseljava morske špilje, polušpilje i prolaze. U Hrvatskoj su, zbog geomorfoloških karakteristika istočne obale Jadrana i holocenske transgresije mora, takvi objekti brojni. U ovom radu istraživane su špilje na jugozapadnoj strani otoka Premude, Dugog otoka i otoka Garmenjaka Velog. Uzorci spužvi sakupljeni su u razdoblju od dvije godine (od 2000. do 2002.), tijekom višekratnih ronjenja pomoću autonomnih ronilačkih aparata. Na temelju mikroskopskih preparata skeletnih struktura utvrđeno je ukupno 76 vrsta spužvi. U špilji Katedrala na otoku Premudi nađene su 32 vrste, u podmorskom prolazu u uvali Brbinjšćica na Dugom otoku 46 vrsta, u špilji Golubinka, također pokraj uvale Brbinjšćica, nađene su 42 vrste, a u polušpilji na otoku Garmenjaku Velom 41 vrsta. Od utvrđenih vrsta 17,1% bilo je prisutno na sve četiri lokacije, 21% na tri lokacije, 19,7% na dvije lokacije, a 42,1% vrsta zabilježeno je u samo jednoj od četiri navedene špilje. Vrste *Thymosiopsis conglomerans* Vacelet i T. cuticulatus Vacelet & Perez, te vrsta *Asbestopluma hypogea* Vacelet & Boury-Esnault

zabilježene su po prvi puta za Jadran. Posebno je značajan nalaz dubokomorske mesojedne vrste A. hypogea jer je to drugi takav nalaz u Sredozemlju. Ova spužva pronađena je na svega 24 m dubine u podmorskoj jami u polušpilji na Garmenjaku Velom, gdje može opstati zbog hladne morske vode, koja se u jami zadržava tijekom čitave godine. Raznolikost vrsta spužvi nađenih u biocenozi polutamnih špilja ukazuje na potrebu daljnjeg istraživanja ovih staništa.

## SPONGES IN FOUR MARINE CAVES ON SOUTHWESTERN PART OF PREMUDA, DUGI OTOK AND GARMENJAK VELI ISLANDS

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Sponges are important group of organisms in the biocoenosis of semi-dark caves, which inhabits marine caves, half-caves and passages. Such phenomena are numerous in Croatia due to geomorphologic characteristics of the eastern coast of Adriatic and Holocene sea transgression. In this work caves on southwestern part of Premuda, Dugi otok and Garmenjak Veli islands were researched. Sponge samples had been collected for two years (2000-2002) during numerous SCUBA dives. Altogether 76 sponge species were determined on the basis of microscopic slides of skeletal structures. In the Katedrala cave (Premuda island) 32 species were recorded, in the submarine passage in Brbinjšćica cove (Dugi otok island) 46 species, in Golubinka cave, also near Brbinjšćica cove, 42 species, and in half-cave on Garmenjak Veli island 41 species. Among found species 17,1% were present on all four locations, 21% on three locations, 19,7% on two locations, and 42,1% species were recorded only in one of the researched caves. *Thymosiopsis conglomerans* Vacelet and *T. cuticulatus* Vacelet & Perez, as well as *Asbestopluma hypogea* Vacelet & Boury-Esnault were recorded for the first time in the Adriatic Sea. Especially important was finding of bathyal carnivorous species A. hypogea since that is the second such finding in the Mediterranean. The sponge lives in the submarine pit in the half-cave on Garmenjak Veli Island, on 24 m of depth, where it can survive due to cold seawater, which stays trapped in the pit throughout the year. Diversity of sponge species in the biocoenosis of semi-dark caves along the Croatian coast deserves further research.

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## USPOREDBA DVA NASELJA POSIDONIJE UZ OTOK KRAPANJ

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Biocenoza naselja posidonije *Posidonia oceanica* (L.) Del. pripada među najvažnije biocenoze Sredozemnog pa tako i Jadranskog mora. U njoj se mnogi

organizmi hrane, razmnožavaju i nalaze zaklon. Ova kompleksna biocenoza, koja ima visoku primarnu produkciju, značajnu produkciju kisika, te stabilizira sediment, u hrvatskom dijelu Jadrana još uvijek je nedovoljno istražena. Cilj ovog istraživanja bio je usporediti dva naselja posidonije izložena različitom utjecaju čovjeka. Uzorci posidonije sakupljeni su na dvije lokacije uz otok Krapanj u rujnu 2002. godine. Na svakoj lokaciji sakupljeno je po 100 izdanaka s dijelom rizoma za daljnju obradu u laboratoriju, te je prebrojan in situ broj izdanaka u 10 kvadrata površine 40 x 40 cm. Na jugozapadnoj strani otoka Krapnja uzorak je uzet na dubini 1-2 m, na mjestu koje je izloženije utjecaju valova ali pod manjim utjecajem čovjeka. Na sjeveroistočnoj strani Krapnja (Brodarica) uzorak je uzet na dubini 3-4 m, u naselju posidonije koje je u posljednje vrijeme izloženo velikom utjecaju turističkog naselja. Obradom uzoraka dobiveni su podaci o broju i tipu listova, dužini i širini listova, kao i indeks površine listova, koeficijent A (% slomljenih listova), te udio lišća pokriven epifitima. Napravljena je lepidokronološka analiza, izmjerena je duljina internodija, te je procijenjena primarna produkcija. Određen je i broj listova po godini te kada je pojedina biljka cvjetala. Utvrđena je također i korelacija između godišnjih cikličnih promjena širine presjeka baze lista i temperature mora. Rezultati su pokazali da postoje razlike između ova dva naselja posidonije, koje ukazuju na izraženiji utjecaj djelovanja čovjeka na Brodarici, kao i razlike koje ukazuju na veći utjecaj valova na naselje s jugozapadne strane Krapnja.

## COMPARISON OF TWO POSIDONIA MEADOWS ALONG THE KRAPANJ ISLAND

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Biocoenosis of Posidonia *Posidonia oceanica* (L.) Del. meadows is one of the most important biocoenosis in the Mediterranean as well as in the Adriatic Sea. Many organisms find food and shelter, and breed there. That complex biocoenosis, with high primary production, important for oxygenating seawater and stabilising the sediment, can still be considered poorly known in the Croatian part of the Adriatic. The aim of this research was to compare two Posidonia meadows exposed to different human impact. Posidonia samples were taken on two locations along the Krapanj Island in September 2002. On each location 100 shoots (fascicles) with accompanying part of rhizome were taken for further laboratory analysis, and shoots in 10 squares (40 x 40 cm) were counted. At the southwestern part of Krapanj sample was taken from 1-2 m of depth, on the place much more exposed to waves but less to human impact. At the northeastern part of Krapanj (Brodarica) sample was taken from 3-4 m of depth, in the Posidonia meadow recently exposed to intense touristic development. Laboratory analysis of samples yielded data about leaves number, type, length, width, as well as leaf surface in-

dex, coefficient A (% of broken leaves), and number of leaves covered with epiphytes. Lepidochronological analysis was done, measurements of internodal length, and primary production was estimated. Number of leaves per year was determined as well as when certain plant flowered. A correlation between annual cyclic changes in width of leaf base cross-sections and sea temperature were noted. Results have shown that there are differences between those two Posidonia meadows, which points towards more pronounced human influence on Brodarica, as well as differences which points towards higher wave impact on the meadow at the southwestern part of Krapanj Island.

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## REZULTATI REPRODUKCIJE I RAZVOJA RANIH STADIJA DROZDA *Labrus viridis* (LINNAEUS 1758)

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Rad prikazuje rezultate laboratorijskog mrijesta drozda *Labrus viridis*, embrionalnog razvoja i uzgoja ličinki. Ova vrsta naseljava priobalno kamenito dno i livade posidonie *Posidonia oceanica* od 2-50 m dubine. Sezona mrijesta drozda je proljeće i ljeto. Ribe su se mrijestile više puta tijekom dva dana krajem ožujka. Prosječan promjer jaja bio je 1018 µm. Inkubacija jaja trajala je 130 sati pri 13.8-15.3 oC i slanosti 38 ‰. Ukupna dužina ličinki nakon izvaljivanja bila je 5020 µm. Žumanjčana vrećica potrošena je nakon 92 sata poslije čega su ličinke prešle na aktivnu ishranu kolnjakom *Brachionus plicatilis*. Ukupna dužina ličinki pri prelasku na aktivnu ishranu bila je 5870 µm. Ličinke drozda su uspješno prošle kritično razdoblje prelaska na aktivnu ishranu s kolnjacima što omogućava lakši uzgoj ove vrste do kasnijih stadija.

## STUDIES OF REPRODUCTION AND EARLY LIFE HISTORY OF GREEN WRASSE *Labrus viridis* (LINNAEUS 1758)

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The paper describes results of spawning, embryonic development and larval rearing of green wrasse *Labrus viridis* in laboratory conditions. The species inhabits rocky bottom and meadows of *Posidonia oceanica* in coastal waters at a depth range from 2 to 50 m. The green wrasse spawns in spring and summer. This species spawned in laboratory conditions few times during two days in last part of

March. Average diameter of eggs was 1018 µm. The incubation period was 130 hours at 13.8-15.3 oC and 38 ‰ salinity. Total length of newly hatched larvae was 5020 µm. The yolk-sac was consumed through 92 hours and after that period larvae started active feeding on rotifers *Brachionus plicatilis*. Total length of larvae at the beginning of active feeding was 5870 µm. Green wrasse larvae were able to survive the critical period of adjustment to active feeding on rotifers. Therefore, these results encourage further culturing of this species.

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## PRVI NALAZ ZELENE ŽELVE *Chelonia mydas* U ISTOČNOM JADRANU

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Zelena želva *Chelonia mydas* jedna je od dvije vrste morskih kornjača koje se razmnožavaju u Sredozemlju s glavnim gnjezdištima u Turskoj i na Cipru. Danas u cijelom Sredozemlju godišnje gnijezdi svega 339 do 360 ženki, pa je sredozemna populacija najugroženija regionalna populacija ove vrsta na svijetu. Iako rijetka, glavata želva bilježena je u Jadranu još od 19. st. kada je uključena i u popise faune jadranskih zemalja, a nekoliko je primjeraka navedeno i u Katalogu vodozemaca i gmazova Hrvatskog prirodoslovnog muzeja (HPM). Predstavljeni podaci o zelenoj želvi u istočnom Jadranu temelje se na pregledu muzejskih zbirki i literaturnih navoda te našim nalazima. Svi primjerci dokumentirani u literaturi i muzejskim zbirkama kao *C. mydas* reidentificirani su kao glavata želva (*Caretta caretta*), s dužinom oklopa od 63.0 do 81.4 cm. Razlog zamjeni ove dvije vrste najvjerojatnije je staro hrvatsko ime za *C. mydas*: golema želva. Pošto manje juvenilne životinje čine većinu glavatih želvi u Jadranu izgleda da su veći (golemi) primjerci glavate želve bili automatski krivo određeni kao *C. mydas*. Četvrtog prosinca 2001. zabilježili smo nalaz juvenilne zelene želve. Želva je nađena mrtva u mreži stajačici 1 km istočno od Trpnja na Pelješcu. Zelena želva do sada nije zabilježena u Sloveniji, ni u Crnoj gori ili Albaniji, dok su se svi prijašnji dokumentirani nalazi u Hrvatskoj zapravo odnosili na glavatu želvu. Stoga je primjerak iz Trpnja prvi dokumentirani nalaz ove vrste u istočnom Jadranu. Želva je preparirana i čuva se u Herpetološkoj zbirci HPM-a. U cijelom je Jadranu do sada zabilježeno svega 12 nalaza zelenih želvi. Svi se odnose na životinje u pelagičkoj fazi s dužinom karapaksa od 28 do 40 cm, a većina ih je nađena u južnom Jadranu. Stoga je moguće da taj akvatorij predstavlja pelagičko razvojno stanište dijela sredozemne populacije zelene želve, pa je neophodno hitno provesti edukaciju ribara u južnom Jadranu kako bi mogli identificirati i prijaviti nalaze ove kritično ugrožene vrste.

## THE FIRST RECORD OF GREEN SEA TURTLE *Chelonia mydas* IN THE EASTERN ADRIATIC SEA

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The green turtle *Chelonia mydas* is one of two species of sea turtles that reproduce in the Mediterranean basin, with the major nesting areas in Turkey and Cyprus. At present, only 339-360 females nest annually in all Mediterranean beaches, making it the most endangered green turtle population in the world. The green turtle is considered rare in the Adriatic Sea, with the first literature information in the eastern Adriatic dated from the 19th century. It was listed in the Catalogue of Amphibians and Reptiles of the Croatian Natural History Museum (CNHM) and included in the indexes of the fauna of the Adriatic countries. The data presented are based upon inspection of museum collections, literature and our records. Re-identification of all documented records of green turtles in Croatia showed misidentification with large loggerhead turtles (*Caretta caretta*) with the carapace length of 63.0-81.4 cm. The reason for this misidentification most likely lies in the old Croatian name for *C. mydas*: "the huge (big) turtle". As most of loggerheads that frequent Adriatic Sea are juveniles, it seems that any "big" loggerheads were automatically and erroneously identified as *C. mydas*. We recovered a dead juvenile green turtle entangled in the gill net, 1 km east from the City of Trpanj, Pelješac Peninsula, Croatia, on the 4th December 2001. As green turtle has not yet been recorded in Slovenia, Montenegro and Albania, and as all of the original identifications of species in Croatia turned out to be wrong wherever we could perform re-identification, the green turtle captured in Trpanj is the first certain record of this species in the eastern Adriatic Sea. The taxidermy of this turtle is kept in the Herpetological Collection of the CNHM in Zagreb. Currently there have been only twelve green turtles recorded in the Adriatic Sea. The majority of records refer to juveniles with a carapace length of 28-40 cm, recovered in the southern Adriatic. It is possible that this region contains pelagic habitats for green turtle; therefore it is important to promote the education of fishermen along the southern Adriatic coasts to identify and report recoveries of this critically endangered species.

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## FAUNA BODLJIKAŠA (Echinodermata) LITORALNOG PODRUČJA NACIONALNOG PARKA "MLJET"

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Istražene su makrobentoske zajednice u obalnom području Nacionalnog parka "Mljet" i izraditi popis biljnih i životinjskih vrsta koje dolaze u podmorju otoka Mljeta. U razdoblju od 1995. do 1998. godine metodom autonomnog ronjenja istraženo je ukupno 55 postaja u podmorju Nacionalnog parka "Mljet" i to: 39 postaja duž jugozapadne i sjeverozapadne obale otoka Mljeta (između rta Tojsti i rta Glavat) i 16 postaja u Malom i Velikom jezeru. Također je pregledan i materijal iz koćarice (do dubine od 150 m) sa postaje udaljene 2 Nm jugoistočno od rta Goli, te materijal sa tri postaje uzorkovan iz ribarskih mreža. U supralitoralnoj, mediolitoralnoj, infralitoralnoj i cirkalitoralnoj stepenici utvrđeno je 14 bentoskih biocenoza. Pronađene su 44 vrste bodljikaša, što iznosi 44 % od ukupnog broja vrsta bodljikaša do sada pronađenih u Jadranu (101 vrsta). Od toga je jedna vrsta stapčara (Crinoidea), osam vrsta trpova (Holothurioidea), četrnaest vrsta zvjezdača (Asteroidea), deset vrsta zmijača (Ophiuroidea) i jedanaest vrsta ježinaca (Echinoidea). Horizontalna rasprostranjenost bodljikaša u istraživanom području ovisi o osobinama dna na kojem su razvijene određene bentoske biocenoze. Najveći broj vrsta pronađen je u livadama morske cvjetnice *Posidonia oceanica* (27 vrsta) i u biocenozi fotofilnih alga (24 vrste). Najvažniji ekološki čimbenik koji utječe na rasprostranjenje bodljikaša je tip morskog dna. Od 44 pronađene vrste na čvrstim je dnima utvrđeno 28, a na pomičnim 38 vrsta.

## ECHINODERMS (Echinodermata) IN THE LITTORAL AREA OF NATIONAL PARK "MLJET"

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We investigated macrobenthic communities in the coastal area of the National park "Mljet" and prepared list of species living there. In the period from 1995. to 1998. 59 transects in the littoral area of the island Mljet were performed. 55 sites were explored by SCUBA-diving, from which 39 sites along the south-western and north-western coast of the National park "Mljet" (between Cape Tojsti and Cape Glavat) and 16 sites in Mljet Lakes (sea lakes "Veliko jezero" and "Malo jezero"). Samples from one site situated 2 Nm south-east from the Cape Goli were collected by trawl from the depth of 150 meters. Samples taken from fishing nets from three sites outside the National park borders were also explored. In supralittoral, mediolittoral, infralittoral and circalittoral zone fourteen benthic communities were noted. 44 species of echinoderms were found, from which one Crinoidea, eight Holothurioidea, fourteen Asteroidea, ten

Ophiuroidea and eleven Echinoidea species. This number represents 44 % of the total number of the echinoderms species found in the Adriatic Sea up to date (101 species). Horizontal distribution of echinoderms in the area explored depends on the bottom quality, on which certain benthic communities are developed. The greatest number of species was found in *Posidonia oceanica* beds (27 species) and biocenosis of photophilic algae (24 species). The most important ecological factor influencing echinoderms distribution is the bottom type. 28 species were found on rocky bottom and 38 species were found on sandy, detritic and muddy bottoms.

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## POREMEČAJ STRUCTURE POPULACIJE PRSTACA (*Lithophaga lithophaga* L.) ZBOG INTENZIVNOG IZLOVA

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Jasno se pokazalo da izlov prstaca (*Lithophaga lithophaga* L.) vrlo značajno poremećuje biodiverzited u zajednicama hridinastog dna s posljedicom velikog osiromašenja svih elemenata tih zajednica. Istovremeno se pokazalo da dolazi do poremećaja, odnosno do slabljenja populacione strukture samih prstaca. Na središnjem dijelu zapadne istarske obale, na području Rovinja izabrane su 18 postaja. Uzorkovani su dijelovi nedirnute hridinaste podloge i drugi uzorci gdje smo mogli identificirati proteklo vrijeme od zadnjeg izlova. Na temelju biometrije nađenih prstaca, njihove gustoće naseljavanja i frekvencije njihovih dužinskih/starosnih razreda uspjeli smo pokazati kako s izlovom dolazi do oslabljenja njihove populacione strukture. U nekoliko uzoraka s kamena uronjenih prije 15 godina u temeljima jednog lukobrana srednja dužina nađenih prstaca iznosila je tek 29 mm, ali je broj prstaca iznad 50 mm, što je najmanja veličina koja se u restoranu može staviti na tanjuru, iznosio jedva 9%.

## DISTURBANCE OF DATE SHELL (*Lithophaga lithophaga* L.) POPULATION DUE TO HARD HARVESTING

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Date shell (*Lithophaga lithophaga* L.) harvesting significantly distress the biodiversity following by a general degradation of the rocky bottom communi-

ties, but it particularly perturb the population structure of the same date shell as well. In the central part of the west istrian coast in the region of Rovinj samples of limestone were picked up from18 sites. Undisturbed sections and further samples of limestone surfaces precedently picked up at different time intervals were collected. On the basis of the date shell biometry, their specific density and relative size/age frequency we demonstrated that the population structure was significantly weakened. In samples of rocks submerged for 15 years as part of a rock-fill dam, the mean length of date shells was 29 mm, but the number of the largest samples, over 50 mm, that represent their lowest size that can be used for commercial use, was at the level of hardly 9%.

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## MORFOLOŠKE ZNAČAJKE I DINAMIKA POPULACIJE OLIGE *Atherina boyeri* RISSO, 1810 (Pisces) NA UŠĆU MALE NERETVE

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Od ožujka 2001. do veljače 2002. na ušću Male Neretve istraživana je populacija olige, *Atherina boyeri*, Risso 1810. Uzorkovanje je obavljano na jednoj postaji, mjesečno po 100 oliga. Malom mrežom pričvršćenom na metalni okvir ulovljeno je ukupno 1200 jedinki.U populaciji olige dominiraju jednogodišnje i dvogodišnje jedinke (preko 90 % populacije), te je izrazit manjak trogodišnjih i četverogodišnjih jedinki. Ukupan godišnji odnos spolova olige na istraživanom području je iznosio mužjaci:ženke=1:1.31, međutim tijekom godine pokazuje značajnu promjenjivost. Tako mužjaci dominiraju od travnja do lipnja. Ženke dominiraju u svim ostalim mjesecima, posebice u razdoblju od srpnja do prosinca. Prosječni gonadosomatski indeks ženki tijekom 12-mjeseci je iznosio 0,762. GSI počinje rasti u ožujku kada iznosi 1,55%, a najveći je u travnju kada dostiže vrijednost od 5,4%. Prirodni mortalitet olige je bio vrlo visok (M=1,847/god za mužjake, 2,070/god za ženke, a za ukupni uzorak 0,896/god). Ukupni mortalitet je također bio vrlo visok , za mužjake 2,954/god, ženke 2,718/god i ukupni uzorak 1,806/god. Omjer iskorištavanja populacije olige s ušća Male Neretve je iznosio E=0,504/god. Visoki omjer iskorištavanja ukazuje na značajnu razinu izlova populacije olige na ušću Male Neretve. Na oligama je zabilježena jedna vrsta ektoparazita, riblja uš, *Mothoeya epimerica*, te dvije vrste endoparazita pronađenih u probavilima: kukaš vrste *Telosentis exiguus* i jedna vrsta botriocefalidne trakavice, red Pseudophyllidea Carus, 1863, koja je još uvijek nedeterminirana. U ishrani olige s ušća Male Neretve dominirali su Copepoda s brojčanim udjelom 62%, Cladocera su bili zastupljeni sa 21%, Decapoda (ličinke) 7%, Gammaridae 5%, a sve ostale vrste plijena sa udjelima do 3%.

## MORPHOLOGICAL CHARACTERISTICS AND POPULATION DYNAMICS OF SAND SMELT *Atherina boyeri* RISSO, 1810 (Pisces) FROM THE MALA NERETVA RIVER ESTUARY

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Population of the sand smelt, *Atherina boyeri*, Risso 1810, was investigated during the period from March, 2001 to February, 2002, in the Mala Neretva river estuary. Sampling was performed on one site and 100 specimens were collected monthly (total app. 1500 specimens), with small net attached on metal frame. This sand smelt population is characterised with a domination of one and two year old fish, with significant lack of three and four year old fish. Sex ratio of total sample was female:male (1.31:1), but with significant changes throughout the year. Males dominated from April to June, and females in all other months, especially in the period from July to December. Average yearly gonadosomatic index of the females (GSI) was 0.762. GSI started to grow in March (1.55%), with the highest value in April (5.4%). Natural mortality was very high (M=1.847/year for males, 0.070/year for females, and all sample 0.896/year). Total mortality was also very high, for males 2.954/year, females 2.718/year and all samples 1.806/year. The exploitation rate was computed as E=0.504/year, indicating high fishing pressure on the sand smelt population in Mala Neretva river estuary. Sand smelt was invaded with one ectoparasite, *Mothoeya epimerica*, and two endoparasites found in the intestines: acantopephalan *Telosentis exiguus* and one cestodes, order Pseudophyllidea Carus, 1863, which is still not determined. Food of sand smelt was dominated with copepods ( 62%), cladocerans (21%), decapods (larvae) 7%, gammarids (5%), and all other preys with 3% only.

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## SEZONSKA RASPODJELA FITOPLANKTONA U ESTUARIJIMA KRŠKIH RIJEA KRKE I ZRMANJE TIJEKOM 2000. I 2001. GODINE

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Krka i Zrmanja su krške rijeke koje utječu u Jadransko more tvoreći visokostratificirane estuarije. U visokostratificiranim estuarijima oštra haloklina dijeli vodeni stupac na gornji bočati i donji morski sloj. Krka čini veći estuarij, sa značajnijim antropogenim utjecajem. Rijeka Zrmanja je znatno manja rijeka, tvori

manji estuarij te je pod zanemarivim antropogenim utjecajem. Razvoj fitoplanktonskih zajednica istraživan je tijekom 2000. i 2001 godine, kako bi se napravila usporedba između estuarija Zrmanje i Krke. Podaci o abundanciji fitoplanktona analizirani su klasterskom analizom, u kojoj su za podatke iz estuarija Zrmanje dobivene četiri grupe, a za estuarij Krke sedam grupa uzoraka. Za svaku od grupa određene su dominantne vrste. Sezonalni razvoj fitoplanktonskih zajednica u plićem estuariju Zrmanje pokazuje jaču ovisnost o dotoku slatke vode nego u estuariju Krke.

## PHYTOPLANKTON SEASONALITY IN KARSTIC ESTUARIES OF KRKA AND ZRMANJA (2000-2001)

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Krka and Zrmanja are karstic rivers, that discharge into Adriatic Sea, forming highly stratified estuaries. In highly stratified estuaries a sharp halocline separates the upper brackish layer from the lower marine layer. Krka forms a bigger estuary, with more considerable anthropogenic influence. River Zrmanja is a smaller river, which forms smaller estuary with negligible anthropogenic influence. Phytoplankton development was investigated during 2000. and 2001, and compared in these two estuaries. Distribution of phytoplankton abundance was analyzed with cluster analysis. Four groups from Zrmanja, and 7 groups from Krka were defined and characterized by dominant species. Results from the Zrmanja Estuary revealed salinity and freshwater inflow having stronger influence on seasonal development of phytoplankton community than in the Krka Estuary.

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## STAROST I RAST JAKOVSKE KAPICE (*Pecten jacobaeus* LINNAEUS, 1758) U SJEVERNOM JADRANU

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Starost i rast jakovske kapice (*Pecten jacobaeus* Linnaeus, 1758) je istraživana na 292 primjerka prikupljena iz komercijalnog lova u sjevernom Jadranu, tijekom siječnja i veljače 2003. Starost školjkaša je određena pomoću ožiljaka na ligamentu i prstena rasta na vanjskoj stani ljušture te su podatci uvršteni u von Bertalanffyjevu

jednadžbu rasta. Nadalje, starost i rast su procijenjeni iz prirasta pomoću Gulland-Holt prikaza te je iz tih podataka izrađena relativna krivulja rasta. Dužina analiziranih primjeraka kretala se od 75,1 do 142,0 mm, dok je procijenjena starost bila između 2 i 13 godina. S obzirom na dvije primijenjene metode, dobivene su slijedeće von Bertalanffyjeve jednadžbe za visinu školjkaša: H=108,79(1-e-0,47t+0,16) i H=110,08(1-e-0,53t). Procijenjene asimptotske dužine školjkaša iznosile su 125,83 i 127,35 mm.

## AGE AND GROWTH OF THE MEDITERRANEAN SCALLOP (*Pecten jacobaeus* LINNAEUS, 1758) IN NORTHERN ADRIATIC SEA

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Age and growth of the scallop (*Pecten jacobaeus* Linnaeus 1758) was investigated on 292 specimens collected from a commercial catch in the northern Adriatic during January and February 2003. Shells were aged based on ligament scars and formation of growth rings on the external shell surface and data was fitted to the von Bertalanffy growth function. In addition, age and growth were estimated from growth increments using Gulland-Holt plot and relative growth function was constructed. Length of analyzed P. jacobaeus ranged from 75.1 to 142.0 mm, while estimated age ranged from 2 to 13 years. With respect to two methods applied, obtained von Bertalanffy equations for shell height were: H=108.79(1-e-0.47t+0.16) and H=110.08(1-e-0.53t). The asymptotic shell lengths were estimated to be 125.83 and 127.35 mm, respectively.

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## PREGLED NALAZA RIBLJIH "LESEPSIJSKIH MIGRANATA" U JADRANU

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Pojam "lesepsijski migranti" označava pojavu jednosmjernog i uspješnog bitoskog procesa prelaženja organizama iz Crvenog mora u istočni Mediteran. Broj riba - lesepsijskih migranata koje uspješno naseljavaju istočni Mediteran se stalno povećava. Glavni cilj ovog rada jest utvrditi sve nalaze tih riba, njihovu raspodjelu i brojnost te kolebanja nekih abiotičkih čimbenika tijekom nekoliko zadnjih godina u Jadranu. Zasada je 8 (9) vrsta dospijelo u Jadran tijekom zadnjih 20 godina. Zabilježene su slijedeće vrste u Jadranu: *Pampus argenteus* (? - ulovljena 1896 (1 jedinka), *Paraexocoetus mento* (bez podataka), *Hemiramphus far* (bez podataka), *Saurida undosquamis* (2 jedinke), *Sphyraena chrysotaenia* (jedna

jedinka), *Epinephelus coioides* (1 jedinka), *Leiognathus klunzingeri* (1 jedinka), *Stephanolepis diaspros* (1 jedinka) i *Siganus rivulatus* (2 jedinke). Tri vrste su utvrđene duž albanske obale, jedna vrsta uz crnogorsku obalu, tri (četiri) uz hrvatsku obalu te jedna vrsta uz talijansku obalu (sjeverni Jadran-Trst). Svi nalazi ovih vrsta ujedno predstavljaju i najsjevernije nalaze ovih vrsta u Mediteranu i uopće. Tijekom posljednjih 10-15 godina uočene su promjene u kvantitativnom i kvalitativnom sastavu jadranske ihtiofaune koje se pak mogu dovesti u svezu s promjenama oceanografskih svojstava Jadrana.

## THE SPREADING OF LESSEPSIAN FISH MIGRANTS INTO THE ADRIATIC SEA: A REVIEW

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The term "Lessepsian migration" characterizes a phenomenon of unidirectional and successful biotic advance from Red Sea to the Eastern Mediterranean. The number of the Lessepsian fish migrants colonizing the eastern Mediterranean increases continuously. The purpose of this paper is to examine the records, distribution and abundance of the fish Lessepsian immigrants in the Adriatic waters taking into account some new data on their presence and abundance and on variation of some abiotic parameters during recent years. At least 8 (or 9) species have reached the Adriatic Sea in last 20 years. The Lessepsian migrants fished in Adriatic waters (all findings were along the eastern Adriatic coast) are: *Pampus argenteus* (?-caught in 1896) (1 specimen), *Paraexocoetus mento* (not known), *Hemiramphus far* (not known), *Saurida undosquamis* (two specimens), *Sphyraena chrysotaenia* (one specimen), *Epinephelus coioides* (one specimen), *Leiognathus klunzingeri* (one specimen), *Stephanolepis diaspros* (one specimen) and *Siganus rivulatus* (two specimens). Three species were found at the Albanian coast, one species at the Montenegro coast, three (four ?) species at the Croatian coast and one species at the Italian coast (eastern north Adriatic). All the records of Lessepsian immigrants represent the northernmost occurrences of mentioned species. During last 10-15 years, changes in the quantitative and qualitative composition of the Adriatic ichthyofauna have been noted. These observations can be related to oceanographic changes in the Adriatic Sea.

