Hrvatsko biološko društvo
SOCIETAS BIOLOGORUM CROATICA
Croatian Biological Society

13. HRVATSKI BIOLOŠKI KONGRES
s međunarodnim sudjelovanjem

13th CROATIAN BIOLOGICAL CONGRESS
with International Participation

Poreč, 19 - 23. 09. 2018.

13. Hrvatski biološki kongres

Poreč

ZBORNIK SAŽETAKA

BOOK OF ABSTRACTS

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Zagreb, 2018.

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Cijenjene kolegice i kolege, dragi biolozi!

PRVI KONGRES BIOLOGA HRVATSKE
Poreč, 21—26. rujna 1981.

Prvi kongres biologa Hrvatske održat će se u Poreču od 21. do 26. rujna 1981. godine u organizaciji Hrvatskoga biološkog društva. Cilj mu je da prikaže naša dostignuća u suvremenoj biologiji i odredi smjernice daljeg razvoja biologije u nas. Kongres će raditi u sekcijama u kojima će biti prezentirana duža plenarna predavanja i kratka saopćenja u trajanju do 10 minuta. Sekcije će biti definirane pošto prispiju prijave za sudjelovanje na Kongresu, a bit će koncipirane tako da se što više prožimaju različita a ipak tematski srodna područja biologije. Planirane su ove sekcije: citologija, genetika, mikrobiologija, molekularna biologija, biokemija, biofizika, radiobiologija, histologija, anatomija, razvojna biologija, fiziologija životinja i čovjeka, fiziologija bilja, botanika, ekologija, zoogeografija, biotehnologija, biologija čovjeka, zaštita prirode, zoologija.

Posebna sekcija bit će posvećena akademiku Zdravku Lorkoviću u povodu 80. godišnjice života.

Posebne sekcije obuhvatit će ove teme: suvremeni način izvođenja nastave biologije, osuvremenjivanje nastave i nastavne tehnologije iz biologije, modeli eksperimentalne nastave biologije, te znanstveni i stručni radovi studenata (uz mentora).

Svi sudionici dobit će knjigu sažetaka referata.

Sve obavijesti o Kongresu mogu se dobiti od predsjednika Organizacijskog odbora prof. dra Oskara Springera, Zavod za animalnu fiziologiju, 41000 Zagreb, Roosveltov trg 6, p. p. 933.

N. Banić

Hrvatsko biološko društvo ima bogatu tradiciju djelovanja i veliku ustrajnost u održavanju do sada 12 kongresa biologa Hrvatske od 1981. godine. Proteklih 37 godina nastoji okupiti i svojim intrigantnim predavanjima privući ne samo biologe Hrvatske već i velik broj suradnika i kolega iz zemalja u okruženju te biologe iz drugih zemalja Europe i svijeta. Kongresi biologa Hrvatske nastoje povezivati izvorna znanstvena postignuća s primijenjenim područjima biološke struke i okupiti sve koji žele podijeliti i prenijeti svoja znanja i iskustva, unaprijediti i razvijati različite grane biologije, a ponajprije održati tradiciju i poduprijeti viziju naprednog razvoja biologije u Hrvatskoj.

Hrabro se noseći sa svim poteškoćama koje donosi organizacija jednog ovako velikog kongresa, ove godine iz znanstveno-istraživačke, stručne i društvene perspektive donosimo pregršt aktualnih i "vrućih" bioloških tema koje će nam prezentirati eminentni biolozi iz tuzemstva i inozemstva. Time ukazuemo na veliki potencijal naših djelatnika, a posebice studenata koji svekoliko razvijaju stečena znanja u Hrvatskoj, postajući tako naša i svjetska sadašnjost i budućnost.

U ime Organizacijskog odbora 13. hrvatskog biološkog kongresa, koji se uz prateće simpozije (7. Simpozij Hrvatskog društva za biljnu biologiju, 4. Simpozij Hrvatskog entomološkog društva, 3. Simpozij edukacije biologije, 2. Hrvatski simpozij biologa u zdravstvu i 2. Balkanski herpetološki simpozij) održava od 19. do 23. rujna 2018. godine u Poreču, zahvaljujemo se na dolasku i potpori svim sudionicima kongresa, a osobito kolegicama i kolegama iz čak 16 zemalja (Albanija, Austrija, Belgija, Bosna i Hercegovina, Crna Gora, Češka, Francuska, Irak, Kanada, Mađarska, Makedonija, Njemačka, Poljska, SAD, Slovenija, Srbija, Turska, Ukrajina i Velika Britanija) koji su dali međunarodnu dimenziju našem kongresu. Sudjelovanje na kongresu prijavilo je 375 kolegica i kolega, a na kongresu će biti održano 9 plenarnih predavanja, 144 usmenih izlaganja i 187 posterskih priopćenja.

Hvala našim pokroviteljima i sponzorima koji su na bilo koji način pridonijeli održavanju 13. kongresa biologa Hrvatske u Poreču, a osobitu zahvalnost upućujemo svim članovima organizacijskog i znanstvenog odbora, tehničkoj potpori, PBZ Card-u, hotelskoj kući Valamar, kao i svima Vama koji ćete svojim prisustvom, predavanjima, posterskim izlaganjima te

međusobnim kontaktima doprinijeti ugodnoj, motivirajućoj i opuštenoj atmosferi održavanja kongresa te pridonijeti da on uspije u svim svojim dimenzijama.
Položaj i značaj biološke struke u suvremenom društvu Hrvatske predodređen je ponajviše zalaganjem svakog pojedinca, a osobito marom strukovnih udruga kao što je Hrvatsko biološko društvo, koje treba brižno čuvati svoju tradiciju i kulturu djelovanja.
Sa štovanjem,

Prof. dr. sc. Mladen Kučinić, Predsjednik HBD-a
Izv. prof. dr. sc. Sanja Gottstein, članica Organizacijskog odbora

Esteemed colleagues, dear biologists!

The Croatian Biological Society (CBS) has a rich tradition in activity and great persistence in maintaining up to 12 Croatian biological congresses since 1981. The Society has been trying to reassemble and attract Croatian biologists as well as a large number of associates and colleagues from the surrounding countries, other European countries and biologists all over the world by intriguing lectures during the past 37 years. The Croatian biological congress is held every three years. It links original scientific achievements with applied areas of biological profession and gathering of all those who want to share and convey their knowledge and experience, as well as to advance and develop different branches of biology, and above all to maintain a tradition and to support the vision of the advanced development of biology in Croatia.
We bravely cope with many difficulties during the organisation of such, a large congress, but in spite of that, we bring numerous current and "hot" biological topics through presentations of eminent biologists from the country and abroad. The scientific, professional and social perspectives of the Congress pointing out the great potential of our employees, and especially students who all develop the acquired knowledge in Croatia, thus becoming our own and the world's bright present and future.
The Organizing Committee of the 13th Croatian Biological Congress and the accompanying symposiums (7th Symposium of the Croatian Society for Plant Biology, 4th Symposium of the Croatian Entomological Society, 3rd Symposium on Biology Education, 2nd Croatian Symposium on Biology in Health Care and 2nd Balkan Herpetological Symposium) is thankful to all participants on their arrival and support. We are sending special gratitude to the colleagues from 19 countries (Albania, Austria, Belgium, Bosnia and Herzegovina, Montenegro, Czech Republic, France, Iraq, Canada, Hungary, Macedonia, Germany, Poland, USA, Slovenia, Serbia, Turkey, Ukraine and the United Kingdom), thus giving an international dimension to our congress. Participation at the Congress was filed by 375 colleagues and at the congress will be held 9 plenary lectures, 144 oral and 187 poster presentations.
We are grateful to the patrons and sponsors who contributed to the 13th Croatian Biological Congress in Poreč, and we express our special gratitude to the Organizing and Scientific Committee, the Technical Team, the PBZ Card, the Valamar Hotel House and to all of you who will attend by lectures and posters, contributing to the pleasant, motivating and relaxed atmosphere on the Congress.
The position and significance of the biological profession in the contemporary society of Croatia are predestined mostly by the efforts of every individual, and in particular by the abundance of professional associations such as the Croatian Biological Society, which should carefully guard their tradition and culture of action.
With regards,

Prof. Mladen Kučinić, President of CBS,
Assoc. Prof. Sanja Gottstein, Member of the Organizing Committee

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PLENARNA PREDAVANJA

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PL-1
THE TECHNOLOGY AND BIOLOGY OF SINGLE CELL RNA SEQUENCING

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Recent advances in genomic technology have led to a shift from traditional methods that measure gene expression in samples containing populations of cells ('bulk') towards profiling of hundreds to thousands of individual cells. As a result, cellular properties that were previously masked in "bulk" measurements can now be observed directly for each single cell. One such approach, single cell mRNA sequencing (RNA-seq), has emerged as a powerful tool for surveying the diversity of cell types in a complex sample and has enabled an unbiased, high-throughput, and high-resolution transcriptomic analysis of single cells. Utilizing a single cell RNA-seq approach, researchers have been able to reveal new biology in terms of the composition of tissues. For example, we can detect distinct subpopulations, study rare cells, and identify novel cell types. In my lab we employed single cell RNA-seq to understand the composition and heterogeneity of diverse samples ranging from human and mouse in vitro cultured cells to in vivo derived normal and malignant tissues. Extensive understanding of sample origin and its nature, as well as its unique preparation, were shown to be critical in enabling efficient sample processing and generating high-quality data. Besides single cell RNA-seq, we developed single nucleus RNA-seq for profiling a variety of challenging tissues resistant to cell disassociation. I will present examples of the technical assessment of single cell and single nucleus RNA-seq and the analysis of data produced on different commercial systems (e.g. Fluidigm, Wafergen, 10x Genomics) to illustrate both the key technological advances and challenges of this methodology. I will also discuss our efforts on utilizing single cell RNA-seq to study complex diseases such as cancer. Other biological systems such as embryonic development, tissue regeneration, and the immune response have also been explored. Taken together, the progress made by single cell RNA-seq has greatly impacted our understanding of diverse biological processes. This technology has an exciting future with broad implication for both biological and medical research.

PL-2
FROM ARCHAEA TO MAN: EVOLUTION AND FUNCTIONS OF AN UNIVERSAL TRNA MATURATION PATHWAY

T. Basta Le Berre
HDR, University Paris-Sud, France

Our laboratory investigates evolution and cell biology of Archaea, the third domain of life. I will first briefly describe the discovery of Archaea in the 1970-ties by Carl Woese and present newest findings on diversity, abundance and importance of these organisms in biogeochemical cycling of matter. I will also present the newest hotly debated work, to which our laboratory participated, concerning evolutionary link between Archaea and Eukaryotes. My current work is on the evolution and function of proteins and protein

complexes catalyzing the synthesis of a modified nucleoside, called t6A, found exclusively in tRNA molecules. t6A is universal, found in all autonomously living cells. It was discovered in early 1960-ties and biochemical and structural work established that it promoted translation fidelity. However, the genes encoding the t6A synthesis machinery were only recently identified. This enabled first biochemical and structural studies using purified proteins and I will describe our contribution to this effort. Based on these data and other published work we are currently investigating the complex evolutionary history of the t6A synthetic pathway. Finally, I will present work to which we contributed, and which shows that mutations in t6A synthetic genes are responsible for a severe genetic disease called Galloway-Mowat syndrome. Further work is now going on to understand why and how these mutations impact the function of kidney and brain cells.

PL-3

ANCIENT POLLEN DNA AS A NOVEL TOOL FOR RECONSTRUCTING PLANT POPULATION HISTORIES

A. Prohaska
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Reconstruction of plant population histories holds a central position in the study of ecological and evolutionary processes. For over a century the most commonly used methods for reconstructing ancient plant populations were based on the morphology of fossilised plant remains (e.g. pollen, seeds, phytoliths). Taxonomic and technical limitations of these methods have prompted a search for novel approaches. Ancient DNA (aDNA) has been recently put forward as a promising new proxy for studying late Quaternary plant populations, allowing direct inquiry into their genetic responses to large-scale environmental and anthropogenic changes. While ancient plant DNA can be retrieved from various types of fossils, pollen represents one of the most common and abundant sources for the majority of plant species. However, there are significant challenges associated with retrieving and analysing this type of aDNA, which will require development of new experimental and computational methods. In this talk, I will give a brief account of the history of fossil pollen DNA research, present the work currently being undertaken by our group in Cambridge (with a focus on the high-throughput single-pollen sequencing and its application to reconstructing plant population histories) and discuss future research directions.

PL-4

HISTONE VARIANTS AND THEIR IMPACT ON CHROMATIN ARCHITECTURE AND FUNCTION

Z. Lorković
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Within eukaryotic cells, DNA is wrapped around octameric nucleosomes that serve as building blocks for higher order DNA packaging in chromosomes. Nucleosomes comprise dimers of the four histone proteins, H2A, H2B, H3, and H4. A distinct level of regulation of chromatin plasticity depends on histone variants. Histone variants are functionally-defined isoforms of core histones and are found primarily in families of histones H3 and H2A Beyond a structural role, histones regulate the accessibility of the adjacent DNA strands and the

transcription of associated genes. For example, at the genome-wide level H3.1 correlates with transcriptionally silent heterochromatin whereas H3.3 regulates the chromatin landscapes over gene bodies and is required for proper transcriptional regulation. These activities of H3 variants are modulated by posttranslational modifications of their N-terminal tails. The roles of H2A variants in chromatin organization are less well understood. We are using genomics, biochemistry, genetics, and high resolution microscopy approaches to reveal how H2A variants contribute to chromatin organization and function in plant lineage with major focus on a flowering plant *Arabidopsis thaliana*.

PL-5

A NOVEL ELECTRON MICROSCOPY APPROACH ON THE RISE: FIB-SEM AND 3D IMAGING IN CELL BIOLOGY

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Focused ion beam (FIB)-scanning electron microscopy (SEM) is an emerging technique for studying ultrastructural organization of cells and tissues in three-dimensional (3D) nanometric resolution. Such 3D information can provide insights on the shape, morphology and spatial relations between organelles and neighboring cells in large volumes. Here, I will present sample preparation techniques for (cryo)FIB-SEM imaging approach and a pipeline for sample preparation, data acquisition and 3D rendering that has been set up at the Max Planck Institute of Colloids and Interfaces. As an example of room temperature FIB-SEM approach, I will present research on the assembly of the arthropod cuticle in locusts. Here, the analysis of 3D volumes of the epidermal cell-cuticle interface has helped us understand the mechanisms underlying alternating arrangement of chitin fibers between unidirectional orientation and helicoidal structure. As an example of cryogenic FIB-SEM approach, I will also present a study on vitrified samples of coccolithophores. This approach helped us identify vital components that control the intracellular biomineralization process in these unicellular algae. Moreover, due to 3D visualization we were able to identify structural reorganizations that take place during progression of biomineralization. Overall, the work presented in this talk will demonstrate (cryo)FIB-SEM as a powerful tool to visualize large volumes and various intracellular processes at high precision and give insights into analysis of the acquired datasets.

PL-6

THE MICROBIAL ECOLOGY OF NITRIFICATION - ON THE DISCOVERY OF NEW PHYSIOLOGIES AND THEIR POTENTIAL INFLUENCE ON OUR EVERYDAY LIFE

P. Pjevac
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Nitrification is the oxidation of ammonia to nitrite and finally nitrate. This microbially mediated process is an essential part of the global nitrogen cycle, with wide reaching consequences for our everyday life. For over a century, nitrification has been considered to be an obligatory two-step process, in which the oxidation of ammonia to nitrite and the consecutive oxidation of nitrite to nitrate are carried out by two separate microorganisms.

Around the globe, the activity of microbial nitrifiers is utilized in drinking and wastewater treatment, facilitating the in- and output of clean water from our households. However, microbial nitrification also causes massive fertilizer loses from agricultural fields, directly or indirectly leading to increased eutrophication of downstream waterbodies and increased greenhouse gas emissions. Due to its broad impact on humans and the environment, nitrification is a comparatively well studied and understood biogeochemical process. It is thus even more surprising that an entire group of microorganisms capable of oxidizing ammonia all the way to nitrate – the so called complete ammonia oxidizers or comammox microorganisms – has only been discovered in 2015. With mounting evidence on the importance of comammox organisms for nitrification in a wide range of environments, and in particular in drinking water treatment, comammox research has become one of the primary focuses in the field of nitrification research. Today, a mere three years after their discovery, an impressive body of knowledge on the physiology, ecology and evolution of comammox organisms is already available, and will be summarized in this lecture.

PL-7

NEW PRECISION MEDICINES IN EGFR-MUTATED NON-SMALL CELL LUNG CANCER

I. Štagljar
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University of Toronto, Canada

During my talk, I will describe MaMTH-DS1, a novel live-cell, small molecule screening platform, based on our previously developed Mammalian Membrane Two-Hybrid (MaMTH) assay2-4, for identification of compounds targeting the functional protein interactions of Receptor Tyrosine Kinases (RTKs). As proof-of principle, we used MaMTH-DS to screen a pilot collection of approximately 10,000 small molecules against an oncogenic RTK, an Epidermal Growth Factor Receptor (EGFR) mutant resistant to osimertinib, the latest generation of tyrosine kinase inhibitor used to treat non-small cell lung carcinomas with a specific mutation in the EGFR gene. Our screen and subsequent follow-up analyses in live cells identified several promising EGFR mutant-specific compounds, one of which would not have been identified by traditional in vitro kinase assays, and all of which are the focus of continuing therapeutic study. The highly sensitive and flexible nature of MaMTH-DS also makes it amenable for use with other integral membrane protein targets, such as ion channels, ABC transporters, SLCs and GPCRs, thus expanding its use beyond RTKs. In summary, we believe that MaMTH-DS represents a notable technical advance in the area of drug screening research, and should greatly facilitate the identification of valuable new therapeutic molecules.

PL-8

REGULATION OF GENE EXPRESSION BY POLYCOMB REPRESSIVE COMPLEX 1 (PRC1)

P. Dobrinić
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Polycomb group proteins (PcGs) play an important role in regulation of transcriptional programs underlying normal mammalian development. PcGs function by forming a number of distinct multi-protein complexes which frequently co-occupy unmethylated CpG islands

and reinforce each other's recruitment and activity, resulting in a formation of transcriptionally repressive chromatin domains. Recent findings from our group emphasize the role of one class of PcG complexes, Polycomb Repressive Complexes 1 (PRC1) in the formation of repressive Polycomb domains. PRC1 acts through monoubiquitylation of histone H2A on lysine 119 (H2AK119ub1) and formation of higher order chromatin structures, although exact mechanisms of transcriptional silencing still haven't been elucidated. Previous studies in mouse embryonic stem cells established that genes involved in differentiation and developmental processes are preferentially upregulated in the absence of the core components of PRC1. However, these findings were based on conventional genetic deletion approaches which preclude discovery of primary targets due to the time needed for complete degradation of both mRNA and protein, usually on a scale of days. We used an alternative perturbation system based on auxin-inducible degradation of the PRC1 catalytic subunit which enabled us to detect transcriptional changes happening on a scale of hours. The core PRC1 subunit depletion resulted in a very rapid loss of H2AK119ub1, and immediate and global gene upregulation. Detailed characterisation of direct targets of a major transcriptional regulator, such as PRC1, is required to understand its function and mechanisms behind establishment and maintenance of cellular identity, as well as the consequences of its malfunction in disease.

PL-9

INQUIRY-BASED LEARNING AND INCLUSION IN SCIENCE CLASSES – TWO ANTAGONISTIC FRIENDS

S. Markić
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In the last years, two main topics are occupying science education in general and biology teaching and learning in particular. On the one side, there is a demand for more inquiry-based teaching and learning in science classes. On the other side, influenced also by the UN Convention, there is a call for inclusive educational system. For most of the teachers the question of inquiry-learning is an antagonist to inclusive educational system since the demand on student-centered learning and students' self-regulated learning is a contrast to inclusive education for most of them. Inquiry-based learning is not a method of teaching and learning science, it is more a form of active learning which can be packed in different methods in science classroom. The range is between confirmation inquiry, which is more teacher-centered to open inquiry where students' initiatives and activities are the core of the inquiry. The presentation will focus on the two aspects of modern science education research. The theory and examples of inquiry-based learning in science and biology classes will be given as well as the role of the teacher while practicing inquiry-based learning discussed. Furthermore, the focus will turn to the discussion about the combination of the two above named requirements on science lessons finding the way of combination and integration.

USMENA PRIOPĆENJA

ORAL PRESENTATIONS

2. Balkanski herpetološki simpozij
2nd Balkan Herpetological Symposium

O-1

SINECOLOGY OF EUROPEAN COMMON SPADEFOOT TOAD, *Pelobates fuscus* (Laurenti 1768) (Amphibia: Anura: Pelobatidae), IN THE AGROECOSYSTEMS OF BOSNIA AND HERZEGOVINA

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The European spadefoot toad (*Pelobates fuscus*) is a lowland, nocturnal, semi-fossorial and secretive anuran species with a narrow ecological niche. The southernmost border of its areal reaches the northern parts of Bosnia and Herzegovina (B&H, in Posavina region) where the species is mostly restricted to agroecosystems located in the vicinity of aquatic habitats. In this study, we analysed the population of *P. fuscus* living in B&H in the following ecological aspects: (1) the population density, (2) the sex ratio and sexual size dimorphism characteristics, (3) (micro) habitat preferences and (4) the diet habits. The number of individuals estimated using the transect method showed to be the highest in the locality of Čardak (703 individuals per hectare), followed by locality Gromiželj (309 individuals per hectare) and lowest at locality Okanovići (191 individuals per hectare). *P. fuscus* clearly prefers arable lands, were most of the individuals can be found on cultivable surfaces. The highest activity of *P. fuscus* occurs during air temperatures that vary between 15 and 18°C, relative humidity values from 60 - 80% and soil temperatures of 11 to 13°C. The diet analysis confirm that *P. fuscus* is a generalist that shows very little specialization in prey choice but proved to be a very effective predator that can be used for integrated pest management (IPM) programs.

Keywords: fossorial species, Pannonian plain, distribution border, agriculture, ecology

O-2

EFFECT OF BIOTIC AND ABIOTIC FACTORS ON ASYMMETRY OF PHYSICAL CHARACTERISTICS IN COMMON WALL LIZARD (*Podarcis muralis*) AND HORVATH'S ROCK LIZARD (*Iberolacerta horvathi*)

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We analysed asymmetry in the common wall lizard (*Podarcis muralis*) and the Horvath's rock lizard (*Iberolacerta horvathi*) from 16 populations in Slovenia and Croatia to understand their connection with potential stress factors: altitude, urbanization and the presence of another species (interspecific competition). We also compared morphological body characteristics between the two species. We used geometric morphometric methods to create landmark coordinates in the photographs of lizards' heads. Modified ANOVAs and an asymmetry index were used to find the differences in four physical characteristics: shape and size of the head, body size and the number of supraciliar scales. We found fluctuating asymmetry in the number of supraciliar scales and the shape of the head in all of our samples. Both species had more asymmetric heads in the intermediate altitudes, which might be connected with lower availability of suitable habitats. Common wall lizards from urban environment did not have more asymmetries compared to those living in natural environments. Contrary to our expectations, we found more asymmetries in allotopic than syntopic populations, indicating limited effect of interspecific interactions. Horvath's rock lizards had more asymmetric heads, which suggests they might be exposed to greater environmental stress, although genetic effects are also possible, since directional asymmetry was also detected.

Keywords: lizards, *Podarcis muralis*, *Iberolacerta horvathi*, stress, asymmetry

O-3

TOWARDS THE END OF FACULTATIVE PAEDOMORPHOSIS IN BALKAN NEWTS? A FOCUS ON MONTENEGRIN POPULATIONS

M. Denoël[1,2], G.F. Ficetola[3], N. Sillero[4], I. Muhovic[5], V. Ikovic[5], M. L. Kalezić[6], G. Džukić[6], B. Lejeune[1]

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Facultative paedomorphosis is a polyphenism that implies the development of two alternative adult phenotypes: the metamorphs that lose their gills at metamorphosis and the paedomorphs, which retain them. This process is much rarer than metamorphosis but in some areas (e.g. in Balkans) some important populations have been described. We aimed to determine whether both phenotypes were declining and what were the causes of decline in eco-cultural landscapes of Montenegro. High population losses were found in both ponds and lakes. The decline concerned first paedomorphs and then metamorphs. The situation is particularly catastrophic with almost all populations of paedomorphic newts now extinct. This includes all lake populations and endemic “paedomorphic” taxa. The main environmental driver of the decline was the presence of introduced fish. More recently, introductions of crayfish added a new pressure, contributing further to population declines. Based on Corine land cover, land use had no significant effect on population losses but care should also be taken to future changes of land use. Consequently, our results show that the status of biodiversity in Montenegrin small waterbodies is alarming with few sites remaining undisturbed. Despite their location in apparently pristine places, waterbodies of Montenegro follow the trends found in other parts of Europe. There is therefore an urgent need to preserve these habitats before their biodiversity belong to the past.

Keywords: amphibians, biodiversity, conservation, Montenegro, paedomorphosis

O-4

KEY HABITATS OF A COLD-ADAPTED SNAKE IN THE WARMING MEDITERRANEAN BASED ON HABITAT SUITABILITY, LAND USE AND CLIMATE CHANGE

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We mapped threats potentially affecting the long term survival of Greek Meadow Viper (*Vipera graeca*) populations to identify the high-importance habitats for the conservation of this species. We used nine variables of three focus points to assess conservation value of habitats: habitat quality (size of suitable habitat, occupancy, climate and vegetation suitability), climate change (persistence, altitudinal shift) and land use (disturbance, habitat degradation and loss). We applied Zonation, a systematic conservation planning algorithm to identify priority areas where the population survivorship and sustainability are at the highest potential. In the foreseeable future, 90% of current habitats occupied by this species will most likely become unsuitable and conservation actions need to be implemented to reduce extinction as this is already an endangered species. Spatial conservation planning tools can be applied to identify and map proposed area networks for the protection and preservation of single species, if threats are known and can be mapped and transformed into conservation value layers.

Keywords: climate change, habitat suitability, land use, spatial conservation planning, protected area

O-5

FOOD SPECTRUM OF THE VIVIPAROUS LIZARD *Zootoca vivipara* (LICHTENSTEIN, 1823) (REPTILIA: LACERTIDAE) FROM ITS SOUTHERN RANGE OF DISTRIBUTION

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The Viviparous lizard is the reptile species with the widest distribution in Eurasia (from Ireland to Japan and from South Europe to north of the Arctic Circle). In the southern parts of its distribution range such as in Bulgaria, this species is a glacial relict, restricted to mountainous areas. In Bulgaria, *Zootoca vivipara* is known from isolated populations in the mountains of Stara Planina, Vitosha, Rila, Pirin, Osogovo and Western Rhodopes, where it inhabits open humid areas from 1200 to 2900 m a.s.l. In spite of its wide distribution, there is still lack of knowledge on its dietary habits, especially from the southern part of the range. The study area embaraced three sites in Bulgaria (Vitosha Mt, Rila Mt and Stara Planina Mt.). There were visited in 2016 and 2017 during the active season (May–September) and 343 Viviparous lizards were captured by hand. To investigate the food preferences faecal samples were used. A total of 166 faecal samples were collect that could be inidivually attached to specimen, age, sex and

ocupied habitat. In adition, to investigate the use of food resources, invertebrates were colected from the field using pit-ful traps. The most preferred invertebrates groups were Auchenorrhyncha, Araneae and Coleoptera, followed by Fomicidae, Opiliones, Orthoptera and Heteroptera. In spring (May and June) the diet were dominated by beetles and spiders, while the sumer diet were more diverse and dominated by cicadas, spiders, ants, opiliones and grasshoppers.

Keywords: food niche, lacertid lizard, mountain habitats

O-6

GEOGRAPHIC VARIATION IN WATER RETENTION IN FRAGMENTED POPULATIONS OF ALPINE SALAMANDERS

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The alpine salamander can be found continuously over most of the Alpine arc, but has a fragmented distribution in the Dinaric Alps. Habitat use exhibits considerable geographic variation: while northern Dinaric populations typically inhabit forested areas, southern populations are entirely restricted to exposed alpine meadows above the tree line. On a daily basis, the above ground activity of the alpine salamanders is strongly dependent on the relative humidity of the air, which is influenced by the water vapor pressure and temperature. We hypothesized that: (a) the crucial parameters for salamanders' daily activity strongly differ between the habitats of the northern and southern Dinaric populations, (b) that populations originating from a drier habitat have a higher water retention to compensate for the increased exposure to drier conditions. We performed a 52 minutes long water loss experiment in a climate chamber in which we manipulated values of air temperature and humidity (a total of 15 different treatments) and followed the body mass loss/gain of animals originating from the northern and southern Dinarides. All our hypothesis revealed true.

Keywords: water loss, amphibians, adaptation, skin resistance

O-7

TOXIN VARIATION AMONG POPULATIONS OF A TERRESTRIAL SALAMANDER AND THE POSSIBLE ROLE OF PREDATION PRESSURE AND INFECTION RISK

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Amphibian poisons provide protection against predators and against microbial infections. It could therefore be expected that geographic variation in toxin composition will reflect variation in predation pressure and infection risk. However, few studies have tested this hypothesis. We studied toxin variation and its possible reasons in four populations of *Salamandra atra* in the Dinaric Alps. Populations differed in the presence of snake species known to predate on S. atra. Toxin samples were collected in the field and analysed using UPLC-MS/MS. Soil samples were taken in each habitat in order to estimate the abundance and diversity of micro-organisms in the environment, by cultivation and DNA-analyses. Salamanders had smaller poison glands, and produced less toxins with lower amounts of samandarine where snakes were scarce, but there was no perfect match between toxin variation and snake presence. Salamanders produced more samandarone in populations with a higher infection risk, however, contributions of samandarone were lower than expected. In conclusion, there was only limited support for the hypothesis that toxin variation is driven by variation in predation pressure and infection risk. Further research on this species, over a broader geographic range, might lead to more conclusive insights in the evolution of toxin diversity.

Keywords: amphibian toxins, geographic variation, *Salamandra atra*, samandarines, snake predation

O-8

TKO JE TKO? INTERAKTIVNI INDIVIDUALNI IDENTIFIKACIJSKI SISTEM (I3S PROGRAM) ALAT ZA NENAMETLJIVU IDENTIFIKACIJU ENDEMSKE MOSORSKE GUŠTERICE, *Dinarolacerta mosorensis*

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Označavanje jedinki je često potrebno prilikom terenskih istraživanja i proučavanja ponašanja za potrebe prepoznavanja pojedinih jedinki. Razvojem tehnologije, starije metode poput rezanja ljuski ili prstiju, žigosanja, bojanja, postavljanja oznaka, označavanja trakama se zamjenjuju identifikacijom upotrebom tehnologije. U posljednjem desetljeću nenametljiva i jeftina metoda digitalne identifikacije jedinki postala je popularna te s njom možemo pravilno prepoznati veliki broj jedinki. I3S program je prvenstveno napravljen za raspoznavanje jedinki morskih psina, ali se uspješno koristi za prepoznavanje gušterica iz porodice Lacertidae. Mosorska gušterica endem je zapadnog Balkana s procijenjenim područjem rasprostranjenosti manjim od 2000 km2. Također, kvalifikacijska je vrsta ekološke mreže Natura 2000. U 2017., opsežno istraživanje populacijskih značajki i biologije započelo je na Biokovu, jednoj od dvije trenutno potvrđene lokacije u Hrvatskoj. Kako bi ispravno identificirali svaku jedinku koristili smo program Interaktivni individualni identifikacijski sistem. Fotografije prva četiri reda trbušnih ljusaka tijela korištene su za raspoznavanje i usporedbu. Preliminarni rezultati temeljeni na 30 jedinki su uspješni i sve jedinke su se mogle razlikovati. S većim uzorcima, moguće je da će za određivanje biti potrebno više vremena, ali u tom slučaju, korištenje dodatnih parametara poput spola ili obojanost olakšati će identifikaciju.

Ključne riječi: Lacertidae, digitalna identifikacija, označavanje jedinki, Natura2000 vrsta, Biokovo

WHO IS WHO? INTERACTIVE INDIVIDUAL IDENTIFICATION SYSTEM (I3S SOFTWARE) AS A TOOL FOR NON-INTRUSIVE IDENTIFICATION OF AN ENDEMIC LACERTID SPECIES, MOSOR ROCK LIZARD, *Dinarolacerta mosorensis*

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Marking individuals is often needed in field and behaviour studies that required repeated identification of individual animals. With the development of technology, older methods like scale or toe clipping, branding, painting, tagging, banding are slowly being replaced by technology facilitated identifications. In last decade non-intrusive and inexpensive digital identification of individual animals become popular and with these method large sets of individuals can be re-identified. I3S Software is originally developed for identification of sharks but has successfully been already used for identification of Lacertidae lizard species. Mosor rock lizard is an endemic species of western Balkans. It's area of occurrence is approximated to be less than 2000 km2. It is also a Natura 2000 species. In 2017, an extensive study of its population parameters and biology has started on Mt. Biokovo, Croatia. To facilitate the correct identification of each individual, we used the program Interactive Individual Identification System. Photographs of first four rows of ventral scales of the lizard body were used for identification and comparison. Preliminary results based on 30 individuals were satisfactory, and all the individuals could be distinguished successfully by the program. With larger datasets, it is possible that the identification could be more time consuming, but in that case, additional parameters like sex or colorations may be used for correct identification.

Keywords: Lacertidae, digital identification, individual marking, Natura2000 species, Biokovo Mt.

O-9

SPATIAL CONSERVATION PRIORITIES FOR THE AMPHIBIANS AND REPTILES IN THE BALKAN PENINSULA: PRELIMINARY RESULTS OF THE BALKAN HERPS PROJECT

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The Balkan Peninsula is a historical speciation centre, refugium and currently a hotspot for biological diversity in Europe. It has more than 120 species of amphibians and reptiles with high level of endemism. Therefore, protection of the Balkans is a high priority and requires effective protected area coverage. The aim of the Balkan Herps project is to assess the representation of the regional herpetofauna under the current protected area network and reveal potential shortfalls. We created a database of distribution records (openbiomaps.org / projects / balkanherps) and built 30' resolution species distribution models using Bioclim and Envirem variables. We used the systematic conservation planning tool Zonation to identify spatial conservation priorities. Generally, conservation values were highest in the Adriatic Coast, Peloponnese, Thrace and Danube Delta and lower in the Central Balkans. We identified the top 17% of the region and calculated the extent of coverage by protected areas. We found one-third coverage with important gaps occurring in non-European Union countries, but we also found gaps in the EU, mostly in the southern Balkans. It is promising that many deficiencies can be solved by the designation of new Natura 2000 areas in EU candidate countries. There are still gaps in the Balkan Herps database, we hope that the ongoing collaboration will lead to a cross-border conservation plan in the Balkan Peninsula for an effective protection of herpetological diversity.

Keywords: herpetology, cross-border analysis, climatic variables, prioritisation, gap analysis

O-10

MORFOLOŠKE ZNAČAJKE PUNOGLAVACA SMEĐE KRASTAČE, *Bufo bufo* (LINNAEUS, 1758) I ZELENE KRASTAČE, *Bufotes viridis* (LAURENTI, 1768) (ANURA, BUFONIDAE) IZ HRVATSKE

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Iako su smeđa i zelena krastača česte vrste vodozemaca u Hrvatskoj, malo je podataka o morfološkim značajkama njihovih punoglavaca u Hrvatskoj. Tijekom travnja i svibnja 2015. godine sakupili smo 63 punoglavca vrste *Bufo bufo* na lokalitetu Rajić blizu Bjelovara i 9 punoglavaca vrste *Bufotes viridis* na lokaciji Hrvace (Dalmatinska zagora). Odredili smo razvojne stadije punoglavaca (prema Gosner, 1960) i proveli mjerenje 26 morfometrijskih mjera u svrhu pronalaska morfometrijskih razlika među njima. Rezultati neparnog dvosmjernog t-testa ukazuju na statistički značajne razlike ($p<0.001$) između punoglavaca smeđe i zelene krastače u ukupnoj dužini, dužini tijela (pogled odozgo i bočno), širini tijela, interorbitalnoj udaljenosti,

oko-rep dužini, visini repnog mišića pri bazi repa kao i u omjerima širine glave i maksimalne visine repa pri bazi repa sa dužinom tijela. Punoglavci vrste *B. bufo* imaju izometričan rast osam tjelesnih mjera između stadija 27, 31 i 41 kao i četiri mjere stražnjih nogu između stadija 38, 39 i 41. Punoglavci smeđe i zelene krastače ukazuju na snažnu korelaciju ukupne dužine sa dužinom repa i tijela. Rezultati ukazuju na to da su punoglavci vrste *B. viridis* elegantniji, izduženiji i, prema većini mjerenih parametara, veći od punoglavaca smeđe krastače, sa većom dužinom repa i tijela od ranih stadija do stadija koji prethode kraju metamorfoze.

Ključne riječi: punoglavci, morfološke značajke, metamorfoza, Hrvatska

MORPHOLOGICAL TRAITS OF *Bufo bufo* (LINNAEUS, 1758) AND *Bufotes viridis* (LAURENTI, 1768) (ANURA, BUFONIDAE) TADPOLES FROM CROATIA

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Although common and green toads present frequent amphibian species in Croatia, there are few data about morphological traits of their tadpoles in Croatia. During April and May 2015, we collected 63 Bufo bufo tadpoles on a locality Rajić near Bjelovar and 9 Bufotes viridis tadpoles on a location Hrvace (Dalmatinska zagora). We determined tadpoles' development stages (according to Gosner, 1960) and conducted a measurement of 26 morphometric measures in order to find the morphometric differences between them. The results from unpaired two-tailed t-tests show statistically significant differences ($p<0.001$) between *B. bufo* and *B. viridis* tadpoles in the total length, body length (dorsal and lateral view), body width, interorbital distance, eye-tail length, tail muscle height at the tail base as well as in the ratios of both the head width and maximum tail height at the tail base with the body length. *B. bufo* tadpoles show the isometric growth of eight corporal measures between stages 27, 31 and 41 as well as of four hind legs' measures between stages 38, 39 and 41. *B. bufo* and *B. viridis* tadpoles show strong correlation between the total length and the tail length as well as with the body length. Results indicate that *B. viridis* tadpoles are more elegant, elongated and, according to most measured parameters, larger in size than B. bufo tadpoles, with bigger tail and body length from early stages to the stages near the end of metamorphosis.

Keywords: tadpoles, morphological traits, metamorphosis, Croatia

O-11

MORFOLOŠKE RAZLIKE KOD OTOČNE I KOPNENE POPULACIJE BLAVORA, *Pseudopus apodus* (SQUAMATA: ANGUIDAE)

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Blavor, *Pseudopus apodus* (Pallas, 1775) je veliki, beznogi gušter koji živi na kopnu, ali naseljava i mnoge mediteranske otoke. U sklopu našeg istraživanja, usredotočili smo se na dvije populacije blavora, kopnenu (s Klisa) te otočnu (s otoka Cresa). Proučavali smo morfologiju i prilagođeni maseni indeks (scaled mass index) te smo ispitali je li otočna populacija blavora različita od populacije s kopna. Također, istražili smo moguće razlike u korištenju staništa između istraživanih populacija. Očekujemo da će jedinke iz otočne populacije imati veće tijelo te izraženiji seksualni dimorfizam nego populacija s kopna, budući da otoci obično imaju specifične okolišne uvjete na koje populacije odgovaraju promjenama u ponašanju, ekologiji, morfologiji ili fiziologiji. Uzorkovali smo 164 jedinke; 92 s Klisa i 72 s otoka Cresa te smo pokazali da kod blavora postoji razlika u veličini među istraživanim populacijama. Kod populacije sa Cresa postoji izražen seksualni dimorfizam koji nije prisutan kod populacije s Klisa. Neki okolišni čimbenici važni za ovu vrstu su ujednačeni u obje populacije (temperatura tla, udaljenost od skloništa) te nisu pod utjecajem tipa staništa. S druge strane, vegetacijski pokrov se razlikuje među lokacijama te je vegetacija razvijenija na Klisu nego na otoku Cresu.

Ključne riječi: Mediteran, Sauria, Anguidae, seksualni dimorfizam, *Pseudopus apodus*

MORPHOLOGICAL DIVERGENCE IN INSULAR AND MAINLAND POPULATIONS OF THE EUROPEAN GLASS LIZARD, *Pseudopus apodus* (SQUAMATA: ANGUIDAE)

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The European glass lizard, *Pseudopus apodus* (Pallas, 1775) is a large, legless lizard that can be found both on the mainland and on many Mediterranean islands. Our research focuses on two populations of the European glass lizard from the eastern Adriatic coast, one from mainland (Klis), and one from an island (Cres). We studied morphology and scaled mass index (SMI) and tested if insular *P. apodus* are different than the mainland population. In addition, we explored possible differences in habitat utilization between these populations. We predict a larger body size and a more pronounced sexual dimorphism in the island population, since islands usually comprise specific conditions to which populations respond by changes in behaviour, ecology, morphology, or physiology. We sampled 164 individuals; 92 from Klis, and 72 from Cres Island and showed that the European glass lizard exhibits size differences between the populations. In addition, the Cres Island population showed clear sexual dimorphism which is lacking in the mainland population. Some ecological characteristics of this species are similar in both populations (soil temperature, distance to the hiding place) and are not influenced by habitat differences. However, the vegetation cover differs between two sites, with more vegetation present in Klis than on Cres.

Keywords: Mediterranean, Sauria, Anguidae, sexual dimorphism, Sheltopusik

O-12

PRAĆENJE STANJA KOPNENE KORNJAČE (*Testudo hermanni*) NA PODRUČJU NACIONALNOG PARKA MLJET

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Kopnena kornjača, *Testudo hermanni* Gmelin, 1789, je mediteranska vrsta koja naseljava priobalni dio Hrvatske i veći broj otoka. Radi se o gotovo ugroženoj vrsti, prema IUCNu, koju primarno ugrožavaju fragmentacija i gubitak staništa. Vrsta je također vrlo popularna na crnom tržištu kućnih ljubimaca što ju čini još ranjivijom na opadanje brojnosti populacije. Za ovu vrstu iznimno je važno provođenje konzervacijskih aktivnosti kako bi se smanjio utjecaj navedenih ugroza na divlje populacije. Ovdje izlažemo rezultate praćenja stanja i procjene brojnosti kopnene kornjače na području Nacionalnog parka Mljet provedenog 2015. i 2018. godine. Prema našim saznanjima, ovo su prvi rezultati te vrste za kopnenu kornjaču u Hrvatskoj. Za procjenu smo koristili 10 nasumično odabranih linearnih transekata, svaki duljine 100 m, raspoređenih na četiri lokaliteta unutar granica nacionalnog parka. Monitoring se provodi na način da dvije osobe hodaju paralelno duž transekta i bilježe okomitu udaljenost svake kopnene kornjače. Prikupljeni podaci su analizirani u program DISTANCE kako bi se izračunala gustoća jedinki i ukupna brojnost na definiranoj površini. Veličina populacije je procijenjena korištenjem dva različita modela, no oba su pokazala gotovo jednaku procjenu od oko 10 000 jedinki kopnenih kornjača na području NP Mljet.

Ključne riječi: veličina populacije, gustoća populacije, zaštićena područja, Mljet

MONITORING OF HERMANN'S TORTOISE (*Testudo hermanni*) IN NATIONAL PARK MLJET

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Hermann’s tortoise, *Testudo hermanni* Gmelin, 1789, is a Mediterranean species that inhabits costal part of Croatia and several islands. It is near threatened species whose major threats are fragmentation and loss of habitat. This species is also very popular on black market for pet trade making it even more vulnerable to population decline. Therefore, conservation measures should be in place to reduce the impact of factors which negatively affect these populations. We started monitoring of this species on Mljet island in 2015 and continued in 2018. This is, to our knowledge, the first population monitoring of this species in Croatia. It was performed using 10 randomly selected line transects, each 100 meters long, on four localities in the National Park Mljet. Monitoring is performed by two people who walk parallelly and mark down the perpendicular distance of each individual they see on the transect. DISTANCE software is later used to calculate the density of Hermann’s tortoise and to estimate the number of animals in specified area. Size of Hermann’s tortoise population in National Park

Mljet was estimated using two models and both gave similar estimate of around 10 000 individuals.

Keywords: population size, density, protected areas, Mljet

O-13

JE LI SPONTANA LOKOMOTORNA AKTIVNOST PONOVLJIVA U UVJETIMA RAZLIČITIH TJELESNIH TEMPERATURA I MEĐU JEDINKAMA MLADIH VODENJAKA?

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Lokomotorna aktivnost jedna je od glavnih značajki živih organizama. Iako ovo obilježje određuju važne ekološke procese, poput sposobnosti rasprostranjenja i interakcija jedinki, nedovoljno je znanja o izvorima njegove varijabilnosti. U našem smo istraživanju ispitivali utjecaj tjelesne temperature (13, 18, 23, i 28°C) i individualnosti na spontanu lokomotornu aktivnost u mladih jedinki planinskog vodenjaka, Ichthyosaura alpestris, tijekom dva tjedna. Spontanu smo lokomotornu aktivnost opisali pomoću četiri svojstva: ukupna pređena udaljenost, maksimalna brzina kretanja, frekvencija kretanja i ukupna aktivnost. Dobiveni rezultati pokazuju kako su tjelesna temperatura, identitet ispitivanih jedinki, ali i vrijeme testiranja imali različiti utjecaj na navedena svojstva. U prvom tjednu ispitivanja svojstva koja su bila pod utjecajem temperature i razlikovale su se među jedinkama, nisu bila podložna njima u drugom tjednu, osim u slučaju frekvencije kretanja. Usprkos razlici u dobivenim apsolutnim vrijednostima za sva svojstva, individualne su vrijednosti iz prvog tjedna bila pozitivno asocirana s onima iz drugog tjedna. Odnosno, jedinke koje su bile aktivnije u prvom tjednu testiranja, bile su aktivnije i tijekom drugog pokazujući tako kratkoročnu ponovljivosti ovih svojstava. Ovi će rezultati biti u budućnosti od velikog značaja prilikom modeliranja utjecaja klimatskih promjena na interakcije vrsta.

Ključne riječi: ponovljivost, utjecaj temperature, vodozemci, lokomotorna aktivnost

IS SPONTANEOUS LOCOMOTOR ACTIVITY REPEATABLE ACROSS BODY TEMPERATURES AND AMONG INDIVIDUALS? A CASE STUDY ON JUVENILE NEWTS?

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Locomotor activity is among major attributes of living organisms. Although this trait determines important ecological processes, such as dispersal and species interactions, the sources of its variation are not fully understood. We repeatedly examined the influence of body temperature (13, 18, 23, and 28°C) and individual identity on spontaneous locomotor activity in juvenile alpine newts, Ichthyosaura alpestris, during two consecutive weeks. Spontaneous locomotor activity was characterized by four traits: total distance traveled, maximum velocity, frequency of movements and total activity. Body temperature, individual identity and the week of measurement had various influence on the examined traits. Those

traits which were affected by body temperature and individual identity during the first round of measurements, were not affected by them during the second round, except for frequency of movements. Despite distinct absolute values in all traits, individual values from one week were positively associated with those from the second week. In other words, more active individuals during the first week were also more active during the second week suggesting short term repeatability of this trait. Thermal dependence of spontaneous locomotor activity diverged from assumptions of current theory. This result has implications for further modeling the effect of climate change on species interactions.

Keywords: repeatability, thermal dependence, amphibians, locomotor activity

O-14

HYBRIDIZATION AND EARLY LIFE-HISTORY TRAITS: *Triturus ivanbureschi* AND *T. macedonicus* CONTACT ZONE

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Large-bodied newts (genus *Triturus*) comprise nine ecologically, morphologically and geographically distinct species with common hybridization at species contact zones. Especially interesting is *Triturus ivanbureschi* and *T. macedonicus* contact zone where *T. macedonicus* expanded and transected *T. ivanbureschi* range. A natural hybrid population consisting of hybrids derived through many years of mutual hybrid crossing and backcrossing with parental species was confirmed at south-eastern Serbia. To explore reproductive potential, viability and survival rate of embryos of parental species and their hybrids we performed controlled, common garden experiment with four experimental crossings: 1) *T. ivabureschi* ♀ × *T. ivabureschi* ♂ ; 2) *T. ivabureschi* ♀ × T. macedonicus ♂ ; 3) *T. ivabureschi* ♂ × *T. macedonicus* ♀ ; 4) *T. macedonicus* ♂ × *T. macedonicus* ♀ . For each crossing we recorded the number of eggs laid per female, the number of developing eggs, the viability of embryos and hatch rate. We found that females of *T. macedonicus* have higher reproductive potential than *T. ivanbureschi*, while no differences in the survival rate between species and among parental species and their hybrids were found. Species and their hybrids did not differ in the viability of embryos, and hatch rate ($P > 0.05$ in all pairwise comparisons). These preliminary results suggest that similar rates of development could contribute to the maintenance of *T. ivanbureschi* and *T. macedonicus* hybrid zone.

Keywords: hybrid zone, reproductive potential, viability, survival rate

O-15

DISTRIBUTION AND POPULATION CHARACTERISTICS OF KARST VIPER (*Vipera ursinii macrops* MÉHELY, 1911) (REPTILIA: SERPENTES, VIPERIDAE) POPULATION FROM LOVĆEN MOUNTAIN (MONTENEGRO)

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Karst Viper is a relict species that is endemic to the Balkan Peninsula. The distribution of this viper in Montenegro is still not precisely defined, while the regionally scientists have been researching it for over a decade. Due to the known vulnerability of this species it was realized that it was imperative to protect populations of Karst Viper. Using common research methods (i.e. performing research of all habitats, describing habitat`s characteristics, marking found samples, taking morphometric and meristic measures), we found three new locations where Karst Viper is present on Lovćen Mt. First location is intersected by a road with a high impacting human traffic as a negative factor. The second and third locations are well preserved and natural. Considering ten found individuals, in our opinion, these locations contain a stable population of this species. They are all part of one population, thus there are no physical barriers between them. Additionally, population on Lovćen Mt. is the only Karst viper population that inhabits Mediterranean biogeographical region. According to earlier studies Karst Viper is genetically divided in “croatian” and “macrops” clade, while, in Montenegro is present only “macrops” clade, but specimens from Lovćen Mt. were not included. Thus, by comparing morphometric and meristic data of individuals from Lovćen Mt. and Bjelasica Mt. it was concluded that both populations belong to the “macrops” clade, but genetic confirmation is still needed.

Keywords: Alpine meadows, Balkan, endemic, relict

2. Hrvatski simpozij biologa u zdravstvu

O-16

NGS U MOLEKULARNOJ DIJAGNOSTICI: PRIMJENE U DIFERENCIJALNOJ DIJAGNOSTICI, PRAĆENJU I PREDIKCIJI BOLESTI

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Masivno paralelno sekvenciranje, češće zvano Next-generation sequencing (NGS), je pristup sekvenciranju koji se oslanja na višestruko paralelnu obradu genskih sekvenci. Takav pristup omogućuje obradu stotine i tisuće puta više podataka od klasičnog Sanger sekvenciranja (Sekvenciranje prve generacije) u istom vremenskom okviru. NGS metode se drastično razlikuju od proizvođača do proizvođača, no sve ih odlikuje obrada od deset pa do više stotina milijuna baza u jednom pokretanju uređaja. U molekularnoj dijagnostici se najčešće koriste ciljani paneli gena. Prilagođeni su testiranje nasljednih ili stečenih bolesti, procjenu rizika od nastanka bolesti kao i određivanje terapije kod onkoloških bolesnika. Najnoviji potencijal NGS-a je tekućinska biopsija. Ta metoda detektira cirkulirajuću tumorsku DNK u plazmi čak i ako je zastupljena u tisuću puta manjoj koncentraciji od ostale DNK iz normalnih stanica. Upravo tu dolazi do izražaja mogućnost paralelnog očitanja svakog nukleotida u više tisuća puta i detekcije mutacija na pojedinim molekulama DNK.

Ključne riječi: NGS, tekućinska biopsija, genski panel, molekularna dijagnostika

NGS IN MOLECULAR DIAGNOSTICS: DIFFERENTIAL DIAGNOSTICS, DISEASE PREDICTION AND PATIENT FOLLOW UP APPLICATION

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Masive parallel sequencing, also known as Next-generation sequencing (NGS), is a high-troughput approach to sequencing. It enables processing of hundreds and thousands times more data than Sanger sequencing (first generation sequencing) in the same timeframe. NGS methods are drastically different depending on the producer, however all are distinguished by processing from ten to several hundreds millions of bases in one run. In molecular diagnostics target gene panels are most common. They are addapted for testing inherited disease, disease risk assessment and oncology therapy selection. Recent NGS potential is liquid biopsy. It is a method that detects circulating tumor DNA in plasma even if it is present in thousand time smaller concentration than the DNA from normal cells. Here we see the importance of reading every nucleotide several thousand times and detecting mutations on single molecules of DNA.

Keywords: NGS, liquid biopsy, molecular diagnostics

O-17
PRVI DOKAZI ARBOVIRUSA U KOMARCIMA NA PODRUČJU HRVATSKE

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Tijekom posljednjeg desetljeća na području Hrvatske dokazane su autohtone arbovirusne bolesti čije uzročnike prenose komarci: dengue groznica (DENV), West Nile (WNV) i Usutu neuroinvazivna infekcija (USUV). Unesene bolesti povremeno su dokazane u putnika: dengue, chikungunya groznica (CHIKV) i Zika virusna infekcija (ZIKV). Na području Hrvatske do sada su pronađene 52 vrste komaraca, a među njima je nekoliko vrsta sa značajnim potencijalom za prijenos bolesti (vektori). Tigrasti komarac, *Aedes albopictus* invazivna je vrsta komaraca do danas udomaćena u priobalnom području, na otocima i dijelovima kontinentalne Hrvatske. Vrsta je visokog potencijala za prijenos DENV i CHIKV te je potencijalni prijenosnik ZIKV. Prirodni ciklus WNV i USUV uključuje ptice kao glavne domaćine virusa i komarce kao vektore. Vrsta komaraca s najvećim potencijalom za prijenos navedenih virusa je *Culex pipiens* kompleks. U razdoblju od 2015. do 2017. godine na području sjeverozapadne Hrvatske obavljeno je uzorkovanje odraslih jedinki komaraca radi testiranja na prisustvo arbovirusa. USUV RNA dokazana je u jednom od ukupno 80 testiranih skupnih uzorka komaraca (2.459 jedinki) vrste *Ae. albopictus* s područja grada Zagreba i u jednom od ukupno 25 skupnih uzoraka komaraca (648 jedinki) vrste *Culex pipiens* kompleks s područja Međimurske županije. U jedinkama vrsta *Aedes vexans* i *Ochlerotatus sticticus* nije dokazana RNA virusa WNV, USUV i Tahyna virusa.

Ključne riječi: komarci, arbovirusi, Hrvatska

FIRST DETECTION OF ARBOVIRUSES IN MOSQUITOES IN CROATIA

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During the last decade, autochthonous mosquito-borne arboviral diseases such as dengue fever (DENV), West Nile (WNV) and Usutu (USUV) neuroinvasive infection were detected in Croatia. Imported dengue, chikungunya (CHIKV) and Zika virus (ZIKV) infections were sporadically reported in travelers. So far, 52 mosquito species were detected in Croatia, several species of which are potential vectors for arboviruses. Asian tiger mosquito, *Aedes albopictus* is an invasive mosquito species established in the coastal areas, on the islands and in some regions of the continental Croatia. The species is a potential vector for DENV and CHIKV as well as ZIKV. The natural cycle of WNV and USUV include birds (reservoirs) and mosquitoes (vectors). *Culex pipiens* complex is the main vector for both viruses. In the period from 2015 to 2017, adult mosquitoes were collected in northwestern Croatia and tested for the presence of arboviruses. USUV RNA was detected in one of the 80 tested *Ae. albopictus* pools (2,459 mosquitoes) from the City area of Zagreb and one of the 25 tested *Culex pipiens* complex pools (648 mosquitoes) from the area of Međimurje County. No one pool of *Aedes vexans* and *Ochlerotatus sticticus* mosquitoes was positive for WNV, USUV or Tahyna virus RNA.

Keywords: mosquitoes, arboviruses, Croatia

O-18
TIPIZACIJA GENA HLA-DRB1 U DIJAGNOSTICI REUMATSKIH BOLESTI

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Određivanje gena HLA (tipizacija HLA) koristi se dijagnostici autoimunih i reumatskih bolest. Cilj istraživanja bio je utvrditi učestalost gena HLA-DRB1 u skupini od 628 bolesnika, upućenih u Odjel radi određivanja gena HLA, tijekom godinu dana, sa slijedećim uputnim dijagnozama: upalna poliatropatija (IPA; N=239), reumatoidni artritis s pozitivnim reuma faktorom (RA/RF+; N=70), reumatoidni artritis s negativnim reuma faktorom (RA/RF-; N=83), psorijatični artritis (PsA; N=112), ankilozantni spondilitis (AS; N=61), spondiloartritis (SpA; N=63). Kontrolnu skupinu su činili darivatelji iz Hrvatskog registra dobrovoljnih darivatelja koštane srži. Geni HLA-DRB1 određeni su svim ispitanicima metodom PCR-SSO. Usporedba svih bolesnika s kontrolom nije pokazala značajne razlike u raspodjeli učestalosti DRB1 gena, no učestalost DRB1*07 (P=0.0106;

OR=0.74) i DRB1*14 gena (P=0.0003; OR=1.16) su bile značajno povećane među PsA bolesnicima, dok je DRB1*11 bio manje prisutan u usporedbi s kontrolom (P=0.0230; OR=0.24). Kod RA/RF+ bolesnika je, u odnosu na kontrolu, utvrđena značajna razlika u učestalosti alela koji nose "zajednički epitop", slijed od pet aminokiselina na poziciji 70-74 HLA-DR lanca. Ovi aleli (DRB1*01:01, *01:02, *04:01, *04:04, *04:05, *10:01) kao i DRB1*16 su bili češće prisutni među bolesnicima (P=0.005, OR=1.74; P=0.0155; OR=1.77). Zaključno, navedeni rezultati govore u prilog važnosti određivanja gena HLA-DRB1 u dijagnostici reumatskih bolesti.

Ključne riječi: reumatske bolesti, dijagnostika, HLA-DRB1

HLA-DRB1 GENE TYPING IN DIAGNOSTICS OF RHEUMATIC DISEASES

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HLA typing is commonly performed in diagnostic process for certain autoimmune and rheumatic diseases. Aim of our study was to investigate HLA-DRB1 genes distribution among 628 patients, referred to our laboratory for HLA typing in a one year period, with following diagnoses: Inflammatory Polyarthropathy (IPA; N=239), Rheumatoid Arthritis RF+ (RA/RF+; N=70), RA/RF- (N=83), Psoriatic Arthritis (PsA;N=112), Ankylosing Spondylitis (AS; N=61) and Spondyloarthritis (SpA; N=63). Individuals from Croatian Bone Marrow Donor Registry were used as controls. HLA-DRB1 typing was performed by PCR-SSO method. Significant difference in DRB1 gene frequencies was not observed between all patients and controls, but frequency of DRB1*07 (P=0.0106; OR=0.74) and DRB1*14 gene (P=0.0003; OR=1.16) were significantly increased among PsA patients, while DRB1*11 appeared less frequently in comparison to controls (P=0.0230; OR=0.24). RA/RF+ patients showed significant difference in comparison to controls for alleles which carry "shared epitope", a five amino acid sequence motif in residues 70-74 of HLA-DRβ chain. These alleles (DRB1*01:01,*01:02,*04:01, *04:04, *04:05, *10:01) as well as DRB1*16 gene were more present among patients (P=0.005, OR=1.74; P=0.0155; OR=1.77, respectively). Our results reinforce the importance of evaluating disease susceptibility alleles in our population and suggest that additional analyses are required before HLA-DRB1 genotyping is incorporated into diagnostic algorithms.

Keywords: rheumatic diseases, diagnostics, HLA-DRB1

O-19
ULOGA ODREĐIVANJA LOKUSA HLA-DQ U DIJAGNOSTICI GLUTENSKE ENTEROPATIJE

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Glutenska enteropatija (GE) je bolest kod koje je utvrđena jedna od najjačih povezanosti sa genima sustava HLA. Približno 95% bolesnika s GE su nosioci HLA-DQ2.5 (HLA-DQA1*05-DQB1*02 u cis ili trans konfiguraciji ovisno o tome da li se HLA-DQA1*05 i -DQB1*02 nalaze na istom ili

različitim kromosomima) i/ili HLA-DQ8 (HLA-DQA1*03-DQB1*03:02) heterodimera. Cilj rada bio je istražiti raspodjelu alela HLA-DQA1 i -DQB1 kod bolesnika (N=118), osoba s s povećanim rizikom za razvoj GE (članovi obitelji bolesnika (N=206) ili osobe s nepotvrđenom GE (N=1685)) i zdravih osoba (ZK) (N=985). U tu svrhu je za sve ispitanike provedena tipizacija gena HLA-DQA1 i -DQB1 metodom PCR-SSP visoke rezolucije. Rezultati su pokazali da je genotip DQ2.5 cis značajno učestaliji među svim ispitivanim skupinama u odnosu na ZK (P<0.0001) te da je znatno učestaliji među bolesnicima u odnosu na članove obitelji bolesnika te osobe s nepotvrđenom GE (P=0.0003 i P=0.0057). Genotip DQ2.5 trans je značajno učestaliji među bolesnicima u odnosu na ZK kao i u odnosu na osobe s nepotvrđenom GE i članove obitelji bolesnika (P=0.0002, P=0.0032 i P=0.0160). Nije utvrđena značajna razlika u učestalosti ovog genotipa kod osoba s nepotvrđenom GE i članova obitelji bolesnika u usporedbi sa ZK. Za genotip DQ8 ne postoji statistički značajna razlika među skupinama. Zaključno, prisutnost HLA-DQ2.5 heterodimera ukazuje na najveći rizik za razvoj GE te je stoga tipizacija gena HLA-DQ je izrazito važna u dijagnostici ove bolesti.

Ključne riječi: HLA-DQ, glutenska enteropatija

THE IMPORTANCE OF HLA-DQ TYPING IN DIAGNOSTICS OF CELIAC DISEASE

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Association of HLA-DQ genes and celiac disease (CD) is one of the best established disease associations of HLA system, with approx. 95% of patients carrying HLA-DQ2.5 (HLA-DQA1*05-DQB1*02) and/or HLA-DQ8 (HLA-DQA1*03-DQB1*03:02) genotype. Aim of study was to investigate HLA-DQA1 and -DQB1 allele distribution among CD patients (N=118), individuals with a higher risk for developing CD (family members of CD patients (N=206) or individuals with unconfirmed diagnosis (N=1685)) and healthy controls (HC) (N=985). HLA-DQ typing of all individuals was performed using PCR-SSP high resolution method. Genotype DQ2.5 cis (HLA-DQA1*05 and DQB1*02 present on the same chromosome) was significantly more present among all groups when compared to HC (P<0.0001). It was also more frequent among patients then family members and unconfirmed patients (P=0.0003 and P=0.0057). Trans configuration of this genotype (HLA-DQA1*05 and DQB1*02 present on different chromosomes) showed a significantly higher incidence among patients when compared to HC, but also unconfirmed patients and family members (P=0.0002, P=0.0032 and P=0.0160). No difference was seen for this genotype among unconfirmed patients and family members when compared to HC. Frequency of DQ8 genotype did not show significant variation among tested groups. In conclusion, the results confirm that presence of HLA-DQ2.5 heterodimer confers the highest risk for developing CD and thus emphasize the importance of HLA-DQ typing in diagnostics of CD.

Keywords: HLA-DQ, celiac disease

O-20
MOLEKULARNA DIJAGNOSTIKA ZARAZNIH BOLESTI U JAVNOM ZDRAVSTVU - PRIMJER IZ VARAŽDINSKE ŽUPANIJE

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Razvoj molekularne biologije potaknuo je revoluciju dijagnostike zaraznih bolesti u kliničkim mikrobiološkim laboratorijima. Nove tehnologije molekularne dijagnostike omogućuju višu razinu brige o bolesnicima pružanjem brže i informativnije mikrobiološke dijagnoze. Djelatnost za medicinsku mikrobiologiju Zavoda za javno zdravstvo Varaždinske županije provodi rutinsku molekularnu dijagnostiku uzročnika spolno prenosivih bolesti (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, HPV) u uzorcima urogenitalnog trakta, kao i analizu prisutnosti toksina A i B bakterije *Clostridium difficile* u uzorcima stolice. Djelatnost za medicinsku mikrobiologiju sudjeluje i s drugim djelatnostima u Zavodu za javno zdravstvo, kao i s liječnicima primarne zdravstvene zaštite i kliničarima u osmišljavanju novih programa molekularne dijagnostike kako bi omogućila korisnicima svojih usluga visoku razinu zdravstvene skrbi dajući pravovremene i precizne rezultate mikrobioloških pretraga. Tehnike molekularne dijagnostike koje se koriste u Djelatnosti za medicinsku mikrobiologiju zahtijevaju pažljivo prikupljanje, transport i rukovanje uzorcima na kliničkoj i laboratorijskoj razini. Briga za pacijente i točnost laboratorijskog nalaza glavni su cilj rada Djelatnosti, stoga je u Djelatnosti uspostavljen sustav upravljanja kvalitetom temeljen na međunarodnom standardu HRN EN ISO 15189:2012, Medicinski laboratoriji - zahtjevi za kvalitetu i osposobljenost.

Ključne riječi: molekularna dijagnostika, zarazne bolesti, sustav upravljanja kvalitetom, HRN EN ISO 15189

MOLECULAR DIAGNOSIS OF INFECTIOUS DISEASES IN PUBLIC HEALTH - AN EXAMPLE FROM THE VARAZDIN COUNTY

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The development of molecular biology has enabled a revolution in the field of molecular diagnostics of infectious diseases in clinical microbiology laboratories. New molecular diagnostics techniques have enabled a higher level of patient care by providing faster and more reliable microbiological diagnosis. Medical Microbiology Unit of the Institute of Public Health of Varaždin County carries out the routine molecular diagnosis of sexually transmitted diseases (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, HPV) in urogenital samples, as well as the detection of *Clostridium difficile* toxins A and B in stool samples. Medical Microbiology Unit cooperates with other Units at the Institute of Public Health, as well as with primary health care physicians and clinicians in the design of new molecular diagnostic programs that would enable their users a high level of health care by providing timely and accurate microbiology testing results. Molecular diagnostics techniques used at the Medical Microbiology Unit require careful collection, transport and handling of samples at both clinical and laboratory level. Care for the patients and the accuracy of the laboratory results are the main aim of the Unit's activity. Therefore, a Quality Management System based on International Standard EN ISO 15189: 2012, Medical Laboratories Medical laboratories - Requirements for quality and competence has been established at the Unit.

Keywords: molecular diagnostics, infectious diseases, quality management, ISO 151899

O-21
ULOGA TELOMERA U KONTROLI STANIČNOG RASTA I KARCINOGENEZE

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Telomere su specijalizirane strukture na krajevima linearnih kromosoma koje imaju presudnu ulogu u zaštiti kromosoma i očuvanju stabilnosti genoma. One sudjeluju u nekim od najvažnijih staničnih funkcija kao što su dioba i kontrola normalnog rasta te održavanju imortalnog stanja tumorskih stanica i jednostaničnih eukariota. Telomere su dinamične strukture tako da se prilikom staničnih dioba kontinuirano skraćuju. Kako je broj skraćivanja ograničen, u normalnim stanicama ono dovodi do prestanka dioba i indukcije staničnog starenja. U tumorskim stanicama prekomjerno skraćivanje telomera inducira genomsku nestabilnost i apoptozu. Enzim telomeraza pak produžava telomere i u imortalnim stanicama kao što su matične i tumorske, procesi skraćivanja i produžavanja telomera su u ravnoteži. U predavanju će biti riječi o molekularnim mehanizmima koji povezuju telomere, aktivnost telomeraze i apoptozu uz osvrt na važnost ovih nalaza za istraživanja antitumorske terapije.

Ključne riječi: telomere, telomeraza, senescencija, karcinogeneza

THE ROLE OF TELOMERES IN CONTROL OF CELL GROWTH AND CARCINOGENESIS

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Telomeres are specialized structures at the ends of linear chromosomes that play an important role in protecting chromosomes and maintaining the genome stability. They take part in some of the most important cellular functions, such as cell division, control of normal cell growth as well as maintenance of immortality of tumor cells and single-celled eukaryotes. Telomers are dynamic structures so that with each cell division they are continuously shortened. As the number of shortenings is limited, in normal cells it results in exit from further divisions and induction of cellular aging. In tumor cells too short telomeres induces genomic instability and apoptosis. Enzyme telomerase extend telomeres and in imortal cells such as stem and tumor, the shortening and extention of telomeres are in balance. The lecture will be about molecular mechanisms linking telomeres, telomerase activity and apoptosis, with reference to the importance of these finds for antitumor therapy research.

Keywords: telomeres, telomeraze, senescence, carcinogenesis

O-22
IMUNOLOŠKI SUSTAV I RAK: KAKO NAJBOLJE ISKORISTITI KOMPLICIRANU VEZU

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Od 2011. kada je ipilimumab odobren za liječenje bolesnika s metastatskim melanomom došlo je do velikog napretka u kliničkoj primjeni imunoterapije raka sa nekoliko novih lijekova koji su pokazali terapijsku učinkovitost kod različitih vrsta raka. Ti lijekovi uključuju inhibitore imunološke kontrolne točke (monoklonska protutijela specifična za CTLA-4, PD1 i PD1-L receptore) te od 2017. godine T stanice sa kimeričnim receptorom za antigen. Djelovanje ovih lijekova se temelji na našem razumijevanju kompleksnih interakcija između imunološkog sustava i raka o kojima će biti riječ u predavanju. Također će biti obrađeni neki izazovi vezani uz trenutnu imunoterapiju raka (npr. nedostatak pouzdanih biljega za predviđanje odgovora bolesnika na liječenje ovim lijekovima, razvoj rezistencije na imunoterapiju raka). Neka najnovija istraživanja u području imunologije raka (npr. uloga makrofaga u poticanju raka i mogućnost imunoterapije raka usmjerene na makrofage, uloga mikrobioma u oblikovanju interakcija između imunološkog sustava i raka te moduliranju odgovora raka na imunoterapiju) će također biti predstavljena u ovom predavanju.

Ključne riječi: imunoterapija raka, imunologija raka, inhibitori imunološke kontrolne točke, T stanice s kimeričnim receptorom za antigen

IMMUNE SYSTEM AND CANCER: HOW TO BENEFIT FROM A COMPLICATED RELATIONSHIP

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Since 2011 when ipilimumab was approved for treatment of metastatic melanoma we have seen a rapid progress in clinical use of cancer immunotherapy with several drugs showing therapeutic efficiency in different types of cancer. These drugs include immune-checkpoint inhibitors, monoclonal antibodies that specifically target CTLA-4, PD1 or PD1-L receptors, and since 2017 chimeric antigen receptor (CAR) T cells. Activity of these drugs is based on our understanding of complex interactions between immune systems and cancer which will be discussed in the lecture. Also some challenges related to current cancer immunotherapy (eg. lack of reliable markers to predict patient response to these drugs, development of resistance to cancer immunotherapy) will be covered. Some cutting-edge research results in the field of cancer immunology (eg. cancer-promoting role of macrophages and potential for macrophage-directed cancer immunotherapy, role of microbiome in shaping interaction between cancer and immune system and modulating cancer response to immunotherapy) will be also presented in the lecture.

Keywords: cancer immunotherapy, cancer immunology, immune-checkpoint inhibitors, chimeric antigen receptor T cells

O-23

PRAĆENJE KIMERIZMA NAKON TRANSPLANTACIJE KRVOTVORNIH MATIČNIH STANICA PRIMJENOM METODE TEMELJENE NA PCR-u U STVARNOM VREMENU

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Određivanje statusa kimerizma je rutinska pretraga koja se izvodi u svrhu praćenja prihvaćanja presatka ili relapsa nakon transplantacije krvotvornih matičnih stanica. Cilj ovog rada je bio istražiti primjenjivost metode temeljene na PCR-u u stvarnom vremenu za praćenje kimerizma. U tu svrhu su testirana 74 para primatelj-davatelj (36 primatelja sa srodnim davateljem i 38 primatelja s nesrodnim davateljem) koristeći komercijalni kit KMRtype Core, Applied Biosystems® 7500 Real-Time PCR aparat i računalni program KMRengine® Chimerism Analysis. Medijan broja informativnih biljega bio je slijedeći: M=4 (raspon 1-7) za primatelje sa srodnim davateljem; M=3 (raspon 0-7) za srodne davatelje; M=6 (raspon 2-10) za primatelje s nesrodnim davateljem; M=6 (raspon 3-12) za nesrodne davatelje. Za četiri primatelja sa srodnim davateljem određen je samo jedan informativni biljeg. Od 30 analiziranih biljega, devet je bilo informativno za primatelja u >20% slučajeva. S druge strane, nekoliko biljega je pokazalo nisku razinu informativnosti kao i visok postotak (≈20%) netipičnih rezultata (KMR011, KMR035, KMR041, KMR057). Zaključno, metoda PCR-a u stvarnom vremenu se pokazala prikladnom za praćenje kimerizma kod većine naših bolesnika. Poboljšanje ove metode, prvenstveno u povećanju razine primjenjivosti koje je značajno u odnosu na početne metode ovog tipa, opravdava njeno uvođenje u rutinski postupak praćenja kimerizma nakon transplantacije krvotvornih matičnih stanica.

Ključne riječi transplantacija krvotvornih matičnih stanica, kimerizam, PCR u stvarnom vremenu

THE USE OF QUANTITATIVE PCR METHOD FOR CHIMERISM MONITORING AFTER HSCT

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Chimerism analysis is a routine test performed in order to monitor engraftment or relapse after hematopoietic stem cell transplantation. Gold-standard method for this test, PCR-STR analysis, is increasingly being replaced with a more sensitive quantitative PCR based methods. Aim of the study was to evaluate applicability of a qPCR based chimerism monitoring method. For that purpose, 74 recipient/donor pairs (36 patiens with related donor, 38 patients with unrelated donor) have been tested using KMRtype Core kit, Applied Biosystems® 7500 Real-Time PCR System and KMRengine® Chimerism Analysis Software. Median number of informative markers (M) was as follows: M=4 (range 1-7) for patients with related donor; M=3 (range 0-7) for related donors; M=6 (range 2-10) for patients with unrelated donor; M=6 (range 3-12) for unrelated donors. Only four patients with related donor had a single informative marker identified. Out of 30 tested markers, nine markers were informative for a patient in >20% of cases. Conversely, several markers showed a low level of informativeness and a high percentage (≈20%) of atypical results (KMR011, KMR035, KMR041, KMR057). In conclusion, qPCR method has shown to be adequate for majority of our patients. Although the level of PCR-STR method's applicability remains unreached, the improvement of this qPCR method's applicability in comparison to initial qPCR based methods is encouraging and justifies its implementation in routine chimerism monitoring procedure.

Keywords: hematopoietic stem cell transplantation, chimerism, real time PCR

3. Simpozij edukacije biologije
3rd Biology Education Symposium

O-24

SPOLNO PRENOSIVE BOLESTI I KONTRACEPCIJA – ISPITIVANJE ZNANJA I STAVOVA STUDENTSKE POPULACIJE

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Cilj istraživanja bio je analiza znanja i stavova studenata preddiplomskih studija Zdravstvenog veleučilišta o spolno prenosivim bolestima i kontracepciji. Poznato je da spolno prenosive bolesti mogu imati vrlo negativan utjecaj na zdravlje oboljelih osoba, posebno ukoliko nisu otkrivene i liječene na vrijeme. Upravo je iz tog razloga važno dobiti uvid u razinu znanja studentske populacije, a posljedično i u eventualnu potrebu za boljom edukacijom tijekom školovanja, budući da je upravo u prevenciji spolno prenosivih bolesti od posebne važnosti edukacija mladih osoba koja uključuje edukaciju o bolestima kao i načinu prenošenja. Iz dobivenih rezultata na području ponašanja i stavova ispitanika vidljivo je kako je većina ispitanika stupila u spolni odnos, te se većina izjasnila kako je koristila neku vrstu zaštite pri prvom spolnom odnosu, a velika je većina koristi i dalje. Manji je broj onih koji je ne koriste, a navedeni je razlog u većini slučajeva to što se nalaze u stalnoj vezi. O spolnosti i kontracepciji mladi najviše razgovaraju s prijateljima, rijetko s roditeljima; pritom mnogo više s majkom nego s ocem, a navode da nikada o navedenom ne komuniciraju s profesorima i liječnicima. Zaključno, gotovo svi ispitanici navode da bi tijekom visokoškolskog obrazovanja trebali dobiti više znanja o navedenim temama, što otvara novu/staru temu o potrebi za boljom edukacijom mladih osoba na području reproduktivnog zdravlja.

Ključne riječi: spolno prenosive bolesti, kontracepcija, studenti

SEXUALLY TRANSMITTED DISEASES (STDS) AND BIRTH CONTROL – RESEARCH ON AWARENESS AND ATTITUDES AMONG THE STUDENT POPULATION

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Purpose of this research was to analyse awareness and attitudes among undergraduate students at the University of Applied Health Sciences about the topics of sexually transmitted diseases (STD) and birth control. It is a known fact that the STDs may adversely influence the health of the diseased individuals, particularly if the STDs are not detected and treated in time. This is a specific reason why it is essential to obtain insight in the student population awareness, and consequently possible need for better education during schooling, since precisely the education of the young plays an important role in the prevention of STDs. The obtained results in the field of behaviour and attitudes of data subjects evidently show that most data subjects have had a sexual intercourse, and most of them reported using some type of protection at the first intercourse, majority claiming continued use thereafter. Fewer data subjects do not use protection, and the

reason they report in majority of cases is they are engaged in a steady relationship. The young discuss sexuality and birth control mostly with their friends, rarely with parents; whereas they report never communicating about the topic with their professors or doctors. In conclusion, almost all data subjects report that they should get more knowledge about these topics during higher education, which opens a new/old issue about the need for better education of the young in the field of reproductive health.