TOKSIKOLOGIJA, EKOTOKSIKOLOGIJA I BIOMONITORING
*TOXICOLOGIA ET OECOTOXICOLOGIA*
TOXICOLOGY, ECOTOXICOLOGY AND BIOMONITORING

IL-19

## CJELOVITOST MOLEKULE DNA KAO BIOMARKER ONEČIŠĆENJA OKOLIŠA

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Citogenetička stabilnost je integralni dio homeostaze svake jedinke. Imajući u vidu važnost DNA za održavanje života (stanice, jedinke i vrste), određivanje jačine oštećenja DNA od primarnog je značaja pri utvrđivanju učinka onečišćenja na živi svijet i ekosistem u cjelini. Najrealniju procjenu antropogenog djelovanja na okoliš moguće je dobiti samo analizom organizama koji se u njemu i nalaze. Takav pristup je ujedno i najteži jer je tumačenje rezultata često vezano uz mnoge faktore čije djelovanje nam je nepoznato i/ili ga ne možemo kontrolirati. Kopnene vode i more posebno su izloženi djelovanju čovjeka jer su krajnji recipijenti većine otpadnih tvari. Genotoksični stres u vodenih organizama moguće je detektirati/procijeniti mikronukleus- i komet-testom. U našim istraživanjima u tu svrhu koriste se stanice tjelesnih tekućina školjkaša i riba. U sklopu projekta «CROWAT», financiranog od strane Norveškog istraživačkog savjeta, a u suradnji s Norveškim institutom za istraživanje voda (NIVA), provode se i ispitivanja oštećenja/cjelovitosti DNA u hemocitima dvije vrste školjkaša: dagnje *Mytilus galloprovincialis* (na više postaja u srednjem Jadranu) i slikarske lisanke *Unio pictorum* (u kopnenim vodama Istočne Slavonije). Raspravit će se značajnost rezultata dobivenih ovim istraživanjima.

## DNA INTEGRITY AS THE BIOMARKER OF ENVIRONMENTAL POLLUTION

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Cytogenetic stability is integral part of the homeostasis of any organism. Keeping in mind the importance of DNA for maintenance of life (cell, organism, and species), evaluation of DNA damage is of primary concern when determining the pollution-related stress in living organisms and ecosystems. The most realistic assessment of anthropogenic impact on the environment is only possible through analysis of the organisms that inhabit it. Such an approach is also the most difficult one since the interpretation of results is accompanied by many unknown/uncontrollable factors. Freshwater and marine environments are especially prone to anthropogenic impact because of their role as the end recipient of the majority of pollutants. The genotoxic stress in aquatic organisms can be detected/evaluated by using the micronucleus and comet assays. For this purpose, blood cells of mussels and fish have been used in our research. Within the «CROWAT» project, funded by the Norwegian Research Council (Project 150-463) and in cooperation with the Norwegian Institute for Water Research (NIVA), damage/integrity of DNA in haemocytes of two species of mussels (Mediterranean mussel *Mytilus galloprovincialis* - few locations in Middle Adriatic Sea, and Painter,s mussel *Unio pictorum* - in freshwaters of Eastern Slavonia), has been evaluated. The importance of results obtained through this research will be discussed.

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## "CROWAT PROJEKT – SUSTAV MONITORINGA OKOLIŠA ZA KOPNENE VODE, ESTUARIJE I MORE U HRVATSKOJ"

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Hrvatska je bogata vodom, ali zbog nedostatka postrojenja za njeno pročišćavanje velike količine otpadnih voda iz gradova i industrijskih postrojenja uzrokuju degradaciju njene kakvoće. Sprečavanje gubitka našeg prirodnog bogatstva iziskuje razvoj i uspostavu specifičnih monitoring programa za procjenu zdravlja biološke komponente i kakvoće voda. Glavni cilj "CROWAT projekta" je, suradnjom hrvatskih i norveških istraživača osmisliti, razviti i uspostaviti biomonitoring hrvatskih kopnenih voda i mora. U ovom interdisciplinarnom projektu upotrebljavaju se većinom biološke metode koje odražavaju promjene u organizmima uzrokovane djelovanjem onečišćivača - biomarkeri. Biomarkeri, kao mjera izlaganja onečišćenju i njegovog učinka su od posebnog značaja jer reagiraju samo na biološki raspoložive koncentracije onečišćivača i pružaju mogućnost rane detekcije promjena uzrokovanih onečišćenjem. Tijekom prve godine projekta (2002) za naša istraživanja analizirali smo prirodne populacije organizama (školjkaše i ribe) kao i jedinke izlagane u kavezima. Istraživanja su provedena na tri postaje na Srednjem Jadranu (Kaštelanski zaljev) i tri postaje u Istočnoj Slavoniji (rijeke Drava i Dunav). Ovaj projekt uključuje mjerenje slijedećih biomarkera: koncentracije metalotioneina, aktivnosti etoksirezorufin-O-deetilaze (EROD), aktivnosti benzo(a)piren monooksigenaze (BaPMO), aktivnosti acetilkolinesteraze (AChE), aktivnosti mehanizma multiksenobiotičke otpornosti (MXR), koncentracije glutationa (GSH), koncentracije vitelogenina, oštećenja DNA (komet-test i mikronukleus-test), koncentracije policikličkih aromatskih ugljikovodika i njihovih metabolita, energetskog statusa organizama i destabilizacije lizosomalne membrane.

## AN INTEGRATED ENVIRONMENTAL MONITORING SYSTEM FOR CROATIAN FRESHWATER, ESTUARINE AND COASTEL MARINE AREAS: THE CROWAT PROJECT

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Croatia is rich on water resources but due to lack of wastewater treatment plants the degradation of the water quality occurs. In order to prevent environmental degradation and the loss of natural wealth specific monitoring programmes are needed to estimate the water quality and the health of its biological resources. The overall objective of the "CROWAT project" is to establish collaboration between Croatian and Norwegian research groups to design, develop and implement a biomonitoring system for Croatian freshwater, estuarine and coastal marine waters. This interdisciplinary project is using mainly biological methods that reflect contaminant exposure and/or health effects in organisms, i.e. biomarkers. Biomarkers have the advantages that they respond to the biologically available contaminant concentration and detect pollutant impact on living organisms at an early stage. During the first year of the project (2002) we used both selected caged organisms and organisms collected from their natural habitat (bivalves and fish) at three locations in the Middle Adriatic (Kaštela bay) and three locations in Eastern Slavonia (river Drava and Dunav). Endpoints in this study include a suite of biomarker methods: metallothionein concentration, ethoxyresorufin O-deethylase (EROD) activity, benzo(a)pyrene hydroxylase (BaPH) activity, acetylcholine esterase (AChE) activity, multixenobiotic resistance (MXR) activity, glutathione (GSH) concentration, vitellogenin concentration, DNA damage measurement (comet assay and micronucleus test), polycyclic aromatic hydrocarbons (PAH) metabolites concentration and lysosomal membrane stability measurement.

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## RIBNJAČARSTVO JADRO: BIOMONITORING OPORAVKA POMOĆU BIOMARKERA

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Policiklički aromatski ugljikovodici (PAH) u vodenom okolišu predstavljaju veliki problem za resursnog upravitelja u odnosu na važnost zaštite i očuvanja ribljih resursa. Budući da ova zagađivala djeluju na povećanje pojavnosti

tumora, smanjenje plodnosti i povećanje razvojnih abnormalnosti u riba, njihova se genotoksičnost mora uzeti u obzir u biomonitoringu zagađenja voda. Cilj istraživanja bio je pratiti oporavak zagađenog ribogojilišta kroz dvije godine nakon prestanka utjecaja zagađenja (izljev mazuta 07. 01. 2002), mjerenjem biomarkera. Odabir ovih metoda temelji se na sljedećim razlozima: (a) nafta u ribama, uključujući i pastrvu, uvećava aktivnost EROD za nekoliko puta i potencijal za bioaktivaciju prokancerogena poput BaP u *S. typhimurium* mutagene; (b) period oporavka omogućava praćenje trajanja i/ili vrijeme-ovisnost opadanja razine djelovanja parametara nakon prestanka izlaganja. Aktivnost EROD (mjerena po metodi Burke i Mayer) u jetri pastrva (prosječne težine 200 g, različitog spola) izloženih mazutu bila je povećana (30,96 pmola resorufina/mg/min). Aktivnost EROD bila je najveća u jetri zagađenih pastrva sakupljenih u siječnju 2002. iz ograđenih bazena u ribnjičarstvu Jadro-Solin. U svibnju 2002. (5 mjeseci nakon incidenta) aktivnost EROD smanjena je za 5,4-puta (5,72 pmola resorufina/mg/min/). Aktivnost EROD u jetri pastrva izmjerena u svibnju 2003. ostala je približno na istoj vrijednosti (6,49 pmola resorufina/mg/min). Aktivnosti EROD u jetri pastrva izmjerene u siječnju 2002. inhibirane su *in vitro* s 100 mM a-naftoflavonom oko 70%. Rezultati su također pokazali da S9 frakcija pripremljena od mazutom zagađenih pastrva ima sposobnost bioaktiviranja BaP u mutagene metabolite kada se upotrijebi u *Samonella typhimurium* muta-testu. Dobivena je jasna korelacija (doza-učinak) između aktivnosti EROD i metaboličke aktivacije benz(a)pirena. Rezultati su pokazali primjenjivost metode biomarkera u biomonitoringu poboljšanja kvalitete okoliša nakon intervencije u okoliš.

## FISH-FARMING JADRO: BIOMONITORING OF RECOVERY USING BIOMARKERS

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The ubiquity of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment poses many serious problems for the resource manager concerned with the protection and conservation of fishery resources. The aim is to study the recovery of polluted fish-farming during two years after closing the source of pollution (masut spill January 7$^{th}$ 2002), using biomarkers. The choice of these methods was based on the following rationale: (a) crude oil induce in fish, including rainbow trout, severalfold enhancements of both the EROD activity and the potential to bioactivate procarcinogens like BaP to *S. typhimurium* mutagens (b) a recovery period allows the determination of the persistence and/or time-dependent decline in the level of impacted parameters after the cessation of

exposure. EROD activity (was determined by the method of Burke and Mayer) in the liver of trout (200 g average weight, both sexes) exposed to masut was increased (30.96 pmoles of resorufin/mg/min). EROD activity shown the maximum induction in the liver of polluted trout collected in January 2002. from the closed ponds of fish-farming Jadro-Solin. Through May 2002 (5 months after the incident) followed by a decrease in EROD activity for 5,4-fold (5.72 pmoles of resorufin/mg/min). EROD activity in the liver of trout measured on May 2003 remained at the same value (6.49 pmoles of resorufin/mg/min). These values, suggest, may well be the normal values of EROD activitiesin this fish species. The EROD activities in trout liver measured on January 2002. were inhibited with 100 mM a-naphthoflavone cca.70%. The results also showed that the S9 fraction prepared from masut polluted trout has the ability to bioactivate BaP to mutagenic metabolites when used in the *Salmonella typhimurium* reverse mutation assay. A clear dose-response relationship was observed between EROD activity and metabolic activation of benzo(a)pyrene. Results have shown to prove applicability of biomarkers in biomonitoring of improvements in environmental quality after remediation activities.

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## ISTRAŽIVANJE ZAGAĐENJA PRIOBALNOG MORA ISTRE I KVARNERA PRIMJENOM BIOMARKERA

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Priobalna područja mora i oceana mjesta su povećane industrijske aktivnosti i urbanizacije. Kao posljedica intenzivnog djelovanja čovjeka morski ekosustavi ovih područja su posebno ugroženi i izloženi različitom zagađivanju antropogenog porijekla. U prošlosti učinci ovog nepovoljnog djelovanja bili su često utvrđeni u završnoj fazi, tj. pojavom masovnog mortaliteta i/ili smanjenjem biološke raznolikosti ekosustava. Primjenom biomarkera moguće je utjecaje zagađenja otkriti u ranoj fazi prije nego dođe do nepopravljive štete po ekosustav. U našim istraživanjima koristili smo histokemijske metode za određivanje stabilnosti lizosomalne membrane, sadržaja neutralnih masti i lipofuscina u probavnoj žlijezdi dagnje (*Mytilus galloprovincialis* Lam.) te pratili mogućnost preživljavanja na zraku. Rezultati mjerenja stabilnosti lizosomalne membrane i mogućnost preživljavanja na zraku, kao biomarkera općeg stresa, pokazali su značajne razlike ($p<0.05$) između postaja u urbanim i lučkim zonama i čistim područjima Istre i Kvarnera. Statistički značajne razlike također su utvrđene mjerenjem količine neutralnih masti i lipofuscina kao pokazatelja izloženosti lipofilnim organskim zagađivalima. Tijekom godinu dana ispitan je i utjecaj tem-

perature, saliniteta, pH, zasićenja $O_2$ i količine klorofila, na korištene biomarkere. Značajne sezonske varijacije utvrđene su u količini neutralnih masti i lipofuscina te sposobnosti preživljavanja na zraku. Promjene u količini lipofuscina te sposobnosti preživljavanja na zraku značajno su bile ovisne ($p<0.05$) o promjenama temperature tijekom godine. Istraživanja su pokazala veliku osjetljivost metode mjerenja stabilnosti lizosomalne membrane i njenu neovisnost o promjenama biotičkih i abiotičkih faktora okoliša. Kod korištenja ostalih biomarkera u biomonitoringu i tumačenju rezultata treba voditi računa o njihovoj podložnosti sezonskim promjenama.

## EFFECTS OF CONTAMINATION ON SELECTED BIOMARKERS IN ISTRIA AND KVARNERINE COASTAL REGION (CROATIA)

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Lysosomal membrane stability, content of neutral lipids and lipofuscin as cellular biomarkers were measured in digestive gland of mussels, *Mytilus galloprovincialis* Lam., during one year survey on selected sites along the coast of northern Adriatic. On the same sites the ability of mussels to survive in air as general physiological response was tested. Annual changes of sea temperature, salinity, $O_2$ saturation, pH and chlorophyll a concentration were measured and effects of these environmental factors on used biomarkers were determined. Survival after aerial exposure of mussels was significantly ($p<0.05$) reduced in contaminated harbours of Pula and Rijeka compared to clean control sites. Lysosomal membrane stability of mussels from urban- and industrial-related areas was also decreased indicating exposure to different environmental contaminants. On the same sites the content of neutral lipids and lipofuscin in digestive cells was significantly higher, suggesting alterations in lipid metabolism. Variations of natural environmental factors greatest influence exerted on aerial survival of mussels and content of neutral lipids and lipofuscin in digestive gland. In conclusion, used biomarkers successfully differentiated contaminated areas from clean ones, and confirmed their usefulness as early warning tools for detection of environmental disturbances caused by anthropogenic influence. Among them, measurement of lysosomal membrane stability showed greatest sensitivity and low dependency to seasonal variations of natural parameters. Other biomarkers were influenced by environmental factors in greater extent, what lowered their ability to detect contamination in all seasons. Therefore, annual variations of particular biomarker should be known prior to its use in biomonitoring studies for correct interpretation of results.

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## CONTAMINATION BY ORGANOCHLORINE PESTICIDES THROUGH VACCARES LAGOON TROPHIC COMPARTMENTS

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Littoral wetlands are ranged amongst most endangered aquatic ecosystems. Even the largest protected coastal wetland in Western Europe, the UNESCO's Reserve of Biosphere of Camargue, is under various stresses due to anthropogenic environmental modifications. In particular, organochlorine (OC) pesticides sprayed on the adjacent agricultural areas get transferred, mainly *via* the irrigation ditches, into the Vaccarčs lagoon causing chronic contamination of this aquatic ecosystem. The objective of this study was to determine the contamination of the Vaccarčs lagoon food-web by prohibited and/or strongly restricted and permanently used OC pesticides. In order to establish the lagoon trophic structure the stable isotopes method was used. The isotopic signatures ($d^{15}N$, $d^{13}C$) and the tissue OC amounts were measured on Vaccarčs organisms from different trophic levels (bivalves, crustaceans, prey and predator fishes). The main goal was to develop pesticide-trophic level relationships in order to estimate the extent of the biomagnification phenomenon. The results demonstrate the presence of investigated pesticides through all trophic compartments. In the up to date research the infrequency of positive relation between $d^{15}N$, $d^{13}C$ and pesticide concentration does not indicate regular trophic level biomagnification and suggests that the contamination by OC pesticides in the Vaccarčs lagoon food web is not due to trophic transfer only but also to other accumulation processes. It highlights the fact that physiological and metabolic parameters play a significant role in determining bioaccumulation in investigated species. Therefore the effects of OCs on metabolic processes such as biotransformation and oxidative stress will also be evaluated on selected Vaccarčs biota.

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## OCJENA PROGRAMA BIOLOŠKOG MONITORINGA KOPNENIH VODA ZA BIOMONITORE-AMATERE: PRIMJER ORGANIZACIJE "PRIJATELJI POTOKA SAUSAL" IZ OAKLANDA, KALIFORNIJA, SAD

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U Sjedinjenim Američkim Državama brojne volonterske skupine građana bave se biomonitoringom kopnenih voda. Ti amateri (*volunteer monitors*) koriste uzorke bentalnih makroavertebrata kao prvenstveni pokazatelj stanja ekosustava. Službene ograde prema kvaliteti tih potencijalno vrijednih amaterskih podataka ostaju prepreka njihovom većem koristenju. U radu je opisan i evaluiran program biološkog monitoringa za biomonitore-amatere iz lokalne organizacije "Prijatelji potoka Sausal" iz Oaklanda u sjevernoj Kaliforniji (SAD). Provjerili smo i ocijenili (1) točnost determinacije avertebrata od strane amatera usporedivši je s našom determinacijom na istom uzorku, i (2) taksonomsku razlučivost (tj. najnižu taksonomsku kategoriju koju amateri koriste pri determinaciji). Taksonomska razlučivost bitna je za korištenje standardnih indeksa. Rezultati pokazuju da pogreška kod amaterske determinacije makroavertebrata iznosi manje od 10%, i da postignuta taksonomska razlučivost omogućava izračunavanje najčešće korištenih indeksa (npr. Hilsenhoff). Podaci koje su amateri prikupili o makroavertebratima dovoljno su detaljni da se na temelju njih mogu po stupnju zagađenja razlučiti lokaliteti s kojih su uzeti uzorci. Zaključujemo da biomonitori-amateri koristeći posebno sastavljeni amaterski protokol za biomonitoring, mogu prikupiti korisne i reprezentativne podatke o stanju ekosustava, unatoč ograničenjima koja nameće određivanje makroavertebrata na terenu. Također želimo istaknuti da su obrada i prezentacija podataka za biomonitore-amatere često najslozeniji dio biomonitoringa, te da je pomoć stručnjaka pri tome najbolji način da se poboljša kvaliteta amaterskih podataka.

## EVALUATING VOLUNTEER STREAM MONITORING: CASE STUDY OF THE "FRIENDS OF SAUSAL CREEK", OAKLAND, CALIFORNIA, USA

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Numerous volunteer groups are involved in monitoring aquatic ecosystems throughout the United States. Many of these groups use benthic macroinvertebrate surveys as their primary monitoring method, and produce potentially valuable data, but concerns about data quality remain a barrier to greater acceptance of volunteer-produced data. We describe and evaluate a volunteer-specific biomonitoring program developed by the Friends of Sausal Creek, a volunteer monitoring organi-

zation in Oakland, California. We evaluate (1) the accuracy of volunteers' macroinvertebrate identification against our own reidentification of the same sample, and (2) the achieved taxonomic resolution with respect to the ability of metrics calculated from volunteer data to allow the detection of the impaired sampling site. We found that taxonomic level achieved by the volunteers allowed the calculation of commonly-used metrics, and that the volunteers' identification of macroinvertebrates are largely accurate, with the metric scores differing by less than 10% when calculated using our identifications. The data also provide enough detail so that the two sampling sites – subject to different levels of anthropogenic impact – can be distinguished from one another. We conclude that, despite the limitations of in-field identification of macroinvertebrates, the volunteer groups using a volunteer-specific monitoring protocol can produce useful and representative biomonitoring data. We also note that summarizing and analyzing data remains the biggest challenge to many volunteer groups, and that help with this aspect of biomonitoring may be the best way to improve data quality.

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## BIOMONITORING OF AIR POLLUTION IN SLOVENIA

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Air pollution as a side effect of man's activities has become well known environmental problem during last two centuries and big efforts have been dedicated to resolve it world-wide by acceptance of international conventions. First phase comprised everywhere chemical and physical measurements of air quality, emission and immisions of pollutants regarding men's health. Soon became clear that the problem could be resolved only when other recipient were considered in regard of ecosystem's function, so the issue of biomonitoring and bioindicators rose up, first in biology, later also in other disciplines. The approach of ecotoxicology, used in agriculture, medicine, animal husbandry and in some other disciplines could never completly fulfiled environmental issues. In Slovenia first activities started with investigations of effects of industrial air pollution on man and vegetation and the use of bioindicators was more widely integrated in environmental studies after the acceptance of CLRTAP convention in former Yugoslavia and especially in period of intesive forest decline studies in eighties. In this time it was realised that only by organisms harmful effetc of air pollutants could be determined and several bioindication methods were implemeted. Screening of general air quality by mapping of epiphytic lichens in forest decline studies within the ICP- Forest and monitoring of ozone effects on agricultural crops and seminatural vegetation within ICP-Vegetation are briefly presented in contribution.

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## ACETILHOLINESTERAZA I ASPARTATAMINOTRANSFERAZA KAO BIOMARKERI KOD DAGNJE *Mytilus galloprovincialis* LAM

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Školjkaši su sjedilački organizmi koji filtriraju velike količine morske vode. Posjeduju izraženu sposobnost nakupljanja različitih ksenobiotika i zato se često upotrebljavaju u programima biomonitoringa. Biomarker je opća ili specifična promjena u biološkom sustavu do koje dolazi zbog izloženosti organizama zagađivalima iz okoliša, a učinak je mjerljiv na svim razinama biološke organizacije. Cilj istraživanja bio je ispitati mogu li inaktivacija acetilholinesteraze i porast aktivnosti aspartat aminotransferaze biti korišteni kao pokazatelji općeg stresa kod dagnji izložene kontaminaciji organofosfornim pesticidima. Dagnje su u laboratorijskim uvjetima bile izložene različitim koncentracijama organofosfornih pesticida diklorovosa i triklorfona, a enzimska aktivnost mjerena je u različitim vremenskim intervalima spektrofotometrijski u homogenatu škrga prema Ellmanovoj metodi, koja je bila prilagođena za rad s mikrotitarskim pločicama. Aktivnost AChE smanjila se u ovisnosti o koncentraciji pesticida, dok se aktivnost AAT nije značajnije mijenjala ($p<0,05$). Aktivnost istih enzima mjerena je i u uzorcima koji su sakupljani na različitim postajama zapadnoistarske i kvarnerske obale. Značajna razlika primijećena je između nekih postaja i procijenjen je potencijal ovih parametara u rutinskim mjerenjima biomonitoringa.

## ACETHYLCHOLINESTERASE AND ASPARTATE AMINOTRANSFERASE AS BIOMARKERS IN MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* LAM

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Bivalve mussels are filter-feeding organisms with a great capacity to accumulate various xenobiotics and they are frequently used as sentinel organisms in biomonitoring programs. A biomarker can be defined as a specific or general change in a biological system due to the exposure of living organisms to environmental contamination and the effects can be measured at the molecular, cellular till to the population and community level. It was our purpose to prove whether the inactivation of acetylcholinesterase (AChE) and the increase of aspartate aminotransferase (AAT) activity can be used as general or specific stress indicators in mussels exposed to organophosphorous pesticide contamination. Mussels were exposed in laboratory conditions to various concentrations of organophosphorous

dichlorovos and trichlorfon pesticides and the enzyme activity was measured at different time intervals according to Ellmans method by spectrophotometry in a gill tissue homogenate, adjusted for use with microtitar plates. The AChE activity decreased in a concentration dependent manner, while the AAT activity showed no significant change ($p<0.05$). The activity of the same enzymes was measured also in various samples from the West Istrian coast and Kvarnerine side. Significant differences have been noted among some stations, and the potential application of these parameters for biomonitoring routine has been evaluated.

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## METALOTIONEINI U PRIRODNOJ POPULACIJI DAGNJI *Mytilus galloprovincialis* UZORKOVANIH NA RAZLIČITIM PRIOBALNIM LOKALITETIMA KAO BIOMARKER IZLOŽENOSTI METALIMA

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Praćene su prostorne varijacije sadržaja metalotioneina u škrgama i probavnoj žlijezdi dagnji *M. galloprovincialis* prikupljenih na četiri priobalna lokaliteta u Kaštelanskom zaljevu (srednja Dalmacija). Podaci za metalotioneine uspoređeni su s koncentracijama Cd, Cu i Zn u toplinski obrađenoj frakciji citosola. Sadržaj metalotioneina određen je spektrofotometrijskom metodom. Istraživanje je napravljeno u sklopu zajedničkog projekta s Norveškim institutom za istraživanje voda (NIVA), projektu "CROWAT" (An integrated environmental monitoring system for Croatian freshwater, estuarine and coastal marine areas, projekt CROWAT, financiran od strane Norveškog istraživačkog savjeta). Utvrđene su statistički značajne razlike u sadržaju metalotioneina među istraživanim lokalitetima za oba ispitivana organa. Najviše vrijednosti metalotioneina zabilježene su u dagnjama uzorkovanim na postaji Trogir-Brodogradilište, a slijede je u padajućem nizu postaje Trogir-Marina, Vranjic i Inavinil. Koncentracije metalotioneina u škrgama kretale su se u rasponu od 26 do 44 µg/g vlažne težine tkiva (vl.t.) i visoko su korelirale sa sadržajem bakra u toplinski obrađenom citosolu ($r=0,76$, $p<0,01$). Koncentracije metalotioneina u probavnoj žlijezdi bile su oko tri puta više u odnosu na koncentracije u škrgama (96-119 µg /g vl.t.) i pokazale su statistički značajnu i pozitivnu korelaciju sa sadržajem cinka ($r=0,66$, $p<0,05$). Od ispitivanih metala, statistički značajne razlike u prostornoj raspodjeli metala među istraživanim lokalitetima zabilježene su za Cu u škrgama te za Zn i Cu u probavnoj žlijezdi. Ovi rezultati pokazuju da razine metalotioneina izmjerene u škrgama i probavnoj žlijezdi prirodne populacije dagnji *M. galloprovincialis* odražavaju različitu biološku ulogu ispitivanih organa u regulaciji metala pa se mogu upotrijebiti za prostorno praćenje izloženosti organizama metalima u priobalnom moru.

## METALLOTHIONEINS IN A NATURAL POPULATION OF MUSSELS *Mytilus galloprovincialis* SAMPLED AT DIFFERENT COASTAL LOCATIONS AS A BIOMARKER OF METAL EXPOSURE

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The spatial variation of metallothionein content was followed in gills and digestive gland of the mussel *Mytilus galloprovincialis* collected at four coastal locations in the Kaštela Bay (Middle Dalmatia) and compared with Cd, Cu and Zn concentrations in the heat-treated cytosolic fraction. Metallothionein concentrations were determined by a spectrophotometric assay. The investigation was performed in the framework of the common project with Norwegian Institut for Water Research (NIVA), CROWAT project (An integrated environmental monitoring system for Croatian freshwater, estuarine and coastal marine areas, CROWAT project, supported by Norwegian Research Council). In general, regardless to the organ investigated, the significant differences between investigated locations in metallothionein content were observed. The highest values were determined in mussels collected at Trogir-Shipyard station, followed in a decreasing order with Trogir-Marina, Vranjic and Inavinil stations. In the gills, metallothionein concentrations ranged from 26 to 44 μg/g wet tissue weight (w.w.) and were highly correlated with Cu concentrations in heat-treated cytosol ($r=0.76$, $p<0.01$). Metallothionein concentrations in the digestive gland were about three times higher than those in the gills (96-119 μg/g w.w.) and showed positive and significant correlation with Zn concentrations ($r=0.66$, $p<0.05$). For the metals measured, Cu concentrations showed the significant spatial variation in the gills whilst in the digestive gland significant differences in Zn and Cu content between sampling stations were detected. These results show that levels of metallothioneins, measured in the gills and digestive gland of a natural population of mussels M. galloprovincialis reflect different biological role of these organs in metal regulation. Therefore, metallothioneins could be used for spatial survey of metal exposure of organisms in coastal areas.

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## MONITORING KONCENTRACIJA KADMIJA U DAGNJI *Mytilus galloprovincialis* IZ KAŠTELANSKOG ZALJEVA, HRVATSKA

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Analiza sadržaja kadmija provedena je na dagnjama *Mytilus galloprovincialis* iz Kaštelanskog zaljeva. Cilj istraživanja bio je saznati postoji li gradijent

zagađenja od istočnog dijela zaljeva koji je najzagađeniji, prema sjeveru i da li je cijeli zaljev više zagađen od referentne postaje na Bistrini, Malostonski zaljev. Koncentracije kadmija u cijelom Kaštelanskom zaljevu su bile slične, te su se kretale od 0,26 do 1,38 µg Cd/g suhog tkiva. Značajno niže koncentracije kadmija bile su na samo jednoj postaji u zaljevu. Začuđujuće je da su koncentracije kadmija bile najviše na referentnoj postaji gdje je izmjereno između 1,99 i 4,14 µg Cd/g suhog tkiva, što je značajno više od koncentracija u zaljevu. Teško je odrediti razlog viših koncentracija kadmija u dagnjama s referentne postaje, ali mogući razlozi su da su dagnje bile manje i imale niže kondicijske indekse, a blizina ušća Neretve bi također mogla imati utjecaj. Postoji negativna korelacija između koncentracije kadmija i težine suhog tkiva dagnje, dakle čim je dagnja manja, tim je viša koncentracija kadmija u suhom tkivu. Analiza indeksa kondicije je pokazala da postoji korelacija između kondicije dagnje i koncentracije kadmija. Rezultat nije pokazivao statističku značajnost, ali postoji trend prema negativnoj korelaciji, gdje dagnje s nižim kondicijskim indeksom sadrže višu koncentraciju kadmija.