Keywords: sexually transmitted diseases (STDs), birth control, students

O-25

USVOJENOST NASTAVNOG SADRŽAJA IZ PRIRODE I BIOLOGIJE U DVOJEZIČNIM RAZREDNIM ODJELIMA

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Škole po B modelu su škole koje nastavu izvode osim na hrvatskom jeziku i na jeziku nacionalne manjine. U takvim se školama nastava izvodi dvojezično (u istom razrednom odjeljenju nastava se istovremeno izvodi na dva jezika). Istraživanje je provedeno s ciljem utvrđivanja utječe li dvojezičnost na rezultate učenja iz Prirode i Biologije. S učenicima petog, šestog i sedmog razreda dvojezičnog razrednog odjela (nastava Prirode i Biologije se izvodi istovremeno na hrvatskom i mađarskom jeziku), odjela u kojem se nastava izvodi na hrvatskom jeziku i odjela u kojima se nastava izvodi na mađarskom jeziku izvršena je analiza ocjena koje su učenici stekli tijekom 2017./2018. školske godine. Analizom je utvrđeno da su učenici u dvojezičnim odjelima bolji u ukupnoj ocjeni (srednja vrijednost ocjena oba elementa ocjenjivanja, $p<0,001$) te u ocjenama pojedinog elementa ocjenjivanja (usvojenost, razumijevanje i primjena programskih sadržaja, $p=0,011$ te element praktični radovi, $p=0,048$) od učenika odjela u kojem se nastava izvodi samo na hrvatskom odnosno mađarskom jeziku. Dvojezični učenici podjednaki su u usmenoj i pisanoj provjeri znanja dok su po ukupnoj ocjeni od svih dvojezičnih najbolji učenici šestog razreda, a najslabiji učenici sedmog razreda. U svim ispitivanim razrednim odjelima učenici aktivno uče, a u dvojezičnim odjelima učenici su govornici oba jezika te čuju učiteljevo i učeničko tumačenje više puta (ovisno o metodi i obliku rada) što pridonosi boljem razumijevanju.

Ključne riječi: dvojezično, usvojenost nastavnog sadržaja, priroda, biologija

CLASS CONTENT ACQUISITION IN SCIENCE AND BIOLOGY IN BILINGUAL CLASS DEPARTMENTS

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B model schools are schools that besides teaching class in Croatian also teach class in national minority language. In those schools the class is taught bilingually. The research was conducted with the goal of confirming if bilingualism affects on the results of studying in Science and Biology. With the students of fifth, sixth and seventh grade in bilingual departments (class is taught in Croatian and Hungarian at the same time), in the department where the class is taught only in Croatian and in department where the class is taught in Hungarian the grades were analyzed. The analysis confirmed that the students in bilingual departments have better total grade (average value of the grades from both grading elements $p<0.001$) and grades of the individual grading element (acquisition, understanding and application of the program content $p=0.011$ and element of practical work $p=0.048$) from students in the department where class is taught only in Croatian, that is, in Hungarian. Bilingual students were equal in the oral and the written exam, when from all the bilingual students the students of the sixth grade were the best in total, where the weakest were the students of the seventh grade. In all of the examined departments the students are actively studying, in the bilingual departments the students are speakers of both languages and they hear teacher's and student's explanation more times (depends on the method and form of work) which contributes to a better understanding.

Keywords: bilingual, class content acquisition, science, biology

O-26

ISTRAŽIVAČKO UČENJE U RADU S VISOKOMOTIVIRANIM UČENICIMA – PRIMJERI DOBRE PRAKSE CENTRA IZVRSNOSTI ZA BIOLOGIJU

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Centar izvrsnosti za biologiju osnovan je 2013. godine i sjedište mu je u Prvoj gimnaziji Varaždin. Polaznici Centra izvrsnosti su visokomotivirani učenici osnovnih i srednjih škola Varaždinske županije. Svake školske godine u rad Centra uključi se 30 učenika osnovnih, 40 učenika srednjih škola i 20 mentora. Rad u Centru temelji se na istraživačkom učenju i učenju otkivanjem te osmišljavanju i provedbi samostalnih istraživanja učenika. Uz aktivno usvajanje sadržaja, takvim načinom rada nastoje se kod učenika razvijati vještine promatranja i uočavanja uzročno-posljedičnih veza u prirodi, donošenja zaključaka, kreativnost i kritički način razmišljanja. Upotrebom modernih tehnologija učenici prikupljaju, statistički obrađuju i grafički prikazuju te analiziraju dobivene rezultate što doprinosi razvoju prirodoslovnog načina razmišljanja i pismenosti koji su neophodni za nastavak školovanja na višoj razini i za kasniju znanstvenu aktivnost. Suvremeno obrazovanje u prirodoslovlju stavlja izraziti naglasak na sudjelovanje učenika u različitim oblicima aktivnog učenja poput istraživačkog učenja te promiče takav način učenja jer osim što kod učenika razvija širok spektar vještina, istovremeno djeluje na povećanje znatiželje te interesa učenika za znanost. Uz kontinuirano eksperimentiranje, Centar učenicima pruža mogućnost aktivne primjene aktualnih sadržaja u biologiji u području bioinformatike, molekularne biologije, mikrobiologije, modeliranja i 3D printanja te GIS-a.

Ključne riječi: istraživačko učenje, Centar izvrsnosti za biologiju

INQUIRY BASED LEARNING IN WORKING WITH HIGHLY MOTIVATED STUDENTS – EXAMPLES OF GOOD PRACTICE OF THE CENTRE OF EXCELLENCE IN BIOLOGY

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Centre of Excellence in Biology was established in 2013 with the headquarters in Prva gimnazija Varaždin. The students in the Centre of Excellence are highly motivated pupils of primary and secondary schools of Varaždin County. Each school year the Centre includes 30 primary school pupils, 40 secondary school students and 20 mentors. Work in the Centre is based on inquiry learning and learning by discovering as well as designing and conducting independent student research. With the active learning of content, such work method aims to develop students' ability to observe and perceive cause-effect connections in nature, to make conclusions, be creative and think critically. By using modern technology, students collect, statistically process and graphically resent and analyse the obtained results, which contributes to the development of scientific way of thinking and literacy necessary for continuing higher education and for later scientific activity. Contemporary education in natural sciences puts emphasis on the participation of students in various forms of active learning such as inquiry based learning and promotes such learning because, apart from the fact that students develop a wide range of skills, at the same time it increases curiosity and interest of students for science. With continuous experimentation, the Centre gives students the opportunity to actively apply the current biology content in the field of bioinformatics, molecular biology, 3D printing and GIS.

Keywords: inqury based learning, Centre of Excellence in Biology

O-27

ISPITIVANJE ZNANJA O MORU I STAVOVA KOD ŠKOLSKE DJECE PRIJE I NAKON PROVEDENE AKTIVNOSTI – POVOD ZA UVOĐENJE „OCEAN LITERACY“ U NASTAVNI RAD

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Morski resursi i ekosustavi su u današnje vrijeme pod velikim pritiskom antropogenog djelovanja. Kako bi se na održiv način upravljalo ovim važnim globalnim resursom te se dugoročno kvalitetno riješili postojeći problemi u morskom okolišu potrebno je svim generacijama pružiti informaciju o međusobnoj povezanosti i ovisnosti čovjeka i mora kako bi donosili odluke na što odgovorniji način prema morskom okolišu. Obrazovanje budućih generacija je važno, kako bi u budućnosti što uspješnije koristili morske resurse na održiv način, i znali donositi ispravne odluke u upravljanju okolišem. Primjena znanja o moru tzv. „ocean literacy“ u nastavi podrazumijeva stjecanje znanja, razvoj umijeća i sposobnosti te razumijevanje i razvoj svijesti o načinima na koje more djeluje na čovjeka i čovjek na more. Također doprnosi postizanju Cilja održivog razvoja 14 te je u skladu sa UN-ovim Desetljećem znanosti o moru za održivi razvoj 2021-2030. Provedeno istraživanje uključit će provođenje anketa među djecom 2.-4. razreda osnovne škole prije i nakon provedene znanstveno-edukativne radionice te će se usporediti rezultati dobiveni prije i nakon aktivnosti. Anketa se sastoji od 3 dijela: općenite informacije o ispitaniku, znanje o moru i stavovi. Istraživanje će se provesti u školama na području Zadra tijekom lipnja 2018. g. Cilj istraživanja je pokazati utjecaj intervencije kroz edukaciju s određenom morskom temom na znanje o moru i shvaćanje prirodnih procesa i pojava u morskom okolišu.

Ključne riječi: znanje o moru, "ocean literacy", obrazovanje, održivi razvoj, SDG 14

PRE AND POST ACTIVITY TESTING OF MARINE KNOWLEDGE AND ATTITUDES IN SCHOOL CHILDREN – IMPLICATION FOR OCEAN LITERACY IMPLEMENTATION IN THE CLASSROOM

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Marine resources and ecosystems are under strong anthropogenic pressure. Sustainable management of this major global resource requires society that is aware of the interconnection between the sea and society. Ocean literacy can help citizens make decisions which are more responsible and friendly towards marine environment. Education of future generations is crucial for future sustainable management of marine resources. Implementation of ocean literacy in education and teaching will help to acquire knowledge, abilities, understanding and awareness of different aspects of relation between humans and the sea. It will also help to achieve SDG14 and is in line with UN Decade of ocean science for sustainable development 20210-2030. Study on marine knowledge and attitudes about the sea will be performed using a questionnaire. It will be used to test pre and post educational activity knowledge in school kids from 2-4 grade of primary school. Pre and post results will be compared. Questionnaire consist of three parts: general information about the respondents, marine knowledge and attitudes. Research will be done in schools in Zadar area during June 2018. Aim of this research is to show the effect of educational intervention with marine topic on marine knowledge and understanding of natural processes and phenomena in marine environment.

Keywords: ocean literacy, education, sustainable development, SDG 14

O-28

METODE POUČAVANJA KOJE OMOGUĆUJ RAZVOJ PROCEDURALNOG I METAKOGNITIVNOG ZNANJA I VJEŠTINA

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Današnji najveći izazov u nastavi biologije je razviti sposobnosti učenika za uspješno rješavanje zadataka koji ispituju proceduralne i metakognitivne dimenzije znanja. Proceduralno znanje omogućuje spoznaju kako uraditi nešto specifično unutar predmeta, metode istraživanja (analizu izvora, odvajanje bitnog od nebitnog, sažimanje, uspoređivanje, klasificiranje) dok je metakognitivno znanje strategijsko znanje o tome kako postupati da bi se riješili problemi i kognitivni zadaci; kontekstualno znanje; svijest o razini vlastitog znanja (Miletić 2012). Tradicionalne predavačke metode su neadekvatan izvor znanja koje ne omogućuju adekvatnu pripremu učenika k rješavanju zadataka ovih viših kognitivnih razina što je do sada najviše došlo do izražaja na ispitima državne mature iz biologije gdje su te vrste pitanja bile najlošije riješene. Obzirom da sam do istih spoznaja došla nakon analiza zadataka Državne mature iz biologije iste koristim kao model u ovom radu. Ovim izlaganjem predložiti ću par metoda rada na konkretnim primjerima s ciljem što uspješnijeg prevladavanja problematike svladavanja koncepata koji uključuju: 1. važnost razumijevanja odnosa površine i volumena u biološkim procesima, 2. broj kromosoma i kromatida u staničnim diobama, 3. shvaćanje pojmova vezanih uz

istraživačke projekte i izradu grafova te njihovu interpretaciju. Nakon korištenja suvremenih metoda poučavanja ovih koncepata učenici su znatno uspješnije savladavali i rješavali iste.

Ključne riječi: proceduralno znanje, metakognitivno znanje, državna matura, metode rada u nastavi biologije

TEACHING METHODS THAT DEVELOPED BETTER PROCEDURAL AND METACOGNITIVE KNOWLEDGE SKILLS

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The biggest challenge in current biology teaching is to develop student`s skills to successfully solve tasks that involve procedural and metacognitive knowledge. The procedural knowledge allows develop of knowledge to do something specific within the object, method of research, (source analysis, separation of relevant from irrelevant, systematisation and classification abilities) while metacognitive knowledge is strategical knowledge that includes abilities to deal with problem-solving and cognitive tasks, contextual knowledge, awareness about level of own knowledge (Miletić 2012). Traditional teaching methods are the inadequate source that does not support developing of knowledge that gives students abilities to solve higher cognitive tasks, and that was highly visible on previous State exams test in biology. Since those kinds of questions had very low results I have used them as the source to suggest teaching methodology in this work. During the lecture, I will suggest few teaching methods on specific examples with the aim for better overcoming issues and master the concept that includes: 1. understanding surface area to volume ratio in biological processes, 2. ability to distinguish chromosome and chromatid numbers in different phases cell divisions, 3. understandings facts and knowledge linked to science investigations and ability to read and create graphical data presentation. In the class, it was visible that students were much successful in problem-solving tasks.

Keywords: procedural knowledge, metacognitive knowledge, State exam , teaching methodology

O-29
SUSTAVI E-UČENJA I OSTVARENOST ISHODA UČENJA UČENIKA

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U kontekstu globalnih društvenih promjena u sustavu odgoja i obrazovanja važno je mijenjati pristup stjecanja temeljnih kompetencija učenika s posebnim naglaskom na razvoj njihove prirodoslovne i digitalne pismenosti. Današnje učenike, pripadnike allways on line generacije, karakterizira intenzivno korištenje Interneta za socijalne interakcije, ali i za obrazovanje. Upravo stoga potrebno je implementirati IKT-u u nastavnu praksu, a jedna od mogućnosti je e-učenje. S ciljem istraživanja ostvarenosti ishoda učenja prema kognitivnim razinama zadataka obzirom na primijenjene postupke poučavanja i učenja, tradicionalnu nastavu i e-učenje, provedeno je istraživanje u nastavi prirode i biologije osnovne škole na uzorku od osam razrednih odjela. Rezultati istraživanja pokazuju podjednaku uspješnost učenika eksperimentalne i kontrolne skupine u ostvarenosti ishoda učenja te se e-učenje pokazalo jednako uspješnijim kao i tradicionalna nastava u kojoj su korištene suvremene nastavne strategije rada. Stoga se e-učenje može koristiti i u situacijama, poput bolesti, odlaska učenika na višednevna natjecanja, života u izdvojenim sredinama, kada ne postoji mogućnost da učenici nazoče redovnoj nastavi.

Istraživanje je pokazalo kako učenici kontrolne i eksperimentalne skupine jednako uspješno/neuspješno rješavaju zadatke prve, druge i treće kognitivne razine. Dobiveni rezultati mogu poslužiti kreatorima obrazovne politike te biti smjernica učiteljima praktičarima za oblikovanje nastave.

Ključne riječi: e-učenje, ICT u nastavi, kognitivne razine, nastava prirode i biologije

E-LEARNING SYSTEMS AND OUTCOMES OF TEACHING STUDENTS

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In the context of global social changes in the education system, it is important to change the approach to acquiring basic students' competencies, with special emphasis on the development of their natural and digital literacy. Today's students, members of always on line generation, are characterized by the intensive use of the Internet for social interaction as well as for education. Precisely for this reason, it is necessary to implement ICT in teaching practice, and one of the options is e-learning. For the purpose of achieving learning outcomes, according to the cognitive levels of applied teaching and learning, traditional learning and e-learning methods, a research in teaching Science and Biology in elementary schools, using a sample of eight classes, was conducted. The research results show the same success of the experimental and control groups of students in achieving the outcomes, and e-learning proved to be just as successful as the traditional curriculum, where contemporary teaching strategies were used. Therefore e-learning can also be used in situations such as illness, student's absence for multi-day competitions, life in distinguished environments, in cases where there is no possibility for students to attend regular classes. The research has shown that both control and experimental groups were equally successful / unsuccessfully in solving the tasks of the first, second and third cognitive level. The results can be used by the creators of educational policy.

Keywords: e-learning, ICT in teaching, cognitive levels, teaching Science and Biology

O-30
NASTAVA BIOLOGIJE USUSRET DIGITALNOM DRUŠTVU

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Tema je nastala kao rezultat edukacije u projektu e-škola. Usvajanjem brojnih različitih alata na edukacijama za nastavnike, izabrano je nekoliko i primijenjeno u nastavi biologije. Alati Linoit, Padlet, One Note, Forms, Plicers, Socrative,… korišteni su za usvajanje, ponavljanje, vježbanje i vrednovanje znanja. Zajedničkim radom s učenicima postignuto je da oni ne budu sami sebi svrha već da aktivnim sudjelovanjem učenika nastavni proces bude podignut na višu kognitivnu razinu. Doprinos tome je i ostvarena korelacija s drugim nastavnim predmetima - informatikom, matematikom, nastavom TZK-a. Primjena različitih metoda i oblika rada značajno je utjecala na motivaciju učenika. Cilj ovakvog rada je da se primjenom IKT-a u nastavi biologije ona usmjeri na učenika i njegove potrebe te da se potiče kreativnost, samostalnost i odgovornost u radu na zajedničkim dokumentima. Važnost ovog rada je i u refleksiji i razmjeni rezultata na stručnim skupovima sustručnjaka.

Ključne riječi: e-alati,nastava biologije,unapređivanje

TEACHING BIOLOGY USED BY DIGITAL TOOLS

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The theme was born as a result of education in the e-school project. By adopting many different tools on teacher education, several have been selected and applied in the teaching of biology. Tools Linoty, Padlet, One Note, Forms, Plicers, Socrates, ... are used for adoption, repetition, exercise, and evaluation of knowledge. By working together with the students, they have not been for themselves, but with the active participation of the students the teaching process is raised to a higher cognitive level. Contribution to this is also the correlation with other teaching subjects - computer science, mathematics, teaching of physical training. The application of different methods and forms of work has significantly influenced student motivation. The aim of this work is to apply ICT in teaching biology to the student and his needs, and to encourage creativity, independence and responsibility in working on common documents. The importance of this work is also in the reflection and exchange of results at expert meetings.

Keywords: e-tools, biology lessons, advancement

O-31
POVEZANOST ISPITA DRŽAVNE MATURE I NASTAVE BIOLOGIJE U SREDNJOJ ŠKOLI

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Na kraju svojega srednjoškolskog obrazovanja učenici u Republici Hrvatskoj polažu državnu maturu. Ispit iz Biologije jedan je od najčešće biranih ispita iz izbornih predmeta te mu svake godine pristupa 5000 do 7000 pristupnika. Stoga je cilj ovoga istraživanja bio ispitati povezanost ispita državne mature i nastave Biologije. Da bi se to ispitalo, provedene su četiri fokus-grupe u četiri hrvatska grada: Osijeku, Rijeci, Splitu i Zagrebu. U svakoj je sudjelovalo osam do jedanaest iskusnih srednjoškolskih nastavnika Biologije te je obrađeno osam tema povezanih s glavnim ciljem ispitivanja. Fokusne grupe bile su snimane te su po završetku napravljeni transkripti audiosnimki. Svaku fokusnu grupu neovisno su analizirala dva procjenjivača te je slaganje među njima bilo vrlo visoko: 98 %. Rezultati upućuju na to da nastavnici smatraju da su ispiti državne mature i nastava Biologije visoko povezani. Uspjeh učenika na ispitu iz Biologije percipira se kao važan indikator kvalitete rada nastavnika. Nadalje, analizom transkripata uočeno da nastavnici ne vladaju dobro terminima povezanim s ishodima učenja. Ovo ispitivanje pokazuje da se kroz poboljšanja ispita iz Biologije može snažno utjecati na nastavni proces.

Ključne riječi: ispiti, državna matura, biologija, nastava

RELATIONSHIP BETWEEN THE STATE MATURA BIOLOGY EXAM AND HIGH-SCHOOL BIOLOGY TEACHING

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At the end of their high school education, Croatian students take State Matura exams. State Matura consists of mandatory and elective parts. The Biology exam is one of the most frequently selected exams within the elective part, and 5k to 7k candidates take it each year. The aim of this study is to examine the relationship between the State Matura Biology exam and high-school Biology teaching. In order to explore this relation, four focus groups in four Croatian regional centers were formed. In each focus group, eight to eleven experienced high-school Biology teachers participated, and eight topics related to the main objective were discussed during each session. These were audio-recorded and then analyzed independently by two trained raters. Consistency between them was 98%. The main results of the focus group study indicate a strong relationship between State Matura exams and Biology teaching. Furthermore, teachers spend lots of time to prepare students for the Biology exam. The students' success in the exam is perceived as an important indicator of the quality of teachers' work. Analyses also show that many teachers have not mastered concepts related to learning outcomes.

Keywords: exam, State Matura, Biology, high-school Biology teaching

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MISKONCEPCIJE U NASTAVI BOTANIKE

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Otkriće učeničkih miskoncepcija, tj. učeničkih spoznaja koje nisu u skladu sa znanstvenim spoznajama, a nastaju nakon učenja ili se ni tijekom učenja nisu promijenile trebalo bi nas uputiti prema traženju metoda za njihovo što ranije otkrivanje i suzbijanje. Cilj istraživanja je utvrditi učestalost miskoncepcija u području botanike kod učenika drugih razreda gimnazije prije i nakon obrade područja botanike. Pretpostavljamo da će se učestalost miskoncepcija različito umanjiti, ovisno o nastavnim metodama. Inicijalno istraživanje obavljeno je pomoću ispita s 20 pitanja otvorenog tipa na uzorku 190 učenika drugih razreda Gimnazije Vladimira Nazora Zadar, Gimnazije dr. Ivana Kranjčeva Đurđevac, Srednje škole Bartola Kašića Pag te Srednje škole Prelog iz Preloga. Inicijalno ispitivanje je obavljeno kako bismo ustanovili najučestalije miskoncepcije. Učenici su podijeljeni u dvije eksperimentalne skupine. Prvoj skupini je održavana frontalna nastava, a drugoj skupini je primjenjivana metoda suradničkog učenja i istraživačka nastava (zavisna varijabla). Nakon obrađenih tema botanike učenici su ponovno odgovarali na niz pitanja. Rezultati istraživanja su obrađeni kvantitativno u excelu. Nakon završne provjere postignuća ustanovljeno je da postoji značajna statistička razlika u korist druge istraživačke skupine. Unatoč tome neke miskoncepcije su i dalje prisutne poput: korijen iz tla crpi hranu, uloga polena je rasprostranjivanje, puči dovode vodu do lista, oplodnja se zbiva na njušci tučka i druge.

Ključne riječi: miskoncepcije, botanika, istraživačko učenje

MISCONCEPTIONS IN BOTANY TEACHING

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The discovery of students' misconceptions, or their cognitions which do not correspond with the scientific knowledge, but still are created after learning or even were not changed during that state, should direct us to find the methods for their early detection and suppression. The aim of this study is to determine the frequency of misconceptions in botanical teaching contents for second graders in gymnasiums (grammar schools) prior to and after the explanations of botanical contents. We assume that the frequency of misconceptions will vary slightly depending on the teaching methods. The initial research was conducted through the examination containing 20 open – type questions on 190 second graders of the following gymnasiums and other schools: High School Vladimir Nazor from Zadar, High School Ivan Kranjčev from Đurđevac, High School Bartol Kašić from Pag and High School Prelog from Prelog. The initial testing was carried out to establish the most common misconceptions. The students were divided into two experimental groups. The first group held frontal classes, while the second group used the method of collaborative learning and research teaching (dependent variables). After processing the botanical content, the students will be tested again with closed-type questions. The results of the research are processed in Excel. After the final check of the achievements, it was found that there was a significant statistical difference in favour of the other research group. In spite of that, some of the misconceptions which are still present such as: the root of the ground draws food, the role of the pollen is spreading, the stomas bring water to the leaf, the fertilization occurs on the muzzle of the tuck and others.

Keywords: misconceptions, botany, research teaching

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BIRDID – EDUKACIJA PREPOZNAVANJA PTICA NA STEROIDIMA

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BirdID edukaciju započeo je norveški Nord-Trondelag University College u 2006. Primarna joj je svrha povećati broj volontera u Monitoringu čestih vrsta ptica (MČVP) i drugim programima praćenja stanja ptica te poboljšati njihove vještine prepoznavanja ptica. BirdID se pokazao uspješnim u Norveškoj te je od tada, s istom svrhom, implementiran u drugim zemljama. Udruga Biom je 2016. započela implementaciju programa u Hrvatskoj te je sada u trećoj godini provedbe. Osim utjecaja BirdID programa na broj i kvalitetu sudionika MČVP-a, provedba ove edukacije u Hrvatskoj se već pokazala visokovrijednom. Udruga Biom organizira i koordinira niz aktivnosti poput promatranja, prstenovanja i monitoringa ptica za koje su uvijek potrebni sudionici i volonteri. Promatranje ptica, za razliku od zapadne i sjeverne Europe, tek postaje popularno u regiji te je jedan od ciljeva Bioma popularizirati promatranje ptica i proširiti zajednicu promatrača u Hrvatskoj. BirdID edukacija je jedinstvena mogućnost za Biom da postigne taj cilj.

Zbog fleksibilnosti BirdID edukacije, pripadajućih resursa (mrežna stranica i mobilna aplikacija) i strukture koje podržava objektivno vrednovanje vještina prepoznavanja ptica, vjerujemo da je BirdID moćan alat u izvannastavnoj nastavi biologije te vrata učenicima prema drugim skupinama živog svijeta.

Ključne riječi: prepoznavanje ptica, edukacija, birdid, volonteri

BIRDID - TEACHING BIRD IDENTIFICATION ON STEROIDS

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The Norwegian BirdID program was initiated by Nord-Trondelag University College in 2006. Its primary purpose is to increase the number of volunteers in the Common Bird Monitoring scheme (CBM) and other monitoring programs, and improve their skills in bird identification. The implementation of the training programme proved successful in Norway, and was since organised in other countries with identical priorities. Association BIOM started with implementation of the program in 2016 and now it is it in third year of implementation. Aside from the BirdID program's influence on the increase in number and quality of CBM participants, implementing this program in Croatia already had high value. Association BIOM is organising and coordinating a number of birdwatching, bird ringing and monitoring activities, for which volunteers and participants are constantly needed. Birdwatching is only beginning to gain some popularity in the region, and one of our aims is to promote recreational birdwatching and expand the community of birdwatchers in Croatia. BirdID program is providing a unique opportunity for us to come closer to fulfilling this aim. Due to the flexibility of the BirdID program, associated resources (website and the mobile app) and structure which supports objective measuring of bird identification skills, we believe it could be a powerful tool in extracurricular biology education and a gateway for pupils to other taxa.

Keywords: bird identification, education, birdid, volunteering

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PROCJENA OPAŽAČKIH SPOSOBNOSTI STUDENATA BIOLOGIJE NA PRIMJERU PROMATRANJA PAPUČICA

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Promatranje i opisivanje odnosa između pojava i organizama temelji su istraživačke nastave u vertikali biološkog obrazovanja. Cilj istraživanja bio je procijeniti sposobnost promatranja bioloških preparata i bilježenja opažanja među studenima druge godine preddiplomskog studija različitih studijskih programa biologije. Istraživanje je provedeno u sklopu nastave u praktikumu, tijekom koje se od ukupno 46 studenata zahtijevalo da, koristeći strukturirani upitnik, bilježe i ilustriraju rezultate promatranja različitih preparata papučica – netretiranih papučica te papučica obojenih kiselim metilenskim zelenilom i metodom srebrne impregnacije. Za potrebe ovog rada,

analizirana su dva pitanja iz upitnika. Većina studenata (91.5 ± 6,4%) je pored odgovora na zadana pitanja priložila i crteže papučica. Pri tome 29.5 (±9.9)% studenata koji su priložili crteže, nije označilo ucrtane strukture, dok je 16.3 (±7.0)% studenata barem djelomično označilo crtež koristeći prethodna znanja. Međutim, kod 44% studenata opažene su miskoncepcije u prethodnim znanjima i/ili je moguće da su označene strukture imenovane nasumce. U dva analizirana pitanja, svega 26 ± 2.8% studenata detaljno opisuje, ali ne imenuje opažene pojave i strukture u papučica. Ovo istraživanje temelj je za daljnje istraživanje opažačkih sposobnosti između različitih dobnih skupina predškolske djece, učenika, studenata i njihovih nastavnika s ciljem da se unaprijedi poučavanje temeljeno na promatranju i zaključivanju učenika.

Ključne riječi: istraživačka nastava, aktivno učenje, sposobnost opažanja

THE ASSESSMENT OF BIOLOGY STUDENTS' OBSERVATION SKILLS ON THE EXAMPLE OF *Paramecium* OBSERVATIONS

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Observing and describing the relationships between natural phenomena and organisms are the basics of integrating research within biology education from preschool to higher education. The aim of our research was to assess observation skills among undergraduate students of the second study year of various biology programs. Our assessment was conducted at student lab lessons, during which a total of 46 students was required to respond to a structured questionnaire, and to record and illustrate the results of their observations of various Paramecium microscopic preparations - untreated Paramecium, and Paramecium treated with acidified methyl green and silver impregnation technique. We have analyzed two questions from the questionnaire. 2% of students did not answer the questions. Most of the students (91.5 ± 6.4%) answered the questions and attached the drawings of Paramecium. 29.5 (± 9.9)% of the students did not label the drawn structures, while 16.3 (± 7.0)% partially labeled the drawings using previous knowledge. In 44% of the students, misconceptions in the previous knowledge were observed and/or they randomly selected the labeling terms. Only 26 ± 2.8% of the students provided detailed descriptions of their observations, but without naming the phenomena and structures observed in Paramecium. This assessment is the ground for further research aimed at comparing the observation skills among various age groups of children and students as well as their teachers.

Keywords: learning by research, active learning, perception skills

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KONCEPTUALNO POVEZIVANJE FAZA ŽIVOTNIH CIKLUSA ODABRANIH ORGANIZAMA UZ PRIMJENU KUMULATIVNOG UČENJA

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Cilj ovog istraživanja je odrediti usvojenost i konceptualno razumijevanje koncepta Životni ciklusi kod učenika osnovne škole i gimnazije. U svrhu ostvarenja cilja učenici su rješavali provjeru, slikovne zadatke primjene znanja i konceptualnog razumijevanja te anketni upitnik o interesima u on-line sustavu e-učenja MoD. Analiza učeničkih odgovora uključivala je kodiranje odgovora i tumačenje biološkog značenja odgovora. Izdvojeni su i objašnjeni problemi i miskoncepcije vezani uz koncept Životni ciklusi te je analizirana uspješnost učinka kumulativnog provjeravanja. Prosječna riješenost provjere OŠ 32% i G 27% za 10 % odudara od srednje riješenosti provjera iz biologije na nacionalnoj razini. Pogrešan odabir slika za pojedine faze unutar životnog ciklusa upućuje na nedovoljnu vizualizaciju bioloških sadržaja od strane učenika, nedovoljnu upotrebu nastavnih pomagala od strane nastavnika te u konačnici slabije snalaženje u univerzalnom obrascu. Utvrđena je statistički značajna umjerena korelacija uspješnosti pri rješavanju provjere i usvajanja kumulativnog obrasca životnog ciklusa s obzirom na razinu škole (ρOŠ = 0,38; ρG = 0,43; $p < 0,05$). Utvrđene miskoncepcije i problemi ukazuju na potrebu uvođenja nastavnih strategija kao što su kumulativno učenje uz izraženiju potrebu za vizualnom i iskustvenom potporom te uspostavljenjem uzročno-posljedičnih veza pri učenju.

Ključne riječi: osnovna škola, srednja škola, koncept životnih ciklusa, konceptualno razumijevanje, kumulativno učenje i provjeravanje

CONCEPTUAL CONNECTING OF LIFE CYCLE PHASES OF CHOSEN ORGANISMS WITH THE APPLICATION OF CUMULATIVE LEARNING

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The goal of this research is to determine the apprehension and conceptual understanding of the Life cycle concept within middle school and high school students. For the purpose of achieving the goal, students were given a test, image tasks with application of knowledge and conceptual understanding and a personal questionnaire in the online e-learning system MoD. The analysis of the answers included coding and interpretation of the biological meaning. The problems and the misconceptions were singled out and explained, and the succes of the cumulative method was analyzed. The average test results of the middle school students were 32% and the high school students 27%, which was 10% higher than the average test results in biology at the national level. The wrong selection of pictures depicting phases within the life cycle indicates insufficient visualization of the biological content by the students, inadequate use of teaching aids by the teacher, and a weaker resourcefulness in the universal model of the life cycles. A statistically significant moderate correlation between the success in determining the assessment and adoption of the cumulative life cycle pattern was established (ρOŠ = 0.38; ρG = 0.43; $p < 0.05$). The established misconceptions and problems point to the need to introduce teaching strategies such as cumulative learning with a more pronounced need for visual and experimental support and the establishment of causal relationships while learning.

Keywords: middle school, high school, Life cycle concept, conceptual understanding, cumulative learning and cumulative method of review

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KORIŠTENJA JEDNOSTAVNIH KLJUČEVA U RAZVOJU PRIRODOSLOVNE PISMENOSTI

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Danas kada se donose odluke poput Okvira /EU legislation establishing the European framework of key competences/ o ključnim kompetencijama (znanja, vještina i stavova) koje bi učenik u 21 st. trebao razvijati te raspravlja jesu li navedene vještine (ne)odvojive od sadržaja i (ne)prenosive na druge koncepte i područja (Hendrick, 2016.), potrebno je, u obrazovnoj praksi i teoriji, vratiti se na početak i osvijestiti važnost razvoja osnovnih vještina. U ovom radu se ističe važnost kognitivne vještine klasificiranja (razvrstavanja), jedne od osnovnih prediktora školskog uspjeha i koja je neophodna u današnjem i budućem vremenu informacijskog doba. Prikazat će se primjeri scenarija učenja pomoću kojih se na zanimljiv način učenike uvodi u osnovne principe klasifikacije uporabom jednostavnih bioloških ključeva za određivanje listova i morskih puževa i školjkaša (Bajd, 2017.). Učenicima je u početku trebala znatna pomoć u snalaženju i korištenju knjižice Moji prvi listovi, ali kako su knjižicu koristili više puta mijenjajući okruženje ona im je postala jednostavno sredstvo za određivanje imena drveća koja su i samoinicijativno istraživali u vrtu. Osim što su imenovali drveća u neposrednom okruženju te ih klasificirali u kategorije listopadno i vazdazeleno, razvijali su i vještinu klasificiranja i temeljne kompetencije važne za prirodoznanstvenu pismenost.

Ključne riječi: klasifikacija organizama, dihotomski ključevi, školjkaši, puževi, listovi, prirodoslovna pismenost

THE IMPORTANCE OF SIMPLE KEYS TO THE DEVELOPMENT OF SCIENTIFIC LITERACY

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Today, when decisions are taken, such as the EU Framework on Key Competences of the 21 century and discuss whether these learning skills are detachable from content and transferable to other concepts and areas (Hendrick, 2016), it is necessary, in education practice and theory, return to the beginning and realize the importance of developing basic skills. This paper highlights the importance of cognitive classification skills, one of the basic predictors of school success, and which is necessary in today's information age and for the future. There will be examples of learning scenarios that will introduce the students in an interesting way to the basic principles of classification by using simple biological keys for determining leaf and sea snails and shellfish (Bajd, 2017). The students initially need help in using the booklet My first leaves, but as the library used more than once it had become a simple means for determining the names of trees that are independently explored in the garden. In addition to naming trees and classifying them in categories, they also developed the skill of classification and basic competence important for natural science.

Keywords: Classification of organisms, dichotomic keys, bivalves, snails, leaves, scientific literacy

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USPOREDBA ZNANJA HRVATSKIH I SLOVENSKIH OSNOVNOŠKOLACA TE GIMNAZIJALACA O EVOLUCIJI ČOVJEKA

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U nastavi Biologije uočeni su brojni problemi učenika u razumijevanju evolucije čovjeka. Cilj rada je bio utvrditi kakvo je znanje hrvatskih i slovenskih osnovnoškolaca te gimnazijalaca o evoluciji čovjeka. Nastojalo se provjeriti postoji li razlika u znanju učenika dviju država ovisno o spolu, školi i dobi, te se željelo utvrditi postojanje miskoncepcija o evoluciji čovjeka. Istraživanje je provedeno 2017. godine u slovenskim i hrvatskim gimnazijama te osnovnim školama. Sudjelovalo je 170 hrvatskih i slovenskih učenika (100 osnovnoškolaca, te 70 gimnazijalaca). Za provedbu istraživanja koristio se anoniman ispit znanja, a podaci su obrađeni u SPSS programu s Mann-Whitney testom. Ukupni prosječni uspjeh na ispitu znanja bio je 64,8%. Najzastupljeniji točan odgovor učenika odnosio se na nalazište neandertalaca u Hrvatskoj, kod kojeg je 96,4% učenika odabralo Krapinu kao točan odgovor. Rezultati su pokazali da postoje razlike u znanju o evoluciji čovjeka između učenika dviju zemalja, različitih škola i razreda. Slovenski gimnazijalci postigli su statistički bolje rezultate u odnosu na hrvatske gimnazijalce (14 statističko značajnih razlika; $p < 0,05$), dok su hrvatski osnovnoškolci bili uspješniji od slovenskih (16 statističko značajnih razlika; $p < 0,05$). Hrvatski gimnazijalci su pokazali lošije rezultate na ispitu od slovenskih osnovnoškolaca. Hrvatski osnovnoškolci su pokazali više znanja o evoluciji čovjeka od hrvatskih gimnazijalaca.

Ključne riječi: evolucija čovjeka, znanje, Hrvatska, Slovenija, osnovna škola, gimnazija

COMPARISON OF THE KNOWLEDGE OF CROATIAN AND SLOVENIAN ELEMENTARY SCHOOL AND GRAMMAR SCHOOL STUDENTS ABOUT THE HUMAN EVOLUTION

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Previous studies show that students have difficulties in understanding the evolution of human beings. The objective of this research was to determine the knowledge of Croatian and Slovenian elementary and grammar school students about human evolution. We wanted to see if there was a difference in knowledge between the students of two countries, genders, ages and to determine students misconceptions. The research was conducted in 2017 in Slovenian and Croatian gymnasiums and primary schools (100 elementary and 70 high school students). An anonymous knowledge test was used for the research, and the data were processed in the SPSS program with the Mann-Whitney test. Research has shown that the knowledge of tested students about human evolution is satisfying (overall average success was 64,8%). The most common correct answer was about the Neandertal site in Croatia, where 96,4% of students selected Krapina as correct answer. Slovenian high school students showed more knowledge than Croatian high school students (14 statistically significant differences, $p < 0.05$), while Croatian elementary students were more successful than Slovenian (16 statistically significant differences, $p < 0.05$). Croatian gymnasiums have shown worse results than Slovenian elementary school students. Primary school knowledge about human evolution differs from grammar school by the frequency of correct answers in the test. Croatian elementary school students have shown more knowledge than Croatian high school.

Keywords: human evolution, knowledge, Croatia, Slovenia, elementary school, gymnasium

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RAZUMIJEVANJE KONCEPTA RAVNOTEŽA I MEĐUOVISNOST U ŽIVOME SVIJETU KOD UČENIKA 7. RAZREDA OSNOVNE ŠKOLE

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Cilj istraživanja je utvrditi razumijevanje makrokoncepta Ravnoteža i međuovisnost u živome svijetu, na temelju odgovora učenika 7. razreda osnovne škole na pitanja sa Županijskog natjecanja iz biologije 2018. godine. Odgovori učenika analizirani su u svrhu procjene sposobnosti učenika u rješavanju pitanja viših kognitivnih razina uz navedeni makrokoncept te uz pitanja koja povezuju više makrokoncepata. Analiza odgovora učenika uključivala je specifično kodiranje odgovora i tumačenje biološkog značenja odgovora učenika. S obzirom da je namjena ispita za natjecanje razlučiti najbolje učenike, ispit je prihvatljiv (α = 0.65) uz prosječnu riješenost ispita 69,53%. Učenici veći uspjeh pokazuju u rješavanju zadataka nižih kognitivnih razina znanja i pri rješavanju zadataka višestrukog izbora. Učenici nižih klasa riješenosti pokazuju poteškoće u interpretaciji grafičkih prikaza. Rješavanje zadataka III. kognitivne razine srednje je povezano s klasom riješenosti (ρ = 0,49; p = 0,001), međutim zadatke rješavanja problema sa 70% uspješnosti rješavaju najbolji učenici (klasa 90% i 100%). Tijekom analize ove provjere znanja utvrđeni su problemi i miskoncepcije osobito vezani uz koncept Međuovisnost živog svijeta i okoliša. Statistički značajna razlika utvrđena je kod miskoncepcija i problema vezanih uz proces disanja, te uz načine i uvjete prijenosa malarije. Miskoncepcije koje su utvrđene tokom analize, ukazuju na nužnu modernizaciju nastave biologije.

Ključne riječi: natjecanje iz biologije, međuovisnost, ravnoteža, osnovna škola, miskoncepcije

UNDERSTANDING THE CONCEPT OF EQUILIBRIUM AND INTERDEPENDENCE IN THE LIVING WORLD FOR ELEMENTARY SCHOOL STUDENTS IN THE 7TH GRADE

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The aim of the research is to determine the understanding of the macroconcept of Equilibrium and Interdependence in the Living World, based on the responses of elementary school students to questions from the County Biology competition in 2018. Student responses were analyzed for the purpose of assessing the ability of students to address the issues of higher cognitive levels along with the stated macroconcept approach and questions that link multiple macroconcepts. The student responses analysis included specific coding of the responses and the interpretation of the biological meaning of the responses. Since the purpose of the competition exam is to distinguish the best students, the exam is acceptable (α = 0.65) with an average exam resolution of 69.53%. Students have greater success in solving the tasks of lower cognitive levels of knowledge and in solving multiple choice tasks. Students of lower classes of solving show difficulties in the interpretation of graphic presentations. Solving tasks of III. cognitive levels shows a medium correlation with the class of resolution (ρ = 0.49, p = 0.001), however, 70% performance problems are solved by the best students. During the analysis of this knowledge assessment, problems and misunderstandings are identified especially in relation to the concept of interdependence of the living world and the environment. The misunderstandings found during the analysis point to the necessary modernization of the teaching of biology.

Keywords: competition in biology, interdependence, equilibirum, primary school, misconceptions

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OSVIJESTIMO NAŠE ISTRAŽIVAČKE MOGUĆNOSTI I POTAKNIMO NAŠU ISTRAŽIVAČKU KREATIVNOST

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Kroz provedbu malih istraživačkih projekata u svom bliskom okruženju, učenici detaljnije upoznaju svoju životnu sredinu i promoviraju sudjelovanje javnosti u znanstvenim istraživanjima. U ovom predavanju se navodi primjer istraživanja potoka u neposrednoj blizini škole i/ili životne sredine djece i mladih, koje se može uklopiti u nastavu Prirode i Biologije, ali i prilagoditi različitim dobnim skupinama djece i mladih - od predškolske do visokoškolske razine. Na primjeru učeničkog istraživačkog projekta, predlaže se kako odabrati postaje i mikrostaništa za uzorkovanje te kako predvidjeti kontrolne skupine i/ili replikatne uzorke u istraživačkom projektu na zadanu temu. Za provedbu projektnih aktivnosti, predlažu se metode uzorkovanja makrobeskralježnjaka i organizama obraštaja, koji ulaze u sastav bentosa tekućica, kao i pojednostavljeni ključevi za taksonomsku determinaciju organizama. Važno je napomenuti da aktivnosti i metodologija, koje se opisuju u sklopu ovog predavanja, mogu biti prilagođene i primijenjene za slična istraživanja svih tipova dostupnih tekućica, ovisno o različitim dobnim skupinama sudionika istraživanja. Cilj ovog predavanja jest osvijestiti dostupnost istraživačkih mogućnosti u našem bliskom okruženju te potaknuti istraživačku kreativnost profesora, nastavnika, odgajatelja i stručnih suradnika prisutnih na različitim razinama odgojno-obrazovnog procesa.

Ključne riječi: istraživački projekt, urbana ekologija, ekološki koncepti

RAISING AWARENESS OF OUR RESEARCH POSSIBILITIES AND ENCOURAGING OUR RESEARCH CREATIVITY

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Through the implementation of small research projects in their close surroundings, students learn more about their environment and promote ‘citizen science’ in scientific research. This lecture highlights an example of an exploration of stream habitats located in the close surrounding of schools and/or children living surrounding. This example can be integrated into the Sciences and Biology classes but also adapted to different age groups - from pre-school to higher education levels. The given example proposes how to select the sampling sites and microhabitats, how to choose reference sites and/or control or replicate samples in the research project on the given topic. The methodology for sampling stream benthic macroinvertebrates and periphyton organisms as well as simplified keys for taxonomic determination of the sampled organisms are further proposed. It is important to highlight that the activities and methodology described in this lecture can implemented within similar research of freshwater habitats, but it should be adapted to the different age groups of the research participants. The aim of this lecture is to raise awareness of the availability of research opportunities in our close environment and to stimulate

the research creativity of professors, teachers, educators and expert associates present at different levels of the educational process.

Keywords: research project, urban ecology, ecological concepts

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ODRŽATI SE POD SVAKU CIJENU – RAZMNOŽAVANJE BILJAKA

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Zbog nerijetko nerazjašnjenih i / ili pogrešnih interpretacija (naročito u školskim udžbenicima) o životnim ciklusima i razmnožavanju biljaka, jedna od najčešćih učeničkih miskoncepcija je poistovjećivanje vegetativnog i nespolnog razmnožavanja biljaka. Svrha ovog predavanja je ukazati na potrebu razrješavanja postojećih pogrešnih tumačenja. Naime, biljke imaju dva načina razmnožavanja: generativno i vegetativno, vezana uz njihov ciklus izmjene generacija. Generativnim razmnožavanjem biljka stvara isključivo haploidne gamete ili spore, koje stvaraju genetički različite potomke. Stvaranje spora naziva se nespolnim generativnim razmnožavanjem jer u izmjeni generacija spore ne ulaze u proces oplodnje, a nastaju mejotičkim diobama i produkt su sporofitske generacije. Njihovim daljnjim mitotičkim diobama razvija se generacija gametofit, koja stvara gamete. Stoga je to spolno generativno razmnožavanje jer u izmjeni generacija gamete uvijek ulaze u proces oplodnje. Vegetativnim pak razmnožavanjem biljka nikada ne stvara spore ili gamete, već umnožava somatske stanice svog vegetativnog tijela ili njegovog dijela, isključivo mitotskim putem. Tako nastaju specifični fragmenti, vegetativna rasplodna tjelešca, bulbili, vriježe, podanci, gomolji i sl. Potomci ovakvog razmnožavanja uvijek su genetički identični matičnoj biljci koja ih je proizvela (klonovi) te je vegetativno razmnožavanje "kolniranje" dominantne generacije biljaka (gametofit kod mahovina, a sporofit kod ostalih biljaka).

Ključne riječi: izmjena generacija biljaka, generativno (spolno i nespolno) razmnožavanje, vegetativno razmnožavanje

SURVIVAL AT ALL COSTS – PROPAGATION OF PLANTS

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Due to often unclear and / or misleading interpretations (especially in school textbooks) about life cycles and plant propagation, one of the most common student misconceptions is the equation of vegetative and semi-reproductive plant reproduction. The purpose of this lecture is to point to the need to resolve the existing misconception. Namely, plants have two basic ways of reproduction: generative and vegetative, related to their life cycles. Generative reproduction of the plant creates only haploid gametes or spores, which produce genetically different descendants. Creating spores is called an asexual generative propagation because in life cycles spores do not enter the process of fertilization and are formed by meiosis by a sporophyte generation. Their further mitotic divisions evolve into the generation of gametophyte, which creates gametes. Therefore, this is a sexually generative propagation because in life cycles gametes always enter the process of fertilization. By vegetative propagation, the plant never

creates spores or gametes, but multiplies the somatic cells of its vegetative body or part thereof, solely mitotically. This kind of reproduction is always genetically identical to the parent plant (clones) and we can conclude that vegetative propagation is the cloning of the dominant generation of plants (gametophyte in mosses, and sporophyte in other plants).

Keywords: plants life cycle, generative (sexual and asexual) propagation, vegetative propagation

4. Simpozij Hrvatskog entomološkog društva
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PRELIMINARY DATA OF ARTHROPOD COMMUNITY STRUCTURE ON JABLANICA MT., SOUTH-WEST R. MACEDONIA

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In this paper, preliminary data of arthropod community structure and composition between four different forest types and one riparian habitat on Jablanica Mountain are presented. The research was carried out in the period May-October 2017, by using pitfall traps placed along a transect line in five different forest types from 1050 to 1300 m a.s.l. In total, 11170 individuals belonging to 14 groups (Pseudoscorpiones, Scorpiones, Ispoda, Araneae, Lithobiomorpha, Scolopendromorpha, Collembola, Blattodea, Orthoptera, Hemiptera, Coleoptera, Diptera, Lepidoptera, Hymenoptera) were registered. Highest values of species richness were recorded in "St. Spas" locality (ass. *Castanetum sative*). Among them, Coleoptera was the most representative group including 34 species from 14 families. The highest relative abundance of arthropods (4610 individuals) was registered in the locality St.Spas, while the lowest (573 individuals) in the riparian locality near "Vevchani Springs". Regarding the seasonal dynamics, for most of the groups, highest activity values were registered in June, as expected. The results demonstrate that different forest types exhibit communities that reflect its characteristics, corroborating that the dominant vegetation type influences community composition and structure.

Keywords: Arthropods community structure, Jablanica Mt. Macedonia

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BIOLOŠKE OSOBINE KRUŠKINE OSE SRČIKARICE, *Janus compressus* FABRICIUS 1793. (HYMENOPTERA: CEPHIDAE)

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Kruškina osa srčikarica (*Janus compressus* Fabricius) je periodični štetnik. Posljednjih godina sve je značajniji štetnik osobito u mladim nasadima kruške. Kukac pripada porodici Cephidae (ose vlatarice). Primarni domaćin je kruška koju i napada najintenzivnije. Sekundarni domaćin je jabuka dok mušmulu, dunju i aroniju napada samo sporadično i ako se nalaze u blizini primarnog domaćina ili u godini masovne pojave. U pogledu morfološko-bioloških osobina kruškine ose srčikarice, prvu obuhvatniju znanstvenu studiju objavili su 1935. godine Balachowsky i Mesnil. Dostupna novija literatura, dosta skromno opisuje ovoga štetnika. Podaci o biološkom ciklusu štetnika su vrlo šturi i nekompletni, dok su pojedini podaci o biološko-morfološkim osobinama dosta kontradiktorni i konfuzni. Opisom bioloških osobina kruškine ose srčikarice nastoji se eliminirati uočene literaturne proturječnosti. Istraživanja bioloških osobina imaga, jaja i ličinki, provedena su na pet lokaliteta, na širem području Đakovštine. Imaga su u nasadima hvatana pomoću žutih ljepljivih ploča i entomološko mrežom. Jaja su proučavana nakon ekstrakcije iz abdomena uhvaćenih ženki ili pažljivom analizom (nakon rasijecanja), mladih izbojaka u koje je ženka odložila jaje. Ličinke su proučavane nakon uzdužnog rasijecanja i vađenja iz zaraženih izbojka. Rezultati istraživanja su pokazali da postoje znatna odstupanja od ionako šturih literaturnih navoda, u svezi nekih bioloških osobina kruškine ose srčikarice.

Ključne riječi: kruškina osa srčikarica, biološke osobine, imago, jaja, ličinke

BIOLOGICAL CHARACTERISTICS OF PEAR SHOOT SAWFLY, *Janus compressus* FABRICIUS 1793. (HYMENOPTERA: CEPHIDAE)

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Pear shoot sawfly is a periodical pest. It has become more significant pest in recent years, especially in young pear orchards. The insect belongs to the Cephidae family. The attacks are the most intense on pear which is its primary host. Secondary hosts are apples while attacks on medlar, quince and aronia are sporadic or if they are near the primary host or it is the year of the mass phenomenon. The first comprehensive scientific study regarding the morphological and biological characteristics of pear shoot sawfly was published in 1935 by Balachowsky & Mesnil. Recent available literature describing this pest is insufficient. Data on the biological cycle of pests are poor and incomplete, while some data on its biological-morphological traits are quite contradictory and confusing. Description of the biological characteristics of pear shoot sawfly can possibly eliminate the observed contradictions found in literature. Research on biological properties of pear shoot sawfly was carried out on five sites in the wider area of Đakovština. Imagos were captured by yellow sticky traps and entomological net. Eggs were studied after extraction from abdomen of females or by careful analysis of young shoots where the female had oviposited the eggs. The larvae were studied after longitudinal dissection and removal from infected shoots. The results have shown that there are significant deviations from already lacking literary allegations regarding to some biological features of this insect.

Keywords: *Janus compressus* F., biological characteristics, imago, eggs, larvae

O-43

DISTRIBUTION OF *Drusus osogovicus* KUMANSKI, 1980 (TRICHOPTERA: LIMNEPHILIDAE) ON THE BALKAN PENINSULA

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The biodiversity of the order Trichoptera on the Balkan Peninsula has been in the focus of research of many entomologists through the years. However, the number of species slowly but constantly is still increasing, which is a strong indicator that this order of insects may soon be classified among the most diverse in the region. Along with the diversity, distribution area of some known present species is also widening. In this research we present the first reliable records of *Drusus osogovicus* Kumanski 1980 in the R. Macedonia. During the intensive entomological investigation conducted in summer 2017, we recorded this species from Osogovo Mountains. Previously it has been reported as *Drusus* cf *osogovicus* from Karadak/SkopskaCrna Gora Mountains in Macedonia, indicating the need for more specimens to be found in order to reliably identify its status. Since *D. osogovicus* was known to be present only in Bulgaria, notes on its limited distribution on the Balkan Peninsula are given. The results obtained in our research enriches the Trichoptera fauna in R. Macedonia and at the same time contributes to the knowledge of caddisflies distribution on the Balkan Peninsula, providing information for future faunistic investigations as well as possible conservation implications towards studied springs and streams.

Keywords: Trichoptera, *Drusus*, distribution, R. Macedonia, Balkan

O-44

PHYLOGEOGRAPHY OF A POTENTIAL BIOCONTROL AGENT, TRIOXYS SUNNYSIDENSIS (HYMENOPTERA: BRACONIDAE: APHIDIINAE)

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The bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus 1758) is a major cereal pest with a near-cosmopolitan distribution, which damages host plants by both feeding on them and by its role as a vector of numerous plant viruses. As one of the main groups of biocontrol agents of aphids, numerous Aphidiinae are associated with *R. padi*, including some species from the

genus Trioxys Haliday 1833. A recently described species parasitizing *R. padi*, Trioxys sunnysidensis Fulbright & Pike 2007, is recorded from Europe for the first time based on morphological and molecular data. Specimens from North America, Europe and New Zealand were used in the analysis of the cytochrome c oxidase subunit I (COI) to explore genetic variation among populations of this parasitoid. The analysis revealed one of the highest haplotype diversities in Aphidiinae so far, with 25 haplotypes detected. Two most common haplotypes are shared across groups of populations, while all others are found either in North America or Europe. This suggests that, while closely related, these populations have been evolving independently for some time. Since different populations of biocontrol agents can have different effects on target pests, genetic structure of populations is an important factor to consider when choosing a biocontrol agent. In that sense, the findings of this study can be used in potential biocontrol attempts.

Keywords: Aphidiinae, Trioxys, phylogeography, biocontrol

O-45

NEGLECTED GENUS HOLDING NUMEROUS CRYPTIC SPECIES: *Lipolexis* FÖRSTER, 1862 (HYMENOPTERA: BRACONIDAE: APHIDIINAE)

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Genus *Lipolexis* Förster is a small member of the subfamily Aphidiinae, represented only by four described species: *L. gracilis* Förster from Europe and *L. oregmae* Gahan, *L. wuyiensis* Chen and *L. myzakkaiae* Pramanik and Raychaudhuri from Asia. Species of this genus are primary parasitoids of numerous pest aphids and therefore possess potential in biological control. However, in spite of their wide presence througout their range, there has been confusion in the systematics and taxonomy of this genus, with taxons being re-described several times and bearing numerous synonym names. Furthermore, *L. wuyiensis* and *L. myzakkaiae* do not appear in the literature after their description. With the aim of establishing taxonomic positions of the species within the genus Lipolexis, we used the barcoding region of the mitochondrial cytochrome oxidase gene subunit I (COI), the molecular marker that proved to be reliable in previous studies of the subfamily Aphidiinae. Specimens used in this study were collected during the past decade throughout Europe, Asia and North America. The results of molecular phylogenetic analysis grouped *Lipolexis* specimens into nine distinct clades, with molecular distance ranging from 3% to 22%. Furthermore, the morphological examination of specimens showed clear variation in important taxonomic characters. This new insight into systematics of *Lipolexis* suggests that several cryptic species could be hidden within what was considered to be a well known group.

Keywords: Aphidiinae, *Lipolexis*, systematics

O-46

PRELIMINARNI REZULTATI BARKODIRANJA FAUNE KOMARACA HRVATSKE

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Komarci predstavljaju često istraživanu skupinu kukaca unutar reda dvokrilaca (Diptera) budući su vektori uzročnika bolesti čovjeka i životinja. Identifikacija komaraca stoga je ključan korak u nadzoru i kontroli bolesti koje oni prenose. Za preciznije determiniranje vrsta uz morfološke metode potrebno je kombinirati i molekularne. U Hrvatskoj je trenutno zabilježeno 53 vrste komaraca od kojih su i dvije invazivne vrste i vrste od javnozdravstvenog značaja (*Aedes albopictus* i *Aedes japonicus*). Utvrđivanje genetskog statusa ove skupine kukaca je od velikog taksonomskog, filogenetskog i filogeografskog značaja. U sklopu projekta „DNK barkodiranje hrvatske faune“ tijekom prve godine provedeno je prikupljanje uzoraka s područja kontinentalne, mediteranske i alpinske regije Hrvatske tijekom lipnja, srpnja i kolovoza 2017. godine. U prikupljenim uzorcima određeno je 14 vrsta komaraca. Jedan dio materijala morfološki je determiniran te adekvatno pohranjen za daljnju molekularnu analizu. Dio materijala je uspješno identificiran i morfološkim i molekularnim metodama, a to su vrste *Culex pipiens komplex, Culex torrencium, Culex hortensis, Culex territans*, te *Culiseta longiareolata*. Tijekom druge godine projekta nastaviti će se prikupljanje materijala kroz sve tri regije Hrvatske s ciljem uzorkovanja ostalih vrsta komaraca kao i rijetkih vrsta te DNK barkodiranje istih.

Ključne riječi: komarci, *Aedes albopictus*, *Aedes japonicus*, DNK barkodiranje, vrsta

PRELIMINARY RESULTS OF THE BARCODING OF THE MOSQUITO FAUNA IN CROATIA

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The mosquitoes represent a frequently investigated group of insects within the order Diptera precisely because they are human and animal disease vectors. Mosquito identification is therefore a key step in supervising and controlling the diseases they transmit. To achieve more precise identification morphological methods should be complemented with molecular methods. Currently, there are 53 mosquito species in Croatia, including two invasive species which are also species of great importance to public health (*Aedes albopictus* and *Aedes japonicus*). Determining the genetic status of this insect group is of great taxonomic, phylogenetic and phylogeographic significance. Within the first year of the project "DNA Barcoding of the Croatian Fauna", mosquitoes were sampled from the continental, Mediterranean and alpine regions of Croatia during June, July and August 2017. Among the sampled mosquitoes fourteen species were determined. One part of the collected material has been morphologically determined and adequately stored for further molecular analysis. The other part has been successfully identified using morphological and molecular methods to the species: *Culex pipiens complex, Culex torrencium, Culex hortensis, Culex territans*, and *Culiseta longiareolata*. During the second year of the project, mosquitoes throughout the three regions of Croatia will continue to be collected with the aim of sampling other types of mosquitoes as well as rare species and their DNA barcoding.

Keywords: mosquitoes, *Aedes albopictus, Aedes japonicus*, DNA barcoding, species

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DANJI LEPTIRI HRVATSKE

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Posljednjih desetak godina naš rad bio je fokusiran na razvoj jedinstvene baze podataka o rasprostranjenosti leptira Hrvatske s ciljem objave nacionalnih popisa leptira te publiciranjem atlasa rasprostranjenosti vrsta. Danas se baza sastoji od objavljenih literaturnih podatka, podataka iz zbirki muzeja te nepubliciranih podataka koja sežu tri stoljeća unatrag. Iako su istraživanja hrvatske faune leptira bila nesistematična i sporadična, a neka su područja pobuđivala veći interes od drugih, tijekom godina uspjeli smo procjeniti ugroženost danjih leptira te publicirati listu i Crvenu knjigu danjih leptira Hrvatske. Procjene govore da je oko 25% faune danjih leptira Hrvatske na neki način ugroženo. Ovdje navodimo samo glavne razloge ugroženosti koji su posljedica korištenja zemljišta uzrokovanih demografskim promjenama i ekonomskom tranzicijom što za posljedicu ima ili intenzifikaciju ili zapuštanje poljoprivredne proizvodnje. Posljednjih godina naš cilj je uspostava monitoring danjih leptira Hrvatske s ciljem prikupljanja podataka o statusu vrsta u Hrvatskoj. Osim intenzivnih istraživanja populacija različitih vrsta od 2017. godine uključeni smo u projekt barkodiranja bioraznolikosti hrvatske faune (CroBarFauna) s primarnim ciljem uspostavljanja nacionalne baze genetičke raznolikosti.

Ključne riječi: danji leptiri, Hrvatska, CroBarFauna

BUTTERFLIES OF CROATIA

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In the last decade, our work has been attempt to create a national database of distribution records in Croatia to publish the national checklist of butterflies and moths with future distribution atlas. Today the database includes published papers, collection data and unpublished records across three centuries. Even though data collecting has been sporadic and uneven, with some areas receiving more attention than others we managed to assess the threat status of butterfly fauna published in the Red Book of threatened butterflies of Croatia. About 25% of Croatia's butterfly fauna is of conservation concern. The main causes of the declines are thought to be changes in rural land use, especially land abandonment and agricultural intensification because of demographic changes and economic transition. Recent activities of the Lepidoptera working group gathered around the EU Natura 2000 Integration Project (NIP) with the main objective of gathering new distributional data and preparation for setting future monitoring of the species gave us a new inside. In 2017 we started a 5-year project of DNA barcoding of Croatian biodiversity (CroBarFauna) with primary goal of establishing a national database of genetic diversity.

Keywords: butterflies, Croatia, CroBarFauna

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PRELIMINARNI PREGLED FAUNE MOLJACA TRAVA (LEPIDOPTERA: CRAMBIDAE) HRVATSKE

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Moljci trava (Crambidae) su baš poput većine drugih porodica skupine Microlepidoptera gotovo u potpunosti neistraženi na području Hrvatske, s tek rijetkim sustavnijim istraživanjima poput otoka Krka (Habeler, 2003) i južne Dalmacije (Klimesch, 1942). Među pripadnike ove porodice ubrajamo mnoge značajne štetnike na poljoprivrednim kulturama i ukrasnome bilju kao i strane i invazivne vrste koje se postepeno šire Europom. U sklopu ovog istraživanja ujedinili smo sve podatke iz literature, internetskih portala te neobjavljenih nalaza prikupljenih na području Hrvatske u zadnjih desetak godina. Svi podatci su upisani u Excel bazu podataka, prilagođeni današnjoj nomenklaturi te georeferencirani, čime je dobivena osnova za daljnja istraživanja faune moljaca trava Hrvatske. Nadalje, izrađen je preliminarni popis moljaca trava Hrvatske koji broji 211 vrsta, odnosno približno 50 % europske faune ove porodice. Pretpostavljamo da će se u budućnosti zabilježiti još dodatnih vrsta, posebno na području južne Dalmacije i planinskim područjima Dinarida. Potrebno je uložiti još mnogo napora, terenskog rada, i pregleda muzejskih zbirki kako bi se dobio potpuni pregled u raznolikost ove porodice na području Hrvatske.

Ključne riječi: microlepidoptera, raznolikost, rasprostranjenost

PRELIMINARY CHECKLIST OF GRASS MOTHS (LEPIDOPTERA: CRAMBIDAE) OF CROATIA

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The grass moths (Crambidae), are just like most other families of the Microlepidoptera almost completely unexplored in Croatia, with only a few systematic surveys like on Krk Island (Habeler, 2003) and southern Dalmatia (Klimesch, 1942). There are many significant pests on agricultural crops and decorative plants among the members of this family, as well as foreign and invasive species that are gradually spreading across Europe. Within this research, we have united all data from the literature, internet portals, and unpublished records collected across Croatia over the last ten years. All the data are contained in an Excel database, adapted to today's nomenclature and georeferenced, thus providing a basis for further research of the Crambidae fauna. In addition, a preliminary list of Croatian grass moths was created, which contains 211 species, ie approximately 50% of the European fauna. We assume that more species will be recorded in the future, especially in southern Dalmatia and the Dinaric Mountains. Additional effort, field work, and reviews of museum collections are needed in order to get a full overview of the diversity of this family in Croatia.

Keywords: microlepidoptera, diversity, distribution

7. Simpozij Hrvatskog Društva za biljnu biologiju

O-49

DOBRI VS. LOŠI MOMCI: GLJIVE

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U ovom istraživanju ispitivali smo kako gljive istog roda *Fusarium* mogu djelovati kao patogeni i unapređivači zdravstvenog stanja biljaka. U prethodnim istraživanjima utvrdili smo kako endofitne *Fusarium* gljive izolirane s korova bez simptoma bolesti mogu pozitivno utjecati na rast i razvoj pšenice i trešnje (Ilic et al. 2012; Ilic et al. 2017). U ovom istraživanju inokulirali smo zrna pšenice s prethodno spomenutim endofitnim gljivama i posijali ih u polje. Nakon žetve zrna su pregledana na prisustvo *Fusarium* sp. te je utvrđena masa 1000 zrna. Endofitne gljive se mogu primjeniti kao biološka kontrola te se na taj način može smanjiti ili potpuno ukinuti primjena klasičnih pesticida koji uzrokuju zagađenje okoliša, ostatke pesticida u tlu, zraku i vodi i otpornost patogena.

Ključne riječi: endofiti, *Fusarium,* pšenica, patogenost

GOOD VS. BAD GUYS: FUNGI

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In this research we investigated how fungi of the same genus *Fusarium* can act both as plant pathogens and fitness improvers. In previous research we have proven that endophytic *Fusarium* isolated from symptomless weed can positively influence growth of wheat and cherry (Ilic et al. 2012; Ilic et al. 2017). In this research we inoculated wheat grains with the previously mentioned endophytic fungi and planted them in the field. After the harvest *Fusarium* disease symptoms were observed and mass of 1000 grains was measured. Endophytic fungi can be used as biological control and in that way reduce or completely replace use of classical chemical pesticides which cause environment polution, residues in soil and water and pathogen resistance.

Keywords: Endophytes, *Fusarium*, wheat, pathogenicity

O-50

MATH-BTB PROTEINI UROČNJAKA SUDJELUJU U RNA USMJERENOJ DNA METILACIJI

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BPM proteini uročnjaka pripadaju porodici MATH-BTB proteina koji sudjeluju u brojnim razvojnim procesima biljaka i životinja, te su se pokazali ključnim regulatorima staničnog ciklusa. Genom uročnjaka sadrži šest gena za BPM proteine (BPM1-6). Uročnjakovi proteini BPM vežu transkripcijske faktore iz nekoliko porodica označujući ih za ubikvitinaciju kulin3 ovisnom E3 ligazom. Preliminarna istraživanja pokazala su da protein BPM1 stupa u interakciju s proteinima DMS3 i RDM1, koji su važne komponente u procesu RNA usmjerene DNA metilacije. Međutim, uloga BPM proteina u RdDM procesu nije poznata. Kako bi razjasnili sudjeluju li i ostali uročnjakovi MATH-BTB proteini (BPM2-6) u procesu RdDM, sustavom dvaju kvašćevih hibrida istražene su interakcije s proteinima DMS3 i RDM1. Također, istražena je interakcija mutiranog BPM1 proteina s proteinima DMS3 i RDM1. Dobiveni rezultati pokazuju da svih šest BPM proteina stupa u interakcije s DMS3 i RDM1. Domena BTB je osobito važna za interakciju s proteinom RDM1. Dobiveni rezultati upućuju na ulogu proteina BPM u regulaciji metilacije DNA koju tek treba istražiti.

Ključne riječi: *Arabidopsis thaliana*, proteinske interakcije, RNA usmjerena DNA metilacija

ARABIDOPSIS MATH-BTB PROTEINS ARE INVOLVED IN RNA DIRECTED DNA METHYLATION

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Arapidopsis BPM proteins belong to MATH-BTB protein family. MATH-BTB proteins partake in many developmental processes in plants and animals and are known as key regulators of the cell cycle. Arabidopsis genome contains six genes encoding for BPM proteins (BPM1-6). BPM proteins bind members of several transcription factor families targeting them for ubiquitination by Cullin3 E3 ligase. Our preliminary results showed BPM1 interaction with DMS3 and RDM1, important components of RNA-directed DNA methylation (RdDM). Still, the role of BPM proteins in DNA methylation remains unknown. To elucidate an involvement of other Arabidopsis MATH-BTB proteins (BPM2-6) in RdDM, interactions with DMS3 and RDM1 were examined in yeast two hybrid system. Additionally, truncated versions of the BPM1 missing an individual domain were used to test domain specificity for interaction with DMS3 and RDM1. All BPM proteins show interactions with both investigated components of RdDM. BTB domain of BPM1 protein proved to be the most important for interaction with RDM1. Results obtained here point to a role of BPM proteins in regulation of DNA methylation that will be further investigated.

Keywords: MATH-BTB, *Arabidopsis thaliana*, protein-protein interactions, RNA-directed DNA methylation

O-51

SASTAV PONAVLJAJUĆE DNA U GENOMU DALMATINSKOG BUHAČA (*Tanacetum cinerariifolium* (Trevir.) Sch.Bip.)

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Dalmatinski buhač (*Tanacetum cinerariifolium* (Trevir.) SCH. Bip.) je endemska vrsta istočne obale Jadrana koja se u svijetu koristi za proizvodnju organskog insekticida piretrina. Dosadašnji znanstveni radovi o dalmatinskom buhaču bili su usmjereni na istraživanje njegovih morfoloških i biokemijskih značajki koje su relevantne za uzgoj. Međutim, malo se zna o evoluciji kromosoma i organizaciji genoma ove biljne vrste. Naše istraživanje ima za cilj prepoznati, klasificirati i karakterizirati ponavljajuće elemente u vrsti *T. cinerarriifolium* upotrebom metode klasteriranja genomskih sekvenci. Ponavljajuće DNA predstavljaju oko 82% sekvenciranog genoma. Vrsta *T. cinerariifolium* pokazuje neuobičajenu konfiguraciju rDNA tzv. L-tip organizacije rDNA. FISH je pokazao intersticijska telomerna ponavljanja koja vjerojatno predstavljaju evolucijski relikt izveden iz kromosomskih rearanžmana i DNA popravka. Tri satelitne DNA porodice prepoznate su u vrsti *T. cinerariifolium*. Satelitne DNA porodice TcSAT1 i TcSAT2 heterogeno su raspodijeljene u subtelomernim područjima na kromosomima, dok je porodica TcSAT3 smještena interkalarno na dužem kraku dva acrocentrična kromosomska para. Gypsy i Copia LTR-retrotranspozoni su najzastupljeniji dijelovi genoma. Dobiveni rezultati ukazuju na značajnu plastičnost genoma vrste *T. cinerariifolium*, te potvrđuju da subtelomerna područja predstavljaju jedno od najdinamičnijih i brzo evoluirajućih regija eukariotskog genoma.

Ključne riječi: *Tanacetum cinerariifolium*, satelitna DNA, L-tip konfiguracije rDNA, transpozoni

REPETITIVE DNA COMPOSITION OF DALMATION PYRETHRUM (*Tanacetum cinerariifolium* (Trevir.) Sch.Bip.) GENOME

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Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.), a plant species endemic to the east Adriatic coast, is used worldwide for production of the organic insecticide, pyrethrin. Most studies concerning Dalmatian pyrethrum were focused on its morphological and biochemical traits that are relevant for breeding. However, little is known about the chromosome evolution and genome organisation processes in this species. Our study aims to identify, classify, and characterize repetitive elements in the *T. cinerarriifolium* genome using clustering analysis of a low coverage genomic dataset. Repetitive DNA represented about 82% of the sequenced genome. *T. cinerariifolium* exhibited an unusual linked rDNA configuration (L-type). FISH analysis has identified interstitial telomeric repeats which are likely evolutionary relics of chromosomal rearrangements and DNA repair. Three satellite DNA families were identified in *T. cinerariifolium*. TcSAT1 and TcSAT2 satellite DNA families were heterogeneously distributed between different chromosome ends, while TcSAT3 family was exclusively located

intercalary on the longer arm of two acrocentric chromosome pairs. Gypsy and Copia LTR-retrotransposons were the most abundant components of the genome. Altogether our data highlights the striking genome plasticity of *T. cinerariifolium* and confirms that subtelomeres represent one of the most dynamic and rapidly evolving regions in eukaryotic genomes.

Keywords: *Tanacetum cinerariifolium*, satellite DNA, L-type rDNA configuration, transposones

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DREVNA DNA IZ POLENA: NOVI PRISTUP REKONSTRUKCIJI POPULACIJSKE POVIJESTI BILJAKA

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Rekonstrukcija populacijske povijesti biljaka igra ključnu ulogu u istraživanju ekoloških i evolucijskih procesa. Tijekom zadnjeg stoljeća najčešće korištene metode za istraživanje drevnih populacija biljaka bile su temeljene na morfologiji fosiliziranih biljnih ostataka (npr. polen, sjemenke, fitoliti). Taksonomska i tehnička ograničenja tih methoda su potaknule potragu za novim pristupima, i drevna DNA je nedavno predložena kao obećevajući novi 'proxy' za istraživanje biljnih populacija kasnog Kvartara, koji omogućava direktan uvid u genetičke posljedice okolišnih and antropogenih promjena velikih razmjera za biljne populacije. Iako se drevna biljna DNA može izolirati iz različitih fosila, polen predstavlja jedan od najučestalijih and najobilnijih izvora drevne DNA za većinu biljnih vrsta. Međutim, izazovi vezani za vađenje i analizu drevne polenove DNA su značajni, i njihovo savladavanje će ovisiti o razvoju novih experimentalnih i bioinformatičkih methoda. U ovom predavanju dat ću kratak pregled povijesti istraživanja DNA iz drevnog polena, predstaviti aktualni rad nase grupe u Cambridge-u (s fokusom na visoko propusno sekvencioniranje individualnih polenovih zrna i primjenu ove methode u rekonstrukciji populacijske povijesti biljaka) i razmotriti buduće istraživačke smjerove.

Ključne riječi: drevna DNA, polen, populacijska povijest biljaka

ANCIENT POLLEN DNA AS A NOVEL TOOL FOR RECONSTRUCTING PLANT POPULATION HISTORIES

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Reconstruction of plant population histories holds a central position in the study of ecological and evolutionary processes. For over a century the most commonly used methods for reconstructing ancient plant populations were based on the morphology of fossilised plant remains (e.g. pollen, seeds, phytoliths). Taxonomic and technical limitations of these methods have prompted a search for novel approaches. Ancient DNA (aDNA) has been recently put forward as a promising new proxy for studying late Quaternary plant populations, allowing

direct inquiry into their genetic responses to large-scale environmental and anthropogenic changes. While ancient plant DNA can be retrieved from various types of fossils, pollen represents one of the most common and abundant sources for the majority of plant species. However, there are significant challenges associated with retrieving and analysing this type of aDNA, which will require development of new experimental and computational methods. In this talk, I will give a brief account of the history of fossil pollen DNA research, present the work currently being undertaken by our group in Cambridge (with a focus on the high-throughput single-pollen sequencing and its application to reconstructing plant population histories) and discuss future research directions.

Keywords: ancient DNA, aDNA, pollen, plant population history

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ULOGA FEREDOKSIN:NADP+ OKSIDOREDUKTAZE U ALTERNATIVNIM PUTEVIMA PRIJENOSA ELEKTRONA U FOTOSINTEZI

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Feredoksin:NADP+ oksidoreduktaza (FNR) je flavoenzim koji katalizira završni korak u linearnom toku elektrona u fotosintezi u kojem se elektroni reverzibilno prenose s feredoksina na NADP+. FNR dolazi u dva oblika; kao topljiv protein lokaliziran u stromi te vezan u komplekse na membrani s proteinima TROL (thylakoid-rhodanase-like protein) i Tic62 (translocon on the inner chloroplast membrane). Smatra se da feredoksin efikasno prenosi elektrone alternativnim putevima kada FNR nije vezan za TROL. Korišteni modelni biljni organizam *Arabidopsis thaliana* L. Col-0 posjeduje dvije lisne izoforme proteina FNR, dok *Zea mays*, C4 biljka, sadrži tri izoforme. U istraživanjima je korištena FNR1 izoforma iz kukuruza za koju je dokazano da snažno interagira s TROL-om iz *A. thaliana* a prisutna je i u štapićastim stanicama i stanicama mezofila. PCR metodom na ZmFNR1 dodani su biljezi FLAG i HA kako bi omogućili kasniju izolaciju i analizu proteinskih kompleksa. Konstruirali smo transgenične C3 biljke *A. thaliana* koje sadrže element C4 fotosinteze. Križanjem tih biljaka s *A. thaliana* koji pojačano eksprimira TROL s promijenjenim specifičnim aminokiselinama i domenama, stvorili smo eksperimentalne platforme koja će poslužiti za otkrivanje dijelova proteina TROL odgovornih za dinamičko vezanje FNR-a u različitim uvjetima osvjetljenja. Ovo istraživanje financira Hrvatska zaklada za znanost, projekt IP 2014-09-1173.

Ključne riječi: fotosinteza, FNR, TROL, proteinski kompleksi, *Arabidopsis thaliana* (L.) Heynh.

IMPORTANCE OF FERREDOXIN:NADP+ OXYDOREDUCTASE IN PHOTOSYNTHETIC ALTERNATIVE ELECTRON TRANSFER PATHWAYS

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Ferredoxin:NADP+ oxidoreductase (FNR) catalyses reversible reaction of electron transfer from ferredoxin to NADP+ in photosynthesis. FNR is present as a soluble protein in stroma, but also as dynamically bound to the protein complexes with the thylakoid rhodanase-like protein (TROL) and the translocon on the inner envelope chloroplast membrane 62 (Tic62). It is envisaged that ferredoxin efficiently passes electrons to alternative pathways when FNR is not sequestered to the TROL. We are using the common C3 model plant organism *Arabidopsis thaliana* L. Col-0 that has two leaf FNR isoforms and *Zea mays*, a C4 plant that contains three isoforms. We have chosen maize FNR1 because it has been shown that ZmFNR1 strongly binds to the TROL. FLAG and HA tags were PCR-added to the 3´-end of the ZmFNR1 gene to enable subsequent isolation and analyses of protein complexes via tandem affinity purification. By using Gateway cloning technology, we have constructed Arabidopsis transgenic plants containing ZmFNR1 bearing C-terminal FLAG and HA tags. By crossing those plants with the Arabidopsis plants that accumulate TROL with amino acid or domain changes we have created specific experimental platforms which will be used to explore TROL structures that are involved in the dynamic FNR binding under different light conditions. This research is funded by Croatian Science Foundation Project IP2014-09-1173

Keywords: photosynthesis, FNR, TROL, protein complexes, *Arabidopsis thaliana* (L.) Heynh.

O-54

UMANJENA STOPA OPLODNJE SORTE VINOVE LOZE GRK (*Vitis vinifera* L.) UZROKOVANA JE AKOLPORATNOM GRAĐOM VANJSKE STIJENKE PELUDNOG ZRNA

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Grk je autohtona hrvatska sorta vinove loze koja se isključivo uzgaja na otoku Korčuli. Jedna je od rijetkih sorti vinove loze s funkcionalno ženskim cvijetom, što uzrokuje određene probleme u proizvodnji grožđa uzrokovane smanjenom stopom uspješne oplodnje. Grozd Grka u punoj zrelosti sadrži kombinaciju potpuno razvijenih bobica sa sjemenkama, ali i značajan udio manjih besjemenih bobica, čiji optimalni udio ima pozitivan utjecaj na kvalitetu vina. I muški i ženski gametofiti Grka razvijaju se normalno te u zrelosti sadrže dvije spermalne stanice u muškom odnosno jajnu i središnju stanicu u ženskom gametofitu. Glavni razlog umanjene stope oplodnje i posljedični razvoj besjemenih plodova je nevijabilonost peludnog zrna uzrokovana akolporatnom građom vanjske stijenke peludnog zrna kao i izostankom uspješne oplodnje nakon strano-oprašivanja zbog čega dolazi do propadanja sjemenog zametka

Ključne riječi: oplodnja, *Vitis vinifera*, Grk, acolporatna pelud

REDUCED FERTILIZATION IN GRAPEVINE VARIETY GRK (*Vitis vinifera* L.) IS INFLUENCED BY ACOLPORATE EXINE MORPHOLOGY OF A POLLEN GRAIN

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Grk is an autochthonous Croatian grapevine cultivar grown almost exclusively on the island of Korčula. It is one of the rare grape varieties with a functionally female flower, which causes certain problems in grape production associated with reduced fertilization. A cluster of Grk at full maturity contains both fully developed seeded berries but also a significant proportion of undersized seedless berries, whose optimal contribution has a positive impact on wine quality. Both the male and the female gametophyte accomplish their development on a regular way giving rise to two sperm cells in male or egg and central cell in female gametophyte. The main reasons behind the reduced fertilization rate and consequent occurance of seedless berries are pollen non-viabillity provoked by acolporate exine morphology and failure of fertilization after cross-pollination that terminate by ovule abortion.

Keywords: fertilization, *Vitis vinifera*, Grk, acolporate pollen

Biologija kopnenih voda i kopna
Biology of freshwater and terrestrial ecosystems

O-55

BIORAZNOLIKOST OKUĆENIH AMEBA (TESTACEA) U ODABRANIM PODZEMNIM KRŠKIM STANIŠTIMA DINARIDA

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Istraživanja polifiletske skupine okućenih ameba (Testacea) u podzemnim krškim staništima na području Hrvatske i Bosne i Hercegovine provođena su u periodu od 2012. do 2018. godine. Ukupno je obuvaćeno 20 prirodnih krških speleoloških objekata. Osim okućenih ameba, bilježena je prisutnost i ostalih pripadnika skupine Protista te sitnih beskralješnjaka koji su bili prisutni u uzorku. Mikrostaništa obuhvaćena ovim istraživanjem su: vlažni zidovi i higropetrici, lokve vode (na stijenskoj podlozi i u sedimentu), špiljski sedimenti (guano, anorganski sediment), sediment i voda endogenih podzemnih tekućica i stajaćica te naplavljeni drvni materijal. Cilj istraživanja bio je razviti metodologiju uzorkovanja koja bi prikazala reprezentativne zajednice okućenih ameba prisutne u krškim podzemnim staništima kao i prikupiti preliminarne rezultate o njihovoj bioraznolikosti. Zabilježeno je preko 30 vrsta okućenih ameba među kojima je 15 vrsta prvi put zabilježeno u podzemnim krškim staništima. Najveća bioraznolikost zabilježena je u špiljama Dahni (BiH), Velikoj peći (HR) i Čakovcu (HR). Bioraznolikost okućenih ameba ovisila je o prisutnosti šišmišjeg guana, heterogenosti špilja i o učestalosti uzorkovanja. Rezultati ovog istraživanja dokazali su konstantnu prisutnost okućenih ameba u podzemnim krškim staništima Dinarida te je stoga nužno nastaviti s daljnim

istraživanjima ove skupine kako bi se bolje razjasnila njihova uloga u podzemnim ekosustavima.

Ključne riječi: okućene amebe, praživotinje, špilje, higropetrik, Dinaridi

BIODIVERSITY OF TESTATE AMOEBAS (TESTACEA) FROM SELECTED SUBTERRANEAN KARST HABITATS OF THE DINARIC ARC

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Research of the polyphyletic testate amoebas group (Testacea) in subterranean karst habitats of Croatia and Bosnia & Herzegovina was performed during the period 2012 to 2018. It included 20 natural karst speleological objects. Along with testate amoebas, presence of other members of Protista group and small invertebrates in examined samples was also noted. Microhabitats investigated were: wet walls and hygropetric, water puddles (formed on bedrock or in cave sediments), cave sediments (guano, inorganic sediments), sediment and water of endogenous subterranean lakes and rivers and wood debris. The goal of this research was to develop a sampling methodology that could show representative communities of testate amoebas present in subterranean karst habitats and to gain preliminary data of their biodiversity. Over 30 species of testate amoebas was recorded, 15 of which were detected for the first time in subterranean karst habitats. The greatest biodiversity was recorded in caves Dahna (BiH), Velika peć (HR) and Čakovac (HR). Biodiversity of testate amoebas depended on presence of bat guano, cave heterogeneity and sampling frequency. Results of this research proved constant presence of testate amoebas in subterranean karst habitats of Dinarides and it is therefore important to continue with future research of this group in order to clarify their role in subterranean ecosystems.

Keywords: Testate amoebas, Protozoa, caves, hygropetric, Dinarides

O-56
POPULACIJA ČAGLJA U HRVATSKOJ NASTAVLJA ŠIRITI PODRUČJE RASPROSTRANJENOSTI

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Tijekom posljednjeg desetljeća razvio se znanstveni interes za čaglja (*Canis aureus*) te se sve više istražuju uzroci i posljedice njegovog širenja. Povijesno je čagalj bio prisutan uz obale Mediteranskog i Crnog mora, no od ranih 1980ih širi se sjeverozapadno, te se danas čaglja može naći zapadno do Švicarske i istočno do Estonije. Slično je i u Hrvatskoj, čagalj je povijesno bio prisutan na jugu Dalmacije, no proširio se do Istre i kontinentalnog dijela. Genetska istraživanja su potvrdila da se kontinentalna populacija razlikuje od one iz Dalmacije, što je vjerojatno posljedica naseljavanja kontinenta iz Bugarske, Rumunjske i Srbije. Prva sustavna analiza rasprostranjenosti čaglja u Hrvatskoj napravljena je na temelju podataka o smrtnosti za razdoblje 2007. – 2010. godine. Budući su podatci s terena upućivali na nastavak širenja

populacije, na temelju podataka o odstrijelu i otpadu čaglja hrvatskim lovištima za razdoblje 2006.- 2015. godine (ustupljeno od Ministarstva poljoprivrede), napravili smo novu analizu rasprostranjenosti. Naši rezultati pokazuju da je u 5-godišnjem razdoblju (2011.-2015.) prijavljena smrtnost porasla za 27,5%, dok je rasprostranjenost porasla za 5,6%, te je 2015. čagalj bio prisutan na 20156 km2 (35,6% teritorija RH). Najviša smrtnost je prijavljena u Zadarskoj, Šibensko-kninskoj i Dubrovačko – neretvanskoj županiji, Osječko – baranjskoj te Vukovarsko – srijemskoj, dok čagalj nije prisutan na području visokih nadmorskih visina Like i Gorskog kotara.

Ključne riječi: *Canis aureus*, smrtnost, odstrijel, rasprostranjenost

JACKAL POPULATION IN CROATIA CONTINUES TO EXPAND ITS DISTRIBUTION

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During the last decade the European golden jackal (*Canis aureus*) population attracts growing scientific attention with drivers and consequences of its expansion to new areas being researched. The species was historically confined to coastal areas of Mediterranean and Black Sea, but since early 1980s it is spreading in a northwestern direction, today reaching west to Switzerland and east to Estonia. Similarly in Croatia, originally present only in southern Dalmatia jackals spread all the way to Istria and continental region. Genetic research confirmed that jackals from continent are distinct from those in Dalmatia, suggesting the continent was inhabited by immigrants from Bulgaria, Romania and Serbia. First comprehensive analysis of golden jackal distribution in Croatia was based on mortality data from 2007-2010 period. As field observations were pointing to further population expansion, we prepared a new analysis of jackal distribution, using 2006-2015 yearly mortality data for all hunting units in Croatia (provided by the Ministry of Agriculture). Our results indicate that in 5 year period (2011-2015) reported mortality increased 27,5%, while jackal distribution in Croatia expanded by 5,6% and in 2015 jackals were present on 20156 km2 (35,6% of Croatian territory). The highest mortality was recorded in Zadar, Šbenik – Knin and Dubrovnik – Neretva, Osijek – baranja and Vukovar - Srijem County, while the species is not present in high altitudes of Lika and Gorski kotar region.

Keywords: *Canis aureus*, mortality, hunting, distribution

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CONTRIBUTION TO THE PROCESS OF IMPLEMENTATION OF THE AQUATIC MACROINVERTEBRATES AS A MANDATORY COMPONENT IN MONITORING STUDIES IN REPUBLIC OF MACEDONIA, A STRUMICA RIVER BASIN EXPERIENCE

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Human have increased water pollution, altered water flow regime and modified the

morphology of rivers. All these,have resulted in multiple pressures on freshwater ecosystems, changing their biodiversity and ecological structure and functioning. Annex V of the EU WFD, state that macroinvertebrates represent mandatory biological elements in the assessment of ecological status / potential. Here, we analyse response of macroinvertebrates on human pressures, as well as their impact on the ecological status of the water bodies in Strumica River Basin, R. Macedonia. Macroinvertebrata samples were collected during 2016-2018 from 11 water bodies and standard methodology for collection of bottom fauna (EN ISO 10870: 2012) was followed. The results showed that water bodies in good ecological status are associated with the presence of natural areas and high number of EPT taxons, while domination of Chironomidae and Oligochaeta species combined with agricultural activities and urbanization are important predictors of ecological degradation and bad/poor ecological status. In Macedonia, assessing of multiple pressures on river ecosystems and implementation of aquatic macroinvertebrates in determination of the ecological status is challenging, especially because there isn't an efficient national system for that. The findings of this study contribute to the process of implementation of the macroinvertebrates as a mandatory component in monitoring studies in Republic of Macedonia.

Keywords: macroinvertebrates, Strumica River Basin, WFD, indicators, pollution

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ZOOPLANKTON – ZANEMAREN ALI VAŽAN SEGMENT U BIOCENOZAMA MEDITERANSKIH LOKVI

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Razvoj turizma u mediteranskom području i napuštanje tradicionalne poljoprivrede dovodi do sukcesije i nestanka više tipova različitih staništa. Mediteranske povremene lokve jedno su od takvih, izrazito ugroženih staništa. Usprkos maloj površini, lokve su prepoznate kao područje velike biološke raznolikosti te su u zakonskoj legislativi EU navedene kao prioritetno stanište zaštite. Zooplankton je jedan od esencijalnih segmenata biocenoza mediteranskih lokvi. Kratko generacijsko vrijeme i brz odgovor na okolišne promjene naglašava ovu skupinu kao potencijalno dobre indikatore ekološkog stanja lokvi. Glavni ciljevi istraživanja bili su utvrditi: (1) utjecaj glavnih okolišnih čimbenika na sastav, raznolikost, brojnost i trofičku strukturu zooplanktona; (2) važnost zooplanktona kao indikatora ekološkog statusa lokvi. Zooplankton je uzorkovan u dvanaest otočnih lokvi, smještenih na Dugom otoku i Rabu (Jadransko more, Hrvatska). Ukupno smo utvrdili 60 svojti: Rotifera su bili najraznolikija skupina (46), zatim Cladocera (10) i Copepoda (6). Od abiotičkih čimbenika, salinitet je negativno korelirao s bioraznolikošću. Predacija riba negativno je utjecala na brojnost zooplanktona, dok su makrofiti pozitivno utjecali na njegovu brojnost i raznolikost. Na mediteranskim otocima lokve su često jedina slatkovodna staništa. Neophodno je imati holistički pristup istraživanjima i poduzeti odgovarajuće mjere konzervacije i restauracije kako bi se spriječila sukcesija ovih staništa.

Ključne riječi: Rotifera, Copepoda, Cladocera, povremene lokve, konzervacija

ZOOPLANKTON – NEGLECTED, BUT IMPORTANT SEGMENT IN BIOCENOTICS OF

MEDITERRANEAN PONDS

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The development of tourism in the Mediterranean area and the abandonment of traditional extensive agriculture lead to the succession of several different habitat types. One of the most endangered habitats is Mediterranean temporary ponds. Despite small size, these ephemeral waterbodies are recognized as reservoirs of biodiversity and they are one of the priority habitat types for conservation, according to the EU Habitats Directive. Zooplankton is one of the essential segments in the Mediterranean ponds biocenosis. Short generation time and their quick respond to environmental changes highlights zooplankton as good indicator of pond ecological status. Main goals of our investigation were to establish: (1) most important biotic and abiotic factors that shaped zooplankton in small ponds; (2) zooplankton as indicator for ecological status of these waterbodies. We sampled zooplankton from twelve small ponds situated on Dugi otok and Rab island (Adriatic Sea, Croatia). Altogether 60 species were found: Rotifera were most diverse group (46), followed by Cladocera (10) and Copepoda (6). Of abiotic factors, salinity negatively correlated with biodiversity. Fish predation negatively affected zooplankton abundance, while macrophytes positively affected abundance. On the Mediterranean islands ponds are often the only freshwater habitats. It is necessary to have holistic research and to undertake appropriate conservation and restoration measures, in order to prevent their succession.

Keywords: Rotifera, Copepoda, Cladocera, temporary ponds, conservation

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ULOGA ZOOPLANKTONA U RESTORACIJI PLITKIH JEZERA – IN SITU MEZOKOZMOS POKUS

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Prema teoriji alternativnih stanja jezera mogu biti u jednom od dva stabilna stanja: turbidnom, u kojem dominiraju alge, te stanju prozirnog stupca vode s dominacijom makrofita. Zooplankton, a osobito veći rašljoticalci, hranjenjem mogu utjecati na brojnost fitoplanktona i time održavati prozirno stanje jezera. Hoće li stanje biti turbidno ili prozirno, određeno je, preko zooplanktona, abiotičkim čimbenicima, ali i biotičkim čimbenicima, osobito prisutnošću makrofita (npr. pružanjem skrovišta) te predacijom riba (vizualni predatori čiji je predacijski pritisak jači na zooplankton većeg tijela). U rukavcu rijeke Sutle ispitan je utjecaj submerznih makrofita i predacije riba na strukturu zooplanktona plitkih eutrofnih jezera umjerenog pojasa. Jezero je bilo u turbidnom stanju, poribljeno i bez submerznih makrofita. In situ mezokozmos pokus proveden je u dva bazena, izgrađenih od mreža različite veličine oka (10 mm, bez riba;

40 mm, s ribama), u koja su dodane stabljike vrste *Ceratophyllum demersum*. U oba bazena zabilježena je veća brojnost rašljoticalaca veće veličine tijela, kao i veća ukupna raznolikost zooplanktona u usporedbi s kontrolnom točkom. Dodatno, obje vrijednosti su bile su najveće za bazen bez utjecaja riba. Ovi rezultati sugeriraju kako prisutnost submerznih makrofita može biti važna za promjenu stanja jezera, odnosno za zadržavanje prozirnog stupca vode kroz njihov utjecaj na strukturu zooplanktona, posebno uz kontrolu populacije riba.

Ključne riječi: zooplankton, restoracija jezera, mezokozmos pokus

ROLE OF ZOOPLANKTON IN SHALLOW LAKE RESTORATION - IN SITU MESOCOSM EXPERIMENT

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According to the alternative state theory, shallow lakes can appear in one of the two states: turbid water with phytoplankton dominance and clear water state with macrophytes dominance. Zooplankton, especially larger bodied Cladocera, due to grazing, affect phytoplankton abundance and maintain clear water state. Turbid or transparent state, through zooplankton, is determined by abiotic factors, but also by biotic factors, especially macrophyte presence (e.g. providing refuge) and fish predation (visual predators exert stronger predation pressure on larger bodied zooplankton). Impacts of submerged macrophytes and fish predation on zooplankton structure in shallow eutrophic lakes of temperate climate was examined in Sutla River backwater. The shallow water body was in the turbid state, fish stocked and without submerged macrophytes. In situ mesocosm experiment was conducted in two enclosures of different mesh sizes (10 mm, without fish; 40 mm, with fish) in which stems of *Ceratophyllum demersum* were added. Greater abundance of larger bodied Cladocera and overall zooplankton diversity were noted for both enclosures in comparison to the control point. Also, both above-mentioned values were the highest for the enclosure without fish influence. These results suggest that presence of submerged macrophytes can be important for maintaining/transition to the clear water state through their impact on zooplankton, especially when combined with the fish population control.

Keywords: zooplankton, lake restoration, mesocosm experiment

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USPOREDBA ZAJEDNICA VODENIH BESKRALJEŠNJAKA DVAJU SUSJEDNIH KRŠKIH IZVORA U HRVATSKOJ

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Krški izvori iznimno su raznoliki u morfologiji, strukturi mikrostaništa, hidrologiji i hidrogeologiji te su posljedično jedinstvenog sastava zajednica beskralješnjaka. Oba istraživana izvora rijeke Gacke, Majerovo i Tonkovića vrilo, su limnokreni izvori odvojenih porječja, različitog vremena zadržavanja vode u podzemlju i kapaciteta samopročišćavanja. Uzorci beskralješnjaka prikupljeni su sezonski tijekom 2014. godine po modificiranom AQEM protokolu u svrhu određivanja sastava i gustoće bentičkih zajednica na različitim mikrostaništima te njihovih okolišnih i prostornih odnosa. Ukupno je prikupljeno 70 poduzoraka na dominantnom anorganskom i organskom supstratu duž 100 metara svakog izvorišta. Većina fizikalno-kemijskih parametara statistički se značajno razlikuje među izvorima, s uglavnom nižim vrijednostima zabilježenim na Majerovom vrilu. SIMPER analize ukazuju na najveću gustoću jednakonožnih rakova i rakušaca u oba izvora tijekom svih sezona uzorkovanja. Bogatstvo vrsta bilo je veće na Tonkovića vrilu, gdje je utvrđena veća koncentracija otopljenih iona. Pomoću analize nemetričkog multidimenzionalnog skaliranja zajednice beskralješnjaka dvaju izvora su se odvojile. Istraživanje je ukazalo kako su složeni ekološki podaci esencijalni za procjenu promjena korištenja zemljišta u različitim porječjima susjednih izvora.

Ključne riječi: rijeka Gacka, krški izvori, vodeni beskralješnjaci, fizikalno-kemijski parametri, mikrostaništa

COMPARISON OF AQUATIC INVERTEBRATE ASSEMBLAGES BETWEEN TWO ADJACENT KARST SPRINGS IN CROATIA

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Karst springs are highly diverse in morphology, microhabitat structure, hydrology and hydrogeology, and consequently they are unique in their composition of aquatic invertebrate assemblages. Both investigated karstic springs of the Gacka River, Majerovo and Tonkovića Vrilo, are limnocrenous springs situated in separate subcatchments, characterized by different subsurface resident time of water and autopurification capacity. The invertebrate samples were collected seasonally in 2014 according to the modified AQEM protocol in order to assess the benthos assemblage structure and abundance on various microhabitats in relation to environmental and spatial correlates in each spring. In total 70 subsamples were collected at dominant inorganic and organic substrates along 100 meters of the each spring head. The majority of physicochemical parameters were significantly different between both springs, with generally lower values at Majerovo Vrilo. SIMPER analyses indicate the great abundance of isopod and amphipod crustaceans in both springs during all sampling seasons. Invertebrate species richness was greater in Tonkovića Vrilo, where the higher concentrations of dissolved ions were recorded. Non-metric multidimensional scaling ordination highlighted a separation of invertebrate assemblages between springs. This research has pointed out essential complex ecological data to assess land use changes in different subcatchments of adjacent springs.

Keywords: Gacka River, karstic springs, aquatic invertebrates, physicochemical parameters,

microhabitats

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UTJECAJ UKLANJANJA PAJASENA NA OSEDRAVANJE PRIRODNIH PODLOGA I DINAMIKU PERIFITONA U NACIONALNOM PARKU KRKA

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Bogat razvoj viših vodenih biljaka na sedrenim barijerama krških hidrosustava može dovesti do tzv. zaraštavanja barijera te rezultirati isušivanjem dijela barijera. Uslijed navedenog, u NP Krka odabrana je pilot ploha na sedrenoj barijeri Skradinski buk, s koje se pokusno uklonila vegetacija invazivne biljke – pajasena (*Ailanthus altissima* (Mill.) Swinge). Po uklanjanu vegetacije u kolovozu i rujnu 2017. godine, promijenila se hidrologija pilot plohe što je rezultiralo formiranjem novih tokova. S obzirom da perifiton (obraštaj) ima ključnu ulogu u procesu osedravanja, cilj istraživanja bio je pratiti sezonsku dinamiku razvoja perifitona novo-formiranih tokova te osedravanja prirodnih podloga. Jednomjesečno uzorkovanje na sedam postaja započeto je u listopadu 2017. godine. Zajednica perifitona bilježila je manju raznolikost u odnosu na prethodna istraživanja perifitona u NP Krka, a njom su dominirale ameboidne praživotinje, trepetljikaši te heterotrofni nanobičaši. Novo-formirani tokovi bilježili su manju abundanciju u odnosu na već postojeće. Promjena hidrologije uzrokovana uklanjanjem pajasena reflektirala se i u razlici fizikalno-kemijskih parametara među postajama. Na osnovu prve faze praćenja dinamike perifitona i osedravanja, može se zaključiti da je započelo naseljavanje mikrofaune u novo-formiranim tokovima. Kontinuirano praćenje ovog staništa od iznimne je važnosti kako bi se dobili detaljni podaci o dinamici ekosustava nakon uklanjanja invazivne drvenaste vrste.

Ključne riječi: Skradinski buk, praživotinje, invazivna vrsta, sedrene barijere, mikrofauna

SPATIO-TEMPORAL PATTERNS OF TUFA DEPOSITION AND PERIPHYTON COMMUNITY DYNAMICS ALONG THE NEWLY FORMED WATER PATHWAYS IN NATIONAL PARK KRKA, CROATIA

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The removal of invasive species *Ailanthus altissima* (Mill.) Swinge)) from an experimental reach at Skardinski buk, during July and August 2017 resulted with occurrence of new water pathways. Samples of periphyton were collected bimonthly at seven sites (five newly formed and two previously known) starting in October 2017 - two months after the removal of the invasive species. The collected live material was analysed under the microscope within 48 h of sampling and protozoans were identified to the lowest possible taxonomic level using the relevant taxonomic literature. Along with amebozoa and heterotrohic nanoflagrllates, ciliates dominated the peripyhton communities displaying generally lower diversity in comparison to prior research of periphyton communities in the Krka National Park. The abundance of the

protozoan organisms within the newly established water pathways was lower than within the previously present streams. The hydrological shifts, which occurred upon A. altissima removal, also reflected in differences of the water physico-chemical characteristics among the study sites. On the basis of presented environmental and protozoan community data, it is likely that the newly established water pathways represent favourable habitats for colonization of microfauna, however continuous research is required since substrate colonization is a very complex process which depends on several environmental and biotic factors.

Keywords: tree of heaven, protected area, tufa barrier, protozoa, Skradinski buk, Croatia

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BRZINA OPORAVKA ZAJEDNICE MAKROBESKRALJEŽNJAKA NA OBNOVLJENOM STANIŠTU SEDRENE BARIJERE

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Fauna sedrenih barijera vrlo je osobita, ali promjene ekoloških značajki okoliša mogu predhoditi nestanku vrsta i sedrenih staništa. Takvu je promjenu izazvalo nekontrolirano širenje invazivne vrste *Ailanthus altissima* u Nacionalnom parku Krka. Korita 3 kanala desetljećima su bila potpuno isušena. Uklanjanje obraštaja ove invazivne vrste omogućilo je revitalizaciju vodenih staništa, ponovni nastanak sedre i obnavljanje zajednice makrobeskralježnjaka. Istraživali smo proces ponovnog uspostavljanja zajednice makrozoobentosa na 4 obnovljena riječna kanala. Pritom smo se fokusirali na sposobnost vrsta rekolonizatora da iznova nasele obnovljena staništa i na vrijeme potrebno za to. Istraživanje je trajalo pet mjeseci, a uzorci su uzimani jednom mjesečno. Usporedno s uzimanjem uzoraka, mjerili smo i pratili promjene u fizikalno-kemijskim svojstvima vode. Broj izoliranih makrobeskralježnjaka pokazao je da su najdominantniji rekolonizatori pripadnici porodica Simuliidae i Chironomidae. Njihov je broj bio čak i veći u obnovljenim kanalima nego u kontrolnoj skupini. Pripadnici reda Coleoptera, bili su drugi po brojnosti u kontrolnoj skupini, ali su obnovljene kanale najslabije rekolonizirali. Također, kanal koji je pretrpio najveću destrukciju korita zbog biljnog obraštaja, pokazao je najslabije rezultate oporavka zajednice makrobeskralješnjaka.

Ključne riječi: makrobeskralješnjaci, revitalizacija, rekolonizacija, sedra

VELOCITY OF MACROINVERTEBRATE REVITALIZATION AT THE REACTIVATED TUFA-BARRIER HABITAT

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The fauna of the tufa-barrier habitats is remarkable in its resilience, but changes in habitats ecological features may lead to habitat and species disappearance. That kind of change was caused in the Krka National Park by uncontrolled growth of the invasive plant species *Ailanthus*

altissima. Three channels were totally dried for decades. Removal of the invasive trees enabled the reactivation of aquatic habitats and re-commencement of tufa forming and re-establishment of benthic assemblages. We examined the re-establishment process of the macroinvertebrate assemblages at the 4 rewetted channels, focussing on agility of recolonizer species to conquer newly restored habitats. The research lasted for 5 months with monthly core sampling. Concurrently we monitored the changes in physico-chemical properties of water. Count of the isolated macroinvertebrate has shown that the most dominant recolonization taxa were Chironomidae and Simuliidae. Their abundance was higher in the revitalized channels than at the control site. Coleoptera were the second most abundant taxon at the control site, but also the taxa that recolonized the least. We observed the least-revitalisation at the channel that was the most affected by the vegetation overgrowth through which it lost almost all characteristics of a stream channel.

Keywords: macroinvertebrates, revitalization, recolonization, tufa

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INKRUSTACIJA, SVJETLOSNA ADAPTACIJA I EPIFITON NA PAROŽINAMA U KRŠKOM JEZERU

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Parožine (Charophyta) su makroalge koje značajno utječu na slatkovodne ekosustave pružajući stanište drugim organizmima, utječući na kruženje hranjivih soli te stabilizirajući sediment - osobito kroz proces kalcifikacije, koji može biti vrlo intenzivan. U ovom radu istražena je svjetlosna adaptacija parožina u litoralu Prošćanskog jezera (NP Plitvička jezera) te međuodnos kalcifikacije i epifitona. Na uzorcima dviju vrsta (*Chara subspinosa* koja je dominirala na 1 i 5 m te *Chara globularis* koja je dominantna na 10 m) analizirani su fotosintetski pigmenti, količina kalcijevog karbonata te epifiton. Zabilježene su razlike između vrsta parožina te između gornjih i donjih dijelova talusa s obzirom na količine fotosintetskih pigmenata i kalcijevog karbonata. Kompleksni obrasci klorofila *a* i *b* te karotenoida odražavaju adaptaciju na svjetlosne uvjete. Najveća raznolikost epifitskih praživotinja i beskralježnjaka zabilježena je na *C. subspinosa* na 5 m, dok je najveća brojnost zabilježena na *C. globularis*, koja je morfološki više razgranata. Gornji dijelovi talusa podržavali su veću raznolikost praživotinja i beskralježnjaka, vjerojatno zbog većih količina dostupne hrane. Razlika u epifitonu koja je zabilježena između dubina ukazuje na gradijent uvjeta mikrostaništa, pri čemu veća količina kalcijeva karbonata negativno utječe na organizme, posebno na sesilne svojte.

Ključne riječi: *Chara subspinosa*, *Chara globularis*, klorofil a i b, karotenoidi

INCRUSTATION, LIGHT ADAPTATION AND EPIPHYTON ON CHAROPHYTES IN A KARSTIC LAKE

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Stoneworts (Charophyta) are macroalgae with the significant effects on freshwater ecosystems by providing habitat for other organisms, affecting nutrient cycles and stabilising the sediment – particularly through the calcification process, that can be very intense. The aim of this work was to investigate light adaptation of stoneworts in littoral of Lake Prošćansko (Plitvička jezera National Park) together with interrelationship between calcification and epiphyton. Photosynthetic pigments, calcium carbonate deposition and epiphyton were analysed from samples of two species (*Chara subspinosa* that dominated on 1 and 5 m and *Chara globularis* that dominated on 10 m) Differences were detected between charophyte species and between upper and lower thallus parts regarding photosynthetic pigments and calcium carbonate deposits. Complex patterns of chlorophyll *a* and *b* and carotenoids reflect light adaptation. The highest diversity of epiphytic protozoans and invertebrates was detected on *C. subspinosa* on 5 m, while the highest abundance was recorded on *C. globularis*, which is morphologically more complex. Upper thallus parts supported higher diversity of protozoans and invertebrates, probably due to more available food. The differences in epiphyton, detected between depths indicate gradient of microhabitat conditions, with the negative effect of higher calcium carbonate deposits on epiphytic organisms, particularly on sessile taxa.

Keywords: *Chara subspinosa*, *Chara globularis*, chlorophyll a and b, carotenoids

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BIOSPELEOLOŠKA ISTRAŽIVANJA KOSINJSKOG POLJA I PONORNE ZONE LIPOVOG POLJA

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Kroz 2016. godinu provedena su ciljana speleološka i biospeleološka istraživanja Kosinjskog polja i ponorne zone rijeke Like na području Lipovog polja, za potrebe nadopune podataka u procjeni utjecaja na okoliš zahvata HE Kosinj. Biospeleološka istraživanja obuhvatila su carstva: Protista, Fungi te Animalia. Na širem istraživanom području utvrđene su 74 prave špiljske vrste (84% troglobionata i 16% stigobionata). Ističu se Natura 2000 i ugrožene špiljske vrste: *Eunapius subterraneus*, *Marifugia cavatica*, *Congeria jalzici*, *Zospeum likanum*, *Z. subobesum*, *Stalita pretneri*, *Hadzinia karamani*, *Niphargus croaticus*, *Monolistra sketi*; uz opis nove vrste i prve špiljske gljive s područja Hrvatske - *Ramgea ozimecii*. Od 50 utvrđenih endemičnih vrsta, 80% su endemi sjeverno-dinarske biogeografske regije. Čak 25 vrsta (34%) opisano je i ima tipski špiljski lokalitet na širem istraživanom području, te su endemi Like. Na području ponorne zone najdetaljnije su istraživane populacije Natura 2000 vrste C. jalzici. Temeljem mofrometrijske analize, omjera spolova, reproduktivnih stadija i starosti, ocjenjeno je stanje populacija. Istraživanjem je otkriveno novo nalazište *C. jalzici* (ponor Dražice) te značajno

otkriće iznimno velike kolonije u preko 200 m potopljenih kanala sifonskog jezera na kraju glavnog kanala Markovog ponora, u kojima obitava s vrstama *M. cavatica* i *E. subterraneus*. Prvi puta su na staništu *C. jalzici* istraživane i zajednice Protista.

Ključne riječi: Natura 2000, biospeleologija, endemi, *Congeria*

BIOSPELEOLOGICAL RESEARC OF KOSINJSKO POLJE AND SINKHOLE ZONE LIPOVO POLJE

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In 2016 has been conducted biospeleolgical and speleological research of the Kosinjsko polje and sinkhole zone of the River Lika. Aim of the research was to collect the additional data for the environmental impact assessment for the HPP Kosinj. Biospeleological researches comprised following kingdoms: Protista, Fungi and Animalia. In all biospeleogical researches in the wider area has been determined 74 strictly subterranean species (84% troglobionts and 16% stygobionts). Following endangered and Natura 2000 species should be notice: *Eunapius subterraneus*, *Marifugia cavatica*, *Congeria jalzici*, *Zospeum likanum*, *Z. subobesum*, *Stalita pretneri*, *Hadzinia karamani*, *Niphargus croaticus*, *Monolistra sketi*; with the description of the new species and first underground fungi from Croatia - *Ramgea ozimecii*. It is determined 50 endemic species, of which 80% of the species are endems of the North Dinaric biogeographical region. Moreover, 25 species (34%) are descirbed (have the type locality) in the wider research area. Populations of the Natura 2000 species *C. jalzici* has been researched in detail in the sinkhole zone. Evaluation of the population status has been conducted according to the morphometric analysis, sex ratio in population, ratio of the reproductive stages of bivalves and the age ratio of the bivalves. During the research has been discovered a new locality of the *C. jalzici*. Noteworthy is the discovery of more than 200 m of the submerged canals in the siphon lake at the end fo the main canal of the Markov ponor sinkhole, where were recorded species *M. cavatica*, *E. subterraneus* and uniquely large colony of *C. jalzici*. In addition, first time were researched species and associations of the protista in the habitat of *C. jalzici*.

Keywords: Natura 2000, biospelological research, endems, *Congeria*

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EKOLOŠKE ZNAČAJKE I STRUKTURA IHTIOFAUNE PLITVIČKIH JEZERA

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U radu su prikazani struktura zajednice i parametri biološke raznolikosti ihtiofaune Plitvičkih

jezera. Uzorkovanje se provodilo elektro-ribolovom i mrežama stajaćicama. Na temelju svih limnoloških relevantnih čimbenika i podataka dobivenih jednogodišnjim intenzivnim uzorkovanjima istraživanog područja definirana je sadašnja ihtiocenoza Plitvičkih jezera. U usporedbi sa starijim istraživanjima, definirane su promjene zajednice riba Plitvičkih jezera u vremenu. Ukupno je na svim postajama ulovljeno 936 jedinki riba. Nađeno je 8 vrsta riba ukupne mase od oko 146 kg. Prisutne vrste su: *Squalius cephalus, Scardinius erythrophthalmus, Salvelinus alpinus, Salmo trutta, Phoxinus phoxinus, Oncorhynchus mykiss* i *Esox lucius*. Na Bijeloj rijeci izlovljena je samo potočna pastrva, na Crnoj rijeci kalifornijska i potočna pastrva. Na Ciganovcu su ulovljene vrste potočna pastrva, pijor, klen i štuka. Na jezeru Gavanovac ulovljeni su kal. pastrva i klen. Najviše (8) vrsta ulovljeno je u Kozjaku. To su klen, pijor, crvenperka, vijun, zlatoovčica, pot. pastrva i štuka i kal. pastrva. U nekim jezerima uočili smo i vrste koje nisu bile prisutne u ulovu. U relativnim odnosima ihtiomase u ulovima prevladavaju potočna pastrva i klen. Najveću brojnost imaju ribe manjeg habitusa. Uz obale većih jezera primijećena su velika jata klena. Postotak najčešće vrste, poznat kao Berger-Parkerov indeks, korišten je kao vrlo praktično oruđe za definiranje promijenjene zajednice. Simpsonov indeks za sve postaje iznosi 0,765. Shannonov indeks za sve postaje kao cjelinu iznosi 1,626. Vrlo slične odnose za cijelu kolekciju daje i Brillouinov indeks. Kod modela za logaritamsku seriju alfa vrijednost za sustav Plitvička jezera iznosi 1,201. Bogatstvo vrsta za cijeli ulov je 1,605.

Ključne riječi: ihtiofauna, Plitvička jezera, ekološke značajke

ECOLOGICAL CHARACTERISTICS AND STRUCTURE OF THE FISH COMMUNITY IN PLITVICE LAKES NATIONAL PARK

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The paper describes the structure of the fish community in Plitvice Lakes National Park. Sampling was carried out by electrofishing and gill net sets. Based on all the relevant limnological factors and data obtained from one year's intensive sampling of the study area, the current fish community of the Plitvice Lakes is described and compared with historic research to provide evidence of temporal changes in the fish community. In total, at all stations, 936 fish specimens were caught. There were 8 fish species captured comprising 146 kg total weight. The present species included *Salmo trutta, Squalius cephalus*, *Scardinius erythrophthalmus*, *Salvelinus alpinus, Phoxinus phoxinus, Oncorhynchus mykiss* and Esox *lucius*. In the Bijela Rijeka, only brown trout were captured; in Crna Rijeka, only brown trout and rainbow trout; and, in Ciganovac three species of fish (brown trout, chub and pike). The most (8) species were caught in Kozjak Lake. In some lakes, we observed some species that were not captured. In terms of fish biomass, trout and chub were most abundant, and large schools of chub was commonly observed along banks of the larger lakes. The percentage of most common species known as Berger Parker's index was used as a practical tool for evaluating community changes. Simpson's index for all stations was 0.765. Shannon's index for all stations, as a whole, was 1,626. Brillouin's index was also very similar for the entire collection. In the model for the logarithmic series, the alpha value for the Plitvice Lakes system was 1,201. The species richnes for the whole catch was 1,605.

Keywords: fish community, Plitvice Lakes, ecological characteristics

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NEOBIČNA ZAJEDNICA VODENGRINJA (ACARI: HYDRACHNIDIA) U SUBLAKUSTRIJSKOM KRŠKOM IZVORU I RIJECI PRITOCI KAO REZULTAT DOSTUPNOSTI PLIJENA

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Vodengrinje su aktivni predatori račića kao što su Copepoda, Cladocera i Ostracoda te ličinke vodenih kukaca (najčešće Diptera). Njihove zajednice uglavnom pokazuju veliku lokalnu raznolikost s relativno velikim brojem svojti, ali malom abundacijom. Upravo zato su istraživanja interakcija vodengrinja i njihovog plijena u prirodnim okruženjima rijetka. Sezonska uzorkovanja od kolovoza 2016. do lipnja 2017. provedena su na sublakustrijskom krškom izvoru (Torak, jezerom potopljeni izvor na 50 metara dubine) te rijeci pritoci (ušće rijeke Čikole) s ciljem utvrđivanja prostorne raspodijele vodengrinja s obzirom na dostupnost plijena. Triplikati uzoraka makrozoobentosa su uzimani Ekmanovim grabilom s tri dubine (litoral, sublitoral i profundal) na oba lokaliteta. Zajednice vodengrinja oba lokaliteta bile su tipične za lentička staništa, bez krenobiontnih i ritrobiontnih svojti. Bogatstvo vrsta, abundancija i raznolikost vodengrinja nije bila uvjetovana dubinskom raspodjelom, već dostupnošću plijena. Abundancija svojti Copepoda, Ceratopogonidae i Chironomidae statistički su značajno pozitivno korelirale s abundancijom vodengrinja. Populacija vrste *Limnesia connata*, prethodno nađena samo u nevapnenačkim vodama, na ovom je staništu vjerojatno opstala upravo zbog velike dostupnosti plijena. Jedinke vrsta *L. connata*, *Neumania vernalis* i *Piona longipalpis* po prvi puta su zabilježene za područje Hrvatske, dok je nalaz vrste *L. connata* ujedno i prvi takav za cijelo područje Dinarida.

Ključne riječi: lentička zajednica, odnosi predator-plijen, Torak, Čikola

PREY ABUNDANCE SUPPORTING UNUSUAL WATER MITE (ACARI: HYDRACHNIDIA) COMMUNITY IN A SUBLACUSTRINE KARST SPRING AND TRIBUTARY RIVER

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Water mites are predators on microcrustations (Copepoda, Cladocera, Ostracoda) and insect larvae (mostly Diptera). Their communities usually have great diversity with high species richness but relatively low abundance. This is why predator-prey interactions of water mites tend to be poorly investigated in natural habitats. A sublacustrine karst spring (Torak, spring mouth 50 meters below water level) and its tributary (the mouth of the Čikola River) were examined seasonally from August 2016 until June 2017, with the aim of determining water mite distribution with regard to prey availability. Triplicate samples of benthic macroinvertebrates were taken with an Ekman dredge on three different depth levels (littoral, sublittoral and profundal zone) at both study sites. Both sites had typical lentic water mite

communities with no crenobiont or rhithrobiont taxa present. Water mite species richness, abundance and alpha diversity were not governed by depth, but rather by prey availability. Greater copepod, ceratopogonid and chironomid abundances were found to significantly correlate with higher abundances of water mites. The population of *Limnesia connata*, previously reported from lime-poor waters exclusively, was most probably supported by vast prey availability, thus unexpectedly surviving in this karst environment. Species *L. connata*, *Neumania vernalis* and *Piona longipalpis* were recorded for the first time in Croatia, while *L. connata* was recorded for the first time in the Dinaric karst region.

Keywords: lentic community, predator-pray interactions, Torak, Čikola

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USPOREDBA PREHRANE TRI STRANE VRSTA RIBA U DVA TIPA STANIŠTA RIJEKE KRKE

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Rijeka Krka je druga najveća rijeka jadranskog slijeva u Hrvatskoj i karakteristična je po velikom broju endemskih vrsta riba. U zadnjih nekoliko godina u Krki je zapažen porast broja stranih vrsta riba. Istraživanje je vršeno tijekom lipnja, srpnja i kolovoza 2017. godine u razdoblju od sedam tjedana na srednjem dijelu toka Krke, na dva tipa staništa: na plitkom i obraslom jezeru trajno povezanom s rijekom i na odsječku rijeke uzvodno i nizvodno od jezera, ukupne duljine pet km. Lov se obavljao vršama, elektroribolovom i mrežama. Cilj je bio utvrditi razliku u prehrani unesenih vrsta, štuke, crnog somića i sunčanice, u staništima s velikom (jezero), odnosno malom (rijeka) gustoćom naseljenosti. Ukupno je ulovljeno 5530 jedinki riba svrstanih u 11 vrsta, od čega su četiri zavičajne, a sedam stranih (dvije translocirane i pet unesenih). Pregledana su probavila ukupno 146 ciljanih jedinki stranih vrsta duljih od 100 mm. Svojte izolirane iz probavila su svrstane u osam kategorija. Rezultati pokazuju da crni somić i sunčanica u jezeru preferiraju ribu, a u rijeci beskralješnjake. Štuka u oba staništa preferira ribu. Crni somić i sunčanica oportunistički biraju najdostupniji plijen, u jezeru su to ribe, dok se u rijeci, gdje je brojnost riba manja, češće hrane dostupnijim slabo pokretnim plijenom poput puževa, pijavica i ličinki kukaca. Štuka se, kao izrazito ribojedna vrsta, hrani ribom bez obzira na njenu brojnost i dostupnost.

Ključne riječi: jadranski slijev, strane vrste riba, štuka, crni somić, sunčanica

DIET COMPARISON OF THREE ALIEN FISH SPECIES IN TWO DIFFERENT HABITATS WITHIN RIVER KRKA

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River Krka is the second largest river of Adriatic drainage in Croatia and it is characterized by high number of endemic fish species. Number of alien fish species in Krka has increased in the last few years. Research was conducted in June, July and August of 2017, over the period of seven weeks. The study area was in the mid part of Krka, on two habitat types: a shallow lake permanently connected to the river and a five km stretch of the river by the lake. Samples were collected using traps, electrofishing and nets. Aim of this research was to determine diet difference for three alien fish species, pike, bullhead and pumpkinseed, in one habitat with high fish abundance (lake) and other with low fish abundance (river). Eleven species represented by 5530 specimens were caught. Out of those species, four were native and seven alien (two translocated and five introduced). A total of 146 individuals of targeted alien species with total length over 100 mm were subjected to stomach content analysis. Taxons extracted from stomachs were classified in eight categories. Results show that bullhead and pumpkinseed prefer fish in the lake and invertebrates in the river. Pike prefers fish in both habitats. Bullhead and pumpkinseed opportunistically choose the most accessible prey, fish in the lake, and in the river, where fish are sparse, the most accessible is slow moving prey like snails, leeches and insect larvae. Pike, as piscivorous species, prefers fish no matter on its abundance.

Keywords: Adriatic drainage, alien fish species, pike, bullhead, pumpkinseed

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MAKROZOOBENTOS IZVORA I SEDRENE BARIJERE: UZRASNI SASTAV OBALČARA IZVORA BIJELE RIJEKE

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Makrozoobentos je važan dio živog svijeta koji nastanjuje slatkovodne ekosustave. Budući da ima važnu ulogu u kruženju tvari i dinamici ekosustava, odličan je pokazatelj ukupnog ekološkog stanja. Poznavanje biologije odnosno životnih ciklusa pojedinih svojta je pak temelj razumijevanja kompleksnih odnosa unutar ekosustava. Stoga je naše istraživanje imalo ciljeve:odrediti i usporediti najzastupljenije svojte makrozoobentosa na izvorišnom području i sedrenoj barijeri u sustavu Plitvičkih jezera te utvrditi uzrasnu strukturu obalčara na ovim staništima. Obalčare smo odabrali kao nezaobilazne bioindikatore za izvrsnu kakvoću vode. Mjerena fizikalno-kemijska obilježja vode konstantna su na izvoru Bijele rijeke, a očekivano oscilirajuća na barijeri Labudovac. Trzalci (Chironomidae) prevladavaju u makrozoobentosu barijere Labudovac, dok su na izvoru Bijele rijeke najbrojniji obalčari (Plecoptera). Na oba lokaliteta obalčari dominantno pripadaju rodu *Protonemura*. Mjerenjem širina glava obalčara iz roda *Protonemura*, pomoću milimetarskog papira i mikrometarskog mjerila, načinili smo ukupnu i sezonske slike uzrasne strukture na razini 10 veličinskih razreda. Sezonske razlike u veličinskim razredima u skladu su s opisanim univoltnim životnim ciklusom obalčara.

Ključne riječi: makrozoobentos, obalčari, uzrasna struktura, sezonska raspodjela, krška voda

MACROINVERTEBRATE COMMUNITIES AT SPRING AND TUFA BARRIER: SIZE STRUCTURE OF

STONEFLIES FROM THE SPRING OF BIJELA RIJEKA

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Macroinvertebrates play an evitabe role in the energy flow of the freshwater ecosystem. Thus, they are great indicator of total ecological condition. It is important to perceive their biology and life cycles to understand the complexity of interactions in the ecosystem. The aim of this study was to compare two functionally and geographically different sites at the Plitvice Lakes, tufa barrier Labudovac and the spring of Bijela rijeka, by the main taxa of macroinvertebrates, seasonal size structure of stoneflies and environmental factors. The samples of macroinvertebrates were taken in spring and autumn of 2016. We chose stoneflies as a remarkable bioindicators of water quality. The measured physical and chemical parameters were stable at the spring of Bijela rijeka through the seasons and very oscillating at the tuffa barrier Labudovac. Stoneflies (Plecoptera) were the most abundant group at the spring of Bijela rijeka and Dipteran family Chrionomidae was dominant at the barrier Labudovac. Stoneflies from both sites mostly belong to *Protonemura* genus. I made the total seasonal size structure of *Protonemura* larvae distinguished into 10 size classes after measuring the width of head capsules. Differences in seasonal size classes are in accordance with the described univoltine life cycle of stoneflies.

Keywords: macroinvertebrates, stoneflies, size structure, seasonal distribution, karstic water

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UTJECAJ STAROSTI UMJETNIH STAJAĆICA NA ZAJEDNICE LITORALNIH MAKROBESKRALJEŠNJAKA

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Umjetne stajaćice su nastale iskopavanjem i potapanjem područja koje se nalazi uzvodno od brana i ustava. Primarno se stvaraju za potrebe navodnjavanja, prihvaćanje jesenskog i zimskog vodnog vala te dobivanja električne energije. Izmijenjenim vodnim tijelima smatramo ona kojima su nestala lotička obilježja, a nastala su lentička. Umjetne stajaćice su kompleksni sustavi koji predstavljaju neku vrstu prijelaza između jezera i rijeka. Naš cilj je bio istražiti utjecaj antropogenih abiotičkih faktora kao što su starost stajaćica i oscilacija vodostaja na zajednicu litoralnih bentičkih makrobeskralješnjaka. Istraživanje je provedeno na 15 umjetnih stajaćica u Panonskoj ekoregiji Hrvatske pri čemu su uzeti uzorci makrozoobentosa litoralnog područja. Površina uzorkovanja je bila 250 m^2 ili manja, ovisno o strmini obale. Na svakoj istraživačkoj postaji su određeni segmenti od 25 cm do 1 m, u 4 dubinska razreda: od 0 do 0,25 m, od 0,25 m do 0,5 m, od 0,5 m do 0,75 m i od 0,75 m do 1 m. Na svakoj postaji je uzeto 10 poduzoraka ručnom bentos mrežom (500 µm), zahvatne površine 25 cm × 25 cm. Starije stajaćice su pokazale manju oscilaciju vodostaja i veću ujednačenost zajednice koja se može

statistički dokazati Pielouovim indeksom ujednačenosti. Možemo pretpostaviti da su razlog tome stabilniji uvjeti koji omogućuju zajednicama litoralnih makrobeskralješnjaka bolje uvjete za život, a postoji i mogućnost doprinosa od prestanka upotrebe starijih stajaćica u svrhe za koje su prvobitno bile izgrađene.

Ključne riječi: umjetne stajaćice, litoralni bentički makrobeskralješnjaci, Pielouov indeks

ARTIFICAL LAKE AGE IMPACT ON LITTORAL MACROINVERTEBRATE COMMUNITY

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Artificial lakes are man-made water bodies that are usually created by digging and using a dam or lock to store water that can be used for production of electric energy, irrigation or flood control. They are heavily modified lentic waters, usually derived from lotic habitats with complex systems that represent a transitional ecosystem between lakes and rivers. Our goal was to test how anthropogenic abiotic factors such as date of artificial lake construction and water fluctuations influence littoral benthic macroinvertebrate community composition.
Macroinvertebrate communities were studied at 15 artificial lakes located in the Pannonian ecoregion of Croatia. The littoral zone was sampled, on a surface area of 250 m^2 or less, depending on the bank incline. At each sampling site, four levels of depth were defined in 25 cm segments to 1 m total depth (0 to 0.25 m, 0.25 m to 0.5 m, 0.5 m to 0.75 m and 0.75 m to 1 m). Ten subsamples were collected using benthos hand net (25 cm × 25 cm; 500 µm) at each site.
Older lakes have shown less water level fluctuations and how their community composition is more uniform, which can be statistically proven by the by Pielou's evenness index. We assume the reason for that is more stable conditions that allow littoral benthic macroinvertebrate communities better living environment and the possibility that older lakes stopped being used for their original purposes might also be beneficial.

Keywords: artificial lakes, littoral benthic macroinvertebrate, Pielou's evenness indeks

Biologija mora
Marine Biology

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MRIJEST PRSTACA – IZNENAĐUJUĆE NEPOZNAT BIOLOŠKI DOGAĐAJ

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Prstac (*Lithophaga lithophaga* Linnaeus, 1758) je jedan od najčešćih školjkaša plitkog stjenovitog dna Sredozemnog mora. Razmnožava se krajem ljeta, a ciklus razmnožavanja mu je istražen na osnovi mjesečnih uzorkovanja i histološke analize gonada. Terenska istraživanja mrijesta obavili smo na području Starogradskog zaljeva (otok Hvar) krajem ljeta 2014. i 2015. godine. Pronašli smo da je mriještenje visoko sinkronizirani događaj za vrijeme kojeg tisuće jedinki istovremeno ispuštaju gamete. Masovno ispuštanje gameta događa se u uvjetima mirnog mora što omogućava dostizanje potrebne koncentracije kemijskih signala kojima se sinkronizira razmnožavanje jedinki uzduž tridesetak metara obalne linije. Zona s jedinkama koje se mrijeste dalje se širi brzinom od oko 20-30 m/12 h uzduž obale. Mrijest je na nekim od promatranih područja trajao preko 70 sati, a zona mrijesta proširila se na više od 400 m obalne linije. Na područjima mrijesta, velika koncentracija ispuštenih gameta uzrokuje da je morska voda potpuno neprozirna. Zbog svog intenziteta, masovno razmnožavanje prstaca može se zasigurno smatrati jednim od najspektakularnijih procesa razmnožavanja beskralješnjaka u Sredozemnom moru. Unatoč tome, mrijest prstaca ostao je začudo nepoznat do današnjih dana.

Ključne riječi: *Lithophaga lithophaga*, mrijest

SPAWNING OF THE DATE MUSSEL – A SURPRISINGLY UNKNOWN BIOLOGICAL PROCESS

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The date mussel (Lithophaga lithophaga Linnaeus, 1758) is one of the most common molluscs of the shallow Mediterranean rocky bottom. The extent of its reproductive cycle is known based on monthly sampling and histological studies of gonad development. These studies have defined that reproduction occurs at the end of the summer. We performed field research of date mussel spawning in Stari Grad Bay (Island of Hvar) at the end of the summers of 2015 and 2016. We discovered that spawning is a highly synchronized event where thousands of mussels simultaneously release gametes. Mass spawning occurs when the sea is calm. It is an important prerequisite to achieve a threshold concentration of chemical cue in the water column. This enables synchronized reproduction of individuals along cca 30 m of the coastline. The spawning area is spreading along the coastline with progress of 20-30 m/12h. On some observed locations, spawning lasted more than 70 h and progressively spread over more than 400 m of the coastline. Spawning is so intensive that it results in zero visibility inside the spawning zone. Due to its intensity, the spawning of the date mussel should be considered as one of the most spectacular reproduction process among invertebrates in the Mediterranean Sea. In spite of this, the date mussel spawning process remains surprisingly unknown until recently.

Keywords: *Lithophaga lithophaga*, spawning

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STRUKTURA INFRALITORALNE NEKTO-BENTIČKE ZAJEDNICE RIBA ZAŠTIĆENOG MORSKOG PODRUČJA LASTOVSKOG OTOČJA I OTOKA MLJETA

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U svrhu istraživanja strukture zajednice riba morskog područja Parka prirode Lastovsko otočje i Nacionalnog parka Mljet u sklopu projekta „Uspostava protokola praćenja stanja (monitoringa) no-take zona unutar PP Lastovsko otočje i NP Mljet“, provedeno je istraživanje u ljetnim mjesecima 2016. i 2017. godine. Istraživanje je obuhvatilo 31 postaju smještenu u morskom zaštićenom području. Istraživanje je provedeno na dubinama između 7 i 12 metara metodom vizualnog cenzusa bilježenjem vrste, broja jedinki i veličinskog razreda. Ukupno je izvršeno 168 vizualnih cenzusa uporabom autonomne ronilačke opreme. Infralitoralne zajednice na kojima je zajednica istraživana su infralitoralne alge i naselja posidonije te tri prijelazna stadija. Na staništima većinski prekrivena zajednicom infralitoralnih alga uz manja naselja posidonije zabilježena je najveća brojnost vrsta i abundancija te biomasa nekto-bentičkih vrsta riba, dok su najmanje vrijednosti zabilježene na staništima većinski prekrivena naseljima posidonije. Također su zabilježene značajne razlike u raspodjeli abundancije i biomase komercijalno važnih vrsta s obzirom na tip staništa što ukazuje na potencijalne preferencije vrsta i njihovih odraslih stadija spram staništa. Znanje o prirodnim varijacijama mogu pomoći odvojiti utjecaj staništa od drugih izvora varijacije, poput utjecaja ribolova te su to nužne spoznaje za kasniju dobru procjenu učinkovitosti zaštitnih mjera u morskim zaštićenim područjima i no-take zonama.

Ključne riječi: vizualni cenzus, struktura zajednice riba, morska zaštićena područja, znanstveno ronjenje, odabir staništa

THE STRUCTURE OF INFRALITTORAL NECTO-BENTHIC FISH COMMUNITY IN MARINE PROTECTED AREAS OF THE LASTOVO AND MLJET ISLANDS

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The structure of necto-benthic fish community was researched in the marine protected area (MPA) of the Lastovo Island and the island of Mljet as part of the "Establishment of the monitoring protocol for no-take zones within Nature Park Lastovo Island and National Park Mljet“. Research was conducted in the summer of 2016 and 2017. The survey included 31 stations located in MPAs. The study was conducted by visual census at depths between 7 and 12 meters. Species, abundance and size of fish were recorded. Total of 168 visual censuses were performed using SCUBA diving equipment. The infralittoral communities in which the fish have been explored are infralittoral algae and posidonia meadows, and three transitional stages. The highest species richness, abundance and biomass was recorded at the habitats of the infralittoral algae community with patches of posidonia, at the same time the lowest was recorded in the posidonia meadows. There were also significant differences in the distribution of abundance and biomass of commercially important species with regard to the habitat type, which indicates the potential preferences of species and their adult stages towards the specific habitat. Knowledge about natural variations can help isolate the impact of habitats from other sources of variation in the community structure, such as the impact of fishing. This is a necessary insight into a later good assessment of the effectiveness of protective measures in MPAs and no-take zones.

Keywords: underwater visual census - UVC, fish community structure, marine protected areas, scientific diving, habitat preference

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INVAZIVNE VRSTE U ESTUARIJU RIJEKE NERETVE: UTJECAJ NA EKOSUSTAVE I TRADICIONALNO RIBARSTVO

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U zadnjih 20-tak godina u vodenim ekosustavima su utvrđene znatne promjene u sastavu živog svijeta i fizičkih (temperatura, slanost) čimbenika koje značajno utječu na autohtone ekosustave i usluge koje oni osiguravaju. Estuarij Neretve predstavlja još uvijek značajan vodeni ekosustav koji osigurava prihode lokalnom stanovništvu kroz aktivnosti tradicionalnoga ribolova. Zadnjih desetak godina je u neretvanskom estuariju opisan niz novih vrsta vodenih organizama. U 2017. godini su utvrđeni novi nalazi smuđa, *Sander lucioperca* u neposrednoj blizini mora. Također je potvrđen i nalaz pastrvskog grgeča, *Micropterus salmoides*, koji je u većem broju ulovljen u Baćinskim jezerima, ali i bliskom morskom okolišu. Od morskih vrsta riba, povećanje populacije je utvrđeno za nekoliko domaćih vrsta poput strijelke, *Pomatomus saltator* i orade, *Sparus aurata*, s nizom negativnih utjecaja na ekologiju ušća, ribarstvo i školjkarstvo, ali i pozitivnih utjecaja kroz povećanje ulova ovih vrsta. Najnoviji nalaz rebraša, *Mnemiopsis leidyi* u luci Ploče kao rezultat introdukcije balastnim vodama, predstavlja veliku prijetnju za ličinke riba, školjkaša i rakova na području ušća Neretve, te se njegovim potencijalnim širenjem predviđaju velike štete. Utjecaji svih ovih promjena na ekosustave i usluge koje na njima počivaju nisu detaljnije istraživane. U ovom radu će se opisati do sada utvrđene promjene estuarskog područja Neretve i procijeniti sadašnji i budući utjecaji na tradicionalno ribarstvo.

Ključne riječi: Invazivne vrste, estuarij Neretve, tradicionalno ribarstvo

INVASIVE SPECIES IN NERETVA ESTUARY: IMPACTS ON ECOSYSTEMS AND TRADITIONAL FISHERY

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In the last twenty years significant changes in structure and physical (temperature, salinity) factors in water ecosystems are documented. Neretva Estuary (Croatia) is significant ecosystem which secures income to local people through traditional fishery. Several new species is reported in this estuary recently. During 2017th new findings of pikeperch, *Sander lucioperca* in vicinity of marine waters is documented. New record of the largemouth bass, *Micropterus salmoides*, in Baćina Lakes and surrounding marine waters is confirmed. Among marine species, increase of populations is documented for autochthonous fish species like bluefish, *Pomatomus saltator* and gilthead sea bream, *Sparus aurata*, with several negative impacts on estuarine ecology, fishery and shellfish culture, but also positive impacts through increase of catch of these species. The newest finding of the sea walnut, *Mnemiopsis leidyi* in Port of Ploče as a result of introduction via ballast waters presents high threat to early stages of fish, cruastaceans and shellfish in Neretva Estuary. Impacts of these changes on ecosystems and their services are not properly investigated. This article will summarize recent changes in estuarine environment of Neretva River and estimate present and future impacts on traditional fishery.

Keywords: invasive species, Neretva Estuary, traditional fishery

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BEHAVIORAL MESOPREDATOR RELEASE? COMMON MESOPREDATORS IN THE CROATIAN ADRIATIC SEEK FRAGMENTED HABITATS, ARE AGGRESSIVE TOWARDS PREY-SIZED MOBILE LURES, AND CONSTITUTE A HIGH CONSUMPTION POTENTIAL ON SMALL AND JUVENILE FISH

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A lure assisted, visual census, belt transect method was applied to evaluate habitat preferences for Croatian Adriatic demersal fish. In 2009- 2016, a snorkeler (CK) presented an artificial lure along 20 000 transects at 150 locations spanning the entire Croatian coast, mainland and islands. More than 200 000 fish belonging to 115 species were observed at the lure or merely present near the lure's path which intersected various homogeneous and heterogeneous groundcover types composed of combinations of rock, algae, sand and seagrass. A group of common mesopredators, including serranids, gobiids, and sparids, exhibited strong preference for fragmented habitats and exhibited significantly higher attention to the lure which they followed and attacked more frequently than other fish. Consumption rates calculated from bite/ingestion frequencies and species abundances suggest an impact on prey communities that is higher than expected by abundance measures alone. Considering that the lure, 3 x 0,5 cm in cylindrical dimensions, is a moving target at a speed of 3-5 meter per 10 seconds, it most resembles mobile and non-substrate-contacting prey within the water column, such as small and juvenile fishes. We discuss our results in the context of mesopredator release from predation and the potentially associated increase in aggressive and bold behaviors which may set off negative bottom-up effects via intra and inter-guild predation on juveniles of both meso- and top-predators.

Keywords: fishes, behavioral traits, mesopredator release, cascades, lure assisted visual census

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VERTIKALNA RASPODJELA MIKROZOOPLANKTONA „VELIKOG JEZERA“ OTOKA MLJETA (2014./2015.)

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U radu su prikazani rezultati istraživanja utjecaja abiotskih čimbenika na vertikalnu raspodjelu mikrozooplanktona na postaji dubine 45 m. Uzorci su sakupljeni Niskin crpcem od 5 L tijekom dana. Stratifikacija vodenog stupca i obilne padalina u ljeto 2014. godine uvjetovale su stvaranje stalnog hipoksičnog sloja ispod 30 m dubine od rujna do kraja prosinca. Zabilježene su značajne pozitivne korelacije s temperaturom za glavninu taksona mikrozooplanktona. Cilijati su bili najbrojnija skupina. Tijekom 2014. godine dominirala je miksotrofna vrsta *Mesodinium rubrum* što ukazuje na eutroficirane uvjete u tom razdoblju. Niske vrijednosti otopljenog kisika imalo je značajno negativni učinak na vertikalnu raspodjelu i brojnost

mikrozooplanktona. Izuzetak su bili kopepodi iz porodice Oncaeidae (*Monothula subtilis* i *Oncaea zernovi*) i ličinke poliheta. Za obje skupine zabilježene su ekstremno visoke gustoće u dubokom hipoksičnom sloju s vrijednostima 10-14% otopljenog kisika. Rezultate možemo objasniti njihovom visokom tolerancijom na hipoksične uvjete i direktnom ishranom organskim detritusom, ili na njemu pričvršćenim heterotrofnim bakterijama. Hiperbentonski kopepod *Mesoioceras hurei* tijekom hipoksičnih uvjeta izbjegavao je pridneni sloj i boravio na višim dubinama s vrijednostima otopljenog kisika između 11 i 25%. Naši rezultati potvrđuju važnost istraživanja zatvorenih zaštićenih područja u svrhu objašnjenja bioloških procesa i predviđanja globalnih promjena morskih ekosustava.

Ključne riječi: ciliati, naupliji, kopepodi, meroplankton, južni Jadran

MICROZOOPLANKTON VERTICAL DISTRIBUTION IN „BIG LAKE"OF THE MLJET ISLAND (2014/2015)

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The present study reports results of the abiotic factors influence on the microzooplankton vertical distribution. Samples were collected by the 5L-Niskin bottle during the day. The water column stratification and plentiful rainfall in the summer of 2014 caused a permanent hypoxic layer below 30 m depth from September to the end of December. Significant positive correlations with the temperature for the majority of microzooplankton taxon were recorded. Ciliates were the most numerous group. During 2014, the mixotrophic species *Mesodinium rubrum* dominated, which indicates eutrophic conditions in that period. Low values of dissolved oxygen had a significant negative effect on the vertical distribution and the microzooplankton abundance. Exceptions were the Poecilostomatoida copepods (*Monothula subtilis* and *Oncaea zernovi*) and polychaete larvae. Extremely high densities were found for both groups in a deep hypoxic layer with 10-14% of dissolved oxygen. These results could be explained by their high tolerance to hypoxic conditions and direct nutrition on organic detritus, or on attached heterotrophic bacteria. Hyper-benthic copepod *Mesoioceras hurei* avoided the bottom layer during hypoxic conditions and balk upper depths with the higher dissolved oxygen values of 11-25%. Our results confirm the importance of investigations of closed protected areas in order to explain biological processes and predict global change of marine ecosystems.

Keywords: Ciliates, nauplii, copepods, merozooplankton, southern Adriatic

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MIKROPLASTIKA U PROBAVILU TRPOVA RODA *Holothuria* (PP TELAŠĆICA)

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Nakon uspješno provedene analize količine mikroplastike u sedimentu u uvalama Parka prirode Telašćica, analizirali smo probavila trpova roda Holothuria koji se hrane organskom tvari iz

sedimenta. Glavno pitanje je bilo ima li mikroplastike u probavnom sustavu trpova, odnosno kakva je korelacija između relativne količine mikroplastike u sedimentu i probavilu trpova. Uzorci su skupljeni 2017. godine na 10 lokacija na kojima su prethodno uzeti uzorci sedimenta. Uzimano je 15 jedinki trpova po lokaciji. Trpovima je odstranjeno probavilo i konzervirano za daljnju analizu. Uslijed poteškoća prilikom ekstrakcije plastičnih čestica iz trpova te nedostatka istraživanja na tu temu, fokus istraživanja preusmjeren je na odabir najbolje metode za izolaciju plastičnih čestica iz probavila trpova. Između 3 različite metode koje su ispitivane uočena je razlika u uspješnosti ekstrakcije mikroplastike. Preliminarni rezultati pokazuju da je metoda u kojoj se koristi 30% KOH:$NaClO_4$ te sušenje i mikroskopiranje sedimenta uspješnija od ostalih u izoliranju mikroplastike iz sedimenta. Istraživanje utjecaja mikroplastike je vrlo aktualno jer se tek nedavno shvatio utjecaj i količina mikroplastike u moru, a ovo je još jedan mali korak u otkrivanju razine ovog problema.

Ključne riječi: mikroplastika, *Holothuria*, sediment, metoda izolacije

MICROPLASTICS IN DIGESTIVE SYSTEM OF HOLOTHURIANS *Holothuria* SPP. IN TELAŠĆICA MPA

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After successful analysis of microplastics in marine sediments of Telašćica Nature Park, we analyzed digestive system of holothurians that feed on organic matter in that sediment. We wanted to test the hypothesis that there is a positive correlation between the amount of microplastics in digestive system of holothurians and in sediment. Samples were collected during 2017 on the same 10 locations where we sampled the sediment. 15 holothurian individuals were taken at every location and their digestive system was extracted and conserved for analysis. During microplastics extraction we encountered great difficulties and due to lack of published research on this subject, focus of our research shifted toward choosing the best methodology for extraction of microplastics from holothurians' digestive system. Three methods were tested and different efficiency of microplastics extraction was noticed. Preliminary results show that the method of using 30% KOH:$NaClO_4$, drying and microscoping the sediment was the most efficient. Only recently we have realized the real impact and the increasing amount of microplastics in the marine environment and microplastics research represents one of the main topics in marine biology – our research represents one small step towards solving this problem.

Keywords: microplastics, *Holothuria*, sediment, extraction methodology

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GUSTOĆA I STRUKTURA POPULACIJE PLEMENITE PERISKE – *Pinna nobilis* U MALOM JEZERU U NP MLJET

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Na području Nacionalnog parka Mljet, u Malom Jezeru, tijekom 2017. godine provedeno je istraživanje strukture populacije i gustoće plemenite periske *Pinna nobilis*. Istraživanje je provedeno pomoću autonomne ronilačke opreme na rasponu dubina od 1.5 do 4 metra. Za određivanje gustoće korišten je transekt duljine 30 i širine 6 do 8 metara, a za određivanje strukture populacije mjerene su tri duljine ljušture školjkaša (uz sediment, na najširem dijelu i visina ljušture od sedimenta) pomoću stolarskog metra. Rezultati prethodnih istraživanja ukazuju na veliku gustoću jedinki na lokalitetu Malo Jezero do dubine od 15 metara. Naši rezultati pokazuju iznimnu gustoću živih jedinki u plitkom dijelu Malog Jezera, najveću ikad zabilježenu za ovu vrstu. Struktura populacije je također neobična jer je većina jedinki slične veličine što ukazuje na približno sličnu starost. Također, istraživanjem je utvrđeno da nema juvenilnih jedinki. Uslijed globalnih klimatskih promjena koje pogoduju razvoju raznih patogena i parazita, u nekim dijelovima Zapadnog Mediterana došlo je do potpunog izumiranja ove vrste. Ovakva iznimna gustoća, približno homogena populacijska struktura te nepostojanje obnove populacije ukazuje na značaj detaljnog istraživanja ovdje primijećenog fenomena.

Ključne riječi: *Pinna nobilis*, struktura populacija, gustoća, morsko zaštićeno područje

DENSITY AND POPULATION STRUCTURE STUDY OF NOBLE PEN SHELL – *Pinna nobilis* IN MLJET MPA

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Density and population structure research of noble pen shell – *Pinna nobilis* was performed in Malo Jezero (Mljet National Park) in 2017. SCUBA gear was used for survey on depth ranging from 1.5 to 4 meters. For density measurements, 30 meters long and 6 - 8 meters wide transect was used. For population structure descriptors, maximum width, minimum width and unburied length were recorded. Results of previous studies indicate high density of P. nobilis individuals down to 15 meters of depth in Malo Jezero. Present results also point to extremely high density of live individuals in shallow part of Malo Jezero, the highest ever recorded for this species. All recorded individuals were approximately the same size which indicates the same age and unusual population structure. During this research juvenile individuals were not recorded at all. Certain pathogens and parasites benefit from current global climate change and there is evidence of mass mortality events of *P. nobilis* in some parts of Western Mediterranean, even to the point of total extinction. High density and unusual population structure with no recruitment recorded during this study indicate the high importance of this phenomena and the need of further research.

Keywords: *Pinna nobilis*, population structure, density, MPA

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RONJENJE U TIŠINI, UTJECAJ ZVUKA NA STRUKTURU RIBLJIH ZAJEDNICA

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Vizualni cenzus pomoću autonomne ronilačke opreme otvorenog kruga uobičajena je metoda u istraživanju strukture zajednica riba, ali za vrijeme ronjenja uočeno je da veći primjerci i plašljivije vrste riba izbjegavaju ronioca uslijed zvuka i vidljivog traga mjehurića zraka. Tijekom

2017. i 2018. godine, duž hrvatskog i crnogorskog dijela Jadrana, u zaštićenim i nezaštićenim morskim područjima proveli smo istraživanje strukture zajednica riba s ciljem usporedbe uspješnosti dva ronilačka sustava - otvorenog i zatvorenog kruga, tzv. rebreather. Za vrijeme istraživanja, osim određivanja vrsta riba, procjenjivali smo i dužinu riba za kasniji izračun biomase kroz dužinsko-masene odnose. Tijekom ronjenja s opremom zatvorenog kruga zabilježeni su veći primjerci i veća brojnost pojedinih vrsta riba u odnosu na rezultate postignute ronjenjem s opremom otvorenog kruga što ukazuje na to da je zatvoreni krug pogodna metoda za istraživanje plašljivih vrsta koje su pod velikim ribolovnim pritiskom, posebno od strane podvodnih ribolovaca. Zabilježene su i jedinstvene vrste koje nisu zabilježene ronjenjem s opremom otvorenog kruga. U tehničkom i sigurnosnom smislu, ronjenje sa zatvorenim krugom daje ogromnu prednost jer je potrošnja plinova mala što omogućava iznimno dugotrajna ronjenja te zbog fiksnog parcijalnog tlaka kisika omogućava duga ronjenja izvan dekompresije.

Ključne riječi: ronilačka oprema zatvorenog kruga – rebreather, vizualni cenzus, struktura ribljih zajednica, nedostaci-prednosti metodologije, znanstveno ronjenje

SILENT DIVING, BUBBLE-NOISE AND DIFFERENCES IN FISH COMMUNITY STRUCTURE

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For assessing fish community structure underwater visual census (UVC) using SCUBA is commonly used method. During diving, bigger and timid fish species were observed to avoid divers due to the noise and visual trail of emitting bubbles. That hypothesis has been tested while parallel assessing fish community structure with open-circuit SCUBA and closed-circuit rebreather – CCR. During research we were recording fish species and fish lengths for biomass calculation based on length-mass relations. Research was performed during 2017 and 2018 along Croatian and Montenegrin Adriatic coastline, in protected and non-protected marine areas. Significant difference was observed in sizes and number of certain species while performing research with SCUBA and CCR. There are some exclusive fish species that we noticed only with CCR. Difference in these results could be explained by disturbance of certain species by the noise of emitting bubbles during SCUBA diving. Additional advantage of CCR is that it is possible to observe timid species which are under high spearfishing pressure. Technically, CCR provides huge advantage in diving by greatly reducing diving gas consumption thus allowing extended bottom times and fixed oxygen partial pressure reduce decompression obligation.

Keywords: closed-circuit rebreather – CCR, underwater visual census – UVC, fish community structure, survey bias, scientific diving

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STRUKTURALNA KOMPLEKSNOST SUSJEDNIH STANIŠTA UTJEČE NA RELATIVNU VRIJEDNOST NASELJA MORSKE CVJETNICE *Posidonia oceanica* KAO STANIŠTA ZA RIBE

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Morske cvjetnice imaju važnu ulogu u strukturiranju zajednice riba te utječu na raznolikost vrsta, brojnost i ponašanje riba. Korištenjem autonomne ronilačke opreme, na dubini od 10 metara, proveden je vizualni cenzus riba uz korištenje mamca, u razdoblju od lipnja do studenog 2011. i od ožujka do studenog 2012. godine na lokacijama duž hrvatske obale Jadranskog mora, s ciljem utvrđivanja razlika u strukturi zajednica riba u naseljima morske cvjetnice *Posidonia oceanica* s obzirom na dostupnost alternativnog strukturiranog staništa. Pretpostavili smo da će strukturalna kompleksnost susjednih staništa značajno utjecati na relativnu vrijednost naselja posidonije kao staništa za ribe te da će pojedine vrste riba pokazati različit stupanj preferencije za naselje posidonije ovisno o tome koje im je alternativno stanište u blizini dostupno. Korištenjem ordinacijske metode neparametrijskog višedimenzijskog grupiranja (nMDS) te neparametrijske permutacijske analize varijance (PERMANOVA) utvrdili smo statistički značajne razlike u sastavu zajednice riba te snažan utjecaj tipa susjednog staništa, čak i kada se u obzir uzmu svi ostali značajni faktori poput razlike u pokrovnosti posidonije, temperaturi mora te kompaktnosti samog naselja.

Ključne riječi: *Posidonia oceanica*, morske cvjetnice, ribe, struktura zajednice, stanište

NEIGHBORING HABITAT DRIVES FISH COMMUNITY STRUCTURE OF *Posidonia oceanica* MEADOWS IN CROATIAN ADRIATIC

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Seagrass habitats play an important role in fish community assemblage, affecting among other variables, taxonomic diversity, abundance and fish behavior. We performed lure assisted visual census transects using SCUBA diving at 10 meters of depth during June 2011 – September 2012 to identify the relative importance of *Posidonia oceanica* meadows as a habitat for fishes. We predicted significant effects of varying structural complexity of Posidonia-neighboring habitats and also of matrix habitats which support Posidonia meadows as the focal habitat, on fish assemblage descriptors. We further predicted that individual fish species will exhibit significant differences in their preference for *P. oceanica* meadows depending on the types of habitats in close proximity. Using unconstrained, non-metric multidimensional scaling (nMDS) and permutational non-parametric MANOVA we demonstrate significant effects of the predictor variable neighboring habitat on fish community structure. These effects were maintained when controlling for other significant variables, including percentage of Posidonia cover, water temperature and compactness of the meadow.