## MONITORING CADMIUM CONCENTRATIONS IN THE MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* FROM THE KAŠTELA BAY, CROATIA

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Mediterranean mussels, *Mytilus galloprovincialis*, were sampled in Kaštela Bay, Croatia, and analysed for cadmium. The aim was to determine if a pollution gradient, where the eastern part of the bay would be the most polluted could be observed, and if the entire bay was more contaminated than the reference site at Bistrina, Mali Ston Bay. Cadmium concentrations were quite uniform in the bay, ranging from 0.26 to 1.38 µg Cd/g dry wt., being significantly lower only at one of the sites in the bay. Surprisingly, cadmium concentrations were highest at the reference station, ranging from 1.99 to 4.14 µg Cd/g dry wt., and significantly different from those in the bay. It is difficult to pinpoint the reasons for the high cadmium concentrations in mussels at this site, but possible explanations are the smaller sizes and lower condition indices, as well as influence from the Neretva river estuary. A negative correlation could be observed between cadmium concentration and tissue dry weight, smaller mussels exhibiting higher concentrations. A condition index (CI) was calculated and used to test for correlation between body condition of mussels and cadmium concentration. No significance was obtained, but a trend towards a negative correlation, where mussels with lower CI exhibited higher cadmium concentrations, could be observed.

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## UČINAK NISKIH KONCENTRACIJA ATRAZINA NA BRZINU FILTRACIJE VRSTE *Dreissena polymorpha*

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Kao rezultat široke upotrebe i perzistencije u okolišu, niske koncentracije atrazina (selektivni s-triazinski herbicid) su često prisutne u površinskim i podzemnim vodama. Kako prirodni areal raznolike trokutnjače (*Dreissena polymorpha* Pallas) obuhvaća slatkovodne ekosustave agrarnih područja Europe i Sjeverne Amerike, postoji mogućnost da ova vrsta bude izložena atrazinu u svom prirodnom staništu. Cilj istraživanja bio je utvrditi učinak okolišno realnih koncentracija atrazina na filtraciju ove vrste školjkaša. Jedinke vrste *Dreissena polymorpha* tretirane su 21 dan s 0,003, 0,05, 0,5 i 5 mg/L atrazina. Učinak atrazina na filtraciju školjkaša utvrdili smo mjerenjem rate filtracije kontrolne i svih tretiranih skupina 7., 14. i 21. dana pokusa. Atrazin bitno utječe na ratu filtracije raznolike trokutnjače samo u najvišoj pokusnoj koncentraciji, kada je smanjio ratu filtracije za 80-89%. Iako je rata filtracije kontrolne skupine bila gotovo uvijek viša od one izmjerene u tretiranim skupinama, razlike u ratama filtracije kontrolnih životinja i životinja tretiranih s 0,003, 0,05 i 0,5 mg/L atrazina bile su vrlo male (78-107% kontrole) i ne mogu se sa sigurnošću pripisati djelovanju atrazina. Ipak primijećeno je da školjkaši tretirani ovim koncentracijama nisu normalno reagirali na povišenu temperaturu te nisu povećali ratu filtracije. To ukazuje na mogućnost da izloženost niskim koncentracijama atrazina ometa reakciju životinja na promjene okolišnih uvjeta. Za potvrdu ovog učinka potrebna su dodatna istraživanja. Ako okolišno realne koncentracije atrazina utječu na filtraciju vrste *Dreissena polymorpha*, vjerojatno utječu i na intenzitet filtracije drugih vrsta školjkaša. Kako filtratori imaju veliku ulogu u regulaciji trofije, utjecaj na ovu vitalnu funkciju školjkaša predstavlja rizik za slatkovodne ekosustave.

## THE EFFECT OF LOW ATRAZINE CONCENTRATIONS ON THE FILTRATION RATE OF ZEBRA MUSSEL (*Dreissena polymorpha*)

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As a result of an extensive use and persistence in environment, low atrazine concentrations (selective s-triazine herbicide) are often recorded in surface- and ground- waters. Since the natural area of zebra mussel (*Dreissena polymorpha* Pallas) includes freshwater ecosystems in agricultural areas of Europe and North America, it is likely that this species will be exposed to atrazine in its natural habitat. The purpose of this work was to study the effect of environmentally realistic atrazine concentrations on filtration of this species. Zebra mussels were exposed to 0.003, 0.05, 0.5 and 5 mg/L of atrazine, for a period of 21 day. The effect on zebra mussels was determined by measurement of the filtration rate of control and all the treated groups, on the 7$^{th}$, 14$^{th}$ and 21$^{st}$ day of experiment. We have established that only the highest used atrazine concentration had a strong effect on the filtration of zebra mussels. It reduced the filtration rate for 80 – 89%. The filtration rate of the control was almost always higher than those measured in the treated groups, but the differences between the filtration rates of control and animals treated with 0.003, 0.05 and 0.5 mg/L of atrazine was small (78-107% of the control) and we can not be certain that it was caused by atrazine. It was noticed, however, that the mussels exposed to those atrazine concentration did not respond normally to the elevated temperature and did not increase the filtration rate. Thus, exposure to the low atrazine concentrations might disturb the reaction of animals to the change of environmental conditions. For this conclusion the additional studies are necessary. If environmentally realistic atrazine concentrations affects the filtration of zebra mussel, probably also affects the filtration intensity of other bivalvian species. Since filtrators have a major role in trophy regulation, the effect on this bivalvian vital function represents a risk for freshwater ecosystems.

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## UČESTALOST MIKRONUKLEUSA U CELOMOCITIMA GUJAVICA *Lumbricus rubellus* I *Eisenia fetida*

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Mikronukleus test je jedna od najčešće korištenih metoda za detekciju genotoksičnost. Najčešće se koristi u stanicama sisavaca *in vivo* i/ili *in vitro*. Posljednjih desetak godina primjenjuje se i u stanicama drugih biljnih i

životinjskih organizama. Kako su različite vrste gujavica česti test organizmi u ekotoksikologiji, te bioindikatori u biomonitoringu, naš cilj je uvođenje mikronukleus testa u celomocitima gujavica kao standardnog biomarkera u praćenju kopnenih ekosustava. Kao prvi korak istražili smo prirodnu pojavnost mikronukleusa (MN) u celomocitima dvije vrste gujavica (*Lumbricus rubellus* i *Eisenia fetida*). Istražene su po dvije populacije svake vrste. U svakoj populaciji uzet je uzorak celomatske tekućine 20 životinja. U svakom uzorku pod UV mikroskopom pregledano je 1000 celomocita te je zabilježen broj stanica s MN-om. Učestalost MN-a u celomocitima vrste *Lumbricus rubellus*, ovisno o populaciji, bila je 0,25‰ i 0,4‰ odnosno 0,25 (±0,5362) i 0,4 (±0,9165) MN-a po uzorku/jedinci. Ovisno o populaciji, u celomocitima vrste *Eisenia fetida* prosječno se javilo 0,15 (±0,3571) i 0,2 (±0,4000) MN-a po uzorku/jedinci tj. učestalost MN-a bila je 0,15‰ i 0,2‰. Iako se može primijetiti razlika u učestalosti MN-a između dvije istraživane vrste, ona nije statistički značajna. Statistički značajnom se nije pokazala niti razlika između populacija iste vrste. Dobiveni rezultati pokazuju da je prirodna učestalost MN-a u celomocitima gujavica niska, te da se bitno ne razlikuju od literaturnih podataka o prirodnoj učestalosti MN-a u sličnim stanicama drugih vrsta beskralješnjaka.

## THE FREQUENCY OF MICRONUCLEI IN COELOMOCYTES OF EARTHWORMS *Lumbricus rubellus* AND *Eisenia fetida*

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The micronucleus test is a common method for the detection of genotoxicity. It is usually performed on mammalian cells *in vivo* and/or *in vitro*. For the last decade its use expands on the cells of other animal and plant organisms. Since the different earthworm species are common test organisms in ecotoxicology and bioindicators in biomonitoring, we set our goal on establishing the micronucleus test on earthworm coelomocytes as a standard biomarker in the monitoring of terrestrial ecosystems. As a first step, we investigated a natural occurrence of micronuclei (MN) in coelomocytes of two earthworm species - *Lumbricus rubellus* and *Eisenia fetida*. Two populations of each earthworm species were examined. In each population, specimens of coelom fluid from 20 animals were taken. In every specimen, 1000 coelomocytes were examined under the UV-microscope and scored for MNi. The MNi frequencies observed in coelomocytes of *Lumbricus rubellus*, depending on population, were 0.25‰ and 0.4‰ e.g. there was 0.25 (±0.5362) and 0.4 (±0.9165) MNi per specimen/individual. Average MN number in coelomocytes of *Eisenia fetida*, depending on population, was 0.15 (±0.3571) and 0.2 (±0.4000) per specimen/individual e.g. the MNi frequencies were 0.15‰ and 0.2‰. Although it exists, the differ-

ence between the MN frequencies in two studied species is not statistically significant. There was no significant difference between two populations of the same species either. Our results showed that the natural frequency of MNi in earthworm coelomocytes is low. They do not differ much from the literature data on natural MNi frequencies in similar cells of other invertebrates.

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## OVISNOST METALOTIONEINA O ODABIRU VRSTE TKIVA, BIORASPOLOŽIVOSTI METALA I BIOMETRIJSKIM POKAZATELJIMA U *Mullus surmuletus* (TRLJA KAMENJARKA) i *Liza aurata* (CIPAL SKOČAC) IZ KAŠTELANSKOG ZALJEVA

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U naseljenom priobalnom području vodni ekosustav je izložen povećanom unosu metala, kao posljedica ljudske djelatnosti. Biološkim akumuliranjem u morskim organizmima metali uzrokuju toksične učinke na različitoj organizacijskoj razini organizama, od stanične (biomarkeri) do populacijske. Izloženost metalima očituje se kao indukcija metalotioneina (MT), proteina koji vežu metale i imaju glavnu ulogu u homeostazi biološki neophodnih (Zn, Cu) i detoksifikaciji toksičnih (Cd, Hg) metala. U manjoj mjeri i drugi endo- i egzogeni stresori mogu pobuditi sintezu MT. Istražili smo utjecaj biometrijskih parametara (dužina, masa, hepatosomatski i kondicijski indeks), uz koncentraciju metala, na razinu MT u nativnim populacijama trlje kamenjarke (*Mullus surmuletus*), bentičke i cipla skočca (*Liza aurata*), pelagičke vrste. Uzorkovanje je provedeno u suradnji s Norveškim institutom za istraživanje voda (NIVA) u studenom 2001. i listopadu 2002. u Dalmaciji u Kaštelanskom zaljevu. MT, diferencijalnom pulsnom polarografijom i metali, atomskim apsorpcijskim spektrometrom s plamenom tehnikom su određeni u citosolskoj frakciji jetre, bubrega i mozga pripremljenoj homogenizacijom tkiva, centrifugiranjem na 50000xg i toplinskom obradom na 85°C. Koncentracije MT i metala su najviše u jetri i bubrezima, kao glavnim detoksifikacijskim organima, s visokim međusobnim korelacijama u jetri, posebno za Zn/MT i Cu/MT u trlje (0,95; 0,49) i cipla (0,55; 0,69). Koncentracija Cd, kao toksičnog metala je značajno niža u odnosu na Zn i Cu. Korelacija s biometrijskim pokazateljima je značajna i najviša za dužinu, starost i masu. Zbir statističkih analiza, te varijacije u koncentracijama MT i metala među različitim organima i vrstama, potvrđuju da je poznavanje metabolizma i rasta tkiva, starosti, veličine i fiziološkog statusa riba neophodno za pravilnu interpretaciju promjena razine biomarkera.

## METALLOTHIONEIN DEPENDENCE ON TISSUE SELECTION, METAL BIOAVAILABILITY AND BIOMETRIC PARAMETERS IN *Mullus surmuletus* (STRIPED RED MULLET) AND *Liza aurata* (GOLDEN GREY MULLET) FROM THE KAŠTELA BAY

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Aquatic environment is exposed to higher amounts of metals in the coastal urban areas as a consequence of anthropogenic activities. Bioaccumulation of metals in aquatic organisms can cause toxic effects at different organism level, from cellular (biomarkers) to population. Metal exposure is reflected as induction of metallothioneins (MTs), metal binding proteins which have the main role in homeostasis of essential (Zn, Cu) and detoxification of toxic (Cd, Hg) metals. Other endo- and exogenous stressors can also induce MT and this study represents influence of biometric parameters (length, weight, hepatosomatic and condition index), besides metals, on MT levels in native populations of *Mullus surmuletus*, benthic and *Liza aurata*, pelagic species. Sampling was performed in collaboration project with Norwegian Institute for Water Research (NIVA) in November 2001 and October 2002 in Dalmatia in Kaštela Bay. MT, by differential pulse polarography and metals (Zn, Cu, Cd), by Flame Atomic Absorption Spectrometer were measured in cytosolic fraction of liver, kidney and brain prepared by tissue homogenisation, centrifugation at 50000xg and heat treatment at 85°C. MT and metal levels were highest in liver and kidney as main detoxification organs and are statistically correlated in liver, especially for Zn/MT and Cu/MT in M. surmuletus (0.95; 0.49) and L. aurata (0.55; 0.69). Concentration of Cd, as a toxic metal is significantly lower comparing to Zn and Cu. Correlation with biometric parameters is significant and highest with length, age and weight. Summary of statistical analysis, tissue and species variations in MT and metal concentrations confirm that knowledge on tissue metabolism and growth, fish age, size and physiology status play an important role for correct presentation of biomarker's response.

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## OLOVO I KADMIJ U TLU I TRČCIMA ŠUMSKOG EKOSUSTAVA – PRELIMINARNO ISTRAŽIVANJE

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Istražena je povezanost između koncentracije olova i kadmija u tlu i trčcima (Carabidae, Coleoptera) na dvije lokacije. Uzorci trčaka sakupljeni su u šumskoj zajednici *Lamio orvalae – Fagetum* na području Parka prirode Medvednica, na lokacijama odabranim na temelju prijašnjih geokemijskih istraživanja. Upotrebljena je metoda lovnih posuda s mesom kao atraktantom tijekom srpnja 2002. godine. Jedinke su analizirane na razini vrste s obzirom da mnogi autori navode da je akumulacija metala u organizmu određena vrstom prije nego položajem u hranidbenom lancu. Trčci su suho spaljeni, a tlo je ekstrahirano s koncentriranom nitratnom kiselinom. Kvantitativna analiza olova i kadmija u trčcima i tlu provedena je metodom atomske apsorpcijske spektrometrije. Rezultati pokazuju da se koncentracije olova i kadmija mjerene u ekstrahiranom tlu i u trčcima korelirano razlikuju između odabranih lokaliteta. Koncentracija olova u uzorcima tla bila je 47,9 µg/g suhe tvari na prvom (S1) lokalitetu i 34,5 µg/g suhe tvari na drugom (S2) lokalitetu. Izmjerene vrijednosti za kadmij iznosile su 0,47 i 0,31 µg/g. Koncentracija olova u trčcima bila je 0,601 na S1 i 0,317 µg/g suhe tvari na S2, a kadmija 1,684 i 1,458 µg/g suhe tvari. Rezultati ovog preliminarnog istraživanja ukazuju da trčci mogu biti pokazatelji kontaminacije tla olovom odnosno kadmijem. Olovo i kadmij se ne mogu metabolizmom uspješno regulirati kao esencijalni metali. Iako neki autori navode da trčci uspješno eliminiraju asimilirane toksične tvari, u ovom istraživanju zabilježena je povećana koncentracija olova i kadmija u trčcima na lokaciji koja je više opterećena navedenim metalima.

## INVESTIGATIONS ON LEAD AND CADMIUM IN THE SOIL AND GROUND BEETLES OF A FOREST ECOSYSTEM – A PRELIMINARY RESEARCH

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The aim of the study was to determine lead and cadmium concentrations in soil and ground beetles at two sampling sites, in order to find out possible correlation of metals in soil and in beetles. Both sites were located in *Lamio orvalae - Fagetum* forest in Medvednica Nature Park and chosen based on previous geochemical surveys of area. The ground beetles were sampled by pitfall trapping (using meat as attractant) during the summer (July) in 2002. Specimens were analysed on species level because many authors suggest that for metal accumulation species-specific differences are more important than

trophic position. Ground beetles were dry ashed and the soil was extracted with nitric acid. Elements were analysed by atomic absorption spectrometry. Results revealed that two locations were different in lead and cadmium concentrations in extracted soil and ground beetles. Lead concentration in extracted soil was 47.9 µg/g dry weight in the first (S1) site and 34.5 µg/g dry weight in the second (S2) site. Respective cadmium values were 0.47 and 0.31 µg/g. Lead concentrations in ground beetles were 0.601 and 0.317 µg/g dry weight, and cadmium 1.684 and 1.458 µg/g dry weight. These initial results showed that ground beetles were sensitive to soil contaminants such as lead and cadmium. Concentration of xenobiotic metals cannot be regulated efficiently like nutritional ones. Although determined by some authors as "deconcetrators" (detoxification of already assimilated toxic chemicals), higher levels of cadmium and lead are detected in ground beetles at the site with higher level of toxic metals.

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## UTJECAJ INDOLNIH SPOJEVA NA DETOKSIFIKACIJSKI SUSTAV STANICE

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Indolni spojevi nemaju nutritivne vrijednosti, no nedavna istraživanja ukazuju da bi ovi široko rasprostranjeni biljni spojevi mogli imati kemoprotektivno djelovanje. Prijašnjim istraživanjima pokazali smo da indol-3-karbinol inhibira policikličke aromatske amine prije njihove modifikacije u elektrofilni oblik. Također smo pokazali da indol-3-karbinol ne može aktivirati mehanizme popravka DNA nakon što je takvo oštećenje već nastalo. U ovom radu odredili smo najviše netoksične koncentracije tri indolna spoja (indol-3-karbinol, 3-indolil acetonitril, indol-3-karboaldehid) na četiri humane stanične linije: humane stanice raka grkljana, humane stanice raka grkljana križno otporne na citostatike, humane stanice raka grlića maternice i humane stanice raka grlića maternice križno otporne na citostatike. U tu svrhu korištena je metoda po Mickischu. Istražen je utjecaj najviših netoksičnih koncentracija ova tri spoja na staničnu razinu glutationa (spektrofotometrijska metoda po Habigu) i na aktivnost glutation-S-transferaza (spektrofotometrijska metoda po Tietzeu). Uočeno je da indolni spojevi imaju značajan utjecaj na razinu staničnog glutationa i na aktivnost glutation-S-transferaza. Nakon tretmana roditeljskih stanica s indolnim spojevima došlo je do značajnog povećanja koncentracije glutationa i aktivnosti glutation-S-transferaza. Za razliku od toga, kod križno otpornih stanica dolazi do smanjenja bazalne koncentracije glutationa i aktivnosti glutation-S-transferaza u usporedbi s kontrolom.

## INFLUENCE OF INDOLE COMPOUNDS ON THE CELL DETOXIFICATION SYSTEM

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Although indoles are non-nutritive dietary compounds, recent data indicate that these widely spread plant constituents have potential to act as chemoprotective agents. Previously, we have shown that indole-3-carbinol inhibited polycyclic aromatic amines prior to their modification to electrophilic form. Also, we have shown that indole-3-carbinol can not activate mechanisms responsible for DNA repair, after DNA damage has already occurred. In this work we have determined the highest nontoxic doses of three indole compounds (indole-3-carbinol, 3-indolylacetonitrile and indole-3-carboxaldehyde) on four human cell lines: human laryngeal cancer cells, human laryngeal cancer cells cross-resistant to cytostatics, human cervix cancer cells and human cervix cancer cells which are cross-resistant to cytostatics. Method described by Mickisch was used for this purpose. The highest nontoxic doses of these three compounds were investigated for their ability to influence cellular level of glutathione (GSH); spectrophotometric method described by Habig and glutathione-S-transferases (GSTs) activity; spectrophotometric method described by Tietze. It was noticed that indole compounds have significant influence on cellular level of glutathione and glutathione-S-transferases activity. Parental cell lines had elevated both GSH and GSTs activity after treatment with indole compounds, while basal level of GSH and GSTs in cross-resistant sublines was decreased compared to the control.

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## PROCJENA TOKSIČNOSTI BLEOMICINA I VINKRISTINA NA HUMANIM LIMFOCITIMA PERIFERNE KRVI PRIMJENOM KOMET TESTA I CITOGENETIČKIH TEHNIKA

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Toksični učinci bleomicina i vinkristina istraženi su na humanim limfocitima perifene krvi nakon primjene obaju lijekova u njihovim terapijskim koncentracijama. Kao osjetljivi biomarkeri izabrani su komet test u alkalnoj izvedbi, analiza strukturnih aberacija kromosoma, mikronukleus test i test izmjena sestrinskih kromatida. Humani limfociti su u $G_0$ fazi izloženi 24-satnom djelovanju citotoksičnih lijekova u

uvjetima in vitro. Komet test proveden je neposredno nakon tretmana te 24 i 48 sati nakon tretmana. Tretirani uzorci krvi također su uvedeni u kulturu in vitro prema standardnim protokolima za analizu strukturnih aberacija kromosoma, mikronukleus test i analizu izmjena sestrinskih kromatida. Rezultati komet testa pokazuju značajni porast vrijednosti oba parametra komet testa u svim tretiranim uzorcima krvi, te povećani broj apoptoza u usporedbi s kontrolom. Dobiveni rezultati potvrđuju izrazito klastogeno djelovanje bleomicina na kromosome limfocita. S druge strane, vinkristin izaziva snažne inhibitorne učinke na diobeno vreteno tretiranih limfocita. Rezultati komet testa i svih citogenetičkih testova ukazuju da oba lijeka sinergistički oštećuju genom limfocita i narušavaju njihovu mitozu u uvjetima in vitro. Na osnovi dobivenih rezultata može se zaključiti da navedeni citostatici u terapijskim koncentracijama izazivaju značajan porast razine oštećenja DNA u somatskim ne-tumorskim stanicama. Stoga se radi smanjivanja citogenetičkih rizika povezanih s mogućnošću pojave sekundarnih karcinoma u pacijenata liječenih primjenom kemoterapije preporuča provođenje neke vrste citogenetičkog nadzora.

## ASSESSMENT OF BLEOMYCIN AND VINCRISTINE TOXICITY ON HUMAN LYMPHOCYTES USING ALKALINE COMET ASSAY AND CYTOGENETIC TECHNIQUES

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Toxic effects of bleomycin and vincristine are assessed on human peripheral blood lymphocytes following administration of both drugs in their therapeutic concentrations. As sensitive biomarkers the alkaline comet assay, analysis of structural chromosome aberrations, micronucleus assay and sister chromatid exchange analysis were selected. In vitro treatment of human lymphocytes with both antineoplastic drugs was performed on $G_0$-cells and lasted for 24 hours. Comet assay was performed immediately after treatment as well as 24 h and 48 h after treatment. Treated samples of whole blood were also cultivated in vitro for the analysis of structural chromosomal aberrations, micronucleus assay and SCE analysis. The results on comet assay on treated samples showed significant increases of both comet parameters and increased number of apoptosis compared to control. The data obtained suggest that bleomycin acts predominantly clastogenic on lymphocyte chromosomes. On the other hand, vincristine exhibits strong and predominant spindle disturbing effects on treated lymphocytes. The results of comet assay and all cytogenetic endpoints confirmed that both drugs synergistically damaged lymphocyte genome as well disrupted lymphocyte mitosis in vitro. Based on the results obtained it could be concluded that antineoplastic drugs in their therapeutic concentrations cause significant increases of DNA damage in somatic non-

cancer cells. Therefore, to minimise cytogenetic risks related to development of secondary cancers, in chemotherapy-treated patients some kind of cytogenetic biomonitiring should be recommended.

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## PROCJENA OŠTEĆENJA DNA U LEUKOCITIMA PERIFERNE KRVI POPULACIJA PROFESIONALNO IZLOŽENIH FIZIKALNIM MUTAGENIMA

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Komet test u alkalnoj izvedbi izabran je kao biomarker izloženosti u procjeni rizika proizašlih iz profesionalne izloženosti različitim fizikalnim mutagenima. Razina primarnih oštećenja DNA utvrđivana je mjerenjem stupnja migracije DNA u leukocitima periferne krvi izloženih i kontrolnih osoba. Parameteri komet testa istraženi su u 50 medicinskih djelatnika profesionalno izloženih ionizirajućem zračenju, 20 medicinskih djelatnika zaposlenih na odjelima nuklearne medicine, 20 liječnika koji rade s ultrazvučnim uređajima te 10 radarskih tehničara svakodnevno izloženih mikrovalnom zračenju. Kako bi se utvrdila osnovna razina primarnih oštećenja DNA u općoj populaciji, u istraživanje je uključeno i 50 zdravih osoba oba spola. Dobiveni rezultati pokazuju da profesionalna izloženost fizikalnim mutagenima izaziva značajno povećanje razina primarnih oštećenja DNA u leukocitima periferne krvi u usporedbi s kontrolnom populacijom. Navika pušenja također je dodatni čimbenik koji u nekih profesionalno izloženih ispitanika utječe na porast stupnja oštećenja DNA u usporedbi s izloženim nepušačima. S druge strane, dob i spol nisu značajno utjecali na razine primarnih oštećenja DNA u većine ispitanika, kako izložene, tako i kontrolne skupine. Rezultati istraživanja potvrđuju primjenjivost komet testa u alkalnoj izvedbi kao moguće nadopune standardnim biodozimetrijskim metodama. Detekcijom primarnih oštećenja DNA i/ili popravka molekule DNA ovaj vrlo osjetljiv test odražava razine oštećenja DNA prisutne u leukocitima periferne krvi u trenutku uzorkovanja.

## ASSESSMENT OF DNA DAMAGE IN PERIPHERAL BLOOD LEUKOCYTES AFTER OCCUPATIONAL EXPOSURE TO PHYSICAL MUTAGENS

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The alkaline comet assay was selected as a biomarker of exposure to evaluate the ongoing occupational exposure to different physical mutagens. The primary

DNA damage was evaluated by measuring the extent of DNA migration in peripheral blood leukocytes. Parameters of comet assay were studied in 50 medical workers occupationally exposed to ionizing radiation, 20 medical workers employed in nuclear medicine department, 20 physicians working with ultrasound and 10 radar facility workers daily exposed to microwave radiation. To evaluate the baseline DNA damage in unexposed population, 50 healthy control subjects of both genders were also selected. The results showed that occupational exposure to physical mutagens induced highly significant increase in levels of DNA damage in peripheral blood leukocytes compared to the control. Smoking habit was also found to be confounding factor in some occupationally exposed subjects who had significantly elevated levels of primary DNA damage compared to exposed non-smokers. On the other hand, the influences of age and gender on the levels of DNA damage could be excluded in the majority of exposed and control subjects. The results obtained confirmed the usefulness of the alkaline comet assay as additional complement to standard biodosimetric methods. By detection of momentary DNA damage and/or repair activity, it reflects the actual levels of DNA damage present in peripheral blood leukocytes at the moment of blood sampling.

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## UČINAK PRENATALNOG IZLAGANJA ATRAZINU I DEETILATRAZINU NA RAZVOJ I PONAŠANJE ŠTAKORA

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Istraživani su učinci herbicida atrazina i njegovog metabolita deetilatrazina na ženke i potomstvo štakora soja Fischer. Trudnim ženkama Grupe A (A) dnevno je injicirana dvadeset i jedna doza atrazina 16,6 mg/kg. s.c. u 0,1 ml parafinskog ulja. Grupa Deetilatrazin (DA) dobivala je na isti način 16,6 mg/kg deetilatrazina. Kontrolna grupa primala je parafinsko ulje (KU), dok je druga kontrola grupa bila intakna (KI). Tijekom trudnoće ženke su normalno napredovale. Učinak na razvoj potomstva ovisio je o vrsti zamjenske postnatalne majke, tj. intaktna kontrola (I) ili tretirana ženka (II). Prenatalni tretman nije utjecao na tjelesnu težinu potomaka kod rođenja. Opažen je porast tjelesne težine postnatalno od 7.-90. dana u grupama A i DA vs. KU, KI $p<0.05$-$0.01$ (I). Naprotiv, drugog dana života ženke grupe A bile su najlaganije vs. KU $p<0.01$ (II). Broj aktivnih izbjegavanja tijekom ekstinkcije u tamnoj kutiji bio je manji u 94. dana starih ženki grupe A vs. KU $p<0.05$, dok je u grupama DA i KU vs. KI bilo obrnuto $p<0.05$ (I). Motorna koordinacija i fizička izdržljivost na

obrtnom kotaču kao i spontana aktivnost u kavezu bila je jednaka u svim grupama (I). Međutim, 91. dan stare ženke grupe A bile su najizdržljivije na kotaču vs. DA, KU $p<0.05$, dok su najviši stupanj spontane aktivnosti postigli 90. dana mužjaci grupe A vs. DA, KU $p<0.01$ i ženke grupe DA vs. A, KU $p<0.01$ (II). Rezultati su pokazali da prenatalno izlaganje s-triazinima djelomice interferira s postnatalnim porastom tjelesne težine i ponašanjem. To ukazuje da je prenatalni tretman imao trajni latentni neurotoksični učinak.

## DEVELOPMENTAL AND BEHAVIORAL EFFECTS OF PRENATAL ATRAZINE AND DEETHYLATRAZINE EXPOSURE ON RATS

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The effects of the herbicide atrazine as well as of its metabolite deethylatrazine were investigated on the Fischer rat females and on their offspring. Twenty one doses of atrazine 16.6 mg/kg, s.c. in 0.1 ml of paraffin oil were injected daily to the pregnant females in Group A (A). Deethylatrazine Group (DA) received in the same way 16.6 mg/kg of deethylatrazine. Control group applied paraffin oil only (CO) while Contr intact group (CI) was left undisturbed. Rat females grew up with normal body weight gain throughout the pregnancy. The effect on the offspring development depended of the characteristics of the postnatal foster mothers, i.e. the intact controls (I) or treated females (II). Prenatal treatments did not have an effect on offspring body weight at birth, although elevated body weight gain during postnatal period from day 7-90 was observed in Groups A and DA vs. CO, CI $p<0.05$-0.01 (I). However, at the 2$^{nd}$ postnatal day females of Group A were the lightest vs. CO $p<0.01$ (II). The extinction trials of the conditioned escape responses in the shuttle box were facilitated in 94-day old females in Group A vs. CO $p<0.05$, while the higher avoidance scores in both DA and CO vs. CI was $p<0.05$ (I). Motor coordination and physical strength in rotaroad treadmill as well as spontaneous activity in the activity cage were the same in all groups (I). However, 91-day old females of Group A had the best performance in the treadmill vs. DA, CO $p<0.05$, while the highest spontaneous activity showed on the 90$^{th}$ day males of Group A vs. DA, CO $p<0.01$, and females of Group DA vs. A, CO $p<0.01$ (II). In conclusion the results revealed prenatal exposure to s-triazines partly interfere with postnatal weight gain as on behaviour indicating that prenatal treatment had the persistent latent neurotoxic effect.

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## PROCJENA GENOTOKSIČNOSTI ATRAZINA U STANICAMA RAZLIČITIH ORGANA MIŠA POMOĆU KOMET TEHNIKE

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Triazinski herbicidi jedni su od najčešće upotrebljavanih herbicida u svijetu. Među njima najviše se koristi atrazin. Prvi puta je proizveden 1957. godine u J. R. Geigy S. A. u Švicarskoj. Atrazin se koristi za suzbijanje širokolisnog korova i jednogodišnjih trava prije i nakon klijanja, a u samoj biljci djeluje inhibirajući fotosintezu. Epidemiološke studije provedene na populacijama profesionalno izloženim djelovanju atrazina pokazale su povećanu učestalost oboljevanja od tumora ovarija i Non-Hodgkinovog limfoma. U ovom radu korištena je komet tehnika u alkalnim uvjetima u svrhu procjene genotoksičnosti atrazina u limfocitima i stanicama različitih organa miša (jetra, bubreg, slezena, koštana srž). Atrazin je miševima injiciran intraperitonealno, jednokratno, u koncentraciji od 540 mg/kg. 24 sata nakon injiciranja, miševi su žrtvovani, a limfociti i stanice jetre, bubrega, slezene i koštane srži obrađene su komet tehnikom. U svim stanicama tretiranih miševa utvrđen je statistički značajan porast migracije DNA u odnosu na kontrolu. Najveće oštećenje pokazivale su DNA jetre i bubrega. Na taj način, primjenom komet tehnike na stanicama različitih organa moguće je procijeniti gentoksičnost pojedinih pesticida u uvjetima *in vivo* te dobiti više informacija o specifičnosti njihovog mutagenog djelovanja na pojedine organe.

## ATRAZINE GENOTOXICITY EVALUATION IN MULTIPLE MOUSE ORGANS BY COMET ASSAY

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Triazine herbicides constitute one of the largest groups of herbicides sold in the world. Among them, for many years atrazine has been the one most used. It was developed and introduced in 1957 by J.R. Geigy S.A. in Switzerland for pre-emergence and post-emergence control of annual grasses and broadleaf weeds. Atrazine is a member of chlorotriazines and inhibits plant photosynthesis. In the epidemiological studies atrazine was found to be able of increasing the incidence of ovary tumor and non-Hodgkin lymphoma in workers employed in its production and application. In this study we applied the comet assay on blood and 4 mice organs (kidney, liver, bone marrow,

blood and spleen) to evaluate possible genome damage caused by atrazine containig pesticide formulation. Five male CBA mice were assigned to 1 treatment group and control group. Atrazine containing pesticide was injected intraperitoneally once at the concentration of 540 mg/kg. Mice were sacrificed 24 hours after treatment. Alkaline comet assay on the blood samples, kidney, liver, bone marrow and spleen was performed. Statistically significant ($p < 0.01$) increase of tail length for all 5 tissues examined in mice treated with atrazine compared to the control was found. DNA of kidney and liver showed largest increase in migration. In conclusion, by using alkaline comet assay on different tissue samples genotoxic effect of pesticide in vivo can be detected and more knowledge on specificity of its mutagenic action for different organs could be gained.

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## UČINAK KVERCETAGETIN-3'-METILETERA NA RAZINU INTRACELULARNOG KALCIJA U NEURONIMA ŠTAKORA

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Kvercetagetin-3'-metileter glukozid (3,4',5,6,7-pentahidroksi-3'-metoksiflavon-7-O-b-D-gluko-piranozid), je prvi flavonoid izoliran iz hrvatske endemične vrste *Centaurea rupestris* L. U našim prijašnjim istraživanjima utvrdili smo protuvirusne, protubakterijske te protugljivične učinke ovog spoja. Istraživanja provedena u okviru ovog rada pokazala su značajan učinak kvercetagetin-3'-metiletera, kao i njemu strukturno srodnog kvercetina na razinu intracelularnog kalcija u primarnoj embrionalnoj kulturi kortikalnih neurona štakora. Kvercetagetin-3'-metileter uzrokovao je jači influks kalcijevih iona u neurone u usporedbi s kvercetinom. Spomenuti učinak ne povećava se proporcionalno sa koncentracijama istraživanih flavonoida. Navedeni rezultati u skladu su s istraživanjima grupe finskih znanstvenika na $GH_4C_1$ stanicama hipofize štakora o učincima nekih flavonoida, među kojima i izoramnetina i kvercetina, na razinu intracelularnog kalcija. S obzirom na značajne strukturne podudarnosti kvercetagetin-3'-metiletera i izoramnetina, moguće je pretpostaviti da se njihovi učinci temelje na istom mehanizmu koji je za izoramnetin već ustanovila spomenuta grupa znanstvenika. Tako se može pretpostaviti da kvercetagetin-3'-metileter uzrokuje povećanje razine cAMP koji posredstvom protein kinaze A uzrokuje otvaranje naponom reguliranih kalcijevih kanala.

## EFFECT OF QUERCETAGETIN-3'-METHYLETHER ON THE LEVEL OF INTRACELLULAR CALCIUM IN RAT NEURONS

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Quercetagetin-3'-methylether glucoside (3,4',5,6,7-pentahydroxi-3'-methoxy-flavone-7-O-b-D-gluko-pyranoside) is the first flavonoid isolated from the Croatian endemic plant *Centaurea rupestris* L. In our previous investigations we established its antiviral, antibacterial and antifungal activities. In present study significant effect of quercetagetin-3'-methylether and structurally related quercetin, on the intracellular calcium level of primary cortical rat neurons was established. The effect of quercetagetin-3'-methylether was stronger in comparison with quercetin. Also, it was shown that the increase in effect was not proportional with applied concentrations of investigated flavonoids. Our results are in accordance with the results recently published by group from Finland regarding the effects of several flavonoids on the level of intracellular calcium in $GH_4C_1$ cells of rat hypophysis. The mechanism of action of isorhamnetin was also established by the mentioned group. Based on the closely related chemical structure of isorhamnetin and quercetagetin-3'-methylether the same mechanism of their action on the level of intracellular calcium could be supposed. Therefore, it is reasonable to assume that quercetagetin-3'-methylether, like isorhamnetin, induces increase in cellular cAMP which, through the action of protein kinase A, causes the opening of voltage regulated calcium channels.

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## TOKSIČNI UČINAK *N*-SULFONIL DERIVATA URACILA KOD C57BL/6 Zgr MIŠEVA

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Toksični učinak ispitan je kod tri novosintetiziranih *N*-sulfonil-derivata pirimidinske nukleobaze uracila i to kod 5-brom-1-(*p*-toluensulfonil)uracila, 5-brom-1-(metansulfonil)uracila i 5-jod-1-(*p*-toluensulfonil)uracila koji su u uvjetima *in vitro* pokazali citotoksični učinak na nekim staničnim linijama humanih karcinoma, ali nisu bili ispitani in vivo. Toksični učinak navedenih supstanci ispitivan je na miševima soja C57BL/6 Zgr. Miševi su bili podijeljeni u veći broj skupina koji su primili različite doze ispitivanih

supstanci. Tijekom cijelog pokusa životinje smo promatrali kako bi uočili i najranije simptome toksičnog učinka i uginuća. Vrijeme promatranja iznosilo je i do 30 dana u svrhu procjene radi li se o akutnoj toksičnosti ili kasnom toksičnom djelovanju. Dobiveni rezultati ukazuju na izrazito jako toksično djelovanje 5-brom-1-(metansulfonil)uracila u odnosu na ostala dva ispitivana derivata. $LD_{50}$ za 5-brom-1-(metansulfonil)uracil iznosila je 150 mg/kg tjelesne težine. Ostala dva derivata koja su na svojoj nukleobazi uracilu imala supstituirana dva različita halogena elementa (Br i J), ali istu sulfonilnu skupinu, toluensulfonil ili tosil pokazivali su puno manju toksičnost. Kod primjene ovih derivata niti kod doze od 3000 mg/kg nije došlo do uginuća miševa.

## TOXIC EFFECTS OF *N*-SULFONIL DERIVATIVES OF URACIL ON C57BL/6 Zgr MICE

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The aim of this study was to examine toxic effects of three newly produced *N*-sulfonil derivatives of uracil: 5-brom-1-(*p*-toluensulfonyl)uracil, 5-brom-1-(metansulfonyl)uracil and 5-jod-1-(*p*-toluensulfonyl)uracil. These derivatives showed cytotoxic effects in cell cultures of some human carcinomas, but have not been examined in vivo. Toxic effects of these derivatives were examined in C57BL/6 Zgr mice. All mice were divided into groups that received different doses of examined derivatives. During all the time of the study animals were observed so that we could monitor first symptoms of toxic effects and death. Monitoring time was up to 30 days which enabled us to evaluate acute or chronic toxicity. The results show that 5-brom-1-(metansulfonyl)uracil has stronger toxic effects than other two derivatives. $LD_{50}$ for 5-brom-1-(metansulfonyl)uracil was 150 mg/kg of body weight. However, 5-brom-1-(*p*-toluensulfonyl)uracil and 5-jod-1-(*p*-toluensulfonyl)uracil, that have Br or J and tosil group (toluenlsulfonyl) as substitutes, were less toxic than 5-brom-1-(metansulfonyl)uracil. By administration of 5-brom-1-(*p*-toluensulfonyl)uracil and 5-jod-1-(*p*-toluensulfonyl)uracil in dose of 3000 mg/kg there were no cases of dying.

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## BIOLOŠKI UČINCI ŽIVINOG KLORIDA

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Živa je toksični element koji se nakon ulaska u organizam apsorbira i odlaže u najvećoj mjeri u bubrezima. Cilj ovog rada bio je procijeniti doza-učinak žive

mjerenjem genotoksičnih učinaka nakon tretmana štakora različitim koncentracijama živinog klorida. Skupina od tridesetdvije ženke štakora starosti 7 tjedana podijeljena je u četiri grupe od po osam životinja. Tri grupe primale su kroz pet dana oralno živin klorid u koncentracijama od 0.068, 0.136 i 0.272 mg/kg tjelesne težine. Četvrta grupa služila je kao kontrola. Životinje su žrtvovane tri dana nakon zadnje doze živinog klorida. Uzorci krvi analizirani su komet testom i mikronukleus testom uz supravitalno bojenje acridine orange-om. Opterećenje živom mjereno je u jetri i bubrezima atomskom apsorpcijskom spektrometrijom. Rezultati pokazuju značajan porast koncentracije žive u bubrezima nakon dvije više doze živinog klorida, dok je koncentracija u jetri značajno povišena samo nakon tretmana najvišom dozom živinog klorida. Rezultati komet testa i učestalost mikronukleusa značajno su povišeni pri svim koncentracijama živinog klorida. Učestalost mikronukleusa u linearnoj je ovisnosti s dozom živinog klorida. Iz dobivenih rezultata može se zaključiti da su primijenjene koncentracije živinog klorida rezultirale mjerljivim genotoksičnim učinkom.

## BIOLOGICAL EFFECTS OF MERCURY CHLORIDE

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Mercury is toxic element, which after inhalation or ingestion absorbs and deposits mainly in the kidney. Present study was performed to evaluate dose-response relationship by measuring genotoxic effects after different doses of mercury chloride given to rats. Thirty-two female rats, aged 7 weeks, were divided into four groups of eight animals each. Three groups received different doses of mercury chloride orally for five consecutive days. The respective daily doses were 0.068, 0.136 and 0.272 mg $HgCl_2$/kg b.w. The fourth group served as control. Three days after the last mercury chloride dose animals were sacrified. Blood samples were analyzed by comet assay and supravitally acridine orange stained micronucleus test. Liver and kidneys were dissected and mercury measured by vapour generation atomic absorption spectrometry. The results show significant increase in mercury concentration in kidney after two higher doses of mercury chloride, while liver mercury burden showed significant increase only after the highest dose. Tail length, tail moment and micronucleus frequency in mercury treated rats showed significantly higher values compared to control at all three concentrations of mercury chloride. Micronucleus frequency was linearly correlated to the dose of mercury given to rats. It can be concluded that the used concentrations of mercury chloride produce measurable genotoxic effect.

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## PRISUTNOST TEŠKIH METALA U BAZGI (*Sambucus nigra* L.)

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U razdoblju od proljeća 2001. do ljeta 2002. godine sakupljeni su uzorci tla i bazge na jedanaest lokacija sjeverozapadne Hrvatske. Četiri uzorka sakupljena su u Zagrebu, po dva u Gorskom Kotaru i Karlovcu, te po jedan u Kutini, Modrušu i Selcu. Bazga je izabrana kao bioindikator onečišćenja i sakupljeni su njezini cvatovi i plodovi. Uzorci su obrađivani metodom spektrometrije atomske apsorpcije i u njima su mjerene masene koncentracije teških metala (olova, kadmija, mangana i talija). Na temelju dobivenih rezultata procijenjen je utjecaj izvora emisije onečišćenja (prometnice, poplavna rijeka i tvornica umjetnih gnojiva) na okolna područja.

Nakon obrade podataka dokazano je da su područja uz izvore onečišćenja pod direktnim njihovim utjecajem, što se očituje u povećanim koncentracijama teških metala u tlu i u biljnim dijelovima. Kao osobito velik izvor onečišćenja pokazale su se velike prometnice u Zagrebu pokraj kojih je tlo više degradirano nego u ostalim dijelovima Hrvatske. Usporedbom rezultata s dopuštenim vrijednostima izvršena je klasifikacija tla na čisto tlo, tlo povećane onečišćenosti, tlo velike onečišćenosti i onečišćeno tlo. Dokazana je i povezanost između onečišćenja u tlu i u bazgi u kojoj su koncentracije teških metala bile povišene, ali još uvijek ispod zakonom propisanih graničnih vrijednosti. Koncentracije veće od dopuštenih izmjerene su samo u Zagrebu uz veće prometnice.

## THE PRESENCE OF HEAVY METALS IN ELDER (*Sambucus nigra* L.)

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The samples of soil and elder were taken from eleven locations in the northwest part of Croatia in the period from spring of 2001 to autumn of 2002. Out of eleven, four were taken in Zagreb, two in Gorski Kotar, two in Karlovac and one in Kutina, Modruš and Selce. Elder was chosen as bioindicator of pollution and her inflorescence and berries were collected. Concentrations of heavy metals (lead, cadmium, manganese and thallium) were measured by the method of spectrometry of atomic absorption. The influence of sources of pollution (roads, flooding river and industry of fertilizers) on surrounding area was determined based on acquired results. After data processing it was established that surrounding area was directly influenced by sources of pollution, which was connected with higher concentrations of heavy metals in soil and plant parts. It is shown that the motorways in Zagreb are extremely high sources of pollution, and that the soil around them is more contaminated than in other parts of

Croatia. The results were compared with permissible concentrations and based on the fact that soil was classified as clean soil, soil of enlarged contamination, soil of large contamination and contaminated soil. The connection between contamination in soil and elder was also proven. The concentrations of heavy metals in elder were enlarged but still according to law beneath permissible concentrations. Concentrations higher than permitted were measured only on the motorways in Zagreb.

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## THE DYNAMICS OF THE HEAVY METALS IN THE PLANT *Plantago lanceolata* IN THE SARAJEVO-FOJNICA REGION

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High increase of the mobile polluting sources has caused the human environment of the Sarajevo-Fojnica region and larger area of Bosnia and Herzegovina as well, to become contaminated with variety of toxic substances, and among them, heavy metals are lately prevailing. This was the reason for examination of the heavy metals concentrations (Cadmium, Zinc and Lead) in the species *Plantago lanceolata,* separately for the overground part of the plant (leaf, stem, bloom), and separately for the underground part (rhizoma with root). Also, the heavy metals concentrations were examined in the soil from where the samples were taken. The examined plant species *Plantago lanceolata* had the increased values of the heavy metals concentrations, particularly of Lead, at the experimental sites situated in the immediate vicinity of the traffic arteries. Values of the heavy metals concentrations at the control sites that were situated around 1000 meters away from the experimental sites ranged within the limits of the acceptable values. The dynamics and content of the heavy metals in the examined species has also depended on the heavy metals concentrations in the soil (positive correlation), on the vegetative part of the plant, as well as on the vegetative season. Chemical analysis of the plant material and soil was conducted according to the method of the Atomic Absorption Spectrophotometer.

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## BIOKEMIJSKE I ANATOMSKE PROMJENE U IGLICAMA SMREKE IZLOŽENIM URBANOM ONEČIŠĆENJU

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Istražen je utjecaj pojačanog onečišćenja u urbanoj sredini, uzrokovan pretežno prometom, na ovosezonske iglice obične smreke (*Picea abies* L.

Karst.). U skladu s podacima o razini onečišćenja, dobivenim iz Hrvatskog zavoda za javno zdravstvo, formirane su dvije kategorije uzoraka iglica: više i manje izložene iglice. Gvajacilne peroksidaze iz apoplasta upotrebljene su kao molekularni biljezi stresa. Viša peroksidazna aktivnost izmjerena je u više izloženim iglicama (0,006 $ÄA_{470}min^{-1}mg^{-1}$ svježe tvari) u usporedbi s manje izloženim iglicama (0,003 $ÄA_{470}min^{-1}mg^{-1}$ svježe tvari). Elektroforetska analiza pokazala je ekspresiju dodatnog izoenzima u uzorcima više izloženih iglica, što bi se moglo pripisati aktiviranju detoksifikacijskih mehanizama. Anatomska istraživanja također su pokazala značajne promjene. Najizraženija značajka bila je nekroza mezofila u više izloženim iglicama, najčešće povezana s pučima. Distorzija sitastih cijevi bila je prisutna u istim uzorcima iglica, što ukazuje na mogući poremećaj u mineralnoj ishrani. Dobiveni rezultati pokazuju strukturne i biokemijske promjene iglica koje su više izložene onečišćenju. Bez obzira na opisane promijene, stabla smreke uspijevaju preživjeti i u okolišu s višom razinom onečišćenja.

## BIOCHEMICAL AND ANATOMICAL CHANGES IN SPRUCE NEEDLES EXPOSED TO URBAN POLLUTION

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The effect of an extensive urban pollution, caused mainly by road traffic, on some biochemical and structural characteristics of current-year Norway spruce (*Picea abies* L. Karst.) needles was investigated. Two categories of needle samples were formed according to the data about the pollution levels obtained from the Croatian National Institute of Public Health: more and less affected. Apoplastic guaiacol peroxidases were used as the molecular stress markers. Peroxidase activity was higher in more affected needles (0.006 $ÄA_{470}min^{-1}mg^{-1}$ fresh weight) compared with the less affected ones (0.003 $ÄA_{470}min^{-1}mg^{-1}$ fresh weight). Also, the electrophoretic pattern of samples extracted from more affected needles revealed the expression of additional isozyme band, which could be attributed to the activation of detoxifying mechanisms. Anatomy of more affected needles was changed as well. The most outstanding characteristic was the necrosis of needle mesophyll, usually connected with the stomata. Also, distortions of sieve cells were present in the same needle samples indicating possible disturbances in mineral nutrition. The obtained results showed that needles of Norway spruce trees that are exposed to the higher pollution level undergoes to both structural and biochemical changes. Besides of the described changes, the investigated spruce trees are able to survive in more polluted environment as well.

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## OPORAVAK IGLICA CRNOG BORA (*Pinus nigra* ARN.) SA SMANJENJEM KONCENTRACIJA $SO_2$ U ZRAKU – STUDIJA UTJECAJA TERMOELEKTRANE PLOMIN (HRVATSKA)

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Utjecaj $SO_2$ koji izbacuje termoelektrana Plomin (Hrvatska) prati se već petnaestak godina mjerenjem S-koncentracija u iglicama crnog bora na različitim udaljenostima od elektrane. Od 1999. godine emisija $SO_2$ značajno je smanjena, ali do očekivanog smanjenja koncentracija S u iglicama nije došlo iste i sljedeće godine (2000.). Cilj ovog pokusa, izvedenog 2001. godine, bio je odrediti vrijeme potrebno za oporavak iglica crnog bora u smislu S-koncentracija. Obavljenim kemijskim i biokemijskim analizama dobivene su koncentracije N, P, K, Ca, Mg i S, postotak suhe tvari, ukupni proteini i sadržaj fotosintetskih pigmenata. Iako su se S-koncentracije smanjile u usporedbi s vrijednostima iz proteklih godina, utvrđene su i dalje povišene koncentracije u ovogodišnjim iglicama, a u prošlogodišnjim iglicama koncentracije koje odgovaraju niskoj imisiji sumpora. Koncentracije K, Ca i Mg su dobre do optimalne dok je sadržaj P na svim plohama nizak. Sadržaj proteina i pigmenata kao i postotak suhe tvari potvrdili su da fiziološki status iglica nije poremećen. Možemo zaključiti da je snižavanje S-koncentracija započelo oko dvije godine nakon pada koncentracija $SO_2$. Mineralnu ishranu ne možemo smatrati glavnim razlogom sporog smanjenja S-koncentracija u iglicama. Izmjereni biokemijski parametri potvrđuju nepostojanje značajnih razlika u fiziološkom statusu iglica crnog bora na različitim udaljenostima od termolektrane Plomin.

## RECOVERY OF BLACK PINE (*Pinus nigra* ARN.) NEEDLES IN RESPONSE TO THE LOWERING OF $SO_2$ IN THE AIR – A CASE STUDY OF THERMAL POWER PLANT PLOMIN (CROATIA)

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The impact of $SO_2$ emitted from the thermal power plant Plomin (Croatia) was monitored for about fifteen years by measuring the S-concentration in the needles of Black pine grown at different distances from the power plant. Since

1999 the emission of $SO_2$ was significantly lowered, but expected decrease of S-concentrations in needles was not observed in the same and the next (2000) year. The aim of present study, conducted in 2001, was to determine the time needed for the recovery of Black pine needles regarding the S-concentration. The performed chemical and biochemical analysis included the measurement of N, P, K, Ca, Mg and S concentrations, percentage of dry weight, total soluble proteins and photosynthetic pigments content. Although S-concentrations decreased in comparison to the values reported for previous years, they were still elevated in current-year needles, while in previous-year needles S- concentrations belong to low level class. Values for K, Ca and Mg were adequate to optimal, while the P-content for all sample plots was low. The results of soluble proteins and photosynthetic pigments content, as well as percentage of dry weight further confirmed that physiological status of investigated needles was not disturbed. It can be concluded that decreasement of S-concentracion in Black pine needles started about two years after decreasement of $SO_2$ concentrations. The mineral nutrition can nto be considered as the main reason for slow lowerin rate of S-concentration in needles. The mineral nutrition can not be considered as the main reason for slow lowering rate of S-concentrations in needles. Measured biochemical parameters confirmed that there were no significant differences in physiological status of black pine needles at various distances from the Plomin thermal power plant.

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## BIOSYNTHESIS AND BIODEGRADATION OF AFLATOXINS ON GREEN COFFEE BEANS CONTAMINATED WITH AFLATOXIGENOUS MOULD

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The influence of selected cultivation parameters on the growth of the aflatoxigenous mould *Aspergillus flavus* ATCC 26949, and the biosynthesis of aflatoxin $B_1$ and $G_1$ ($AFB_1$ and $AFG_1$) was investigated. Green coffee beans – regular and decaffeinated – served as substrate. Experiments were carried out in stationary culture, at temperatures from 20-40°C and water content in the substrate from 15-40%. The amount of biomass was estimated by measuring the chitin content, and the aflatoxin concentration by means of a fluorodensitometer. It was found that caffeine inhibits the synthesis of both investigated toxins whereas it had practically no influence on biomass growth. After 28 days of cultivation at 30° the concentration of $AFB_1$ was reduced by 15-22%, and at 40°C by 18-28%, relatively to the highest values obtained in the course of biosynthesis. Under the same growth conditions the concentration of $AFG_1$ was reduced by 12-25% at 30°C, and by 20-35% at 40°C.

KONZERVACIJSKA BIOLOGIJA, ZAŠTITA PRIRODE I OKOLIŠA I ZAKONODAVSTVO
*BIOLOGIA CONSERVATIONIS, PROTECTIO NATURAE ET LEGISLATIO*
CONSERVATION BIOLOGY, CONSERVATION OF NATURE, ENVIRONMENTAL PROTECTION AND LEGISLATION

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## KAKO VALORIZIRAMO I KORISTIMO BOGATSTVO FLORE I VEGETACIJE U HRVATSKOJ?

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Područje Hrvatske, usprkos maloj površini od 56.000 km, prostire se kroz nekoliko fitogeografskih regija, provincija i sektora: mediteranska (eumediteranska i submediteranska), sjevernoameričko-eurosibirska (ilirska, pontsko-panonska, srednjoeuropska), te lokalno i alpsko-nordijska regija. Današnji ekološki uvjeti kao i oni u geološkoj prošlosti, napose u glacijalno i postglacijalno doba, razlog su velike bioraznolikosti ovoga područja. Flora Hrvatske broji više od 4000 biljnih vrsta, dok se vegetacija sastoji od nekoliko stotina asocijacija. Zahvaljujući sadašnjim kriterijima za evaluaciju flornih istraživanja, ostaje činjenica da je oko 80% flornih podataka vrlo staro i, s obzirom na današnje metode i kriterije, često neupotrebljivo. U tom smislu Hrvatska je (uz BiH) jedina europska država koja nema svoje tiskane flore. No ovaj prilog ne raspravlja o tome problemu već o praktičnom korištenju bioraznolikosti u Hrvatskoj? Čini se da bi taj segment bio najkorisniji u turizmu. No jeli to tako? Ne (uz vrlo rijetke suprotne primjere). Imamo li mi u turizmu školovane animatore za taj segment? Ne (uz vrlo rijetke iznimke). Nasuprot tome, puni smo primjera da ilegalni sakupljači biljaka, bez obzira na zakonsku regulativu, odnose u inozemstvo velike količine materijala, bilo kao ljekovite biljke, bilo kao primjerke za strane herbarske zbirke. Pitanje je kako možemo riješiti ovu situaciju s obzirom na zaštitu prirode ali i ekonomske efekte u turizmu.

## HOW DO WE EVALUATE AND USE FLORA AND VEGETATION DIVERSITY IN CROATIA?

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Croatian territory, in spite of the small area of 56.000 km, spreads over several phytogeographical regions, provinces and sectors: Mediterranean (Eu- and Submediterranean), Northamerican-Eurosiberian (Illyric, Ponto-Pannonic, Middleeuropean) and touches the Alpine-Nordic region as well. Recent ecological conditions and those in the past, especially in glacial and postglacial age, caused the huge biodiversity of the area. Flora of Croatia number more than 4000 plant species, while vegetation consists of few hundred plant associations. Due to very doubtful scientific evaluation of flora investigations, about 80% of floristic data are very old and according to new methods and criteria they are not compatible

with some new investigations. The result is that Croatia is, together with BiH, the unique European country without printed Flora. But the main question of this contribution is not the scientific evaluation but practical use of our biodiversity. The most prospective use seems to be in tourism. But, do we offer it to visitors? No (with only rare exceptions). Do we have educated animators? Definitely not (with only rare exceptions). On the contrary, we do have many examples of illegal plant collecting, either as medicinal plants or as specimens for foreign herbariums. It is a question how we can manage this situation for the sake of both nature protection as well as of financial effect.

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## MOŽEMO LI OČUVATI RAZNOLIKOST FAUNE U HRVATSKOJ?

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Pregledom stupnja poznavanja taksonomije, rasprostranjenosti, ekologije, populacione genetike i ugroženosti pretežno kralježnjaka, te nekih očito ugroženih staništa, pokušava se odgovoriti na pitanje o uspješnosti zaštite raznolikosti faune u Hrvatskoj. Kritički se raspravlja o usvojenoj strategiji zaštite bioraznolikosti i njenoj dosadašnjoj provedbi. Angažiranje države u zaštiti isključivo preko utvrđivanja popisa ugroženih vrsta, pokušaja brige za neke izdvojene vrste, te uspostavljanja stvarne zaštite u postojećim zaštićenim područjima neće značajno pridonjeti zaustavljanju uočenog trenda gubitka bioraznolikosti. Glavni nedostaci su u nedovoljno znanstveno odabranim prioritetima, izostanku uspostavljanja mehanizma stručne kontrole rada i planiranja, izostanku ozbiljnijeg pristupa ugradnje zaštite bioraznolikosti u prostorno planiranje, te izostanku ugradnje mjera zaštite u planove upravljanja i praksu poljoprivrede, šumarstva i zaštite voda, izostanku osiguravanja stalnog prihoda za zaštitu prirode. Pristup očuvanju raznolikosti faune mora se postaviti na sasvimdruge temelje ako to stvarno želimo, a ne samo deklarativno prihvaćamo.