Keywords: *Posidonia oceanica*, seagrass, fish, community structure, habitat

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THE REVIEW OF OPISTHOBRANCH MOLLUSCS FAUNA IN THE BOKA KOTORSKA BAY, MONTENEGRO

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Opisthobranch molluscs fauna in Montenegro is still not very well studied. Our aim was to improve the knowledge about these marine organisms in the Boka Kotorska Bay, specific fjord-like entity in the South Adriatic Sea. We have compiled a checklist with new sampling records, analysis of grey and published literature and underwater photographs that were taken during 2011-2017. New records of eight opisthobranch species for the Boka Kotorska Bay (*Elysia viridis*, *Boselia mimetica*, *Aplysia parvula*, *Aplysia punctata*, *Paradoris indecora*, *Favorinus branchialis*, *Rubroamoena amoena* and *Trinchesia genovae*) were provided by our survey in 2017, while four new records (*Caloria elegans, Felimare villafranca*, *Flabellina ischitana* and *Phyllidia flava*) were provided by analysis of underwater photographs. The updated checklist summarizes the knowledge of the diversity of opisthobranch species from the oldest record in 1967 to the present day and with our 12 new records includes 69 opisthobranch species for the Boka Kotorska Bay. A comparison of our results with those of neighbouring areas is provided as well as the analysis of habitat and feeding preferences of the opisthobranch molluscs in the Boka Kotorska Bay.

Keywords: opisthobranch molluscs, Boka Kotorska Bay, Montenegro

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GLAVOČI MEDITERANA: TAKSONOMSKA, ZOOGEOGRAFSKA I FILOGENETSKA OTKRIĆA POSLJEDNJEG DESETLJEĆA

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U ovom pregledu predstavljaju se objavljeni rezultati kao i rani uvid u tekuća istraživanja na glavočima Mediterana. Kroz skoro desetljeće koje je prošlo od zadnjeg sažetka o sistematici i biološkoj raznolikosti glavoča sjeveroistočnog Atlantika i Mediterana naše znanje o ovim ribama je naraslo. Šest novih mediteranskih vrsta je opisano. Također pet alohtonih vrsta je kroz to vrijeme po prvi put zabilježeno u Mediteranu. S jednom vrstom izbrisanom iz ranijeg popisa, ukupna biološka raznolikost glavoča Mediterana dostiže 70 vrsta, zadržavajući glavoče kao vrstama daleko najbogatiju porodicu riba u Mediteranu. Poznata biološka raznolikost glavoča narasla je u skoro svim dijelovima Mediterana, s najvećim promjenama u sjeverozapadnom i jugoistočnom Mediteranu. Filogenetska istraživanja zadnjeg desetljeća otkrila su da se mediteranski glavoči, kao i svi europski glavoči pripadaju trima srodstvenim granama, Pomatoschistus-, Aphia-, i Gobius-grupama. Svaka od ovih filogenetskih grana ima

svoju sestrinsku grupu među indopacifičkim glavočima. Aphia- i Gobius-grupe pripadaju potporodici Gobiinae, a Pomatoschistus-grupa potporodici Gobionellinae.

Ključne riječi: Gobiidae, Mediteran, sistematika, zoogeografija, filogenija

MEDITERRANEAN GOBIES: TAXONOMIC, ZOOGEOGRAPHIC AND PHYLOGENETIC DISCOVERIES IN THE LAST DECADE

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The review presents published results as well as the preview on the researches now in progress on Mediterranean gobies. Almost a decade past since the last summary on taxonomy and biodiversity of the North-Eastern Atlantic and Mediterranean gobies and the knowledge on this taxon in the Mediterranean has increased. The six new species were described in that period in the area. In addition, five more alien gobies were recorded in the Mediterranean during the same time. With one species deleted from the earlier Mediterranean check-list, the total Mediterranean gobiid diversity has reached 70 species, keeping Gobiidae as the far species richest fish family in this sea. The known gobiid biodiversity increased in almost all Mediterranean areas, with larger changes happened in Nord-west Mediterranean and South-east Mediterranean. The phylogenetic researches of the last decade have revealed that Mediterranean gobies, as well as other European gobies, cluster in three independent lineages, the Pomatoschistus-, Aphia-, and Gobius-lineages. Each of this clade has its sister groups among Indopacific gobies, with Aphia- and Gobius-lineages being part of Gobiinae subfamily, while gobies of Pomatoschistus-lineage belong to another subfamily, Gobionellinae.

Keywords: Gobiidae, Mediterranean, taxonomy, zoogeography, phylogeny

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NOVE METODE KORIŠTENE PRILIKOM IZRADE KARTE STANIŠTA NA SIDRIŠTIMA SPLITSKO-DALMATINSKE ŽUPANIJE

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Negativan utjecaj sidrenja na biljne i životinjske vrste bentosa dugo je već poznat. U želji da se on smanji u akvatoriju Splitsko-dalmatinske županije krenulo se u izradu studije sidrišta na pomorskom dobru Splitsko-dalmatinske županije. Definiranjem mjesta uređenih sidrišta negativan utjecaj sidrenja bi se ograničio. Studija je obuhvaćala i utvrđivanje postojećeg stanja kroz kartiranje staništa i biocenoza te kroz popisivanje zaštićenih vrsta na lokacijama mogućih sidrišta. Tijekom biocenoloških istraživanja na terenu korištena je metoda direktnog opažanja (in situ) i uzorkovanja pomoću autonomnih ronilaca uz fotodokumentiranje podvodnom video kamerom i foto aparatom. U radu je korištena standardna ronilačka oprema za ronjenje s komprimiranim zrakom. Novost predstavlja korištenje geopozicioniranog podvodnog skutera s video kamerom. Ronilac na skuteru s videokamerom je imao zadatak bilježiti prijelaze staništa i

zajednica, a njegova pozicija definirana je pomoću GPS uređaja koji je vukao na bovi iznad sebe. Ostali ronioci imali su zadaću fotografiranja zaštićenih vrsta. Glavni rezultati ovog stručnog rada su karte staništa izrađene u geoinformacijskom sustavu ArcGis 9.3 i zabilježene strogo zaštićene vrste za 29 lokacija sidrišta. Ovaj rad pokazuje kako se stručno-znanstvenom metodologijom i inovativnim multidisciplinarnim pristupom, može bez povećanih financijskih opterećenja izraditi daleko pouzdanije i detaljnije karte za lokacije manjeg obuhvata (<25 ha).

Ključne riječi: sidrište, Splitsko-dalmatinska županija, prostorni plan, ekološka mreža Natura 2000, podvodno kartiranje

NEW METHODS USED IN HABITATS MAPING AT THE ANCHORS OF THE SPLIT DALMATIA COUNTY

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Negative impact of anchoring on plant and animal species of benthos has long been known. In attempt to reduce the impact in the marine area of the Split-Dalmatia County studing of such negativ impact is initiated. By defining the places of the anchorages, negative impact would be reduced to the smallest possible extent. Study incorporated determination of the present condition of protected species at possible anchoreige sites. During the biocenological field research, the method of direct observation (*in situ*) and sampling using SCUBA divers and photodocumentation with underwater video and photo cameras. The standard diving equipment for compressed air SCUBA diving was used in this paper. Novelity of the present study is geopositioning of underwater scooter with video camera. The diver on the scooter with the camcorder had the task of recording the habitat transitions, and his position was defined by a GPS device that was above on the surface. Remaining of the divers had the task of photographing protected species. The main results of this expert work are maps of habitats made in the ArcGis 9.3 geoinformation system and recorded strictly protected species for 29 anchorage sites. Conclusively, it can be shown that by using professional-scientific methodology and inovative multidisciplinary approaches, much more reliable and detailed maps for midle size locations (<25 ha) can be made without significant financial burdens.

Keywords: underwater mapping, anchorage, Split-Dalmatia County, spatial plan, Natura 2000 ecological network

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FACTORS INFLUENCING THE HOME RANGES OF COMMON BOTTLENOSE DOLPHINS (*Tursiops truncatus*) IN THE CRES AND LOŠINJ WATERS (NORTH ADRIATIC SEA, CROATIA)

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This study investigates the influence of association patterns, gender, natal phylopatry and anthropogenic pressure on the home range size of resident bottlenose dolphins inhabiting Cres-Lošinj waters. Photo-identification data has revealed this area is home to about 200 common bottlenose dolphins. High sighting frequency and regular re-sightings of known individual dolphins indicate their long-term fidelity to this region. Due to the importance of this habitat for the resident bottlenose dolphin population, in 2014 this area was declared a Natura 2000 Site of Community Importance (SCI). The home range sizes (MCP, 95% KDE and 50% KDE) of 44 most resident dolphins were calculated by combining the use of adehabitatHR package in programs R and Home Range Tool in ArcView GIS. Results indicate that differences in gender reflect most on the home range patterns. Females were found to use significantly wider areas than males and form associations that are more fluid, while males show strong bonds and territorial alliances between them. Moreover, results confirm that the human activities, related to nautical tourism, affect both female and male dolphins through seasonal changes in their home range sizes. The overall results provide a better understanding of ranging patterns of resident bottlenose dolphins and improve current knowledge on the main threats that should be considered when developing conservation measures for this Natura 2000 SCI.

Keywords: Home range, *Tursiops truncatus*, minimum convex polygon, kernel density, association, gender, nautical tourism

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RAZUMJETI KAKO NADMETANJE ZA OGRANIČAVAJUĆE NUTRIJENTE OBLIKUJE FITOPLANKTONSKU ZAJEDNICU U MORU

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Sjeverni dio Jadranskog mora je poluzatvoreni planktonski ekosustav u kojem se za fitoplanktonsku planktonsku zajednicu često javlja nedostatak fosfata u odnosu na soli dušika i silikata. Izraženi, prostorno-vremenski ekološki gradijenti omogućuju nam različite strategije uzorkovanja s relativno dobrom prostorno vremenskom pokrivenošću, duž zemljopisne dužine i zemljopisne širine, kao i kroz vodeni stupac. Sjeverni Jadran stoga je posebno pogodan za ekološko istraživanje fitoplanktona, ovdje možemo promatrati njegovo ponašanje i sukcesiju dok putuje kroz mnoštvo ekoloških uvjeta. In situ mjerenja pokazala su da im aktivacija enzima alkalne fosfataze omogućuje pristup organskom fosfatu što je ključno za preživljavanje fitoplanktona u uvjetima limitacije fosforom. Za ključne vrste pratile su se stope rasta i morfološke promjene, stope unosa nutrijenta, profili lipida, sadržaj klorofila i izražavanje aktivnosti alkalne fosfataze (AP) kao reakcije na ograničenje fosfora. Karakterizirali smo

dinamiku ekspresije alkalne fosfataze kroz različite faze rasta, kao i kinetičke parametre enzima. Kombinirajući sve naše rezultate formulirali smo strategije koje objašnjavaju kako se vrste natječu za resurse. Uspjeh u natjecanju za ograničene resurse (fosfor), kao i relativni performans s obzirom na temperaturu i svjetlo može pojasniti sukcesiju i sastav morske fitoplanktonske zajednice.

Ključne riječi: fitoplankton, sjeverni Jadran, limitacija fosfatom, životne strategije, kompeticija

UNDERSTANDING HOW COMPETITION FOR LIMITING NUTRIENTS SHAPES MARINE PHYTOPLANKTON COMMUNITIES

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The northern Adriatic Sea is a semi-separated planktonic ecosystem that is generally characterized by phosphorous limitation. Steep, spatiotemporal ecological gradients allow us sampling strategies with reasonably good spatiotemporal coverage both, across longitude and latitude as well as throughout the watercolumn. The northern Adriatic hence is particularly suited for ecological observations of phytoplankton, here we can observe its behavior and succession as it travels through a multitude of ecological conditions. In situ measurements demonstrated that the expression of alkaline phosphatase activity as a tool to access the organic phosphate pool is a key reaction of the phytoplankton community to phosphorous limitation. For key species we characterized growth curves and morphological alterations, nutrient uptake rates, lipids profiles, chlorophyll content and the expression of alkaline phosphatase (AP) activity as a reaction to phosphorous limitation. We characterized the dynamics of AP expression across growth phases as well as the kinetic parameters of the enzyme activity. Combining all our results we formulated strategies that explain how species compete for resources. Success in competition for the limiting nutrient salt (phosphorous) as well as relative performance with respect to temperature and light availability explains the succession and composition of marine phytoplankton.

Keywords: phytoplankton, Northern Adriatic, alkaline phosphatase, phosphate limitation, life strategies

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PROMJENA OBILJA HLAPA (*Homarus gammarus*) U JADRANU KAO POSLJEDICA POVEĆANJA TEMPERATURE MORA

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Hlap (*Homarus gammarus*) je rasprostranjen u cijelom Jadranu iako je brojniji u sjevernom dijelu. Kao borealna vrsta, njegov mrijest, naseljavanje i novačenje može biti snažno pogođeno globalnim trendom povećanja površinske temperature mora i desetogodišnjom termohalinom varijabilnosti koja se javlja u Jadranskom moru, upravljane režimom jadransko-jonskog bimodalnog oscilacijskog sustava (BiOS). U ovom istraživanju uspostavili smo odnos između pridnenih temperatura mora u prošlom desetljeću s podacima o ulovu hlapa. Podaci o temperaturi su uzeti s redovito uzorkovanih oceanografskih postaja, dok su podaci o ulovu hlapa dobiveni od Uprave ribarstva za 10 ribolovnih zona u razdoblju od 2008 do 2017. Rezultati pokazuju pad ulova u južnom i srednjem Jadranu, dok se ulov povećava u sjevernom Jadranu nakon 2012. Ove se promjene podudaraju s povećanjem pridnene temperature mora nakon 2012. Kako se povećanje temperature podudara s izmjenom negativnog i pozitivnog BiOS režima, od kojih potonji uzrokuje ulazak toplije i slanije vode u Jadran, pretpostavljamo da BiOS mehanizam snažno utječe na životni ciklus hlapa, posebice u južnom i srednjem Jadranu. Povrh toga, daljnje povećanje temperature može dovesti do istaknutijih migracija hlapa prema sjevernom Jadranu, uzrokujući pad ulova hlapa u južnom i srednjem Jadranu.

Ključne riječi: hlap, *Homarus gammarus*, Jadransko more

TEMPERATURE-DRIVEN ABUNDANCE CHANGE OF THE EUROPEAN LOBSTER (*Homarus gammarus*) IN THE ADRIATIC SEA

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European lobster (*Homarus gammarus*) is distributed throughout the whole Adriatic Sea, although it is more abundant in the northern part. As it is a boreal species, *H. gammarus* spawning, settlement and recruitment success might be strongly affected by both global trend of increasing sea surface temperatures and decadal thermohaline variability which occurs in the Adriatic Sea, driven by the Adriatic-Ionian Bimodal Oscillating System (BiOS) regimes. We related sea bottom temperatures measured during last decade with landings data of *H. gammarus*. The temperature data were taken from regularly sampled oceanographic stations, while landings data were obtained by Directorate of Fisheries for 10 fishing zones in a period from 2008 to 2017. The results show a decline in landings in the south and the middle Adriatic, while landings increase in the northern Adriatic after 2012. Those changes coincided with the increase of bottom sea temperature after 2012. The temperature increase coincides with the change between negative and positive BiOS regimes, of which the latter is known to advect warm and salty waters to the Adriatic, and thus we hypothesize that the BiOS mechanism has a strong effect to the lobster life cycle, particularly in the southern and middle Adriatic. The overall increase in temperatures in the future climate might result in more prominent migration of *H. gammarus* towards the northern Adriatic, causing a decline in lobster catch in south and middle Adriatic.

Key words: European lobster, *Homarus gammarus*, Adriatic Sea

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PROMJENE ZVIŽDUKA DOBRIH DUPINA (*Tursiops truncatus*) KOD PRIMJENE OPORTUNISTIČKIH STRATEGIJA HRANJENJA NA PODRUČJU SARDINIJE I SJEVERNOG JADRANA

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Korištenje zvuka je važno za životinje koje žive u složenim društvenim zajednicama. Ovaj rad predstavlja istraživanje komunikacijskih signala - zvižduka koje dobri dupini proizvode za vrijeme različitih aktivnosti hranjenja. U obalnim područjima aktivnosti hranjenja uključuju primjenu oportunističkih strategija koji uključuju interakciju s ribarstvom. Mi smo istražili zvižduke koje dobri dupini proizvode za vrijeme depredacije pridnenih mreža koča u Sardiniji i na cresko-lošinjskom području. Ovo ponašanje uključuje hranjenje na mreži koja predstavlja bogati izvor plijena i može zahtijevati korištenje specifičnih komunikacijskih signala. S ciljem utvrđivanja ovisnosti frekvencijskih i vremenskih komponenti zvižduka o načinu na koji se dupini hrane, razmotrili smo tri situacije: hranjenje iza koča, hranjenje bez motornih plovila u blizini te hranjenje u prisustvu motornih plovila. Rezultati su pokazali da dupini u Sardiniji, za vrijeme hranjenja iza koča proizvode kraće zvižduke s manje modulacija i s višim početnim i minimalnim frekvencijama u odnosu na zvižduke proizvedene za vrijeme drugih scenarija hranjenja. Suprotno tome, u cresko-lošinjskom području, značajne razlike u zvižducima utvrđene su kod hranjenja ovisno o prisutnosti motornih plovila u blizini dupina. Ovo istraživanje predstavlja prvi izvještaj o tome kako dvije zajednice dupina primjenjuju različite akustične strategije pri istim načinima hranjenja.

Ključne riječi: *Tursiops truncatus*, koče, zvižduk, frekvencija, plovila

VARIABILITY IN COMMON BOTTLENOSE DOLPHIN (*Tursiops truncatus*) WHISTLES IN RELATION TO OPPORTUNISTIC FEEDING STRATEGIES IN SARDINIAN AND NORTHERN ADRIATIC WATERS

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The use of sound is of particular importance for animals that live in complex social communities. In this study we assessed communication calls (whistles) that bottlenose dolphins emit during feeding activities. In coastal areas, these may include appliance of opportunistic strategies that often involve dolphin interaction with fisheries. In the present study, we investigated the emissions of whistles during dolphin depredation on bottom trawlers in Sardinian (Italy) and Cres and Lošinj waters (Croatia). This behaviour involves feeding on a concentrated food source, represented by the fishing net, and may require the emission of distinctive communication calls. We assessed the whistle characteristics and investigated if their frequency and time variables change depending on the three feeding contexts: feeding behind trawlers, feeding in the presence of motor boats and feeding in motor boat absence. In Sardinia, when feeding behind the trawlers dolphins emit shorter whistles with less modulations and a higher minimum and start frequency in comparison to whistles produced on other feeding scenarios. Conversely, in Cres and Lošinj waters

significant variations in whistles were found to be related to the presence of motor boats during feeding. This study represents the first report on how two different dolphin communities adopt different acoustic strategies in the context of same feeding tactic.

Keywords: *Tursiops truncatus*, bottom trawler, whistle, frequency, boat presence

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NASELJA KORALJA NA KORALIGENU KAMPANELA (PP TELAŠĆICA)

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U ovom istraživanju opisana su naselja koralja na pučinskom podvodnom grebenu („dimnjak“ ili Kampanel) uz Dugi otok (Park prirode Telašćica) unutar koraligenske biocenoze na dubini od 28 do 80 metara. Istraživanje je napravljeno pomoću ROV uređaja i autonomnim ronjenjem. Na dubinama gdje je razvijen koraligen prevladavaju jake morske struje. Temperatura mora na 40 metara dubine je između 12 i 17°C kroz veći dio godine, uz visoke oscilacije do 24°C zabilježene u kolovozu, rujni i listopadu kada je termoklina na najvećoj dubini. Ukupno je utvrđeno 34 vrste koralja za vrijeme istraživanja na Kampanelu. Koralji koji prevladavaju ukupnom biomasom su gorgonije *Paramuricea clavata*, *Corallium rubrum* i *Eunicella cavolini*, kameni koralji *Leptopsammia pruvoti, Caryophyllia inornata, Caryophyllia smithii* i *Madracis pharensis*, zadružne moruzgve *Parazoanthus axinellae* i *Savalia savaglia*, te meki koralji *Alcyonium acaule* i *Alcyonium coralloides*. Populacije crvene gorgonije *Paramuricea clavata* su najgušće na okomitim zidovima Kampanela. Upravljanje ovakvim područjima je veliki problem, koji još nije u potpunosti riješen u Jadranskom moru. Ipak, dubina na kojoj se nalazi Kampanel dobar je čimbenik zaštite od negativnog utjecaja ribarenja i ilegalnog ronjenja zbog teškog lociranja samog položaja Kampanela. Kočarenje bi trebalo biti u potpunosti zabranjeno u blizini koraligenske biocenoze kako bi se izbjegla šteta na koraligenu i smanjio negativan utjecaj od povišene sedimentacije. Ovaj rad prikazuje preliminarne rezultate istraživanja Kampanela i doprinosi poznavanju dubokog koraligena u istočnom dijelu Jadranskog mora.

Ključne riječi: fauna koralja, pučinski greben, PP Telašćica, Jadransko more

CORAL FAUNA OF THE CORALLIGENE COMMUNITY ON KAMPANEL BANK (NATURE PARK TELAŠĆICA)

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In this study we characterized coral assemblages of an off-shore coralligene bank (“steeple”) dwelling at 28-80 m depth near southern part of Island of Dugi otok (Nature Park Telašćica). Surveys of this bank were made by scuba diving and ROV-imaging. Flowing currents dominate at depths where the coralligenous develops. Water temperature at 40 meters depth ranges between 12 and 17°C for most of the year, but high peaks of 24°C are detected during August, September and October, when the thermocline is at its deepest. The total of 34 anthozoans were found during the survey on the bank Kampanel. Anthozoan species contributing the most to the total biomass are gorgonians *Paramuricea clavata*, *Corallium rubrum* and *Eunicella*

cavolini, scleractinians *Leptopsammia pruvoti*, *Caryophyllia inornata*, *Caryophyllia smithii* and *Madracis pharensis*, cluster anemones *Parazoanthus axinellae* and *Savalia savaglia*, followed by soft corals *Alcyonium acaule* and *Alcyonium coralloides*. Populations of *P. clavata* are the most abundant in steep rocky walls. The management of these areas is big issue, still lacking of an effective solution in the Adriatic Sea. However, depth acts as a protective factor decreasing the effects of fishing, given the difficulty inherent in locating from the surface the coralligene bottoms isolated from the coast. Trawling must be totally forbidden in areas with coralligene outcrops and their vicinity, to avoid not only the physical damage of trawling over the coralligene but also the indirect effects due to increased turbidity and high sedimentation rates. This report represents a preliminary data of the whole study and contributes to the knowledge of deep coralligene banks in the eastern Adriatic Sea.

Keywords: coral fauna, off-shore coralligene bank, Nature Park Telašćica, Adriatic Sea

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OBALNE LAGUNE – USPOREDBA MORINJSKOG ZALJEVA I UVALE MAKIRINA

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Obalne lagune su plitka, kompleksna staništa definirana fluktuacijama u salinitetu i temperaturi. Prema Direktivi o staništima, prioritetno su stanište na razini Europske Unije što upućuje na njihovu rijetkost i ugroženost. U proljeće 2018. godine provedena su inicijalna istraživanja dvije obalne lagune u okolici Šibenika - Morinjski zaljev i uvala Makirina, s ciljem kartiranja staništa i utvrđivanja ekološkog stanja. Oba područja su pod utjecajem slatke vode te su uskim kanalom povezana s okolnim morem. Kartiranje je izvršeno vizualnim pregledom terena i pomoću orto-foto snimki, a ekološko stanje je procijenjeno analizom klorofila *a,* zajednice fitoplanktona i sastava riblje zajednice. Iako se prema geološkoj podlozi i samom staništu radi o sličnim sustavima, provedena istraživanja pokazuju razlike u ekološkom stanju uspoređivanih lokacija, te su potrebna daljnja istraživanja kako bi se ono detaljnije utvrdilo.

Ključne riječi: obalne lagune, Natura 2000, kartiranje staništa, ekološki status

COASTAL LAGOONS – A COMPARISON OF MORINJE BAY AND MAKIRINA INLET

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Coastal lagoons are shallow, complex habitats defined by varying salinity and sea water temperature. According to the Habitats Directive, they are a habitat of priority conservation importance for the whole European Union, which indicates their level of endangerment. In the spring of 2018, an initial research of two coastal lagoons near Šibenik was conducted – Morinje bay and Makirina inlet, with the aim of habitat mapping and ecological status assessment. Both areas are under constant freshwater influence, connected only by a narrow channel to the surrounding sea. Habitat mapping was conducted by insitu area observation and orthophoto analysis, while the ecological status was assessed from chlorophyll *a*, phytoplankton and fish assemblage analysis. Even though both areas share similar geological features and support same habitats, conducted research indicates a difference in their ecological status. In order to gain a more detailed insight, further research is needed.

Keywords: coastal lagoon, Natura 2000, habitat mapping, ecological status

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MAKROBENTOSKA FLORA OTOKA I PLIČINE JABUKE – PRELIMINARNI REZULTATI

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Bentoska flora područja otoka Jabuke (srednji Jadran) rijetko je istraživana. Usprkos tome, uzimajući u obzir sastav, veliki broj svojti i veliku biomasu, poznata je po svojoj jedinstvenosti. Tijekom pedesetih godina 20. stoljeća, dredžanjem je na širem području Jabuke utvrđeno 268, prvenstveno duboko-morskih, svojti makroalgi. Naša istraživanja makrobentonske flore provedena su u rujnu 2016. i svibnju 2017. godine. Uzorci su sakupljeni autonomnim ronjenjem na dubinskim transektima jugozapadnog dijela otoka i pličine Jabuke, a protezali su se u smjeru SI - JZ do dubine od 35 m. Iz uzoraka sakupljenih u jesen odredili smo 240 svojti od kojih 202 uz otok Jabuku i 171 na pličini. Na pličini biomasom prevladavaju svojte rodova *Cystoseira* (četiri svojte) i *Sargassum* (dvije vrste), a iste nisu zabilježene uz otok Jabuku. Njihovo odsustvo pripisujemo utjecaju ribe salpe (*Sarpa salpa*) i ježincima koji uzrokuju potpuni golobrst između 1 i 10 m dubine. Od stranih vrsta zabilježene su *Caulerpa cylindracea*, tetrasporofit vrste *Asparagopsis armata* i *Womersleyella setacea*, koja je prevladavajuća epifitska vrsta u uzorcima sakupljenim na dubinama većim od 10 m. Ovo istraživanje, zajedno s povijesnim podacima, bi moglo biti osnova za vrednovanje područja kao jedinstvenog hot-spot mjesta biološke raznolikosti makroalgi u Jadranskom moru. Ekološka mreža Natura 2000 uključuje samo podmorje otoka Jabuke dok pličina nije uvrštena ni u jednu kategoriju zaštite.

Ključne riječi: makrobentoska flora, Jabuka, srednji Jadran, popis vrsta

MACROBENTHIC FLORA OF ISLET AND SHOAL OF JABUKA - PRELIMINARY RESEARCH

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The macrobenthic flora of Islet Jabuka (Middle Adriatic Sea) has rarely been investigated. Despite this, it is known for unique composition, a large number of species and high biomass. During the 50's of the 20th century, 268 mostly deeper macroalgae taxa were recorded by dredging along the wider area of Jabuka Islet. Our research of macrobenthic flora was carried out in September 2016 and May 2017. Samples were collected by scuba diving up to 35 m deep on SW part of the Islet and Shoal of Jabuka, along transects in NE-SW direction. From the samples collected in the fall, we identified 240 taxa, 202 of them on the Islet of Jabuka and 171 on the Shoal of Jabuka. On the Shoal, biomass was dominated by genera of *Cystoseira* (four taxa) and *Sargassum* (two species). Those taxa were not recorded on the Islet of Jabuka. We consider that the reason for their absence may be grazing of fish *Sarpa salpa* and sea urchins which cause barrens between 1 and 10 m deep. *Caulerpa cylindracea*, tetrasporophytes of Asparagopsis armata and *Womersleyella setacea*, were noted alien species. *W. setacea* was dominant epiphytic algae in the samples deeper than 10 m. Data from our investigation, together with historical information would serve to evaluate this area as a unique area and hotspot of biodiversity for macroalgae in the Adriatic Sea. Natura 2000 network consider only the Islet of Jabuka while the Shoal is not included in any category of protection.

Keywords: macrobenthic flora, Jabuka, Middle Adriatic Sea, checklist

Evolucija, sistematika, filogenija i biogeografija
Evolution, systematics, phylogeny and biogeography

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GENOMIKA I TAKSONOMIJA: DELIMITACIJA VRSTA KOD NEOTROPSKIH MRAVA GLJIVARA

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Mravi neotropskog roda *Sericomyrmex* u svojim mravinjacima uzgajaju gljive za hranu. Za razliku od mrava rezača, svojih dobro istraženih bliskih srodnika, biologija roda *Sericomyrmex* je nedovoljno poznata. Zbog homogene morfologije i nepoznatih evolucijskih odnosa granice Sericomyrmex vrsta su nejasne, takosonomski ključ je zastario, a o gljivi koju uzgajaju i o njihovoj biologiji općenito znamo jako malo. U ovoj studiji koristili smo nove filogenetske markere, Ultrakonzervirane elemente (UCEs) i tradicionalnu morfologiju kako bi odredili granice između vrsta roda *Sericomyrmex*. Uzorkovali smo *Sericomyrmex* mrave u južnoj i središnjoj Americi i sekvencirali u prosjeku 990 UCE lokusa po uzorku, za 93 uzoraka. Koristeći maximum likelihood i species tree metode na različitim setovima podataka rekonstruirali smo gotovo identične, dobro podržane filogenije. Integrirali smo te rezultate sa opsežnim istraživanjem morfologije svih vrsta na više od 1800 uzoraka, uključujući značajke i mjerenja radilica, matica, mužjaka i ličinki. Molekularni i morfološki rezultati ukazuju da se rod Sericomyrmex sastoji od manjeg broja vrsta nego što je ranije smatrano. Odredili smo jedanaest linija na razini vrste, opisujući i tri nove vrste. Naše analize datiranja nude moguće objašnjenje nižeg broja vrsta: procjena starosti crown grupe

roda *Sericomyrmex* je 4.3 milijuna godina, što ukazuje na recentnu, rapidnu radijaciju.

Ključne riječi: filogenomika, ultrakonzervirani elementi, *Sericomyrmex*, *Attina*, simbioza

GENOMICS AND TAXONOMY: SPECIES DELIMITATION IN THE NEOTROPICAL FUNGUS-FARMING ANTS

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Ants in the Neotropical genus *Sericomyrmex* cultivate fungi for food, and both ants and fungi are obligate, coevolved symbionts. Unlike leaf-cutter ants, their well-researched close relatives, not much is known about *Sericomyrmex* biology. Because of the homogeneous morphology and unknown evolutionary relationships the species limits in *Sericomyrmex* are vague, taxonomic key is outdated, and its fungal cultivar or other aspects of its natural history are poorly known. To address these issues we use novel phylogenomic markers, Ultraconserved Elements (UCEs), and traditional morphology, to infer species boundaries in *Sericomyrmex*. We sample *Sericomyrmex* ants across South and Central America, and we sequence an average of ~990 UCEs loci per sample for 93 samples. Using maximum-likelihood and species-tree approaches, we recovered nearly identical, well supported phylogenies across different data sets. We integrated these results with comprehensive morphological studies of over 1800 specimens, including characters and measurements of workers, queens, males, and larvae. Both molecular and morphological data show that *Sericomyrmex* consists of a lesser number of species than previously described. We identify 11 species-level lineages in *Sericomyrmex,* describing three new species. Our divergence-dating analyses offer possible explanation for low species number: we recovered 4.3 million years as the crown-group age estimates for *Sericomyrmex*, indicating a recent, rapid radiation.

Keywords: phylogenomics, Ultraconserved elements, *Sericomyrmex*, symbiosis

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GENOMSKA DIVERGENCIJA BRZO EVOLUIRAJUĆIH POPULACIJA PRIMORSKE GUŠTERICE

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Razumijevanje mikroevolucijskih procesa kao osnove brze adaptivne evolucije te načina na koji selekcija modulira fenotipsku divergenciju jedni su od glavnih ciljeva evolucijske biologije. 1971. g

proveden je transplant eksperiment u kojem je pet parova primorske gušterice *Podarcis siculus* introducirano s otočića Pod Kopište na obližnji otočić Pod Mrčaru (Jadransko more). Kasnija istraživanja pokazala su da je tijekom samo 35 godina došlo do izraženih promjena u ekologiji i morfologiji introducirane populacije, vjerojatno uzrokovanih prijelazom na omnivorni način prehrane. No, još uvijek je nepoznata relativna uloga genetičkih promjena i fenotipske plastičnosti u toj brzoj fenotipskoj evoluciji. U tu svrhu, u našim istraživanjima kombiniramo procjenu nasljednosti u eksperimentima križanja i asocijacije genotip-okoliš-fenotip istražujući te dvije eksperimentalne, i još 18 nativnih populacija *P. siculus* i *P. melisellensis*. Ovdje predstavljamo rezultate genomske diferencijacije tih 20 populacija koje nastanjuju otoke duž okolišnog gradijenta u regiji. 374 jedinke genotipizirane su metodom genotipiziranja putem sekvenciranja (genotype-by-sequencing approach). Populacijske genomske analize omogućuju nam procjenu genomske divergencije na razini cijelog genoma, kao i specifičnih lokusa među populacijama duž ekološkog gradijenta, te kvantifikaciju varijacije genotipa uzrokovane geografskim i okolišnim varijablama.

Ključne riječi: brza evolucija, *Podarcis siculus*, evolucijska ekologija

GENOMIC DIVERGENCE OF RAPIDLY EVOLVING POPULATIONS OF ITALIAN WALL LIZARDS

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Understanding the microevolutionary processes underlying rapid adaptive evolution and the way selection shapes phenotypic divergence in natural populations remains one of the major goals of evolutionary biology. In a transplant experiment in 1971, five pairs of Italian wall lizards *Podarcis siculus* from the islet of Pod Kopište were introduced on the nearby islet Pod Mrčaru (Adriatic Sea). Follow up study on these populations has revealed that in only 35 years distinctive changes in ecology and morphology have occurred in the transplanted population, probably linked to dietary change towards omnivory. However, the relative the role of genetic changes vs. plasticity in this rapid phenotypic evolution remains unknown. To address this question, we will combine estimates of heritability from crossing experiments with genotype-environment-phenotype associations using two experimental and 18 native populations of *P. siculus* and *P. melisellensis*. Here, we present the results on genomic differentiation among these 20 populations inhabiting islands along environmental gradient in the region. We genotyped 374 individuals using a genotype-by-sequencing approach. Population genomic analyses allows us to estimate genome-wide and loci-specific genomic divergence among populations distributed along the ecological gradient and to quantify the amount of variation in genotype that is explained by geographic and environmental variables.

Keywords: rapid evolution, *Podarcis siculus*, evolutionary ecology

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MORFOLOGIJA PELUDI NEKIH ALPSKO-DINARSKIH SVOJTI RODA *Crocus* L. - TAKSONOMSKI ZNAČAJ

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Cilj ovog rada bio je istražiti morfološke značajke peludi roda *Crocus* L. i njihovo potencijalno taksonomsko i evolucijsko značenje. U tu svrhu odabrano je nekoliko taksonomski zanimljivih alpsko-dinarskih skupina ovog roda. Njihov pelud istraživan je skenirajućim elektronskim mikroskopom, prethodno pripremljen prema standardnoj palinološkoj proceduri (Halbritter 1998). Terminologija i opisi peludi u skladu s Hesse i sur. (2009). Rezultati su pokazali da su peludna zrnca ispitivanih svojti roda *Crocus* spiraperturatne monade, s ehinatnom i perforatnom ornamentacijom. Pelud je sferoidan i velik (51-100 µm) te s različitim i promjenjivim brojem apertura. Suhi pelud je ovalan s udubljenim aperturama. Može se razlučiti nekoliko različitih tipova peludi, taksonomski razgraničenih do specifičnih taksonomskih kategorija. Nadalje, neke posebne palinološke značajke upućuju na moguće hibridno podrijetlo pojedinih svojti. Palinološka obilježja roda *Crocus* koja bi mogla imati taksonomsku važnost na različitim sistematskim razinama su: oblik suhe peludi; oblik, obris i aproksimativna veličina hidratizirane peludi, ornamentacija membrane apertura i eksine. Predložit će se i ukratko raspraviti o mogućem taksonomskom i evolucijskom značenju morfologije peludi roda *Crocus*.

Ključne riječi: SEM, por. *Iridaceae*, evolucijski značaj morfologije peludi

POLLEN MORPHOLOGY OF SOME ALPINE-DINARIC TAXA OF THE GENUS *Crocus* L. - TAXONOMICAL IMPLICATIONS

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The objective of this study was to research pollen morphological features of the Genus *Crocus* L. and their possible taxonomical and evolutionary significance. For that purpose several taxonomically interesting Alpine-Dinaric groups of the genus *Crocus* were selected. Their pollen grains were studied by scanning electron microscopy, prepared according to standard

palynological procedure (Halbritter 1998). Pollen terminology and descriptions follow Hesse et al. (2009). Results showed that pollen grains of the researched taxa of the genus *Crocus* are spiraperturate monads, in general with echinate and perforate exine ornamentation. All investigated pollen grains are spheroidal, large (51-100 µm), and with different and variable number of apertures. Generally, dry pollen grains are circular with sunken apertures. However, several different pollen types were recognized and taxonomically delimited to the specific taxonomic sublevels. Furthermore, some peculiar palynological features suggested possible hybridogenous origin of some taxa. Palynological features of the genus *Crocus* which could have taxonomical importance on different classification levels are: shape of dry pollen grains; shape, outline and approximate size of hydrated pollen grains, ornamentation of the aperture membrane and ornamentation of the exine. Possible taxonomic and evolutionary implications of pollen morphology of the genus *Crocus* will be suggested and briefly discussed.

Keywords: SEM, fam. *Iridaceae*, evolutionary significance of pollen morphology

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POVEZANOST I ADAPTACIJSKI POTENCIJAL RIBARSTVENIH POPULACIJA HOBOTNICE I KOZICE U MEDITERANU

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U današnjem vremenu brzih globalnih promjena, određivanje povezanosti i adaptivnog potencijala izlovljavanih morskih vrsta ključan je čimbenik za održivo ribarstvo. Iako korištenje genomičkih alata nije posebice raširenu u ribarstvenim znanostima, evolucijski koncepti koji proizlaze iz njihove upotrebe mogu pružiti prijeko potrebne informacije o strukturi ribljih fondova i njihovoj mogućnosti adaptacije. U ovom istraživanju ispitali smo integraciju znanja temeljenog na evolucijskoj biologiji u zaštiti i održivom upravljanju dvije ribolovne vrste: obične hobotnice *Octopus vulgaris* i crvene kozice *Aristeus anntenatus*. U tu svrhu, uzorkovano je 19 populacija hobotnice i 12 populacija crvene kozice sa područja cijelog Mediterana. Populacije su genotipizirane sekvenciranjem (eng. genotyping by sequencing, GBS) DNA nove generacije, čime je za svaku vrstu i populaciju određen veliki broj polimorfnih genetskih markera. U svrhu određivanja populacijske strukture, F-statistikom je procijenjena genetička diferencijacija između populacija. Povezanost istraživanih populacija potvrđena je analizom obrazaca genomske

varijacije u prostornim razmjerima. Podaci su testirani na evoluciju uz selekciju kako bi istražili pojavu lokalne adaptacije. Ovdje predstavljamo visoko-rezolucijski uvid u strukturu, povezanost i lokalnu adaptaciju hobotnice i crvene kozice u Mediteranu, te ukazujemo na područja koja je neophodno zaštititi u svrhu održivog upravljanja mediteranskim ribolovnim populacijama.

Ključne riječi: hobotnica, kozica, genomika, povezanost, adaptacija

CONNECTIVITY AND ADAPTATION CAPACITY OF EXPLOITED OCTOPUS AND SHRIMP POPULATIONS ACROSS THE MEDITERRANEAN

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In this era of fast global change, defining connectivity and adaptive potential of exploited marine stocks is a key requirement towards sustainability of fisheries. Though the use of genetics tools is not very pervasive in fisheries management, evolutionary concepts that derive from its use provide the much needed information regarding stock structure and its adaptation capacity. In our study, we examine the integration of evolutionary-based knowledge in fisheries sustainable management and conservation of two target species: common octopus, *Octopus vulgaris* and red shrimp *Aristeus antennatus*. To that end, we sampled 19 octopus and 12 red shrimp populations across the Mediterranean. Populations were genotyped using genotyping by sequencing (GBS) approach and a number of polymorphic genetic markers (SNPs) was validated for each species and population. In order to assess stock structure, genetic differentiation among populations was estimated with F-statistics and patterns of genomic variation across spatial scales were obtained, providing evidence of connectivity. To investigate occurrences of local adaptation, the data set was tested for evolution under selection. Here we provide high resolution perspective on stock structure, connectivity and local adaptation of octopus and red shrimp in the Mediterranean and indicate fishery areas that are critical to preserve, contributing directly to the sustainable management of Mediterranean fishery populations.

Keywords: octopus, shrimp, genomics, connectivity, adaptation

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FILOGENETIČKA ANALIZA FITOPLAZMATSKIH DNAB, DNAG I SSB PROTEINA UPUĆUJE NA

NJIHOVO SLOŽENO EVOLUCIJSKO PODRIJETLO

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Rod '*Candidatus* Phytoplasma' obuhvaća unutarstanične bakterije bez stanične stijenke koje žive unutar biljnog floema i u stanicama kukaca koji su ujedno i njihovi prirodni vektori. Taksonomski, fitoplazme pripadaju razredu Mollicutes zajedno s aholeplazmama, mikoplazmama i srodnim bakterijama, a za razliku od spomenutih bakterija, nije ih moguće uzgajati u čistoj kulturi in vitro. Jedna od glavnih karakteristika njihovih malih i reduciranih genoma jest postojanje potencijalnih mobilnih jedinica (potential mobile units; PMUs), genetičkih elemenata nalik transpozonima za koje se smatra da imaju ulogu u prilagodbi domaćinu. Replisomski geni, dnaB, dnaG i ssb, često se nalaze na takvim dijelovima genoma. U ovom radu izvršili smo opsežnu filogenetičku analizu DnaB, DnaG i SSB proteinskih sekvenci unutar članova razreda Mollicutes. Rezultati upućuju na složeno evolucijsko podrijetlo fitoplazmatskih replisomskih proteina s potencijalnih mobilnih jedinica. Analize sekvenci proteina DnaB pokazale su jasnu podjelu na dva klastera: DnaB1 i DnaB2, s dodatnim razdvajanjem PMU i ne-PMU sekvenci unutar DnaB1 klastera. Grupiranje sekvenci podrijetlom s PMU u zaseban klaster potvrđeno je i kod proteina DnaG i SSB. Dobiveni rezultati ukazuju na razdvojenu evoluciju PMU i ne-PMU replisomskih sekvenci, te česte rekombinacije i miješanje PMU gena između različitih fitoplazmatskih vrsta, što ovim bakterijama omogućava bolju prilagodbu i opstanak u različitim životnim uvjetima.

Ključne riječi: evolucija, filogenija, fitoplazme, potencijalne mobilne jedinice, replisom

PHYTOPLASMA DNAB, DNAG AND SSB REPLISOME PROTEINS PHYLOGENY REVEALS THEIR COMPLEX EVOLUTIONARY HISTORY

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Genus '*Candidatus* Phytoplasma' encompasses uncultivated endocellular bacteria without cell wall that reside within plant phloem and insect cells. In nature, these pathogens are transmitted between plants by phloem-feeding insects. Taxonomically, they belong to the class Mollicutes together with acholeplasmas, mycoplasmas and related bacteria. One of the main characteristics of small and reduced phytoplasma genomes is the existence of potential mobile units (PMUs), transposone-like elements which are suggested to contribute to the ability of these pathogens to colonize wider range of hosts. Replisome genes, dnaB, dnaG and ssb are frequently found on PMUs and we suggest their possible role in independent PMU replication. Herein, the extensive phylogenetic analysis of DnaB, DnaG and SSB replisome PMU sequences within Mollicutes class was performed. Our results point to a complex evolutionary history of phytoplasma PMU replisome proteins. DnaB sequence analysis revealed division into DnaB1 and DnaB2 clusters with additional clustering of PMU and non-PMU sequences within DnaB1. Separate grouping of the PMU sequences was also confirmed for DnaG and SSB sequences. These results reveal independent evolution of non-PMU and PMU replisome sequences and frequent intermixing and recombination of PMU genes among different phytoplasma species enabling their better adaptation to different enviroment.

Keywords: evolution, phylogeny, phytoplasma, potential mobile unit, replisome

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APPLICATION OF BONE ARCHITECTURE IN TAXONOMIC DISTINCTION BETWEEN GUDGEON SPECIES FROM R. MACEDONIA

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Osteological characters are considered as important diagnostic traits for inferring phylogenetic relationships within Cyprinidae. In this study for the first time is provided a detail description of the osteological features of four gudgeon taxa from R. Macedonia. Based on the results, *os kinethmoideum*, *os mesethmoideum*, *os orbitosphenoideum*, *os basioccipitale*, *os pterosphenoideum*, *os parasphenoideum*, *os maxilla*, *os ceratohyale* posterior are some of the skeletal elements that provide morphological character states which enable taxonomic distinction of *Gobio ohridanus*, *Gobio balcanicus*, *Romanogobio stankoi* and *Romanogobio banarescui*. The detail analyses of the bone architecture, crystallized new character states useful as additional osteological differences between *Gobio* and *Romanogobio*.

Keywords: *Gobio*, *Romanogobio*, skull osteology, taxonomy

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SVIJETLA TICALA: ISTRAŽIVANJE FUNKCIONALNE MORFOLOGIJE POMOĆU DRUŠTVENIH MREŽA

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Živimo u tehnološki naprednom dobu, u kojem su na društvenim mrežama dostupne ogromne količine podataka koje se mogu koristiti i u znanstvenim istraživanjima. Svijetla boja ticala uočena je kod kukaca iz redova Orthoptera, Hemiptera, Neuroptera, Coleoptera, Hymenoptera, Lepidoptera i Mantodea. Kako bi utvrdili geografsku rasprostranjenost, filogenetske odnose te funkcionalnu morfologiju svijetlih ticala kod kukaca, koristili smo društvene mreže (iNaturalist, Facebook Entomology Group i Flickr) kao primarni izvor podataka. Testirali smo postoje li morfološke i ekološke značajke koje su povezane s pojavnosti svijetlih ticala. Odabrali smo ukupno 76 vrsta kukaca iz navedenih redova, sa i bez svijetlih ticala. Proveli smo kladističke analize sličnosti i udaljenosti vrsta prema morfološkim (78) i ekološkim (12) značajkama u programu Mesquite i analizirali SEM fotografije ticala. Permutacijskim testom i analizom glavnih komponenti dobivene su značajne razlike u obliku ticala (1% značajnosti), te svojstvima povezanim s vizualnom komunikacijom i ritualima parenja. Svijetla boja je opažena na ticalima različitih oblika. Na ticalima skakavaca je strukturno, a kod leptira je pigmentno obojenje. Vrste sa svijetlim ticalima nastanjuju širi areal pa time vjerojatno zauzimaju više različitih ekoloških niša. Pretraživanje društvenih mreža pokazalo se metodološki pogodnim u provođenju in silico istraživanja funkcije mofoloških značajki kukaca na velikom geografskom području.

Ključne riječi: geografska rasprostranjenst, morfologija, ekologija, kladistika, kukci, pretražni elektronski mikroskop - SEM

PALE-COLORED ANTENNAE: INVESTIGATING FUNCTIONAL MORPHOLOGY USING SOCIAL MEDIA

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Tremendous amounts of unsystematically categorized data on insects can be found online, especially in photos. Pale anttene have been observed in insects within several orders: grasshoppers, true bugs, lace-winged insects, beetles, butterflies and moths, and praying mantids. To test whether there are morphological and/or ecological features correlated with the presence of pale-colored antennae, we decided to use data from online social media (iNaturalist, Facebook Entomology Group, i Flickr) as the primary material for our research. We selected 76 species with and without pale-colored antennae from the seven insect orders and provided cladistic analyses of species similarity and distance according to morphological (78) and ecological (18) characters in Mesquite, SEM and permutation tests on character states' variability between two control groups. Permutation test and PCA analysis showed significant difference between pale and black antennae morphology (1% significance level), and between traits related to visual communication and courtship. Pale-colored antennae resulted from the structural morphology (grasshoppers) and by the presence of pale pigments (butterflies). Pale coloration was found on antennae with different structures. Species with pale antennae inhabit a larger array of habitats and ecological niches.Oonline social media proved to be suitable for large data collection and can be a powerful tool in conducting large scale scientific research.

Keywords: geographic range, morphology, ecology, insects, cladistics, scanning electron microscope

O-96

INSIGHTS FROM GENOTYPE-ENVIRONMENT ASSOCIATION AND OUTLIER METHODS TO THE EXPLORATION OF LOCAL ADAPTATION OF PEDUNCULATE OAK (*Quercus robur* L.) POPULATIONS NEAR THE SPECIES' SOUTHERN RANGE MARGIN

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We here investigate genetic variation of *Quercus robur* L. along water availability gradient in 19 populations (n = 380 trees) residing near the species southern range margin in Croatia. We explored variation at 160 SNPs from candidate genes including 58 related with drought stress and 20 for bud phenology, and combined FST-based outlier tests with genotype-environment association (GEA) tests between SNP allele variation and local environmental factors with the aim of detecting potential drivers of genetic divergence. Overall genetic differentiation among populations was very low (FST ~ 0.013). We identified 11 significant outlier SNPs across methods,

which had a divergence on average nine times stronger than the rest of the markers, while 38 SNPs (of which six outliers) showed significant associations with at least one environmental variable, resulting in a total of 65 significant associations. Single-locus and multilocus GEA tests showed that SNP variation is most frequently and/or strongly related with precipitation seasonality and water availability related variables, suggesting that these are the main factors putatively involved in local adaptation of these marginal populations. Our results provide possible benefits for developing genetically informed guidelines for a climate-change integrated conservation and management of *Q. robur* forests exposed to ongoing increasing drought stress.

Keywords: Genotype-Environment Association, SNP, local adaptation, *Quercus robur*

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VARIJABILNOST ABCB1 GENA U ROMSKOJ POPULACIJE HRVATSKE

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Glikoprotein P (P-gp) velik je transmembranski prijenosnik raznih lijekova: kemoterapeutika, antibiotika, imunosupresanata, itd. Ovaj visoko polimorfan pripadnik proteinske obiteljirezistentne na više lijekova produkt je ABCB1 gena. Među često istraživane polimorfne varijante u kodirajućoj regiji gena spadaju rs1128503 i rs1045642, kao i rs3213619 na 5`-UTR kraju. U ovom su istraživanju određeni kod 440 osoba, pripadnika triju socio-kulturno različitih i geografski udaljenih skupina Roma. Učestalost rjeđeg alela (MAF) lokusa rs1128503 je bila najviša kod Balkanskih Roma (69,4%), u odnosu na skupine iz Baranje (62,5%) i Međimurja (54,5%) (p<0,001). MAF idućeg polimorfnog lokusa u protein kodirajućoj regiji, rs1045642, također je bila značajno veća u skupini Balkanskih Roma (63,6%), nego li kod Baranjskih (52,3%) i Međimurskih Roma (48,8%) (p=0,0005). rs3213619 nije bio polimorfan u Međimurskoj skupini, a učestalosti rjeđeg alela kod Balkanskih Roma i kod Baranjskih Roma su bile vrlo niske (1,5% tj. 1,9%). Analizirani polimorfni lokusi potvrđuju genetsku različitost triju testiranih skupina Roma koja bi mogla biti rezultat reproduktivne izolacije. Nadalje, činjenica da se učestalosti rjeđih alela lokusa rs1128503 i rs1045642 kod Roma razlikuju od onih u populacijama projekta 1000 Genomes upućuje na genetsku specifičnost ove populacije, što treba uzeti u obzir prilikom odabira i doziranja farmakoterapije.

Ključne riječi: ABCB1, rs1128503, rs1045642, farmakogenetika, Romi

VARIABILTY IN ABCB1 GENE IN THE ROMA POPULATION FROM CROATIA

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A large transmembrane P-glycoprotein (P-gp) has an important role in the bioavailability of various drugs: chemotherapeutic drugs, antibiotics, immunosupressants, etc. It is a product of ABCB1 gene, a highly polymorphic member of multidrug resistance protein family. Some of the most common polymorphisms in the protein coding region are rs1128503 and rs1045642, and rs3213619 at 5`-UTR end, which were analyzed in this study in 440 subjects who belong to three socio-culturally different and geographically distant Roma (Gypsy) groups. Minor allele frequency (MAF) of rs1128503 was the highest in the Balkan Roma (69.4%) when compared to the Baranja (62.5%) and the Međimurje Roma (54.5%) (p<0.001). The MAF of the other polymorphic locus in

the protein coding region, rs1045642, was also significantly higher in the Balkan group (63.6%) than in the Baranja (52.3%) and the Međimurje group (48.8%) (p=0.0005). rs3213619 was not polymorphic in the Međimurje group, but the MAFs in the Balkan (1.5%) and in the Baranja Roma (1.9%) were very low. The analyzed polymorphic loci confirm genetic distinctiveness of three tested Roma groups, which may be the result of reproductive isolation. Furthermore, the fact that Romani MAFs of rs1128503 and rs1045642 differ from 1000 Genomes populations indicates this population's genetic specificity, which should be taken into account in modulating pharmacotherapy.

Keywords: ABCB1, rs1128503, rs1045642, pharmacogenetics, Romani (Gypsy)

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DISTRIBUCIJA TRI NOVE VRSTE RODA *Phoxinus* ZA HRVATSKU

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Unatoč velikom povećanju primjene molekularno - filogenetskih istraživanja u kombinaciji s tradicionalnom taksonomijom i sistematikom, inter- i intraspecifični odnosi unutar roda Phoxinus i dalje nisu dovoljno poznati. Rod *Phoxinus* je široko distribuiran u cijeloj palearktičkoj regiji, a do nedavno se smatralo da samo dvije vrste roda *Phoxinus* nastanjuju Hrvatsku (*Phoxinus phoxinus* i *Phoxinus lumaireul*). Genetske analize ukazuju na nekoliko novih vrsta na zapadnom Balkanu koje su dovele do nedavnog opisa dvije nove vrste u Hrvatskoj - *Phoxinus likai* (sustav rijeke Otuče), *Phoxinus karsticus* (Neretvanski slijev) te postojanja nove, neopisane vrste iz rijeke Krke. Nedavno je objavljena nova distribucija vrsta roda *Phoxinus* u Hrvatskoj i Bosni i Hercegovini, sugerirajući prisutnost vrste *Phoxinus marsilii* u Hrvatskoj, ali i upućujući na široku rasprostranjenost vrste *P. lumaireul*. U ovom se radu daju najnovije informacije o distribuciji vrsta roda *Phoxinus* u Hrvatskoj. Prikazuje se distribucija vrste *P. marsilii* na sjevernim obroncima planine Papuk (slijev rijeke Drave). Status *P. likai* ne može se potvrditi jer nema svježeg materijala iz tipskog lokaliteta i ta vrsta vjerojatno više nije prisutna na tom lokalitetu. „Krški pijor", *P. karsticus*, raširen je samo u sustavu rijeke Neretve u Hrvatskoj, dok je nova, neopisana vrste iz rijeke Krke vjerojatno endem u gornjim dijelovima rijeke Krke i njezinih pritoka.

Ključne riječi: distribucija, pijor, *Phoxinus*, Hrvatska

CURRENT DISTRIBUTION OF THREE NEW *Phoxinus* SPECIES IN CROATIA

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Despite the discernible increase in research integrating molecular phylogenetic approaches with traditional taxonomy and systematics, inter- and intraspecific relationships in the genus *Phoxinus* are still poorly known. The genus *Phoxinus* has a wide distribution range throughout the Palaearctic region and until recently it was thought that only two *Phoxinus* species inhabit Croatia (*Phoxinus phoxinus* and *Phoxinus lumaireul*). Genetic analyses suggest several new species in the Western Balkans which led to the recent description of two new species in Croatia – *Phoxinus likai* (Otuča river system), *Phoxinus karsticus* (Neretva drainage) and existence of new, unnamed species from Krka river. Recently, an update has been made on the distribution of *Phoxinus* in Croatia and Bosnia and Herzegovina, suggesting *Phoxinus marsilii* as new species for Croatia and wide distribution of *P. lumaireul*. Here we give an update on distribution of *Phoxinus* in Croatia. We also present the distribution of *P. marsilii* on northern slopes of Papuk mountain, Drava drainage. Status of *P. likai* can not be confirmed as there is no fresh material from type locality and it is likely that this species is no longer present at that locality. Karst minnow, *P. karsticus*, is distributed only in Neretva drainage system in Croatia, while new, unnamed species from Krka is endemic to upper parts of Krka river and its tributaries.

Keywords: new species, *Phoxinus*, distibution, Croatia

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PRVO NACIONALNO ISTRAŽIVANJE EPIGEJSKE RAZNOLIKOSTI MALACOSTRACA U SLATKIM I BOČATIM VODAMA HRVATSKE TEMELJENO NA DNA BARKODIRANJU

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Naše istraživanje na temelju DNA barkodiranja je rezultiralo s prvom pouzdanom procjenom raznolikosti i endemizma razreda Malacostraca u općenito ugroženim epigejskim slatkovodnim ekosustavima u Hrvatskoj. U istraživanju je analiziran materijal sakupljen na 196 lokaliteta koji uključuju izvore, potoke, rijeke i jezera širom Hrvatske. Ukupno je dobiveno 764 COI sekvenci od 47 morfoloških vrsta iz redova Amphipoda, Isopoda, Mysida i Decapoda. Sekvence su učitane u Barcode of Life Database (BOLD) kako bi im se dodijelili indentifikatori poznati kao Barcode Index Numbers (BIN-ovi). Prepoznali smo 80 BIN-ova od kojih je 58 bilo unikatno (nisu zabilježeni u BOLD sustavu). Amphipoda dominiraju u analiziranom materijali s obzirom na broj jedinki (404 sekvence u BOLD sustavu) i na broj rodova. Red Isopoda je zastupljen s 202 sekvence, red Decapoda sa 108 sekvenci i red Mysida s 50 sekvenci. Izvanredna genska varijabilnost je zabilježena u kompleksu vrsta *Gammarus fossarum*, s brojnim BIN-ovima endemičnim za Hrvatsku. Kompleksnu evolucijsku povijest *G. fossarum* Hrvatskoj povezuje se s razvojem središnjeg Paratetisa tijekom Neogena. Ovakva povijest diversifikacije je u prethodnim istraživanjim uočena kod kompleksa vrsta *Gammarus balcanicus* u Hrvatskoj. U ovom istraživanju smo zabilježili nove BIN-ove za *G. balcanicus*. Dobiveni podaci potvrđuju veliku skrivenu raznolikost faune epigejskih Malacostraca u Hrvatskoj i potrebu za detaljnijim istraživanjima ove

grupe organizama.

Ključne riječi: Amphipoda, Isopoda, Decapoda, Mysida

FIRST COUNTRY-WIDE SURVEY OF EPIGEAN MALACOSTRACAN DIVERSITY IN CROATIAN FRESH AND BRACKISH WATERS BASED ON DNA BARCODING

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We provide a first reliable assessment of malacostracan diversity and endemism in the generally endangered epigean freshwater ecosystems in Croatia based on the country-scale DNA barcoding survey. The survey included material from 196 localities in springs, streams, rivers and lakes all over Croatia. In total, 764 COI sequences for 47 morphospecies of Amphipoda, Isopoda, Mysida and Decapoda were successfully obtained during the study and submitted to Barcode of Life Database (BOLD) to receive the identifiers, known as Barcode Index Numbers (BINs). We have recognized 80 BINs out of which 58 are unique (not reported before). Amphipods dominated in the analysed material both in terms of number of individuals (404 sequences in BOLD) and the number of genera. Isopods are represented by 202 sequences, order Decapoda by 108 sequences, and order Mysida by 50 sequences. An outstanding genetic variability was detected in *Gammarus fossarum* species complex, showing numerous BINs endemic for Croatia. This result highlights a complex evolutionary history of *G. fossarum* in Croatia related to the development of the Central Paratethys basin in Neogene. This history of diversification is shared by *Gammarus balcanicus* species complex from the country, for which current study provided new BINs. The obtained data confirmed the high level of hidden diversity of epigean malacostracan fauna in Croatia. However, analyses revealed severe gaps in available genetic barcode data on some groups of Malacostraca.

Keywords: Amphipoda, Isopoda, Decapoda, Mysida

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FIZIOLOŠKE I MORFOLOŠKE PRILAGODBE DVA RODA DIJATOMEJA NA LIMITACIJU NUTRIJENTIMA

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Sjeverni Jadran je plitko područje pod jakim utjecajem dotoka rijeke Po. Karakteriziraju ga brojni prostorno-vremenski gradijenti (npr. temperatura, salinitet, nutrijenti). To rezultira izrazito

kompleksnom strukturom planktonskog ekosustava koja nam omogućuje praćenje prilagodbi planktonskih organizama duž navedenih gradijenata. U ovom istraživanju analizirali smo vrste roda *Leptocylindrus* i vrstu *Chaetoceros peruvianus*. Posebno smo se usredotočili na njihove fiziološke odgovore na limitaciju fosfatima. Analizirali smo njihovo ponašanje *in situ* te proveli eksperimentalna mjerenja *ex situ*. U ovom radu prikazati ćemo promjene brzine rasta pojedine vrste pri različitim koncentracijama nutrijenata, brzine unosa fosfata, podatke o aktivnosti, lokalizaciji i načinima aktivacije enzima alkalne fosfataze, te morfološke prilagodbe pojedine vrste na stres fosfatima. Uočene fiziološke promjene uključuju promjene dužine generacijskog vremena i različitu ekspresiju enzima alkalne fosfataze. Uz fiziološke, uočene su i morfološke promjene kao reakcija na stres fosfatima. Kod roda Leptocylindrus uočene su promjene u dužini stanica i lanaca u uvjetima smanjene koncentracije fosfata, dok su kod *C. peruvianus* uočene morfološke promjene koje nalikuju prijelazu između dva već opisana oblika te vrste (*C. peruvianus* i *C. peruvianus* var. *gracilis*). Naši rezultati upućuju na to da morfološke analize in situ uzoraka mogu biti dobar pokazatelj trenutnog stanja u okolišu kao što je limitacija fosfatom.

Ključne riječi: dijatomeje, *Chaetoceros*, *Leptocylindrus*, APA, limitacija fosfatom

PHYSIOLOGICAL AND MORPHOLOGICAL ADAPTATIONS TO NUTRIENT LIMITATION IN TWO DIATOM GENERA

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The northern Adriatic is a shallow basin highly influenced by the river Po as a major freshwater input and is characterized by a multitude of spatiotemporal gradients (e.g. temperature, salinity, nutrients). This results in a highly structured planktonic ecosystem that allows us to investigate the performance of planktonic species along those gradients. We analyzed the species from genus *Leptocylindrus* and *Chaetoceros peruvianus* with a special focus on their metabolic reaction towards phosphate limitation. We compared their performance *in situ* as well as performed phosphate limitation experiments *ex situ*. Here we report data on species specific growth rates under different nutrient regimes, phosphate uptake rates, alkaline phosphatase activity, localization and activation patterns and characteristics of alkaline phosphatase activity as well as on morphological reactions to phosphate stress. Physiological changes observed include changes in generation time, expression of alkaline phosphatase as well as morphological reactions to phosphate limitation. Species from the genus *Leptocylindrus* showed cell and chain elongation in phosphate limitation, while *C. peruvianus* showed morphological changes resemble the transition between two described variabilities, (*C. peruvianus* and *C. peruvianus* var. gracilis). Our results show that careful morphological analysis of in situ samples can be a good indicator for the detection of complex ecological circumstances like phosphate limitation.

Keywords: diatoms, *Chaetoceros*, *Leptocylindrus*, APA, phosphate limitation

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RAZVOJ TEHNIKA ZA EKSTRAKCIJU MAKROMOLEKULA IZ DREVNIH KOSTI I ZUBI

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Drevni i povijesni ostaci, poput kosti i zubi, od interesa su za različite grane arheoloških znanosti, uključujući istraživanja radioizotopa i genetička istraživanja. Ovakav multidisciplinarni pristup proučavanju arheoloških materijala može nam pomoći u razrješavanju povijesti populacija i drugih događaja u prošlosti. Međutim, proučavanje makromolekula ekstrahiranih iz takvih ostataka, prvenstveno DNA i proteina, predstavlja izazove koji nisu prisutni kod rada sa suvremenim biološkim materijalima. Drevne kosti i zubi sadrže vrlo male količine molekula od interesa, dok većina makromolekula potječe od mikroorganizama koji koloniziraju materijal nakon smrti, te od ljudi koji su došli u kontakt sa uzorcima tijekom ili nakon iskopavanja. Takvi ostaci su ujedno vrlo rijetki i izuzetno vrijedni, osobito uzorci drevnih hominida, što ograničava opseg molekularnih analiza koje se mogu provesti, budući da takve analize zahtijevaju destruktivno uzorkovanje koje može ugroziti njihov morfološki integritet. Kako je očuvanje arheološke i povijesne baštine izuzetno važno za buduće generacije, razvoj metoda za molekularnu analizu skeletnih ostataka, s naglaskom na maksimiziranju prikupljanja informativnih molekula bez potrebe za višestrukim destruktivnim uzorkovanjem, je ključni korak ka tom cilju, kao i ujedinjavanje molekularnih tehnika i istraživanja iz različitih područja arheologije u jedno.

Ključne riječi: drevna DNA, drevni proteini, arheološki urorci, kosti i zubi

DEVELOPING TECHNIQUES FOR THE RETRIEVAL OF MACROMOLECULES FROM ANCIENT BONES AND TEETH

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Ancient and historical remains such as bones and teeth are of great interest to different fields of archaeological research, including radioisotope studies and genetics. This multidisciplinary approach of studying archaeological material can help resolve population histories and past events. However, the analysis of macromolecules from ancient remains, primarily DNA and proteins, brings forth challenges that are not present when studying contemporary biological material. Ancient bones and teeth contain very low quantities of target molecules, while the vast majority of molecules originates from microorganisms that colonized the material after death, as well as humans that handled the specimens during or after excavation. Furthermore, such remains are often rare and extremely valuable, especially those of ancient hominins, thereby limiting the extent of molecular analyses that can be conducted, as they often require destructive sampling which could compromise the morphological integrity of the specimen. In order to preserve the archaeological and historical heritage for future generations, it is therefore crucial to develop methods for the molecular analysis of skeletal remains with a focus on maximizing the retrieval of informative molecules without the need for multiple destructive samplings, as well as to combine molecular techniques from different archaeological research fields into one.

Keywords: ancient DNA, ancient proteins, archaeological samples, bones and teeth

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ŠTO SVE OTKRIVA OBLIK TIJELA? PRIMJENA GEOMETRIJSKE MORFOMETRIJE ZA UTVRĐIVANJE INTRASPECIJSKE RAZNOLIKOSTI DVIJU ENDEMSKIH VRSTA RODA *Carabus* (COLEOPTERA: CARABIDAE) DUŽ DINARIDA

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Razvojem tehnika geometrijske morfometrije proučavanje morfologije uzdiglo se na novu razinu, a dobiveni podaci pokazali su se korisnima i kod rješavanja taksonomskih odnosa na razini podvrsta. Zbog toga smo, po prvi puta, analizirali oblik pronotuma, glave i edeagusa dviju sestrinskih vrsta roda *Carabus* – *C. croaticus* Dejean 1826 i *C. caelatus* Fabricius 1801, uzorkovanima na većem dijelu njihovog areala duž Dinarida. Obje vrste obiluju fenotipskim varijantama koje su rezultat izoliranog planinskog areala koji nastanjuju. Analiza je obuhvatila 192 jedinke vrste *C. caelatus* i 223 jedinke vrste *C. croaticus*. Koristeći analizu glavnih komponenti (PCA) provjerili smo varijacije oblika glave, pronotuma i edeagusa na cijelom skupu podataka. Zbog velikog broja populacija koristili smo srednje vrijednosti za svaku populaciju kod kanonske varijacijske analize (CVA) i PCA u MorphoJ-u. Geometrijska morfometrija pokazala je dobro interspecijsko razdvajanje na temelju oblika pronotuma, glave i edeagusa. Na intraspecijskoj razini, GM je rezultirala brojnim preklapanjima populacija sa susjednih planinskih lanaca upućujući na postojanje djelomičnog protoka gena između geografski bliskih populacija.
Dobiveni podaci morfoloških analiza, uz primjenu molekularnih analiza, mogu dati uvid u evolucijske procese specijacije ovih endemskih vrsta i pomoći kod utvrđivanja statusa nekih podvrsta.

Ključne riječi: geometrijska morfometrija, intraspecijska raznolikost, carabidae

WHAT HIDES THE BODY SHAPE? APPLICATION OF GEOMETRIC MORPHOMETRICS FOR REVEALING INTRASPECIFIC DIVERSITY WITHIN THE TWO ENDEMIC SPECIES FROM THE GENUS *Carabus* (COLEOPTERA: CARABIDAE) ALONG DINARIC ALPS

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With the development of geometric morphometrics (GM), the study of morphology has grown to a new level, and the obtained data have proved to be useful in solving taxonomic relationships on the intraspecific level. Thus, for the first time, we decided to analyse the shape of pronotum, head and edeagus of the two sister species belonging to the genus *Carabus*, from the major part of their distributional range along Dinaric Alps – *C. croaticus* Dejean 1826, and *C. caelatus* Fabricius 1801. Both species abound in phenotypic variants as a result of more or less isolated mountain habitats. For GM analyses, a 2D data set consisting of images of 192 *C. caelatus*, and 223 *C. croaticus* specimens were used. Principal components analyses (PCA) were performed to analyse shapes variations in the entire dataset. Due to a great number of population, average values for each one were used for PCA, and canonical variate analysis (CVA) in MorphoJ.

Geometric morphometrics showed good interspecific delimitation based on the shape of pronotum, the shape of head, and the shape of edeagus. At intraspecific level, GM showed many overlaps within the population of both species indicating the existence of partial gene flow between some geographically closed populations. Obtained data from GM analyses, combined with molecular analyses, may provide insight into evolutionary processes of speciation of these endemic species, and help to confirm the status of some subspecies.

Keywords: geometric morphometrics, intraspecific diversity, carabidae

Genetika, stanična i molekularna biologija
Genetics, cell and molecular biology

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GENOME-WIDE DIVERGENCE AS A CONSEQUENCE OF SYMPATRIC SPECIATION OF *Rhagoletis cerasi* HOST PLANT ECOTYPES

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Phytophagous insects with differentiated host-plant ecotypes, which represent early stages of the speciation continuum are excellent systems for the study of sympatric speciation. An important criterion for host-plant ecotype formation is being able to prove that the two ecotypes do not interbreed randomly. This is readily shown through genomic differentiation between host-plant ecotypes that do exchange gene flow. Some extensively studied examples of host plant mediated evolutionary shifts include the apple and hawthorn ecotypes of the apple maggot fly, *Rhagoletis pomonella*, and the striped vs. unstriped walking stick insect, *Timema christinae*, residing on two different host plants. The European cherry fruit fly, *Rhagoletis cerasi*, is suitable for sympatric speciation research because it has adapted to two drastically different host plant families, *Prunus* spp. (Rosaceae) and *Lonicera* spp. (Caprifoliaceae). We used a ddRADSeq approach to obtain thousands of genome-wide single nucleotide polymorphisms (SNPs) spread throughout fly's genome. In total, 185 SNPs were identified as Fst outliers under host-plant related diversifying selection in multiple sympatric sites. Fst outlier BLAST search inquiries resulted in excess *R. zephyria* predicted mRNA sequences. We show repeated diversification in four different Prunus-Lonicera sympatric sites. Although host-plant ecotypes of *R. cerasi* resemble progress along the speciation continuum, populations still cluster more by geography.

Keywords: *Rhagoletis cerasi*, host-plant ecotypes, sympatric speciation, radseq, outlier-loci

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HRVATSKO NAZIVLJE MOLEKULARNE I STANIČNE BIOLOGIJE

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Hrvatski standardni jezik prolazi kroz razdoblja širenja i rasta na temelju potrebe proizašle iz društvenih promjena, tehnološkoga razvoja i globalizacije. U području strukovnoga nazivlja ta je potreba posebno izražena, no prati je i česta pojava zaobilaženja potrebnih preduvjeta i stvaranja vlastitih termina koji unutar zatvorenih profesionalnih zajednica često prerastaju u žargonizme. Razvoj i usustavljivanje hrvatskoga leksika molekularne i stanične biologije, s posebnim naglaskom na segmentu genetike, značajna je perspektiva očuvanja hrvatskoga jezika, razvoja znanstvene komunikacije i obrazovanja budućih naraštaja stručnjaka ovoga područja. Na Zavodu za molekularnu biologiju Biološkoga odsjeka Prirodoslovno-matematičkoga fakulteta Sveučilišta u Zagrebu započeta su zbog toga dva projekta vezana uz strukovno nazivlje. Njihov je cilj na jednome mjestu okupiti najvažnije pojmove i sustavno razrađeno hrvatsko nazivlje molekularne i stanične biologije. Na temelju višegodišnje suradnje jezikoslovaca i biologa ovim izlaganjem želimo pružiti uvid u nekoliko osnovnih zaključaka koje stručnjaci i znanstvenici mogu primijeniti u svome svakodnevnom radu bez obzira na područje biologije koje je u njihovu fokusu. Poseban naglasak izlaganja stavljen je na učestalo, i vrlo rasprostranjeno, pogrešno korištenje simboličkih imena DNA i RNA kao i pogrešno korištenje pridjeva „genski“, „genetski“, „genomski“ i „genetički“.

Ključne riječi: genetika, molekularna i stanična biologija, hrvatsko nazivlje

CROATIAN VOCABULARY OF MOLECULAR AND CELL BIOLOGY

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Standard Croatian vocabulary passes through periods of expansion and growth based on the needs arising from social change, technological development and globalization. In the field of scientific terminology, this need is particularly pronounced, but it is also accompanied by the frequent negligence of the necessary terminological prerequisites and the creation of colloquial terms within the closed professional communities, which often result in development of jargon. The development and systematization of the Croatian vocabulary of molecular and cell biology, with a particular emphasis on the genetics, is a significant part of the preservation of Croatian language, the development of scientific communication and education of future generations of experts in this field. For this reason, two projects related to

professional terminology were conducted at the Division of Molecular Biology, Faculty of Science, University of Zagreb. Their goal is bringing together the key terms and a systematically elaborate Croatian vocabulary of molecular and cell biology. Based on the few years' experience and cooperation of linguists and biologists, we would like to give an overview of several guidelines that scientists can apply in their everyday work, regardless of the biology field, which is their specialty. Special emphasis is placed on the frequent and widespread misuse of symbolic names of DNA and RNA as well as misuse of adjectives„genski", „genetski", „genomski", and „genetički".

Keywords: genetics, molecular and cell biology, Croatian vocabulary

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SATELITNE SEKVENCE DNA U CENTROMERNIM KROMOSOMSKIM PODRUČJIMA

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Satelitne DNA su nekodirajuće sekvence DNA čiji su dugi nizovi uzastopno ponovljenih monomera uglavnom smješteni u heterokromatinskim odjeljcima eukariotskih kromosoma. One predstavljaju uobičajenu genomsku sekvencu u pericentromernim kromosomskim područjima, a također se nalaze i u funkcionalnoj centromeri. Svaki genom sadrži puno satelitnih DNA, koje se razlikuju po nukleotidnoj sekvenci, broju kopija i kromosomskoj lokalizaciji. Prema modelu biblioteke, brzi nastanak vrsno specifičnih razlika u sadržaju satelitnih DNA prvenstveno je posljedica promjena broja kopija u biblioteci satelitnih DNA, a ne promjena u samoj nukleotidnoj sekvenci. Ova osobitost dinamike sekvenci satelitnih DNA može predstavljati prednost u održavanju DNA-proteinskih interakcija u centromerama. Kako ne postoji određena centromerna DNA, najizrazitiji vrsno-specifični biljeg centromerne funkcije je varijanta histona H3, protein CenH3, čiji je N-terminalni kraj u asocijaciji sa sekvencom DNA. CenH3 i centromerna sekvenca DNA stoga moraju biti u suglasju te evolucija jedne komponente dovodi do evolucije druge. Nakupljanje razlika u DNA i proteinskim komponentama između skupina organizama može uzrokovati poteškoće u sparivanju kromosoma kod hibrida, dovesti do reproduktivne izolacije te u konačnici do specijacije. (Peri)centromerna područja su u smislu poznavanja genoma najslabije istražena, jer su zbog poteškoća u sekvenciranju i preklapanju dugih nizova ponovljenih sekvenci DNA podzastupljena u rezultatima genomskih projekata. Napredak u sekvenciranju metodom NGS i u analizi sekvenci DNA otvorili su mogućnosti proučavanja satelitoma, cjelokupnog genomskog kompleta satelitnih DNA, što zajedno s metodom imunoprecipitacije omogućuje detaljno proučavanje sekvenci DNA u centromernom području.