## CAN WE MAINTAIN FAUNAL DIVERSITY IN CROATIA?

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The achievement in conservation of faunal diversity in Croatia is analyzed on the example of taxonomy, distribution, ecology and population genetics of verte-

brates, and some threatened habitats. The existing strategy of biodiversity conservation and its implementation is critically discussed. The State engagement restricted only on a legal protection of species, compiling of red lists, limited efforts focused on conservation of some species and establishing protected areas will unlikely prevent the trend of biodiversity loss. The lack of scientifically based priority settings, mechanisms of expert control, implementation of biodiversity protection into physical planning and management plans, agriculture, forestry and water protection, as well as insufficient funding, are the main problems in conservation of biodiversity in Croatia. Instead of a declarative acceptance of protection of faunal diversity, a new approach should be urgently adopted in order to increase the efficiency of conservation.

IL-22

## UGROŽAVAJU LI «GENETIČKI MODIFICIRANE» KULTURE BIORAZNOLIKOST?

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Utjecaj poljoprivrednih GM kultura na bioraznolikost izrazito je složeno područje istraživanja. Zbog toga je u procjeni rizika važno postavljati prava pitanja. Budući da poljoprivredna praksa sama po sebi predstavlja jedan od najvećih ekoloških problema, jer se prirodni ekosustavi pretvaraju u oranice, interesantno polazno pitanje moglo bi biti da li GM kulture ugrožavaju okoliš više ili manje u odnosu na konvencionalne kulture. U tom slučaju procjena rizika bi se temeljila na uspoređivanju svake pojedine GM sorte s najsličnijim konvencionalnim sortama. Na taj se način provodi procjena učinka na okoliš u svim zemljama u kojima se biljke oplemenjuju genetičkim inženjerstvom, prije nego se dopusti istraživanje na pokusnim poljima i komercijalni uzgoj svake pojedine sorte. Budući da neke GM sorte sadrže ista ili vrlo slična svojstva nekim tradicionalnim sortama, iskustva stečena uzgojem takvih tradicionalnih sorti olakšavaju procjenu potencijalnog rizika pojedinih GM sorti. U cjelokupnoj složenosti međudjelovanja poljoprivrednih kultura i okoliša, križanje između kultiviranih sorti i divljih srodnika ističe se kao jedan od najznačajnijih potencijalno negativnih učinaka na bioraznolikost. Križanje između određene poljoprivredne kulture i divljeg srodnika moguće je ako se staništa tih biljaka preklapaju, ako cvjetaju u isto vrijeme i ako su spolno kompatibilni. Za 12 od 13 najvažnijih svjetskih poljoprivrednih kultura utvrđeno je da se mogu križati barem s jednom divljom vrstom barem u jednom dijelu područja uzgoja. Posljedice prijenosa novih gena iz poljoprivrednih kultura (tradicionalnih i GM) u divlje vrste ovisit će o svojstvima koja određuju ti geni, te o biologiji i ekologije pojedine divlje vrste. Neka svojstva, koja se nastoje postići u poljoprivrednih kultura stotinama godina (npr. otpornost na bolesti i nametnike, te snošljivost različitih abiotičkih činitelja), mogu povećati prilagodbene sposobnosti divljih vrsta. Zbog toga bi u

okviru procjene rizika trebalo istražiti dobivaju li križanci novim genima (svojstvima) prednosti koje bi im omogućile povećanje brojnosti. Budući da je mogućnost križanja između pojedine poljoprivredne kulture i divljih srodnika zemljopisno uvjetovana, u tom bi kontekstu i zakonska regulativa trebala obuhvatiti procjenu svake pojedine kulture (tradicionalne i GM) imajući u vidu zemljopisno područje u kojem se pojedina kultura uzgaja.

## DO GENETICALLY MODIFIED (GM) CROPS AFFECT BIODIVERSITY?

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The impact of GM crops on biodiversity is a complex and complicated issue. Therefore, only when we ask the right questions we might be able to reach an informed judgement on the risk and benefits of GM plants. Since a major threat to biodiversity is habitat loss due to conversion of natural ecosystems to agricultural land, a valuable question would be whether GM crops pose threats to biodiversity that are qualitatively and/or quantitatively different from conventional crops. Assessing the influence of GM crops on biodiversity should then involve a comparison of the perceived benefits and potential threats of such crops to the conventional crops they are intended to replace. The assessment of the environmental impact of GM crops is a fundamental part of the international regulatory process undertaken before GM crops can be grown under field conditions experimentally or commercially. Since some of the modifications made by GM plant breeding have direct parallels in conventional plant breeding, experience of the impact of conventional crop varieties on biodiversity is highly instructive in regard to the interpretation of the impact of GM crops. Hybridisation between GM plants and their wild relatives has emerged as one of the primary risks associated with the commercialisation of GM crop plants. In order for crop genes to be transferred to wild populations via hybridisation, crop plants and their wild relatives need to occur sympatrically, overlap in flowering time and be sexually compatible. It has been demonstrated that 12 of the world's 13 most important food crops hybridise with at least one wild relative in at least part of their range of cultivation. The consequences of the transfer of novel genes from GM or conventional crops to wild relatives depend on the nature of the novel genes and the biology and ecology of the recipient wild species. Some traits that are the target of genetic manipulation for hundreds of years – such as disease or insect resistance and tolerance of various abiotic stresses – may confer a strong fitness advantage in the wild. Therefore, research on the risks associated with hybridisation should focus on the fitness effects of the genes (traits) in question. In the regulatory process, all classes of crops should be assessed on a case-by-case basis in the context of the geographical locations where they are to be grown.

OP-80

## TAKSONOMIJA I ELEMENTI AKCIJSKOG PLANA ZAŠTITE HRVATSKIH POPULACIJA PRIMORSKE I KRŠKE GUŠTERICE (Reptilia, Lacertidae)

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Na osnovi rezultata sustavnih filogeografskih istraživanja primorske gušterice (*Podarcis sicula*) i krške gušterice (*P. melisellensis*) temeljenih na analizi mitohondrijskih DNK biljega, raspravlja se o mogućem raspletu neophodne taksonomske revizije. Uz dvije iznimke, utvrđena je mala genetička varijabilnost između populacija antropohorne primorske gušterice, dok je kod krške gušterice utvrđena alopatrička izolacija i diferencijacija na dva zasebna udaljenija otočja u odnosu na populacije na obali s bližim otocima. Iako rezultati istraživanja mitohondrijskih biljega nisu ključ za taksonomiju unutar vrsta, broj od 27 dosad na hrvatskom dijelu areala opisanih podvrsta primorske gušterice prijeći će potpuno u sinonimiju ili će se svesti na najviše jednu ili dvije podvrste, a u krške gušterice od 20 na samo tri podvrste (*P. m. melliselensis, P. m. fiumana, P. melisellensis* ssp. n). Navedena istraživanja su u velikoj mjeri riješila nedoumice oko potreba zaštite intraspecijskog biodiverziteta ove dvije vrste. Osigurala su znanstvenu podlogu za pokretanje zaštite dvije endemične otočne podvrste krške gušterice, te potrebu očuvanja reliktnih populacija *P. sicula campestris* na otocima Mala Palagruža i Sušac, kao i u historijsko doba doseljene populacije *P. sicula sicula* kod Dubrovnika. Predlažu se elementi akcijskog plana zaštite za sve svojte i grupe populacija.

## TAXONOMY AND ELEMENTS OF ACTION PLAN FOR PROTECTION OF CROATIAN POPULATIONS OF ITALIAN AND DALMATIAN WALL LIZARDS (Reptilia, Lacertidae)

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On the basis of the results of systematic phylogeographic research of Italian wall lizard (*Podarcis sicula*) and Dalmatian wall lizard (*P. melisellensis*), founded on the analysis of mtDNA markers, there has been a discussion on the possible outcome of a necessary taxonomic revision. Apart from two exceptions, a minor genetic variability has been established between populations of antropochore Italian wall lizard, while in case of Dalmatian wall lizard

on two separate distant archipelago allopatric isolation and differentiation has been established as compared to populations on the coast and associate islands. Although mtDNA investigations are, of course, not the key for intraspecific classification of species, on the Croatian part of distribution of Italian wall lizard the number of 27 described subspecies will entirely transfer into synonymy or will be reduced to one or two valid subspecies. In case of karst lizard it will be reduced from 20 to only three subspecies (*P. m. melliselensis*, *P. m. fiumana, P. melisellensis* ssp. n.,). The mentioned researches have to a great extent solved the dilemma over the need to protect the intraspecific biodiversity of these two species. They have insured a scientific basis for launching the protection only of two endemic insular subspecies of karst lizard, and they have stressed the need for preservation of relict populations of *P. sicula campestris* on the islands of Mala Palagruža and Sušac, as well as of population of *P. sicula sicula* which in ancient times was introduced to the area of Dubrovnik city. We propose the elements of action plan of protection for all threatened taxa and populations.

OP-81

## EKOLOŠKI STATUS AKUMULACIJA JEZERO I PONIKVE NA OTOKU KRKU: SADAŠNJE STANJE I DUGOTRAJNE PROMJENE

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Istraživanje makrozoobentosa u plitkim (srednja dubina oko 2,5 m) akumulacijama Jezero (0,37 $km^2$) i Ponikve (0,75 $km^2$) na najvećem (409,9 $km^2$) jadranskom otoku Krku (45° 10' N, 14° 33' E) provedeno je tijekom 2000. i 2001. godine kao dio biološko-ekoloških istraživanja podobnosti ovih akumulacija za vodoopskrbu. Uzorkovanje je obavljano jednom mjesečno od studenog 2000. do listopada 2001. godine. Kvantitativni uzorci makrozoobentosa sabirani su grabilom tipa Ekman (zahvatne površine 0,01 $m^2$) na pet postaja u akumulaciji Jezero (s 0,5-11 m dubine) i na četiri postaje u akumulaciji Ponikve (s 0,5-6 m dubine). Mjereni su i fizikalno-kemijski parametri (koncentracija, zasićenje i biološka potrošnja kisika; temperatura; pH; ukupno otopljene soli; elektroprovodljivost; količina nitrata, nitrita, amonijaka, ortofosfata, aluminija i klorofila *a*) te prozirnost vode. Usporedbom s rezultatima prethodnih istraživanja (iz 1980-ih i 1990-ih) uočene su neke promjene u akumulacijama. Zamjetno je postupno povećanje količine nutrijenata i ukupne produkcije. Uočene su i značajne razlike u sastavu i brojnosti zajednica makrozoobentosa i podvodne makrovegetacije između istraživanih akumulacija. Nekad raznolika i brojna (šest svojti 1980-ih) podvodna makrovegetacija potpuno je nestala u

akumulaciji Jezero. Raznolikost i srednja godišnja gustoća makrozoobentosa mnogo su manje u akumulaciji Jezero (26 svojti, 563 jedinke $m^{-2}$) nego u akumulaciji Ponikve (53 svojte, 9347 jedinki $m^{-2}$). Uočene promjene i razlike između istraživanih akumulacija mogle bi biti rezultat prirodnih eutrofikacijskih procesa (akumulacija Jezero mnogo je starija) i prijelaza plitkog jezera iz stanja bistre vode s bujnom podvodnom vegetacijom u stanje mutne vode bez vegetacije. Dodatan uzrok, mogla bi biti i dugogodišnja visoka koncentracija aluminija u vodi i sedimentu akumulacije Jezero, kao dokaz utjecaja čovjeka na akumulaciju od 1970-ih do danas.

## ECOLOGICAL STATUS OF THE LAKE JEZERO AND THE LAKE PONIKVE ON KRK ISLAND, CROATIA: PRESENT OVERVIEW AND LONG-TERM CHANGES

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Investigation of the macrozoobenthos in two shallow (mean depth approx. 2.5 m) Mediterranean lakes, Jezero (0.37 $km^2$) and Ponikve (0.75 $km^2$), was done during 2000 and 2001 as a part of biological-ecological study of their suitability for water supply on Krk Island (45° 10' N, 14° 33' E, 409.9 $km^2$). Sampling was done monthly from November 2000 to October 2001. Quantitative samples of macrozoobenthos were collected at five stations in Lake Jezero (from 0.5-11 m depth) and at four stations in Lake Ponikve (from 0.5-6 m depth) using an Ekman grab (sampling area 0.01 $m^2$). Physico-chemical parameters (dissolved oxygen, oxygen saturation, biological oxygen demand, temperature, pH, total dissolved solids, conductivity, nitrate, nitrite, ammonium, orthophosphate, aluminium and chlorophyll *a*) and transparency were also measured. Comparing to previous investigations (from 1980s and 1990s) some changes, like gradual increase of dissolved nutrients and total production, were noticed in both lakes. Considerable differences between Lake Jezero and Lake Ponikve were noticed in the community structure of macrozoobenthos and submerged vegetation. Once diverse and lush (six taxa in 1980s), submerged vegetation is recently totally absent in Lake Jezero. Macrozoobenthos diversity and mean annual abundance are recently much lower in Lake Jezero (26 taxa, 563 ind. $m^{-2}$) than in Lake Ponikve (53 taxa, 9347 ind. $m^{-2}$). Natural eutrophication processes (Lake Jezero is much older) in the shallow lake and its shift from vegetation-dominated clear water state to turbid non-vegetated state could be the reason for differences between investigated lakes. An additional reason could be the long-lasting high water and sediment concentration of aluminium in Lake Jezero, as evidence of anthropoid influence from 1970s to the present.

OP-82

## USPOSTAVA NACIONALNE EKOLOŠKE MREŽE

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Uspostavljanje ekološke mreže jedna je od najvažnijih obveza u zaštiti prirode europskih zemalja koje su članice ili su u postupku pristupanja Europskoj uniji. Ovim konceptom osigurava se zaštita najvrijednijih prirodnih predjela te mreža manjih važnih staništa i njihova međusobna povezanost prirodnim koridorima. Ministarstvo zaštite okoliša i prostornog uređenja u suradnji s tvrtkom OIKON d.o.o. i uz sudjelovanje Europskog centra za zaštitu prirode iz Tilburga, Nizozemska, provodi u razdoblju od prosinca 2002. do lipnja 2005.godine projekt *Uspostava nacionalne ekološke mreže (CRO-NEN)*, kojega sufinancira Europska komisija iz fonda LIFE-Treće zemlje. Cilj projekta je uspostaviti nacionalnu ekološku mrežu kao dio Sveeuropske ekološke mreže i EU programa NATURA 2000 uz istovremeno razvijanje sustava prikladno obučenih suradnika u prikupljanju podataka o biološkoj raznolikosti. Očekivani rezultat je nacionalna ekološka mreža s GIS orijentiranom bazom podataka njezinih područja te uspostava *Nacionalnog sustava monitoringa biološke raznolikosti.* Primjenjuje se međunarodno propisana metodologija. Najprije se prikupljaju podaci o odabranim ugroženim vrstama i staništima. Kartiranjem i analizom njihove rasprostranjenosti utvrđuju se područja važna na europskoj i nacionalnoj razini koja sadrže najveći broj ugroženih vrsta i staništa te čine jezgre ekološke mreže. Analiziranjem njihovog prostornog rasporeda i mogućnosti međusobne komunikacije njihova živog svijeta, kreira se sustav koridora i zaštitnih zona. Također se analizira postojeći i predlaže potrebni status zaštite pojedinih područja. Paralelno s izradom karte ekološke mreže, popunjava se odgovarajuća baza podataka o njezinim područjima. Važan dio projekta je razrada mehanizama uključivanja različitih skupina suradnika, a posebice nevladinih udruga i škola, u prikupljanje podataka o biološkoj raznolikosti.

## BUILDING-UP THE NATIONAL ECOLOGICAL NETWORK

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Building-up the ecological network is one of the most important nature protection obligations of European countries that are EU members or are in the process of

EU accession. This concept ensures that the most valuable natural areas as well as the network of smaller important sites are protected and interconnected by natural corridors. Ministry of Environmental Protection and Physical Planning in collaboration with the firm OIKON Ltd. and with participation of the European Centre for Nature Conservation, Tilburg, Netherlands, is implementing the project co-funded by European Commission from the LIFE-Third Countries fund *Building-up of national ecological network (CRO-NEN)* in the period from December 2002-June 2005. The project's objective is to set-up the national ecological network as a part of the Pan-European Ecological Network and the EU NATURA 2000 Programme, together with developing a system of adequately trained collaborators in biodiversity data collecting. Expected results are the national ecological network with GIS-oriented database of network's sites as well as establishment of the *National Biodiversity Monitoring System*. The methodology used is internationally well defined. Firstly the data about chosen threatened species and habitats are being collected. Through mapping and analysing their distribution sites are being identified that are important on European and national level, comprising the highest number of threatened species and habitats and representing core areas of the ecological network. By studying their spatial distribution and needs for inter-communication of their species, a system of corridors and buffer zones is being created. Also analysis of current and needed conservation status of individual sites is being performed. Parallel with mapping, a database related to sites is being filled-in. An important part of the project is developing mechanisms to include different groups of collaborators, especially NGO's and schools, into systematic biodiversity data collecting.

OP-83

## PETROFILNA ORNITOFAUNA KANJONA VELIKE PAKLENICE

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Nacionalni park Paklenica smješten je u sjevernoj Dalamciji i u južnom dijelu Velebita. Proteže se na površini od 96 km$^2$. Ornitološki najzanimljiviji dijelovi parka su kanjoni Velike i Male Paklenice, u kojima obitava iznimno bogata stijenjačka fauna ptica. Istraživanje petrofilne ornitofaune u kanjonu Velike Paklenice obavljeno je u travnju, svibnju i lipnju 1999. i 2000. godine metodom transekta. Zabilježeno je ukupno 14 petrofilnih ptičjih vrsta, od kojih je najbrojnija vrsta bila modrokos (*Monticola solitarius*). U ovom razdoblju zabilježeno je i nestajanje bjeloglavog supa (*Gyps fulvus*) s tog područja. Ovim radom nastoji se prikazati utjecaj posjetitelja, šetača i penjača na brojnost gnijezdarica stjenjačkog tipa ornitofaune. Pokazalo se da je taj utjecaj mnogo veći na ptice grabljivice, npr. bjeloglavog supa

(*Gyps fulvus*), sivog sokola (*Falco peregrinus*), sove, primjerice sovu ušaru (*Bubo bubo*) i gavrana (*Corvus corax*). Većina vrsta pjevica nije pokazivala promjene u brojnosti parova. Iz rezultata ovog rada zaključujemo da je osjetljivim vrstama ptica potrebno osigurati pravovremenu i adekvatnu zaštitu.

## PETROPHILIC ORNITHOFAUNA OF THE VELIKA PAKLENICA CANYON

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NP Paklenica is situated in the north part of Dalmatia and in south part of Velebit Mountain. The size of Paklenica NP is 96 km$^2$. Ornithologicaly most interesting areas are canyons of Velika and Mala Paklenica with very rich petrophilic ornithofauna. The investigation of the petrophilic ornithofauna in the Velika Paklenica canyon was done in April, May and June of 1999 and 2000 by transect method. Total 14 petrophilic bird species were recorded, from which the most abundant was Blue Rock Thrush (*Monticola solitarius*). During this period, the Griffon Vulture (*Gyps fulvus*) disappeared from this area. In this work we attempted to show the impact of visitors, walkers and climbers, especially climbing activities on the breeding of this type of ornithofauna. This impact is bigger on birds of prey, ex. Griffon Vulture (*Gyps fulvus*), Peregrine Falcon (*Falco peregrinus*), owls, like Eagle Owl (*Bubo bubo*) and Raven *(Corvus corax*). The majority of song birds didn't show changes in the number of pairs. From the results of this work we conclude that the sensitive species need adequate protection.

OP-84

## RAZNOLIKOST PODZEMNIH KOPNENIH JEDNAKONOŽNIH RAKOVA U HRVATSKOJ

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Terestrički jednakonošci se u Hrvatskoj istražuju od druge polovice 19. stoljeća. Prikupili smo literaturne podatke i podatke iz vlastitih istraživanja. Regionalnim razmatranjem podataka za Hrvatsku, do 2001. godine utvrđeno je 300 speleoloških

objekata s podzemnim kopnenim jednakonožnim rakovima. Provedeno istraživanje rezultiralo je s 45 novih lokaliteta. U Hrvatskoj je do sada zabilježeno 115 vrsta i 31 podvrsta kopnenih jednakonožnih rakova, od čega je 29 vrsta i 8 podvsta kavernikolno. Među kopnenim jednakonožnim racima utvrđene su 74 endemične svojte za Hrvatske, a od kavernikolnoh taksona 18 endema Hrvatske i 7 endema Dinarida. Rasprostranjenost je prikazana UTM kartom s kvadrantima 10×10 km. Utvrđen je udio podzemnih, endemičnih, trogofilnih i troglobiontnih jednakonožnih rakova u ukupnom broju kopnenih vrsta jednakonožnih rakova. Prikazani su položaji tipskih lokaliteta (utvrđen je 21 tipski lokalitet) te centri endemizma u Hrvatskoj, kao i rasprostranjenost rijetkih i ugroženih vrsta. Usporedba faune Hrvatske sa susjednim zemljama dinaridskog krša pokazuje da Hrvatska ima najveći broj podzemnih svojti kopnenih Isopoda. Fauna ove skupine je potpuno neistražena u nekoliko regija Hrvatske. Buduća istraživanja ekologije, taksonomije i rasprostranjenosti podzemnih kopnenih Isopoda je neophodno zbog adekvatne zaštite jedinstvenog krškog podzemja.

## THE ANNOTATED CHECKLIST OF SUBTERRANEAN TERRESTRIAL ISOPODS IN CROATIA

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Terrestrial isopods in Croatia have been researched since the second half of the 19th century. We gathered data from literature and those from our own research. Until 2001, 300 speleological localities with subterranean terrestrial isopods were recorded in a regional survey conducted in Croatia. Presently executed research made in the area resulted in 45 new localities. In Croatia 115 species and 31 subspecies of terrestrial isopods have been recorded up to date, out of which 29 species and 8 subspecies in 3 families and 13 genera are cavernicolous. Among terrestrial isopods in Croatia there are 74 endemic taxa to Croatia. Of these, 18 cavernicolous taxa are endemic to Croatia and 7 cavernicolous taxa are endemic to Dinarids. In this paper we analyse spatial distribution of the subterranean terrestrial isopods through UTM grid map of Croatia (10 x 10 km). A percentage of subterranean, endemic, troglophilous and troglobitic species are calculated out of total number of terrestrial isopod species. Both the type localities (21 type localities are recorded so far) and centres of endemism are mapped, as well as distribution of rare and endangered species. The comparison of Croatian subterranean terrestrial isopods fauna with the fauna of dinaric karst of neighbouring countries shows that Croatian karst is the richest in subterranean terrestrial isopods taxa. The fauna of this group is still completely unexplored in several regions in Croatia. The future research of the ecology, taxonomy and spatial distribution of this group in Croatia is vital in terms of conservation of the unique karstic underground.

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## ODNOS SPOLOVA JUVENILNIH GLAVATIH ŽELVI, *Caretta caretta*, U ISTOČNOM JADRANU I IMPLIKACIJE ZA ZAŠTITU

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Odnos spolova jedna je od najznačajnijih demografskih značajki koje utječu na održivost i zaštitu ugroženih vrsta. Kod morskih kornjača, kao i kod većine gmazova, spol nije genetski određen već ovisi o temperaturi inkubacije, a spolni dimorfizam vidljiv je isključivo kod odraslih životinja. Kako je spol nezrelih želvi moguće ustanoviti jedino indirektno, podaci o odnosima spolova glavate želve (*Caretta caretta*) u Sredozemlju vrlo su oskudni. Istraživanja na gnjezdištima ukazala su na dominaciju izleženih ženki (81,6-99%), dok podaci o odnosima spolova u morskim staništima nedostaju. Morfološkom i histološkom analizom gonada utvrdili smo spol 33 juvenilne glavate želve. Vizualnim pregledom gonada pri sekcijama utvrđen je odnos spolova 1:1, dok je histološka analiza rezultirala malom prevagom u broju mužjaka, ali na značajno različitom od odnosa 1:1 ($\div^2$ = 2,00, p = 1,157). Ti su rezultati suprotni utvrđenoj dominaciji izleženih ženki na gnjezdištima, ali potvrđuju rezultate iz središnjeg Mediterana, podupirući teoriju o imigracijama juvenilnih mužjaka iz sjeverozapadno-atlantskih populacija u Sredozemlje. Kako oko 50% pelagičkih nezrelih želvi u Sredozemlju potiče iz SZ Atlantika, imigracije juvenilnih mužjaka u morskim staništima smanjuju dominaciju nezrelih ženki iz regionalnih populacija. Odnos spolova u našem istraživanju ukazuje na imigracije atlantskih mužjaka i u Jadransko more, određujući ga kao zajedničko stanište juvenilnih glavatih želvi iz Sredozemne i SZ-atlantske populacije. Kako nezreli mužjaci glavate želve migriraju dalje od svojih reproduktivnih staništa nego ženke, a s obzirom na veću reproduktivnu vrijednost ženki, ljudske djelatnosti, kao npr. ribarstvo, imat će veći utjecaj na populacije s bližih gnjezdišta. Stoga visok slučajni ulov želvi u ribolovne alate u Jadranu ima veći negativni utjecaj na regionalne populacije glavate želve.

## SEX RATIOS OF JUVENILE LOGGERHEAD SEA TURTLES, *Caretta caretta*, IN THE EASTERN ADRIATIC SEA AND IMPLICATIONS FOR CONSERVATION

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Sex ratio is among the most important demographic parameters that significantly affect the viability and conservation of endangered species. As with most reptiles, the sex of sea turtles is environmentally determined by temperature. Sea turtles exhibit sexual dimorphism only as adults; hence diagnosing the sex of hatchlings and juveniles request employment of different techniques. Data on sex ratios of the loggerhead turtle (*Caretta caretta*) in the Mediterranean are scarce. A few studies on the nesting beaches suggested female-biased hatchling production (81.6-99%), while data for marine habitats are lacking. Therefore, we examined sexes of 33 juvenile loggerheads by morphological and histological analysis of gonads. Visual examination of gonads resulted in a 1:1 ratio, while histology gave a slightly male-biased sex ratio, but not significantly different from a 1:1 ratio ($\div^2 = 2.00$, $p = 1.157$). Our results are in discordance with the strong female-biased loggerhead hatchlings production on the Mediterranean nesting beaches. However, they confirm results on male-skewed sex ratios of juvenile loggerheads from central Mediterranean, supporting the theory on male-biased immigration of juveniles from north-western Atlantic populations into the Mediterranean. If we consider that about 50% of pelagic juveniles in the Mediterranean originate from NW Atlantic, most of them being males, such male-skewed immigration is likely to buffer the original female-bias of the Mediterranean juvenile populations. The sex ratio in our study suggests that the male-biased dispersal of NW Atlantic juveniles reaches the Adriatic Sea, classifying these waters as a habitat shared by juveniles belonging to both Mediterranean and NW Atlantic populations. As in juvenile loggerheads males seem to disperse farther than females, considering a higher reproductive value of females, human impact, such as fishery, would affect more populations from closer nesting sites than the distant ones. Therefore, high bycatch in Adriatic fisheries would have the most detrimental effect for juvenile loggerheads from the regional nesting grounds.

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## AKTIVNA ILI PASIVNA ZAŠTITA ŠUMA - PRIMJER NACIONALNI PARK «RISNJAK»?

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Šume su najvrjedniji obnovljivi prirodni resurs i opće su dobro iznimnog interesa za Republiku Hrvatsku. U većini zemalja svijeta ljudskom djelatnošću

značajno je promijenjen prirodni sastav šuma. Hrvatska je danas zapravo «otok» očuvanih prirodnih šuma velike biološke raznolikosti u Europi. U općem očuvanju prirode gdje je šuma najvažniji čimbenik, posebice kojih je temeljni fenomen šumski ekosustav, treba intenzivirati zaštitarsku, socijalnu i edukativnu ulogu šume te i dalje podržavati sve ekološke uloge (hidrološka, protuerozijska, vodozaštitna, klimatska, protuimisijska) primjenjujući sofisticirane postupke uzgajanja šuma. Rad ukazuje na stanje i vrijednosti nekih strukturnih elemenata koji utječu na prirodnu obnovu i opstojnost negospodarenih šuma na primjeru Nacionalnog parka «Risnjak». Primjenom digitalizacije izrađen je trodimenzionalni digitalni dinamički model i vertikalni profil sastojine s konkretnim mjerenim stablima na terenu. Model omogućuje računalno-digitalno praćenje promjena strukture sastojine, simuliranje istih i otvara nove mogućnosti u izučavanju strukture sastojina. Preveliki broj prezrelih stabala, potpuni izostanak mlađih stabala u najtanjim debljinskim stupnjevima, kao i manjak srednje debelih stabala jele, ukazuju na narušenost prirodne strukture. Prevelika zastrtost tla krošnjama od 88,46%, uz nekvalitetan i zastarčen pomladak bukve u sloju grmlja, u konačnici je rezultirao izostankom prirodne obnove. Prepustiti ove sastojine isključivo prirodnom tijeku razvoja, potpuno eliminirajući gospodarske radnje ili opreznim radnjama potpomagati pozitivne prirodne procese uzgojnim zahvatima pomlađivanja i njege sastojina? Rezultati istraživanja ukazuju kako pasivni način zaštite dugoročno šteti razvoju ovih sastojina te kako ipak treba naći pravu mjeru u smislu aktivne i pasivne zaštite šuma nacionalnih parkova. Uvjet da se šumski ekosustavi na području Nacionalnih parkova održe u ravnoteži, a da pri tom fiziološki ne oslabe, zahtijevaju adekvatno gospodarenje. Samo tako ćemo očuvati temeljnu postavku šumarstva u smislu trajne samoobnovljivost (potrajnosti, održivog razvoja) ovih sastojina.

## ACTIVE OR PASSIVE FOREST PROTECTION - EXAMPLE OF THE NATIONAL PARK "RISNJAK"

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Forests are the most valuable regenerable natural resource and represent a common wealth of exceptional interest for the Republic of Croatia. In the majority of countries of the world man's activity has considerably changed the natural forest structure. However, today Croatia is an "island" of preserved natural forests of great bio-diversity in Europe. In the general preservation of nature, in which the forest is the most important factor (with forest ecosystem

as a fundamental phenomenon), the protective, social and educational role of the forest should be intensified. Furthermore, all ecological functions (hydrological, counter-erosional, water-protective, climatic, counter-emissional) should be maintained by continuous application of sophisticated methods of silviculture. The paper indicates the state and values of some structural elements, which influence natural reforestation, and viability of non-managed forests, taking the National Park "Risnjak" as an example. By the method of digitalisation a 3-dimensional digital dynamic model and a vertical profile of a stand was realised with specific measuring trees in the field. The model enables computerised-digital monitoring of changes in the stand structure and their simulation, and opens up new possibilities in the studies of stand structure.

The excessive number of over-matured trees, absence of younger trees with the smallest diameter degrees, and the lack of medium fir trees, indicate the disruption of natural structure. Excessive cover of soil by tree-crowns, amounting to 88.46 %, together with poor and suppressed young growth of beech in the shrubs layer, resulted in the absence of natural reforestation. The dilemma is whether to leave these stands exclusively to natural developmental processes entirely eliminating management methods, or by cautious work to assist positive natural processes by applying silvicultural interventions for regeneration and tending of stands. Investigation results show that in the long run passive methods of protection harm the development of these stands, and that the right measure of active and passive protection of national park forests should be defined. The condition for maintaining balance in forest eco-systems, and at the same time avoiding their physiological weakening, requires adequate management. This is the only way of preserving the fundamental postulate of forestry with regard to permanent self-regeneration (sustainability of growth) of all stands.

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## BIORAZNOLIKOST HRVATSKIH PASMINA DOMAĆIH ŽIVOTINJA

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Bioraznolikost u poljoprivredi, jedna od vitalnih komponenti opće biološke raznolikosti, izrazito je ugrožena uslijed globalizacije i razvoja neodržive industrijske proizvodnje hrane. Mnoge pasmine domaćih životinja ne mogu izdržati ekonomsku utrku s modernim, visokoproizvodnim pasminama i hibridima, te dolazi do drastičnog smanjenja njihovih populacija, pa čak i izumiranja. Danas u svijetu 12 % svih pasmina smatramo izumrlim, dok ih je 30

% ugroženo. Pošto Hrvatska nije pošteđena ovog procesa, neke naše pasmine su izumrle, a neke su pred nestajanjem i traže hitnu akciju zaštite. U skladu s FAO kriterijima, 7 lokalno adaptiranih pasmina i 5 egzotičnih smatramo izumrlim, te se danas koristi ukupno 68 pasmina, od kojih 27 lokalno adaptiranih, 37 egzotičnih, dok su četiri u procesu nastanka (*status nascendi)*. Samo 10 lokalno adaptiranih pasmina ima zadovoljavajuću populaciju, dok ih je čak 17 ugroženo. Osim očuvanja biološke raznolikosti pasmina domaćih životinja, tradicionalni sustav uzgoja i držanja domaćih životinja utječe na razvoj i održanje krajobraza, te ukupne bioraznolikosti, kako kroz očuvanje staništa, tako i direktno kroz opstanak gljivljih, biljnih i životinjskih vrsta vezanih uz domaće životinje. Danas, u doba preispitivanja *high input, high output* tehnologije, koja u sebi nosi brojne moralne dvojbe, velika prilika pruža se za ponovno širenje domaćih pasmina. Minimalna ulaganja za optimalne rezultate; očuvanje ekoloških sustava i bioraznolikosti; trajna upotreba obnovljivih izvora uz stalni nadzor i kontrolu kvalitete, predstavljaju potencijalnu budućnost naše poljoprivrede.

## DOMESTIC ANIMAL BIODIVERSITY IN CROATIA

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Biodiversity in agriculture is a vital component of overall biodiversity, highly threatened by globalization and the spread of unsustainable industrial food production. Many breeds and varieties of domestic animals were not able to endure the economic run and have become either endangered or extinct. Today in world 12% of all breeds were extinct and additional 30% of breeds of domestic animals are endangered. As Croatia was not deprived of the process of extinction, some of breeds have become extinct and some of the remaining ones are facing extinction and it is required to take urgent actions for their preservation. According to FAO criteria, 7 locally adapted and 5 exotic breeds have been lost and today there are 68 breeds utilized in Croatian agriculture, out of which 27 are locally adapted, 37 exotic and the last 4 are breeds in *status nascendi*. Only 10 locally adapted breeds develop actively, while 17 are endangered. Beside conservation, traditional breed and keeping of domestic breeds influence on develop and wealth of general biodiversity, throw conservation of habitats and survival of fungi, plants and animals which are directly connected on domestic animal. Today, in time of high *input, high output, high output* technology which bear ethic suspicions, big chance is for reexpand of domestic breeds. Minimal input for optimal results, conservation of ecosystems and existence of biodiversity, using of sustainable resources with permanent survey and quality control, present potential future of Croatian agriculture.

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## UPOTREBA MOLEKULARNIH BILJEGA U ODREĐIVANJU VELIČINE POPULACIJE VUKOVA U GORSKOM KOTARU – PRELIMINARNO ISTRAŽIVANJE

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Poznavanje veličine populacije neophodno je u svrhu učinkovitog gospodarenja njome, posebice ukoliko se radi o ugroženoj vrsti kao što je vuk. Napretkom molekularnih metoda omogućeno je određivanje veličine populacije iz uzoraka prikupljenih neinvazivnim metodama, primjerice analizom DNA iz uzoraka izmeta. Uz upotrebu polimorfnih genetskih biljega, poput mikrosatelita, moguće je identificirati pojedine jedinke u populaciji. Svrha ovog preliminarnog istraživanja bila je odrediti broj vukova u dijelu Gorskog Kotara pomoću mikrosatelitskih biljega i procijeniti upotrebljivost metode. Tijekom 2002. i 2003. na području Gorskog kotara sakupljena su 24 uzorka izmeta i dva uzorka tkiva vuka. Uzorci su genotipizirani na 11 mikrosatelitskih lokusa. Kod oba uzorka tkiva i 22 uzorka izmeta uspješno je PCR metodom umnoženo dva do osam lokusa. Dva su uzorka imala jedinstvene genotipove, a još je pet različitih genotipova utvrđeno kod preostalih uzoraka. Ovim istraživanjem utvrđeno je postojanje najmanje sedam različitih jedinki vuka, a da bi se napravila točnija procjena potrebne su detaljnije analize. Dobiveni rezultati pokazuju da se ova metoda može koristiti u određivanju veličine i genetičke strukture populacije vuka u Hrvatskoj.

## APPLICATION OF MOLECULAR MARKERS IN ESTIMATING THE SIZE OF WOLF POPULATION IN GORSKI KOTAR – A PILOT STUDY

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Efficient management of wildlife, especially when endangered as the grey wolf *Canis lupus* in Europe, requires knowledge of its population size. Very recent ad-

vances in molecular methods now allow to estimate population size using non-invasively sampled DNA sources such as fecal samples. Individuals can be identified by using polymorphic molecular markers such as microsatellite loci. This pilot study aimed to estimate the number of individual wolves in a study site in the Gorski Kotar region by microsatellite genotyping and to evaluate the applicability of the method. Twenty-four wolf faecal samples and two tissue samples were collected in the field. Samples were genotyped using 11 microsatellite loci. Between two and eight microsatellite loci amplified successfully in both tissue samples and in 22 faecal samples. Two samples yielded unique genotypes which were not present in the other samples. Five genotypes were found more than once amongst the remaining samples. The study provided a minimum estimate of seven wolves and further analysis is required to provide a more accurate estimate. Our results demonstrate that we can use this method to estimate the size of the Croatian wolf population in the future. The data obtained can also be used in population genetic studies.

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## THE LOŠINJ DOLPHIN RESERVE – PROMOTING HABITAT PROTECTION AND SOCIO-ECONOMIC DEVELOPMENT THROUGH THE PRESENCE OF FLAGSHIP SPECIES

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The proposed Lošinj dolphin reserve (LDR) is unique in the fact that it will be both the first dedicated dolphin reserve within Europe, but outside the European Union, and the first dolphin reserve in the Mediterranean. The establishment proposal is based on research data collected by Adriatic Dolphin Project (ADP) since 1987, which identifies the Lošinj-Cres archipelago as a critical habitat for bottlenose dolphins. The main threats to this population are fishing competition and disturbance by tourist boats. The education and public awareness activities of BW have been aimed at the reduction of the main stresses the dolphins face and conservation of habitat they live. The establishment of the LDR will be the next logical step in this process. Although the primary focus of the proposal is dolphin conservation, the societal needs of the local people are regarded as an important aspect to be incorporated into the designation and management of the reserve. Support for the reserve by the local community shows how cooperation can limit conflict in areas of conservation. Raising the profile of cetaceans and their socio-economic worth in this area has shown that protection and the sustainable development of the economy can work when all groups are involved. As research in other areas of the Croatian Adriatic identifies critical habitats for other populations of flagship species the designation and management techniques developed and tested in the LDR can be used as a best practise model for the creation of similar reserves through out Croatia.

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## IDENTIFICATION OF CRITICAL FACTORS AFFECTING THE DISTRIBUTION AND HABITAT USE OF BOTTLENOSE DOLPHINS IN KVARNERIĆ, CROATIA

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The Kvarnerić zone experiences heavy human exploitation, seasonally by tourism and year-round by small-scale fisheries, increasing yearly. This study analyses dolphin distribution and habitat use with an aim to provide information for the management of possible critical factors affecting the distribution of bottlenose dolphins in this area. The distribution of sightings and dolphin habitat use were investigated between March 1995 and September 2002. GIS was used to integrate data from a total of 332 sightings with submarine slope and variability, distance from the coast, and the distance to sites affected by high intensity human use, such as trawling areas, marine petrol stations, and tourist boaters' 'highways'. These parameters were considered within cells of 1000 and 2000 $m^2$ size. Habitat types were defined by using Cluster analysis and the spatial analysis tools of ArcView 3.2. Correlation with environmental and other factors were analysed by using Generalised Linear Models (GLMs). The number of sightings varied greatly between years, without any apparent trend, except for a significant decrease, of almost 50%, in 1996. The distribution of sightings showed highly significant annual and seasonal variability. GLMs indicated a significant negative impact of the variable related to the transit of speedboats ('highway') on the presence/absence of bottlenose dolphins. Two possible prime critical factors were suggested to cause such avoidance reaction: a) increasing level of noise, and/or b) high speed moving objects, inducing an anti-predator like response.

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## UZROCI SMRTNOSTI VUKOVA U HRVATSKOJ TIJEKOM 1986-2001

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Od 1986. do 2001. zabilježena je smrt ukupno 92 siva vuka (*Canis lupus*) u Hrvatskoj: 60 (65,2%) su ustrijeljena, 18 (19,6%) je poginulo u prometnim nezgodama, za 6 (6,5%) vukova je uzrok smrti ostao nepoznat, 5 (5,4%) je imalo bjesnoću, a po jedan su ubijeni sjekirom, vilama ili od drugih vukova. Udio ženki među mrtvim vukovima je bio 56%, prosječna dob je bila 1,9 godina, a prosječna masa 31,4 kg. Posebno je analizirano posljednjih 10 godina (1986-1995) prije, te prvih 6 godina (1996-2001) nakon zakonske zaštite vrste (15. svibanj 1995.), a u

kojim je razdobljima stradalo 30 odnosno 62 vuka. Godišnja smrtnost je porasla sa 3,0 prije zaštite na 10,3 nakon zaštite (3,4 puta). Ovaj porast pokazuje da se zakon ne provodi, kao i porast neprihvaćanja vuka od lokalnog stanovništva. DIjelom je porast smrtnosti povezan i sa porastom populacije vuka, te se čini da je veličina zabilježene smrtnosti podnošljiva za populaciju. Predlaže se prihvaćanje propisa koji bi omogućavali ograničeni i kontrolirani lov vukova.

## CAUSES OF WOLF MORTALITY IN CROATIA DURING 1986-2001

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We recorded a total of 92 dead grey wolves (*Canis lupus*) in Croatia during 1986-2001: 60 (65.2%) were shot, 18 (19.6%) died in traffic accidents, the causes of death of 6 (6.5%) wolves remained unknown, 5 (5.4%) had rabies, and one each was killed by axe, by hay-fork, and by other wolves. Among dead wolves the share of females was 56%, the mean age was 1.9 years, and the mean mass of adult wolves was 31.4 kg. We analyzed separately the last 10 years (1986-1995) before, and in the first 6 years (1996-2001) after the legal protection of the species (15 May 1995), when 30 and 62 wolves died, respectively. The yearly mortality grew from 3.0 before protection to 10.3 after protection (3.4 times). This increase in mortality indicates no enforcement of legal protection, as well as the increase of the animosity of local people. It was also partly influenced by a wolf population growth and seems that the recorded mortality is sustainable. We propose to adapt the legislation to allow controlled hunting of wolves.

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## BIORAZNOLIKOST, KONZERVACIJSKA BIOLOGIJA I ZAŠTITA PRIRODE U HRVATSKOJ

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Hrvatska je među zemljama s najvećom bioraznolikošću u Europi. Ona uključuje inter- i intra-specijsku raznolikost, raznolikost ekosistema i visok stupanj

endemizma. Svjetski trend smanjenja biološke raznolikosti uzrokovan gubitkom, fragmentacijom i onečišćenjem staništa, prekomjernom eksploatacijom te unošenjem novih vrsta i bolesti doveo je do drastičnog povećanja brzine izumiranja. U Hrvatskoj su npr. samo od kralješnjaka nestali sredozemna medvjedica, *Monachus monachus*, kudravi nesit *Pelecanus crispus*, atlantska jesetra *Acipenser sturio* i drugi. Postojeći koncept zaštite prirode, zasnovan na formalnoj zakonskoj zaštiti vrsta i osnivanju zaštićenih područja pokazao se nedovoljno učinkovitim. Obzirom na brz gubitak svjetske bioraznolikosti, krajem 70-tih započelo se s novim znanstvenim pristupom u njenom očuvanju – konzervacijskom biologijom. Zasnovana na zakonitostima ekologije, konzervacijska biologija usredotočuje istraživanja i očuvanje prirode, vrednujući relativnu učinkovitost određene promjene ili mjere zaštite kroz odgovor populacije ili ekosistema. Iako disciplina u razvoju, konzervacijska biologija danas se smatra znanošću 21. stoljeća i pretpostavka je suvremenog koncepta očuvanja biološke raznolikosti. Dok je u mnogim zemljama svijeta konzervacijska biologija sastavni dio visokoškolskog obrazovanja, a njeni se rezultati praktično koriste, u Hrvatskoj to nije slučaj. To se očituje kako u dosadašnjem nedostatku konzervacijske biologije kao definiranog pojma u hrvatskom prirodoslovlju, tako i u poistovjećivanju konzervacijske biologije s ekologijom i/ili zaštitom prirode. Imajući u vidu sve veći utjecaj čovjeka na ekosisteme te ograničena sredstva i vrijeme, neophodno je uvesti konzervacijsku biologiju kao zasebnu znanstvenu disciplinu, te koristiti njena ciljana istraživanja kao i osnovu pri planiranju očuvanja bioraznolikosti Hrvatske.

## BIODIVERSITY, CONSERVATION BIOLOGY AND NATURE PROTECTION IN CROATIA

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Croatia is among European hot-spot countries with high biodiversity. This biodiversity includes intraspecific, interspecific and ecosystem diversity and high endemism rate. A rapid decrease of world biodiversity caused by loss, fragmentation and disturbance of habitats, overexploitation, and introduction of alien species and diseases recently resulted in dramatically increased extinction rates. In Croatia, just among vertebrates, the list of extinct species includes the monk seal *Monachus monachus*, Dalmatian pelican *Pelecanus crispus*, Atlantic sturgeon *Acipenser sturio* etc. The existing concept of the nature conservation based upon formal protection of species and establishing of protected areas showed to be

globally insufficient. Challenging world biodiversity loss, a new scientific approach to biodiversity conservation appeared in late 1970's – the conservation biology. Based on ecological principles, it focuses research and management efforts in conservation, evaluating the relative effectiveness of specific changes on population and ecosystem responses. Although still a developing discipline, conservation biology is considered the science of the 21st century and a prerequisite for effective conservation of nature. While in many countries worldwide the conservation biology is included in university curricula and employed in the field of biodiversity conservation, it is not the case in Croatia. This reflects in confusion of conservation biology with ecology or nature protection. Considering increased human impact on ecosystems, limited resources and the time constraint, the conservation biology should be recognized as a "new" scientific discipline in Croatia whose analysis should become a baseline in planning effective conservation of Croatian biodiversity.

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## NUŽNOST SPELEOLOŠKE STUDIJE ZA PLANIRANJE UPRAVLJANJA TURISTIČKOM SPILJOM - PRIMJER VETERNICE KRAJ ZAGREBA

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Špilja Veternica predstavlja kompleksni višeetažni speleološki objekt u neposrednoj blizini Zagreba, dužine preko 7 km, kroz koji protječe 15 vodenih tokova. Od 1979. godine zaštićena je kao geomorfološki spomenik prirode. Pregled špiljske faune izrađen je na temelju literaturnih podataka i vlastitih terenskih istraživanja. Zabilježene su dvije vrste gljiva, 18 vrsta beskralješnjaka i 13 vrsta šišmiša. Troglobiontna i stigobiontna fauna je zastupljena s 8 vrsta, a troglofilna i stigofilna s 25. Od mikoflore dolaze predstavnici skupina Basidiomycete i Ascomycete. Troglofilna fauna je zastupljena predstavnicima redova Hirudinea, Acari, Collembola, Coleoptera Orthoptera, Trichoptera, Lepidoptera i Diptera. Troglofilna dvojenoga *Schubartia lohmanderi* u Hrvatskoj je prvi puta zabilježena u Veternici. Troglobiontna i stigobiontna fauna zastupljena je predstavnicima iz skupina Gastropoda, Crustacea, Pseudoscorpiones, Aranea, Collembola, Diplura i Coleoptera. Veternica je tipsko nalazište troglobiontnog lažištipavca *Chthonius jalzici* i troglobiontnog skokuna *Pseudosinella dallaii*. Ove dvije vrste do sada su nađene samo u svom tipskom nalazištu. U špilji Veternici zabilježeno je 13 vrsta šišmiša. U najveće kolonije zimi okupljaju se *Rhinolophus ferrumequinum* i *Rh. euryale*, a ljeti *Miniopterus schreibersii* i *Rh. euryale*. To su i jedine veće kolonije šišmiša poznate za Park prirode Medvednica. Potrebno je ograničiti pristup i posjete špilji kako bi se zaštitile osjetljive i ugrožene vrste šišmiša. Predstavljen je popis faune

sa ekološkim statusom, endemizmom i stupnjem ugroženosti, kao i osnovni ekološki parametri objekta. Potrebna je zaštita cijelog sljevnog područja uz održivo turističko gospodarenje objektom.

## NECESSITY OF SPELEOLOGICAL ELABORATE FOR TOURISTIC CAVES MANAGEMENT - EXAMPLE VETERNICA CAVE NEAR ZAGREB

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Veternica Cave near Zagreb is a complex multilevel underground system more than 7 km long, with 15 subterranean waterflows. It is legally protected as Geomorphological Nature Monument since 1979. The overview of cave fauna is based on literary data as well as recent fieldwork data. It has been recorded 2 fungi belong to Ascomyceta and Basidiomyceta divisions, 18 invertebrate and 13 bat species by now. Troglobitic and stygobitic fauna is represented by 8 species and troglophilic and stygophilic fauna by 25 species. Troglophilic fauna is represented by orders Hirudinea, Acari, Collembola, Coleoptera, Orthoptera, Trichoptera, Lepidoptera and Diptera. Troglophilic diplopod, *Schubartia lohmanderi* has been recorded here for the first time in Croatia. Troglobitic and stigobitic fauna is represented by: Gastropoda, Crustacea, Pseudoscorpiones, Aranea, Collembola, Diplura and Coleoptera. The Veternica cave is the type locality for troglobitic pseudoscorpion *Chthonius jalzici* and troglobitic springtail *Pseudosinella dallaii*. These 2 species have been recorded only in the type locality so far. In the Veternica Cave 13 bat species have been recorded so far. The cave is an important hibernaculum for large colonies of *Rhinolophus ferrumequinum* and *Rh. euryale* bats. In summer it provides shelter for nurseries of both *Miniopterus schreibersii* and *Rh. euryale* bats. The cave is the most important bat locality known in the Medvednica Nature Park. Fauna list is given along with ecological status, endemism and endangerment level for all species. Described are basic cave ecological parameters. Protection of the whole drainage area along with sustainable management of the cave is necessary.

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## PREGLED TIPSKIH LOKALITETA PODZEMNE FAUNE HRVATSKE

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Pregledom tipskih lokaliteta podzemne faune Hrvatske i svojti koje su iz njih opisane zabilježeno je. 216 tipskih lokaliteta. Iz njih je opisano 340 svojti, od čega 87 svojti vodenih staništa i 253 svojte kopnenih staništa. Popis sadržava slijedeće skupine životinja s brojem svojti: Demospongia (2), Turbellaria (3), Gastropoda (46), Clitellata (2), Crustacea (66), Arachnida (97), Myriapoda (22), Insecta (101) i Osteichthyes (1). Gotovo svi organizmi predstavljaju endeme Hrvatske. Najviše, čak 13 svojti, opisano je iz špilje Šipun kod Cavtata. Među tipskim lokalitetima s najvećim brojem opisanih svojti ističu se i: Špilja Pišurka na otoku Korčuli (9), Gornja i Donja Cerovačka špilja kod Gračaca (8), Balićeva špilja (7) i Vranjača (5) kod Dugopolja, Ozaljska špilja kod Ozlja (5), Špilja Tounjčica kod Tounja (5), Jama Čampari na otoku Cresu (5), Špilja Movrica (5) i Špilja Ostaševica (5) na otoku Mljetu, izvor Ombla kod Dubrovnika (5), špilja Ledenica kod Lokvi (4), Pčelina špilja kod Buljmiza (4), Manita peć kod Starigrada (4) i Jama Ješkalovica na otoku Braču (4). Važnost ovakvog pregleda je u pripremi podloge za akciju zaštite tipskih objekata, ali i širih područja gdje su smješteni tipski lokaliteti.

## AN OVERVIEW OF TYPE LOCALITIES OF SUBTERRANEAN FAUNA IN CROATIA

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An overview of 216 type localities of subterranean fauna in Croatia was recorded. In type localities 340 taxa are listed, including 87 taxa from water habitats and 253 taxa from terrestrial habitats. The list contains the following groups: Demospongia (2 taxa), Turbellaria (3 taxa), Gastropoda (46 taxa), Clitellata (2 taxa), Crustacea (66 taxa), Arachnida (97 taxa), Myriapoda (22 taxa), Insecta (101 taxa) and Osteichthyes (1 taxon). Almost all of them are Croatian endemic taxa. The most, even 13 species are described from the cave Šipun near Cavtat. Among type localities stand out these with the highest number of described species, too: the Cave Pišurka on the island of Korčula (9), Gornja and Donja Cerovačka špilja cave near Gračac (8), Balićeva špilja cave (7) and Vranjača cave (5) near Dugopolje, Ozaljska špilja cave near Ozalj (5), the Cave Tounjčica near Tounj (5), Čampari cave on the island of Cres (5), the Cave Movrica (5) and the Cave Ostaševica (5) on the island of Mljet, the Ombla

spring near Dubrovnik (5), Ledenica cave near Lokve (4), Pčelina špilja cave near Buljmize (4), Manita peć cave near Starigrad (4) and Ješkalovica cave on the island of Brač (4). Importace of this overview is in guidance for further protection of type localities and the regions around them.

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## BIOLOŠKA I GEOLOŠKA VALORIZACIJA OBALNOG PODRUČJA I PODMORJA OTOKA ĆUTIN VELI I ĆUTIN MALI S CILJEM USPOSTAVE ZAŠTIĆENOG PODRUČJA

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Ovim programom obuhvaćena su geološka, sedimentološka i biološka istraživanja podmorja s ciljem biološke i geološke valorizacije te prijedloga uspostavljanja zaštićenog područja. Metodom direktnog opažanja uz uporabu autonomne ronilačke opreme obrađeno je 8 transekata od površine do 50 m dubine. Mjerena je količina svjetlosti, temperature, kisika, saliniteta i pH vrijednosti. U podmorju istraženog područja nalaze se tri osnovna tipa morskoga dna: golo kamenito morsko dno nepokriveno sedimentom, morsko dno prekriveno grubozrnatim pjeskovitim do šljunkovitim sedimentom i morsko dno prekriveno sitnozrnatim muljevitim sedimentom. Kamenito dno je vidljivo do dubine -8,5 m, a dublje je osnovna stijena pokrivena šljunkom i pijeskom. Sitnozrnasti sedimenti nalaze se u bazenu Kvarnerića, na dnu dubljem od 40 m. Na istraživanom području ustanovljeno je 53 vrsta makroflore (Cyanophyta 1, Rhodophyta 26, Phaeophyta 13, Chlorophyta 12, Angiospermae 1) i 195 vrsta makrofaune (Sarcodina 1, Porifera 25, Cnidaria 22, Placophora 2, Gastropoda 21, Bivalvia 17, Cephalopoda 1, Echiura 1, Sipuncula 1, Polychaeta 8, Crustacea 23, Bryozoa 9, Echinodermata 12, Tunicata 5, Pisces 46). Mediteranskim akcijskim planom, koji je dio programa Ujedinjenih naroda, ustanovljena je lista zajednica, asocijacija i facijesa te lista vrsta za odabir područja koja se uključuju u nacionalni popis prirodnih područja od interesa za zaštitu. U obalnom području otoka Ćutin Veli i Ćutin Mali ustanovljeno je 8 bentoskih biocenoza od kojih su 5 prema MAP-u kalsificirane u posebno vrijedne zajednice s 19 vrsta flore i faune. Pri uspostavljanju zaštićenog područja važno je koristiti međunarodno priznate i postavljene kategorije jer se time olakšava i na bolji način povezuje nacionalni ili međunarodni sustav zaštićenih područja istih kategorija. Izmjena informacija i iskustva često su bitna karika u provođenju zaštite, a to se osobito odnosi na zaštitu mora koje je zahtjevnije u odnosu na zaštitu kopna.

## BIOLOGICAL AND GEOLOGICAL VALORISATION OF THE COASTAL LINE AND SUBMARINE AREA OF THE ISLANDS ĆUTIN MALI I ĆUTIN VELI AIMING TO ESTABLISH A PROTECTED AREA

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The program encompasses geological, sedimentological and biological investigations of the submarine zone with the aim of biological and geological assessment, and the proposal for establishing a protected area. Eight transects, from the surface up to 50 meters in depth were investigated employing the method of direct observation and using autonomous diving equipment. The quantity of light, temperature, oxygen, water salinity and pH values were measured. In the submarine zone of the area researched there are three basic types of sea bottom: bare rocks with no sediment layer, coarse sands to gravel, and finely grained muddy sediment. Rocky bottom can be observed up to -8.5 m in depth, while base rock is covered by gravel and sand in deeper areas. Fine-grained sediments are situated in the Kvarnerić basin, on the bottom exceeding 40 m in depth. In the investigated area 53 macrofloral species (Cyanophyta 1, Rhodophyta 26, Phaeophyta 13, Chlorophyta 12, Angiospermae 1) and 195 macrofaunal species (Sarcodina 1, Porifera 25, Cnidaria 22, Placophora 2, Gastropoda 21, Bivalvia 17, Cephalopoda 1, Echiura 1, Sipuncula 1, Polychaeta 8, Crustacea 23, Bryozoa 9, Echinodermata 12, Tunicata 5, Pisces 46) were determined. Mediterranean Action Plan, which is part of the United Nations program, established a list of communities, associations and faciae, as well as a list of areas to be included in the national list of areas of interest for protection. In the coastal area of Ćutin Veli and Ćutin Mali islets 8 biocenoses were determined, 5 of which, according to the MAP are classified as especially valuable communities with 19 floral and faunal species. In the process of establishing a protected area it is important to use internationally recognized and set categories, thus improving the link between national or international systems of protected areas of same categories. The exchange of information and experience is often crucial in the actual implementation of protection, which especially refers to the protection of the sea which is more demanding then the protection of the land.

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## UTJECAJ PROMETNICE NA POPULACIJU OBIČNIH KRASTAČA (*Bufo bufo*) KOD KOPRIVNICE U VRIJEME MIGRACIJA NA MRIJEŠĆENJE

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Istraživanje je provedeno tijekom ožujka 2003. godine na prometnici u selu Gabajeva Greda, oko 22 km sjeveroistočno od Koprivnice. Istraživali smo utjecaj prometa na populaciju krastača u vrijeme migracija na mriješćenje i procijenili veličinu populacije krastača. Za istraživanje smo konstruirali dvije zapreke duljine 60 m i 33 cm visine. Zapreke smo postavili uz cestu, između prometnice i desetak metara udaljene močvare u kojoj se žabe mrijeste. Krastače su prelazile prometnicu i zaustavljale se na zapreci, te su nastavljale put uz zapreku do kanti ukopanih na kraju zapreke. Žive krastače smo sakupljali uz zapreku i u kantama, te ih prebrojavali. Također smo prebrojavali i sve krastače pregažene na prometnici nasuprot zapreke. Rezultat je bio 9% pregaženih krastača. Na toj prometnici u noćnim satima prođe manje od 5 automobila kroz sat vremena. Istovremeno smo prebrojavali sve krastače pregažene tijekom prelaska prometnice u smjeru močvare, prije nego što su se počele vraćati s mriješćenja. Iz tog broja i postotka pregaženih krastača dobivenog prvim mjerenjem procijenili smo populaciju krastača na 14 000 jedinki.