Ključne riječi: satelitne DNA, centromera, kromosomi

SATELLITE DNA SEQUENCES IN CENTROMERIC CHROMOSOMAL REGIONS

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Satellite DNAs are non-coding DNA sequences that build long arrays of tandem repeats usually located in heterochromatic compartments of eukaryotic chromosomes. They represent preferred genome component in pericentromeric regions, spanning also the functional

centromere. Each genome harbors many satellite DNAs which differ in sequence, copy number and chromosomal localization. According to the library model, species-specific differences in satellite DNA composition evolve rapidly as a consequence of copy number alterations within a library of satellite DNAs, rather than as a consequence of changes in nucleotide sequences. This feature of satellite DNA sequence dynamics might be advantageous in maintaining DNA-protein interactions in centromeres. Since there is no particular centromeric DNA, the most prominent species-specific marker of centromere function is histone H3 protein variant CenH3, N-terminal part of which is associated with the DNA sequence. As a consequence, CenH3 and centromeric DNA sequences must remain in accord, and they therefore drive each other's evolution. Accumulation of differences in DNA/protein components among groups of individuals can cause incompatibilities in chromosome pairing in hybrids, trigger reproductive isolation and ultimately speciation. From the genomic point of view, (peri)centromeres are the least known regions, underrepresented in outputs of every genome project because of difficulties in sequencing and assembly of long arrays of repetitive DNAs. Advances in NGS sequencing and in analysis of repetitive sequences opened possibility to study the satellitome, the whole-genome complement of satellite DNAs, and together with the immunoprecipitation method to focus onto detailed composition of DNA sequences in the centromere.

Keywords: satellite DNA, centromere, chromosomes

Komparativna fiziologija, imunobiologija i biologija čovjeka
Comparative physiology, immunobiology and human biology

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ISTRAŽI, POVEŽI I PRIMIJENI-BIOLOGIJA ČOVJEKA U KONCEPTUALNOJ MAPI

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Istraži, poveži i primijeni je projekt nastao kao rezultat dvogodišnje eksperimentalne realizacije nastave biologije u 8. razredu. Navedena je nastava bila koncentrirana u jednom obrazovnom razdoblju s cjelogodišnjom satnicom. Cilj projekta bio je kontinuirano uključiti učenike u nastavni proces s idejom da gradivo koje obuhvaća anatomiju i fiziologiju čovjeka usvoje trajno i kvalitetno. Rješavajući redom problemske zadatke, od starta do cilja, svaki je učenik samostalno izradio vlastiti razvojni plakat koji je po završetku rada prezentirao. Sljedeća generacija osmaša učila je na isti način, izrađujući plakate u digitalnom obliku u formi konceptualne mape, koristeći web alat Prezi. Digitalna verzija omogućila je korištenje mape i na nastavi drugih predmeta čime je ostvaren interdisciplinarni pristup u nastavi. Projekt je omogućio učenicima neprestano povezivanje prethodnih sadržaja (o sustavima organa) te podizanje razine znanja od usvajanja sadržaja prema konceptualnom razumijevanju. Aktivnim učenjem omogućeno je učenicima trajno usvajanje znanja, kreativnost u radu, povezivanje ideja, suradničko učenje te učenje procesa samovrednovanja. Provedena evaluacija i istraživanja školskoga psihologa pokazala su povećanje učeničke motivacije i porast korištenja različitih strategija učenja.

Ključne riječi: biologija čovjeka, konceptualna mapa, razvojni plakat, interdisciplinarna nastava, strategije učenja

EXPLORE, CONNECT AND APPLY – HUMAN BIOLOGY IN A CONCEPTUAL MAP

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Explore, connect and apply is a project which emerged from an experimental, two-year implementation of biology classes in 8th grade. The mentioned classes were concentrated in an educational period with the all year school timetable. The aim of the project was to include the students into the teaching process, with the idea that they master the matter, consisting of human anatomy and physiology, in a permanent and quality manner. By successively solving the problem tasks from start to finish, every student has created a development poster on her/his own, which was then presented upon the work completion. The next generation of eight-graders acquire knowledge in the same way, creating digital posters in form of conceptual maps, using the Prezi tool. The digital version enabled the use of the map in other classes, thus accomplishing the interdisciplinary approach. This project enabled the students to combine the previous contents (about organ systems) and it also raised the knowledge level from acquiring the contents to conceptual understanding. Through active learning, students are provided with a long-term knowledge acquisition, creativity in work, linking ideas, cooperative learning and learning the self-evaluation process. The evaluation and studies, conducted by the school psychologist, have shown an increase of student's motivation and the rise of using various learning strategies.

Keywords: human biology, conceptual map, development poster, interdisciplinary school classes, learning strategies

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"OSOBNOSTI NA RAZINI SKUPINE" U OBIČNIH MARMOZETA: VAŽNOST DRUŠTVENOG OKRUŽENJA KOD INDIVIDUALNIH VARIJACIJA U KOGNICIJI

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Osobnosti u životinja, definirane kao stalne inter-individualne razlike u ponašanju tijekom vremena i konteksta, nedavno su pronađene kod običnih marmozeta, koristeći kombinaciju bihevioralnih testova i opažanja. Štoviše, pokazalo se da je stalna bihevioralna varijacija u istraživanju i smjelosti-sramežljivosti manja među pojedincima iste skupine u usporedbi s pojedincima različitih skupina. Marmozeti su poznati po svojim izvanrednim sposobnostima učenja, no čini se da među pojedincima postoje značajne inter-individualne razlike u tim sposobnostima. Varijacije u učenju dosad nisu bile proučavane u smislu određenih osi osobnosti, tj. niti jedna studija do danas nije proučavala utječe li i kako inter-individualna varijacija u istraživanju i smjelosti-sramežljivosti na sposobnost samostalnog učenja kod marmozeta. Testirali smo zato 28 običnih marmozeta u standardnim testovima osobnosti (opća aktivnost, nova hrana, novi objekt, hranjenje pod rizikom, predator) te smo im dali niz kognitivnih zadataka podijeljenih na jednostavne motorne zadatke te zadatke raspoznavanja.

Ovdje ćemo izvijestiti: i) koji čimbenici predviđaju stupanj sličnosti u osobnostima na razini skupine i ii) utječu li sličnosti u osobnostima na razini skupine na vjerojatnost samostalnog učenja (te kasniji prijenos tih novostečenih informacija unutar grupe). Razmotrit ćemo pojam "osobnosti na razini skupine" i procijeniti relativnu važnost društvenog okruženja na individualne varijacije u obradi informacija.

Ključne riječi: osobnosti, osobnosti na razini skupine, ponašanje životinja, kognitivni testovi, obični marmozeti

"GROUP PERSONALITY" IN COMMON MARMOSETS: IMPORTANCE OF THE SOCIAL ENVIRONMENT ON INDIVIDUAL VARIATION IN COGNITION

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Animal personality, defined as consistent inter-individual differences in behaviours across time and contexts, has recently been found in common marmosets, using a set of personality tests and observations. Interestingly, consistent behavioural variation in exploration and boldness-shyness is smaller between individuals of the same group as compared to the variation among individuals of different groups. Marmosets are apt learners and they have also been reported to display substantial inter-individual variation in these tasks. However, consistency and direction of such variation has never been studied in terms of certain axes of marmoset personality, i.e. no study to date asked whether and how inter-individual variation in exploration and boldness-shyness affects marmosets' propensity for individual learning. Thus, we tested 28 common marmosets in standard personality tests (general activity, novel food, novel object, foraging under risk, predator) and a series of cognitive tasks, divided in simple motor tasks and discrimination learning tasks. Here, we will report i) which factors predict the degree of group-level similarity in personality and ii) whether the degree of such within-group similarity affects the likelihood of individually mediated learning (and subsequent social information transmission in the group). We will discuss the concept of "group personality" and evaluate the relative importance of social environment on individual variation in information processing.

Keywords: animal personality, group personality, animal behaviour, cognitive tests, common marmosets

Konzervacijska biologija, zaštita prirode i okoliša
Conservational biology, nature and environment protection

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PROMATRANJE SREDOZEMNE MEDVJEDICE U ŠPILJAMA / STANIŠTIMA PUTEM KAMERA 2009-2017

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Sredozemna medvjedica (*Monachus monachus*) ugroženi je morski sisavac. Do sada smo istražili putem kamera pojedine špilje sjevernog Jadrana (Istra, otok Cres) za koje smo sigurni da su aktivna staništa vrste. Kamera je podešena da se aktivira na pokret, i bežično prosljeđuje informacije sa slikama u bazu podataka. Promatranje sredozemne medvjedice putem kamera omogučilo nam je brzo prikupljanje bioloških uzoraka, izmeta, sline iz nosa i dlake. Uzorci su prikupljeni nakon odlaska jedinki iz staništa, te su u roku od 48 sati poslani na analizu. Studija je provedena na ograničenom broju uzoraka (48) u razdoblju 2010. – 2014. godine. Uzorci izmeta su sadržavali riblje kosti, ljuske i jajašca parazita *Diphylolobotrium latum*, dok je bakteriološka obrada negativna. Virusološka obrada sluzi iz nosa je negativna, a bakteriološkom obradom smo dokazali prisutnost bakterije *Pseudomonas aeurigonosa*. Forenzička obrada dlake na teške metale je negativna. Promatranje špilja/aktivnih staništa putem kamera omogučava bolje upoznavanje vrste, ponašanja, te pravovremeno prikupljanje uzoraka.

Ključne riječi: sredozemna medvjedica, biološki uzorci, kamera

MONITORING OF THE MEDITERRANEAN MONK SEAL IN CAVES / HABITATS VIA CAMERA FROM 2009 TO 2017

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The Mediterranean Monk Seal (*Monachus monachus*) is an endangered marine mammal species. So far, we have explored specific caves in the northen Adriatic (Istria, Cres island) via camera for which we are sure that are active habitats of the species. The camera has been set to activate by movement in the cave, and wirelessly transmits the information with the images to the database. The monitoring of the Mediterranean Monk Seal via cameras enabled us to quickly collect biological samples, feces, nose mucus and hair. The samples were collected after departure of a species specimen from the habitat, and were sent for analysis within 48 hours. The study was conducted on a limited number of samples (48) in the period 2010 - 2014. Feces samples contained fish bones, shells and parasites eggs *Diphylolobotrium latum*, while bacteriological treatment was negative. Viral analysis of nose mucus was negative and bacteriological analysis has demonstrated the presence of *Pseudomonas aeurigonosa* bacteria. Forensic hair treatment on heavy metals is negative. Monitoring caves/active habitats via cameras enables better understanding of the species, their behaviour, and promptly collection of samples.

Keywords: Monk Seal, biological samples, camera

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EKOLOŠKA KARTA GRADA ZAGREBA - OD IDEJE DO REALIZACIJE

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Zdrav okoliš temeljna je pretpostavka za očuvanjem zdravlja ljudi i kvalitete života. Sam učinak čimbenika okoliša na zdravlje ovisan je o prostoru i vremenu njihova djelovanja, intenzitetu izloženosti, međusobnim interakcijama, kao i o ciljanoj populaciji na koju djeluje. Ideja izrade ekološke karte realizirana je 2017. godine te je uspostavljena web GIS aplikacija „Ekološka karta Grada Zagreba“. Ekološka karta predstavlja interaktivan operativni alat s inovativnim pristupom u kojem su podaci prikazani na jednostavan vizualan način, lako razumljiv svim građanima grada Zagreba. Integrirani pristup prezentacije podataka okolišnog monitoringa u realnom vremenu omogućuje praćenje promjena stanja okoliša u definiranom vremenskom intervalu. Stvorena je podloga za učinkovito upravljanje, planiranje i unaprjeđenje životne i radne sredine, praćenje utjecaja okolišnih čimbenika na zdravlje s ciljem povećanja povjerenja građana te transparentnost i dostupnost podataka. Osim navedenog, u slučaju incidentnog događaja dostupno je „nulto stanje” onečišćenja čime se omogućuje promptna procjena utjecaja na okoliš i smjernice za mjere postupanja i sanaciju. Projekt je realiziran zahvaljujući suradnji Nastavnog zavoda za javno zdravstvo „Dr. Andrija Štampar“, Agronomskog fakulteta Sveučilišta u Zagrebu, Instituta za medicinska istraživanja i medicinu rada, Državnog hidrometeorološkog zavoda te nadležnih gradskih ureda Grada Zagreba.

Ključne riječi: ekološka karta, Zagreb, web GIS aplikacija

ECOLOGICAL MAP OF THE CITY OF ZAGREB - FROM IDEA TO REALIZATION

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A healthy environment is a fundamental premise for the preservation of human health and the quality of life. The effect of environmental factors on health depends on the space and time of their activity, the intensity of exposure, interactions, as well as the targeted population. The idea of creating environmental map was implemented in 2017 and the same year a web GIS application "Ecological map of the City of Zagreb" was created. The ecological map represents an interactive operating tool with an innovative approach in which the data is presented in a simple visual way, easily understood to all citizens of Zagreb. An integrated approach to the presentation of data monitoring system in real time enables the monitoring of environment changes in a defined time interval. This provides creating a basis for effective management, planning and the improvement of living and working environment, monitoring of environmental factors impact on health to increase citizens' trust and transparency and availability of data. In addition, in case of accidental events zero state of pollution is available allowing prompt environmental impact assessment and guidelines for treatment and recovery measures. The project was implemented thanks to the cooperation of the Andrija Stampar Teaching Institute of Public Health, Faculty of Agriculture University of Zagreb, Institute for Medical Research and Occupational Health, Meteorological and hydrological institute of Croatia and the City of Zagreb.

Keywords: ecological map, Zagreb, web GIS application

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IZVJEŠTAVANJE KAO OBVEZA SUKLADNO EU DIREKTIVAMA ZA OČUVANJE PRIRODE - SAMO PODACI ILI POTICAJ ZA ZNANSTVENI RAD?

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Hrvatska, kao zemlja članica EU, mora ispuniti obveze proizašle iz Direktive o staništima (DS, 92/43/EC) i Direktive o pticama (2009/147/EU). Jedna od obveza je i praćenje stanja (monitoring) stanja očuvanosti vrsta i stanišnih tipova koji se nalaze na popisu priloga DS te svih vrsta ptica koje prirodno obitavaju u Hrvatskoj. Stanje očuvanosti je varijabla koju definira zbroj utjecaja koji utječu na rasprostranjenost, strukturu i funkciju, te dugoročni opstanak tipičnih vrsta pojedinog stanišnog tipa, te na rasprostranjenost i brojnost vrsta. Svaka država članica obvezna je svakih šest godina pripremiti opsežno izvješće, tj. ocjenu stanja očuvanosti vrsta i stanišnih tipova, a koje treba biti rezultat odgovarajućeg monitoringa. Hrvatska će takvo izvješće po prvi puta pripremiti 2019. godine. U izlaganju ćemo predstaviti proces uspostave nacionalnog sustava monitoringa vrsta i stanišnih tipova. Predstaviti ćemo izazove i mogućnosti za nadogradnju monitoringa iz „samo još jedne“ EU obveze u jedinstvenu priliku za razvitak učinkovitog alata za očuvanje prirode Hrvatske. Završno, prezentirajući rezultate provedbe shema monitoringa iz drugih država pokazati ćemo kako prikupljanje podataka o vrstama i stanišnim tipovima znanstvenim metodama može biti istovremeno i ispunjavanje zakonskih obveza države i znanstveno istraživanje u svrhu poboljšanja razumijevanja promjena u populacijama te utjecaja koji te promjene uzrokuju.

Ključne riječi: monitoring, vrste, staništa, ptice

REPORTING AS AN OBLIGATION ARISING FROM THE EU NATURE CONSERVATION DIRECTIVES – ONLY DATA OR OPPORTUNITY FOR SCIENTIFIC WORK?

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Croatia, as an EU member, has to meet obligations arising from the Habitats Directive (HD, 92/43/EC) and the Birds Directive (2009/147/EU). One of obligations is the surveillance (monitoring) of the conservation status of the habitats and species listed on the Annexes of the HD and all naturally occurring bird species. Conservation status is defined as a variable reflecting the sum of influences acting on species or habitat that may affect its distribution, structure and functions as well as the survival of its typical species (as regards habitat types) and abundance of species populations. Every six years Member States are obliged to prepare a comprehensive report, i.e. assessment of the species and habitats conservation status that should be result of appropriate habitat types/species monitoring. Croatia will prepare such report for the first time in 2019. In this talk we will present the process of establishment of the national species and habitats monitoring system. We will show the challenges and opportunities of upgrading of the monitoring as „just another“ EU duty into unique opportunity for development of the strong tool for the nature conservation. Finally, by presenting policy driven long lasting monitoring schemes from other countries we will present how gathering scientifically sound species and habitats data can be used for policy purposes as well as for researches aimed to improve our understanding of species population changes and their driving forces.

Keywords: monitoring, species, habitats, birds

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ANALIZA POSTOJEĆE PROSTORNE ZAŠTITE U HRVATSKOJ U KONTEKSTU OČUVANJA SLATKOVODNIH RIBA

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Slatkovodne ribe jedna su od najugroženijih skupina kralješnjaka. Kopnene vode Hrvatske nastanjuje oko 140 svojti riba, među kojima više od 50 svojti ima status endemske vrste. Zakonskim okvirom zaštite prirode omogućeno je njihovo očuvanje kroz mehanizme stroge zaštite vrste i prostorne zaštite područja u smislu zaštićenih područja i područja ekološke mreže Natura 2000. Kategorije zaštićenih područja nacionalni park, park prirode, regionalni park, posebni rezervat i značajni krajobraz obuhvaćaju oko 46% slatkovodnih staništa u Hrvatskoj. 50 vrsta koštunjača i 4 vrste kružnousta nalaze se na referentnoj listi Hrvatske te su za njih izdvojena područja očuvanja, ukupno 67. Rad analizira prostorni razmještaj zaštićenih područja i područja ekološke mreže u odnosu na podatke o distribuciji slatkovodnih riba. Osim područja ekološke mreže u kojima je neka riblja vrsta ciljna za očuvanje razmatrana su ona u kojima provedba mjera za očuvanje ciljnih staništa ili vrsta direktno doprinosi očuvanju staništa riba u povoljnom stanju. Analiza je tako obuhvatila područja u kojima su neki od slatkovodnih stanišnih tipova ili jedna od dvije vrsta dekapodnih rakova s referentne liste ili školjkaš obična lisanka ciljni za očuvanje. Uz prostorne podatke o distribuciji i bogatstvu vrsta analizom su obuhvaćeni podaci o endemizmu i ugroženosti riba. Rezultati ove analize dati će uvid u doprinos prostorne zaštite očuvanju slatkovodne ihtiofaune u Hrvatskoj te će istaknuti potencijalne nedostatke.

Ključne riječi:
ugrožene ribe, očuvanje, zaštićena područja, ekološka mreža, Natura 2000

ANALYSIS OF EXISTING SPATIAL PROTECTION IN CROATIA IN THE CONTEXT OF FRESHWATER FISH CONSERVATION

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Freshwater fishes are one of the most threatened groups of vertebrates. Inland waters of Croatia are inhabited by approximately 140 fish species of which more than 50 are endemic. In Croatia legal mechanisms for their conservation are strict protection of species, various categories of protected areas and Natura 2000 ecological network. Protected areas designated in the categories of national park, nature park, regional park, special reserve and significant landscape include about 46% of freshwater habitats in Croatia. As 50 teleosts species and 4 lampreys are included on the Croatian referent list, in total 67 areas of conservation have been designated for them. This paper analyses the spatial distribution of protected areas and Natura 2000 sites in relation to available data about range of freshwater fish. In this analyses were

considered sites in which fish are target species and sites in which implementation of conservation measures directly contributes to the conservation of habitats important for fish in a favourable state. That also included sites designated for some freshwater habitat types, crayfish species and thick shelled river mussel. In addition to spatial data on distribution and richness of fish species, the analysis included data on endemism and level of threat. The results of this analysis will provide an insight into the contribution of spatial conservation to the conservation of freshwater ichthyofauna in Croatia and point out potential gaps.

Keywords: threatened fish, conservation, protected areas, ecological network, Natura 2000

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BIHEVIORALNI ASPEKTI DVIJE KOMPETITIVNE VRSTE *Podarcis*: PRIMORSKA I KRŠKA GUŠTERICA

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U Hrvatskoj je zabilježeno 55 međunarodno važnih skloništa za šišmiše predano Areali dvije vrste lacertida: *Podarcis siculus* i *Podarcis melisellensis* se ponekad preklapaju. U slučaju kada se to dogodi dominatniji kompetitor *P. siculus* nadjača slabijeg *P. melisellensis*. U svrhu istraživanja bihevioralne razlike koje mogu potencijalno pomoći *P. siculus* da nadjača *P. melisellensis*, testirali smo 28 jedinki svake vrste, oba spola, u open field i radial maze testu. Ovim eksperimentima smo testirali stopu habituacije u svakoj vrsti kako bi odredili koliko brzo i kako se mogu prilagoditi novim situacijama. Oba ekperimenta su trajala između 15 i 23 minute i ponovljeni su 3 puta kako bi se gušteri habituirali na novi okoliš. Parametri koje smo pratili su: zbroj udaljenosti, brzina, vrijeme provedeno u centralnoj zoni vs. vrijeme provedeno u perifernoj zoni, latencija izlaska iz skloništa, postotak vremena proveden na mjestu i dizanje na stražnje noge. Preliminarni rezultati pokazuju da postoji značajna razlika u gotovo svim parametrima koje smo testirali. Generalno, *P. siculus* se kretao više i bio je oprezniji. U isto vrijeme, adaptirao se puno brže od *P. melisellensis*, što mu omogućuje da brže iskoristi nove situacije i okoliš.

Ključne riječi: interspecijska kompeticija, open field, ponašanje životinja, *Podarcis*, radial maze

BEHAVIOURAL ASPECTS OF TWO COMPETITIVE *Podarcis* SPECIES: ITALIAN AND DALMATIAN WALL LIZARD

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In nature, the areals of two lacertid species *Podarcis siculus* and *Podarcis melisellensis* sometimes

overlap. When that happens the more dominant competitor *P. siculus* overpowers *P. melisellensis*. In order to investigate if some behavioural differences potentially help *P. siculus* to overpower *P. melisellensis*, we tested 28 individuals of each species, both sexes, in open field test and radial maze. We used these experiments to test the rate of habituation in each species to assess how fast they can adapt and how they react to a new situation in general. Both experiments lasted 15 to 23 minutes and were repeated 3 times in order to habituate the lizards to the new environment. The parameters we tracked were cumulative distance, velocity, time spent in central zone vs. time spent in peripheral zone, latency time of exiting tube, percent of time spent immobile and lifting on hind legs. Preliminary results show that there is significant difference in almost all groups of parameters we tested. In general, *P. siculus* moved more and was more cautious. At the same time, it adapted a lot faster than *P. melisellensis*, thereby allowing it to exploit new situations and environments faster.

Keywords: animal behaviour, interspecies competition, open field, *Podarcis*, radial maze

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MORFOMETRIJSKA ANALIZA I GENETSKA KARAKTERIZAICJA POPULACIJA MEDITERANSKE DAGNJE *Mytilus galloprovincialis* UZDUŽ HRVATSKE OBALE JADRANA

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Dagnje *Mytilus* spp. pripadaju skupini ključnih vrsta u morskim obalnim ekosustavima. Ovaj rad je pokazao dominantnu prisutnost mediteranske dagnje *Mytilus galloprovincialis* Lmk. 1819 na istočnoj obali Jadrana, od Limskog zaljeva do Dubrovnika. Morfološke varijacije mjerenja ljuštura bile su unutar normalnih vrijednosti za vrstu *M. galloprovincials* (omjer v/d 55,89 ± 5,08%) s nekoliko morfotipova određenih analizom kontura. Međutim, molekularna analiza dala je novi uvid u genetsku raznolikost jadranskih dagnji *M. galloprovincialis*. Prethodnom analizom jezgrinog biljega Me 15/16, koji može razlikovati dagnje *Mytilus edulis* kompleksa (*M. edulis*-EE, *M. trossulus*-TT i *M. galloprovincialis*-GG), među dagnjama s 22 lokacije identificirali smo samo 2 od 110 uzoraka kao heterozigote GE i GT genotipova, dok su svi ostali identificirani kao homozigoti GG. AFLP analizom našeg novog jezgrinog biljega gena prekolagen-D (preCol-D) utvrdili smo da je većina prevladavajućih veličina preCol-D fragmenta jadranske dagnje *M. galloprovincialis* 239/240 bp. Uspoređujući AFLP rezultate populacija dagnji Jadranskog mora *M. galloprovincialis* s dagnjama *Mytilus* spp. (GG, GE, EE, ET i TT) iz okruženja i šire (Sredozemlje, Atlantik, Baltik i južni Pacifik) moguće je razlikovati populacije dagnji *M. galloprovincialis* Jadransko-mediteranskog (239/240) i Atlantskog podrijetla (236/237 bp), kao i identificirati sve ostale vrste *M. edulis* kompleksa.

Ključne riječi: oblik ljušture, morfotipovi, jezgrini biljezi, Me15/16, preCol-D, AFLP

MORPHOMETRIC ANALYSES AND GENETIC CHARACTERIZATION OF MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* POPULATIONS ALONG THE CROATIAN ADRIATIC COAST

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Mussels *Mytilus* spp. belong to a group of key species in marine coastal ecosystems. This work demonstrated the dominant presence of Mediterranean mussel *Mytilus galloprovincialis* Lmk. 1819 along the eastern Adriatic coast, from Lim bay to Dubrovnik. Morphological variations based on mussel shell measurements were within normal values for *M. galloprovincials* (h/l ratio 55.89 ± 5.08%) with several morphotypes determined by outline analysis. However, molecular analyses gave new insight into the genetic diversity of Adriatic mussel *M. galloprovincialis*. Previously, with the application of nuclear marker Me 15/16 analysis that distinguishes mussels of *Mytilus edulis* complex species (*M. edulis*-EE, *M. trossulus*-TT and *M. galloprovincialis*-GG), among mussels from all 22 sites, only 2 of 110 specimens were identified as heterozygote GE and GT genotypes, while all others were identified as homozygote GG. The amplified fragment length polymorphism (AFLP) analysis using our new nuclear precollagen-D gene marker (preCol-D) showed that the most prevalent Adriatic *M. galloprovincialis* preCol–D fragment size is 239/240 bp. Comparing the AFLP results of Adriatic *M. galloprovincialis* populations with *Mytilus* spp. (GG, GE, EE, ET and TT) from other locations (Mediterranean, Atlantic, Baltic, southern Pacific) it was possible to distinguish Adriatic-Mediterranean (239/240) and Atlantic (236/237 bp) *M. galloprovincialis* mussel populations as well as to identify all other species of *M. edulis* complex.

Keywords: shell shape, morphotypes, nuclear markers, Me15/16, preCol-D, AFLP

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KOPNENA STANIŠTA OTOKA LOKRUMA – KOLIKE SU SE PROMJENE DOGODILE U POSLJEDNJIH 60 GODINA?

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Otok Lokrum nalazi se u blizini Dubrovnika te je uglavnom pokriven vazdazelenom šumom i makijom, a samo manji dio čine otvorena i kultivirana staništa te morska obala. Od 1976. godine zaštićen je kao posebni rezervat šumske vegetacije te od tada prirodna sukcesija mijenja sastav biljnih zajednica. Ovim istraživanjem cilj je bio usporediti vegetacijske podatke iz 1959. odnosno 1979. s recentnim podacima kako bi se utvrdile eventualne promjene u sastavu i zastupljenosti zajednica. Prilikom istraživanja 2016. godine na otoku je zabilježen 21 stanišni tip pri čemu prevladavaju šumska staništa vazdazelenih i mješovitih šuma i makija sa sedam stanišnih tipova i njihovih kombinacija. Usporedbom povijesnih i recentnih podataka vidljivo je da je sukcesija zahvatila cijeli otok. Površine pod klimazonalnom zajednicom *Fraxino orni-Quercetum ilicis* blago se povećavaju dok su garizi potpuno nestali odnosno postupno su prešli u makiju, a djelomično i u šumu. Odnos zajednica *Myrto-Quercetum ilicis* i *Querco ilicis-Pinetum halepensis* nastavio se mijenjati u korist zajednice *Myrto-Quercetum ilicis* jer se zbog izostanka požara, a time i mogućnosti pomlađivanja borova broj borova iz godine u godinu smanjuje.
Vrlo male površine preostalih travnjaka kojima je sastav znatno izmijenjen u odnosu na očekivani pod velikim su pritiskom ispaše kunića koji uzrokuju velike štete na vegetaciji.

Najmanje promjene dogodile su se u obalnoj vegetaciji te vegetaciji stijena pošto se radi o trajnom stadiju.

Ključne riječi: vegetacija, Braun-Blanquet, sukcesija

TERRESTRIAL HABITATS OF ISLAND LOKRUM - WHAT ARE THE CHANGES THAT OCCURRED IN THE LAST 60 YEARS?

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The Island Lokrum is located near Dubrovnik and is mainly covered with evergreen forests and macchia. Only a small part of it consists of open habitats and sea shore. Since 1976 it has been protected as a special reserve of forest vegetation and since then the natural succession has been changing the composition of the plant communities. The purpose of this research was to compare vegetational data from 1959 and 1979 with recent data to determine possible changes in the composition and representation of communities. During the research in 2016, 21 habitat types were found with seven dominant habitats of evergreen and mixed forests as well as macchia and their combinations. By comparing historical and recent data, it is apparent that the succession affected the entire island. Area of climazonal community *Fraxino orni-Quercetum ilicis* is slightly increasing and the garrigue has completely disappeared i.e. has gradually changed to macchia and partly to forest. The ratio between the communities *Myrto-Quercetum ilicis* and *Querco ilicis-Pinetum halepensis* continued to change in favour of the community *Myrto-Quercetum ilicis* due to continuous decrease in number of old pines and lack of fire needed for rejuvenation of pine forest. Small area of the remaining grasslands, whose composition is significantly changed, is heavily influenced by rabbits causing great damage. Almost no changes have occurred in coastal vegetation because it is more or less permanen stage of vegetation.

Keywords: vegetation, Braun-Blanquet, succession

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VAŽNOST BESKRALJEŽNJAKA ZA ZAŠTITU PRIRODE: SLUČAJ MRAVA

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Taksonomska pristranost je sveprisutna tendencija da se nekim skupinama organizama, posebice kralježnjacima i biljkama, pridaje više pažnje u istraživanjima i planiranju očuvanja. Posljedica toga je da najmanje znamo o gubitku bioraznolikosti i stopi izumiranja skupina s najvećom biomasom i raznolikošću, kao što su primjerice kukci. Nedostatak znanja o tim manje karizmatičnim skupinama nije lako rješiv u praksi očuvanja vrsta, npr. određivanjem surogat svojti – stanišni uvjeti jedne skupine često se ne preklapaju s uvjetima drugih skupina, što

pogotovo vrijedi za beskralježnjake. Kako bi se suočili s ovim problemom istražili smo koju ulogu mravi, jedna od zanemarenih skupina kukaca, mogu imati u očuvanju kukaca u Hrvatskoj. Revidirali smo faunu mrava Hrvatske na temelju terenskih istraživanja provedenih od 2009.-2016. godine i literaturnih podataka, te predložili vrste koje su dobar model za očuvanje kukaca. Važnost mrava za zdravlje ekosistema, te njihova dominantnost i brojnost u kopnenim staništima ih čini praktičnim za uzorkovanje i praćenje, a njihov brz odgovor na okolišne promjene u okolišu ih čini korisnim indikatorima klimatskih i drugih okolišnih promjena. Skupine mrava koje je potrebno zaštititi i posvetiti im daljnja istraživanja su: 1) endemske i rijetke vrste, 2) specijalisti hladnih klima na rubnim staništima, 3) vrste roda *Myrmica* čiji mravinjaci su obligatno stanište za druge zaštićene vrste, 4) šumski mravi roda *Formica*.

Ključne riječi: očuvanje kukaca, zadružni kukci, *Formica*, *Myrmica*

THE IMPORTANCE OF INVERTEBRATES IN CONSERVATION: THE CASE OF THE ANTS

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Taxonomical bias is an omnipresent tendency to favor some groups of organisms, mainly vertebrates and plants, over others in research programs and conservation planning. Consequently, we know the least about the biodiversity loss and extinction rates of the groups with the highest diversity and abundance, insects in particular. Our poor knowledge of less charismatic taxa is not easily solved in conservation practices, e.g., by designation of surrogate species – habitat requirements for one taxon frequently do not overlap with requirements of other taxa, and this is particularly true for invertebrates. To address this problem, we investigate what role could ants, one such neglected group of insects, have for conservation in Croatia. We review the ant fauna of Croatia based on the field work conducted from 2009-2016 and literature data, and we identify species that could serve as a model for insect conservation. The ecological importance of ants, their biomass and dominance in terrestrial habitats makes them practical for collecting and monitoring, and their fast response to environmental change makes them useful as indicators of climate change. We identify ant taxa that would benefit from conservation actions and further research in Croatia: 1) endemic and rare species, 2) cold climate specialists, 3) species from genus *Myrmica* which are hosts of other endangered species, 4) wood ants from genus *Formica*.

Keywords: insect conservation, social insects, *Formica*, *Myrmica*

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PRELIMINARNI REZULTATI TELEMETRIJSKOG PRAĆENJA GREGULE (*Puffinus yelkouan*) - PRVI KORAK PREMA MORSKIM POP-OVIMA U HRVATSKOJ

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U Hrvatskoj trenutno postoji 38 područja očuvanja značajnih za ptice (POP), od kojih tek maleni postotak uključuje morsku komponentu. koja je uvijek vezana uz kopenena područja kao što su gnijezdeće kolonije ptica. Jedno takvo područje je i POP Lastovsko otočje koje predstavlja jedno od dva područja na kojima gnijezdi gregula (*Puffinus yelkouan*). Prema dostupnim podacima, čak 80% nacionalne populacije gregule gnijezdi na Lastovskom otočju. Korištenje morskog prostora ove vrste prilikom odhranjivanja mladih u sezoni gniježđenja je do ovog istraživanja bilo potpuno nepoznato. Kako bi došli do podataka o kretanju gregule u sezoni gniježđenja i poduzeli prvi korak prema "ocrtavanju" granica prvih morskih POP-ova, proveli smo istraživanje na Lastovskom otočju 2016. i 2017. godine. Koristeći GPS telemetriju, prikupili smo po prvi puta za Hrvatsku podatke o kretanju gregula. Prikupljeni su podaci kretanja 16 jedinki pomoću kojih smo definirali važna područja koja koriste za hranjenje i odmaranje. Prikupljeni podaci jasno upućuju u činjenicu kako gregule u sezoni gniježđenja koriste puno šire područje za hranjenje i odmaranje od trenutnih granica POP-a Lastovsko otočje.

Ključne riječi: gregula, Lastovo, POP, telemetrija

TOWARDS FIRST MARINE IBAS IN CROATIA – PRELIMINARY RESULTS OF YELKOUAN SHEARWATER GPS TRACKING

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In Croatia there are currently 38 Important Bird Areas (IBAs) and only a small percentage of their total surface includes a marine component, which is always adjacent to terrestrial areas such as breeding colony sites. One such IBA is the Lastovo Archipelago, which represents one of only two nesting areas of the Yelkouan shearwater (*Puffinus yelkouan*) in Croatia. According to available data over 80% of the national population of Yelkouan shearwater breeds in this IBA. However their use of marine area for feeding and rafting were completely unknown up until this study. In order to fill this knowledge gap and take the first step towards the designation of the first marine IBAs and SPAs in Croatia, research was carried out by Biom staff in 2016 and 2017 on Lastovo archipelago. During the study, data on the distribution of the Yelkouan shearwaters on sea was collected using GPS telemetry. After preliminary surveys, tracks of 16 individual birds were collected and were used to pinpoint their main feeding and rafting areas. Collected data clearly indicate that breeding birds use a much broader area for feeding and rafting during their breeding season than the actual surface of the Lastovo Archipelago SPA and IBA.

Keywords: Yelkouan shearwater, Lastovo, IBA, SPA, telemetry

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UTJECAJ KLIMATSKIH PROMJENA NA BUDUĆU RASPROSTRANJENOST INVAZIVNIH RAKOVA U SLATKOVODNIM EKOSUSTAVIMA HRVATSKE

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Brojnost i veličina populacija autohtonih europskih vrsta slatkovodnih rakova su u stalnom opadanju. Uzrok tome su negativni pritisci na njihova staništa, klimatske promijene te prisutnost i širenje alohtonih invazivnih vrsta rakova. Invazivni rakovi istiskuju autohtone kroz kompeticiju za prostor i hranu te kroz prenošenje letalne bolesti račje kuge. U Hrvatskoj su prirodno rasprostranjene četiri autohtone vrste rakova koje su doživjele značajan pad u brojnosti između ostalog i zbog širenja tri invazivne vrste rakova. Kako bi se zaštitilo ugrožene autohtone rakove te kontroliralo/nadziralo brzo širenje dviju invazivnih vrsta (signalni i bodljobradi rak), cilj ovog rada je bio predvidjeti potencijalno pogodna područja (trenutna i buduća) rasprostranjenosti ove dvije vrste u kontinentalnom dijelu Hrvatske. U tu svrhu su razvijeni modeli distribucije vrsta (eng. SDM) pri čemu su korišteni podaci o zabilježenoj prisutnosti dviju invazivnih vrsta i set bioklimatskih prediktora odabranih na temelju njihove ekološke relevantnosti. Dobiveni modeli omogućuju evaluaciju potencijalnih trenutno pogodnih staništa, kao i onih koja će biti pogodna u budućnosti prema različitim scenarijima klimatskih promjena. Rezultati će olakšati prioritizaciju onih područja u kojima će negativni utjecaj invazivnih vrsta biti najizraženiji, a isto tako će poslužiti kao kvalitetna osnova za izradu adekvatnih konzervacijskih strategija i planova upravljanja s ugroženim autohtonim vrstama.

Ključne riječi: *Pacifastacus leniusculus*, *Orconectes limosus*, SDM

EFFECTS OF CLIMATE CHANGE ONTO FUTURE DISTRIBUTION OF INVASIVE CRAYFISH SPECIES IN FRESHWATERS OF CROATIA

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Indigenous European freshwater crayfishes (ICS) have undergone significant declines in populations' numbers and sizes across their ranges. Apart from being threatened by climate change and habitat loss and deterioration, ICS are endangered by non-indigenous crayfish species (NICS). NICS displace ICS through both competitive exclusion and transmission of diseases such as crayfish plague. In Croatia all 4 ICS species have undergone severe declines in population abundance and numbers, in part due to the spread of the 3 NICS species. In order to protect threatened ICS, and manage fast dispersal and invasion range expansion rates of the two NICS recorded in Croatia (the signal crayfish and the spiny- cheek crayfish), the aim of this study was to predict their potential current and future distribution in continental Croatia. We developed Species distribution models (SDMs) using presence only records of the two NICS species and a set of bioclimatic predictors selected based on their ecological relevance, excluding highly correlated ones. Resulting SDMs enabled us to evaluate the potential contemporary range of two NICS as well as to predict the impact of ongoing climate change onto their future distribution in Croatia. Obtained results will facilitate prioritisation of locations where NICS will probably establish and where their negative impacts on ICS will be most pronounced. Application of acquired results in the future ICS conservation and NICS management programs is discussed.

Keywords: *Pacifastacus leniusculus*, *Orconectes limosus*, SDM

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PRIRODOSLOVNA OSNOVA KRAJOBRAZA

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Iako se krajobrazom bave propisi iz područja zaštite prirode i prostornog uređenja, a javlja se i kao kategorija zaštićenog područja, odnosno kao prostorno planska kategorija zaštite, učinkovit i suvremeni sustav očuvanja krajobraza u Hrvatskoj još nije uspostavljen. Krajobraz, kao kompleksan prirodni fenomen i predmet posebne grane biologije – krajobrazne ekologije, područje je interesa agronomije, šumarstva i krajobrazne arhitekture te ostaje uglavnom izvan domene biološke znanosti. Rad razmatra važnost krajobraza za očuvanje biološke raznolikosti i predlaže izokretanje sadašnjeg pristupa izučavanju krajobraza u Hrvatskoj naglašavajući ulogu biologije i drugih prirodnih znanosti za njegovo istraživanje i vrednovanje. Također, krajobraz je moguće koristiti kao indikator očuvanja ekoloških funkcija nekog prostora. Izučavanje ekosistemskih usluga ima veliki potencijal za valorizaciju brojnih funkcija krajobraza te je u tom kontekstu važno razmatrati njegove estetske i kulturne vrijednosti.

Ključne riječi: krajobraz, krajobrazna ekologija, prirodne znanosti, biologija, bioraznolikost

NATURAL BASIS OF LANDSCAPE

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Although landscape is an element of the nature protection and physical planning legislation, and also presents a type of protected area and physical planning protection category, efficient and modern system of landscape protection has not been established yet in Croatia. Landscape, as a complex natural phenomenon and subject of a particular biological discipline – landscape ecology, is a research subject of agronomy, forestry and landscape architecture, staying in the greater part outside the domain of biological science. This paper elaborates significance of the landscapes for the conservation of biological diversity, and suggests inversion of the present approach to landscape studies in Croatia emphasising role of biology and other natural sciences as a basis for any landscape research. Also, landscape could be used as an indicator of the ecological status of certain area. Study of ecosystem service has great potential for valorisation numerous landscape functions, and in this context it is important to consider its aesthetic and cultural values.

Keywords: landscape, landscape ecology, natural sciences, biology, biodiversity

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POSJEĆIVANJE ŠPILJA UNUTAR EKOLOŠKE MREŽE NATURA 2000 - PRIMJER ŠPILJE VRLOVKE

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Osim tradicionalne zaštite, jedan od mehanizama očuvanja prirodnih vrijednosti je i uklapanje područja, vrsta i stanišnih tipova u europsku ekološku mrežu Natura 2000. Takve primjere nalazimo i kod špilja, gdje ciljevi očuvanja mogu obuhvaćati podzemno stanište, vrste koje u njemu obitavaju, ali i okolni nadzemni prostor koji te vrste koriste. Špilja Vrlovka dio je Natura 2000 mreže kao važan objekt za hibernacijske i porodiljne kolonije četiri vrste, a dodatno se u njoj pojavljuju još dvije vrste šišmiša. Vrlovka je i geomorfološki spomenik prirode, uređen u turističke svrhe 1928. godine do 330m glavnog kanala, dok je preostalih 50m kanala urušeno. Kako bi se spriječio nekontrolirani ulazak, na ulaz špilje su 2016. godine postavljena vrata prilagođena prolasku šišmiša. No je li moguće pomiriti ideju učinkovite zaštite značajnih populacija šišmiša s turističkom namjenom špilje? Istraživanja provedena tijekom 2017. godine ukazuju na prirodne periodičke izmjene populacija šišmiša u objektu tijekom njihova godišnjeg ciklusa, pri čemu su prisutna razdoblja iznimno velike i iznimno male brojnosti jedinki. Dodatno, njihov sezonski prostorni raspored i ponašanje u prisutnosti ljudi u objektu ostavljaju mogućnost za promišljanje i planiranje posjećivanja. Sveobuhvatnim pristupom moguće je propisati adekvatne mjere zaštite šišmiša, a ujedno i kreirati okvir održivog korištenja špilje u edukacijske svrhe te na taj način pomiriti ove dvije različite uloge istog speleološkog objekta.

Ključne riječi: špilje, šišmiši, zaštita, posjećivanje

VISITING CAVES WHICH ARE PART OF NATURA 2000 ECOLOGICAL NETWORK - EXAMPLE OF VETERNICA CAVE

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Beside traditional protection, another mechanism of conservation of natural values is inclusion of areas, species and habitat types in the European ecological network Natura 2000. Such examples can be found with caves, where conservation objectives may include underground habitat, species which live inside it, but also surrounding overground area used by those species. Vrlovka cave is part of Natura 2000 network as an important site for hibernating and maternity colonies of four bat species and additional two species which occur in the cave. Also, Vrlovka is a geomorphological natural monument, adapted for tourism in 1928 up to 330m of the main channel, whereas other 50m of the channel is partly collapsed. To prevent the uncontrolled entry, “bat friendly” door was installed in 2016. But is it possible to reconcile the idea of effective conservation of important bat populations with touristic use of cave? Monitoring during 2017 showed natural periodical fluctuations of bat populations in cave during their yearly cycle, with periods of very large and very small bat numbers. Additionally, seasonal spatial distribution and reaction to presence of people leave some possibilities to deliberate and plan visiting. With comprehensive approach it is at the same time possible to prescribe adequate conservation measures for bats and to create a framework for sustainable use of cave for educational purposes. In that way two different roles of one speleological object can be harmonised.

Keywords: caves, bats, protection, visiting

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MOGUĆNOST ZAŠTITE BIOLOŠKE RAZNOLIKOSTI I SMANJENJE KLIMATSKIH PROMJENA KROZ UPRAVLJANJE ZELENOM INFRASTRUKTUROM GRADA ZAGREBA

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Zelena infrastruktura (ZI) je detektirana kao izuzetno vrijedna sastavnica prostora, kvalitete života i okoliša te važan identitetski element gradova EU. Jedna od glavnih karakteristika ZI je povezanost svih njenih elemenata kako bi se omogućila komunikacija živih organizama, njihovo širenje, razmjena genetskog materijala, a samim time i održanje i/ili povećanje bioraznolikosti. Stoga jedna od najvažnijih analiza ZI, je analiza povezanosti, koja je napravljena GIS analizama povezanosti staništa i fragmentiranosti područja s visokom vegetacijom te vizualnom analizom neprekinutosti elemenata ZI. Ove analize istaknule su područja u kojima je potrebno osigurati veću povezanost staništa različitim elementima zelene infrastrukture. Osim očuvanja biološke raznolikosti ključan je doprinos ZI u smanjenju učinka klimatskih promjena. Kombinacija razvoja i globalnog zagrijavanja uzrokuje više temperature u gradu, a temperaturne razlike mogu iznositi i 10 °C između urbanih i ruralnih sredina. Fenomen viših temperatura u gradovima i njihovoj okolini zove se „urbani toplinski otok“, a u sklopu izrade Strategije zelene infrastrukture Grada Zagreba ovaj fenomen je kartiran analizom satelitske snimke. Rezultati pokazuju da manji udio izgrađenih površina te prisutnost zelenila (pogotovo šume) smanjuje temperaturu površine na području grada odnosno da se povećanjem zelene vegetacije (pogotovo stabala) smanjuje učinak „urbanih toplinskih otoka“.

Ključne riječi: zelena infrastruktura, Grad Zagreb, GIS, urbani toplinski otok, biološka i krajobrazna raznolikost

POSIBILITES OF THE BIODIVERSITY CONSERVATION AND MITIGATION OF THE CLIMATE CHANGES THROUGH THE MANAGEMENT OF THE GREEN INFRASTRUCTURE OF THE ZAGREB CITY

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Green Infrastructure (GI) is recognised as excaptionaly valuable feature of the space, life quality and environment as well as the elelent of the identity of the cities in EU. On of the mayor characteristics of the GI is connectivity of all of its elements to provide the communication of the living organisms, their dispersal, genetic material exchange as well as conservation and/or increase of the biodiversity. Therefore one of the main analysis of the GI s. s conectivity habitat analysis and fragmentation of the high vegetation areas and the visual analysis of the uniteruptedness of the GI elements. The analyses highlited the areas where diverse elements of GI could increase the habitats connectivity. In addtion to the biodiversity

conservation GI has importance in the reduction of the climate change effects. Developments and global warming cause higher temperatures in the cities and temperature variations between urban and suburban areas could be 10°C. Urban heat island is phenomenon of the higher temperatures in the cities and their souranding areas. In the Strategy of the Green Infrastructure of Zagreb City the urban heat islands phenomenon is mapped through the satelite image analyses. Results show that smaller poportion of the built area and existance of green vegetation (specificaly forests) are reducing the surface temperature in the City, respectively the increase of green vegetation (specificaly trees) decrese the urban heat island effect.

Keywords: green infrastructure, Zagreb City, GIS, urban heat island, biological and landscape diversity

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OČUVANJE PROPUSNOSTI ŽELJEZNIČKE PRUGE HRVATSKI LESKOVAC-KARLOVAC ZA DIVLJE ŽIVOTINJE NAKON DOGRADNJE NOVOG KOLOSIJEKA

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Glavni negativni utjecaj razvoja prometne infrastrukture na prirodu predstavlja fragmentacija i izolacija prirodnih staništa koja koriste divlje životinje. One rezultiraju povećanom učestalosti stradavanja jedinki, otežavanjem migracije te smanjenjem areala kretanja i kvalitete staništa. Kako bi se osigurala propusnost prometnice za kretanje životinja i smanjili posljedični negativni utjecaji, potrebna je konstrukcija prolaza ili prijelaza za životinje. Postojeća željeznička pruga od Hrvatskog Leskovca do Karlovca prolazi staništima koja koristi divljač, mali glodavci i vodozemci. Trasa ujedno prolazi ekološkom mrežom Natura 2000, što području daje dodatnu važnost u smislu zaštite prirode. Pretpostavka je da životinje za prolazak ispod pruge koriste neke od 40 postojećih objekata poput propusta za vodu, mostova i vijadukata. Na dionici je planirana dogradnja novog kolosijeka koji će povećati efekt barijere za životinje, stoga je pri njenom planiranju potrebno nastojati očuvati postojeću propusnost. Prolazi za životinje potrebni su na mjestima gdje pruga siječe migracijske koridore, a svojim dimenzijama predstavlja prepreku kretanju jedinki. Analiziran je prostoran raspored staništa, dimenzije pružnog nasipa te prikladnost postojećih objekata koji bi nakon planirane rekonstrukcije potencijalno, mogli funkcionirati kao prolazi. Na temelju analize određeni su prostorni raspored i dimenzije 55 rekonstruiranih i novih prolaza koji bi osigurali postojeću propusnost za životinje.

Ključne riječi: prometna infrastruktura, fragmentacija, propusnost, prolazi za životinje

PRESERVING THE HRVATSKI LESKOVAC-KARLOVAC RAILWAY PERMEABILITY FOR WILDLIFE AFTER THE CONSTRUCTION OF A NEW TRACK

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The main negative impact of transport infrastructure on nature is fragmentation and isolation of natural wildlife habitats. The consequences include increase in wildlife-traffic collisions,

cutoff of migration routes and decrease in movement range and habitat quality. In order to assure transport infrastructure permeability for animal migrations and to reduce the ensuing negative impacts, construction of wildlife crossings and passages is required. The existing Hrvatski Leskovac-Karlovac railway passes through habitats used by game, small rodents and amphibians. The route also cuts through the eNatura 2000 network, which gives the area an additional importance in terms of nature protection. It is assumed that wildlife species use some of the 40 objects including culverts, bridges and viaducts to pass under the rails. From H. Leskovac to Karlovac, a new additional railway track is to be built, which will increase the barrier effect on wildlife. To reduce the negative effect of the barrier, it is important to preserve its permeability. Wildlife passages are required at spots where railway cuts off migration routes and where it represents an obstacle because of its size. The spatial distribution of habitats, embankment size and suitability of existing objects, which could function as passages after planned construction, were analyzed. Based on the analysis, spatial distribution and dimensions of 55 reconstructed and new passages for preserving wildlife permeability were defined.

Keywords: transport infrastructure, fragmentation, permeability, wildlife passages

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MONITORING PTICA POLJOPRIVREDNIH STANIŠTA U HRVATSKOJ

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Ptice su prepoznate kao ključna indikatorska skupina kojom se procjenjuje stanje prirode i biološke raznolikosti u raznim sektorima, pa tako i u poljoprivredi. U Hrvatskoj se od 2015. provodi monitoring populacija čestih vrsta ptica na poljoprivrednim staništima. Podatke na godišnjoj razini prikupljaju brojni terenski istraživači (oko 30) na ukupno 55 kvadranata veličine 10 X 10 km diljem cijele Hrvatske ravnomjerno raspoređenih u tri biogeografske regije: kontinentalna, alpinska i mediteranska. Svake godine se na istim kvadrantima ptice prebrojavaju standardnom ornitološkom metodom istraživanja, na dva točkasta transekta s po devet točaka. Na tim točkama ptice se bilježe u tri pojasa udaljenosti (0-30 m, 30-100 m, >100 m) dvaput tijekom jedne sezone gniježđenja od početka travnja do sredine lipnja. 30 vrsta ptica poljoprivrednih staništa čine indeks čestih vrsta ptica poljoprivrednih staništa (Farmland Bird Index) preko kojeg se prati trend populacija. Rezultati (2015.- 2018.) upućuju na to da indeks oscilira oko početne vrijednosti.

Ključne riječi: ptice poljoprivrednih staništa, monitoring, indeks, Hrvatska

FARMLAND BIRD MONITORING IN CROATIA

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Birds are widely accepted as key indicators for assessing the state of nature and biodiversity in different sectors, among them in the agricultural sector. Since 2015 a farmland bird monitoring has been conducted in Croatia. Data are gathered with help of numerous field

researchers (around 30) in 55 quadrants 10 x 10 km across Croatia evenly distributed in three biogeographic regions: Continental, Alpine and Mediterranean. Each year birds are being sampled and counted in the same quadrants using standardized field ornithology methods: sampling along two point-count transects. Birds are counted in three distance belts (0-30 m, 30-100 m, >100 m) twice per breeding season from beginning of April to mid-June. 30 farmland bird species are encompassed by the Croatian farmland bird index monitoring their population trend. Results (2015-2018) indicate that the farmland bird index oscillate around the initial value.

Keywords: farmland birds, agriculture, monitoring, index, Croatia

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SASTAV I BROJNOST MORSKOG OTPADA NA PLAŽAMA U ZADARSKOJ ŽUPANIJI

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Otpad u moru predstavlja veliku prijetnju obalama, morskom okolišu i životu u njemu. Najveći dio otpada (80%) dolazi u more sa kopna zbog neodgovarajućeg gospodarenja otpadom. Sredozemno more je jedno od najonečišćenijih područja svijeta. Kao dio Sredozemlja, i Jadransko more je izloženo onečišćenju otpadom. Na području Zadarske županije provodi se istraživanje o količini i vrsti morskog makro otpada na plažama. Istraživanje se provodi na tri plaže od ožujka do svibnja 2018.g. Istraživanje se provodi prema DeFishGear protokolu za monitoring makro otpada (> 2,5 cm) na plažama. Sav makro otpad prikuplja se na transektu duljine 100 m i širine 10 m od morske linije. Svi prikupljeni predmeti se važu i kategoriziraju u neku od osam kategorija materijala: umjetni polimeri, guma, tkanina/tekstil, papir/karton, obrađeno drvo, metal, staklo/keramika i neidentificirani otpad/kemikalije. Svaka kategorija ima svoje podkategorije. Izračunava se indeks čistoće obale (Clean Coast Index (CCI)) za svaku lokaciju. Vrsta i količina otpada usporedit će se za različite lokacije i odredit će se mogući izvori otpada. Ovo istraživanje pružit će uvid u moguće izvore otpada u moru te će moći doprinijeti gospodarenju otpadom na istraživanim područjima. Rezultati također mogu doprinijeti razvijanju svijesti u lokalnoj zajednici o problem otpada u moru.

Ključne riječi: otpad u moru, plaža, Jadransko more, onečišćenje, Zadar

COMPOSITION AND ABUNDANCE OF MARINE LITTER ON ZADAR COUNTY BEACHES

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Marine litter presents a big threat to coastline, marine environment and marine life. Most of it (approx. 80%) enters the sea from land sources due to improper waste management on land. The Mediterranean Sea has been described as one of the areas most affected by marine litter in the world. As a part of the Mediterranean, Adriatic Sea is also exposed to high pressure of marine litter. To tackle this problem in Zadar area, research is done on the amount and type of marine macro litter on beaches. Marine macro litter is going to be

analysed on 3 beaches in Zadar County from March to May 2018 following DeFishGear protocol for monitoring macro litter (> 2.5 cm) on beaches. All macro litter will be collected from transects 100 m long and 10 m wide, from the water line. All items will be collected, weighted and categorized into 8 possible categories of material: artificial polymer materials, rubber, cloth/textile, paper/cardboard, processed/worked wood, metal, glass/ceramics and unidentified and/or chemicals. Each category has different subcategories. Clean Coast Index (CCI) will be calculated for each location. Type and amount of litter will be compared for different locations, and possible sources of litter identified. This study will give an insight to possible sources of litter hence it could be used to improve waste management in the studied area. Results could also contribute to increasing awareness of this problem in the local community.

Keywords: marine litter, beach, Adriatic Sea, pollution, Zadar

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PRIMJENA MOLEKULARNIH BILJEGA KOD MUZEJSKIH UZORAKA ZA REKONSTRUKCIJU PODRIJETLA DIVOKOZA SJEVERNOG VELEBITA

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Dvije od sedam priznatih podvrsta sjeverne divokoze (*Rupicapra rupicapra*) nastanjuju Dinarske planine: alpska divokoza (*R. r. rupicapra*) i balkanska divokoza (*R. r. balcanica*). Tijekom ranih 1900-tih populacije divokoza na sjevernom Velebitu su iskorijenjene, međutim, 1970-ih godina dogodilo se nekoliko translokacija sa različitih područja koja su nastanjena bilo sa alpskom ili balkanskom divokozom. Taksonomska klasifikacija autohtone populacije divokoze sa sjevernog Velebita nije poznata i jedini dostupni uzorci su četiri muzejske lubanje od mužjaka koje datiraju iz godina 1886., 1893., 1895. i 1939. Izolirali smo DNA iz srednjeg sloja i vanjskog dijela rožine muzejskih uzoraka. Koristili smo mikrosatelite i mitohondrijske biljege da istražimo genetsko podrijetlo autohtone populacije divokoze i da odredimo južni distribucijski doseg alpske divokoze i sjeverni distribucijski doseg balkanske divokoze. STRUCTURE (sa q vrijednostima između 0.6 i 0.7) i Geneclass (sa svim rezultatima iznad 95%) analize su dodijelile muzejske primjerke alpskim divokozama. Suprotno analizama mikrosatelita, analize mitohondrijskih biljega su potvrdile da muzejski uzorci pripadaju balkanskim haplotipovima, pokazujući prisutnost balkanske divokoze na Velebitu prije izumiranja vrste. Stoga ovo istraživanje razjašnjava filogenetski status nekadašnji populacija divokoza na sjevernim Dinarskim planinama.

Ključne riječi: divokoza, mikrosateliti, mtDNA, genetsko podrijetlo, Velebit

HISTORICAL RECONSTRUCTION OF THE CHAMOIS ORIGIN FROM THE NORTH VELEBIT

MOUNTAIN REVEALED BY OLD MUSEUM SAMPLES

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Two of the seven recognized subspecies of Northern chamois (*Rupicapra rupicapra*) occupy the Dinaric Mountains: Alpine chamois (*R. r. rupicapra*) and Balkan chamois (*R. r. balcanica*). During the early 1900s, chamois populations in the north Velebit Mountain were extirpated, however, in 1970s there were several translocations from different areas inhabited either by Alpine chamois or Balkan ones. Taxonomic classification of the autochthonous north Velebit Mountain chamois population is not known and the only available samples are four museum male chamois skulls, dating from years 1886, 1893, 1895, and 1939. We extracted DNA from middle layer and outer sheath of horns of these museum samples. Microsatellite and mitochondrial markers were used in order to investigate genetic origin of autochthonous chamois population and to determine the southern distribution range of Alpine chamois and the northern distribution range of Balkan chamois. STRUCTURE analysis (with q values between 0.6 and 0.7) and Geneclass (with all scores above 95%) assigned museum samples to the Alpine chamois. But in contrary to microsatellite analyses, mitochondrial analyses confirmed Balkan haplotypes in museum samples, indicating the presence of Balkan chamois on Velebit Mountain even before the extinction. Therefore, this study clarifies the phylogenetic status of former chamois populations in northern Dinaric Mountains.

Keywords: chamois, microsatellite, mtDNA, genetic origin, Velebit

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STRADAVANJE VODOZEMACA NA PROMETNICAMA MEĐIMURSKE ŽUPANIJE

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Fragmentacija staništa gustom cestovnom mrežom Međimurske županije neposredno utječe na stradavanja vodozemaca na prometnicama, posebno za vrijeme mriješćenja. Najznačajnija stradavanja zabilježena su na osam lokaliteta ukupne duljine 7.800 metara prometnica. Lokaliteti se poklapaju s značajnijim prostornim promjenama proteklih 30-ak godina (mjestima formiranja retencija i ribnjaka, izgradnje autoceste, mreže kanala i sl.). Kako bi smanjili stradavanje vodozemaca na prometnicama u proteklih pet godina JU Međimurska priroda je organizirala akcije spašavanja vodozemaca metodom postavljanja zaštitne ograde te prenošenjem jedinki preko ceste na dva lokaliteta, u Selnici i Črečanu. U akciju se uključuju lokalne udruge i preko 30 volontera te se svake godine educira preko 100 učenika

iz lokalnih škola. Zabilježeno je šest vrsta vodozemaca, od čega preko 90% otpada na smeđe krastače (*Bufo bufo*), a ostatak na smeđe šumske žabe (*Rana dalmatina*), zelene žabe (*Pelophylax* kl. *esculentus*), male vodenjake (*Lissotriton vulgaris*), gatalinke (*Hyla arborea*) te kvalifikacijsku vrstu ekološke mreže Natura 2000, češnjaču (*Pelobates fuscus*). Navedena aktivnost pridonosi senzibilizaciji javnosti na problematiku stradavanja vodozemaca, ali i drugih životinjskih vrsta na prometnicama kao posljedica fragmentacije staništa. Potrebno je izvršiti opsežno kartiranje crnih točaka na području županije, postaviti prometne znakove upozorenja te istražiti načine trajnog rješavanja problematike.

Ključne riječi: fragmentacija staništa, stradavanje vodozemaca, smeđa krastača, Međimurska županija, edukacija

AMPHIBIAN ROADKILLS IN MEĐIMURJE COUNTY

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Habitat fragmentation through dense road network in Međimurje County directly affects the amphibian roadkills, especially during the mating season. The most significant casualties were recorded at eight sites of a total length of 7,800 meters of roads. Locations coincide with significant spatial changes over the past 30 years (retention sites, pond formations, highway construction, canal network, etc.). To reduce the amphibian roadkills Međimurje Nature - Public Institution for Nature Protection has organized over the past five years rescue operations using the protective fence method and transferring the animals across the road in two locations: Selnica and Črečan. Local NGOs and over 30 volunteers are involved in the action, and each year over 100 students from local schools are educated. Six amphibian species have been recorded, of which over 90% is the species *Bufo bufo*, and the rest are species *Rana dalmatina*, *Pelophylax* kl. *esculentus*, *Hyla arborea*, *Lissotriton vulgaris* and one qualification Natura 2000 species *Pelobates fuscus*. This activity contributes to public awareness on the issue of amphibian and other animal species as roadkills as a result of the habitat fragmentation. Extensive mapping of the black spots in the County, setting up traffic signs and research the best ways to solve this problem are needed.

Keywords: habitat fragmentation, amphibian roadkills, common toad, Međimurje County, education

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STATUS DVIJE VRSTE VELIKIH PLAVACA (*Phengaris* spp.) U HRVATSKOJ

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Cilj rada bio je utvrditi ključne faktore koji uzrokuju pad brojnosti dviju vrsta velikih plavaca u Hrvatskoj, *Phengaris teleius* i *Phengaris nausithous*. Za razliku od zapadnog dijela rasprostranjenja *P. teleius* je šire rasprostranjena i brojnija vrsta od *P. nausithous*. Poznavanje njihove ekologije ključni je faktor za uspješnu zaštitu vrste u Hrvatskoj. U tu svrhu je na izabranom području, zaštićenom spomeniku prirode Bedekovićeve grabe, provedeno

višekratno markiranje, puštanje te ponovno hvatanje leptira da bi se utvrdili populacijski parametri i korištenje staništa. Podaci o brojnosti gusjenica, biljke hraniteljice, zajednica mravi te različiti abiotski faktori također su prikupljani. Zaključno možemo utvrditi da su populacije obje vrste stabilne, ali slaba mobilnost i loše gospodarenje staništem mogli bi ih dovesti do ruba opstanka. Stoga mjere zaštite, a predlaže se košnja, mora biti fleksibilna i s ciljem zadržavanja raznolikosti mikrostaništa.

Ključne riječi: Veliki plavci, Hrvatska

STATUS OF TWO LARGE BLUE BUTTERFLIES IN CROATIA

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The aim of this study was to define the key factors affecting the decline of two highly endangered large blue butterflies, *Phengaris teleius* and *Phengaris nausithous*. In Croatia *P. teleius* is more widespread and abundant than *P. nausithous*. Understanding their ecology on the study site is the key factor of effective conservation. A mark-release-recapture study has been conducted to obtain detailed information on population structure and habitat occupancy in the protected landscape Bedekovićeve grabe near Čakovec, northern Croatia. Information on caterpillars' numbers, food-plant density, ant community and different abiotic factors including soil chemistry were additionally studied. It can be concluded that populations of both species are stable within the site, but low mobility and bad management could reduce the effective population size. Habitat management should be focused on the maintenance of different microhabitats that hold both species and resources, demanding some flexibility in mowing regimes.

Keywords: large blue butterflies, Croatia

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USPOSTAVA NACIONALNOG SUSTAVA PRAĆENJA POPULACIJE RISA NA TEMELJU FOTOZAMKI

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Unatoč činjenici da je ris (*Lynx lynx*) jedna od najugroženijih vrsta u Hrvatskoj, do sada nije bio uspostavljen nacionalni sustav praćenja populacije te nisu dostupni detaljni podatci o području rasprostranjenosti, niti znanstveno utemeljena procjena brojnosti populacije. Istraživanja risa u Hrvatskoj provode se posljednjih dvadesetak godina, tijekom kojih su prikupljeni podatci o morfološkim obilježjima, kretanju i aktivnosti, prehrani, uzrocima smrtnosti te genskoj raznolikosti populacije. Najučinkovitija metoda praćenja populacije risa je pomoću automatskih fotoaparata (fotozamki). Budući svaki ris ima jedinstvenu boju i uzorak krzna, analizom fotografija se mogu razlikovati jedinke. Tako se sustavno postavljenim fotozamkama mogu

prikupiti podaci o rasprostranjenosti populacije, a na temelju metodologije „označavanje i ponovno hvatanje“ se može procijeniti i brojnost. Potencijalno područje rasprostranjenosti risa u Hrvatskoj podijeljeno je na kvadrante dimenzija 10 x 10 km, te je u suradnji s javnim ustanovama i ovlaštenicima prava lova, u svaki kvadrant postavljena najmanje jedna fotozamka. Postavljene su na markirališta, šumske ceste i druge lokacije na kojima je viđen ris ili su nađeni znakovi prisutnosti risa (svi zabilježeni podatci o prisutnosti risa javno su dostupni u bazi http://vef.lynx.hr). U pojedinim područjima, poput NP Plitvička jezera i PP Velebit, je postavljena znatno gušća mreža fotozamki. Prve procjene brojnosti očekuju se krajem 2019. godine.

Ključne riječi: *Lynx lynx*, rasprostranjenost, brojnost ppulacije

ESTABLISHMENT OF NATIONAL MONITORING SYSTEM FOR EURASIAN LYNX BASED ON CAMERA TRAPS

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In spite of the fact that Eurasian lynx (*Lynx lynx*) is one of the most endangered species in Croatia, until recently national monitoring system was not established. Scientific data about the distribution and estimation of population size are not available. Lynx research in Croatia started about 20 years ago and until now morphology, movement and activity, feeding habits, mortality and genetic diversity were researched. The most efficient method for lynx monitoring are camera traps. Each lynx has unique pelt color and pattern, so individual animal can be identified based on photographs. So using a network of camera traps population distribution can be monitored and based on „capture – recapture“ methodology minimal population size can be estimated. Potential area of lynx distribution in Croatia was divided into 10 x 10 km squares and in cooperation with public institutions and hunters at least one camera trap was placed in each quadrant. In certain areas, like National park Plitvice lakes and Nature park Velebit, a significantly higher density of traps were placed. Camera traps were placed on locations used by lynx for marking their territory, on forest roads and other locations where lynx were seen or signs of their presence were noticed. All signs of lynx presence are archived in publicly available database http://vef.lynx.hr. First estimations of lynx population size in Croatia are expected in the end of year 2019.

Keywords: *Lynx lynx*, distribution, population size

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ZAJEDNICE PRIMARNIH I SEKUNDARNIH DUPLJAŠICA U POPLAVNIM NIZINSKIM ŠUMAMA UZ RIJEKU DRAVU U SJEVEROZAPADNOJ HRVATSKOJ

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Sastav zajednica ptica šumskih staništa ovisi o florističkim i strukturalnim svojstvima šume. U Hrvatskoj su dosad provedena istraživanja ornitofaune nizinskih šuma hrasta lužnjaka te gorskih i planinskih šuma i ukazala su na različit utjecaj florističkih i strukturalnih svojstava šume na sastav zajednica ptica i izbor staništa pojedinih vrsta. Među asocijacijama sjeverozapadnih nizinskih predjela Hrvatske osim prevladavajućih šuma hrasta lužnjaka postoje i relativno dobro očuvane poplavne šume johe, topole i jasena. Provedeno istraživanje obuhvaća zajednice primarnih i sekundarnih ptica dupljašica u poplavnim nizinskim šumama uz rijeku Dravu u sjeverozapadnoj Hrvatskoj. Istraživanje sastava zajednice ptica provedeno je metodom prebrojavanja u točki u različitim asocijacijama poplavnih šuma u kojima prevladavaju joha, topola i jasen na lokacijama Varaždinske podravske šume, Đurđevačke nizinske šume i Repaš tijekom 2016., 2017. i 2018. godine. Utvrđeno je postojanje ukupno 12 vrsta dupljašica, od čega pet vrsta primarnih i sedam sekundarnih. Najčešća primarna dupljašica je vrsta veliki djetlić, a najčešća sekundarna velika sjenica. Na istraženim staništima zabilježeno je ukupno 15 vrsta drvenastog bilja. Prevladavaju topola, joha, jasen i vrba. Utvrđena je pozitivna korelacija između ukupnog broja zabilježenih parova i starosti staništa, što je u skladu s dosadašnjim istraživanjima na drugim tipovima staništa.

Ključne riječi: dupljašice, poplavne šume

COMMUNITIES OF PRIMARY AND SECONDARY HOLE-NESTING BIRDS IN FLOODED FORESTS ALONG DRAVA RIVER, NORTH-WEST CROATIA

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Floristic and structural characteristics have a pronounced impact on forest bird communities. In Croatia, only research on lowland pedunculate oak, hillside and mountain forests have been conducted. Among lowland forest associations beside dominant pedunculate oak, there are still preserved alder, poplar and ash stands near the river Drava. This research includes communities of primary and secondary hole-nesting birds in these stands along the river Drava in north-west Croatia. The point-count method was used for analyzing bird communities and circular plot method for habitat mapping in different lowland associations with dominant alder, poplar and ash stands. The research was carried out in three locations during breeding season in 2016, 2017 and 2018: Varaždin lowland forest, Đurđevac lowland forest and Repaš. 12 species of hole-nesting birds were recorded, five of which primary and seven secondary. In total 15 plant species were recorded. Most of the stands had poplar, ash, alder or willow dominating. A positive correlation was found between total number of breeding pairs and stand maturity which is consistent with research done so far in other stand types.

Keywords: hole-nesting birds, lowland forest

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PROGRAM LIFE I OČUVANJE PRIRODE

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Program LIFE je financijski instrument EU namijenjen financiranju projekata iz područja klimatskih aktivnosti i zaštite prirode i okoliša te je jedini program EU namijenjen isključivo okolišnim pitanjima. Od svog početka 1992. godine LIFE je sufinancirao preko 4500 projekata s preko 5 milijardi eura. Glavni cilj LIFE projekata kojima je u fokusu zaštita prirode je provedba EU politika iz tog područja: Direktiva o pticama i staništima i EU Strategije o bioraznolikosti. Iako LIFE nije namijenjen financiranju znanstvenih istraživanja, tu ulogu ima drugi program EU – Horizon 2020, kroz LIFE projekte ona se često provode kao prvi korak u planiranju i provođenju konkretnih mjera očuvanja ugroženih vrsta i staništa te su u takvim projektima znanstveni članci važan proizvod. U ovom predavanju predstavljene su osnovne značajke Programa LIFE, važnost EU politika čije se provođenje njime podupire, osnovne razlike između LIFE-a i Horizona te primjeri uspješnih projekata u kojima je znanstveni rad imao važnu ulogu, a sve u svrhu povećanja vidljivosti LIFE-a i poticanja na prijavu LIFE projekata što će posljedično dovesti do poboljšanja stanja u zaštiti prirode i znanosti u RH.

Ključne riječi: LIFE, očuvanje prirode, projekti

THE LIFE PROGRAMME AND NATURE CONSERVATION

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The LIFE Programme is the EU's financial instrument supporting environmental, nature conservation and climate action projects and it is the only EU programme exclusively dedicated to environmental issues. Since its start in 1992, LIFE has co-financed more than 4500 projects with more than 5 billion euro. The main objective of LIFE projects focused on nature protection is the implementation of EU nature protection policies: Birds and Habitats directives and EU Biodiversity Strategy. Although research funding is the role of another EU programme – Horizon 2020, in many LIFE projects research activities have to be undertaken as a first step in planning and implementation of concrete conservation actions on threatened habitats and species. Science papers are important outputs in such projects. This lecture presents the basic information on the LIFE Programme, the importance of EU policies whose implementation it supports, differences between LIFE and Horizon programmes and successful projects examples in which scientific study had an important role. All of it in order to increase the visibility of the LIFE Programme and encourage potential applicants to apply for LIFE funding which will lead to the improvement of nature protection and science in Republic of Croatia.

Keywords: LIFE, nature conservation, projects

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ODGOVORI FENOTIPSKE PLASTIČNOSTI RIBA U ANTROPOGENO UVJETOVANOM MORSKOM OKOLIŠU

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Istraživanja adaptivnih svojstava ribljih vrsta u okolišima pod antropogenim pritiscima predstavljaju brojne izazove s obzirom da fenotipska plastičnost riba pod utjecajem različitih ekoloških prilika ispoljava različita fenotipska obilježja. Kavezno-uzgojni sustavi tuna u morskom okolišu čine umjetne strukture oko kojih se okupljanju morski organizmi. Nepojedena hrana predstavlja jedan od glavnih čimbenika koji utječe na brojnost i vrijeme zadržavanja divljih riba u okolici uzgajališta. Predmetno istraživanje ima za cilj ustanoviti razinu morfološke i fiziološke prilagodile populacija komarči (*Sparus aurata*) u antropogeno uvjetovanom okolišu u odnosu na populacije iz prirodnih staništa. Primjenom homolognih anatomskih točaka geometrijske morfometrije, kvantificirana su morfološka obilježja dok je fiziološki odgovor promatran kroz trofički status i spolnost protandričnih populacija. Uočene morfološke razlike u obliku glave i položaju usta posljedica su različite ishrane kavezno asociranih naspram divljih jedinki. Spolnost je uvjetovana energetskim statusom dostupne hrane (prirodna-školjkaši vs. kavezna-sitna plava riba) gdje jedinke koje obitavaju u blizini kaveza podliježu promjeni spola pri nižim dužinskim veličinama u odnosu na prirodne populacije. Navedeni rezultati upućuju da uzgajališta tuna formiraju nove ekološke niše te su nužna daljnja istraživanja kako bi se ustanovio razmjer i valorizirao njihov utjecaj na lokalne ihtiopopulacije.

Ključne riječi: ribe, fenotipska plastičnost, geometrijska morfometrija, ishrana, spolnost

PHENOTYPIC PLASTICY RESPONSES OF FISH IN HUMAN-MEDIATED MARINE ENVIRONMENT

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Adaptive trait research of fish species in anthropogenic pressured environments poses many challenges since phenotypic plasticity of fishes exhibits different phenotypic characteristics under different environmental conditions. In marine environment, tuna farms structure attract a variety of mobile organisms. Uneaten feed is one of the main factors influencing the abundance and retention time of wild fish in the farm vicinity. The aim of this research was to investigate the extent of morphological and physiological adaptation of the gilthead seabream (*Sparus aurata*) in the anthropogenic conditioned environment in relation to the natural habitats adaptations. Homologous anatomical points of geometric morphometrics were used for morphological feature quantification while the physiological response was evaluated through trophic status and sex change of protandrous populations. Observed morphological differences in head shape and mouth position were linked with different diet composition of farm-associated and wild populations. Sex change is conditioned by the energy status of available food (natural-shellfish vs. farm associated-small pelagic fish) where farm-associated individuals are subjected to sex change at smaller body sizes relative to the natural populations. These results indicate that tuna farms may represent new ecological niches and further studies are needed to valorize their impact on local ichthyopopulations.

Keywords: fish, phenotypic plasticity, geometric morphometrics, diet, sex

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MODELIRANJE KORIDORA KRETANJA VUKOVA (*Canis lupus* LINNAEUS, 1758) U HRVATSKOJ

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Prometnice u današnjem svijetu najviše utječu na fragmentaciju staništa, uznemiravanje i neposrednu smrt velikih zvijeri te tako predstavljaju jednu od najvećih prijetnji za njihov opstanak. Konstrukcije prikladnih struktura za prijelaz, koje moraju biti dovoljno prostrane, široke i u prirodnom okruženju, na ekološki značajnim mjestima izrazito su potrebne. Model pogodnosti staništa vuka napravljen je u Maxent programu koristeći telemetrijske položaje vukova, mjesta opažanja vukova, lokacije nađenih mrtvih životinja, glasanja i otisaka šapa u kombinaciji sa podacima o staništu (GIS slojevi). Podaci o staništu su: nagib terena, broj vrsta parno-prstaša, udaljenost od naselja, udaljenost od ruba šume i ceste, udio pašnjaka, gustoća stanovnika, Shannonov indeks raznolikosti, udio poljoprivrednih površina, nadmorska visina, gustoća ceste, indeks razvedenosti terena i udio šume. Dobiveni model (vrijednost AUC > 0.8) pokazao je staništa pogodna za prisutnost vuka. Na kvalitetu staništa najviše su utjecale udaljenost od naselja (59.6%), nadmorska visina (27.2%) te broj vrsta parnoprstaša (6.8%). Koristeći model u GIS-u, napravljeni su potencijalni koridori kretanja s obzirom na moguću rasprostranjenost vuka, postojeće autoceste i objekte na njima koji su pogodni za korištenje od strane vuka. Model i kartografski prikaz mogu se koristiti za procjenu utjecaja budućih prometnih infrastruktura na koridore kretanja vuka, a time i pridonositi samoj zaštiti vuka.