## THE ROAD INFLUENCE ON THE COMMON TOAD (*Bufo bufo*) POPULATION NEAR KOPRIVNICA DURING THE BREEDING MIGRATIONS

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Investigations were made in March 2003 on the road situated in Gabajeva greda village, 22 km NE from Koprivnica (northern Croatia). We explored influence of traffic on the common toad population during the breeding migrations, and estimated size of that population. We constructed two fences 60 m long and 33 cm high. Fences were placed between the road and the nearby oxbow lake just a few meters away, the aim of migration. Migrating common toads passed the road and then stopped at the fence, forced to continue along the fence until the bucket that was dug in the ground. We collected them in the bucket or along the fence. Then we numbered all toads that crossed the road, and the toads that have been killed on the road in the length of the fence. Result was about 9% killed common toads. Traffic on that road was under 5 cars per hour during the night. At the same time we numbered all toads killed on the road during the breeding migration, but before they came back from the pond. From this number and percent calculated by first measuring we estimated the common toad population to be about 14 000 individuals.

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## PRELIMINARNA EKOLOŠKA MREŽA – TEMELJ USPOSTAVE NACIONALNE EKOLOŠKE MREŽE

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S porastom svijesti o potrebi zajedničkih međudržavnih aktivnosti sa ciljem sačuvanja biološke raznolikosti i okoliša, raste i broj inicijativa i projekata koji objedinjuju podatke više država u jedinstvene baze podataka. Takva inicijativa proširene Višegradske skupine (Mađarska, Češka, Slovačka, Poljska + Ukrajina i Hrvatska) iz 2001 pokrenula je razvoj nacionalne ekološke mreže. Koristeći europske kriterije i metodologiju za razvoj Paneuropske ekološke mreže (PEEN) razvijena je, na temelju raspoloživih podataka, preliminarna ekološka mreža RH. Ekološka mreža sastavljena je od jezgri (područja od međunarodnog i nacionalnog značaja za zaštitu značajnih krajobraza, staništa i/ili vrsta), koridora (povezuju jezgre i smanjuju negativan učinak fragmentiranosti staništa), zaštitnih zona (štite mrežu od nepovoljnih vanjskih utjecaja) i tzv. "stepping stones" (područja od sezonskog značaja za migratorne vrste). U preliminarnu ekološku mrežu uključeno je 32% kopnenog teritorija RH i 6,5% morske površine, odnosno 22,9% ukupne površine RH. Analizirajući po pojedinačnim elementima ekološke mreže, u njenom daljnjem razvoju očekuje se porast udjela morske površine u svim elementima ekološke mreže, kao i porast zaštitnih zona u kopnenom dijelu, te promjenu udjela kopnenih koridora nakon dodatnog prikupljanja podataka i uključivanja podataka iz projekta "Karta staništa RH". Tako projektirana ekološka mreža omogućit će učinkovitiju zaštitu okoliša i organizama koji u njemu žive. Rad na uspostavi nacionalne ekološke mreže nastavljen je u sklopu LIFE - Treće zemlje projekta "CRO-NEN".

## PRELIMINARY ECOLOGICAL NETWORK – FOUNDATION FOR THE DEVELOPMENT OF NATIONAL ECOLOGICAL NETWORK

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With the increase of awareness for necessity of joined international efforts with the aim of preserving biodiversity and the environment, there is also increase in initiatives and projects that combine data from several countries into unique data-

bases. Such an Initiative from the extended "Višegrad 4" countries (Hungary, Czech Republic, Slovakia, Poland + Ukraine and Croatia) from 2001, initiated the development of Croatian ecological network. Using the criteria and methodology developed for creation of the Pan-European ecological network (PEEN), preliminary ecological network was created based on available data. The ecological network consists of international and national core areas (contains habitats, species or landscapes of importance), corridors (interconnect the core areas, diminish the problem of habitat fragmentation), buffer zones (protect the network from outer potentially damaging impacts) and stepping-stones (areas of seasonal importance for migratory species). All in all, 32% of land, and 6.5% of sea was included in the preliminary ecological network, i.e. 22.9% of overall state territory. Analysing the econet through its components, in its further development we are expecting increase of included portion in all econet elements in sea, as well as increase in terrestrial buffer zones and change of portion of terrestrial corridors upon inclusion of data on habitats. That way created econet will ensure more efficient protection of the environment and its living organisms. Further work on the development of national ecological network is ensured through the LIFE – Third countries project "CRO-NEN".

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## FAUNA PTICA GNJEZDARICA KOPAČKOG RITA I BARANJE

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Od 1853. godine do danas na području Parka prirode Kopački rit i šire u Baranji ukupno je zabilježeno gniježđenje 144 vrsta ptica. U periodu 1998.-2003. godine zabilježeno je gniježđenje 130 vrsta ptica od kojih 119 smatramo redovitim gnjezdaricama koje se gnijezde svake godine, a daljnjih 11 povremenim. Četiri nove vrste su započele s gniježđenjem nakon 1998. godine – crvenokljuni labud *Cygnus olor*, kulik slijepčić *Charadrius dubius*, crvenonoga vlastelica *Himantopus himantopus* i modronoga sabljarka *Recurvirostra avosetta*. U proteklom razdoblju 14 vrsta ptica je prestalo s gniježđenjem - ražanj turkoč *Plegadis falcinellus*, bijela žličarka *Platalea leucorodia*, jastrebac cipolaš *Pandion haliaetus*, orao zmijar *Circaetus gallicus*, orao kliktaš *Aquila pomarina*, patuljasti orao *Hieraaetus pennatus*, veliki potrk *Otis tarda*, šljuka bena *Scolopax rusticola*, šljuka kokošica *Gallinago gallinago*, crna čigra *Chlidonias nigra*, sovuljaga buljina *Bubo bubo*, sova močvarica *Asio flammeus*, zlatovrana modrulja *Coracias garrulus* i mali svračak *Lanius minor*. Nekadašnje gnjezdarice bile su vezane za tri tipa staništa: velike tršćake i močvarna staništa, vlažne livade i pašnjake, te stare šume s obiljem starih stabala s dupljama. Intenzivno šumarstvo, lovstvo, poljoprivreda i melioracije dovele su do nestanka ili degradacije ovih staništa, prestanka gniježđenja

gore navedenih vrsta, kao i opadanja brojnosti mnogih drugih današnjih gnjezdarica. Stoga je očuvanje preostalih ugroženih staništa, te restauracija nestalih staništa imperativ zaštite biološke raznolikosti ovog područja.

## BREEDING BIRD FAUNA OF KOPAČKI RIT AND BARANYA

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Since 1853, breeding of 144 bird species was recorded in the Kopački rit and Baranya region. In the period 1998-2003 we have recorded nesting of 130 bird species. We consider 119 species as regular, and further 11 as irregular breeders. Four species have started with nesting since 1998 – *Cygnus olor*, *Charadrius dubius*, *Himantopus himantopus* and *Recurvirostra avosetta*. Within the past period 14 species ceased with nesting: *Plegadis falcinellus*, *Platalea leucorodia*, *Pandion haliaetus*, *Circaetus gallicus*, *Aquila pomarina*, *Hieraaetus pennatus*, *Otis tarda*, *Scolopax rusticola*, *Gallinago gallinago*, *Chlidonias nigra*, *Bubo bubo*, *Asio flammeus*, *Coracias garrulus* and *Lanius minor*. Former nesters were typically related to three types of habitats – extensive reedbeds and marshlands, wet meadows and pastures, as well as old forests with numerous tree-holes. Intensive forestry, hunting, agriculture and improper water management resulted in the disappearance or degradation of these habitats, termination of breeding of above-mentioned species and decrease in the breeding populations of many other species. Thus, protection of remaining threatened habitats and possible restoration of lost habitats is the crucial for the biodiversity protection of this area.

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## ISTRAŽIVANJE POPULACIJE PJEGAVOG DAŽDEVNJAKA (*Salamandra salamandra* L.) NA SJEVEROZAPADNOM DIJELU KALNIKA

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Populacija pjegavog daždevnjaka, *Salamandra salamandra* (L.), istraživana je od jeseni 2001. do proljeća 2003. g. u bukovoj šumi na sjeverozapadnim obroncima Kalnika (sjeverozapadna Hrvatska). Cilj istraživanja bio je odrediti utjecaj redovitog

ljetnog presušivanja potoka Stiper na populaciju pjegavog daždevnjaka. Potok presušuje tijekom ljeta zbog korištenja glavnog izvora za vodovod. Odabrane su dvije površine uz potok Stiper veličine 1 ha (Ploha 1) i 0,5 ha (Ploha 2). Terestrički dio populacije istraživan je tijekom dana nakon kiše, a noću su u potoku uz istraživane površine prebrojavane ličinke. Istraživane površine su podijeljene na kvadrate veličine 10x10 m. Prilikom pretraživanja ploha mjerene su temperatura i relativna vlažnost zraka, a svaka ulovljena životinja je izmjerena, te joj je određen spol i položaj na plohi. Budući da je leđni uzorak svake jedinke jedinstven, one nisu označavane nego su fotografirane digitalnim fotoaparatom. Od ukupno ulovljene 431 jedinke (288 na Plohi 1 i 143 na Plohi 2), ponovo ih je ulovljeno 121. Gustoća populacije procijenjena pomoću metode označi-ponovo ulovi (model Jolly-Seber, program Ecological Methodology 5.2) iznosila je: 275-671 jed./ha na Plohi 1, 170-610 jed./ha na Plohi 2. Od ukupnog broja ulovljenih jedinki 57% su bili mužjaci, 35% ženke, a 8% juvenilne jedinke. Omjer spolova je bio 1,6. Maksimalnu brojnost ličinke su postigle u proljeće, a zbog presušivanja potoka početkom ljeta najveći broj ih je uginuo prije preobrazbe. Time objašnjavamo mali udio juvenilnih jedinki u populaciji, što bi u budućnosti moglo dovesti do njezinog smanjenja.

## THE POPULATION STUDY OF FIRE SALAMANDER (*Salamandra salamandra* L.) IN THE NORTHWESTERN PART OF THE KALNIK MOUNTAIN (NW CROATIA)

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The population of fire salamander *Salamandra salamandra* (L.) was researched from autumn 2001 to spring 2003 in a beech forest in NW part of the Kalnik Mountain (NW Croatia) The aim of this study was to establish the impact of summer dry-ups of the stream Stiper on the fire salamander population. These dry-ups are caused by using the main spring of the stream for water supply. The research was carried out at two plots (1 and 0.5 ha) by the stream Stiper. The terrestrial part of the population was studied during daytime after the rain and larvae were counted nightly. During the field trips meteorological conditions were monitored. Captured animals were measured and their sex was determinated. The plots were divided into quadrants (10 x 10 m) to note the cartographic position of captured specimens. Since dorsal pattern of every specimen is unique, they were not marked but photographed. 431 salamanders were captured and 121 recaptured. The estimation of the population density made with mark-recapture method (Jolly-Seber model, program Ecological Methodology 5.2) was 275–671 individuals/ha on Plot 1, and 170–610 individuals/ha on Plot 2. We estimated 57% of males, 35% of females and 8% of juveniles. Sex ratio was 1.6 males / 1 female. The larvae were most numerous during spring, but most

of them have not metamorphosed because of drying up of the stream at the beginning of summer. We think that this is the cause of the small percentage of juveniles and that it could eventually cause the population decline.

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## STANIŠTE GNIJEŽĐENJA CRNE RODE, *Ciconia nigra*, U PARKU PRIRODE KOPAČKI RIT

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Crna roda *Ciconia nigra* je redovita gnjezdarica Parka prirode Kopački rit. Procjenjuje se da je u periodu 1999-2003. ukupna gnjezdeća populacija iznosila između 5-7 pari godišnje. Tijekom istraživanog perioda provedena su mjerenja osnovnih parametara staništa na kojima crne rode izrađuju svoja gnijezda. Gnijezda su najčešće izgrađena na hrastu lužnjaku *Quercus robur* (50%), bijeloj topoli *Populus alba* (33%), te po jedno (8,5%) na jasenu *Fraxinus sp.* i crnom orahu *Juglans nigra.* Gnijezda su u prosjeku smještena na 12,22 m visine od tla (n = 14, raspon = 7,34 - 21,60 m) na stablima čiji je prsni promjer u prosjeku je iznosio 270,55 cm (n = 12, raspon = 129,20 - 660,00 cm) i čija je prosječna visina iznosila 21,06 m (n = 12, raspon = 11,5 - 30,0 m). Gnijezda su najčešće izgrađena na bočnoj grani neposredno uz deblo (64%) ili u rašljama horizontalne bočne grane (28%). Ekspozicija gnijezda je u najvećem broju slučajeva bila jugo-zapadna (35%), sjeverna ili istočna (14%) što je u skladu s dominantnim vjetrovima ovog područja koji pušu sa sjevero-zapadnog ili jugo-istočnog smjera. Na području Parka prirode Kopački rit crne rode gotovo isključivo gnijezde u više od 80 godina starim, poplavnim šumama. Stoga je za njihovo očuvanje nužno zaštiti ove preostale šumske sastojine od uznemiravanja i sječe.

## NESTING HABITAT SELECTION BY BLACK STORKS, *Ciconia nigra*, IN KOPAČKI RIT NATURE PARK

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Black Stork *Ciconia nigra* is the regular breeding bird in the Kopački rit Nature Park. During 1999-2003 period the breeding population was estimated to 5-7 pairs/year. During the study period the basic nesting habitat parameters were measured. Nests were built on *Quercus robur* (50%), *Populus alba* (33%), as well as *Fraxinus sp.* and *Juglans nigra* (8.5%), respectively. Nests were built in average 12.22 m from the ground (n = 14, range = 7.34 – 21.60 m) on trees with average diameter of 270.55 cm

(n = 12, range = 129.20 – 660.00 cm) and average height of 21.06 m (n = 12, range = 11.5 – 30.0 m). In most cases nests were situated on the primary branch near the trunk (64%) or horizontal branch (28%). The nests had south-western (35%), northern or eastern (14%, respectively) exposition that is consistent with prevailing winds in the area coming from northwest and southeast direction. Black Storks in Kopački rit are almost exclusively nesting in old, over 80-years old, alluvial forests. Thus, all remaining suitable forests should be protected from disturbance and cutting in order to protect their nesting population.

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## BIOSPELEOLOŠKA ISTRAŽIVANJA GROMAČKE I MOČILJSKE ŠPILJE NA PODRUČJU GRADA DUBROVNIKA

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Tijekom 2003. godine članovi Hrvatskog biospeleološkog društva obavili su prva sustavna biospeleološka istraživanja Močiljske i Gromačke špilje – zaštićenih spomenika prirode na području grada Dubrovnika. Zbog svojih geomorfoloških, paleontoloških i arheoloških vrijednosti špilje su zaštićene 1963., odnosno 1986. godine. Močiljska špilja ukupne je dužine kanala preko 500 m i predstavlja tipsko nalazište za vrstu vodenog jednakonožnog račića *Proasellus anopthalmus dalmatinus*. Gromačka špilja najimpozantniji je speleološki objekat Dubrovačko-neretvanske županije, velikih dimenzija kanala ukupne dužine preko 2 km. Za znanstvenu javnost otkrivena je tek 1985. godine, a u njoj su zabilježeni jedinstveni nalazi otiska stope neolitskog čovjeka. Predstavlja tipsko nalazište za amfipodnog rakušca *Accubogammarus algor jalzici*, te ze Hrvatsku prvo nalazište troglobiontnog kornjaša filtratora *Hadesia vasiceki*. Utvrđeno je nulto stanje objekata, određeni najvažniji ekološko-klimatski parametri, obavljena osnovna fizikalno-kemijska analiza pedološkog supstrata i vode, sakupljena podzemna fauna i obavljena fotografska i video dokumentacija. Ovom prilikom predstavljeni su preliminarni rezultati istraživanja s popisom faune u obliku Check liste.

## BIOSPELEOLOGICAL RESERCH OF GROMAČA AND MOČILJSKA CAVE IN DUBROVNIK REGIONE

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During year 2003. members of Croatian Biospeleological Society make first systematic research of Močiljska and Gromačka cave – protected speleological ob-

jects in region of Dubrovnik city. Because of its geomorphological, palentological and arheological values, Močiljska cave is protected since 1963., while the Gromača cave since 1986. year. The total lenght of Močiljska cave is more than 500 m. The cave presents locus typicus for the subterranean isopod *Proasellus anopthalmus dalmatinus*. The most impressive speleological object for the Dubrovnik-Neretva County is Gromača cave, big size channels, more than 2 km long. This cave is discovered in 1985. with its unique finding of the neolitic human footprints. The cave presents locus typicus for the subterranean amphipod *Accubogammarus algor jalzici* and in Croatia first habitat for troglobitic coleoptera *Hadesia vasiceki*. We defined the most important ecological and climatological parameters, also physical and chemical analyses of sediment and water, collecting subterranean fauna while the photo and video material was taken. This paper presents preliminary results of investigation and Checklist of subterranean fauna from both caves.

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## METODE PRIKUPLJANJA I SPREMANJA UZORAKA IZMETA VUKOVA (*Canis lupus*) ZA GENETIČKA ISTRAŽIVANJA

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Nezaobilazan dio istraživanja i upravljanja divljim životinjama je pronalaženje i identifikacija pojedinačnih jedinki. Istraživanje zvijeri (Carnivora) promatranjem u divljini vrlo je teško, jer su to životinje koje se skrivaju, teško ih je uloviti, a često su i vrlo rijetke. Zato se u istraživanjima takvih vrsta sve češće koriste neinvazivne molekularne tehnike, kao što je analiza uzoraka izmeta. Međutim, DNA izolirane iz takvih uzoraka je vrlo malo i loše je kvalitete, pa je vrlo važno koje metode prikupljanja i spremanja uzoraka se koristi da bi se kasnije uspješno provela genetička analiza. Ovdje prikazujemo istraživanje u kojem procjenjujemo učinkovitost triju metoda spremanja DNA vuka iz uzoraka izmeta vuka (*Canis lupus*) za genetičku analizu, u ovom slučaju genotipizaciju mikrosatelita. Tijekom prošle godine je na području Gorskog Kotara sakupljeno 9 svježih uzoraka izmeta vukova. Na terenu je svaki uzorak podijeljen na tri dijela koji su stavljeni u 95% etanol, u epruvetu s desikatorom i smrznuti (-20°C). DNA je izolirana korištenjem komercijalnog kita, te je provedena genotipizacija na 8 mikrosatelitskih lokusa, što znači da su za svaku metodu spremanja uzoraka provedene 72 lančane reakcije polimerazom (9 uzoraka x 8 lokusa). Uspješno je dobiveno 59 genotipova (82%) iz uzoraka spremljenih u etanol, 43 genotipova (60%) iz uzoraka spremljenih u epruvetu s desikatorom, te samo 41 genotip (57%) iz

smrznutih uzoraka. Stoga preporučujemo spremanje uzoraka izmeta vukova u 95% etanol, što je i najpraktičnije na terenu.

## COLLECTION AND PRESERVATION METHODS OF WOLF (*Canis lupus*) FAECAL SAMPLES FOR USE IN GENETIC RESEARCH

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An integral part of wildlife research and management is detection and identification of individual animals. It is very difficult to study free-ranging carnivores (Carnivora) by direct observation, because they are secretive, difficult to capture or rare. Noninvasive molecular techniques, such as the analysis of faecal samples, are increasingly valuable in studies of such species. However, DNA extracted from these samples is typically of low quantity and quality. Further, the method of collection and preservation can be crucial for successful genetic analysis. Here we evaluate the performance of three methods for preserving endogenous DNA in wolf (*Canis lupus*) faecal samples for genetic analysis, namely microsatellite genotyping. Nine fresh wolf faecal samples were collected in Gorski Kotar during last year. Faecal samples were divided in the field in three parts and each part subsequently preserved either in 95% ethanol, placed in a tube with a desiccator or frozen (-20°C). DNA was extracted from each sample using commercially available extraction kit and microsatellite genotyping on 8 loci was performed. From samples preserved in ethanol we successfully obtained 59 (82%) genotypes out of 72 possible genotypes (i.e. 9 samples times 8 loci). Samples preserved by desiccation yielded 43 (60%) genotypes and frozen samples yielded only 41 (57%) genotypes. Hence, we would recommend preservation of wolf faecal samples in 95% ethanol, which is also the most practical method in the field.

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## ČIMBENICI PROCJENE UTJECAJA CESTOVNE INFRASTRUKTURE NA OKOLIŠ

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Suvremene cestovne prometnice, posebno autoceste, zbog svojih prometnotehničkih i građevinskih zahtjeva, te zbog svoje ograničene fleksibilnosti, odnosno

nemogućnosti da se prilagode u prostoru kroz koji prolaze, zahvat su koji znatno utječe na okoliš, prvenstveno zauzimanjem velikih površina, prenamjenom zemljišta, devastiranjem krajobraza, bukom, onečišćenjem zraka, tla i voda, te remećenjem ekosustava (uništenjem, fragmentacijom i smanjenjem kakvoće staništa i dr.). Procjena utjecaja na okoliš osigurava ostvarivanje načela preventivnosti usklađivanjem i prilagođavanjem namjeravanog zahvata s mogućnostima okoliša na određenom prostoru. U prošlosti se procjena utjecaja na okoliš cestovnih prometnica vršila bez cjelovite informacije o stanju okoliša uz prvenstveno zadovoljavanje tehničko-prometnih uvjeta. Time je izostalo sagledavanje uzročno-posljedičnih veza i ocjena sinergističkog djelovanja, te rezultati tako propisanih mjera i uvjeta nisu uvijek osiguravali odgovarajuću zaštitu okoliša. Danas se procjenom utjecaja sveobuhvatno sagledava mogući nepovoljni utjecaj na glavne sastavnice okoliša u cilju propisivanja adekvatnih mjera zaštite i monitoringa. Procjena utjecaja zahvata na okoliš i procjena budućih međusobnih utjecaja vrši se pomoću pokazatelja odnosno čimbenika okoliša. Čimbenici okoliša su mjera stanja okoliša koji daju izraz međuodnosa različitih procesa u okolišu i njihovih posljedica na sveukupnu prirodu odnosno čovjeka u određenom vremenskom periodu. Kako bi se što cjelovitije prosudio utjecaj zahvata na okoliš potrebno je utvrditi čimbenike koji ovisno o vrsti zahvata utječu na jačinu, trajanje i rasprostiranje tog utjecaja. Čimbenici na temelju kojih se prosuđuje i ocjenjuje utjecaj cestovnih prometnica na okoliš prvenstveno su meteorološki, klimatološki, hidrološki, hidrogeološki, geološki, geotehnički, seizmološki, pedološki, bioekološki, krajobrazni, zdravstveni, sociološki, prometni i dr.

## FACTORS RELEVANT TO THE ENVIRONMENTAL IMPACT ASSESSMENT OF ROAD INFRASTRUCTURE

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Due to their traffic, technical and civil engineering requirements, and limited flexibility, i.e. impossibility to adapt to the area they traverse, modern road infrastructure projects, especially motorways, significantly influence our environment, primarily by taking up large areas of land, changing its use, devastating natural landscape, generating noise, polluting air, soil and water, and by their disturbance to natural ecosystems (through destruction, fragmentation and deterioration of the quality of habitats, etc.). Environmental impact assessment studies ensure fulfillment of the prevention principle by harmonizing and adapting planned road schemes to environmental capabilities of a particular area. In the past, the environmental impact assessment of road projects was conducted without complete information about actual condition of the environment, and the primary emphasis was on meeting technical and transport-related criteria. In this way, the full

understanding of cause-and-effect relationships and synergic interactions was lacking, and the results of measures and requirements specified in this way did not always ensure adequate protection of our environment. Present day environmental impact assessment procedures enable us to gain full insight into possible harmful effects on major components of natural environment so that appropriate protection and monitoring measures can be set. The assessment of impact an infrastructure project has on environment, and the estimation of future interactions, is carried out by means of environmental indicators or factors. These environmental factors depict condition of the environment and give information about interaction of various environment-related processes and their effects on the overall natural environment and human health within a specified period of time. In order to estimate in the best possible and complete way the environmental influence exerted by a project, it is necessary to determine factors which, depending on the type of the project, influence the intensity, duration and spreading of such influence. The environmental impact of road projects is primarily assessed by means of meteorological, climatic, hydrological, hydrogeological, geological, geotechnical, seismological, pedological, bioecological, landscaping, health, sociological, transport-related and other factors.

BIOLOGIJA U SUVREMENOJ HRVATSKOJ ŠKOLI
*BIOLOGIA ET EDUCATIO*
BIOLOGY IN MODERN CROATIAN SCHOOL

IL-23

## SUVREMENA HRVATSKA ŠKOLA – CILJ I ODREDNICE REFORME

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Suvremeno obrazovanje mora odgovoriti izazovima društvenih promjena (posebno brzim promjenama na tržištu rada), razvoju novih tehnologija i brzom povećanju fundusa znanstvenih spoznaja. To je razlog provođenju reformi školstva u većini zemalja. U tim se procesima polazi od ljudskih i građanskih prava, kao temeljnih tekovina civilizacije i novih spoznaja s područja psihologije i pedagogije. U dokumentima UNESCO-a, načela škole za 21. stoljeće definirana su kao 4 potpornja ili kamena temeljca obrazovanja: *učiti znati; učiti činiti; učiti biti; učiti živjeti zajedno*. Promišljanje o ulozi učenja prirodoslovlja u tom kontekstu, dovodi do zaključka da je težište na usvajanju osnovnih prirodoznanstvenih principa, na razvoju vještine logičkog zaključivanja i kritičkog razmišljanja, na oblikovanju pozitivnih stavova, na osposobljavanju za samostalno učenje te za pronalaženje i prepoznavanje znanstveno utemeljenih informacija. S promjenom životnog okruženja škole, mijenja se i uloga nastavnika, od kojeg se traže nove kompetencije, šire od poznavanja matične struke. Stoga je cjeloživotno obrazovanje nastavnika danas imperativ. U Hrvatskoj se u smislu priprema reforme školskog sustava započelo s uvođenjem kurikulumskog pristupa planiranju i programiranju nastavnih sadržaja. *Tradicionalni pristup planiranju i programiranju* usmjerava se na sadržaj učenja. Postignuća učenika mjere se prema stupnju reprodukcije predviđenih sadržaja. Učenici i nastavnici trebaju se prilagoditi realizaciji propisanih programskih sadržaja. *Suvremeni kurikulumski pristup* stavlja učenika u središte zanimanja, što ponajprije podrazumijeva jasno utvrđivanje cilja obrazovanja, dok se sadržaji, organizacija i metode učenja prilagođavaju učeničkim sposobnostima, potrebama i interesima (veća izbornost). Potrebna je veća interdisciplinarnost, odnosno cjelovitost i međusobna povezanost te životna primjenjivost nastavnih sadržaja, naglašena je potreba razvoja vještina i oblikovanja stavova.

## MODERN CROATIAN SCHOOL – THE GOALS AND GUIDELINES IN SCHOOL REFORMING

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Modern education has to respond to the changes in society (specially in labor market), to the development of modern technologies, as well as to rapid enlargement of scientific knowledge. These are the reasons of school system reforming

in majority of the countries. The reforming processes are rooted in human and civic rights, which are the fundaments of modern civilization, as well as in new psychological and pedagogical achievements. In UNESCO's documents, the pillars of education in 21. century are shortly defined as: *learn to learn; learn to do; learn to be; learn to live together.* In that context, the roll of science education is aimed to learning and understanding of basic science concepts, on logical and critical thinking skills developement, on positive attitudes, on preparations for self-education and ability to find and recognize scientificly relevant information. Changes in school social environment, reflect on the wider demands for teachers' competences. Therefore, life-long learning becomes the imperative for teaching profession. In Croatia, the implementation of curriculum based aproach in planning of the teaching contents started last year, as the school reform preparation. *Traditional approach* in planning is focused on the subjects' content. Students' achievements are measured according to the student's ability to reproduce those contents. Students and teachers must adapt to this given program. *Modern curriculum approach* makes student the center of all school activities. Clearly defined educational goals must be the priority of that approach, while the contents, organization and teaching methods are adjusted to students' abilities, needs and interests (wider offer of elective programs). That model demands more of interdisciplinary and holistic approaches, as well as more life related contents, while the need of skills and attitudes development is emphasized.

OP-89

## UDŽBENIK I NJEGOVA UPORABA U POČETNOJ NASTAVI PRIRODOSLOVLJA

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Udžbenik je jedan od najraširenijih izvora znanja u školi. Možemo ga najjednostavnije odrediti kao knjigu u kojoj je na poseban način prema pedagoškim, psihološkim, didaktičkim i metodičkim načelima prerađena pojedina znanost ili struka i prilagođena izobrazbi mladih. Za razliku od svake druge knjige koja je najjednostavnije, najpogodnije, najraznovrsnije i najjeftinije sredstvo prenošenja misli, udžbenik bi trebao voditi učenike do otkrivanja novih spoznaja te bi prestao biti samo "knjiga za učenje" i postao bi "knjiga koja uči učiti". Suvremeni udžbenik najčešće sadrži naglašenu "didaktičko-metodičku aparaturu": određenje zadaće nastave jedinice - naslov, prethodne zadatke za učenike/učenice, sažeto tekstualno i ilustrativno objašnjenje problema zadataka nastave, naglašene temeljne pojmove koje učenici trebaju spoznati, naputke za korištenje vježbenice, zadatke za vrednovanje učenikova postignuća, pojmovnik s kazalom, popis znanstvenopopularne literature za određeni razred i dr. Suvremeni udžbenik ima posebnu zadaću u suradničkom učenju u nastavi prirodoslovlja.

## THE ROLE OF THE TEXTBOOK IN THE INITIAL TEACHING OF NATURAL SCIENCES

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The textbook is one of the most prevalent sources of knowledge in school. It can simply be defined as a book in which, in a particular way, by applying pedagogical, psychological, didactic and methodological principles a particular science or field of study is rewritten and adapted to be used in the education of young people. Unlike any other book, which is the simplest, most suitable, most varied and cheapest means of conveying thoughts, the textbook should lead students into discovering new ideas and in such a way it stops being just "a book for learning" but becomes "a book that teaches to learn". A modern textbook usually has an accentuated didactic and methodological apparatus: it defines the objectives of a teaching unit, it contains preliminary tasks for students as well as a concise textual and illustrative explanation of the aim of teaching, it stresses the basic topics and notions that the students should acquire and understand, it contains instructions how to use the workbook, tasks to be used to assess the students

performance, a glossary with a table of contents, a list of popular-science references that can be used at a particular stage, etc. A modern science textbook plays a particular role in cooperative learning.

OP-90

## POKUS - NEZAOBILAZAN PRAKTIČAN RAD U NASTAVI PRIRODE I BIOLOGIJE

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Pokus, kao jedan od oblika praktičnog rada mora biti povezan za perciptivnu i misaonu aktivnost, te će kao takav znatno doprinjeti učinkovitosti nastave. Eksperimentator (učenik/učenici ili nastavnik) mijenjajući pojedine čimbenike tijekom pokusa ispituje pretpostavke, otkriva uzročne veze i donosi zaključke. Školski pokusi mogu biti kvalitativni i kvantitativni. Uz kvalitativni pokus obično se postavljaju učenicima pitanja na koje se očekuje kratki odgovor, DA ili NE. Primjerice: "Je li sjemenki potrebna voda da bi proklijala?" Rezultati kvantitativnih pokusa u pravilu se iznose u brojčanim vrijednostima. Primjerice, traži se odgovor na pitanje: "Koliko je biljci potrebno vode da bi proklijala?" U učenika mlađe dobi prevladavati će kvalitativni pokusi , a u učenika viših razreda osnovne škole i u srednjoj školi kvantitativni pokusi. Tijekom izlaganja ovog priopćenja govoriti će se o kratkotrajnom i dugotrajnom pokusu, te o sastavnicama pokusa, od postavljanja problema do interpretacije rezultata s mogućim transferom novih spoznaja na ostale učenike. O važnosti pokusa govore nam rezultati istraživnja Nijemca Grupea: "Ono što čujemo na nastavi ostaje u pamćenju do 20%, ono što vidimo do 30%, ono što čujemo i vidimo do 50%, a od samostalnog pokusa (praktičnog rada) do 90%".

## EXPERIMENT - UNAVOIDABLE PRACTICE WORK IN NATURE AND BIOLOGY CLASSES

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Experiment, as one of practice work forms must be connected with perceptive and mental activity and, as such, it contributes significantly to the class efficiency. By changing certain elements during experiment, the experimenter (student/students or teacher) tests presumptions, discovers cause-effect relationship and shapes conclusions. We distinguish between qualitative and quantitative school-experiment in the class practice. The questions asked with qualitative experiment are usually answered with short answers - YES and NO. For example: "Does a seed need water to

sprout?" The results of quantitative experiments are generally expressed in figures. For example: "How much water does a seed to sprout?" Qualitative experiments should prevail in lower classes, while quantitative experiments should be used in higher classes in the elementary school and in the secondary school. During this lecture, I shall talk about short and long experiments and about their elements, from questions asked to observing and interpreting results with possible transfer of new understanding to other students. The importance of experiment is best described by German Grupe: "We remember up to 20% of what we hear in classes, up to 30% what we see, up to 50% of what we hear and see, but we remember up to 90% of what we experience in a self-performed experiment

OP-91

## KORELACIJE RAZLIČITIH PREDMETA KROZ PROJEKTNU NASTAVU BIOLOGIJE

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U Gimnaziji u Čakovcu već niz godina izvodi se fakultativna nastava u obliku tzv. "nastave bez zvona". Takva nastava omogućuje korelacije sadržaja kemije, biologije, geografije i drugih nastavnih područja. Naglasak je pri tome na praktičnoj primjeni teoretski usvojenih znanja različitih područja izradom samostalnih istraživačkih radova ili kroz nastavnu u prirodi. Učenici tako primjenjuju usvojena teoretska znanja te trajnije i kvalitetnije usvajaju nova znanja i shvaćaju svrhu učenja. Kod pripreme i provođenja nastavnih ekskurzija potrebna je suradnja nastavnika kod kojih dolazi do korelacije sadržaja (kemije, biologije, geografije). Kao primjer možemo navesti nastavnu ekskurziju u kamenolom Marčan (kod Vinice) gdje povezujemo sadržaje biologije, kemije i geografije jer učenici sami traže fosile, ispituju vrstu i karakteristike tla, izrađuju fitocenološku snimku, a obzirom na blizinu lokacije dio ispitivanja radi usporedbe provode u arboretumu Opeka. Učenici rade timski i samostalno organiziraju rad unutar grupe ovisno o njihovom predznanju i mogućnostima. Na taj način učenici različitih dobi i predznanja jedni drugima prenose svoja iskustva i spoznaje. U radovnoj nastavi teško je ostvariv ovakav rad no zbog kvalitete usvojenog znanja, trebao bi ipak biti više prisutan i u osnovnim i u srednjim školama, bilo u fakultativnoj ili izbornoj nastavi. Predlažemo da svojim iskustvom u terenskim oblicima rada nastavnici biologije, kemije i geografije pomognu učiteljima razredne nastave u pripremi "nastave u prirodi" za niže razrede osnovne škole. Na taj način pomogli bismo uvođenju drukčijeg pristupa usvajanju znanja koji kod učenika potiče i razvija sposobnosti uočavanja i zaključivanja o uzročno-posljedičnih vezama u prirodi te shvaćanja povezanosti svih prirodnih znanosti što bi svakako pridonijelo povećanju kvalitete kasnijeg obrazovanja učenika.

## THE CORRELATIONS AMONG DIFFERENT SUBJECTS THROUGH BIOLOGY PROJECT TEACHING

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For many years, Grammar School in Čakovec has been organizing optional subjects, in the "no-bell classes" form. Such lessons enable correlations among chemistry, biology, geography and many other subjects. A special importance is given to practical use of knowledge gained through theoretical education of different subjects, by conducting independent research projects or by lessons in the nature. In that way, students can use the knowledge gained through theoretical education, gain long-lasting and quality knowledge and also recognize the essence of studying. While preparing and going on excursions, a cooperation among teachers is needed in order to correlate contents of chemistry, biology, geography etc. For example, on an excursion to quarry Marčan (near Vinica) we interconnect biological, chemical and geographical contents: students search for fossils, examine the type and characteristics of the soil, make the study of species' distribution. Also, for comparison, a part of the research is made in the nearby arboretum Opeka. Students work in teams and organize work individually inside the team according to their prior knowledge and capabilities. In this way students of different age and prior knowledge can inform one another of their experience and perception. This work plan is not easy to be carried out in regular lessons, but because of the gained knowledge quality it should be used more in primary and high schools in the form of optional classes. We suggest that biology, chemistry and geography teachers help lower grade teachers with their experience in field - work, to prepare lessons in the nature for lower grades of primary school. In this way we can help introduce a different approach to learning that encourages and develops students' capabilities of noticing and drawing conclusions about cause and effect relations in nature and enables them to realize the connection of all natural sciences, which will contribute to the increase of quality of later part of students' education.

OP-92

## OBILJEŽAVANJE «DANA BEZ DIMA» U DUBROVAČKIM SREDNJIM ŠKOLAMA

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Ekološka grupa gimnazija-Dubrovnik pridružila se akciji prevencije pušenja u mladih obilježavanjem «DANA BEZ DIMA» 5.03.2003. prigodnom izložbom i

analizom ankete o raširenosti pušača u dubrovačkim srednjim školama. Anketirano je ukupno 1500 učenika ugostiteljske, pomorske, ekonomske, matematičke, jezične, opće i klasične gimnazije u Dubrovniku. Anketa je provedena u pisanom obliku. Svrha ankete bila je utvrditi postotak pušača među učenicima srednjoškolske populacije, prepoznati važnost problema pušenja i svih posljedica koje ono nosi za zdravlje, te poticati mlade na razmišljanje o štetnosti pušenja. Analiza ankete pokazuje da je od ukupno 1500 anketiranih 33,3% pušača. Najveći postotak pušača zabilježen je u II. razredima Medicinske škole (55,8%). Drugo mjesto na ljestvici pušača zauzeli su II. razredi Ugostiteljske škole (49,5%), a treće maturanti Pomorske škole (48,6%). Najniži postotak pušača zabilježen je u I. razredima Medicinske škole (4,3%), I. razredima Ekonomske škole (22,2%), II. razredima gimnazije (24,7%) i I. razredima Ugostiteljske škole (27,7%). Na temelju poraznih rezultata ankete o pušenju zaključili smo da bi više pažnje trebalo posvetiti edukaciji učenika o najvećoj epidemiji dvadeset i prvog stoljeća – pušenju cigareta.

## MARKING OF «DAY WITHOUT SMOKE» IN DUBROVNIK SECONDARY SCHOOLS

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Ecological group of grammar school – Dubrovnik joined the campaign of smoking prevention of the young by marking of «Day Without Smoke» on 5th March, 2003. which was an appropriate exhibition and at the same time an analysis of the survey result on expansion of smokers in Dubrovnik secondary schools. A total of 1500 pupils of Tourist and Catering, Navy school, Business school and Grammar school (all its divisions) were inquired. The survey was carried out in written form. Its aims were to establish the percentage of smokers among the pupils of the secondary school population, to recognize the importance of the smoking problems and all the consequences it has for the health and also to include the young to think about the affects of smoking. The greatest percentage of smokers was recorded in the 2nd classes of Medical school (58.8%). The second place on the smokers' scale was taken up by the pupils of Tourist and catering school (49.5%) and the third one by the final classes of Navy school (48.6%). The lowest percentage of smokers was recorded in the 1st classes of Medical school (4.3%), the 1st classes of Business school (22.2%), the 2nd classes of grammar school (24.7%) and finally, the 1st classes of Tourist and catering school (27.7%). On the basis of these disastrous survey results on smoking we concluded that more attention should be drawn to education of pupils about one of the greatest epidemic of the 21st century – cigarette smoking.

OP-93

## SPOLNI ŽIVOT I KONTRACEPCIJA -ANKETA

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Anketa je provedena među učenicima 3. i 4. razreda gimnazije, a sastoji se od 24 pitanja vezana za spolni život i kontracepciju. Anketirano je 245 učenika (145 učenica i 100 učenika). Iz ankete je vidljivo da je 33 ispitanika stupilo u spolni odnos do sada, 21% ispitanika je imalo spolni odnos sa partnerima koji spadaju u rizične skupine. Oko 46% ispitanika zna nabrojati 3 spolno prenosive bolesti, a 82% ih se izjasnilo da je metoda računanja plodnih i neplodnih dana nepouzdana. 33% ispitanika razgovara o spolnosti i kontracepciji s razrednikom, prof. biologije ili sa drugim profesorima u školi. 30% djevojaka je odabralo svog ginekologa ili ga je posjetilo, a 99% ispitanika nije tražilo savjet o kontracepciji ili spolno prenosivim bolestima svog liječnika. Iz navedenih rezultata možemo zaključiti da bi se ovoj problematici trebala posvetiti još veća pažnja, sa više edukacijskih predavanja u svrhu informiranja kako učenika, tako i roditelja.

OP-94

## SPOLNOST MLADIH - POGLEDI I ISKUSTVA

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Anketa je provedena u razdoblju od 2000. do 2002. godine na skupini od 315 učenica u rasponu od 16. do 18. godine. Sadrži 8 ciljanih pitanja na koja se odgovara slobodnim pisanim stilom. Anketom ovog tipa željelo se isključiti utjecaj sugestivnosti, a povećati intenzitet kognitivne i emocionalne komponente stava i uvjerenja. Od ukupnog broja anketiranih učenica, 11,75% koristilo je kontracepciju, a 87,94% nije. Od učenica koje su koristile kontraceptive, čak 75,7% navodi kondom, 10,8% pilule, a 13,5% gotovo sva sredstva. Vrlo je interesantno da 96% učenica nije znalo ili je djelomično točno znalo objasniti plodne i neplodne dane u ciklusu žene. Nadalje, 64,13% anketiranih misli da su kontracepcijska sredstva nezdrava, dok 8,25% misli da su zdrava, a 10,48% misli da su neka zdrava, a neka nisu. Ohrabruje činjenica što se 92% anketiranih negativno izjasnilo glede abortusa, a samo 8% pozitivno. Na pitanje što je umjetna oplodnja samo 5% anketiranih znalo je odgovor, dok 26,03% učenica podržava tu metodu. Potrebno je točna, istinita i pravovremena edukacija mladih o spolnosti. Pri obradi sadržaja o spolnosti potreban je holistički pristup, a on zahtijeva integraciju i interdisci-plinarnost biologije, vjeronauka, psihologije, pa i sociologije.

## YOUNG PEOPLE'S SEXUALITY

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The survey was carried out in the period from 2000 until 2002 within the group totalling 315 students from 16 to 18 years of age. It contains eight target questions to which the answers are given in the free written style. With the survey of this type any influence of suggestiveness was pointed to be excluded, whereas the intensity of the cognitive and emotional component of the attitude and conviction was meant to be increased. From the total number of polled students, 11.75 % of them used contraception and 87.94 % of the girls have never used it. From the students who used contraceptives even 75.7 % of them used condoms, 10.8 % of students used pills and 13.5 % of girls used almost all contaceptives. It is very interesting that 96 % of students were not able or only partially to explain the fertile and infertile days in the female cycle! Moreover, 64.13% of the polled students think that the contraceptives are unhealthy, while 8.25 % of them think that they are healthy and 10.48% of the students think that some of them are healthy and some are not. The encouraging fact is that 92% of our students declared their negative opinion about abortion and only 8% of them have a positive attitude about that. Only 5 % of the girls knew what the artificial insemination was, while 26.03 % of them support that method! The correct, true and timely young people's sexuality education is needed. While working on sexuality, a holistic approach is necessary, which demands integration and interdisciplinarity of biology, religion, psychology and sociology.

OP-95

## REZULTATI ANKETIRANJA SAMOPROCJENE RADA NASTAVNIKA

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Uz uvriježene oblike praćenja i procijenjivanja rada nastavnika (ravnatelji, pedagozi, roditelji, učenici, prosvjetne vlasti pa i novinari), u posljednje se vrijeme ističe potreba sustavne i cjelovite samoprocjene, kao najučinkovitije metode unapređivanja nastavnog rada. Primarni cilj anonimne ankete koja je provedena na sastancima županijskih stručnih vijeća biologije, bio je pomoći nastavnicima u sagledavanju elemenata za kvalitetnu i cjelovitu samoprocjenu vlastitog rada, posebno u kontekstu provedbe projekta rasterećenja učenika/kurikularnog pristupa planiranju i programiranju nastavnog sadržaja. Ponuđeni

elementi samoprocjene bili su sljedeći: stručnost, trajno osobno usavršavanje, programiranje ciljeva, organizacija nastavnog procesa, vrednovanje učeničkih postignuća, odgojni stil, suradnja. Ukupni rezultati pokazali su visoke ocjene u samoprocjeni vlastitog rada nastavnika. Detaljnija analiza pojedinih elemenata samoprocjene po razinama sustava OŠ i SŠ, te po dobnim skupinama (godinama staža nastavnika) pokazala je varijacije rezultata, primjerice u poznavanju propisa i zakona, ne redovito praćenje područja psihologije, pedagogije, didaktike te neke neusklađenosti iskazanih ocjena za pojedine aspekte suradnje. Ovi rezultati mogu biti jedan od pokazatelja u evaluaciji provedbe projekta rasterećenja učenika u kontekstu osuvremenjivanja nastave biologije. O njima bi trebalo voditi računa pri organizaciji kako dodiplomskog, tako i trajnog stručnog usavršavanja nastavnika.

## SURVEY OF SELF-ASSESSMENT OF TEACHER'S WORK – RESULTS

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Along with the ingrained patterns of monitoring and assessing of teacher's work (headmasters, educationists, parents, pupils, educational authorities and journalists), the need for systematic and complete self-assessment makes its mark lately as the most efficacious method for advancement of teaching work. The primary goal of the anonymous survey that was carried out on counties' biology experts councils was to help teachers in observing elements for quality and complete self-assessment of one's own work, especially in the context of the implementation of the project based on disburdening of pupils/ curriculum approach to planning and programming of teaching contents. The following elements of self-assessment were given: competence, permanent personal improvement, organization of teaching process, evaluation of pupils' achievements, educational style, and cooperation. The total results showed high appraisals in self-assessment of teacher's own work. More detailed analyses of the specific elements of self-assessment on the levels of primary and secondary schools, and of age groups (teacher's years of service) showed variation in results, for example in knowledge of regulations and law, irregularly observing the field of psychology, pedagogy, didactics and some mismatches of the stated grades for particular aspects of cooperation. These results can be an indicator in the pupils' disburdening evaluation project in the context of the modernization of biology teaching. They should be taken into consideration while organizing undergraduate and permanent personal improvement of teachers.

OP-96

## IZOBRAZBA NASTAVNIKA O KOPAČKOME RITU – ZA KOPAČKI RIT

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Cilj istraživanja je unapređivanje znanja nastavnika o zavičajnim prirodnim znamenitostima za primjenu u nastavi. Dan je primjer dopune nastavnih programa osnovne škole sadržajima o Kopačkome ritu. Za potrebe istraživanja priređena je anketa kojom su prikupljeni podaci o posjećenosti Kopačkoga rita, mogućnosti izvedbe nastave u Kopačkome ritu, interesom za obrazovanjem o Kopačkome ritu i poznavanje osobitosti Kopačkoga rita. Istraživanje obuhvaća 109 nastavnika četiriju osnovnih škola Županije osječko-baranjske i 57 studenta Pedagoškoga fakulteta u Osijeku (šk. god. 2001/2002). Dobiveni rezultati ukazuju na nisku razinu poznavanja osobitosti Kopačkoga rita, nisku razinu posjećenosti (22,08 % nastavnika i 21,21 % studenata). Većina nastavnika (83,48 %) i studenata (91,92 %) zainteresirana je za izobrazbu o Kopačkome ritu, kako bi bolje ostvarivali ciljeve i zadatke ekološkog odgoja i obrazovanja.

## TEACHING TEACHERS ABOUT KOPAČKI RIT – FOR KOPAČKI RIT

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The aim of this research is to improve the teachers˘ knowledge about their native natural importance and then to use the acquired knowledge in the teaching process. This paper includes an example of primary school curricula expanded by some subject-matter on Kopački rit. A survey was constructed in order to collect data on Kopački rit. The survey includes the Kopački rit attendance rate, the possibilities of teaching in Kopački rit, the teachers˘ interests in being taught about Kopački rit and the teachers˘ level of knowledge about the distinctive features of Kopački rit. The research includes 109 teachers from 4 different primary schools in Osječko-baranjska district and 57 students from the Pedagogical Faculty in Osijek (school year 2001/2002). The given results show the low level of knowing Kopački rit˘s distinctive features as well as its low attendance rate (22.08% of the teachers and 21.21% of the students). The majority of the teachers (83.48%) and the students (91.92%) would like to learn more about Kopački rit, all in order to achieve better goals and tasks in the ecological training and education.

OP-97

## POTICANJE STUDENATA NA AKTIVNO UČENJE I KRITIČKO RAZMIŠLJANJE U NASTAVI FIZIOLOGIJE

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U namjeri da potaknemo aktivno sudjelovanje studenata u nastavi fiziologije na PMF-u, počeli smo primjenjivati novu metodu poučavanja poznatu kao ERR okvir. Metoda podrazumijeva izvedbu nastavnog sata u tri faze: evokacija, razumijevanje značenja i refleksija. U fazi evokacije studente se potiče na prisjećanje postojećeg znanja o temi nastavnog sata; u fazi razumijevanja značenja studenti usvajaju novo znanje; u fazi refleksije od studenata se traži da razmisle o tome što su saznali i povežu nove činjenice s prethodnim znanjem. U svakoj od ovih faza nastavnik primjenjuje konkretne edukacijske tehnike koje uključuju individualni rad studenata, suradničko učenje, čitanje, pisanje, raspravu, razmjenu mišljenja i slično. Na taj način potiče se interes studenata i njihovo aktivno sudjelovanje u nastavi. Od jeseni 2002. godine metodu ERR primjenjujemo u praktičnoj nastavi Animalne fiziologije s tri skupine od 15 do 18 studenata. Naša iskustva u primjeni ove metode pokazuju da je ovakva nastava dinamična i učinkovita, kako za studente tako i za nastavnika.

## MOTIVATING STUDENTS TO LEARN ACTIVELY AND THINK CRITICALLY IN PHYSIOLOGY CLASS

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In order to encourage students to participate actively in physiology class at the Faculty of Science, we have started implementing a new teaching method known as the ERR frame. According to this method, each lesson consists of three phases: evocation, realisation of meaning and reflection. In the evocation phase, students are stimulated to evoke their previous knowledge about the topic; in the realisation phase students gain new knowledge; in the reflection phase students rethink what

they have learned in the previous phase and combine new facts with their existing knowledge. In each of these phases the teacher implements specific educational techniques, including student's individual approach, cooperative learning, reading, writing, discussion, exchange of ideas, etc. In this way, students' interest and their active participation in the lecture are encouraged. Since autumn 2002 we have been implementing the ERR teaching method in Animal physiology practical course with three groups of 15 to 18 students. Our experience with this method suggests that lectures are very dynamic and efficient for the students and for the teacher as well.

OP-98

## BIOKEMIJSKI EDUKACIJSKI "SOFTWARE" – ZAŠTO I KAKO

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U Zavodu za biologiju Sveučilišta J. J. Strossmayer u Osijeku pripremljen je elektronički nastavni materijal s biokemijskim sadržajima. Na ovome skupu prezentirat će se digitalni udžbenik "Metabolizam ugljikohidrata", kao drugi dio projekta s općim naslovom "Biokemijske teme". Udžbenik je objavila izdavačka djelatnost Pedagoškog fakulteta u Osijeku. Recenzenti su bili prof.dr.sc. Tihana Žanić-Grubišić (Farmaceutsko – biokemijski fakultet u Zagrebu), te prof.dr.sc. Ivana Weygand – Đurašević (Prirodoslovno – matematički fakultet u Zagrebu). Kao i ranije objavljeni prvi takav udžbenik ("Oksidacijska fosforilacija", 2002. god.), i ovaj sadrži klasične informacije u obliku teksta, te uz njih brojne ilustracije, animacije molekularnih mehanizama i procesa, kao i neke priloge u programu Chime. Uz osnovna poglavlja pripremljena u Microsoft-ovu programu PowerPoint, također sadrži i iscrpne upute za korištenje, jednako kao i instalacijske verzije programa koji su nužni za cjelovito korištenje svih priloga. Popis literature navodi svjetski poznate, nove i popularne udžbenike, jednako kao i odabrane najzanimljivije WEB-stranice iz ovog područja. Neka su poglavlja specifično organizirana (npr. *Glikoliza* i *Ciklus limunske* kiseline), što će se demonstrirati u prezentaciji. Također će se pojasniti razlozi koji su nas usmjerili ka pripremi elektroničkih nastavnih materijala, ukratko iznijeti dosadašnja nastojanja, kao i najaviti neke buduće projekte.

## EDUCATIONAL SOFTWARE IN BIOCHEMISTRY – WHY AND HOW

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Educational software dealing with Biochemistry has been developed at the Department of Biology, University J. J. Strossmayer in Osijek. On this meeting, it will be

presented digital textbook "Carbohydrate metabolism", published as the second part of the project with general title "Biochemical Themes", by the Faculty of Education in Osijek. The reviewers were prof. Tihana Žanić–Grubišić (Faculty of Pharmacy and Biochemistry, Zagreb), and Ivana Weygand–Đurašević (Faculty of Sciences, Zagreb). Like the first one ("Oxidative phosphorilation", published in the year 2002.), it includes classical textual informations, combined with numerous illustrations, animations of molecules and molecular mechanisms and processes, as well as some tutorials in the program "Chime". In addition to the main chapters prepared in Microsoft program Power Point, it contains comprehensive instructions for use, as well as necessary programs for the extended applications. Literature site quotes several world-known recent popular textbooks as well as a number of the most interesting WEB-pages dealing with subjects of interest. Some chapters are organized in a specific way (i.e. Glycolysis, Citric acid cycle), what will be demonstrated in the presentation. Also, the reasons for orientation to electronic form will be explained, the history of the work in this field until now outlined, and some future projects indicated.

OP-99

## ANALIZA DOKTORATA IZ BIOLOGIJE OBRANJENIH NA PRIRODOSLOVNO-MATEMATIČKOM FAKULTETU U ZAGREBU OD 1950. – 2000. GODINE

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Scientometrijskom analizom 344 doktorske teze koje su obranjene na Biološkom odsjeku Prirodoslovno-matematičkog fakulteta u Zagrebu u razdoblju od 1950. – 2000. godine te demografskom analizom doktoranada, istraženo je stanje bioloških znanosti u Hrvatskoj u posljednjih 50-ak godina. U analizi teza korišteni su slijedeći scientometrijski pokazatelji: godina izdanja, područje istraživanja, mjesto izrade i mentori teze. Broj se teza povećavao tijekom vremena, te je trećina obranjena 1990-ih. Istraživanje je pokazalo da je područje najvećeg interesa bila biologija mora te da je većina teza izrađena na sveučilištima i javnim institutima. Najproduktivniji mentori bili su ujedno i najugledniji hrvatski biolozi. Potpunija demografska slika doktora biologije u Hrvatskoj dobivena je analizom doktoranada na temelju spola, dobi, zaposlenja te sudjelovanja u međunarodnoj suradnji. Više od polovice doktoranada bilo je ženskog spola (55%), a prosječna dob stjecanja doktorata iznosila je 37 godina. Nakon stjecanja doktorata većina je doktora biologije (64%) ostajala na sveučilištima i javnim institutima, a 8% ih je otišlo iz zemlje. Istraživanje je pokazalo da se međunarodna suradnja povećala od 1990-ih. Jedini način da doktorske teze budu dostupne u izvornom obliku jest zastupljenošću u specijaliziranoj bibliografskoj bazi podataka *Dissertation Abstracts International*. Pretraživanje ove baze pokazalo je da analizirane teze u njoj nisu zastupljene te ih ne možemo smatrati dostupnima znanstvenoj zajednici.

## ANALYSIS OF THE DOCTORATES IN BIOLOGY DEFENDED AT THE FACULTY OF SCIENCE IN ZAGREB FROM 1950 TO 2000

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The situation in biological sciences in Croatia during the last 50 years has been investigated by the scientometric analysis of 344 doctoral theses defended at the Department of Biology, Faculty of Science in Zagreb, from 1950 to 2000, as well as by the demographic analysis of the PhD candidates. The theses were assessed by the following scientometric indicators: year of publication, field of research, institutions where they were carried out and supervisors. The number of theses rose over time, with the 1990's accounting for a third of all the theses defended. The study found that marine biology was the area of main research, with theses work carried out primarily in universities and public institutes. The most productive supervisors were the most eminent Croatian biologists. A greater understanding of the demographic situation among Croatian biologists with a doctoral degree was obtained by the analysis of their gender, age, employment and participation in international collaboration. Over half of those who were awarded a doctorate were women (55%), and the average age was 37 years. The majority (64%) remained in universities and public institutes, with 8% ultimately leaving the country. The study also found that international collaboration has increased since the 1990's. The only way for the doctoral theses to be made accessible in their original form is to be included in *Dissertation Abstracts International,* specialised bibliographic database. The search of the database showed that analysed theses are not represented in it and thus can not be considered accessible to the scientific community.

OP-100

## ZNAČAJ MUZEJSKOG POSTAVA U LAKŠEM USVAJANJU NASTAVNIH SADRŽAJA PRIRODE U OSNOVNIM ŠKOLAMA

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Izvođenje nastave prirode u prirodoslovnim muzejima provodi se izuzetno rijetko. Posjet muzeju, kako učenika osnovnih tako i srednjih škola, svodi se uglavnom na površno razgledanje eksponata muzeja uz ili bez stručnog vodstva. Kako kvalitetno razgledati muzej i na koji ga način najbolje iskoristiti? Primarno je

potrebna dobra priprema nastavnika koji tijekom obilaska postava komunicira ne samo sa učenicima već i s kustosom pedagogom, te učenicima na taj način omogućava lakše usvajanje nastavnih sadržaja na primarnom izvoru znanja. Hrvatski prirodoslovni muzej u Zagrebu svojim bogatim muzejskim zbirkama pruža velike mogućnosti obogaćivanja i lakšeg usvajanja pojedinih sadržaja. U radu se prezentiraju istraživanja provedena na uzorku učenika 6. razreda OŠ Bogumila Tonija iz Samobora. Eksperiment je obuhvatio grupu od 27 učenika koji jednu nastavnu jedinicu obrađuju u školi, drugu u prirodi i treću u muzeju. U sva tri dijela eksperiment se odvijao istim metodama, a realizator eksperimenta bio je isti nastavnik. Od instrumenata ispitivanja primjenjeni su inicijalni i konačni zadaci objektivnog tipa, te ankete za učenike, nastavnike i kustose pedagoge.

## THE IMPORTANCE OF THE CROATIAN NATURAL HISTORY MUSEUM'S DISPLAY IN TEACHING NATURAL HISTORY SUBJECTS IN PRIMARY SCHOOLS

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Practice of teaching natural history subjects in natural history museums is rarely used by school teachers. Even if schools organize a visit to the Museums for their students, it is mostly done in an inadequate, unsatisfying way, with or without a professional museum guide. The question is how to improve this situation and make the best of such a visit? Firstly, teachers should primarily be well prepared for the visit and be able to communicate both with students and with museum educators, in order to make the lesson more understandable. The Croatian Natural History Museum in Zagreb, with its variety of collections, provides a lot of possibilities for enrichment and easier understanding of certain topics and lessons. In this paper we present the research made among the 6th grade pupils of the Primary school Bogumil Toni from Samobor. The group of 27 pupils was included in this research. They had one lesson at school, the second one in the field, and the third one in the museum. All three parts of the experiment were realized by using same methods, and performed by the same teacher. The methods used in this research were initial and final tasks of the objective type, and questionnaires for pupils, teachers and museum educators.