Ključne riječi: Maxent, rasprostranjenost vuka, fragmentacija staništa

MODELLING OF WOLF (*Canis lupus* LINNAEUS, 1758) MOVEMENT CORRIDORS IN CROATIA

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Modern day roads have the biggest impact on habitat fragmentation, disturbance and on the immediate death of large carnivores which makes them one of the biggest threats for their survival. Suitable crossings that ought to be wide enough, spacious and in natural surroundings, on ecologically relevant places are more than necessary. The model of wolf habitat suitability was created in Maxent using wolf movement locations, locations of animals found dead, wolf vocalization sites and foot prints, combined with GIS environmental layers. Layers used were: terrain inclination and elevation, number of ungulate species, distance from settlements, forests and roads, percent representation of grassland, forest and agricultural land, human population density, road density, Shannon diversity index and terrain ruggedness index. The Maxent model (with AUC value >0.8) determined probability of wolf presence or suitability of habitat for wolves. Habitat quality was mainly affected by the distance from settlements (59.6%), altitude (27.2%) and number of ungulate species (6.8%). Using GIS software with the habitat suitability map, potential movement corridors were created. They consider probability of wolf presence, existing highways and objects on the highways suitable as crossing structures for wolves. The model and cartographic representation can be used to evaluate the impact of future transportation infrastructure on wolf movement corridors, as

well as contribute to protecting the wolf.

Keywords: Maxent, wolf distribution, habitat fragmentation

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PREGLED SISAVACA NA PODRUČJU NACIONALNOG PARKA "KRKA"

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Na području Nacionalnog parka Krka nalazimo 46 vrsta sisavaca. Vrste koje nalazimo, a uvrštene su u crvenu knjigu sisavaca su vjerojatno ugrožene vrste vidra (*Lutra lutra*), potencijalno ugrožene vrste vjeverica (*Sciurus vulgaris*), vrtni puh (*Eliomys quercinus*), vuk (*Canis lupus*) i zec (*Lepus europaeus*). Divlja mačka (*Felis sylvestris*) koja je ugrožena vrsta, također je zabilježena na području. Tijekom lipnja i srpnja 2017. godine intenzivno su prikupljani podaci o sisavcima, izuzev šišmiša, zajedno sa dvjesto deset internacionalnih studenata u sklopu programa istraživanja biološke raznolikosti u Nacionalnom parku „Krka“. Metoda snimanja fotokamerama kontinuirano je korištena za snimanje srednjih i velikih sisavaca na području Parka. Za uzorkovanje dlake postavljeni su drveni štapovi premazani uljem valerijane dok su se mali sisavci lovili živolovkama. Tragovi kretanja, viđanja, sakupljeni izmeti, pješčani transekti i tuneli sa tintom, dodatne su metode korištene za utvrđivanje prisutnosti vrsta. Kombinacijom metoda potvrđeno je 16 vrsta, od toga najčešće divlja svinja (25.1%), srna (12.5%), kuna bjelica (10.5%), euroazijski jazavac (9.9%), zec (9.9%) i divlja mačka (3.9%). Sisavci na području parka nisu dovoljno istraženi a literaturnih podataka je malo, stoga nam ovo istraživanje daje kvalitetniji pregled vrsta za kojih nema dovoljno podataka i pokazuje potrebu za kombinacijom više metoda kako bi se efikasnije potvrdila prisutnost naših ugroženih vrsta.

Ključne riječi: sisavci, Nacionalni park "Krka", fotokamere

MAMMALS IN “KRKA” NATIONAL PARK- AN OVERVIEW

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There are 46 species of mammals in “Krka” National Park. The ones that have been included in the Red Book of Mammals in Croatia are near threatened species the otter (*Lutra lutra*), red squirrel (*Sciurus vulgaris*), garden dormouse (*Eliomys quercinus*), wolf (*Canis lupus*) and brown hare (*Lepus europaeus*). The wild cat (*Felis sylvestris*), which is an endangered species, is also recorded in the area. During 2017 (June and July) mammal data, except for bats, was intensively collected, along with two hundred and ten international students as part of the biodiversity research program at “Krka” National Park. Camera trapping is a method that was continuously used for recording medium and large mammals in the Park area. For hair

sampling, wooden rods were coated with valerian oil while small mammals were captured by live traps. Mammal fieldsign including tracks and scat, sightings, sand traps and ink tunnels were additional methods used to determine the presence of species. By combining all methods, 16 species were confirmed, most commonly wild boar (25.1%), roe deer (12.5%), beech marten (10.5%), european badger (9.9%), brown hare (9.9%) and wild cat (3.9%). Mammals in the Park are not sufficiently explored and literature data is scarce, so this research provides a better insight of species for which there is not enough data and shows the need to combine multiple methods so we can more effectively confirm the presence of our endangered species.

Keywords: mammals, Krka National Park, camera trapping

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PROSTORNA PRIORITIZACIJA ZA MITIGACIJU ELEKTROKUCIJE PTICA U HRVATSKOJ

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Prepoznata je hitna potreba za smanjenjem negativnog učinka kritične infrastrukture na populacije divljih ptica u Hrvatskoj. Te napore otežava činjenica da, čak i za ograničeno predmetno područje, sveobuhvatni projekti mitigacije na velikim infrastrukturnim zahvatima iziskuju izuzetno visok istraživački napor i novčani trošak. Kako bismo postigli što veći učinak s ograničenim sredstvima, naš pristup je bio kombinirati modeliranje rasprostranjenosti ciljnih vrsta i kvantitativnu procjenu rizika kako bismo mogli rangirati područja prema prioritetu zaštite i stupnju rizika, a zatim na odabranim prioritetnim područjima provesti terenske provjere smrtnosti. Rezultati te provjere korišteni su kao temelj za izradu specifičnih preporuka za mitigaciju elektrokucije ptica na postojećim srednjenaponskim vodovima. Na temelju naših rezultata možemo zaključiti kako bi isti pristup mogao biti koristan za istraživanje i kvantifikaciju raznih problema vezanih za konflikt ljudi i prirode, poput krivolova ili gubitka staništa.

Ključne riječi: ptice, elektrokucija, kartiranje osjetljivosti, mitigacija, procjena rizika

SETTING SPATIAL PRIORITIES FOR MITIGATING BIRD ELECTROCUTION IN CROATIA

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There is a recognised urgent need in Croatia to reduce the negative effect of critical infrastructure such as medium voltage power lines on bird populations. However, even for a relatively small area, comprehensive mitigation projects on large-scale infrastructure can be extremely labour intensive and prohibitively costly. To circumvent these limitations, our approach was to use a combination of species distribution modelling and quantitative risk assessment to rank the intervention areas by conservation priority and level of risk, followed by a field survey of mortality in the identified priority areas. Our findings, presented here, were used as a basis for producing recommendations for specific mitigation measures on existing medium voltage power lines. Our experience suggests that the same approach could also be

successfully applied to investigate and quantify many other areas of human-wildlife conflict, such as illegal killing or habitat loss.

Keywords: birds, electrocution, sensitivity mapping, mitigation, risk assessment

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MOLEKULARNA IDENTIFIKACIJA PODRIJETLA KOMARČE (*Sparus aurata* L. 1758) NA PODRUČJU ISTOČNOG JADRANA

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Posljednjih godina zamijećeno je značajno povećanje ulova divlje komarče u istočnom dijelu Jadranskg mora. Smatra se kako ubrzani porast kaveznog uzgoja doprinosi povećanju populacija komarči kroz zbjegove iz akvakulture (Chavanne i sur., 2008). Zbog zaštitile bioraznolikosti, nužna su genetska istraživanja koja podrazumijevaju određivanje genetske raznolikosti i strukture divljih i uzgojenih populacija. Stoga, osim procjene genetske strukture populacija od interesa, predmetno istraživanje je imalo za cilj procijeniti razinu introgresije uzgojnog genotipa u prirodnim populacijama komarče primjenom standardiziranog seta dvadeset i jednog neutralnog mikrosatelitnog lokusa i tri mikrosatelitna lokusa na genima kandidatima (hormon rasta, prolaktin i receptor za aktivaciju-modifikaciju proteina). Indeks genetske diferencijacije FST između istraživanih populacija bio je nizak i statistički značajan (0.023 za neutralne lokuse, 0.021 za kodirajuće lokuse) što je u skladu sa dosadašnim znanstvenim istraživanjima populacija komarče u Jadranu. Bayesian klaster analizom je prepoznat broj nezavisnih genetskih grupa (K) u uzorku na osnovi genetskih profila jedinki, te je primjenom neutralnih mikrosatelitnih lokusa utvrđeno pet grupa, a mikrosatelitnim lokusima na genima kandidatima četiri. Zbog izražene genetske razlike između uzgojenih i divljih populacija, Bayesian analiza je omogućila identifikaciju prebjega s vrlo visokom razinom pouzdanosti.

Ključne riječi: populacijska struktura, komarča, identifikacija podrijetla, mikrosateliti

MOLECULAR TRACEABILITY OF THE GILTHEAD SEABREAM (*Sparus aurata* L. 1758) ORIGINS IN THE EASTERN ADRIATIC SEA

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Over the last few years, a significant increase of wild gilthead seabream in fisheries landings have been documented in coastal areas of Adriatic Sea. It has been suggested that rapid expansion of sea-cage culture contributes to increasing of seabream populations through aquaculture escapes (Chavanne et al., 2008). In order to protect biodiversity and reducing the effect of escapes, population genetic studies are necessary for the determination of genetic structure of wild and farmed stocks. The aim of this work was to assess the population genetic structure of wild and farmed populations along the eastern Adriatic coast and to investigate the impact of genetic introgression of escaped farmed seabream to wild seabream populations. Origin traceability has been assessed by using standardized panel of 21 neutral

microsatellites loci and three loci linked to three candidate genes (growth hormone, prolactin, and receptor activity modifying protein 3 gene). The global FST value among all populations was relatively small (0.023 with neutral microsatellite loci, 0.021 with candidate genes) and it was in line with already reported values for the Adriatic Sea. Results of the Bayesian clustering analysis suggested 5 clusters (inferred with neutral microsatellite) and 4 clusters (inferred with candidate genes) as the most likely number of populations.

Keywords: population structure, gilthead seabream, origin traceability, microsatellites

Mikrobiologija
Microbiology

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GROWTH PERFORMANCE AND MEAT QUALITY OF PHEASANTS SUPPLEMENTED WITH PAENIBACILLUS ALVEI DZ-3

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According to the definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". The present study was carried out to evaluate the effects of biological (*Paenibacillus alvei* DZ-3) supplementation on productive performance and quality of meat of pheasant. Methods: Total of 50 two-week old of pheasant were randomly divided into two groups. The first group- control was fed a basal diet, while the second group was fed the basal diet that supplemented with the probiotic bacterium (1 gm/kg of diet), in concentration of 1010 CFU/ml, until 10 weeks of age. Experimental diets and drinking water were provided ad libitum. During the experiment lasting 10 week individual body weight were measured on a weekly basis. At the end of the experimental period it was determined quality of meat and the concentrations of *Escherichia coli* in caecum. Results: Treated group with *Paenibacillus alvei* DZ-3 showed significant effect on final body weight gain, slightly better quality of meat (increased protein contents, and decreased fat content in breast fillet), but extremely reduction of the number of *E. coli* in caecum as compared to the control one. Conclusion: Biological supplementation can be used as one of important additive for enhancing the productive efficiency and health conditions of growing pheasants. *Paenibacillus alvei* DZ-3 seem to be a good alternative to the use of antibiotics as growth promoter.

Keywords: biological supplementation, *Paenibacillus alvei* DZ-3, pheasant

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GROWTH PERFORMANCE AND MEAT QUALITY OF PHEASANTS SUPPLEMENTED WITH PAENIBACILLUS ALVEI DZ-3

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Acinetobacter baumannii is a gram negative non-fermentative coccobacillus and its commonly called multi drug resistant (MDR) because has resistant to many types of antibiotics. The *A. baumannii* has become a significant challenge to health care systems. *A. baumanii* survives for prolonged periods and wide range of environmental conditions. Wide spread environmental contamination is often demonstrated and outbreak of infections have been traced to respiratory case equipment, wound care procedures, humidifiers and patient care items. Total of 50 samples were collected from different hospitals and services in Bosnia and Herzegovina and 15 different antibiotics for antibiotic susceptibility testing. The laboratory experiment performed Kirby–Bauer disk diffusion method and the VITEK 2 Compact System (bioMe´rieux) methods. All data were organized and presented as percentages. The results showed that antibiotic resistance profiles of *A. baumannii* strains by this study. All of the samples had a Colistin sensitivity but 28% of them Tobramycin and 94% of them Trimethoprim resistance showed according to our study. All of our samples had a resistance rest of the tested antibiotics (12/15). The antimicrobial agents have saved the human race from many disease and infection. Increasing antimicrobial resistance leaves very few options for treatment, and till now there is no well-designed clinical trials to compare treatment opportunity for multidrug resistant *Acinetobacter* infections.

Keywords: antibiotic resistance, *Acinetobacter baumannii*, resistance profiles

Toksikologija i ekotoksikologija
Toxicology and ecotoxicology

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SEWAGE SLUDGE ANALYSIS – IS IT SAFE FOR AGRICULTURE?

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Wastewater treatment produces a great amount of sewage sludge on daily basis and it presents the problem of disposing it. The aim of this study was to analyze the concentrations of heavy metals in sewage substrates and plants (*Lactuca sativa*) to consider possible plant accumulation. The study involved collection of sewage samples (fresh sewage sludge (FS), elder sewage sludge (ES)), heavy metals analysis of sewage sludge and control substrates (CS), seedling of plants with various sewage substrates (FS and ES) and CS, analysis of heavy metals in seeded plants. Heavy metals analysis of Cr, Co, Ni, Cu, Zn, Mo, Cd was determined in all substrates and plants by ICP MS except for Hg that was determined by AAS technique. ES contained 103.58 mg/kg of Pb while CS contained 7.26 mg/kg. Zn concentrations ranged from 751.46 mg/kg in FS, 640.76 mg/kg in ES, 623.9 mg/kg in 50%ES + 50%CS to 35.42 mg/kg in CS. Hg concentrations were 0.19 mg/kg in CS and in FS substrate were 1.95 mg/kg.

According to EU regulation for agriculture use of sewage sludge (86/278/EEZ) none of metal concentrations in sewage substrates exceeded maximum permitted levels as well as heavy metals concentrations measured in plants when compared to Commission regulation (EC) 1881/2006 maximum levels for certain contaminants in foodstuffs. The results point out possible usage of sewage sludge in agriculture as compost for food production, implying the need for further research of impacts to human health.

Keywords: sewage sludge, heavy metals, ICP MS, AAS, food

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PRESENTATION OF RELEVANT NANOSAFETY INFORMATION IS CRUCIAL FOR ACCURATE PUBLIC PERCEPTION OF NANOTECHNOLOGY: EXPERIENCE FROM DANA 2.0. PROJECT

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During the last decade nanosafety research has advanced considerably as evidenced by a large number of funded projects (19 under EU Horizon 2020, 50 within the EU 7th Framework Programme). It is now a big challenge how to deal with a large quantity of obtained information and even more important how to convey relevant and quality information to the public. We shall present a successful approach achieved within the project DaNa 2.0 (Data and knowledge on nanomaterials - processing of socially relevant scientific facts). It is a German public funded project, and comprises of experts from 10 institutions across Europe, among which University of Ljubljana plays an active role as external expert for nanoecotoxicology. An interdisciplinary team of experts from different research areas covering all aspects of nanosafety research (human and environmental toxicology, biology, physics, chemistry and pharmacy) is working together to provide a non-biased, quality-approved and up-to-date knowledge base for more transparency. The central tool of the integrated application-based database, allows the user to quickly access the information between an application of nanoproducts, the used nanomaterial and its individual possible outcome / toxicological effect(s) (https://www.nanopartikel.info/en/). The expert team developed methodology, the «Literature Criteria Checklist» which facilitate the evaluation process of scientific publications for discriminating between high- and low-quality data.

Keywords: nanotoxicity, nanosafety, quality data, public communication

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MULTIVARIJANTNI PRISTUP ODREĐIVANJA FIZIOLOŠKOG STANJA DAGNJI U ODNOSU NA OKOLIŠNE UVJETE, ONEČIŠĆENJE I SEZONALNOST

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Zbog složenosti sezonski i prostorno promjenjivog okoliša i kompleksne veze s biološkim faktorima, nije jednostavno utvrditi odgovor organizama u specifičnim, stresnim uvjetima, kao što je onečišćenje. Kako bismo razdvojili uzročne čimbenike i utvrdili odgovore na njih, primijenili smo kompleksne multivarijatne metode na aktivnost biomarkera 15 nativnih populacija Mediteranske dagnje *Mytilus galloprovincialis*, uzorkovanih u proljeće i jesen. Dodatno smo dio jedinki u transplant eksperimentu izložili čistim i onečišćenim postajama u prirodi i kontroliranim uvjetima. Analizirali smo set biomarkera; indikatora oksidativnog stresa (katalaza, glutation reduktaza, glutation S-transferaza, sadržaj malondialdehoda i karbonila), genotoksičnosti (komet test), i neurotoksičnosti (acetilkolinesteraza), te set okolišnih varijabli. Rezultati su potvrdili vezu između sezonski promjenjivog fiziološkog odgovora *M. galloprovincialis* i razine onečišćenja. Fiziološki status dagnji se razlikuje između Jadranskih regija ovisno o okolišnim čimbenicima te između čistih i onečišćenih postaja ovisno o metalima u tkivu. Multivarijatna analiza aktivnosti biomarkera i primjena eksperimenata omogućila nam je da fiziološki odgovor dagnje ne interpretiramo kao samostalni razvoj događaja već kao strategiju razvoja istog.

Ključne riječi: biomarkeri, *Mytilus galloprovincialis*, okolišni čimbenici, metali, sezonalnost

MULTIVARIATE DATA IMPLEMENTATION IN DEFINING MUSSEL'S PHYSIOLOGICAL STATUS REGARDING ENVIRONMENTAL CONDITIONS, POLLUTION STATUS AND SEASONAL EFFECTS

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The complexity of seasonally and spatially variable environments, coupled with complex biological interactions, makes it difficult to pinpoint biological responses to specific environmental stressors, including a chemical pollution. To disentangle causative factors and reveal specific responses, we applied biomarker-based multivariate approaches to 15 native populations of Mediterranean mussel *Mytilus galloprovincialis* in spring and autumn. In addition, we used a subset of these populations in transplant experiments between clean and polluted sites in natural environments and in lab mesocosms. We analysed a battery of biomarkers, indicators of oxidative stress (catalase, glutathione reductase, glutathione S-transferase, content of malondialdehyde and carbonyls), genotoxicity (Comet assay), and neurotoxicity (acetylcholinesterase), and assessed a set of environmental variables. Results confirmed the relationship between seasonally differing physiological status of *M. galloprovincialis* and environmental pollution. Physiological status of mussels varies between Adriatic regions dependent upon the set of environmental variables, and between clean and polluted sites depending on measured concentrations of metals in mussel's tissue. Multivariate description of biomarkers activity and application of specific experiments allowed us to

characterize the mussel's physiological responses as a strategy rather than a single, self-contained event development.

Keywords: biomarkers, *Mytilus galloprovincialis*, environmental factors, metals, seasonality

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EKOTOKSIKOLOŠKE KARAKTERISTIKE PROTUTUMORSKOG LIJEKA 5-FLUOROURACILA

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Primjena protutumorskih lijekova u stalnom jer porastu. Jedan od razloga je produljenje prosječne životne dobi kada je vjerojatniji nastanak zloćudnih tumora. 5-fluorouracil (5-FU) je široko primjenjivan protutumorski lijek i u kliničkoj praksi je već više od 50 godina za liječenje različitih vrsta zloćudnih tumora. Nakon terapeutskog postupka 5-FU i njegovi metaboliti putem sustava za izlučivanje ulaze u okoliš, površinske i podzemne vode u kojima je moguć učinak na vodene organizme. Ispitan je utjecaj 5-FU na inhibiciju rasta slatkovodne alge (*Pseudokirchneriella subcapitata*) i inhibiciju pokretljivosti *Daphnia magna* kao i biološka razgradnja 5-FU. Rezultati ispitivanja pokazali su višestruko veću osjetljivost jednostanične zelene alge u odnosu na organizam *D.magna*, a rezultati biološke razgradnje potvrdili su njegovu biološku nerazgradivost u vodenom okolišu. Iako se 5-FU u površinskim vodama može naći u nanogramskim koncentracijama ne treba podcijeniti njegov toksični učinak na vodene organizme, a osobito na alge kao fotosintetske organizme te perzistentnost u vodenom okolišu.

Ključne riječi: 5-FU, *Daphnia magna*, *Pseudokirchneriella subcapitata*, biološka razgradnja

ECOTOXICOLOGICAL CHARACTERISTICS OF THE CYTOTOXIC DRUG 5-FLUOROURACIL

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Application of cytotoxic drugs has increased in the last few decades. One of the factors is longer expected life time which leads to the higher probability of cancer development. The 5-fluorouracil (5-FU) cytotoxic drug is widely applied in clinical practice for various cancer treatment for more than fifty years. After the treatment the 5-FU and metabolites are released through the excretory system to the environment, surface and groundwater with possible impact on aquatic organisms. The influence of the 5-FU on the freshwater algal (*Pseudokirchneriella subcapitata*) growth inhibition, and inhibition of the mobility of *Daphnia magna* is presented in this study. Also the biodegradability of 5-FU is analyzed. Test results indicate considerably larger sensitivity of the unicellular green algae compared to *D. magna*, and biodegradability test results confirmed the 5-FU as nonbiodegradable in aquatic environment. 5-FU can be found in surface waters only in ng/l range concentrations, but its

toxic effect on aquatic organisms and photosynthetic algae specifically should not be underestimated. Also the persistency of 5-FU in aquatic environment should be taken into consideration.

Keywords: 5-FU, *Daphnia magna*, *Pseudokirchneriella subcapitata*, biodegradability

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EFFECT OF ORAL MODIFIED CITRUS PECTIN (MCP) ON AL-INDUCED LIVER TOXICITY

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We are living in the aluminium age and unwitting exposure to Al can cause serious impact on human health. Al induces oxidative stress (OS) by changes in the levels of superoxide dismutase (SOD), catalase (CAT) and biomarkers of cellular peroxidation. In recent years, increasing attention has been paid to the application of nutritional antioxidants (such as herbal products) in diseases related to OS. Modified citrus pectin (MCP) is a complex polysaccharide obtained from the inner peel pulp of citrus fruit. MCP is known with antioxidant, immune modulator and cancer preventive properties. The present study investigated protective effect of MCP against Al induced OS and hepatocyte proliferation in the liver of Sprague Dawley rats. The study was consisted of a control and three experimental groups with a total number of 24 rats. All applications were carried out orally in a period of 30 days. Group I (control) was administered physiological salin (% 0.9); group II AlCl2 (34 mg/kg/day); group III MCP (50 mg/kg/day); group IV AlCl2 (34 mg/kg/day) and MCP (50 mg/kg/day) with 2 hour intervals respectively. SOD, CAT activities and lipid peroxidation were measured. Histopathological changes were investigated. Proliferating cell nuclear antigen immunofluorescence was assessed to evaluate hepatocyte proliferation. According to the findings, MCP modulated antioxidant parameters, alleviated LP and tissue damage resulted from Al toxicity. In parallel, hepatocyte proliferation was decreased.

Keywords: aluminium, MCP, liver

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DOVODI LI KO-IZLAGANJE URBANE SREDINE NANOČESTICAMA I INSEKTICIDIMA DO NJIHOVE POJAČANE TOKSIČNOSTI

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Usporedno s brzinom rasta nanotehnologije sve je više potrošačkih proizvoda koji sadržavaju nanočestice. U gradovima i mjestima s velikom gustoćom naseljenosti očekuje se znatno opterećenje nanočesticama, posebice u tokovima otpadnih voda. Nadalje, piretroidni insekticidi, koji su danas pronašli široku primjenu u vrtnim rasadnicima, gradskim

strukturama i krajobrazima sa svrhom suzbijanja stambenih i vrtnih štetočina (npr. komaraca), također imaju potencijal za ulazak u otpadne vode. Potencijalna interakcija između ko-prisutnih nanočestica i insekticida mogla bi modulirati toksičnost potonjih. U radu se ispituje učinak srebrnih nanočestica (AgNP) u kombinaciji s dva često korištena piretroidna insekticida, cipermetrina i deltametrina, na modelu dagnje *Mytilus galloprovincialis*. Standardni testovi toksičnosti koji su korišteni uključuju određivanje aktivnosti acetilkolinesteraze (AChE) i glutation S-transferaze (GST) u škrgama te koncentracije proteina metalotioneina (MT) u probavnoj žlijezdi školjkaša. AgNP u kombinaciji s cipermetrinom pokazao je povećanu aktivnost enzima GST iako nije utjecao na aktivnost AChE te veću koncentraciju proteina MT. Nadalje, kombinacija AgNP i deltametrina izazvala je znatno povećanje aktivnosti GST. Kompleksno ponašanje temeljeno na međusobnom djelovanju između AgNP i insekticida upućuje na činjenicu da se scenariji zajedničke izloženosti mora uzeti u obzir zbog važnosti njihova toksičnog djelovanja na ljude i okoliš.

Ključne riječi: srebrne nanočestice, acetilkolinesteraza, glutation S-transferaza, metalotioneini, *Mytilus galloprovincialis*

DOES CO-EXPOSURE TO NANOPARTICLES AND INSECTIDICES IN URBAN ENVIRONMENTS LEAD TO ENHANCED TOXICITY?

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In parallel with the rapid growth of the field of nanotechnology, there is an ever increasing number of consumer products that contain nanoparticles. In towns and cities where there is a large population density, the nanoparticle load in waste water streams is expected to be significant. Further, pyrethroid insecticides, which are widely used today in a diverse range of applications from garden nurseries, urban structural and landscaping sites, residential garden pest control and mosquito abatement, also have the potential to enter wastewater streams. The potential interaction between co-present nanoparticles and insecticides may modulate the toxicity of the latter. This work focuses on investigating the effect of silver nanoparticles (AgNP) in combination with two commonly used pyrethroid insecticides, cypermetrin and deltametrin, in bivalve *Mytilus galloprovincialis* as a model. Standard toxicity tests used in this study include determination of acetylcholinesterase (AChE) and glutathione S-transferase (GST) activity in mussel gill and metallothionein (MT) concentration in the digestive gland. AgNP in combination with cypermethrin showed increased GST, though not AChE activity and higher MT concentrations. Further, AgNP in combination with deltamethrin caused a significant increase in GST activity. Complex behaviour based on the interplay between AgNP and insecticides indicate that co-exposure scenarios must be considered due to relevance in human and environmental toxicity.

Keywords: silver nanoparticles, acetylcholinesterase, glutathione S-transferase, metallothioneins, *Mytilus galloprovincialis*

O-143

HYDROXYTHYROSOL MODULATES AL INDUCED OXIDATIVE STRESS IN MICE LIVER

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Aluminium (Al) is the most abundant metal and the third most abundant element in the earth's crust. The highest levels of Al are found in the bone and liver and to a certain extent the brain. Al is a pro-oxidant that disrupts biological membranes, enhances ROS formation, and damages DNA. Natural antioxidants, which alleviate the oxidative stress (OS) or induce the cellular antioxidant milieu would most probably treat and/or protect against Al poisoning. The present study investigated the effects of hydroxythyrosol (HT), a powerful antioxidant and metal chelator found in the composition of extra virgin olive oil, in prevention of OS and hepatocyte proliferation resulted from Al toxicity in mice liver. Swiss albino mice were randomized into four groups (n=4) as: Group I (control:0.9% NaCl); Group II ($AlCl_3$: at one time, 25 mg/kg; Grup III (HT:10 mg/kg/day - 7 days); Grup IV ($AlCl_3$+HT: 25 mg/kg and 10 mg/kg/day - 7 days respectively). All applications were carried out intraperitoneally. Superoxide dismutase, catalase and lipid peroxidation (LP) were measured in order to determine OS. Histopathological alterations were evaluated. Proliferating cell nuclear antigen immunofluorescence was assessed to evaluate hepatocyte proliferation. According to the findings, HT modulated OS, LP and histopathological changes resulted from Al toxicity. In parallel, hepatocyte proliferation was decreased. It is suggested that HT acted by neutralising Al-derived radicals while fulfilling those effects.

Keywords: aluminium, HT, liver

POSTERSKE PREZENTACIJE

POSTERS

P-1

COLOUR VARIATIONS IN THE EUROPEAN TREE FROG *Hyla arborea* (Linnaeus, 1758)

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The colour of amphibian skin results from the relative abundance and distribution of chromatophores (pigment-containing and light-reflecting cells). Skin colour changes can be quite common in amphibians, especially in species that manifest crypsis. The European tree frog or *Hyla arborea* is one of the amphibian species that is known to change colours in response to different factors such as ambient temperature, background colour and environment light intensity for purpose of predatory avoidance, thermoregulation and sexual selection. Colour variations in *H. arborea* are given according to the field investigations from five different localities in Bosnia and Herzegovina in period of 2015 to 2017. Herein we describe 16 main coloration types (yellow-green, light green, green, dark green, light olive, olive, dark olive, green-brown, light brown, brown, dark brown, brown-grey, light grey, grey, dark grey and turquoise) and numerous transitional coloration such as different colour gradients and marbled colourations (bicolour, tricolour) in *H. arborea*. Typical light green was the most common observed, while other colouration were present in one to five individuals. Yellow-green was observed only in females. Besides our results, we compared them with other available literature data concerning the coloration of tree frogs and present all known colour variations.

Keywords: skin, chromatophores, pigment, crypsis, Bosnia and Herzegovina

P-2

THE CHECKLIST OF REPTILE FAUNA (Chordata: Vertebrata: Reptilia) IN BOSNIA AND HERZEGOVINA

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Bosnia and Herzegovina (B&H), as a specific region in the north western Balkan Peninsula, has a high level of genetic, species and ecosystem diversity primary due to the presence of different climatic zones that extend from north to south of the country (in cca 400 km). Multiple numbers of refugia and relict landscapes in this area enabled the survivorship for species during several glaciation events in the past and allowed the existence of a rich biodiversity. Even though that herpetofauna research in B&H can be traced back to the O. Möellendorf PhD dissertation (1873), studies of reptiles have been rare and sporadic in this area (e.g. Werner

1898, Bolkay 1924, Đurović et al. 1979, Radovanović 1951) until this time. The main aim of this work is to give new distribution maps for all existing reptile species in B&H (N=31) with revised taxonomic updates. Besides native species (N=29), two allohotous species (*Trachemys scripta* and *Pelodiscus sinensis)* are described in the herpetofauna of B&H. Authors did not confirm the existence of just two species (*P. sinensis* and *Dolichopis caspius*) that are reported to occur in B&H (literature data). Some species are extremely rare with only one (*Ablepharus kitaibeili*) or several findings (*Telescopus fallax*, *Zamenis situla*, *Platyceps najadum*), and most of the species (42%) have still an unclear distribution due to lack of data.

Keywords: distribution, hot spots, Balkan Peninsula, biodiversity, species

P-3

MAKING TOO MANY MISTAKES: CASES OF ABNORMAL AMPLEXI IN EUROPEAN ANURANS

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Even though that reproductive isolation of anurans is well developed, amphibians perform many “mistakes” during the mating period. Herein we present 65 cases of abnormal/unusual/wrong amplexi in European species of Anura collected mostly from the period 2005-2017. We generally divided the wrong amplexi into intraspecific or interspecific and furthermore as: (i) heterosexual (male with non-gravid female), (ii) homosexual (male with male), (iii) pedosexual (male with sexually immature, juvenile individuals or eggs), (iv) necrosexual (with dead individuals), (v) objectosexual (with inanimate objects), (vi) group (mating balls with more than two individuals or (vii) combined. Erroneous amplexi in European taxa can also include variable positions like abdominal, lateral, semi-axillary, semi-iguinal etc. We discuss how well reproductive isolation of different anuran species are developed. The results show that *Bufo bufo* is the species who makes the most mistakes (45% of all cases), followed by *Bombina variegata* and *Rana temporaria* (both with 11,5%). The species that suffers the most as a consequence of wrong amplexus are *B. bufo* females (38,8%) followed by *B. variegata*, *R. temporaria*, *Pelophylax species*, *Salamandra salamandra* (all with 8,6%). Of all Anura species, only *Pelophylax esculentus, P. ridibundus* and *P. perezi* were species not observed in mistaking amplexi.

Keywords: mistake, wrong, mating, mismatch, sexual

P-4

BEHAVIOURAL DEFENCES OF THE GREEN TOAD *Bufotes viridis* (Laurenti, 1768) FROM BOSNIA AND HERZEGOVINA

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Anti-predator adaptations are mechanisms developed through evolution that assist prey organisms in their constant struggle against predators. The most common defensive strategy of mobile animals is to remain motionless or to flee from potential predators. However, there is a wide array of defensive strategies that are alternatively used to cope with the risk posed by a predator. As common prey for a variety of predators Anurans, display a wide range of behaviours between remaining motionless and fleeing extremes (Toledo et al., 2011). It little is known about behavioural biology of *Bufotes viridis* (Laurenti, 1768). Herein we present 10 behavioural defences of *B. viridis*, ascertained by field research from 2013 to 2018 in Bosnia and Herzegovina: (1) Immobility or remaining motionless, (2) Crouching down, (3) Chin-tucking, (4) Puffing up the body, (5) Eye-protection, (6) Hiding, (7) Digging, (8) Fleeing, (9) Cloacal discharge and (10) Production of secretions. As this presents 30% off all described defensive mechanisms of Anurans, it can be argued that B. viridis shows a high diversity of behavioural defences.

Keywords: adaptation, anti-predator, defensive strategy, prey, defences

P-5

WIDESPREAD IN BOSNIA AND HERZEGOVINA? THE SMOOTH SNAKE *Coronella austriaca* Laurenti, 1768, APPEARANCE IN THE MEDITERRANEAN BIO-CLIMATIC REGION OF BOSNIA AND HERZEGOVINA

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The smooth snake (*Coronella austriaca* Laurenti, 1768) is a small, ovoviviparous, non-venomous colubrid that is relatively widely distributed throughout most of Europe: from the Iberian Peninsula to the Ural Mountains. It can be found in different habitats types ranging from forests to open meadows depending on latitude and altitude. Towards the south, it reaches the Balkan, Italian, and Iberian peninsulas, where it shows a rather more fragmented distribution than the homogeneous distribution found in Euro-Siberian zone (Santos et al., 2008; Llorente et al., 2011). In this paper we present recorded cases of *C. austriaca* from Mediterranean bioclimatic region of Bosnia and Herzegovina according to the field research. Most of the presented data (80 %) are linking the existence of *C. austriaca* populations above 1000 m, however we found individuals on much lower altitude: (1) Hutovo Blato (12 m a.s.l.), (2) Popovo polje (605 m a.s.l.) and (3) Humilišani (600 m a.s.l.). Up to this research, distribution of the species was referred to the central and northern part (continental, alpine and pannonian region) of Bosnia and Herzegovina. These data indicate that *C. austriaca* is probably widespread in Bosnia and Herzegovina, both horizontally and vertically.

Keywords: distribution, horizontal, vertical, fragmentation, smooth snake

P-6

LOOKING INTO THE MOUTH OF GIANT TADPOLES – MORPHOLOGICAL AND MERISTIC DESCRIPTION OF *Pelobates fuscus* (LAURENTI, 1768) ORAL DISC

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European common spadefoot toad is the only representative species of Pelobatidae family in Croatia. Species is wide spread all over the Europe and many papers are based on gathering biological description of metamorphs, ecological and distributional data. Interesting facts concerning giant tadpole biology are still one of intriguing questions that needs to be studied. This study is based on population from Lijeva Luka, Sisak-Moslavina Country, Croatia, where two clutches of eggs were collected. Total of 249 individuals were sampled and determine in stages by Gosner (1960). From each stage oral disc in several representatives was photographed using Leica EZ4 HD stereo microscope. Oral disc formula are presented by Altig & McDiarmid 1999. Results of this study are presented with: 1) detailed morphological and meristic description of oral disc by stages by Gosner (1960), 2) precise anatomical drawings of each stage, 3) oral disc formula for each stage. Determine tadpole stages were from 23-41 with exception of stages 25, 32-34, 36-39. Oral formula for stages 29-34 is 4 (1-2-3) / 4 [1-2-3] and stages for 39-40 is 5 (1-2-3-4) / 5 [1-2-3-4], which proved out to be the best stages for *Pelobates fuscus* tadpole determination by oral disc. With this paper results have additional goal to help determine *P. fuscus* species with certainty from other tadpoles of the same genus and lookalike tadpoles, and help answering the diet questions in different ontogenic tadpole stages.

Keywords: oral disc, Pelobates, European common spadefoot toad, tadpoles

P-7

ŽIVOT NA RUBU - PRIJETNJE OPSTANKU RIJEČNE KORNJAČE U HRVATSKOJ

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Riječna kornjača, *Mauremys rivulata* kritično je ugrožena vrsta na području Hrvatske. Ima vrlo usko područje rasprostranjenja i dolazi samo u Dubrovačko-neretvanskoj županiji na tri geografski izolirana lokaliteta. Budući da se nalazi na Dodatku II i IV Direktive o staništima, proglašena je strogo zaštićenom vrstom te su sve tri lokacije na kojima dolazi proglašene dijelom ekološke mreže Natura 2000. Terenska istraživanja ove vrste provode se zadnjih desetak godina, što je omogućilo stjecanje dugoročnog uvida u stvarne i potencijalne prijetnje opstanku riječne kornjače u Hrvatskoj. Sve opažene prijetnje određene su sukladno IUCN standardnoj shemi uzroka ugroženosti. Dodatno, svaka je prijetnja rangirana i izračunat je njezin utjecaj na populaciju (engl. Threat impact score), kako bi se mogle prioritizirati već

postojeće mjere očuvanja, ali i po potrebi definirati nove. Ukupno je prepoznato devet prijetnji riječnoj kornjači, dok su najznačajnije nestanak i degradacija staništa kanaliziranjem vodotokova te prirodnim zaraštanjem staništa kao posljedica nekorištenja i neodržavanja. Dodatno, ilegalna uporaba vrša na nekim područjima rezultira visokom smrtnošću riječnih kornjača, ali i popratne faune. Za dugoročnu zaštitu, potrebno je izraditi Akcijski plan zaštite riječne kornjače u Hrvatskoj, definirati mjere očuvanja te iste aktivno provesti u djelo.

Ključne riječi: *Mauremys rivulata*, ugroženost, mjere zaštite, Natura 2000

LIVIN' ON THE EDGE - THREATS TO THE SURVIVAL OF THE BALKAN TERRAPIN IN CROATIA

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The Balkan Terrapin, *Mauremys rivulata* is critically endangered species in Croatia. It has very small distribution area and can be found only in Dubrovnik-Neretva county on three geographically isolated localities. It is listed on Annexes II and IV of the Habitats Directive and is a strictly protected species in Croatia. Also, all three localities on which terrapin occurs are declared a part of the Natura 2000 ecological network. Long-term field studies, lasting for more than ten years, enable estimation and understanding of real and potential threats to this terrapins survival in Croatia. All observed threats were defined according to the IUCN Threats Classification Scheme. Additionally, each threat was ranked and its threat impact score was calculated in order to prioritize existing conservation measures and define new ones if necessary. Altogether nine threats were recognized. The most significant ones were degradation and habitat loss caused by channelization of watercourses and by ecological succession due to abandonment. Additionally, illegal use of fish traps in some localities results in high mortality of the Balkan Terrapin and other fauna. For the long term protection of the species it is necessary to create Action plan for the protection of the Balkan Terrapin in Croatia and to define and implement conservation measures.

Keywords: *Mauremys rivulata*, endangerement, conservation measures, Natura 2000

P-8

CAN WE SURVEY *Elaphe quatuorlineata* USING A PHEROMONE TRAP? HYPOTHESIS TO SET-UP THE MONITORING SCHEME FOR SPECIES WITH LOW-DETECTABILITY

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Slovenia has 22 species of native reptiles. Two are listed on Annex 2 of the EU Habitat directive, including the four-lined snake (*Elaphe quatuorlineata*). Despite the country's obligations to monitor the population, this has not yet started and there is a need to develop a national monitoring scheme. In Slovenia, *E. quatuorlineata* is limited to the Dragonja river valley and its surroundings, which represents the northern limit of the species' distribution. Due to small population size, we propose to develop a monitoring using traps with attractants to ensure higher detectability success. This is based on ecology of larger snakes, like boas, which are

known to use chemical communication to help males and females finding each other during copulation period. We hypothesise that also females of *E. quatuorlineata* produce trail pheromones to attract males. Based on this hypothesis we propose the following steps: (i) analyse the compound composition of pheromones excreted by females, (ii) perform behavioural experiments with males in captivity to select the most attracting compound, (iii) synthesize the pheromonal compound to be used for trapping, (iv) design the traps and (v) set-up and test traps in the field. In this way, we aim to gradually develop an effective pheromone traps for *E. quatuorlineata* and use it for the future monitoring. We will present the project scheme and review of available knowledge of using pheromone traps for monitoring snakes and other species.

Keywords: *Elaphe quatuorlineata*, pheromone, monitoring, conservation, Slovenia

P-9

FEMALE BIASED POPULATION OF DALMATIAN TORTOISE (*Testudo hermanni hercegovinensis*) AT PELJEŠAC PENINSULA

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We studied the population of Dalmatian Tortoise in central Pelješac Peninsula in South Dalmatia. In a systematic field survey, 29 wild tortoises were found, of which 2 were dead. The population was found to be female biased with 89% of individuals females (dead specimens excluded); 90% of females were full-grown adults (N=20) and 86% of females were juveniles < 5 years old (N=7). Found high sexual bias is not common in Hermann's Tortoises and might be caused by increased temperatures during egg incubation since bias was found already at the level of juveniles. According to growth bars the average age of adults were 15.4±4.7 years with the oldest individual being 27 years old. The main taxonomic characters for the subspecies *T. h. hercegovinensis* is absence of inguinal scutes, but this character was found in less than half tortoises (48%), and in one specimen the inguinal scute was present only at one side indicating this character is substantially variable among individuals. Most tortoises were found in agricultural landscape with vineyards and hedgerows (relative abundance 0.75±0.24 ind. / search hour; N=34 search hours), while the species was less abundant in bushy and hilly landscape (0.1±0.1 ind. / search hour; N=9 search hours). We also found that more than 70% of adults were injured (mainly carapace) by agricultural machinery. Overall, the population of tortoises in Pelješac seems relatively abundant but with a strong female bias and apparent pressure of agriculture.

Keywords: *Testudo hermmani*, tortoise, population structure, Pelješac, Croatia

P-10

MORFOLOŠKE ZNAČAJKE BARSKE KORNJAČE *Emys orbicularis hellenica* IZ NACIONALNOG PARKA KRKA

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Cilj rada je bio odrediti prisutnost spolnog dimorfizma kod primorskih populacija barske kornjače (*Emys orbicularis hellenica*). Istraživanje je provedeno na jedinkama s područja NP Krka (Kistanje), te uspoređene s dostupnim muzejskim jedinkama iz HPM u Zagrebu. Za lov kornjača korišteno je 15 šesterokutnih vrša s 4 nasuprotna ulaza. Ulovljeno je trideset i devet jedinki vrste *Emys orbicularis hellenica*, od čega 15 mužjaka i 24 ženke. Za svaku jedinku je provedena deskriptivna statistička obrada podataka na 38 morfometrijskih varijabli i 37 indeksa morfometrijskih obilježja. Determiniran je spol i sve su označene jedinstvenim ID brojem (urezan na oklop). Nakon fotografiranja jedinke su puštene na mjestu ulova. Prikupljeni podatci su uspoređeni i s muzejskim materijalom (12 ženki s otoka Paga). Analize pokazuju da kod populacije barskih kornjača, ženke se od mužjaka primarno razdvajaju po masi, opsegu oklopa, zakrivljenoj dužini karapaksa i duljini plastrona od vratne do početka analne ploče. Rezultati prikazuju najveće srednje vrijednosti za ženke iz Krke, s najvećom maksimalnom vrijednosti, dok mužjaci imaju manju srednju i manju minimalnu vrijednost, što ukazuje na prisutnost spolnog dimorfizma u veličini tijela. Izlaganje nepovoljnim uvjetima za vrijeme suhih ljetnih perioda, uzrokuje patuljasti rast u populaciji. Uzorci jedinki iz muzeja, čije je nalazište na Pagu, imaju "patuljast" rast i maksimalnu duljinu koju mogu dosegnuti i do 120 mm.

Ključne riječi: morfometrijska obilježja, spolni dimorfizam, Pag, distribucija

THE MORPHOLOGICAL CHARACTERISTICS OF A TERRAPIN *Emys orbicularis hellenica* FROM NATIONAL PARK KRKA

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The aim of the paper was to determine the presence of sexual dimorphism in populations of terrapins (*Emys orbicularis hellenica*). The research was carried out on units from the area of the NP Krka (Kistanje), and compared with the available museum units from HPM in Zagreb. For turtle hunting, 15 hexagons were used with 4 opposite inputs. Thirty-nine individuals of the species *Emys orbicularis hellenica* were caught, of which 15 were males and 24 females. For each individual, a descriptive statistical data processing was performed on 38 variables and 37 indexes of morphometric features. Gender is determined and all are marked with a unique ID number (carved on to the shield). After taking pictures, the animals were released at the site of the catch. The collected data were compared with the museum material (12 females from the island of Pag). Analyses show that in the terrapin population, males are primarily separated by

weight, shield range, curved length of carapace and length of the plastron from the neck to the beginning of the anal plate. The results show that females from Krka have the highest mean, with the highest maximum value, while males have lower mean and lower minimum values, which indicates the presence of sexual dimorphism in body size. Exposure to unfavorable conditions during dry summers, causes dwarf growth in the population. Samples from the museum, located on the island of Pag, have a "dwarf" growth and a maximum length that can reach up to 120 mm.

Keywords: meristics, morphological differences, sexual dimorphism, distribution

P-11

AMPHIBIAN MALFORMATIONS FROM BALKANS AND THE CZECH REPUBLIC

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Amphibian populations are worldwide declining. These animals are the most threatened vertebrate group due to numerous factors including body malformations, that are being considered as significant. There are numerous records of amphibian malformations reported lately from all over the world. Plenty of types of malformations are currently recognized (ectrodactyly, polydactyly, syndactyly, oligodactyly, clinodactyly, ectromely, clinomely, phocomely, polymely, symmely, taumely, polyphalangy, microcephaly, anophthalmy, various atypical colorations or translucent skin). However, causal factors responsible for such malformations remain often unclear. Herein we present several cases of amphibian malformations collected during field work in various parts of the Balkan peninsula and the Czech Republic. In some cases we examined the individuals by x-ray to better understand the character of particular malformation. Single reports of amphibian malformations provide us an opportunity to build up a greater knowledge and understanding of this phenomenon. As possible reasons for occurrence of body malformations in amphibians are considered (1) chemical pollutants used in agriculture, (2) parasite infestation or microbes, (3) UV-B radiation and (4) previous attempts of predation. However, without further complex research of amphibian populations, it is unlikely to uncover the origin of these malformations.

Keywords: Amphibian, malformation, ecology, morphology

P-12

INSIGHTS INTO THE PHYLOGENY AND PHYLOGEOGRAPHY OF THE STREAM FROG (*Rana graeca* Boulenger, 1891) IN THE BALKAN PENINSULA

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Among Western Palearctic frogs, the Stream Frog (*Rana graeca* Boulager, 1891) is a Balkan endemic. The phylogenetic position of *R. graeca* is well studied in respect to other *Rana* species but evolutionary relationships within *R. graeca* populations are completely unknown. By analyzing a partial sequence of the cytochrome-b (cyt-b) of samples collected along the species range, phylogenetic and phylogeographic relationships within the Stream Frog are reported in this study. Bayesian phylogenetics revealed the existence of two distinct lineages: the northern lineage - characterized with three haplotypes, and the southern lineage - defined with two additional, more diverged haplotypes. The diversity of inferred haplotypes is suggesting different colonization origin of the two lineages. Tajima's test confirmed recent population expansions that cause low differentiation that is especially registered in samples from the northern part of the range that are represented by a single haplotype. Additional genetic markers and a wider sampling are needed to further clarify the evolutionary relationships within *R. graeca* populations inferred from this study.

Keywords: Balkan brown frogs, evolution, Glacial refugia, colonization, expansion

P-13

FOOD COMPOSITION OF EUROPEAN POND TURTLE (*Emys orbicularis*) IN VLORA BAY, (WESTERN ALBANIA)

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The diet of the European pond terrapin (*Emys orbicularis*) is poorly known in Albania. Through this study, we aim to provide some data on the food composition of the species in the area of Vlora Bay (Western Albania). We examined 15 individuals of *E. orbicularis* (10 ♀, 4 ♂ and one juvenile) from two different habitats (Zverneci pond and freshwater channel of Panaja) during 2015. The faecal samples collected (after keeping the animals in small boxes for 24-48 hours) were stored in 70% alcohol and examined under a simple stereomicroscope. Prey items were identified down to the lowest possible systematic level, depending on their digestion state, using aquatic macroinvertebrate keys and a reference collection. Among the prey taxa, insects (frequency% = 100%), cnidaria (6.6%), gastropods (20%) and plant material (6.7%), were frequently consumed by European pond turtles. Food consisted primarily of insects (dominated by dipteran larvae = 80%) and in a low percentage of other invertebrates and plant materials. Our result is in line with some other studies conducted in different regions of Europe, that shows that the European pond turtle is a generalist opportunistic omnivore, whose diet is most strongly influenced by prey availability.

Keywords: European pond turtle, *Emys orbicularis*, diet, Vlora Bay, Albania

P-14

NA VRHU ILI DNU? SUŽIVOT DVIJE VRSTE PETROFILNIH GUŠTERICA NA PLANINI BIOKOVO, HRVATSKA

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Mosorska gušterica endemska je te klasifikacijska vrsta ekološke mreže Natura 2000 s fragmentiranom rasprostranjenošću na planinama Dinarida u Hrvatskoj, Bosni i Hercegovini te Crnoj Gori. Saznanja o rasprostranjenosti u Hrvatskoj bazirana su na nepotvrđenim literaturim podatcima. Kako bi odredili trenutnu rasprostranjenost, tijekom 2017. posjetili smo povijesne lokalitete ove vrste. Na planinama Mosor i Biokovo vrstu smo zabilježili, ali je nismo uspjeli potvrditi na području Opora i Kozjaka. Ipak najviše jedinki je uočeno na Biokovu te smo zbog toga za populacijska istraživanja izabrali poligon na tom području gdje smo proveli istraživanje suživota ove vrste s drugim vrstama gušticama te njihov odabir staništa. Na poligonu samo jedna je petrofilna vrsta prisutna, a radi se o zidnoj gušterici, *Podarcis muralis* koja je simpatrijska vrsta *Dinarolacerta mosorensis*. Općenito, *D. mosorensis* je češća na staništu od P. muralis s omjerom vrsta 2:1 u korist *D. mosorensis*. Većina jedinki (88%) *D. mosorensis* je pronađena na velikim stijenama ili blokovima u prosjeku 2 m od tla dok je *P. muralis* zabilježena na tlu ili na malim stijenama (64%) u prosjeku 0,78 m od tla. Pogledamo li dnevnu aktivnost obje vrste su bile aktivne od 8:00 sati ujutro do 19:00 sati popodne. Naši preliminarni rezultati ukazuju da je *D. mosorensis* još brojna na Biokovu te da korištenje različitih mikrostaništa omogućava joj suživot s *P. muralis* na tom području.

Ključne riječi: Lacertidae, *Dinarolacerta mosorensis*, stanište, *Podarcis muralis*

TOP OR BOTTOM? COEXISTENCE OF TWO PETROPHILOUS LIZARD SPECIES ON MT. BIOKOVO, CROATIA

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The Mosor rock lizard is endemic Natura 2000 species with fragmented distribution in Dinaric Mountains of Croatia, Bosnia & Herzegovina and Montenegro. Knowledge about its occurrence in Croatia is based almost exclusively on unconfirmed historical data. In order to assess its current distribution, during 2017 we visited historical localities for this species. We recorded it on Mosor and Biokovo Mts., but we were not able to find it at Opor and Kozjak Mt. Biggest number of the individuals were observed on Mt. Biokovo, and accordingly, we chose it as a polygon for population studies. There we conducted the survey of species coexistence with other lizard species and their habitat preferences. On the site, only one another petrophileus species, common wall lizard, *Podarcis muralis* occur sympatrically with *Dinarolacerta mosorensis*. In general, *D. mosorensis* is more common on the habitat than *P. muralis*, with

species ratio being 2:1 in favour of *D. mosorensis*. Most of the individuals (88%) of *D. mosorensis* were found on large stone rocks and boulders in average 2 m from the ground while *P. muralis* lizards were found on the ground or on the small rocks (64%) in average 0,78 m from the ground. Regarding daily activity both species were active from 8:00 AM to 7:00 PM. Our preliminary results show that *D. mosorensis* is still numerous on Mt. Biokovo, and the utilization of different microhabitats allows its coexistence with *P. muralis* in the area.

Keywords: Lacertidae, *Dinarolacerta mosorensis*, habitat, *Podarcis muralis*

P-15

URBANI STIL BARSKE KORNJAČE (*Emys orbicularis*)

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Javna ustanova Maksimir za upravljanje zaštićenim područjima Grada Zagreba

Širenjem gradskih područja širom svijeta javlja se potreba zaštite urbane bioraznolikosti. Zaštićeni krajobraz Savica najveće je močvarno područje Grada Zagreba te je naseljeno populacijom ugrožene barske kornjače (*Emys orbicularis*). Kako bi se pojačala zaštita barske kornjače temeljno je poznavati njezinu rasprostranjenost i način života pa je cilj ovog istraživanja dobiti uvid u značajke populacije barske kornjače na Savici. Tijekom sezone populacija je praćena na 5 jezera ukupne površine 18.06 ha, a veličina je procijenjena koristeći Schnabelovu metodu za zatvorene populacije. Ulovljeno je i označeno 78 jedinki, procijenjena im je starost, određen spol te je mjereno 15 morfoloških karakteristika. Godišnja aktivnost barske kornjače na Savici traje od kraja veljače do početka studenog. Populacija je procijenjena na 219 jedinki na promatranoj površini s relativnom gustoćom od 11 kornjača/ha. Omjer spolova je značajno pomaknut u korist mužjaka (1.85 mužjaka : 1 ženka). Uglavnom sve ulovljene jedinke su bile odrasle te su prema abrazijama oklopa svrstane u tri dobna razreda (mlade odrasle 21.33%, srednje stare odrasle 49.33% i stare odrasle 29.33%). Također su utvrđene dvije glavne ugroze za barsku kornjaču na Savici: korištenje jezera za sportski ribolov i prisutnost invazivne vrste *Trachemys scripta*. Stoga je budućim istraživanjima važno utvrditi dinamiku populacije barske kornjače te njeno korištenje staništa.

Ključne riječi: urbana bioraznolikost, ugrožena, populacija

URBAN LIFESTYLE OF EUROPEAN POND TURTLE (*Emys orbicularis*)

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Constant spreading of urban areas urges for the increased preservation of urban biodiversity. Protected landscape, Savica lakes, is the largest wetland habitat in the City of Zagreb and it's inhabited by the population of endangered European pond turtle, *Emys orbicularis*. To intensify its conservation it is crucial to understand its distribution and natural history. Thus, the aim of this study is to get insights into population parameters of pond turtle on Savica lakes, Zagreb, Croatia. For this purpose the population was monitored on the area of 5 lakes (in total 18.06 ha) trough season, and population size was estimated using Schnabel mark-recapture method. We caught and marked 78 individuals, scored their age, sex and 15 morphometric characteristics. The annual activity of pond turtles on Savica lasts from the late February to the beginning of November. Population size of 219 adult turtles was estimated (95% CI, 133-426)

on research area, with relative density of 11 turtles/ha. The sex-ratio was significantly skewed in favour of males (1.85 males : 1 female). Almost all recorded specimens were adults and distributed in 3 age classes based on shield abrasion (young 21.33%, middle aged 49.33% and old 29.33%). Two main threats for the Emys orbicularis population in Savica that we detected are: the use of lakes for sport fishing and the presence of *Trachemys scripta*. For this reason, it is important to determine their habitat use and population dynamic in following studies.

Keywords: urban biodiversity, endangered, population

P-16

CURRENT STATUS OF INTRODUCED SPECIES OF REPTILES IN UKRAINE

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Introduction of species in addition to the impact of climate change and particular anthropogenic factors, has led to significant changes in the biodiversity of certain world regions. For today several reptile species are introduced in the Ukrainian territory: rock lizards *Darevskia armeniaca* (Mehely, 1909) and *Darevskia dahli* (Darevsky, 1957) (Zhytomyr region); geckos *Tenuidactylus fedtschenkoi* (Strauch, 1887) and *Hemidactylus* sp.; wall lizards *Podarcis muralis* (Laurenti, 1768) (Odessa region); pond sliders *Trachemys scripta* (Schoepff, 1792) in different cities of Ukraine. In 2015-2017 a number of expeditions were carried out to the different regions of Ukraine. As a result, *P. muralis* was found in only 3 localities near the city of Reni (Odessa region): abandoned concrete complex in front of the sea port, the first the second road turns across the water canal. In 2016 rock lizards (*D. armeniaca* and *D. dahli*) spreading for more than 9 km along the riverbank from the place of first introduction. *D. armeniaca* juveniles were noticed on the right bank of the Teteriv river for the first time. Well-established population of *T. fedtschenkoi* was confirmed for Odessa, while *Hemidactylus* sp. was found in the seaport of Chornomorsk (Odessa region). Different subspecies of *Trachemys scripta* were repeatedly found to survive wintering in Odessa and other cities (publications are based on the research SFFR F76).

Keywords: climate change, anthropogenic factors, introduced, reptiles, Ukraine

P-17

ZNAČAJKE FAUNE VODOZEMACA I GMAZOVA BIOKOVA, MOSORA I KOZJAKA S ASPEKTA ZAŠTITE PRIRODE

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Tijekom razdoblja od 2006. do 2011. godine provedena su opširna istraživanja faune vodozemaca i gmazova šireg područja planinskog masiva Biokova, Mosora i Kozjaka te je zabilježeno prisustvo ukupno 35 vrsta. Od tog broja zabilježeno je 9 vrsta vodozemaca i 26

vrsta gmazova. Bogatstvu vrsta značajno doprinose visoke planine koje na svojim vršnim dijelovima imaju vrlo karakterističnu endemsku faunu. Najznačajniji herpeto endem ovog područja jest *Dinarolacerta mosorensis* koja bi trebala biti i krovna vrsta za zaštitu. Za područje Parka prirode Biokovo zabilježeno je ukupno 7 vrsta vodozemaca i 21 vrsta gmazova te se zajedno s Mosorom sa 8 vrsta vodozemaca i 21 vrstom gmazova može smatrati najbolje istraženim dijelom našeg obuhvatnog područja. Za područje Kozjaka zabilježeno je svega 5 vrsta vodozemaca i 15 vrsta gmazova. Ipak, ovaj masiv (Biokovo, Mosor i Kozjak) potrebno je promatrati kao kontinuirano stanište i jednaku pozornost posvetiti i zaštiti preostalog područja (južni masiv Biokova, Mosor i Kozjak). Navedeni prostor obuhvaća i vodene mase kao što su rijeke Cetina, Žrnovnica i Jadro koje su važna staništa za vrste poput *Emys orbicularis* i *Natrix tessellata*. Alohtona invazivna vrsta *Trachemys scripta* bilježi se u manjim vodenim masama poput lokvi, a *Chelonia mydas* i *Caretta caretta* zabilježene su u priobalju. Prve korake u zaštiti ovoga preostalog područja donosi i njihovo uključivanje u ekološku mrežu NATURA2000 na području Republike Hrvatske.

Ključne riječi: herpetofauna, zaštita, NATURA2000, „hotspot“

FEATURES OF AMPHIBIANS AND REPTILES FAUNA OF BIOKOVO, MOSOR AND KOZJAK IN TERMS OF NATURE CONSERVATION

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During the period of 2006-2011 we carried out an extensive research of amphibians and reptiles in mountain massive of Biokovo, Mosor and Kozjak and recorded the presence of 35 species.Of this number there were 9 species of amphibians and 26 species of reptiles. High mountains which have a very characteristic endemic fauna at their peak areas significantly contribute to species richness.The most significant endemic species of this area is *D. mosorensis* which should be an umbrella species for protection. By the number of species recorded it is obvious that the area of Biokovo NP with 7 species of amphibians and 21 species of reptiles together with Mosor area with 8 species of amphibians and 21 species of reptiles are the best surveyed parts of our research area.Kozjak area follows with only 5 species of amphibians and 15 species of reptiles recorded.However, this whole area of Biokovo, Mosor and Kozjak should be considered as a continuous habitat and the same level of attention should be given to the areas outside the NP (southern Biokovo, Mosor and Kozjak). This area also includes the water bodies such as Cetina, Žrnovnica and Jadro rivers that provide important habitats for *E. orbicularis* and N*. tessellata*. Allochthonous invasive species *T. scripta* was only recorded in few very small ponds and *C. mydas* and *C. caretta* only sporadically in coastal areas. First steps to wider protection of the areas outside the NP was made by their inclusion in the ecological network NATURA2000.

Keywords: herpetofauna, protection, NATURA2000, „hotspot“

P-18
HERPETOFAUNA ZNAČAJNOG KRAJOBRAZA „SUTINA“

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Tijekom 2016. i 2017. godine provedena su istraživanja faune vodozemaca i gmazova na području značajnog krajobraza „Sutina“ te je zabilježeno ukupno 15 vrsta. Od tog broja zabilježeno je 5 vrsta vodozemaca (*Salamandra salamandra* (Linnaeus, 1758), *Bombina variegata* (Linnaeus, 1758), *Bufo bufo* (Linnaeus, 1758), *Pelophylax ridibundus* (Pallas, 1771), *Rana dalmatina* Fitzinger in Bonaparte, 1838)) i 10 vrsta gmazova (*Algyroides nigropunctatus* (Duméril & Bibron, 1839), *Dalmatolacerta oxycephala* (Duméril & Bibron, 1839), *Lacerta bilineata* Daudin, 1802, *Podarcis melisellensis* (Braun, 1877), *Podarcis muralis* (Laurenti, 1768), *Coronella austriaca* Laurenti, 1768, *Zamenis longissimus* (Laurenti, 1768), *Natrix natrix* (Linnaeus, 1758), *Malpolon insignitus* (Geoffroy Saint-Hilaire, 1827), *Vipera ammodytes* (Linnaeus, 1758)). Od 15 zabilježenih vrsta njih 2 se nalaze u Crvenoj knjizi vodozemaca i gmazova Hrvatske: *Bombina variegata kolombatovici* i *Podarcis melisellensis* u kategoriji najmanje zabrinjavajuće vrste (LC).

Ključne riječi: herpetofauna, Sutina, vodozemci, gmazovi

HERPETOFAUNA OF SIGNIFICANT LANDSCAPE “SUTINA”

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During 2016 and 2017 the reserch of amphibaian and reptilian fauna in the area of significant landscape „Sutina“ was carried out and the presence of 20 species was recorded. Of this number, there were 5 species of amphibians - (*Salamandra salamandra* (Linnaeus, 1758), *Bombina variegata* (Linnaeus, 1758), *Bufo bufo* (Linnaeus, 1758), *Pelophylax ridibundus* (Pallas, 1771), *Rana dalmatina* Fitzinger in Bonaparte, 1838)) – and 10 species of reptiles – (*Algyroides nigropunctatus* (Duméril & Bibron, 1839), *Dalmatolacerta oxycephala* (Duméril & Bibron, 1839), *Lacerta bilineata* Daudin, 1802, *Podarcis melisellensis* (Braun, 1877), *Podarcis muralis* (Laurenti, 1768), *Coronella austriaca* Laurenti, 1768, *Zamenis longissimus* (Laurenti, 1768), *Natrix natrix* (Linnaeus, 1758), *Malpolon insignitus* (Geoffroy Saint-Hilaire, 1827), *Vipera ammodytes* (Linnaeus, 1758)). Two (2) of the 15 species recorded are included in the Red Book of amphibians and reptiles of Croatia: *Bombina variegata kolombatovici* and *Podarcis melisellensis* in the category least concern species (LC).

Keywords: herpetofauna, Sutina, amphibia, reptilia

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CONTRIBUTION TO THE KNOWLEDGE OF DISTRIBUTION OF HERPETOFAUNA IN KOSOVO

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In this field report we present the results of a 9-day field trip to Kosovo during the months of April and May 2018. The trip was organized by DŠB, Biology Students' Society. During the trip we sampled different localities in Kosovo focusing on the area around Prizren. The fieldwork was carried out in two groups of a mentor and volunteers, one group focused on the group Reptilia the other on Amphibia. The main focus of the field trip was to present Slovene students with the diversity of the Balkan herpetofauna and variety of the ecosystems found in Kosovo. We focused our fieldwork on localities with an already known high diversity of herpetofauna as well as potential localities for various reptile and amphibian species as presented in previous studies by Tomović et al. (Reptiles in Serbia: Distribution and diversity patterns. Bulletin of the Natural History Museum, 2014) and Vukov et al. (Amphibians in Serbia: distribution and diversity patterns. Bulletin of the Natural History Museum, 2013). Here we present our findings by general localities. We believe that the work of volunteering students has significant value in collecting the distributional knowledge of reptiles and amphibians in the Balkans and gives a unique perspective that adds additional value to the individual interest in studying biology in this region as well as provides robust data for further analysis of distributional patterns.

Ključne riječi: Reptiles, Amphibians, Kosovo, distribution

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THE POPULATION CHARACTERISTICS OF THE YELLOW-BELLIED TOAD (*Bombina variegata*) IN THE MRTVICA RIVER CANYON

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Southern and central parts of Montenegro are one of the richest biodiversity parts of the Balkans. However, systematic studies of distribution and diversity patterns of batrahofauna are still lacking. These are one of the most important studies in determining protected areas, and the planning of conservation activities of species and habitats. During the last decade in Montenegro, there is an increase in urbanization and infrastructure development at the expense of destruction and degradation of habitats. If such a trend continues habitats of many species will be reduced to minimum. For this reason research and protection of biodiversity are a priority, especially of sensitive batrahofauna. This is part of the larger research that deals with the research and protection of the biodiversity of the Morača River. The focus of this research was the yellow-bellied toad in the canyon of the Mrtvica River, one of the tributaries of a larger Morača river. During the field research yellow-bellied toad was found at 7 locations along the river. In order to determine the density of yellow-bellied toad populations, the linear transect methodology was used. The results showed that the population of yellow-bellied toad in the Mrtvica River is relatively large and healthy. Canyon of Mrtvica River is an extremely important location for this Natura 2000 species, and therefore it should be legally protected. We suggest inclusion of Morača Canyon into proposed Natura 2000 ecological network.

Ključne riječi: Montenegro, protection, river, canyon, research

2. HRVATSKI SIMPOZIJ BIOLOGA U ZDRAVSTVU

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ULOGA MOLEKULARNE KARIOTIPIZACIJE U RAZJAŠNJAVANJU ETIOLOGIJE RAZVOJNOG ZAOSTAJANJA

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Intersticijske delecije dugog kraka kromosoma 14 vrlo su rijetke ako se isključe slučajevi ring kromosoma 14 ili translokacije. Klinička slika ovisi o veličini i mjestu delecije genetičkog materijala, a prema dostupnoj literaturi bolesnici dijele zajedničke fenotipske osobine, ponajprije blagu dismorfiju lica i razvojno zaostajanje. Primjenom metode molekularne kariotipizacije (ArrayCGH), koja ima znatno veću moć razlučivanja od metode klasične kariotipizacije, znatno je olakšano istraživanje učinka genotipa na fenotip zbog mogućnosti točnog određivanja mjesta loma. Prikaz slučaja: Djevojčica u dobi od 7 godina upućena je na obradu razvojnog zaostajanja. Indicirana je molekularna kariotipizacija, kojom je u regiji 14q31.3-q32.13 nađena de novo intersticijska delecija kromosoma 14 veličine 8.3Mb. Usporedba fenotipa naše bolesnice s ranije objavljenim slučajevima potvrđuje prisutnost zajedničkih fenotipskih karakteristika te naglašava važnost upotrebe metode aCGH kao dijagnostičkog alata u razjašnjavanju etiologije razvojnog zaostajanja.

Ključne riječi: 14q intersticijska delecija, Komparativna genomska hibridizacija na čipu (aCGH), Razvojno zaostajanje

THE ROLE OF MOLECULAR KARYOTYPING IN CLARIFYING THE ETIOLOGY OF DEVELOPMENTAL DELAY

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If we exclude the cases of ring chromosome 14 or translocation, interstitial deletions of the long arm of chromosome 14 are very rare. The clinical picture depends on the size and location of deletion of genetic material. According to the available literature, patients share common phenotypic characteristics, primarily mild facial dysmorphia and developmental delay. By applying the method of molecular karyotyping (array CGH), which compared with the

conventional karyotype has a much higher resolution, the possibility of accurate breakpoint determination improved the analysis of genotype-phenotype correlations. Case presentation: A 7-year-old girl was referred due to developmental delay. Molecular karyotyping was indicated and de novo interstitial deletion of chromosome 14 was found in the region 14q31.3–q32.13 with the size of 8.3Mb. Comparison of our pateint´s phenotype with previously reported cases confirme the presence of common clinical features and highlights the utility of aCGH as a diagnostic tool in clarifying the etiology of developmental delay.

Keywords: Chromosome 14q interstitial deletion, Array CGH, Developmental delay

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VAŽNOST MULTIDISCIPLINARNOG PRISTUPA U BOLNIČKOM SUSTAVU: UTJECAJ MIKROKLIME I UVJETA RADA NA OSJETLJIVOST MIKROBNOG EKOSUSUSTAVA U BOLESNIKA NAKON OPSEŽNIH OPERATIVNIH ZAHVATA NA GLAVI I VRATU

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Većina operacija u otorinolaringologiji spada u kategoriju čistih-kontaminiranih operacija. Učestalost infekcija kod ovih operacija je 8-11%, a kod velikih, opsežnih zahvata na glavi i vratu čak 28-87%. Zbog toga je za ovu skupinu operacija indicirana rutinska antimikrobna profilaksa (AP: metronidazol i cefazolin/klindamicin). Cilj istraživanja je bio procijeniti utjecaj promjene mikroklime i uvjeta rada na osjetljivost mikrobnog ekosustava (ME) u bolesnika nakon preseljenja s lokacije Šalata na lokaciju Rebro. U ovom istraživanju je korištena baza podataka 89 pacijenata operiranih u KBC-u Zagreb (Klinika za bolesti uha, nosa i grla i kirurgiju glave i vrata). U poslijeoperativnom razdoblju (nakon 24/72/102 sata) praćeni su upalni parametri (lokalni, CRP, WBC) i mikrobiološki (MB) nalazi (bris traheostome, rane i usne šupljine). U prva 2 mjeseca od preseljenja nalazi: (a) WBC i CRP-a ukazuju na značajan porast ($p<0,0001$); (b) MB ukazuju na promjenu osjetljivosti bakterija: G (-) 93% na gentamicin i 18% na cefazolin; G (+) 19% na cefazolin i klindamicin. Revidirani su protokoli za prevenciju bolničkih infekcija, te AP u gentamicin i klindamicin. Tijekom 7 mjeseci sustavnog praćenja zabilježen je značajan pad WBC i CRP-a u odnosu na prethodno razdoblje ($p<0,00001$). Promjena lokacije je značajno narušila mikroklimu stacionara s čime se promijenio ME stacionara, a u skladu s time i osjetljivost ME bolesnika na AP korištenu na lokaciji Šalata.

Ključne riječi: mikroklima, mikrobni ekosustav, bolničke infekcije, otorinolaringologija

THE IMPORTANCE OF MULTIDISCIPLINARY APPROACH IN THE HOSPITAL SYSTEM: INFLUENCE OF MICROCLIMATE AND WORKING CONDITIONS ON THE SENSITIVITY OF THE MICROBIAL ECOSYSTEM IN PATIENTS WHO HAD EXTENSIVE SURGERY ON THE HEAD AND NECK

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Most of the operations in otorhinolaryngology belong to the category of clean-contaminated operations. The frequency of infections in these operations is 8-11%, and in large, extensive interventions on the head and neck 28-87%. Therefore, is indicated routine antimicrobial prophylaxis (AP: metronidazole and cefazolin/clindamycin). The aim of this study was to assess the influence of microclimate changes and working conditions on microbial ecosystem sensitivity (ME) in patients after relocation from location Šalata to Rebro site. This study used database of 89 patients who had surgery at Department of Otorhinolaryngology. In the postoperative period (after 24/72/102 hours), inflammatory parameters (local, CRP, WBC) and microbiological (MB) analysis (smear of tracheostomy, wounds and mouth cavities) were observed. In the first 2 months after changing the location: (a) WBC and CRP indicate a significant increase ($p < 0.0001$); (b) MB indicate changes in bacterial sensitivity: G (-) 93% on gentamicin and 18% on cefazolin; G (+) 19% on cefazolin and clindamycin. We have revised protocols for prevention of hospital infections, also AP in gentamicin and clindamycin. During the 7 months of systematic monitoring, there was a significant decrease of WBC and CRP compared to the previous period ($p<0.00001$). The change of location has significantly disrupted microclimate and ME of stationary and consequently the sensitivity of the ME patients to the AP used at location Šalata.

Keywords: microclimate, microbial ecosystem, hospital infections, otorhinolaryngology

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NACIONALNI MONITORING INVAZIVNIH VRSTA KOMARACA U HRVATSKOJ OD 2016 DO 2018. GODINE

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Inicijativom Hrvatskog zavoda za javno zdravstvo u 2016. godini je započet, a tijekom 2017. i 2018. nastavljen nacionalni monitoring invazivnih vrsta komaraca. Determinacija komaraca je provedena u Zagrebu, Puli, Splitu, Rijeci i Osijeku. Ovipozicijskim klopkama su od svibnja do studenog, dva puta mjesečno prikupljani podaci o nalazima invazivnih komaraca roda *Aedes*, osobito u područjima za koje nisu postojali podaci. Ovipozicijske klopke su relativno jednostavna i jeftina metoda za nadzor i rano otkrivanje invazivnih vrsta komaraca na mjestima ulaska. Podaci o zastupljenosti komaraca na području Hrvatske služe za izradu karte

rasprostranjenosti žarišta, izradu jedinstvene nacionalne baze podataka te procjenu rizika za vektorske zarazne bolesti. Provedeni nacionalni monitoring potvrdio je prisutnost azijskog tigrastog komarca - *Aedes (Stegomyia) albopictus* (Skuse, 1895) u svim županijama. Potvrđen je i nalaz komarca *Aedes (Finlaya) japonicus japonicus* (Theobald) u županijama gdje tijekom 2016. nije bio zabilježen. Postojeći uvjeti potrebni za razvoj i razmnožavanje te prisutni vektorski potencijal determiniranih komaraca uz eventualnu pojavnost uzročnika mogu imati značajan utjecaj na širenje zaraznih bolesti. Prekomjerna brojnost jedinki komaraca postaje sve izraženiji problem za ljudsko zdravlje te je potrebno osvijestiti javnost o važnosti i načinima sprječavanja razmnožavanja i širenja komaraca. Provedba monitoringa je odličan primjer suradnje uključenih institucija.

Ključne riječi: Hrvatska, nacionalni monitoring, invazivne vrste komaraca, ovipozicijske klopke, baza podataka, procjena rizika

NATIONAL MONITORING OF INVASIVE MOSQUITO SPECIES IN CROATIA FROM 2016 TO 2018

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Based on the Croatian Institute of Public Health initiative in 2016 started and during 2017 and 2018 continued national monitoring of invasive species of mosquitoes. Determination was conducted in Zagreb, Pula, Split, Rijeka and Osijek. By oviposition traps data of *Aedes* species were collected from May to November particularly in areas where there is no data yet. Oviposition traps are simple and inexpensive method for monitoring and early detection of invasive species of mosquitoes at points of entry. Collected data will be used for creation of distribution maps, national database and vector surveillance and risk assessment of the disease. Conducted national monitoring confirmed the presence of the Asian tiger mosquito *Aedes (Stegomyia) albopictus* (Skuse, 1895) in all counties. Also, during the monitoring through oviposition traps the eggs of another invasive mosquito species *Aedes (Finlaya) japonicus japonicus* (Theobald) were sampled. Existing conditions, necessary for the development and reproduction, with present vector potential of determined mosquitoes with the possible occurrence of pathogens, can have a significant impact on the spread of infectious diseases. Excessive number of mosquitoescan become a problem for human health and it is necessary to educate the public about importance and ways of suppressing breeding of mosquitoes and their expansion. Implementation of monitoring is an excellent example of cooperation of all included institutions

Keywords: Croatia, National monitoring, invasive mosquitoe species, ovitraps, databases, risk assessment.

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OTKRIVANJE POREMEĆAJA GENOMSKOG UTISKIVANJA METODOM MS MLPA

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Utisnuti geni eksprimiraju se samo s jednog alela, ovisno o roditeljskom porijeklu. Temelj genomskog utiskivanja je DNA metilacija. U ovom se radu kod pacijenata sa sumnjom na poremećaj genomskog utiskivanja, kao Beckwith-Wiedemann (BWS) i Russell-Silver (RSS) sindromi te Prader-Willi (PWS) i Angelman (AS) sindromi, primjenjivala metoda metilacijski-specifičnog višestrukog umnažanja vezanih probi (MS MLPA) za otkrivanje promjena u broju kopija ciljanih regija genoma i metilacijskog statusa. Setovi MS-MLPA proba za ME030 BWS/RSS i ME028 PWS/AS koriste se od proizvođača MRC-Holland, Amsterdam/NL. ME030 BWS/RSS set sadrži 26 proba specifičnih za regiju 11p15, od kojih 10 proba sadrži prepoznatljivo Hha1 metilacijski-specifično mjesto. ME028 PWS/AS set sadrži 34 probe specifične za gene u PWS/AS kritičnoj regiji 15q11-q12. Među njima je šest proba koje sadrže Hha1 restrikcijsko mjesto za otkrivanje aberantnog metilacijskog obrasca. Od 43 pacijenta u dva pozitivna slučaja RSS nađena je H19 hipometilacija, dok su četiri slučaja hipometilacije gena KCNQ1 potvrdila BWS. U sedam je slučajeva setom PWS/AS nađena hipermetilacija SNRPN i MAGEL2 gena i/ili promjena u broju kopija gena. MS MLPA je korisna metoda za precizno i brzo otkrivanje te razlikovanje promjena u broju kopija i metilacijskog statusa kod bolesnika kod kojih postoji sumnja na poremećaje BWS/RSS i PWS/AS. Genetičko savjetovanje neophodno je za odabir genetičkog testiranja.

Ključne riječi: MS MLPA, metilacijski status, poremećaji genomskog utiskivanja

DIAGNOSTICS OF GENOMIC IMPRINTING DISORDERS BY MS MLPA

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Imprinted genes are monoallelically expressed depending on the parental origin of the alleles. The basis of the genomic imprinting is the DNA methylation. In this study we applied methylation-specific multiplex ligation-dependent probe amplification (MS MLPA) in patients with suspected imprinting disorders such as Beckwith-Wiedemann (BWS) and Russell-Silver Syndrome (RSS), Prader-Willi (PWS) and Angelman (AS) Syndrome, to detect copy number changes and methylation status. The MS-MLPA probe mixtures ME030 BWS/RSS and ME028 PWS/AS were used from the MRC-Holland, Amsterdam/NL manufacturer. ME030 BWS/RSS probemix contains 26 probes specific for 11p15 region, so 10 of these are methylation-specific since they contain an Hha1 recognition site. ME028 PWS/AS probemix contains 34 probes specific to the genes in the PWS/AS critical region (15q11-q12). Six of these probes contain a

sensitive Hha1 restriction site for detection aberrant methylation patterns in the 15q11 locus. Out of 43 patients, in RSS two cases were positive for H19 hypomethylation, four cases with KCNQ1 hypomethylation confirmed BWS. The diagnosis was confirmed in seven cases with PWS/AS probemix, hypermethylation of SNRPN and MAGEL2 genes and/or copy number changes.
MS MLPA is a useful method for precise and rapid detection and for distinguishing copy number variation and methylation status in patients with BWS/RSS and PWS/AS disorders. Genetic counseling is essential for the selection of genetic testing.

Keywords: MS MLPA, Methylation status, Genomic imprinting disorders

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MUTACIJE GENA ZA NASLJEDNU HEMOKROMATOZU KOD PACIJENATA S INFARKTOM MIOKARDA

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Nasljedna hemokromatoza je poremećaj nakupljanja željeza u tkivima s kojim su povezane koronarne bolesti srca. Nakupljanjem željeza nastaju slobodni radikali i reaktivni kisikovi radikali koji potiču oksidaciju LDL kolesterola što može dovesti do razvoja ateroskleroze. Istraživanja su pokazala da nositelji mutacija gena HFE mogu imati veći rizik za razvoj kardiovaskularnih bolesti od onih bez mutacija. Cilj ovog istraživanja je utvrditi učestalosti mutacija gena HFE za nasljednu hemokromatozu kod pacijenata s infarktom miokarda u odnosu na zdravu skupinu u istočnoj Slavoniji. Provedeno je retrospektivno istraživanje slučajeva i kontrola na populaciji od 400 ispitanika. U prvoj skupini je bilo 200 pacijenata (114 muškaraca i 86 žena) koji su preboljeli infarkt miokarda. Drugu skupinu su činili kardiološki zdravi ispitanici (103 muškaraca i 97 žena). Svi pacijenti su genotipizirani na tri najčešće mutacije gena HFE karakteristične za nasljednu hemokromatozu: Cys282Tyr, His63Asp i Ser65Cys, metodom PCR-a u stvarnom vremenu. Učestalosti nositelja ove tri mutacije između pacijenta i kontrola nisu bile značajne (Cys282Tyr: 4,5 nasuprot 8,1%; His63Asp: 19 nasuprot 24,5%; Ser65Cys: 3,5 nasuprot 4%), kao ni učestalost i raspodjela mogućih haplotipova gena HFE u dvije skupine. Ovim istraživanjem nije pronađena povezanost mutacija gena HFE za nasljednu hemokromatozu s infarktom miokarda u populaciji istočne Slavonije.

Ključne riječi: HFE, hemokromatoza, infarkt miokarda, PCR u stvarnom vremenu

HEREDITARY HEMOCHROMATOSIS GENE MUTATIONS IN PATIENTS WITH MYOCARDIAL INFARCTION

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Hereditary hemochromatosis is a disorder of iron accumulation in tissues, which is related to coronary heart diseases. Free radicals and reactive oxygen species, created because of iron deposition, promote oxidation of LDL cholesterol and could lead to the development of atherosclerosis. Studies have shown that HFE gene mutation carriers might be at higher risk of developing cardiovascular diseases compared with non-carriers. This study aimed to determine the frequency of HFE gene mutations in hereditary hemochromatosis in patients with myocardial infarction compared to a healthy group in eastern Slavonia. A retrospective case-control study was carried out on a population of 400 participants. In the first group were 200 patients (114 males and 86 females) with myocardial infarction. The second group consisted of 200 controls (103 males and 97 females) without a history of cardiovascular diseases. All patients were genotyped for the three most common mutations of the hereditary hemochromatosis in the HFE gene: Cys282Tyr, His63Asp, and Ser65Cys, by real-time PCR. The frequency of carriers of these mutations between patients and controls was not significant (Cys282Tyr: 4.5 vs. 8.1%; His63Asp: 19 vs. 24.5%; Ser65Cys: 3.5 versus 4%), as well as frequency and distribution of possible HFE gene haplotypes. In this study was not found an association of the HFE gene mutation for hereditary hemochromatosis with myocardial infarction in the population of Eastern Slavonia.

Keywords: HFE, hemochromatosis, myocardial infarction, real-time PCR

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CITOGENETIČKA ANALIZA SPONTANIH POBAČAJA: PETOGODIŠNJA RETROSPEKTIVNA STUDIJA

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Otprilike 10-15% klinički prepoznatih trudnoća završi spontanim pobačajem, dok se najčešćim uzrokom smatraju kromosomske abnormalnosti koje se nađu u više od 50% ranih gubitaka trudnoće. Kariotipizacija spontanih pobačaja rutinski se provodi klasičnom citogenetičkom analizom uzoraka kože ploda i/ ili korionskih resica. Cilj ovog istraživanja bio je odrediti učestalosti pojedinih kromosomopatija kod spontanih pobačaja, usporediti uspješnost različitih metoda kultivacije, odrediti utječe li dob trudnica na pojavu pojedinih kromosomopatija i gestacijsku dob pobačenog ploda. Citogenetička analiza je uspješno provedena u 214 od ukupno 253 uzorka spontanih pobačaja (84,6%). Abnormalan kariotip nađen je kod 48,6% plodova, dok su najčešće kromosomopatije bile monosomija X (15,38%), triploidija (11,54%), te trisomije 18 (12,5%), 16 (9,62%) i 22 (9,62%). Utvrđeno je da ne postoji povezanost dobi majke i gestacijske dobi pobačenog ploda. Nadalje, potvrđeno je da je dob majke u pozitivnoj korelaciji s pojavom trisomija, dok povezanost s pojavom monosomije X i triploidije ne postoji. Usporedbom uspješnosti različitih metoda kultivacije uočena je 100%-tna uspješnost kod paralelne analize tkiva posteljice i tkiva kože, dok je u slučaju samostalne kultivacije korionskih

resica uspješnost bila 93,85%, a u slučaju tkiva kože 35,14%. Također, metoda dugotrajne kulture stanica mezenhimalne strome pokazala se kao bolji izbor od kratkotrajne kulture stanica citotrofoblasta.

Ključne riječi: citogenetička analiza, kromosomopatije, metode kultivacije tkiva, spontani pobačaj

CYTOGENETIC STUDIES OF SPONTANEOUS MISCARRIAGES: A RETROSPECTIVE ANALYSIS OF FIVE-YEAR DATA

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About 10-15% of all recognized pregnancies end in spontaneous abortion, while chromosomal abnormalities are considered as the most common cause, found in more than 50% of first-trimester miscarriages. Chromosomal analysis of products of conception is performed by conventional karyotyping of cultured chorionic villi and/or fetal skin samples. The aim of this study was to determine the incidence of chromosomal abnormalities in spontaneous abortion, to compare the success of different tissue cultivation techniques, and to determine possible effect of maternal age on the occurrence of individual chromosomopathy and gestational age of aborted fetus. Out of 253 samples cytogenetic analysis was successful in 214 cases (84.6%). Chromosomal abnormalities were found in 48,6% fetuses, while the most commonly observed where monosomy X (15.38%), triploidy (11.54%), and trisomies 18 (12.5%), 16 (9.62%) and 22 (9.62%). It was found that there is no correlation of maternal age and gestational age at abortion. Positive correlation of maternal age with the occurrence of trisomies, but not with monosomy X and triploidy was confirmed. Comparison of different tissue cultivation methods showed 100% success rate when both skin and placental tissues were cultivated, 93.85% when only chorionic villi were available, and 35.14% for isolated skin sample culturing. Furthermore, long-term mesenchymal stroma culture has been shown as a technique with higher success in comparison with short-term culture.

Keywords: cytogenetic analysis, chromosomal abnormalities, tissue cultivation techniques, spontaneous abortion

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DNA METILACIJA I PRIROĐENE SRČANE GREŠKE U DJECE S DOWN SINDROMOM

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DNA metilacija predstavlja ključan epigenetički mehanizam koji ima značajnu ulogu u regulaciji genske aktivnosti tijekom razvoja srca. Prirođene srčane greške (PSG) jedne su od najčešćih abnormalnosti koje se javljaju u 40%-60% slučajeva s Down sindromom (DS). Osnovni cilj ovog rada bio je utvrditi povezanost globalne DNA metilacije u djece s DS i prisustva prirođenih srčanih grešaka. Materijali i metode: Globalna DNA metilacija analizirana je u 42 osobe s DS-om u limfocitima periferne krvi, kvantifikacijom metilacije LINE-1 pomoću MethyLight metode. Rezultati: Nema statistički značajne razlike u vrijednosti globalne DNA metilacije u ispitanika s DS-om ovisno o prisutnosti PSG-a (P=1,000), ali ju statistički značajno opisuje spol. Utvrđena je značajna vjerojatnost od 19,1% da će ispitanici s DS-om ženskog spola imati niže vrijednosti globalne DNA metilacije u odnosu na ispitanike s DS-om muškog spola (P<0,05) (R2=19,1%; P=0,025). Zaključak: Spol se pokazao kao statistički značajan prediktor globalne DNA metilacije. Iako razlika nije bila statistički značajna,osobe ženskog spola imale su niže vrijednosti globalne DNA metilacije (P=0,068). Studije na ispitanicima s DS-om su pokazale veću učestalost PGS-a u ženskog spola nego u muškog spola. Daljnja istraživanja na većem broju ispitanika razjasniti će ulogu niže globalne DNA metilacije u osoba s DS-om ženskog spola u nastanku prirođenih srčanih grešaka.

Ključne riječi: globalna DNA metilacija, prirođene srčane greške, Down sindrom

DNA METHYLATION AND CONGENITAL HEARTH DEFECTS IN CHILDREN WITH DOWN SYNDROME

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DNA methylation is a key epigenetic mechanism that plays a significant role in regulating gene activity during cardiac development. Congenital heart defects (CHD) are one of the most common abnormalities occurring in 40% -60% of cases with Down Syndrome (DS). The main aim of this study was to establish the association of global DNA methylation in children with DS and the presence of CHD. Materials and methods: The global DNA methylation was investigated on a sample of 42 people with DS in peripheral blood lymphocytes by quantification of LINE-1 methylation using MethyLight method. Results: No significant differences in global DNA methylation were found according to the presence of CHD (P=1.000), but values of global DNA methylation were significantly influenced by gender (R2=19.1%; P=0.025). Significant probability of 19.1% was found in women with DS who had lower global DNA methylation values than DS male (P <0.05) (R2 = 19.1%; P = 0.025). Conclusion: Gender was statistically significant predictor of global DNA methylation, although the difference was not statistically significant, female subjects had lower global DNA methylation values (P = 0.068). Studies on DS patients showed higher incidence of CHD in female than in male. Further researchs on a large number of subjects will clarify the role of lower global DNA methylation in DS women in the formation of CHD.

Keywords: global DNA methylation, congenital heart defects, Down syndrome

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ISPITIVANJE LEGIONELA U VODI

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Ispitivanje vode na prisutnost legionela provodi se prema standardiziranoj metodi kultivacije propisanoj ISO standardom (HRN EN ISO 11731:2017- Brojenje Legionella) koja je primjenjiva na sve vrste voda. Izolacija legionela uključuje postupke direktnog nacjepljivanja i membranske filtracije te upotrebu selektivnih i visokoselektivnih podloga za kultivaciju i potvrđivanje. Standardizirani postupak bira se na temelju 4 osnovna koraka; određivanje matriksa, odabiru tehnike ispitivanja, vrste tretmana i mikrobiološke podloge koja se koristi za kultivaciju. Postupak identifikacije nije strogo propisan samim standardom, a u okviru prihvatljivih metoda za identifikaciju u Laboratoriju za mikrobiološke analize voda koriste se lateks aglutinacijski test i MALDI-TOF. U procesu je i ispitivanje efikasnosti komercijalnog testa za dokazivanje i brojenje *Legionella pneumophila*, temeljenog na MPN metodi. Problematika ispitivanja tiče se karakteristika legionela i kompleksnosti njihovog uzgoja, nedostatka precizne i jasne legislative po pitanju maksimalno dopuštenih koncentracija legionela i ocjene sukladnosti, neusklađenosti iskazivanja rezultata molekularnih metoda s metodom kultivacije, i samim time cjenovne i praktične neprihvatljivosti molekularnih metoda u službenim akreditiranim laboratorijima.

Ključne riječi: *Legionella*, voda, mikrobiološka analiza

TESTING WATER FOR LEGIONELLA

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Testing water for *Legionella* is based on cultivation method prescribed by the ISO standard (HRN EN ISO 11731: 2017- Enumeration of Legionella) and is applicable to all kinds of water samples. Legionella isolation involves techniques of direct plating and membrane filtration, and the use of selective and highly selective mediums for cultivation and confirmation of this bacteria. The method procedure is chosen based on 4 main steps; determining the type of water to be analyzed, the selection of the examination technique, the type of the treatment that will be used and selection of the medium to be used for cultivation. The identification procedure is not strictly prescribed by the standard. Laboratory for Microbiological Analysis of Water uses latex agglutination test and MALDI-TOF as procedures for identification of *Legionella*. At his moment the Laboratory is testing the effectiveness of a commercial test for the detection and counting of *Legionella pneumophila* that is based on the MPN method. Issues arising during testing water for Legionella are caused by Legionella's characteristics and complexity of cultivation, the lack of precise and clear legislation on maximum permitted concentrations and conformity assessment, the mismatch between the results of molecular

methods with the cultivation method, and thus the pricing and practical unacceptability of molecular methods in official and accredited laboratories.

Keywords: *Legionella*, water, microbiological analysis

3. SIMPOZIJ EDUKACIJE BIOLOGIJE
3rd BIOLOGY EDUCATION SYMPOSIUM

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BILJKO, JESI LI ŽEDNA? ISTRAŽIVAČKO UČENJE KORIŠTENJEM BBC MICRO:BITA

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Ovo je analiza učeničkog istraživačkog projekta u kojem se koristio BBC micro:bit. Cilj je postavljen dvojako, s jedne strane to je analiza potrebe biljke za vodom u trima različitim uvjetima, odnosno iskustvene spoznaje o ekologiji i fiziologiji jedne biljne vrste, ali s metodičke strane ciljevi su bili: testirati mogućnosti i primjenu micro:bita u nastavi biologije, primijeniti metodologiju istraživačkog učenja (IBL) te povećati digitalne kompetencije učenika. Promatran je razvoj dvaju lončanica iz roda Spathyphillum u različitim uvjetima. Jedna je zalijevana automatski (micro:bit set za zalijevanje) (A), a drugu su zalijevali učenici (B) uz korištenje senzora za vlažnost tla. Rezultati su pokazali da je automatski zalijevana biljka (A) u svim uvjetima trošila više vode. Na sunčanom i toplijem mjestu obje su biljke mirovale, a na sjenovitom mjestu biljka (A) je procvjetala i trošila više vode. Nakon zamijene humusnog tla pijeskom obje su biljke trošile više vode. Većina rezultata su očekivani, ali iskustveni uvid omogućio je šire shvaćanje važnosti pojedinog zaključka. Primjerice, danas je značajna učinkovitost automatiziranih procesa kao i globalna nužnost navodnjavanja poljoprivrednih površina. Zatim, potrebno je poznavati ekološke uvjete koji odgovaraju određenoj biljnoj vrsti (u ovom slučaju visoka vlažnost i sjenovito stanište), kao i osnove njene fiziologije (ovdje povećana potrošnja vode za vrijeme cvatnje), u svrhu suvremenog i tehnologijom poduprtog uzgoja.

Ključne riječi: BBC micro:bit, istraživačko učenje, set za zalijevanje biljke, Spathiphyllum

PLANT, ARE YOU THIRSTY? INQUIRY-BASED LEARNING USING BBC MICRO:BIT

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This is an analysis of a student research project where BBC micro:bit was used. The objective is set in two ways: on the one hand, this is an analysis of plant water needs in three different conditions and achievement of experiential knowledge on the ecology and physiology of a plant species, but from the other hand, methodical objectives were: to test the application of micro: bit in biology projects, apply the IBL methodology and increase students digital competences. The development of two Spathiphyllum specimens in different conditions was observed. One was watered automatically (micro: bit watering set) (A), and the other by students (B) using a soil moisture sensor. The results showed that automatically watered plant (A) used more water in all conditions. In the sunny and warmer place both plants stagnated, and in a shady place, the plant

(A) flourished and therefore consumed more water. After replacing humus soil with sand, both plants consumed more water. Although most of the results were expected, the students experience gained a wider understanding of a particular conclusion. For example, the great importance of automated processes in the global need for irrigation of agricultural surfaces. Also, it is useful to understand the ecological conditions preferred by specific species (in this case, high humidity and shady habitat), as well as the basics of its physiology (here, increased water consumption during flowering), for the purpose of modern cultivation.

Keywords: BBC micro:bit, inquiry-based learning, plant watering set, Spathiphyllum

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PRIRODOSLOVNE TEME I EDUKATIVNE IGRE KAO PRIMJER UČENJA DJECE PREDŠKOLSKE I RANE ŠKOLSKE DOBI

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Dijete je socijalno biće koje se od rođenja razvija i raste na slojevima kulture i okruženo je predmetima koji su oblikovani kulturom, znakovnim sustavom i specifičnom ljudskom interakcijom. Djeca su aktivni istraživači u potrazi za informacijama o svom okruženju. Svijet oko sebe pokušavaju razumjeti promatranjem, uspoređivanjem, mjerenjem, opisivanjem, eksperimentiranjem i drugim oblicima i metodama rada. Prirodoslovne teme i edukativne igre kao primjer učenja djece predškolske i rane školske dobi dio su projekta UNICEF-a „Škole za Afriku“ kojim pomažu djeci koja žive u iznimno teškim uvjetima, djeci bez roditelja, kao i o djevojčicama koje su u nekim sredinama često dodatno uskraćene za mogućnost obrazovanja te pilot-projekt „(Ne)obične ptice“ u okviru kolegija Prirodoslovlje, Odsjek u Čakovcu kojim se upozorava na kontinuirani gubitak prirodnih staništa. U pilot-projekt su uključeni studenti Učiteljskog fakulteta i djeca predškolskog i ranoškolskog uzrasta. U radu je prikazan dio provedbe projekta „Škole za Afriku“ započet godine 2009., volonterski doprinos kolegija Prirodoslovlje Učiteljskog fakulteta, Odsjek u Čakovcu osmišljenoga u svrhu educiranja učenika predškolske i rane školske dobi te edukativni pilot-projekt „(Ne)obične ptice“ uspješno započet 2017. godine. Projekti se nastavljaju i tijekom 2018. godine.

Ključne riječi: dijete, edukacijske igre, prirodoslovne teme, projekt

NATURAL SCIENCE TOPICS AND EDUCATIONAL GAMES AS AN EXAMPLE OF TEACHING PRESCHOOL AND EARLY SCHOOL CHILDREN

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A child is a social being who, from birth onwards, develops and grows on layers of culture and is surrounded by objects shaped by culture, sign system and specific human interaction. Children are active researchers looking for information about their environment. They try to understand the world around by observing, comparing, measuring, describing, experimenting, as well as by using other forms and methods of work. Natural Science topics and educational games as an example of learning of preschool-age and young school-age children are part of the UNICEF

Project "Schools for Africa", which help children living in extremely difficult conditions and girls who are in some areas often deprived of the right to education as well as of the pilot project "(No) Ordinary Birds" within the course of Natural History at the Department of Čakovec that warns of the continuous loss of natural habitats. Participants in this pilot project are university students from the Faculty of Teacher Education and preschool and young school-age children. This paper presents a part of the implementation of the "School for Africa" project. It started in 2009 as a contribution of volunteers from the Department in Čakovec of the Faculty of Teacher Education that was designed for the purpose of educating young school-age children and an educational pilot project "(No) Ordinary Birds" that was successfully initiated in 2017. Projects have been continuing in 2018.

Keywords: children, educational games, Natural Science topics, project

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PRAKTIČNI RADOVI UČENIKA - IZRADA 3D BIOLOŠKIH MODELA

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Praktični rad je jedna od sastavnica praćenja i ocjenjivanja učenika sa ciljem što trajnijeg usvajanja znanja. Kao jedan od mogućih praktičnih radova učenicima je ponuđena izrada 3D modela o temi i u tehnici po želji, uz uvjet da izrađeni model mogu povezati s usvojenim gradivom. Od 117 učenika sedmih razreda 42 učenika je izradilo 3D model. Najzastupljenija tema prikazana 3D modelima bila je tema Kralježnjaci (17 modela), a slijede teme Geološka doba - Dinosauri (9), Kukci (6), Mekušci (3), Bodljikaši (3) i Virusi (1 model). Tema Kritosjemenjače bila je zastupljena u manjoj mjeri (3 modela), možda zato što je izrada herbara obvezna tema praktičnog rada šestog razreda. Najzastupljeniji materijal izrade bio je glinamol (21 model) i papir-origami (10), a u manjoj mjeri perlice (5), keramika (3) platno (3) i vruće ljepilo (1 model). U 8. razredu praktičan rad izrade modela DNA bio je zadan svim učenicima. Najviše je izrađenih modela iz žice (35), plastelina (34), slijede plastične slamke (24), drvene kuglice (8), savitljive trake (6), papir-origami (4), stiropor kuglice (3), drvene daščice (1) i spužvice za posuđe (1). Model koji je privukao najviše pažnje među učenicima izrađen je od bombona Haribo medvjedića (1), pri čemu su npr. raspored dušičnih baza učenici usvojili konzumiranjem“ istih“. Izrada 3D modela pridonosi duljem fokusiranju učenika na sadržaj kojeg treba usvojiti, a privlači pažnju i ostalih učenika, budući da modeli ostaju izloženi u učionici biologije.

Ključne riječi: učenički praktični rad, 3D biološki modeli

PRACTICAL WORKS OF STUDENTS - DESIGNING 3D BIOLOGICAL MODELS

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Practical work is one of the components of student assessment with the aim of obtaining more permanent knowledge acquisition. As one of the possible practical works, students are offered a 3D model designs in the subject and technique as desired. Out of the 117 students of the 7th grade, 42 students developed a 3D model. The most prominent feature presented in 3D models was the theme Vertebrates (17), followed by the topics Geological age – Dinosaurs (9), Bugs (6), Molluscs (3), Echinoderms (3) and Viruses (1 model). The topic Angiospermae (3) was represented to a lesser extent, since making herbars is a mandatory topic of practical work in the 6th grade. The most widely used material in seventh grade was glinamol (21) and paper-origami (10), and to a lesser extent beads (5), ceramics (3), canvas (3) and hot glue (1). In the 8 th grade, the practical work of creating a DNA model was given to all students. Most of the models are made of wire (35), plasticine (34), followed by plastic straps (24), wooden beads (8), flexible strips (6), paper origami (4), balloon styrofoam (3), wooden darts (1) and dishwashing sponges (1). The model that attracted the most attention was made out of the candy Haribo Bears (1), whose arrangement of nitrogen bases was grasped by students through their consumption. The creation of 3D models contributes to student's longer focus on the content and attracts the attention of other students as models remain exposed in the biology classroom.

Keywords: practical work, 3D models

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PREVENCIJOM DO ZDRAVLJA OČIJU I ZAŠTITE VIDA - PRIMJERI IZ PRAKSE

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Kako bi potaknuli učenike na redovitije nošenje naočala i leća te educirali učenike i roditelje o potrebi zaštite vida, u suradnji sa Zavodom za Javno zdravstvo Sv. Rok Virovitica proveden je tijekom školske 2016./2017. godine zajednički projekt učenika i učitelja OŠ Ivane Brlić-Mažuranić Virovitica i OŠ Gradina. Istraživanjem je obuhvaćeno 109 učenika petih razreda i 96 učenika osmih razreda virovitičke škole i 36 učenika petih i 34 učenika osmih razreda OŠ Gradina. Na satovima Biologije i SRZ-a kroz temu Građanskog i Zdravstvenog odgoja "Živi zdravo - Čuvaj zdravlje očiju i vid" provedeno je testiranje, tzv. screening, vidne oštrine i test za pojavu astigmatizma. Učenici dviju škola ispunjavali su i upitnik o životnim navikama i stavu prema osobama koje nose naočale. Vidljive su razlike u zastupljenosti tipa poremećaja refrakcije u obje škole. Učenici škole iz Gradine nemaju slabovidnih učenika, a u virovitičkoj školi ona se javlja kod 2% učenika. Kratkovidnost i dalekovidnost učenika gradinske škole upola je manja u odnosu na učenike virovitičke škole. Učenici obiju škola smatraju da suvremeni način života šteti zdravlju očiju, no ipak aktivno ne pridonose zaštiti jer većina koriste mobitele i gledaju TV više od tri sata dnevno. Većinom se zdravo hrane, a odnos prema učenicima koji nose naočale im je pozitivan.

Ključne riječi: zaštita vida, životne navike

PREVENTION FOR HEALTHY EYES AND EYESTIGHT PROTECTION - PRACTICAL EXAMPLES

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To encourage pupils to wear glasses and contact lenses more regularly and to educate pupils and parents on the need for eyesight protection, in cooperation with the Sv. Rok Institute for Public Health Virovitica, a joint pupil and teacher project was implemented during the school year of 2016/2017 at the Ivana Brlić-Mažuranić Elementary School in Virovitica and the Gradina Elementary School. 215 pupils from the school in Virovitica, 109 fifth-grade and 96 eighth-grade pupils, and 36 fifth-grade and 34 eighth-grade pupils of the Gradina school participated in the research. Visual acuity and astigmatism screenings were conducted in biology and homeroom classes through the Civil and Health Education topic Live Healthily – Protect Eye Health and Eyesight. Pupils from both schools also filled out a questionnaire on their life habits and attitudes to persons who wear glasses. Differences in the frequency of certain types of refractive disorders are evident in both schools. No pupils from the Gradina school and 2% of the pupils from the Virovitica school are visually impaired. Short-sightedness and long-sightedness affect twice as many pupils from the Virovitica as those from the Gradina school. Pupils from both schools consider that the contemporary way of life harms eye health – but they do not actively protect their eyes because most of them use mobile phones and watch TV more than 3 hours a day. They mostly eat healthily and they have a positive attitude to the pupils who wear glasses.

Keywords: eyesight protection, way of living

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PROJEKT PRIMORSKA MAKOVICA (*Glaucium flavum* Crantz)

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Cilj projekta Primorska makovica promocija je zaštite ove rijetke biljke. Zbog sve većeg broja turista i njihovih aktivnosti na obali nestaje s naših plaža te ima status ugrožene i strogo zaštićene vrste. U medulinskom akvatoriju pronađena je na obali na tri lokaliteta: na otoku Ceji i rtu Škara te u Ližnjanu u uvali Marlera. Projektu smo pristupili interdisciplinarno kroz predmetnu nastavu i slobodne aktivnosti. U prvom polugodištu učenici šestih razreda istraživali su literaturu i izrađivali edukativne plakate. U drugom polugodištu na nastavi Likovne kulture i likovnoj grupi oblikovali su modele makovice od gline i žice te oslikavali platnene vrećice. Dramska skupina osmislila je tekst i predstavu na temu makovice, novinarska je izradila strip, a produženi boravak lutkarsku predstavu. Osigurali smo i novac za izradu edukativne ploče koju smo postavili na Otok Ceju. Ploča sadržava fotografije i tekst koji su s hrvatskog, učenici jezičnih grupa preveli na engleski, talijanski i njemački jezik. Sve navedene aktivnosti prezentirane su na izložbi, u našoj Općini Medulin, povodom Dana biološke raznolikosti. Time smo postigli da o makovici učenici saznaju i uče na zanimljiv i kreativan način a širu smo javnost potakli na razmišljanje o potrebi razvoja turizma u smjeru suživota s prirodom i njenim vrijednostima.

Ključne riječi: Žuta makovica, zaštićena vrsta

PROJECT YELLOW HORNED POPPIES (*Glaucium flavum* Crantz)

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The aim of the project is promotion of this rare plant. Due to the growing number of tourists and their coast activities, it has been disappearing from our beaches and has the status of an endangered and strictly protected species. We found it on three sites in Medulin sea coast, on Ceja island, Škara promontory and in Marlera cove. The Project has been approached in an interdisciplinary way of learning. During the school year 2017/18. the sixth-graders were examining literature and making educational posters. In art classes, as well as in Art group, they were making models of poppies in clay and wire, and they were also painting poppies on linen bags. The drama group has thought of a text for a performance having the poppy as the topic, the journalist group has made a comics, and children of the extended day program have made a puppet show. We have also made an educational board which was put on the island of Ceja for which the students translate the text from Croatia into English, Italian and German. All the above mentioned activities were presented at the exhibition in Medulin, on the Day of Biological Diversity. In this way we have taught the students how to learn in an interesting and creative way, and we have encouraged people to think about developing tourism in coexistence with nature and its values.

Keywords: Yellow horned poppies, protected species

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KAKO RADITI S POTENCIJALNO DAROVITIM UČENICIMA U OSNOVNOJ ŠKOLI?

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U svrhu ispitivanja uspješnosti usvajanja nastavnog sadržaja iz biologije i kemije kod potencijalno darovitih učenikatijekom šk.god. 2017/2018. u OŠ Bogumila Tonija Samobor provodi se istraživanje na populaciji učenika 7. razreda. Na početku istraživanja izvršena je inicijalna provjera znanja nastavnih sadržaja biologije i kemije 1. polugodišta 7. razreda, prikupljene su ocjene iz pisanih provjera, prosječne ocjene iz biologije i kemije, a VARK upitnikom utvrđen je stil učenja kod odabrane skupine učenika. Nakon provedenog istraživanja provodi se pisana provjera obrađenih nastavnih jedinica. Identifikaciju potencijalno darovitih učenika provela je školska psihologinja koristeći standardizirani psihodijagnostički instrumentarij, točnije test za ispitivanje opće kognitivne sposobnosti ispitanika, Ravenove Standardne progresivne matrice (SPM, Standard progressive matrices, John C. Raven). Potencijalno daroviti učenici su suradničkim oblikom učenja obrađivali dvije nastavnejedinice u biologiji i dvije u kemiji uz obogaćenje nastavnog sadržaja. Tijekom obrade nastavne jedinice, u oba predmeta, učili su u homogenim skupinama nastavnim metodama koje odgovaraju njihovom stilu učenja, dok su tijekom obrade druge nastavne jedinice radili u heterogenim skupinama koje su činili učenici s različitim stilovima učenja. Istraživanjem želimo utvrditi kako učenici učeći nastavnim metodama koje odgovaraju njihovom stilu učenja uz obogaćenje nastavnog sadržaja postižu bolje rezultate.

Ključne riječi: daroviti učenici, biologija, kemija, stilovi učenja

HOW TO TEACH POTENTIALLY GIFTED STUDENTS IN PRIMARY SCHOOL

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For the purpose of examining the success of the teaching content of biology and chemistry in potentially gifted students during the school year 2017/18 in the Primary School B. Toni in Samobor a research is conducted on the population of the 7th grade students. At the beginning of the research the initial validation of the teaching content of the biology and chemistry knowledge of the 7th grade, the written exams results as well as the average grade of biology and chemistry were collected. The VARK questionnaire is used to determinate the learning style in the chosen student group. After the research written validation of the processed teaching units is conducted.The identification of potentially gifted students was carried out by a school psychologist using standardized psychodiagnostic instruments, more specifically the test for the general cognitive ability of the respondent, Raven's Standard Progressive Matrix (SPM, John C. Raven).Potentially gifted students cooperatively studied two teaching units in biology and two in chemistry with the enrichment of teaching content. During the teaching of the teaching unitthey learned in homogeneous groups by teaching methods that correspond to their learning style while working on the second unit was arranged in heterogeneous groups made of pupils with different learning styles.Using this research, we want to determine that students learning with teaching methods that match their learning style content achieve better results.

Keywords: gifted students, biology, chemistry, learning styles

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BIOLOGY OF BLEACHING HAIR

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Today, healthy, shiny and tidy hair is an essential component of everyday life. The concept of beauty has significantly changed through time, but the beautiful hair has somehow remained its most important element. In order to maintain the health of your hair it is very important to know its structure. This lecture aims to present what we know about the structure of hair and the structure of molecules that give natural and artificial hair color. In order to understand why hair colors among humans differ, we need to study three basic parts of the hair shaft: the outer layer - the cuticle, the central layer - cortex and the inner layer of the hair-the medulla. The cuticle is made of several types of keratin proteins. The outer keratin is called "hard keratin" which is comprised of from 2 to 11 layers of overlapping scales that are sticked together with "soft keratin". This kind of keratin provides the thin and transparent cuticle with the protective role of the inner layer of hair. Likewise, this knowledge enables the implementation of chemical processes on the hair. When bleaching and dyeing hair hard keratin scales must be openedso that active substances can get in to the cortex.The cortex contains pigments that give hair its color.The melanin pigment in the cortex is seen through the scales of the cuticle and dark color of hair is formed. The medulla is the innermost layer of the hair shaft. It is a line that in most people stretches through the entire length of the hair.

Keywords: hair, bleaching, cuticula, cortex, medulla, keratin, pigments

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ISTRAŽIVANJE USVOJENOSTI BOTANIČKIH SADRŽAJA NA SATU BIOLOGIJE RAZLIČITIM NAČINIMA POUČAVANJA

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U radu je istraživana usvojenost znanja botaničkih sadržaja na satu biologije iz nastavne jedinice „Kritosjemenjače“ u drugom razredu gimnazije. U istraživanju je sudjelovalo 170 učenika drugih razreda, od toga 64 učenika i 106 učenica. Razredi su podijeljeni u dvije skupine, eksperimentalnu i kontrolnu. U svakoj školi jedan je razred predstavljao eksperimentalnu, a jedan kontrolnu skupinu. Učenicima je na početku sata dan inicijalni test sastavljen se od sedam zadataka kojim se ispitivalo predznanje učenika Ukupna rješenost inicijalnog testa iznosi 60,31%, i pokazuje bolju riješenost testa kod eksperimentalne skupine. Test za ponavljanje se sastojao od osam zadataka, a rješavan je nakon obrade nastavne jedinice Ukupni rezultat testa za oonavljanje iznosi 56,43% i pokazuje bolju riješenost testa kod kontrolne skupine. Provedenom anketom prepoznato je nezadovoljstvo učenika učestalošću i zastupljenošću izvanučioničke nastave iz Biologije. Ukupno 77,50% anketiranih učenika nisu imali niti jedan put izvanučioničku nastavu tijekom godine. Obzirom na rezultate testova utvrđuje se da u ispitivanim razredima izvodi predavačka nastava Biologije bez aktivnog sudjelovanje učenika. Iz cijelog istraživanja može se zaključiti da samo upornošću i konstantnim radom i kurikularnim pristupom može postići vizija moderne i suvremene škole u Hrvatskoj.

Ključne riječi: nastavni sustavi, nastavne metode, socijalni oblici rada, biologija, učenik

RESEARCH OF THE BOTANICAL CONTENT ACQUIRED DURING BIOLOGY LESSONS WITH THE APPLICATION OF DIFFERENT TEACHING METHODS

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The objective of this work is the research on the acquisition of botanical content during biology lesson “Angiosperm” which is part of the regular curriculum of the second grade of grammar school. The study involved 170 students of second grade. Of the total number of pupils there were 64 male pupils and 106 female pupils. The classes were divided into two groups, experimental and control group. In each school, one class represented an experimental and other one control group. At the beginning of the lesson the initial test was given to the students, which was the same for both groups. It consisted of seven assignments and the task of the initial test was to determine foreknowledge of the pupils. The results of the initial test show (60.31%) a better resolution of the test in the experimental group. After processing the teaching unit, the students solved the test for repetition. The repetition test consisted of eight tasks. The repetition test results show a better resolution of the control group (56,43%). The survey conducted revealed the dissatisfaction of students (77,50%) with the frequency and attendance of extra-curricular teaching in Biology. Given the results of the tests, it is concluded that lecturing classes in Biology without the active participation of the students are taking place in the tested classes.

From the whole research we can conclude that only by perseverance and constant work we can achieve the vision of a modern school in Croatia.

Keywords: teaching systems, teaching methods, social forms of work, biology, disciple

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PARK PRIRODE MEDVEDNICA - DOM ZA MNOGOBROJNE VRSTE VRETENACA

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Park Prirode Medvednica nalazi se u blizini Zagreba, što je neposredno uvjetovalo istraživanja flore i faune planine, koja traju od 19. stoljeća pa sve do danas. Unatoč tome, mnoge skupine kukaca nikada nisu bile istraživane, a jedna od takvih skupina su i vretenca. Prilog tome govori činjenica da je do sada u Hrvatskoj zabilježena 71 vrsta, dok su za područje Medvednice literaturnim pregledom utvrđene samo tri. Područje Medvednice iznimno je bogato potocima i izvorima te oni čine većinu staništa pogodnih za razvoj vretenaca. Stajačice, poput lokvi i akumulacija, nalazimo uglavnom na rubnim dijelovima Parka prirode, no one predstavljaju iznimno važna staništa za razvoj mnogih vrsta vretenaca. Između 2012. i 2014. provedeno je prvo sustavno istraživanje faune vretenaca PP Medvednica. Utvrđene su 32 vrsta od čega je 29 vrsta po prvi puta zabilježeno za PP Medvednica. Šest pronađenih vrsta nalazi se u Crvenoj knjizi vretenaca Hrvatske (NT - *Lestes barbarus, Coenagrion ornatum, Aeshna isoceles, Sympetrum vulgatum, S. meridionale*; DD - Orthetrum coerulescens). Dodatno, dvije vrste *Cordulegaster ornatum* i *C. heros*, kvalifikacijske su vrste ekološke mreže Natura 2000. Nakon ovoga istraživanja Medvednica postaje jedno od najbolje istraženih područja u Hrvatskoj što se tiče faune vretenaca. Daljnja istraživanja trebala bi se usmjeriti na praćenje stanja populacija Natura 2000 i ostalih ugroženih vrsta na području PP Medvednica.

Ključne riječi: Odonata, raznolikost, popis vrsta, zaštićeno područje, Natura 2000

NATURE PARK MEDVEDNICA - A HOME FOR NUMEROUS DRAGONFLY SPECIES

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Nature park Medvednica is located in the near vicinity of Zagreb, which resulted in surveys of flora and fauna of the mountain, spanning from the 19th century until today. Despite of that, many insect groups have never been surveyed, and one of such groups are dragonflies. So far, 71 dragonfly species have been recorded in Croatia, while for Medvednica only three species are known. The area of Medvednica is extremly rich in streams and springs, habitats most suitable for the development of dragonflies. Still water bodies like ponds and acumulations can

be found only at the border area of the Nature park, but represent important habitats for the development of many dragonfly species. Between 2012 and 2014 a first systematic survey of dragonflies of NP Medvednica was carried out. Alltogether 32 species have been recorded, of which 29 are newly recorded in the area. Six recorded species are listed in the Red book of dragonflies of Croatia (NT - *Lestes barbarus, Coenagrion ornatum, Aeshna isoceles, Sympetrum vulgatum, S. meridionale*; DD - Orthetrum coerulescens). Additionally, two species, *Cordulegaster ornatum* and *C. heros*, are qualification species for the ecological network Natura 2000. After this survey, the area of Medvednica can be considered as one of the best surveyed areas in Croatia in respect to the dragonflies fauna. Further surveys should be aimed at the monitoring of the populations of Natura 2000 and other endangered species in the area of NP Medvednica.

Keywords: Odonata, diversity, species list, protected area, Natura 2000

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TAXONOMY OF A BIOCONTROL AGENT DIAERETIELLA RAPAE (HYMENOPTERA, BRACONIDAE, APHIDIINAE)

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Diaeretiella rapae (M'Intosh, 1855) is a cosmopolitan aphid parasitoid with more than 60 aphid hosts. It is a significant parasitoid of some economically important aphids such as: cabbage aphid *Brevicoryne brassicae* (L. 1758); green peach aphid *Myzus persicae* Sulzer, 1776; Russian wheat aphid *Diuraphis noxia* (Kurdjumov, 1913); cotton aphid *Aphis gossypii* Glover, 1877; bird cherry-oat aphid *Rhopalosiphum padi* (L. 1758); corn leaf aphid *Rhopalosiphum maidis* (Fitch, 1856); mustard aphid *Lipaphis erysimi* (Kaltenbach, 1843). *Diaeretiella rapae* is considered as main primary parasitoid of *B. brassicae* and it is frequently used as an agent of biological control in North America and Australia. Beside *B. brassicae*, it is also used for control of *D. noxia*. Although there was a great number of various studies considering usage of *D. rapae* as a biocontrol agent (ecological studies, behavioral studies, studies of its population genetics etc.) there is a knowledge gap considering its taxonomy. *Diaeretiella rapae* was first described as *Aphidius rapae* by M'Intosh in 1855 and since then a long list of synonyms appeared in literature putting it in different genera such as *Trioxys, Trionyx, Lysiphlebus* etc. In 1960 Starý described the new genus *Diaeretiella* with type species *D. rapae*. We examined the generic status of *Diaeretiella rapae* based on morphological and molecular data (barcoding region of mtCOI gene) and found no significant differences between *D. rapae* and *Aphidius* species.

Keywords: *Diaeretiella*, taxonomy, parasitoid, *Aphidius*

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FAUNISTIČKE ZNAČAJKE DANJIH LEPTIRA (INSECTA, LEPIDOPTERA) PODRUČJA BANOVINE

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Regija Banovina je brežuljkasto područje smješteno u kontinentalnom dijelu Hrvatske, između rijeke Save i donjih dijelova rijeka Kupe, Gline i Une. To se područje odlikuje velikom raznolikošću kopnenih staništa uključujući i različite vrste travnjaka i livada. Istraživanje danjih leptira na području Banovine provode se nekoliko godina. U ta istraživanja uključeni su i studenti molekularne biologije Prirodoslovno-matematičkog fakulteta (Biološki odsjek), Sveučilišta u Zagrebu, na svojoj terenskoj nastavi. Dosadašnjim istraživanjima na tome je području utvrđeno više od 80 vrsta danjih leptira, uključujući i neke vrlo zanimljive vrste: *Zerynthia polyxena, Pieris brassicae, Aporia crataegi, Leptidea* sp. *Lycaena dispar, Maculinea arion*,… Istraživanje ove skupine nastavlja se i dalje u cilju utvrđivanja još neutvrđenih vrsta (smatramo da fauna danjih leptira Banovine broji oko 100 vrsta), te točne rasprostranjenosti svih vrsta na ovome području. Jedan od ciljeva ovih istraživanja je i izdavanje knjige: Danji leptiri Banovine – raznolikost, rasprostranjenost i zaštita.

Ključne riječi: danji leptiri, biološka raznolikost, Banovina

FAUNAL FEATURES OF BUTTERFLIES (INSECTA, LEPIDOPTERA) OF THE BANOVINA REGION

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The Banovina region is hilly landscape situated the continental part of Croatia, between the Sava River and the lower parts of the Kupa, Glina and Una Rivers. This area is characterized by very different types of land habitats including various types of lawns and meadows. The research of the butterfly's fauna in the Banovina region conducts for a several years. In these researches are also included the students of molecular biology of the Faculty of Sciences (Department of Biological), University of Zagreb, on their field teaching. In the present research, more than 80 species of butterflies have been identified in this area, including some very interesting species: *Zerynthia polyxena, Piersi brasicae, Aporia crataegi, Leptidea* sp*., Lycaena dispar, Maculinea arion*,... The research of this group of insects continues to identify some new species for this area (we think that the butterfly fauna of the Banovina have about 100 species), and knowledge of the distribution of all species in this region. One of the results of these researches is the publication of the book: Butterflies of Banovina – diversity, distribution and protection.

Keywords: butterflies, biodiversity, Banovina region

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POPIS VRSTA JELENAKA (COLEOPTERA, LUCANIDAE) PRISUTNIH U HRVATSKOJ, S LITERATURNIM I RECENTNIM PODACIMA NA DISTRIBUCIJSKIM KARTAMA

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Jelenci (Coleoptera, Lucanidae) su saproksilni kornjaši s važnom ulogom u šumskim ekosustavima. Unatoč činjenici da su neke vrste prisutne na dodatcima Direktive o staništima i da su procijenjeni u Europskom crvenom popisu saproksilnih kornjaša, ova porodica je slabo istražena u Hrvatskoj. Cilj ovog istraživanja bio je sastaviti popis vrsta jelenaka prisutnih u Hrvatskoj s njihovom distribucijom prikazanom na kartama, kako bi se u budućnosti istraživanja mogla usredotočiti na trenutne rupe u znanju. Dostupna literatura je provjerena i podatci recentnih entomoloških istraživanja udruge Hyla su ujedinjeni. Rezultat je popis sedam vrsta porodice Lucanidae (Coleoptera) i prikazi njihove distribucije na kartama: *Aesalus scarabaeoides, Ceruchus chrysomelinus, Sinodendron cylindricum, Dorcus parallelipipedus, Lucanus cervus, Platycerus caprea, P. caraboides*.

Ključne riječi: saproksilni, jelenci, Lucanidae, distribucija

LIST OF STAG BEETLES (COLEOPTERA, LUCANIDAE) SPECIES PRESENT IN CROATIA WITH LITERATURE AND RECENT RECORDS ON DISTRIBUTIONAL MAPS

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Stag beetles (Coleoptera, Lucanidae) are saproxylic beetles with an important role within forest ecosystems. Despite the fact that some of these species are listed on Annexes of the Habitats Directive and are evaluated in the European Red List of Saproxylic Beetles, this family is poorly researched in Croatia. The aim of this study was to make a list of stag beetle species of Croatia with their distribution presented on maps, so more research can be focused on gaps in knowledge in the future. Available literature was checked and records from Association Hyla's recent entomological surveys were assembled. Altogether, a list of seven species of Lucanidae from Croatia is given and displayed on maps: *Aesalus scarabaeoides, Ceruchus chrysomelinus, Sinodendron cylindricum, Dorcus parallelipipedus, Lucanus cervus, Platycerus caprea, P. caraboides*.

Keywords: saproxylic, stag beetles, Lucanidae, distribution

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KLIŠNJACI (COLEOPTERA, ELATERIDAE) S EUROPSKOG CRVENOG POPISA SAPROKSILNIH KORNJAŠA – PODATCI O RASPROSTRANJENOSTI U HRVATSKOJ

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Značajan dio entomološke bioraznolikosti šumskih ekosustava predstavljaju klišnjaci (Coleoptera, Elateridae). Neki od njih su prisutni na nekoliko popisa saproksilnih vrsta veće konzervacijske vrijednosti, poput popisa reliktnih prašumskih vrsta, popis Urwald reliktnih vrsta, čak i na dodacima Direktive o staništima. Ovim istraživanjem nastojali smo otkriti koje vrste saproksilnih klišnjaka su prisutne u Hrvatskoj, koja je njihova trenutno poznata rasprostranjenost i koji od njih su više rangirani prema Europskom crvenom popisu saproksilnih kornjaša. Sastavljen je popis od 49 vrsta koristeći dostupnu literature i podatke udruge Hyla s recentnih entomoloških istraživanja. Trideset vrsta smatra se najmanje zabrinjavajućima (LC),

tri su nedovoljno poznate (DD), 12 ih je gotovo ugroženo (NT), jedna je osjetljiva (VU), a tri su ugrožene (EN). Potrebno je provesti više istraživanja u budućnosti kako bi se procijenio status ovih vrsta na nacionalnoj razini u Hrvatskoj.

Ključne riječi: saproksilni, klišnjaci, Elateridae, rasprostranjenost, crveni popis

DISTRIBUTIONAL RECORDS OF EUROPEAN RED-LISTED SAPROXYLIC CLICK BEETLES (COLEOPTERA, ELATERIDAE) IN CROATIA

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A significant part of the forest ecosystem entomological biodiversity belongs to the click beetles (Coleoptera, Elateridae). Some of them are present on several lists of saproxylic species of great conservation value, such as the primeval forest relict species list, the Urwald relict species list, even the Annexes of the Habitats Directive. The aim of this study was to determine which saproxylic click beetle species are present in Croatia, what is their currently known distribution and which of them have a higher rank according to the European Red List of saproxylic beetles. A list of 49 species was made using literature as well as the records from Association Hyla's recent entomological surveys. Thirty of these species are categorized as least concern (LC), three as data deficient (DD), 12 as near threatened (NT), one as vulnerable (VU) and three as endangered (EN). More research is necessary in the future to evaluate these species' national status in Croatia.

Keywords: saproxylic, click beetles, Elateridae, distribution, red list

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EFFECT OF CADMIUM ON PHENOTYPIC PLASTICITY IN THE GYPSY MOTH (*Lymantria dispar* L.)

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As one of the most potent pollutants, cadmium enters the environment through different sources and into the living organisms though the food chain thus realizing its toxic effect. This work examines cadmium effects at 10 (C1), 30 (C2) and 50 µg Cd/g (C3) concentrations of dry food on cost of plasticity of *Lymantria dispar* L. gypsy moth, polyphagous, invasive species who has detrimental influence on forest ecosystems. We used regression analysis to determine cost of phenotypic plasticity in duration of larval development (LD), pupal mass (PM) and pupal development (PD) in relation to adult lifespan (AL), which was taken as a fitness value measure, also differences within gypsy moth females and males for cost of phenotypic plasticity, and compared the standardized coefficients from a linear regression for fitness-related traits in relation to AL. Results showed that genotypes pay the price for cost of maintaining homeostasis in control environment (C0) which secure normal LD in relation to AL. Regression

coefficient for PM in group C3 indicates that, in the presence of cadmium, genotypes pay the cost of plastic responses, i.e. reduced PM does not influence the increase of fitness in stressful conditions. Maintaining homeostasis is not significantly different between genders and between C0 and cadmium-treated groups. Significant negative regression coefficient in males was observed for LD in group C3 and for PD in C0 and C1 group where males pay the cost of plasticity for PD.

Keywords: cadmium, gypsy moth, phenotypic plasticity, fitness-related traits

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PRELIMINARY DATA OF ARANEAE ON MOUNTAIN KOZUF

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Preliminary data concerning Araneae abundance and community characteristics were compared along an altitudinal gradient (from 89 to 2080 m a.s.l.) on Kozuf Mt. in Republic of Macedonia. The research was carried out in the period May – October 2016 with monthly dynamics, in 17 localities, with pitfall, ramp and arboreal traps as well as collected by hand. In total 2000 specimens belonging to 173 species, 101 genera and 26 families were registered. Species richness was highest at 550 m a.s.l. (48 species) while the lowest value was registered at 89 m a.s.l. (12 species). The highest values of abundance (281 ind.) were recorded at 1672 m a.s.l. and the lowest at 89 m a.s.l. The species *Inermocoelotes karlinskii* (Kulczynski, 1906) was the most abundant species throughout the gradient. In general, there were noticeable differences of the araneocoenosis from 17 different habitats. The increasing altitude and consequently differences in vegetation type affected species richness, composition and abundance of spider communities on Kozuf Mt.

Keywords: preliminary data, Araneae, Kozuf Mt., ecology, altitudinal gradient

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POPIS ŠAVOLJIKA (Insecta: Coleoptera: Hydraenidae) HRVATSKE: TRENUTNO STANJE, DNA BARKODIRANJE I BUDUĆE SMJERNICE

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Šavoljike (Insecta: Coleoptera: Hydraenidae) su široko rasprostranjena skupina kukaca koja uglavnom naseljava izvore i tekuće vode, ali ih se može pronaći i u malim jezerima, hipersalinim lokvicama te higropetriku. Često se koriste kao bioindikatori u procjeni kakvoće voda te su od

posebnog interesa iz dva aspekta: 1) lokalni endemizam je vrlo čest; 2) prisutnost još neopisanih vrsta je vrlo moguća. Za razliku od drugih dijelova Europe, u Hrvatskoj je skupina šavoljika, do nedavno, samo sporadično istraživana, a uglavnom u sklopu istraživanja bentičkih zajednica. Naše istraživanje uključuje nekoliko ciljeva: i) provesti preliminarni popis (check-lista) šavoljika Hrvatske; ii) provesti dodatno skupljanje šavoljika uzduž Hrvatske; iii) provesti integrativnu taksonomiju šavoljika Hrvatske. Stoga, provedena je detaljna analiza literaturnih podataka, muzejskih i privatnih zbirki te do sada prikupljenih jedinki. Usporedno, započeli smo DNA barkodiranje uzorkovanih jedinki. Očekujemo da će naši rezultati uvelike doprinijeti dosadašnjem znanju o ovoj neopravdano zapostavljenoj porodici vodenih tvrdokrilaca te otkrivanju taksonomski zanimljivih svojti.

Ključne riječi: integrativna taksonomija, endemske vrste, specijacija, vodeni tvrdokrilci

CHECKLIST OF CROATIAN MINUTE MOSS BEETLES (Insecta: Coleoptera: Hydraenidae): CURRENT STATUS, DNA BARCODING AND PERSPECTIVES

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Minute moss beetles (Insecta: Coleoptera: Hydraenidae) are widely distributed small sized insects that mostly inhabit springs and running waters but can also be found in small ponds, hypersaline pools or hygropetric. They are often used as bioindicators in water quality assessments, and are of particular interest from two aspects: 1) the local endemism is very frequent; 2) presence of still undescribed species is highly expected. In contrast to other parts of Europe, in Croatia Hydraenidae were until recently only sporadically investigated, mainly as a part of benthic communities. Our study included several aims and scopes: i) to conduct preliminary checklist of Hydraenidae in Croatia; ii) to sample additional material of the family throughout the country; iii) to conduct integrative taxonomy of the family in Croatia. Thereby, we did comprehensive analysis of literature, museum and private collections, and sampled new specimens throughout Croatia. Simultaneously, we started with DNA barcoding of sampled specimens. We expect that our results will greatly contribute to the knowledge of this so far unduly neglected water beetle family, as well as reveal taxonomically interesting taxa.

Keywords: integrative taxonomy, endemism, speciation, water beetles

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***Odontocerum hellenicum* MALICKY, 1972 (TRICHOPTERA: ODONTOCERIDAE) AS A HOST OF *Agriotypus armatus* CURTIS, 1832 (HYMENOPTERA: ICHNEUMONIDAE) – THE FIRST FINDING FOR SERBIA**

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Odontocerum Leach, 1815 is a single European caddisfly genus within family Odontoceridae, containing three species, *O. albicorne* (Scopoli, 1763), *O. hellenicum* Malicky, 1972 and *O. lusitanicum* Malicky, 1975. First two species are registered in Serbia, but *O. hellenicum* has a much narrower area (southeastern part of the country). Species of Odontoceridae, Goeridae and Uenoidae in a pupal stage can be a host for ectoparasitoid wasp *Agriotypus armatus* Curtis, 1823. Distribution of *A. armatus* was recorded during the macrozoobenthos research of the streams of Serbia in 2011–2012. The caddisfly species *Silo pallipes* Fabricius, 1781 (fam. Goeridae) is known to be as the most common host of this parasitoid wasp. Out of eight rivers involved in the study, we recorded parasitized pupae of *O. hellenicum* only in the Vrla River, which is the first record of this host–parasitoid relation on the territory of Serbia. Among 36 *O. hellenicum* cases examined, seven cases were empty (19.44%), 27 were non-parasitized (75%) and only two were parasitized (5.56%). Parasitoid wasps we found were in pupal stage. As known so far, *O. hellenicum* is the second recorded host species for *A. armatus* in Serbia. Having in mind that *Silo pallipes* is more common species compared to *O. hellenicum*, and also the fact that no infected cases of more frequent species *O. albicorne* were found in our survey, we can assume that *A. armatus* has a higher preference for the species of Goeridae family in Serbian streams.

Keywords: aquatic insects, parasitoid, host, distribution, Serbia

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POPULATION GENETICS OF INVASIVE *Aedes japonicus japonicus* (DIPTERA: CULICIDAE) IN SLOVENIA

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Asian bush mosquito, *Aedes japonicus japonicus* (Diptera: Culicidae) is an invasive species, which is spreading rapidly around the world. In 2011, the species has been found near the Slovenian-Austrian country border, for the first time. To determine its distribution and to get a better insight into its dispersal mechanisms a survey for invasive mosquito larvae in artificial water containers was conducted in 2013 and 2015. The predictive maps were built from the occurrence data, bioclimatic variables from CHELSA dataset and the altitude variable in Maxent software. Built models performed great in the prediction of the species distribution. According to collected field data, the accuracy of built model was almost 100%. We suspect that the species is spreading passively as well as actively. Additionally, we performed a genetic study to characterize the variation in *Ae. j. japonicus* population. We used seven microsatellite and mitochondrial marker NAD4 to analyse the genetic structure. Genetic assignment of samples was performed using methods implemented in two software STRUCTURE and GENELAND. The obtained results suggested separation of population from different geographical areas. *Ae. j. japonicus* can serve as a vector of mosquito-borne diseases. We gained important information on patterns of its distribution, which is crucial for the management of the species and implementation of a national mosquito monitoring program.

Keywords: *Aedes japonicus japonicus*, Slovenia, distribution modeling, population genetics

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PRELIMINARNO ISTRAŽIVANJE BRANIČEVKI (DIPTERA: SIMULIIDAE) U ŽUPANJSKOJ POSAVINI

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Katastrofalna poplava koja je u svibnju 2014. godine pogodila područje županjske Posavine omogućila je razvoj velikog broja komaraca. Monitoring koji je prethodio provođenju javnozdravstvenih mjera poslužio je i za preliminarno istraživanje faune braničevki (Diptera: Simuliidae) navedenoga područja. Uzorkovanje odraslih jedinki braničevki obavljeno je pomoću CDC klopki uz suhi led kao atraktant, na 16 postaja: Babina Greda, Štitar, Županja sjever, Županja jug, Bošnjaci, Gunja, Rajevo Selo 1, Rajevo Selo 2, Drenovci, Soljani, Strošinci, Vrbanja, Spačvanska šuma, Durići, Posavski Podgajci i Račinovci. Izvršena su 34 terenska izlaska i ukupno je prikupljeno 212 jedinki braničevki od kojih su determinirane dvije vrste: *Simulium (Boophthora) erythrocephalum* De Geer, 1776 i *Simulium (Wilhelmia) balcanicum* Enderlein, 1924. Udio vrste *S. balcanicum* iznosio je 78%, s najvećim brojem pojavljivanja na lokacijama Babina Greda i Gunja. Iako su obje vrste braničevki u literaturi opisane kao agresivne i antropofilne, za istraživano područje nisu predstavljale potencijalnu zdravstvenu prijetnju.

Ključne riječi: braničevke, županjska Posavina, CDC klopka

PRELIMINARY RESEARCH OF BLACK FLIES (DIPTER: SIMULIIDA) IN ŽUPANJSKA POSAVINA

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The catastrophic floods that in May 2014 hit territory of Županjska Posavina in Brod-Posavina County enabled the development of a large number of mosquitoes. The monitoring that was preceded of implementation of public health measures also served for the preliminary investigation of the black flies fauna (Diptera: Simuliidae) in that area. Sampling of adult black flies was performed using dry ice baited CDC traps at 16 locations: Babina Greda, Štitar, Županja sjever, Županja jug, Bošnjaci, Gunja, Rajevo Selo 1, Rajevo Selo 2, Drenovci, Soljani, Strošinci, Vrbanja, Spačvanska šuma, Durići, Posavski Podgajci and Račinovci. There were 34 field surveys and total of 212 adult black flies were collected from which two species were determined: *Simulium (Boophthora) erythrocephalum* De Geer, 1776 and *Simulium (Wilhelmia) balcanicum* Enderlein, 1924. The species *S. balcanicum* was recorded in a larger number with a share of 78%, with the highest number of appearance on the locations Babina Greda and Gunja. Although both species are described in the literature as highly aggressive and anthropophilic they did not represent potential health threat to the investigated area.

Keywords: Black flies, Županjska Posavina, CDC trap

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MOLEKULARNA IDENTIFIKACIJA VRSTA RODA HYDROCHARA (COLEOPTERA: HYDROPHILIDAE)

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Vodeni kornjaši su sastavni dio biotičke komponente vodenih sustava te predstavljaju raznoliku skupinu koja ima niz karakteristika koje ih čine dobrim biološkim indikatorima. Unutar reda Coleoptera porodica Hydrophilidae ima velik broj vrsta čije predstavnike karakterizira velika raznolikost i prilagođenost na različite tipove vodenih staništa. Rod *Hydrochara* je zastupljen s 23 vrste, a Hrvatska je područje rasprostranjenosti dvije vrste *H. caraboides* i *H. flavipes*. Kako su razlike u morfologiji jedinki roda *Hydrochara* primijećene samo u veličini jedinki, pristupilo se molekularnom istraživanju upotrebom dva mitohondrijska markera: geni za citokrom oksidazu podjedinica I (COI) i za manju podjedinicu ribosomalne RNA (16S rRNA). Cilj ovog istraživanja je molekularnom identifikacijom i analizom COI i 16S rRNA gena odrediti vrste unutar roda *Hydrochara* te utvrditi raznolikost i rasprostranjenost kornjaša roda *Hydrochara* na području kontinentalne Hrvatske. Dobiveni rezultati se podudaraju s opće prihvaćenom sistematikom baze podataka Fauna Europaea prema kojoj je Hrvatska područje rasprostranjenosti dvije vrste iz roda Hydrochara, vrste *H. caraboides* i vrste *H. flavipes*. Različitost uzorka s otoka Virje (OV) u filogenetskim analizama ukazuje na prisutnost nove vrste unutar roda Hydrochara na području Hrvatske što se mora dodatno potvrditi budućim istraživanjima.

Ključne riječi: vodeni kornjaši, *Hydrochara*, molekularni markeri COI i 16S rRNA, filogenetski odnosi

MOLECULAR IDENTIFICATION OF A SPECIES IN GENUS *Hydrochara* (COLEOPTERA: HYDROPHILIDAE)

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Water beetles are an integral part of biotic component of water systems and they represent a diverse group with features that make them good biological indicators. Within the order Coleoptera Hydrophilidae is family of water beetles with morphologically diverse representatives that live in various habitats. The genus *Hydrochara* comprises 23 species that occur in Europe and there are 2 species known in the Croatia, *H. caraboides* and *H. flavipes*. Differences in the morphology of the *Hydrochara* were observed only in the size and this is the reason why we approached to molecular research using two mitochondrial markers: genes for cytochrome oxidase subunit 1 (COI) and for smaller subunit of ribosomal RNA (16S rRNA). The main objective of this study was to determine the species within the *Hydrochara* genus and to determine the diversity and distribution of the *Hydrochara* genus in the continental Croatia. The results of research match with the systematics of Fauna Europaea database according to which Croatia is area with two species of *Hydrochara* genus, *H. caraboides* and *H. flavipes*. The diversity of samples from the island of Virje (OV) in the phylogenetic analyzes indicates the presence of a new species within the genus *Hydrochara* in Croatia, which must be confirmed in

future research.

Keywords: water beetles, *Hydrochara*, molecular markers COI and 16S rRNA, phylogenetic relationship

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CHAETOMARGOREICHEIA IN CROATIA – ON THE DISCOVERY OF A NEW EYELESS SCARITINE GROUND BEETLE SPECIES

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Recently established genus *Chaetomargoreicheia* Margini & Bulirsh, 2005 (Scaritinae: Clivinini) includes endemic hypogean ground beetles found only in the Dinaric Alps (near Adriatic Sea coast). So far, this genus consisted of two species, one from Montenegro – *C. lakotai* (Magrini & Bulirsch, 2005) and another from Bosnia and Herzegovina – *C. zoufali* (Reitter, 1913). As a result of multiple cave explorations in the region of Dalmatia conducted over recent years, we found a single specimen of the first representative of the aforementioned genus in Croatia. The only specimen found is a male, which allowed us to carry out detailed morpho-anatomical comparisons with other known congeners. Since it is clearly separated from the most related taxa, we have realized that we have a species unknown to science and described it as *C. gljevensis* Ćurčić, Pavićević, Vesović & Rađa, 2018. It has been collected by hand in the Jama u Ljučici Cave, village of Gljev, near Sinj, on the southwestern slopes of Mt. Kamešnica. The three Chaetomargoreicheia stenoendemic species are for sure among the rarest known ground beetles based on the number of specimens collected (three in total). The new species has a long body, no sublateral pronotal discal setae and 27-28 small teeth on each lateral elytral margin. The Dinarides are definitely one of the world's underground biodiversity hotspots and our finding confirms the high level of endemism present in the local entomofauna.

Keywords: Carabidae, Clivinini, *Chaetomargoreicheia*, new species, Croatia

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NEW HIGHLY-SPECIALIZED APHAENOPSOID GROUND BEETLES (CARABIDAE: TRECHINAE) FROM SERBIA

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There are 11 aphaenopsoid trechine genera from the Dinaric Alps described so far. All of them are cave-dwelling and endemic to certain regions of Croatia, Bosnia and Herzegovina, Montenegro and Albania. A new contribution to the rich subterranean fauna of the Dinarides appeared in the form of a recent discovery of a new monotypic highly-evolved genus, the first from Serbia (*Velesaphaenops*), and a new species (*Acheroniotes lethensis*). Three specimens of *Velesaphaenops tarensis* (one male and two females) are collected during 2013 and 2015 by rotten meat-baited pitfall traps in the Vučja Jama Pit, Mt. Tara, western Serbia. The new genus is characterized by the presence of a dense body pubescence (on genae, rest of head and pronotum) and two pairs of elytral discal setae. It is morphologically closest to the genera *Acheroniotes, Adriaphaenops* and *Minosaphaenops*. *Velesaphaenops* tarensis has submentum with four pairs of setae, mentum with anterior median convexity well-expressed, very elongated mandibles and densely pubescent genae. Two females of *A. lethensis* are found during 2009 in the Bezdan Pit, Kamena Gora, southwestern Serbia. The species differs from other congeners by the presence of three pairs of setae on submentum and three pairs of elytral discal setae. These great discoveries definitely confirm that the Dinaric karst of Serbia is still insufficiently explored.

Keywords: Carabidae, *Velesaphaenops*, Acheroniotes, cave-dwelling, Serbia

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FAUNA TULARA (TRICHOPTERA) IZVORSKIH PODRUČJA NACIONALNOG PARKA PLITVIČKA JEZERA

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Nacionalni park Plitvička jezera nalazi se u središnjem dijelu Dinarskog krša Hrvatske, u ličkoj regiji. Ovaj prostor odlikuje oštra kontinentalna klima s elementima planinske klime, iako se uočava i neposredan utjecaj blizine Mediterana. Područje se odlikuje iznimnim hidrološkim značajkama koje se očituju u čitavom spektru različitih tipova staništa. Zahvaljujući biodinamičkom procesu osedravanja formiran je oligotrofni kaskadni sustav od 16 jezera međusobno odvojenih sedrenim barijerama. Te značajke uvjetovale su razvoj specifičnosti Nacionalnog parka Plitvička jezera kada je u pitanju skupina Trichoptera, jer sa 89 zabilježenih vrsta to je jedno od žarišnih točaka ne samo Hrvatske nego i Dinarskog krša uopće. Posebni segment specifičnosti u toj fauni daju različiti tipovi izvora koji su u velikom broju zastupljeni na području Parka. Dosadašnjim istraživanjem izvora na ovom području zabilježeno je 46 vrsta tulara, a daljnja detaljna istraživanja ovih staništa su u tijeku.

Ključne riječi: Plitvička jezera, jezera, tulari, izvori

CADDISFLY FAUNA OF SPRING AREAS IN PLITVICE LAKES NATIONAL PARK

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Plitvice Lakes National Park is located in the central part of the Dinaric karst of Croatia, in Lika region. This area is characterized by a sharp continental climate with elements of mountain climate, although the immediate impact of the proximity of the Mediterranean is evident. The area is characterized by exceptional hydrological features that are manifested in a wide spectrum of different types of habitats. Thanks to the biodinamic process of tufa formation, an oligotrophic cascade system of 16 lakes was formed, separated by sedentary barriers. These features caused the development of the National Park Plitvice Lakes specificity when it comes to Trichoptera, since with 89 recorded species it is one of the HOT spots not only of Croatia but of the Dinaric karst in general. A special segment of specificity in this fauna is given by various types of springs that are represented in large numbers in the Park area. There are 46 types of Caddisfly species (Kučinić, 2017) in this area, and further detailed research of these habitats is in process.

Keywords: Plitvice Lakes, lakes, caddisflies, springs

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USPOSTVA MONITORING LEPTIRA U HRVATSKOJ

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Nedavne aktivnosti radne skupine za Lepidoptera okupljene oko EU Natura 2000 projekta integracije (NIP) imale su dva glavna cilja: prikupljanje novih distribucijskih podataka i uspostavljanje sustava monitoringa. Tijekom dvogodišnjeg istraživanja u 124 ravnomjerno raspoređena 10x10 km kvadrata tijekom 744 terenska dana utvrdili smo 1220 taksona i 11622 nalaza. Od toga su 163 vrste danjih leptira (s 4487 nalaza i 23 267 jedinki), što čini preko 80% poznate raznolikosti vrsta. Tijekom istraživanja znatno je povećana količina znanja o raznolikosti i rasprostranjenosti vrsta, no ipak su informacije o trendu populacija vrsta i dalje nedostupne obzirom na dvogodišnje trajanje istraživanja. Budući planovi za monitoring pomoći će nam u razumijevanju promjena u okolišu, osobito kako gubitak staništa i klimatske promjene utječu na vrste.

Ključne riječi: monitoring, Lepidoptera, Hrvatska

ESTABLISHING A LEPIDOPTERA MONITORING SCHEME IN CROATIA

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Recent activities of the Lepidoptera working group gathered around the EU Natura 2000 Integration Project (NIP) had two main objectives: gathering new distribution data and setting up a monitoring scheme. During the two-year study within 124 10 x 10 km grid-squares, we made 744 fieldtrips all over the country and observed 1220 taxa with 11622 observations. Butterflies were encountered with 163 species (4487 observations and 23 267 counts) which represents over 80% of the known species diversity. During the study the level of knowledge on diversity and distribution of species has raised considerably but still information on species trends are poor. Future monitoring plans will help us to understand impacts of environmental change, particularly how habitat loss and climate change is affecting our wildlife.

Keywords: monitoring, Lepidoptera, Hrvatska

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FAUNA OBADA HRVATSKOG DIJELA BARANJE (DIPTERA: TABANIDAE)

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Sustavna uzorkovanja obada (Tabanidae) u hrvatskom dijelu Baranje tijekom proteklih 17 godina (od 2001. do 2017.) rezultirala su determinacijom 29 vrsta svrstanih u 7 rodova. Nalazi četiri vrste i jedne podvrste iz ranijih istraživanja nisu potvrđena tijekom ovih uzorkovanja. Ukupan broj vrsta obada na istraživanom području iznosi 34. Najbrojniji rod je *Haematopota* s devet vrsta, slijede rodovi *Hybomitra* i *Tabanus* sa sedam vrsta, *Chrysops* s pet vrsta, *Atylotus* s četiri vrste, te *Silvius* i *Heptatoma* s jednom vrstom. Najbrojnija vrsta obada na istraživanom području je *Tabanus bromius* L. 1758 s 54.65%, slijede vrste *Tabanus sudeticus* Zeller, 1842 s 12.60%, *Haematopota pluvialis* (L. 1758) s 9.05%, *Tabanus tergestinus* Egger, 1859 s 8.66% i *Tabanus maculicornis* Zetterstedt, 1842 s 5.15%. Ovih pet vrsta čini 90.11% uzorkovanih obada. Većina utvrđenih vrsta (26) u hrvatskom dijelu Baranje pripada Borealno-euroazijskom tipu faune, slijedi šest vrsta iz južnoeuropske skupine te dvije vrste iz Afroeuroazijsko aridne skupine.

Ključne riječi: Tabanidae, Baranja, Hrvatska

HORSE FLY FAUNA OF THE CROATIAN PART OF BARANJA (DIPTERA: TABANIDAE)

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Systematic samplings of horse flies (Tabanidae) in Croatian part of Baranja during the last 17 years (from 2001 to 2017) have resulted in the determination of 29 species classified in 7 genera. Findings of four species and one subspecies from earlier studies haven't been confirmed during these samplings. The total number of Tabanidae species currently known in the study area amounts to 34. The most numerous is the genus *Haematopota* with 9 species, followed by *Hybomitra* and *Tabanus* with 7 species each, *Chrysops* with 5 species, *Atylotus* with 4 species, *Silvius* and *Heptatoma* with 1 species each. The most abundant species of horse flies on the studied area is *Tabanus bromius* L. 1758 with 54.65 %, followed by *Tabanus sudeticus* Zeller, 1842 with 12.60%, *Haematopota pluvialis* (L. 1758) with 9.05%, *Tabanus tergestinus*

Egger, 1859 with 8.66% and *Tabanus maculicornis* Zetterstedt, 1842 with 5.15%. A total 90.11% of collected horse flies belong to these 5 species. The majority of the species (26) determined in Croatian part of Baranja belong to the Boreal-Eurasian type of fauna, followed by species from South-European group (6) and Afro-Eurasian arid group (2).

Keywords: Tabanidae, Baranja, Croatia

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FAUNA DANJIH LEPTIRA PARKA PRIRODE ŽUMBERAK-SAMOBORSKO GORJE

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U razdoblju između svibnja i rujna 2017. godine istraživali smo danje leptire sjeverozapadnog dijela Parka prirode Žumberak-Samoborsko gorje. Sveukupno smo zabilježili 89 vrsta danjih leptira na 25 istraživanih suhih brdskih travnjaka. Najmanji broj vrsta pronađenih na lokaciji bio je 17, a najveći 45. Zabilježene su i neke od najrjeđih vrsta u fauni Hrvatske poput velikog topolnjaka, *Limenitis populi* i amandinog plavca, *Polyommatus amandus*. Osim toga, pronađena je jedna od zasigurno najvećih populacija Natura 2000 vrste močvarne riđe, *Euphydryas aurinia*. Pregledom literature utvrđeno je 49 vrsta danjih leptira za područje Parka prirode, dok smo ovim istraživanjem po prvi puta zabilježili čak 63 vrste. Izrađen je i prvi preliminarni popis danjih leptira Parka prirode Žumberak-Samoborsko gorje, koji broj 111 vrsta (56 % faune Hrvatske), 12 od kojih nismo uspjeli potvrditi dosadašnjim istraživanjima na području Parka. Daljnja istraživanja potrebno je usmjeriti na istočni i sjeveroistočni dio Parka, kako bi se do kraja istražila raznolikost danjih leptira toga područja.

Ključne riječi: Lepidoptera, raznolikost, *NATURA* 2000, popis vrsta

BUTTERFLY FAUNA OF THE ŽUMBERAK-SAMOBORSKO GORJE NATURE PARK

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In the period between May and September 2017, we studied the butterfly fauna of the northwestern part of the Žumberak-Samoborsko Gorje Nature Park. Overall, we recorded 89 species of butterflies on 25 investigated dry grasslands. The lowest number of species found on the site was 17 and the highest 45 species. Some of the rarest species in the Croatian fauna were also noted, such as the poplar admiral, *Limenitis populi*, and Amanda's blue, *Polyommatus amandus*. In addition, one of the largest populations of the Natura 2000 species of wetland reeds, *Euphydryas aurinia*, has been found. The overview of the available literature revealed that 49 species have boon recorded in the park, while additional 63 species were recorded for the first time in the area during this survey. The first preliminary list of Žumberak-Samoborsko Gorje Nature Park was created, containing 111 species (56% of the fauna of Croatia), of which 12 have not be confirmed by this survey. Further research should focus on the eastern and northern part of the Nature Park, to gain a complete picture of the butterfly diversity in the area.

Keywords: Lepidoptera, diversity, NATURA 2000, species list

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NANOČESTICE SREBRA STABILIZIRANE S OMOTAČIMA UTJEČU NA ISKLIJAVANJE I RAST DUHANA

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Nanočestice srebra (AgNPs) se zbog izvrsnih protubakterijskih i protugljivičnih svojstava koriste u sve većem broju potrošačkih proizvoda. Pojačana proizvodnja neizbježno će dovesti do njihova ispuštanja u okoliš. Biljke, kao ključne komponente bioloških sustava, mogu poslužiti kao potencijalni put za prijenos, bioakumulaciju i unos AgNPs u prehrambene lance. U ovom istraživanju usporedili smo utjecaj AgNPs, stabiliziranih s tri različita omotača [citrat, polivinilpirolidon (PVP) i cetiltrimetilamonijev bromid (CTAB)], primijenjenih u tri koncentracije (25, 50 i 100 µM), na klijanje i rani rast duhana (*Nicotiana tabacum* L.). AgNPs-citrat nisu uzrokovale značajne promjene u postotku klijavosti, dok je klijavost biljaka tretiranih s AgNPs-PVP i AgNPs-CTAB bila značajno smanjena. Uz to, zabilježen je i negativan utjecaj na duljinu korjenčića i rast klijanaca, što je rezultiralo smanjenjem svježe i suhe mase te smanjenim indeksom tolerancije na stres. Kako bi utvrdili je li toksičnost AgNPs-PVP i AgNPs-CTAB posljedica disocijacije iona Ag+ ili samih nanočestica, u podlogu je dodano 125, 250 i 500 µM cisteina, jakog liganda srebra. Cistein je značajno smanjio štetne učinke AgNPs, ukazujući da je fitotoksičnost AgNPs barem djelomično rezultat disocijacije Ag+ iona.

Ključne riječi: nanočestice srebra, cistein, *Nicotiana tabacum*, isklijavanje, indeks tolerancije na stres

COATED SILVER NANOPARTICLES AFFECT GERMINATION AND DISRUPT TOBACCO GROWTH

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Silver nanoparticles (AgNPs) are being used in an ever increasing number of consumer products due to their excellent antibacterial and antifungal properties. Increased production will

inevitably increase the potential for their release into the environment. Plants, as key components of biological systems, may serve as a potential pathway for AgNPs uptake, bioaccumulation and a route into the food chain. In this study we compared the effects of three differently coated AgNPs [citrate, polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)], applied in three concentrations (25, 50 and 100 µM), on germination and early growth of tobacco (*Nicotiana tabacum* L.). AgNPs-citrate did not cause any significant changes in germination percentage, but the germination rate of plants treated with AgNPs-PVP and AgNPs-CTAB was significantly reduced. Root elongation and seedling growth were also negatively affected, resulting in reduction of fresh and dry mass and a decreased stress tolerance index. To examine whether the toxic effects of AgNPs-PVP and AgNPs-CTAB originate from dissolved Ag+ or nanoparticles themselves, 125, 250 and 500 µM of cysteine, a strong silver ligand, have been applied. Cysteine significantly reduced the harmful effects of AgNPs, thus showing that phytotoxicity of AgNPs at least partially derives from dissolved silver.

Keywords: silver nanoparticles, cysteine, *Nicotiana tabacum*, germination, stress tolerance index

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FOTOSINTETSKI ODGOVORI U DVIJE VRSTE RODA *Brassica* NA KRATKOTRAJNI SOLNI STRES

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Kako bi istražili rane fotosintetske odgovore kineskog kupusa (*B. rapa* ssp. *pekinensis*) i raštike (*B. oleracea* var. *acephala*) na povišeni salinitet, određeni su: fotosintetska učinkovitost, koncentracija klorofila i relativna razina akumulacije fotosintetskih proteina (Rubisco LSU, Cyt f, LHC b II i D1). Biljke su uzgojene hidroponski te kratkotrajno (tijekom 24 sata) tretirane s natrijevim kloridom u koncentracijskom rasponu od 0-200 mM. Rezultati su pokazali da dvije istraživane vrste imaju različitu toleranciju na solni stres, što je utemeljeno na različitim strategijama njihove fotosintetske prilagodbe. Raštika je, kao tolerantnija vrsta na solni stres, pokazala značajno bolju fotosintetsku učinkovitost u uvjetima povišenog saliniteta, kao posljedicu boljeg prijenosa elektrona. Suprotno, kineski kupus, kao umjereno tolerantna vrsta, pokazala je značajno smanjenje fotosintetske učinkovitosti u uvjetima povišenog saliniteta, iz razloga slabijeg prijenosa elektrona, povišene apsorpcije svjetlosti, te učinkovitijeg hvatanja ekscitona u reakcijskom središtu PSII. Posljedično, u biljkama kineskog kupusa došlo je do povišenja rasipanja suviška apsorbirane svjetlosti u obliku topline te do modifikacije određenog broja reakcijskih središta iz aktivnih u disipacijske. Uočene razlike u fotosintetskoj učinkovitosti dvije istraživane vrste bile su u skladu s razlikama u akumulaciji klorofila i fotosintetskih proteina uključenih u regulaciju prijenosa elektrona i apsorpcije svjetlosti.

Ključne riječi: kineski kupus, raštika, kinetika fluorescencije klorofila *a*, PSII, kratkotrajni solni stres

THE PHOTOSYNTHETIC RESPONSES OF TWO BRASSICA SPECIES TO THE SHORT-TERM SALINITY STRESS

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In order to investigate early photosynthetic responses of Chinese cabbage (*B. rapa* ssp. *pekinensis*) and kale (*B. oleracea* var. *acephala*) to the increased salinity, photosynthetic performance, chlorophylls concentration and relative accumulation levels of the photosynthetic proteins (Rubisco LSU, Cyt f, LHC b II and D1) were analyzed. Plants were grown hydroponically, treated with NaCl in a range of conc. 0-200 mM for a short-term (24 hours). Results showed that investigated species had different tolerance to the salt stress what was based on their different photosynthetic adjustment strategies. Kale, as a more salt tolerant species, revealed considerably better photosynthetic performance under increased salinity conditions due to more competent electron transport, in comparison to the moderately sensitive Chinese cabbage. On the other hand, Chinese cabbage plants decreased considerably their photosynthetic performance under the salt stress conditions. This was due to the less competent electron transport as well as increased light harvesting and trapping properties of PSII. This led to the increase in dissipation flux and the modification of a certain number of RC from active to dissipative ones. The observed differences in the photosynthetic performance of two investigated species corresponded to the differential accumulation of chlorophylls and photosynthetic proteins that regulate electron transport and light harvesting.

Keywords: Chinese cabbage, kale, chlorophyll *a* fluorescence kinetics, PSII, short-term salinity stress

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FUZIJSKI PROTEINI KU70-CENH3 INDUCIRAJU STVARANJE HAPLOIDA U VRSTI *Arabidopsis thaliana* I POKAZUJU GRADIJENT FUNKCIONALNOSTI KINETOHORNOG HISTONA CENH3

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Centromerni histon CENH3 nalazi se u kinetohorama viših biljaka gdje omogućuje ispravnu segregaciju kromosoma tijekom mitoze i mejoze. Potpuna genetička inaktivacija ovog gena u *A.thaliana* ima za posljdicu letalan fenotip. Različite CENH3 varijante, uključivši i fuzijske proteine, , mogu komplemetirati cenh3 mutaciju. Nadalje, takve komplementirane linije mogu u križanju s divljim tipom dati abortirano sjeme, aneuploide ili haploide. Dosadašnja istraživanja testirala su križanja u kojima se kinetohora s modificiranim CENH3 susreće s intaktnom kinetohorom. Ovim istraživanjem željeli smo ispitati ishode križanja gdje sučeljavamo dvije različito modificirane varijante CENH3.
Konstruirali smo seriju fuzija između CENH3 i Ku70, proteina koji sudjeluje u popravku dvolančanog loma DNA nehomolognim sparivanjem krajeva. Svaki konstrukt imao je cjeloviti CENH3 te Ku70 domenu različitih dužina. Od sedam konstrukata, samo dva konstrukta su

mogla komplementirati cenh3 mutanta i inducirati nastanak haploida u križanjima s divljim tipom. Dva novootkrivena induktora haploida križana su međusobno te s dvije prethodno opisane haploid-inducirajuće linije. U potomstvu takvih križanja uspjeli smo izolirati haploide kod kojih je prevladao samo jedan tip modificiranog CENH3 proteina. „Slabija“ varijanta CENH3 je bila izgubljena. Time smo pokazali da se za odabrani set dizajniranih CENH3 varijanti principijelno može opisati njihov gradijent funkcionalnosti.

Ključne riječi: CENH3, haploidi, *Arabidopsis*

KU70-TAGGED CENH3 FUSIONS INDUCE HAPLOIDS IN *Arabidopsis thaliana* AND REVEAL A FUNCTIONALITY GRADIENT OF THE KINETOCHORE HISTONE CENH3

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CENH3 is a histone found at kinetochores of higher plants promoting proper chromosome segregation. Its loss-of-function is embryo-lethal. CENH3 fusion proteins can complement the cenh3 knockout mutant in *A.thaliana*. Furthermore, such complemented lines when cross-pollinated with wild type result in seed abortion and aneuploid or haploid progeny. Recent studies predominantly tested outcomes of crosses where lines with CENH3-modified kinetochores were crossed to lines with wild type kinetochores. In this study, we performed crosses in which each parent had a different non-wild type CENH3 variant. We hypothesized that such crosses would produce haploids with the more functional CENH3 variant. We constructed a series of *A.thaliana* CENH3 fusion proteins with Ku70, a member of the non-homologous end joining DNA repair pathway. Each construct had a full-length CENH3 protein tagged with a differently-sized Ku70 domain. Only two of seven Ku70-CENH3 constructs complemented the cenh3 loss-of-function phenotype and could induce haploids in crosses with wild type. These two novel haploid-inducing lines were cross-hybridized with each other and with the previously characterised haploid-inducing variants. We could identify haploid plants among the progeny in which just one type of modified CENH3 prevailed, whereas the other „weaker“ CENH3 variant was lost. This indicated that a functionality gradient of a given set of engineered CENH3 variants could be defined for *A.thaliana*.

Keywords: CENH3, haploids, *Arabidopsis*

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UČINCI ZARAZE VIROIDOM VRETENASTOGA GOMOLJA KRUMPIRA NA SADRŽAJ ENDOGENIH HORMONA I AKTIVNOST ANTIOKSIDACIJSKIH ENZIMA U ASIMPTOMATSKIM BILJKAMA VRSTE *Solanum laxum*

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Viroidi su uzročnici biljnih bolesti čiji genom karakterizira mala (400 nt), jednolančana, nekodirajuća molekula RNA. Viroid vretenastoga gomolja krumpira (Potato spindle tuber viroid, PSTVd) uglavnom uzrokuje bolest u vrstama iz porodice Solanaceae, a veliku štetu može izazvati na nasadima krumpira i rajčice. Ukrasne vrste kao što je *Solanum laxum*, ne pokazuju vidljive simptome zaraze viroidom PSTVd te mogu predstavljati rizik od širenja bolesti na osjetljive vrste putem vegetativnog umnažanja zaraženih biljaka. Bolje razumijevanje latentnih infekcija važno je za kontrolu i suzbijanje biljnih bolesti, ali i programe oplemenjivanja s ciljem razvoja otpornosti/tolerancije biljaka na patogene. Da bi saznali više o fiziologiji latentnih infekcija viroidima, analizirali smo promjene u sadržaju endogenih fitohormona i aktivnosti antioksidacijskih enzima, koje nastaju tijekom infekcije vrste *S. laxum* viroidom PSTVd. Rezultati su pokazali da je u listovima inficiranih biljaka sadržaj endogene jasmonske kiseline (JA) i kastasterona (CS) značajno porastao, dok je sadržaj apscisne kiseline značajno smanjen 18 tjedana nakon inokulacije. Usljed infekcije došlo je i do povećanja aktivnosti askorbat peroksidaze i superoksid dismutaze, dok je aktivnost katalaze ostala nepromijenjena. Rezultati istraživanja upućuju na aktivaciju JA i CS signalnog puta kao dio obrambenog odgovora *vrste S. laxum* na infekciju viroidom PSTVd, iako se čini da ove promjene ne utječu na pojavu simptoma bolesti.

Ključne riječi: *Solanum laxum*, PSTVd, latentna zaraza, fitohormoni, antioksidacijski odgovor

EFFECTS OF POTATO SPINDLE TUBER VIROID INFECTION ON THE LEVELS OF ENDOGENOUS PHYTOHORMONES AND ANTIOXIDANT ENZYME ACTIVITY IN SYMPTOMLESS *Solanum laxum* PLANTS

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Viroids are plant pathogens characterized by a small (400 nt) single–stranded noncoding RNA genome. Potato spindle tuber viroid (PSTVd) mainly infects species belonging to the family Solanaceae with the potential to cause substantial damage to potato and tomato crops. In ornamental species including *Solanum laxum*, PSTVd infections are mostly symptomless, and therefore present the risk of propagating infected plants and spreading the disease to related crop plants. Latent infections are important in the control of plant diseases but also in breeding for resistance/tolerance to a pathogen. To learn more about the physiology of latent infections with viroids, changes in the endogenous phytohormones content and antioxidant enzyme activity in *S. laxum* infected by PSTVd were examined. Results showed that the endogenous jasmonic acid (JA) and castasterone (CS) significantly increased, while abscisic acid content significantly decreased in leaves of systemically infected plants compared to that of mock–inoculated control plants 18 wpi. PSTVd infection also enhanced the activity of ascorbate peroxidase and superoxide dismutase, while the activity of catalase remained steady. Results suggest the activation of the JA and CS signaling pathway in *S. laxum* as a part of defense response to PSTVd infection, although it seems that these changes have no effect on the onset of visible symptoms.

Keywords: *Solanum laxum*, PSTVd, latent infection, phytohormones, antioxidant response

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UTJECAJ PROTEINA DJELOMIČNO NEUREĐENE TERCIJARNE STRUCTURE U EKOTIPOVIMA UROČNJAKA *Arabidopsis thaliana* L. NAKON IZLAGANJA SUŠI

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Nedostatak vode jedan je od glavnih čimbenika okolišnog stresa, a s obzirom na klimatsku regiju, on može biti dugotrajan ili kratkotrajan i nasumičan. Sesilni organizmi poput biljaka, morali su razviti posebne strategije kojima prebrođuju i preživljavaju sušna razdoblja. Neke od tih strategija variraju s obzirom na genotip. U sklopu ovog rada, suši su izložena četiri ekotipa biljke *Arabidopsis thaliana*, Col, Oy, Ita i Van porijeklom iz četiri različite klimatske regije. Proučavane su razlike u toleranciji nedostatka vode na razini proteoma, pogotovo proteina djelomično neuređene strukture (IDPs). Mjerili smo aktivnost enzima peroksidaze (GPX) kao indikator oksidativnog stresa i relativnu koncentraciju proteina Hsp70, indikatora općenitog stresa. Elektroforezom razdvojili smo te mjerili i uspoređivali koncentraciju proteina IDPs, uključeni su u signalizacije puteve i dio su mnogih biljnih metaboličkih procesa, uključujući i u odgovor na abiotski stres. U odnosu na kontrolu, ekspresija peroksidaze i proteina Hsp70 je povećana. Ekspresija većine proteina IDPs se promijenila nakon suše. Dvadeset i četiri IDPs je identificirano spetrometrijom mase a bioinformatičkim alatima evaluirana je promjena strukture proteina. Dvadeset i jedan IDP ima produženu regiju bez strukture. Različita ekspresija proteina nakon suše unutar ekotipova ukazuje na genetsku adaptaciju biljaka u pokušaju da ovlada novim arealom.

Ključne riječi: neuređenost proteina, Hsp70, gvajakol peroksidaza, abiotski stres

THE INVOLVEMENT OF INTRINSICALLY DISORDERED PROTEINS (IDPs) IN DIFFERENT ECOTYPES OF *Arabidopsis thaliana* L. AFTER DROUGHT

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is a genus of carnivorous plants known by leaves modified into pitchers which lore, hunt and Water deficit is one of the crucial factors causing environmental stress and it can be in a particular climate region, either long-term or short-term and random. Sessile organisms like plants had to develop strategies in order to overcome and survive periods of drought some of which vary depending on the genotype. Our work involves exposure to drought four different ecotypes of *Arabidopsis thaliana*, Col, Oy, Ita and Van, native to different climate regions, study of their relative tolerance to water deficit on the level of proteome, especi ally analyzing intrinsically disordered proteins (IDPs). As indicators of general and biotic stress, we compared peroxidase activities (GPX) and relative concentrations of heatshock protein (Hsp70). We have separated and afterwards determined relative concentrations of IDPs as they are involved in integration of various signaling pathways and therefore take part in many plant processes in general, including abiotic stress responses. Compared to control, a rise was shown in GPX activity and Hsp70 expression during drought conditions. Expression

of several IDPs also changed after exposure to drought in comparison to control. A 25 IDPs were analyzed by mass spectrometry, and bioinformatics tools have evaluated their sequence structure disorder. Most of IDPs were confirmed to have at least one long disordered region. Difference in protein expression after drought was among ecotypes, which points to a genomic adaptation of plants to new area.

Keywords: protein disorder, Hsp70, guaiacol peroxidase, abiotic stress

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TEMPERATURNO OVISNA STABILNOST PROTEINA BPM1 U UROČNJAKU *Arabidopsis thaliana*

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Proteini MATH-BTB sadrže 2 domene, MATH (Meprin and TRAF Homology) i BTB (Bric-A-Brac, Tramtrack, Broad Complex) i djeluju kao adapterski proteini u kompleksu Cul3-ovisnih E3 ligaza uključenih u proteasomalnu degradaciju ciljnih proteina. U genomu uročnjaka (*Arabidopsis thaliana*) postoji 6 gena MATH-BTB (AtBPM1-6). Kroz interakciju s članovima nekoliko obitelji transkripcijskih faktora (R2R3 MYB, ERF/AP2 i razred I HD-Zip), proteini BPM sudjeluju u regulaciji cvjetanja, razvoja sjemena i odgovoru na abiotički stres. Nedavno je pokazano da proteini BPM reguliraju stabilnost transkripcijskog faktora DREB2A uključenog u odgovor na toplinski stres i isušivanje. S ciljem istraživanja fiziološke uloge proteina BPM1, proizvedene su transgenične biljke *A. thaliana* koje prekomjerno eksprimiraju fuzijski protein GFP-BPM1. Klijanci transgenične linije uročnjaka izlagani su povišenim temperaturama i toplinskom stresu. Ukupni topivi proteini izolirani iz tkiva klijanaca analizirani su imunodetekcijom uporabom antitijela osjetljivog na GFP. U odnosu na detektiranu degradaciju proteina BPM1 u kontrolnim uvjetima, izlaganje povišenim temperaturama i toplinskom stresu potaknulo je značajnu stabilizaciju proteina BPM1 in planta. Ovi rezultati ukazuju na potencijalnu fiziološku ulogu proteina BPM1 u odgovoru uročnjaka na toplinski stres.

Ključne riječi: *Arabidopsis*, BPM1, toplinski stres, imunodetekcija

BPM1 PROTEIN STABILITY IN *Arabidopsis thaliana* IS DEPENDENT ON ENVIRONMENTAL TEMPERATURE

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MATH-BTB proteins are comprised of 2 domains, MATH (Meprin and TRAF Homology) and BTB (Bric-A-Brac, Tramtrack, Broad Complex). They have been shown to interact with Cul3-based E3 ligase complex which promotes proteasomal degradation of target proteins. The *Arabidopsis thaliana* genome encodes 6 MATH-BTB genes (AtBPM1-6). Through interaction with members of at least three families of transcription factors (R2R3 MYB family, ethylene response factor/Apetala2 and class I homeobox-leucine zipper transcription factors), BPMs are involved in plant flowering, seed development and abiotic stress response. According to recent findings, BPM proteins are

responsible for degradation of transcription factor DREB2A involved in heat- and drought-stress response. To examine physiological roles of BPM1 in heat stress reponse, *A. thaliana* plants were transformed to obtain transgenic plants overexpressing GFP-tagged BPM1. Homozygous seedlings were exposed to elevated temperature and heat stress followed by whole soluble protein extraction. Anti-GFP monoclonal antibody was used for immunodetection of transgenic GFP-BPM1 protein. In control conditions, recombinant BPM1 exhibited low stability despite high overexpression rates. However, elevated temperatures and heat stress caused significant stabilization of transgenic BPM1. This result could indicate the physiological role of AtBPM1 in response to heat stress in *Arabidopsis*.

Keywords: *Arabidopsis*, BPM1, heat stress, immunodetection

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RUŽE (*Rosa* ssp.): TRADE-OFF IZMEĐU FOTOSINTETSKIH PIGMENATA, FLAVONOIDA I FENOLNIH KISELINA

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Listovi i latice ruža (genus *Rosa* L.) koriste se u prehrambenoj i kozmetičkoj industriji zbog bioaktivnih specijaliziranih metabolita koje sadrže, međutim nema usporednih podataka o kvalitativnom i kvantitativnom sadržaju ovih spojeva u kultivarima različitih boja. Fotosintetski pigmenti, flavonoidi i fenolne kiseline imaju ključnu ulogu u fotosintezi, metabolizmu i zaštiti biljaka od biotičkog i abiotičkog stresa, te predstavljaju glavne bioaktivne spojeve ruža. Stoga smo u sklopu ovog istraživanja kvantitativno i kvalitativno odredili glavne spojeve listova i latica triju različito obojenih kultivara prikupljenih u Botaničkom vrtu u Zagrebu s ciljem usporedbe njihovog bioaktivnog potencijala. Rezultati su pokazali da je u listovima ruže prisutna veća količina bioaktivnih spojeva nego u laticama. Ruže (listovi i latice) različitih boja pokazuju specijalizaciju u zastupljenosti pojedinih skupina bioaktivnih spojeva. Rezultati su pokazali da je u kultivarima različitih boja omjer fotosintetskih pigmenata, flavonoida i fenolnih kiselina različit, osobito u listovima.

Ključne riječi: bioaktivni spojevi, ferulična kiselina, kempferol, kvercetin, ružmarinska kiselina

ROSES (genus *Rosa* L.): A TRADE-OFF BETWEEN PHOTOSYNTHETIC PIGMENTS, FLAVONOIDS AND PHENOLIC ACIDS IN CULTIVARS OF DIFFERENT COLOURS

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Roses (genus *Rosa* L.) leaves and petals are used in food and cosmetic industry due to the bioactive specialized metabolites they contain, however there are no comparative data on the

qualitative and quantitative content of those metabolites in the cultivars of different colors. Photosynthetic pigments, flavonoids and phenolic acids play a key role in photosynthesis, metabolism and plant protection against biotic and abiotic stress, and are main bioactive components of roses. Therefore, we screened for these components in leaves and petals of three differently coloured cultivars collected in the Botanical Garden in Zagreb with the aim of comparing their bioactive potential. The results revealed that more bioactive components were present in leaves than in petals of roses. Roses (both leaves and petals) of different colours showed a specialisation in bioactive components representation. The results showed that in cultivars of different colours a trade-off between amount of photosynthetic pigments, flavonoids and phenolic acids, especially in leaves, exists.

Keywords: bioactive components, ferulic acid, kaempferol, quercetin, rosmarinic acid

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SADRŽAJ KLOROFILA I FOTOSINTETSKA UČINKOVITOST U IGLICAMA ALEPSKOG BORA

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Alepski bor (*Pinus halepensis* L.) je mediteranska drvenasta vrsta čije prirodne i sađene sastojine učestalo nalazimo u obalnom području Jadranskog mora. Iglice najčešće otpadaju nakon druge godine, što u ekstremnim slučajevima koji se javljaju u uvjetima okolišnog stresa može dovesti do značajnijeg ogoljenja krošnje. Cilj ovog istraživanja bio je usporediti sadržaj klorofila i fotosintetsku učinkovitost u iglicama različite starosti (ovosezonske i prošlosezonske iglice). Koncentracije klorofila određene su spektrofotometrijski, a fotosintetska učinkovitost određena je mjerenjem porasta fluorescencije klorofila *a* (JIP test). Rezultati su pokazali kako su koncentracije klorofila *a* i klorofila *b* bile skoro dvostruko više i statistički značajne u prošlosezonskim iglicama u odnosu na ovosezonske, dok omjer koncentracija klorofila *a* i *b* nije bio značajno različit. Također, značajne razlike nije bilo niti u maksimalnom prinosu kvanta fotosustava II (Fv/Fm). Indeks fotosintetske učinkovitosti (PIABS) bio je značajno viši u ovosezonskim iglicama. Iz priloženih rezultata zaključujemo da mlađe iglice, usprkos znatno nižem sadržaju klorofila pokazuju bolju fotosintetsku učinkovitost. Analiza parametara JIP testa pokazuje kako je glavni razlog za bolju fotosintetsku učinkovitost mlađih iglica njihova veća sposobnost iskorištenja apsorbirane svjetlosne energije u smislu kompetentnijeg prijenosa elektrona, u odnosu na starije iglice.

Ključne riječi: Alepski bor, fotosinteza, fotosustav II, fluorescencija klorofila a, *Pinus*

CHLOROPHYLLS CONTENT AND PHOTOSYNTHETIC PERFORMANCE IN ALEPPO PINE NEEDLES

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Aleppo pine (*Pinus halepensis* L.) is widely spread Mediterranean woody species. Its natural and cultivated populations can be frequently found in the coastal area of the Adriatic Sea. Usually, needles fall down after the second year, what can be the reason for substantial crown defoliation under extreme environmental stress. The aim of this investigation was to compare the chlorophylls content and photosynthetic performance in current-year (CY) and previous-year (PY) *A. pine* needles. Chlorophylls concentrations were determined spectrophotometrically and the photosynthetic performance was determined by measuring the increase in chlorophyll *a* fluorescence (JIP test). Results revealed that concentrations of chlorophylls *a* and *b* were almost two folded in PY needles compared to CY ones, what was statistically significant. Chlorophylls *a* to *b* ratio and values of the maximum quantum yield of the photosystem II (Fv/Fm) showed no statistical significance between CY and PY needles. However, performance index (PIABS) was significantly higher in CY needles compared to PY ones. Based on the presented results it can be concluded that despite of considerably lower chlorophylls content, CY needles reveled enhanced photosynthetic performance than older needles. The analysis of JIP test parameters showed that the main reason for this was better utilization of absorbed light energy in CY needles due to their more competent photosynthetic electron transport, in comparison to PY needles.

Keywords: *Aleppo pine*, chlorophyll *a* fluorescence, photosynthesis, photosystem II, *Pinus*

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UČINAK RAZLIČITIH OBLIKA SELENA NA OKSIDACIJSKI STRES I ANTIOKSIDACIJSKI ODGOVOR KLIJANACA PŠENICE (*Triticum aestivum* L.)

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Nedostatak selena (Se) u prehrani ljudi i životinja može dovesti do pojave različitih patofizioloških stanja. Stoga, biofortifikacija pšenice, jedne od najvažnijih prehrambenih usjeva, sa Se predstavlja održivu strategiju za rješenje ovog problema. Iako nije esencijalan za biljke, Se pokazuje dvostruki utjecaj na njihov metabolizam, tako pri niskim količinama pokazuje blagotvoran učinak, dok pri višim koncentracijama ima toksično djelovanje. Cilj je ovog istraživanja odrediti utjecaj povećanih koncentracija selenata i selenita na oksidacijski stres i antioksidacijski odgovor u kijancima pšenice (*Triticum aestivum* L.). Kao pokazatelji oksidacijskog stresa određivani su količina H_2O_2 i razina lipidne peroksidacije, koja je izražena mjerenjem količine reaktivnih spojeva tiobarbiturne kiseline, dok je antioksidacijski status određen mjerenjem količine ukupnog glutationa kao i određivanjem aktivnosti antioksidacijskih enzima kao što su katalaza i askorbat-peroksidaza. Rezultati su pokazali kako je učinak Se na oksidacijski stres i antioksidacijski odgovor u klijancima pšenice ovisio o njegovu kemijskom obliku i primijenjenoj koncentraciji. Oba oblika selena imala su značajniji utjecaj na mjerene parametre u korijenu u odnosu na izdanak pšenice, što upućuje na tkivno-specifičan odgovor. Dobiveni rezultati mogu poslužiti kao osnova za daljnja istraživanja utjecaja Se na

oksidacijski i antioksidacijski status, u svrhu dobivanja boljeg uvida u detoksikacijske mehanizme pšenice.

Ključne riječi: antioksidacijski enzimi, glutation, oksidacijski stres, pšenica, selen

IMPACT OF DIFFERENT SELENIUM FORMS ON THE OXIDATIVE STRESS AND ANTIOXIDATIVE RESPONSE IN WHEAT SEEDLINGS (*Triticum aestivum* L.)

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Selenium (Se) deficiency in human and animal nutrition could lead to development of different pathophysiological conditions. Accordingly, biofortification of wheat, as one of the most important food crop, with Se is sustainable strategy for overcome this problem. Although not essential for plants, Se shows dual impact on their metabolism, at low levels it has a beneficial effect, while it is toxic at higher levels. The aim of this study was to elucidate the impact of increased concentrations of selenate and selenite on the oxidative stress and antioxidative response in wheat seedlings (*Triticum aestivum* L.). As an indicators of oxidative stress, content of H_2O_2 and lipid peroxidation levels expressed in terms of thiobarbituric acid reactive substances were determined, while antioxidative status was determined by the content of total glutathione and by the activities of antioxidative enzymes such as catalase and ascorbate peroxidase. Results showed that the effect of Se on oxidative stress and antioxidative response in wheat seedlings depended on its chemical form and applied concentration. Both forms of selenium had more significant impact on the measured parameters in the root compared to wheat shoots, suggesting tissue-specific response. Obtained results can serve as a basis for further research on the Se impact on oxidative and antioxidative status in order to get better insight into the wheat detoxification mechanisms.

Keywords: antioxidative enzymes, glutathione, oxidative stress, selenium, wheat

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BIOKEMIJSKI ODGOVOR MUTANTI UROČNJAKA SA NARUŠENOM FUNKCIJOM ENZIMA AUKSIN-AMIDOHIDROLAZA NAKON IZLOŽENOSTI SOLNOM STRESU

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Biokemijski odgovor uslijed povišenog saliniteta (100 mM NaCl) u trajanju od 7 dana praćen je na mutantama *Arabidopsis thaliana* sa narušenom funkcijom enzima auksin-amidohidrolaza (jednostrukim ilr1, iar3, ill2; dvostrukoj iar3 ill2 i trostrukoj ilr1 iar3 ill2) te odgovarajućem divljem tipu (wt) ekotipa Wassilewskija. Auksin –amidohidrolaze su enzimi koji hidroliziraju konjugate auksina s aminokiselinama oslobađajaući aktivne forme, te tako sudjeluju u

homeostazi biljnih hormona auksina. Praćen je sadržaj malondialdehida, prolina i askorbata te sadržaj reaktivnih oblika kisika (superoksidni radikal - SO· i vodikov peroksid - H_2O_2) i glutationa primjenom fluorescentnih proba dihidroetidija (DHE), diklorodihidrofluorescein diacetata (DCFH-DA) i monoklorobimana (MCB). Solni stres izazvao je statistički značajno sniženje sadržaja askorbata kod mutante ill2 te povišenje kod mutanti iar3, iar3 ill2 i ilr1 iar3 ill2. Sadržaj prolina bio je statistički značajno povišen u wt i svim mutantama izloženim solnom stresu. Kao odgovor na solni stres došlo je do statističkog značajnog povećanja u sadržaju MDA (ilr1, iar3, iar3 ill2 i ilr1 iar3 ill2), glutationa (wt, ill2 i ilr1) i reaktivnih oblika kisika (SO·; ilr1 i H2O2; ill2). Najviši sadržaj askorbata, prolina i MDA uslijed solnog stresa izmjeren je u trostrukoj mutanti. Na temelju dobivenih rezultata možemo zaključiti da je trostruka mutanta, koja ima najmanju mogućnost hidrolize auksin-konjugata najosjetljivija na solni stres.

Ključne riječi: *Arabidopsis thaliana*, mutante, solni stres, biokemijski parametri

BIOCHEMICAL RESPONS OF *Arabidopsis thaliana* TRANSGENIC PLANTS WITH DISTURB FUNCTION OF ENZYME AUXIN AMIDOHYDOLASE AFTER SALT STRESS EXPOSURE

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Biochemical response of *Arabidopsis thaliana* mutants with impaired function of the enzyme auxin amidohydolase (single ilr1, iar3, and ill2; double iar3 ill2 and triple ilr1 iar3 ill2) and the corresponding wild type (wt) of the Wassilewskii ecotype to high salinity (100 mM NaCl) was evaluated after period of 7 days. Auxin amidohydrolases are enzymes that participate in homeostasis of plant hormone auxin by hydrolyzing auxin amide-conjugates and releasing active auxins. The contents of malondialdehyde, proline and ascorbate were determined as well as content of reactive oxygen species (superoxide radical SO· and hydrogen peroxide - H_2O_2) and glutathione using fluorescent probes dihydroethidium (DHE), dichlorodihydrofluorescein diacetate (DCFH-DA) and monochlorobimane (MCB). Salt stress induced significant decrease of ascorbate in ill2 and increase in iar3, iar3 ill2 and iar1 iar3 ill2. Accumulation of proline was significant not only in wt but also in all mutants exposed to salt stress. There was a statistically significant increase in the content of MDA (ilr1, iar3, iar3 ill2 and ilr1 iar3 ill2), glutathione (wt, ill2 and ilr1) and reactive oxygen species (SO·; ilr1 and H2O2; ill2) upon salt stress. The highest content of ascorbate, proline and MDA after exposure to salt stress was measured in triple mutant. Based on the obtained results we can conclude that the triple mutant, which has the lowest ability to hydrolyze auxin conjugates, is the most sensitive to salt stress.

Keywords: *Arabidopsis thaliana*, transgenic plants, salt stress, biochemical parameters

BIOLOGIJA KOPNENIH VODA I KOPNA
BIOLOGY OF FRESHWATER AND TERRESTRIAL ECOSYSTEMS

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FOOD WEB STRUCTURE AND FISH COMMUNITY RELATIONSHIPS IN PLITVICE LAKES NATIONAL PARK

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Plitvice Lakes National Park in Croatia represents an ecologically significant example of a karstic dominated ecosystem. Despite its recognition as a UNESCO World Heritage site and its consequent protection, the aquatic environment continues to experience anthropogenic disturbances in the form of water quality deterioration and non-native species introductions (primarily European chub). The objectives of this study were to use stable isotope analysis to establish food web structure, and to identify important food sources and potential dietary overlap between native and non-native fishes. Representative members of the fish community, benthic macroinvertebrates (BMI), zooplankton, macrophytes, mosses, and terrestrial leaf litter were collected from two lakes (Prošće and Kozjak) in Plitvice between June and October of 2017. Samples were subsequently oven-dried prior to carbon and nitrogen stable isotope analysis. Results indicated that the food web structure in both lakes was very similar. Benthic littoral production, likely from allochthonous leaf litter, appears to be the primary energy source. Most BMI and fishes had carbon isotope ratio values between -30 and -35‰ indicating that the fish were primarily eating BMI with plankton contributing relatively little to fish biomass. Native brown trout and pike were the top predators in each lake, and isotopic signatures indicated strong dietary overlap with European chub.

Keywords: web structure, fish community, Plitvice lakes

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INFLUENCE DIFFERENT AGRICULTURAL PRACTICIES ON CARAPACE SHAPE IN *Oedothorax apicatus* (BLACKWALL, 1850)

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The effects of pesticides and enviromental conditions on morphological variations among spiders have been rarely investigated. We explored influence different agricultural practices

on carapace shape in *Oedothorax apicatus* (Blackwall, 1850) specimens obtained from conventional, integrated and organic OSR fileds located in Ahlum, Germany. Carapace shape variation among individuals from these experimental fields was analyzed in MorphoJ software by canonical variate analysis (CVA). We showed that females from conventional and integrated fields had wider posterior part of carapace and were less cephalically protruding by CV 2 axis in addition to those from the organic field. Significant differences in carapace shape were not obtained among males from conventional field on the one side and integrated and organic fields on the other side, nor among males from organic and integrated fields. Difference in carapace shape among *O. apicatus* females may arise from the higher number of molting (and consequently the higher level of growth) on conventional and integrated fields because rigid structures in spiders like carapace can only grow by that way. It can be concluded that agricultural practice, especially on conventional OSR field, indeed have some impact on carapace shape of *O. apicatus* females. Male individuals were not affected by this phenomenon which can be the consequence of their higher mobility.

Ključne riječi: carapace shape, pesticides, spiders

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ZAJEDNICA FITOPLANKTONA U UJEZERENIM STANIŠTIMA VELIKE KRŠKE RIJEKE – SUSTAV PROTOČNOG I KASKADNOG JEZERA

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Taksonomski i funkcionalni sastav fitoplanktona pokazuje jasne sezonske promjene u različitim lotičkim i lentičkim staništima. Promjene u sastavu zajednice fitoplanktona, zajedno s fizikalno-kemijskim pokazateljima vode praćeni su od travnja do rujna 2017. godine u dva lentička staništa na rijeci Krki, u jezerima Visovac i Brljan, s ciljem boljeg razumijevanja funkcioniranja i strukturnih karakteristika ovih specifičnih staništa. Temperatura i koncentracija kisika mjereni su u vertikalnim profilima tijekom svakog uzorkovanja, čime je dobivena informacija o stratifikaciji i dubinskom maksimumu klorofila a. U jezeru Visovac je, za razliku od jezera Brljan, utvrđena ljetna stratifikacija. Dubinski maksimum klorofila *a* nije utvrđen u oba jezera, a jasna anoksija bila je prisutna u ljetnim mjesecima u jezeru Visovac. Rezultati vertikalnih profila temperature i kisika ukazuju na to da je jezero Brljan dio riječnog sustava, dok jezero Visovac ima znatno duže vrijeme zadržavanja vode. Taksonomski i funkcionalni sastav fitoplanktona jasno odražava hidrološka svojstva istraživanih staništa. Centrice, jedan od morfoloških oblika algi kremenjašica, pripadnice funkcionalnih grupa (FG) D i P, dominirale su u jezeru Brljan kao tipičan potamoplankton. Jezero Visovac je karakterizirala kodominacija i sezonske promjene

centrica, zlatnožutih algi i dinoflagelata. Sezonske promjene sastava FG (C-X2,U-L0) uz fizikalno-kemijske pokazatelje vode, ukazuju na mezotrofan status stratificiranog jezera.

Ključne riječi: fitoplankton, funkcionalne grupe, trofički status, stratifikacija, klorofil *a*

PHYTOPLANKTON COMMUNITY IN LENTIC HABITATS OF LARGE KARSTIC RIVER – RIVERINE AND BARRAGE LAKE SYSTEM

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Taxonomical and functional composition of phytoplankton show clear seasonal patterns in the different lotic and lentic habitats. Compositional changes in phytoplanktic communities, with physical and chemical parameters of water were studied from April to September in 2017 in two lentic habitats on Krka River, Visovac and Brljan lakes, for deepening the knowledge about the functioning and structural characteristics of these specific habitats. During sampling, temperature and oxygen were measured in depth profiles providing stratification curve and the position of deep chlorophyll *a* (Chl*a*) maximum. Stratification was detected in the summer months in Lake Visovac, while there was no stratification in Lake Brljan. Deep Chla maximum was not detected in these lakes, but clear anoxia was present in summer in Lake Visovac. Temperature and oxygen profiles indicated that Lake Brljan is a riverine system, while Lake Visovac is lake with much longer retention time. Taxonomic composition and Functional groups (FGs) clearly reflected to hydrological characteristics of the habitat. Centric diatoms belonging to FGs D and P as typical potamoplankton dominated in riverine Lake Brljan. Barrage Lake Visovac was characterised by the co-dominance and seasonal changes of centric diatoms, chrysophytes and dinoflagellates. Seasonal change in FGs composition (C-X2,U-L0) indicated the mesotrophic status of the stratified lake what was also confirmed by the results of physical and chemical parameters.

Keywords: phytoplankton, functional groups, trophic state, stratification, chlorophyll *a*

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ANTI-FUSARIUM EFFECT OF *Megaphyllum unilineatum* (C. L. KOCH, 1838) (DIPLOPODA: JULIDA: JULIDAE) DEFENSIVE SECRETION

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Millipedes (Diplopoda) of the order Julida are known to produce mixtures of quinones (usually as dominant compounds), phenolics, alcohols, esters, aldehydes, ketones, and anthranilate derivatives as agents of antipredator defense. Apart from the mentioned role, data on other biological activities of these secretions are scarce. Being soil organisms, millipedes are in frequent contact with many pathogenic microorganisms. This study assessed the in vitro susceptibility of seven phytopathogenic *Fusaruim* species against ethanol, methanol, hexane, and dichloromethane extracts of defensive secretions of *Megaphyllum unilineatum* (C. L. Koch, 1838) (julidan which secretions are blends of p-benzoquinones, esters, and ketones) according to the broth microdilution method. Commercial antimycotic Nystatin was used as a positive control. The strongest antifungal activity was observed for the ethanol extract, with minimum inhibitory (MICs) and fungicidal concentrations (MFCs) in the range of from 0.15 to 0.30 mg/mL. All tested extracts were significantly stronger compared to Nystatin (MICs and MFCs >0.60 mg/mL). *Fusarium lateritium* was the most susceptible isolate, while *F. sporotrichoides* and *F. verticillioides* were the most resistant. Our data highlight that tested extracts contain antifungal components and broaden our knowledge about chemical ecology of millipedes.

Keywords: millipedes, defensive secretions, *Fusarium*, microdilution, antifungal activity

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CHIRONOMID LARVAE FAUNA (DIPTERA, CHIRONOMIDAE) IN THE FUTURE ACCUMULATION BOSHKOV MOST – REPUBLIC OF MACEDONIA

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Chironomid larvae fauna to date has been widely researched, especially in natural lakes (Ohrid, Prespa and Dojran Lake), while in the reservoirs it’s poorly explored. This is our contribution to the research of the chironomid larvae fauna from the zoobenthos for determining the water quality of the future accumulation Boskov Most, which is from I category because the dominant species, *Prodiamesa olivacea*, is a bioindicator for clean water. Quantitative samples were collected in duration of 1 and a half year with Surber samplers from 6 different localities of the littoral zone and that: Mala River (T1), Lazaropolska River (T2), Garska River (T3), Rosocka River (T4), Tresonecka River (T5), Jadovska River (T6) and then preserved in 70% ethanol. The samples were collected from macrophyte vegetation from depth of 50 cm to 110 cm. Then the samples were transported to the laboratory and were classified and determined. During the research period of the future accumulation Boshkov most, were found 11 taxa of the chironomid larvae fauna belonging to 4 subfamilies: Chironominae, Tanypodinae, Orthocladiinae and Prodiamesinae. The most abundant family is Orthocladiinae with total of 4 taxa. Dominant species is *Prodiamesa olivacea* Meigen 1818 (Prodiamesinae) represented with total of 17 taxa, and

least represented species is Eukiefferiela alpestris Goetghebuer, 1934 (Orthocladiinae) with total of 7 species. According to the seasons, most

Keywords: Boshkov Most, chironomid larvae fauna, *Prodiamesa olivacea*

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MOPRHOLOGY AND ULTRASTRUCTURE OF DEFENSIVE GLANDS OF A MILLIPEDE *Pachyiulus hungaricus* (KARSCH, 1881) (MYRIAPODA: DIPLOPODA: JULIDA)

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Most millipedes possess segmentally arranged defensive glands (ozadenes). In the order Julida, ozadenes are distributed laterally, in pairs, on all, but the first five and the last one or two segments. Defensive fluid is a mixture of quinones and non-quinonic compounds, such as esters, alcohols and other chemicals. Here, we describe the morphology of the ozadenes of *Pachyiulus hungaricus* (Karsch, 1881) analyzed by light and electron microscope. Glands were isolated in 2.5% glutaraldehyde droplets, fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer, post-fixed in 1% osmium tetroxide, and then routinely dehydrated and embedded in Araldite. Ultrathin sections were mounted on copper grids and examined on CM12 transmission electron microscope. Semithin sections were routinely stained by toluidine blue, and observed using a Leica DMLB microscope. Julidan-type gland consists of a reservoir of a secretory nature, and an efferent duct which opens through an ozopore. The entire gland is encompassed by cuticular lining, which is more developed in the duct than toward reservoir lumen. Interdigitations are formed among secretory cells. The cell cytoplasm contains Golgi apparatus, granular endoplasmic reticulum, mitochondria, lipid droplets, and many various types of secretory and pigment granules of different density. These findings are a part of ongoing research that should help in elucidation the functional morphology and mode of secretion of julidan-type defensive glands.

Keywords: defensive glands, transmission electron microscopy, secretory cells, Julida

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***Heribaudiella fluviatilis* (ARESCHOUG) SVEDELIUS – PRVA SLATKOVODNA SMEĐA MAKROALGA ZABILJEŽENA U HRVATSKOJ**

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Monitoring makrofita u kopnenim vodama proveden na području Hrvatske u razdoblju od 2009. do 2017. rezultirao je velikom količinom novih podataka o pojavnosti, sastavu i strukturi vodene vegetacije. Također, omogućio je sustavan uvid u mnoge slabo istraživane skupine među koje se mogu uvrstiti i slatkovodne makroalge. Tako je po prvi puta zabilježena i slatkovodna smeđa makroalga *Heribaudiella fluviatilis* na sveukupno četiri lokacije područja dinaridske ekoregije. Smeđa alga zabilježena je prvi puta 2017. godine na utoku potoka Loskun u rijeku Unu, što je potaklo naknadni pregled materijala iz herbarske zbirke ZA. Time je utvrđeno da je alga sabrana na rijeci Cetini već 2009. te na dva lokaliteta na rijeci Kupi tijekom 2017. Vrsta je toliko dugo ostala nezamijećena zbog neupadljivog korastog smeđeg talusa, te zbog toga što standardni protokol monitoringa kopnenih voda od makroalgi uključuje samo parožine. Ovaj rad donosi detaljne mikrodijagnostičke značajke vrste *H. fluviatilis* te opis njezinih staništa s fizikalno-kemijskim značajkama vode i podacima o ukupnoj vodenoj vegetaciji.

Ključne riječi: Phaeophyta, kopnene vode, prvi nalaz, Hrvatska

Heribaudiella fluviatilis (ARESCHOUG) SVEDELIUS – FIRST FRESHWATER BROWN MACROALGA RECORDED IN CROATIA

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The monitoring of macrophytes in Croatian freshwaters, conducted during the period 2009-2017, resulted with the large amount of new data of the occurrence, composition and abundance of water vegetation. It also provided a systematic insight into the poorly studied groups, among which freshwater macroalgae can be highlighted. In addition, for the first time, the freshwater brown macroalga *Heribaudiella fluviatilis* was recorded on four localities in the Dinaric ecoregion. The brown alga was firstly recorded in 2017, at the mouth of the Loskun stream into the Una River, provoking the subsequent review of the material from ZA herbarium collection. The revision revealed that the alga was already collected in the Cetina River in 2009, and in two locations in the Kupa River during 2017. The species was unrecognized until now because of its inconspicuous, brown crusty talus, and also because the research protocols of the freshwater monitoring project include only members of Characeae. This paper provides the detailed micro-diagnostic characteristics of *H. fluviatilis* and the description of the corresponding habitat, with the physico-chemical preferences and the associated water vegetation.

Keywords: Phaeophyta, freshwater, first record, Croatia

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A CONTRIBUTION TO THE KNOWLEDGE OF FRESHWATER SUBTERRANEAN CRUSTACEANS IN SERBIA AND THE BALKANS

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The crustacean fauna of the Balkans has been extensively studied by various authors over the last century. During our research, still in progress, on the subterranean fauna of Serbia, a number of crustaceans were collected from the caves or groundwater habitats. Samples were captured by hands and hand-net, and preserved in 70% and 96% ethanol, respectively. Based on our recent field studies, we can confirm that the Serbian part of the Balkan Peninsula is characterized by the presence of a very rich troglobiotic and stygobiotic fauna. The most common and most explored were representatives of the amphipod families Gammaridae and Niphargidae. Among them, the most numerous and diverse were representatives of the genus *Niphargus* Schiödte, 1849, with high number of endemic taxa. The isopod fauna specialized for life in groundwater was also diverse and widely distributed. The Asellota (Asellidae, Stenasellidae) was often founded group of stygobiotic crustaceans. Together with the troglobiotic cave-dwelling fauna of the Oniscidea (Trichoniscidae), they have colonized almost all kinds of underground habitats. Although we analyzed the subterranean fauna mainly within the existing administrative territory of Serbia, our findings confirmed the richness of both troglobiotic and stygobiotic crustacean fauna elsewhere in the Balkans, since the populations are still in some manner connected together, and are distributed beyond current political borders.

Keywords: crustaceans, stygofauna, troglofauna, freshwaters, Balkan Peninsula

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PRAĆENJE STANJA BOLESTI RAČJE KUGE U NACIONALNOM PARKU PLITVIČKA JEZERA

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Nacionalni park Plitvička jezera prirodno je stanište zakonom zaštićenih zavičajnih vrsta rakova (riječnog i potočnog). Slatkovodni deseteronožni rakovi ključne su vrste slatkovodnih ekosustava, ali se njihova brojnost smanjuje, između ostalog zbog smrtonosne bolesti račje kuge uzrokovane patogenom *Aphanomyces astaci* (Oomycetes). Prethodnim istraživanjima u NP Plitvička jezera je utvrđena prisutnost patogena, ali je u istraživanje bio uključen samo mali broj jedinki riječnog raka. Stoga je cilj ovog istraživanja bio pratiti prisutnost *A. astaci* na većem broju jedinki obje vrste: *A. astacus* je uzorkovan na lokacijama Burgeti (68 jedinki) i Prštavci (10), a *A. torrentium* na potocima Prijeboj (22) i Sartuk (15). U prikupljanju uzoraka korištena je novorazvijena neinvazivna metoda detekcije patogena temeljena na uzimanju briseva kutikule. Iz briseva je izolirana DNA te je standardnim PCR-om provjerena prisutnost patogena. Od ukupno 115 testiranih rakova, patogen je utvrđen samo u riječnih rakova (9 od 78) na obje istraživane lokacije. Uzorci pozitivni na *A. astaci* analizirani su i kvantitativnim

PCR-testom kako bi se utvrdio intenzitet infekcije (A0A7). Za jedinke visokog stupnja zaraze (>A4) uzorci su genotipizirani te je utvrđeno da patogen pripada starom europskom As soju, što se podudara s rezultatima prethodnih istraživanja. Dobiveni rezultati će biti upotrijebljeni u razvoju planova zaštite ugroženih slatkovodnih rakova u NP i prevencije širenja patogena u okolne vodotoke.

Ključne riječi: *Astacus astacus*, *Austropotamobius torrentium*, *Aphanomyces astaci*

MONITORING OF CRAYFISH PLAGUE IN THE PLITVICE LAKES NATIONAL PARK

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Two indigenous crayfish species, noble and stone crayfish, inhabit waterbodies of the Plitvice Lakes National Park. Crayfish are keystone species, but their numbers are declining, amongst others due to the lethal disease crayfish plague caused by pathogen *Aphanomyces astaci* (Oomycetes). Presence of this pathogen has already been recorded in the Plitvice Lakes but previous research included only small *A. astacus* sample. Thus, the main goal of this research was to analyse the presence of *A. astaci* on both species, using larger sample size, at multiple locations within the Park; *A. astacus* was sampled at Burgeti (68 individuals) and Prštavci (10), and *A. torrentium* at Prijeboj (22) and Sartuk (15). New, non-invasive method, based on collecting swabs of mixed epibiotic microbial communities from crayfish carapace was applied for *A. astaci* detection. DNA was isolated from swab samples and PCR was used to detect presence of pathogen's DNA. Pathogen was detected only in noble crayfish (9/78) from both localities. Positive samples were classified into semi-quantitative categories of pathogen load by quantitative PCR. Microsatellite genotyping identified presence of As strain of *A. astaci* which is consistent with previous results. Obtained results will be used in future management plans aiming to protect vulnerable native species in NP, with focus on prevention of unintentional spread of the pathogen to adjacent streams and crayfish populations.

Keywords: *Astacus astacus, Austropotamobius torrentium, Aphanomyces astaci*

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NOVE I ZNAČAJNE EFEMERNE VRSTE U FLORI MAHOVINA HRVATSKE

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Efemerne vrste mahovina su kolonisti koji mogu završiti životni ciklus unutar kratkog dijela vegetacijske sezone kada nastupe za njih povoljni uvjeti. Značajan broj ovakvih specijaliziranih vrsta vezan je uz staništa kao što su rubovi jezera, akumulacija i rijeka. Tijekom provođena nacionalnog monitoringa površinskih voda u 2016. i 2017. na području čitave Hrvatske istraživane su i obale prirodnih i umjetnih vodnih tijela kako bi se zabilježila eventualna prisutnost efemernih mahovina. Zabilježena je nova vrsta za floru mahovina Hrvatske - *Physcomitrium eurystomum* Sendtn. na obali Biljskog jezera, nekadašnjeg meandra rijeke Drave u blizini Osijeka. Nadalje, za floru Hrvatske je potvrđena rijetka efemerna vrsta *Physcomitrella patens* (Hedw.) Bruch & Schimp. Zabilježena je u zoni povlačenja vode na Biljskom jezeru, akumulaciji Kruščica u Lici, jezeru Sakadaš u Baranji i tri rijeke u Moslavini – Rajić, Ilova i Trebež. Tijekom istraživanja također su zabilježene i rijetke efemerne vrste *Riccia cavernosa* Hoffm., *R. bifurca* Hoffm., *R. fluitans* L. i *Leptobryum pyriforme* (Hedw.) Wilson. Iako se navedene vrste i njihova staništa u Europi smatraju relativno rijetkim i ugroženim, mali broj nalaza u Hrvatskoj je u znatnoj mjeri posljedica nedovoljne istraženosti efemernih staništa i mahovinske flore općenito.

Ključne riječi: *Efemerne mahovine*, *Physcomitrium eurystomum*, *Physcomitrella patens*, *Riccia cavernosa*

NEW AND NOTEWORTHY EPHEMERAL SPECIES IN THE BRYOPHYTE FLORA OF CROATIA

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Ephemeral bryophytes are short-lived colonists that can germinate and finish their whole life cycle within a brief period during the vegetation season when favourable conditions occur. A significant number of these specialist species are associated with habitats such as margins of lakes, reservoirs and rivers. While conducting the national monitoring of the surface waters in 2016 and 2017 natural and artificial water bodies throughout Croatia were surveyed and water margins were inspected for eventual ephemeral bryophytes. We recorded a new species for the bryophyte flora of Croatia - *Physcomitrium eurystomum* Sendtn. on the margins of the Lake Biljsko Jezero, an oxbow lake of the Drava River near Osijek. Furthermore, a rare ephemeral species *Physcomitrella patens* (Hedw.) Bruch & Schimp. was confirmed for the flora of Croatia. *Physcomitrella patens* grew in the drawdown zone of the Lake Biljsko Jezero, the Kruščica Reservoir in Lika, the Lake Sakadaš in Baranja and three rivers in Moslavina – Rajić, Ilova and Trebež. During our research rare ephemeral species *Riccia cavernosa* Hoffm., *R. bifurca* Hoffm., *R. fluitans* L. and *Leptobryum pyriforme* (Hedw.) Wilson were also recorded. Although mentioned species and their habitats are considered relatively rare and threatened in Europe, the low number of records in Croatia is to a considerable extent the result of insufficient research done into ephemeral habitats and bryophyte flora in general.

Keywords: *Ephemeral bryophytes*, *Physcomitrium eurystomum*, *Physcomitrella patens*, *Riccia cavernosa*

P-75

AN UPDATED CHECKLIST OF AQUATIC AND TERRESTRIAL INVERTEBRATES AND FISH FAUNA FROM DOJRAN LAKE AND ITS SURROUNDING (R. MACEDONIA)

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The presented checklist is a first critical list of aquatic and terrestrial invertebrates and fish fauna from Dojran Lake and its surrounding (R. Macedonia). The list is based on historical literature data and field research by the authors during August 2016. In addition, identification and registration of errors from previous studies have been also indicated. Results show that Dojran Lake and the terrestrial habitats around it support extremely high diversity of species, including numerous local, national and Balkan endemic species, confirming that the lake meets the Key Biodiversity Areas criteria in the country.

Keywords: checklist, invertebrates, fish, biodiversity, Dojran Lake, terrestrial habitats

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PAROŽINE (CHARALES, CHARACEAE) HRVATSKE

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Parožine ili alge iz porodice Characeae složene su alge makroskopskog talusa koji izgledom podsjeća na preslice. Uvrštene su na popis Natura 2000 staništa pod nazivom Tvrde oligo-mezotrofne vode s dnom obraslim parožinama. Kako parožine Hrvatske do danas nisu sustavno popisane, ovim je radom proveden detaljan uvid literaturnih podataka, neobavljenih podataka iz istraživanja koautora, kao i podataka iz studija i elaborata kojima su parožine bile obuhvaćene. U Hrvatskoj je ukupno utvrđeno 36 vrsta parožina iz svih šest do sada opisanih rodova: *Chara* (19), *Lamprothamnium* (2), *Lychnothamnus* (1), *Nitella* (10), *Nitellopsis* (1) i *Tolypella* (4). Prema geografskoj rasprostranjenosti, najviše su rasprostranjene u Dinarskoj ekoregiji, ali određeni broj vrsta dolazi i u Panonskoj ekoregiji. Kao Natura 2000 stanište, dominantna su skupina vodene vegetacije u većini hrvatskih prirodnih krških jezera pa ih tako nalazimo u Plitvičkim jezerima, Vranskom jezeru na otoku Cresu, Vranskom jezeru u Dalmaciji i Baćinskim jezerima. Kao pioniri vegetacije dolaze i u povremenim lokvama ili jezerima na hrvatskim otocima poput otoka Mljeta, Paga, Krka i mnogih drugih te duž cijele obale. Ovaj prvi sistematičan popis parožina Hrvatske temelj je

zaštite prirode i značajan doprinos poznavanju bioraznolikosti Hrvatske, posebice jer su njime obuhvaćene neke od najrjeđih pa sve do vrlo čestih vrsta.

Ključne riječi: parožine, popis, rasprostranjenost

STONEWORTS (CHARALES, CHARACEAE) OF CROATIA

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Stoneworts or algae from family Characeae are complex algae with macroscopic habitus that recall to horsetails. They are listed as Natura 2000 site under the name of Hard oligo-mesotrophic waters with benthic vegetation of Chara spp. Since diversity or presence of Croatian stoneworts was never systematically investigated, detailed insight in literature data, unpublished data from co-authors researches, as well as data from environmental studies that comprised stoneworts was done. According to all available sources, there are 36 species of stoneworts in Croatia in total that belongs to all six genera: *Chara* (19), *Lamprothamnium* (2), *Lychnothamnus* (1), *Nitella* (10), *Nitellopsis* (1) and *Tolypella* (4). Geographically, stoneworts are mostly distributed in Dinaric Ecoregion, with minor number of species in Pannonian Ecoregion. They are dominant aquatic vegetation in most of Croatian natural karstic lakes as Natura 2000 site, therefore we find them in large abundance in Plitvice Lakes, Lake Vransko on Island of Cres, Lake Vransko in Dalmatia and in Baćina Lakes. As pioneers of vegetation, stoneworts are often found in temporary ponds and lakes on islands throughout all Adriatic coast, like on islands Mljet, Pag, Krk and many others. This is the first systematic list of stoneworts in Croatia and a foundation for nature protection as well as significant contribution to knowledge of Croatian biodiversity, especially because it brings data about some of the rarest and very common specie

Keywords: charophytes, check list, distribution

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HELMINTH FAUNA OF PRESPA BLEAK (*Alburnus alburnus belvica* KARAMAN, 1924) (PISCES: CYPRINIDAE) FROM MACEDONIAN PART OF LAKE PRESPA

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Eight out of the eleven indigenous fish species of Prespa are endemic, i.e. they exist only in the Prespa region and in no other place on earth. One of these endemic fish species is Prespa bleak (*Alburnus alburnus belvica*). The parasitological examination from the Macedonian part of the Lake Prespa showed that all of 147 examined specimens of Prespa bleak fishes were infested (100.0%). In our case study the presence of 13 parasite species was established: *Dactylogyrus alatus* f. *typica*, *Dactylogyrus sphyrna*, *Paradiplozoon alburni*,

Allocreadium markewitschi, *Phyllodistomum* sp., *Posthodiplostomum cuticola* (larva), *Ligula intestinalis* (plerocerkoid), *Cystidicoloides tenuissima*, *Philometra ovata*, *Raphidascaris acus*, *Contracaecum microcephalum* (larva), *Metechinorhynchus truttae* and *Pomphorhynchus bosniacus*. Individually, by the parasite species, the highest prevalence was with *Posthodiplostomum cuticola* (larva) (100.0%), *Dactylogyrus alatus* f. *typica* (59.18%) and *Raphidascaris acus* (34.69%). The lowest one was with *Paradiplozoon alburni*, *Allocreadium markewitschi* and *Phyllodistomum* sp. (1.36%). In our case study the parasites *Dactylogyrus alatus* f. *typica*, *Dactylogyrus sphyrna* and *Paradiplozoon alburni* are recorded for the first time in the ichthyoparasitofauna of Lake Prespa and Macedonia.

Keywords: Helminth fauna, bleak, Lake Prespa

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DEVELOPMENTAL AND THE OTHER-INDUCED ABNORMALITIES IN MORPHOLOGY OF *Lepidurus couesii* PACKARD, 1875 (CRUSTACEA: BRANCHIOPODA) FROM SERBIA

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Morphological abnormalities are well known phenomena in crustaceans and other groups of arthropods. They usually originate as aberrations during the molting process, or can be attributed to genetic factors. Researchers also assume fixation and preservation of individuals as causers of deformation of bodies or certain body parts. The aims of this study were to observe the variety of the body changes in the large branchiopod crustacean *Lepidurus couesii* Packard, 1875, sampled from natural habitats in Serbia, and to discuss their etiology. In our samples, abnormalities may be divided into two main groups: 1) atypical changes in natural form of certain body parts, including the occurrence of shorter or incomplete body segments, as well as the fused spiral-growthed abdominal segments, and 2) changes due the predatory injury (and uneven regeneration thereafter), physical harm, or disease (invasion of parasites, lesions, changes in body color). In majority of cases, morphological abnormalities were identified on the carapace, abdominal part, and the telson. Some of the observed deformities influenced symmetry and the functional morphology of individuals. In terms of potential causes, most of abnormalities are caused by disturbed development or incomplete molting. Some of them can be attributed to the unpredictability and ephemerality of their extreme habitats and occurred due to the predatory, and environmental stress and contamination.

Keywords: morphological abnormalities, *Lepidurus couesii*, Serbia

P-79

HPLC ANALIZA - BRZA METODA ZA PRAĆENJE DINAMIKE FITOPLANKTONA

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Iako su istraživanja fitoplanktona tradicionalno usmjerena prema određivanju sastava, brojnosti i biomase zajednice korištenjem metode mikroskopiranja, u novije se vrijeme razvijaju brze metode, poput analize pigmenata putem tekuće kromatografije visoke djelotvornosti (HPLC). Cilj istraživanja bio je opisati fitoplanktonsku zajednicu Visovačkog jezera (NP Krka, Hrvatska) pomoću metoda mikroskopije i analize pigmenata (HLPC) te usporediti rezultate metoda s ciljem procjene primjenjivosti i učinkovitosti analize pigmenata na slatkovodnim krškim sustavima. Uzorci vertikalnih profila prikupljeni su na svakih 5 metara dubine sa 7 postaja tijekom jednodnevnog terena u srpnju 2012. i svibnju 2013. godine na Visovačkom jezeru. Udio pigmenata u uzorcima određen je HPLC metodom, pri čemu su indikatorski pigmenti odabrani na temelju kemotaksonomskih značajki: fukoksantin kao indikator dijatomeja, peridinin kao indikator dinoflagelata, aloksantin kao indikator kriptofita, zeaksantin kao indikator cijanobakterija te klorofil *b* kao indikator zelenih algi. Udio pigmenata podudarao se s udjelom biomase pripadajućih skupina. Navedena se metoda pokazala usporedivom metodi mikroskopiranja u opisu fitoplanktonske zajednice jezera Visovac. Zbog svoje jednostavnosti i brzine uporaba HPLC analize u budućim bi istraživanjima krških slatkovodnih ekosustava, u kombinaciji s mikroskopiranjem, omogućila detaljniji i obuhvatniji uvid u prostornu i vremensku dinamiku fitoplanktonskih zajednica.

Ključne riječi: pigmenti, krška jezera, fitoplankton, HPLC

HPLC ANALYSIS – RAPID METHOD FOR PHYTOPLANKTON ASSESSMENT

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Phytoplankton investigations are traditionally focused on determining the composition, abundance and biomass by means of microscopy. However, several rapid methods for characterization of phytoplankton communities, such as pigment-based chemotaxonomy using high-performance liquid chromatography (HPLC) were developed in the recent years. The aim of this research was to describe the phytoplankton community of Lake Visovac (NP Krka, Croatia) by using microscopy, as well as the HPLC, and to compare the results of both methods in order to test the applicability and efficiency of the HPLC in the karstic freshwater ecosystems. Lake Visovac was sampled at the vertical profile on 7 sampling points during one-day fieldtrips in July 2012 and May 2013. Phytoplankton pigments were determined and quantified by the HPLC method. Indicator pigments were selected according to following chemotaxonomic features: fucoxanthin for diatoms, peridinin for dinoflagellates, alloxanthin for cryptophytes, zeaxanthin for cyanobacteria and chlorophyll *b* for chlorophytes. Results showed that indicator pigments correlated with the biomass of the associated phytoplankton groups. Both microscopy and HPLC were comparable in describing the phytoplankton community of Lake Visovac. Due to simplicity and speed the HPLC could, in combination with microscopy, enable a more detailed and comprehensive insight into the spatial and temporal dynamics of the phytoplankton communities in freshwater karstic ecosystems.

Keywords: pigments, karstic lakes, phytoplankton, HPLC

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ANTHROPOGENIC INFLUENCE ON THE REED BELT FROM LAKE OHRID

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The reed, *Phragmites australis* forms natural discontinuous belt around the Lake Ohrid comprised of many complexes with different shape and size. In recent years the anthropogenic influence in Lake Ohrid is increasingly present, and provokes significantly changes in the reed belt. Namely, the reed is cuted, burned and covered with land and different waste for obtaining beaches, hotel complexes, parking spaces and other. In this paper are presented the changes in the reed belt from the Lake Ohrid over the last 30 years (comparison of the reed belt in 2016 with 1986 year was made). The obtained results indicated that there have been changes (in both the number of complexes and the total area of the belt). Namely, the total number of reed complexes in 2016 (80) is lower than the one recorded in the period 1986 (105), and the total surface area of the reed belt is about 2 ha reduced than the one measured 30 years ago. With the uncontrolled destruction of the reed belt of Lake Ohrid are destroys and the natural habitats of the populations of organisms that live in it (algae, invertebrates, and vertebrates). Therefore, one of the priority tasks in the next period is the taking of measures for the protection of the reed belt.

Keywords: reed belt, changes, Lake Ohrid

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AUTOHTONOUS AND ALOHTONUS FISH SPECIES IN THE REPUBLIC OF MACEDONIA

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This paper is result of long-term researches of ichthyofauna from R. Macedonia. The waters from R. Macedonia belong to 3 watersheds: Aegean, Adriatic and Black Sea. The River Vardar and its tributaries (rivers Treska, Lepenec, Pcinja, Crna, Bregalnica and other) and River Strumica belong to the Aegean Sea watershed. Also in this watershed belongs and the Lake Dojran. This area covers 86.9 % of the Macedonian territory. The Crn Drim catchment area (Adriatic Sea watershed) includes lakes Prespa and Ohrid and River Crn Drim with its tributaries on the Macedonian side (River Radika). This area covers 13% of the Macedonian territory. The catchment area of River Binacka Morava belongs to the Danube watershed, respectively to Black Sea. At territory of R. Macedonia are present total about 80 fish species. In family Petromyzonidae belong 2 species, Acipenseridae - 1 species, Anguillidae - 1 species, Clupeidae - 1 species, Cyprinidae - 46 species, Cobitidae - 5 species, Nemacheilidae - 2 species, Ictaluridae - 1 species, Siluridae - 1 species, Esocidae - 1 species, Salmonidae - 12 species, Poecilidae - 1 species, Cottidae - 1 species, Centrarhidae - 1 species, Percidae - 3 species and at Blennidae belongs 1 species.

Keywords: Aegean watershed, Adriatic watershed, fish biodiversity, R. Macedonia

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COMPARISON OF ZOOPLANKTON FUNCTIONAL RESPONSE TRAITS IN DEEP LAKES ACROSS EUROPE

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Freshwater zooplankton has a central position in lake food webs and plays key role in the matter cycling and energy transfer from phytoplankton to top predators. The use of zooplankton functional traits has been increasingly described to characterize and understanding of zooplankton assemblage in different waterbodies. Population dynamics, abundance fluctuations and food web interactions are useful traits to assess zooplankton response mechanisms to environmental changes and internal ecosystem functioning. Since functional response traits are still not commonly used in zooplankton ecology, the aims of this study were: (i) to assess the latitudinal patterns of the zooplankton functional feeding guilds in deep lakes in Europe, and (ii) to compare trophic structure related to internal nutrient loading and latitudinal climate conditions, from south to north: oligotrophic Lake Ohrid (Macedonia), mesotrophic lakes Shkodra (Albania) and Visovac (Croatia) and eutrophic Góreckie Lake (Poland). Domination of raptorial (algivorous and predator) species suggests negative relationship with higher trophic level, and dominance of microphagous zooplankton was observed in more productive lakes. Our results suggest further application of functional traits in the assessment of zooplankton within aquatic biocenosis, as well as combination with species-specific analysis related to particular ecosystems.

Keywords: Rotifera, Copepoda, Cladocera, lake trophy, guild ratio

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ZNAČAJKE RASTA KRUPATICE U HIDROAKUMULACIJAMA RIJEKE DRAVE

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Krupatica, *Blicca bjoerkna* (L., 1758), fitofilna je vrsta iz porodice šaranki, Ciprinidae. Široko je rasprostranjena u Europi gdje se njezin areal prostire od istočne Engleske do Kaspijskog mora (Slastenenko 1956; Kottelat and Freyhoff 2007). U Hrvatskoj je rasprostranjena u rijekama i jezerima dunavskog slijeva. Dominantna je u svim protočnim akumulacijama na Dravi starosti veće od 10 godina. Vrsta nije ekonomski značajna, ali zbog svoje brojnosti čini važan plijen predatorima kao što su štuka, *Esox lucius* (L.,1758) i grgeč, *Perca fluviatilis* (L.,1758). U Europi dosad je objavljeno nekoliko radova o rastu i strategiji hranjenja (Specziár et al. 1997), rastu i reprodukcijskim značajkama (Balık et al. 1999; Hamalosmanoğlu 2003), sazrijevanju i fekunditetu (Gürsoy, 2001) i odnosu mase i dužine (Tarkan et al. 2006) krupatice. Na rijeci Dravi i njenim protočnim akumulacijama nisu još provedena istraživanja na krupatici. Cilj ovog istraživanja bio je istražiti potencijal rasta, odnos duljine i mase kao i drugih parametara rasta krupatice koja nastanjuje akumulacije Varaždin i Dubrava. Uzorkovano je 879 jedinki - ukupna dužina i masa riba kretala se od 181 mm do 326 mm i od 76 g do 987 g. Starost jedinki određena je pomoću ljusaka uzorkovanih s bočne strane tijela ribe (Ricker, 1968, p. 101-104). Povećanjem starosti jezera rast krupatice postaje ovisan o gustoći.

Ključne riječi: *Blicca bjoerkna*, rast, starost, Dubrava

THE GROWTH OF WHITE BREAM (*Blicca bjoerkna* L.) IN RIVER DRAVA RESERVOIRS

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White bream, *Blicca bjoerkna* (L., 1758), a phytophile species of the cyprinid family, is a widespread species whose distribution in Europe extends from eastern England to the Caspian Sea (Slastenenko1956; Kottelat and Freyhoff 2007). Its distribution areas in Croatia include rivers and lakes of the Danubian watershed. In the Drava river, it is dominant in all reservoirs older than 10 years. It is not an economically important species, however, because of its abundance, it is an important prey for top predators such as the northern pike, *Esox lucius* (L., 1758), and European perch, *Perca fluviatilis* (L., 1758). In Europe, several papers on its feeding strategy and growth (Specziár et al. 1997), its growth and reproduction aspects (Balık et al. 1999; Hamalosmanoğlu 2003), maturity and fecundity (Gürsoy 2001), and LWR (Tarkan et al. 2006), have been published. However, no study has previously been performed on this species in Drava reservoirs. The studies' aim was to investigate the growth parameters, age-composition, size-distribution, LWR, and growth both in length and in weight. A total of 879 specimen, collected in reservoirs of Varaždin and Dubrava, was sampled using gill nets. Total lengths and weights varied between 181 mm and 326 mm and 76 g and 987 g, respectively. Age was estimated using scales sampled from the middle region of the side of the body (Ricker, 1968, p. 101-104). White bream density increased with lake aging and growth becomes density dependent.

Keywords: *Blicca bjoerkna*, relative condition, growth, age, Dubrava

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DISTRIBUTION OF ENDANGERED FRESHWATER MUSSEL SPECIES *Unio crassus* PHILIPSSON, 1788 IN SERBIA

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The Thick Shelled River Mussel, *Unio crassus* Philipsson, 1788 is one of Europe's most endangered freshwater mussel taxa – according to the IUCN criteria assessed as endangered (EN) The aim of this study is to present the current distribution of this species in Serbia, based on the research performed between 2001 and 2013. Previous studies in Serbia indicated only sporadic findings of the species in a restricted area over the last several decades (BAES database). Our study covered the entire territory of Serbia. Out of 500 sites covered by the study, the mussels were recorded at 23 sites. *U. crassus* has been recorded at the Velika and Zapadna Morava rivers, the Kolubara River Basin and the Danube, the Tisa and Sava rivers. The species has been sporadically found in Serbia, with low frequency of occurrence and low abundance along the Danube and Tisa rivers (up to 1.75% of the total mussel community), while it was found almost along entire stretches of the Velika Morava and Sava Rivers, with higher relative abundance compared to the Danube and Tisa rivers (up to 15.49% of the total mussel community). Based on our study, a positive population trend, reflected in extension of the known distribution range and increasing of population density has been recorded in Serbia.

Keywords: Unionidae, distribution range, endangered species

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STANDARDISING THE METHODS FOR SAMPLING AQUATIC MACROINVERTEBRATES OF THE LARGE LOWLAND RIVERS

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This paper discusses the use of different methods (multihabitat - MSH, Kick & Sweep method, Air lift sampling, multcorer, Van Veen grab, dredging) for aquatic macroinvertebrates sampling. Material was collected from the Danube River as a part of the three research programs: Joint Danube Survey 2, performed in period August-September 2007 from Regensburg to Danube delta (96 sampling sites), Joint Danube Survey 3, performed in period August-September 2007 (68 sites) and national monitoring program performed in period September-November 2014 along Serbian stretch of the Danube River (12 sites). The aim of the study was to contribute to the standardization of the sampling procedures for the large lowland rivers. We tested the effectiveness of sampling methods in respect to macroinvertebrate taxa richness and metrics typically used for ecological status assessment. The selection of sampling approach is also discussed in relation to objectives of the survey – e.g. research, or routine monitoring, as well in respect to target taxa. Based on the our results we concluded that it is necessary to employ more than one sampling technique and to cover all available regions of large rivers, including the deeper parts in the case of research programs. For the routine monitoring we recommend to use standardized K&S method. Studies of the specific group of organisms require detailed monitoring of available habitats, and use of additional sampling methods (e.g. mussels or crayfish studies).

Keywords: aquatic macroinvertebrates, large rivers, sampling technique, sampling efficiency, standardization

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WATER QUALITY AT LAKE OHRID BAY BASED ON PHYSICAL-CHEMICAL AND BIOLOGICAL PARAMETERS DURING THE TOURISTIC SEASON

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The littoral region of the lakes are most exposed to anthropogenic influence. Human activities in the watershed area contribute to the increased flow of nutrients, especially nitrogen and phosphorus, which can accelerate the process of eutrophication and can result in a change of the lake trophic state. Since Ohrid is a desirable tourist destination, the increased number of accommodation facilities and restaurants on the shore, vessels and motor boats negatively affect the lake environment as well as the lake living world. The aim of this paper is to estimate the trophic status of the water in several localities (Mazija, Pristanishte and Saraishte) in Lake Ohrid Bay during the touristic season 2017, based on physical-chemical parameters (biodegradable organic matter, biochemical oxygen demand-BOD5, total phosphorus and total nitrogen concentrations) and rotifers as biological indicators. Carlson's trophic State index (TSI), based on the concentration of total phosphorus, showed oligotrophic state of the water in all localities, with exception of period june and july 2017, when TSI for Saraishte and Pristanishte showed mesotrophic state. Saprobiological analyses showed that the identified Rotifera species mainly belong to the waters of I, I, II and II category. Saprobity Index during the research ranged from 1.54 to 1.78, which correspond to waters of I, II and II category.

Keywords: Lake Ohrid, trophic status, nutrients, bioindicators

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ZAJEDNICE VODENCVJETOVA (EPHEMEROPTERA) KRŠKIH IZVORA

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Izvori su specifična staništa velike konzervacijske važnosti naseljena brojnim rijetkim i endemskim organizmima, a istovremeno i važan izvor pitke vode. Unatoč tome, još su uvijek relativno zanemarena u ekološkim istraživanjima, a fauna vodencvjetova u krškim izvorima Dinarida još uvijek je relativno slabo poznata. Stoga je sastav i brojnost njihovih zajednica istraživana na 13 velikih izvora Dinarskog krša u Hrvatskoj. Uzorci su prikupljani pomoću standardne bentos mreže prema AQEM proceduri na svim dominantnim mikrostaništma tijekom 4 sezone. S 24 zabilježene vrste (30 % faune vodencvjetova Hrvatske) ovo istraživanje predstavlja značajan doprinos poznavanju faune vodencvjetova krških izvorskih područja. S velikom brojnosti jedinki ističu se izvori Krčić i Krupa dok su izvori Čikola i oba izvora Gacke obilježeni niskom brojnošću. NMDS analiza je pokazala kako se povremeni i stalni izvori uglavnom odvajaju prema sastavu zajednica vodencvjetova, pri čemu je manja raznolikost zabilježena za povremene izvore. S obzirom na korištenje vodencvjetova diljem svijeta kao indikatora kvalitete slatkovodnih ekosustava, ovo istraživanje je potvrdilo njihov indikatorski potencijal i na izvorskim staništima. Iako izvori nisu slatkovodna staništa najbogatija vrstama vodencvjetova, njihova fauna je zanimljiva i iziskuje dodatna daljnja istraživanja. Također, budućim analizama provedenim u sklopu ovog projekta istražit će se i utjecaj fizikalno-kemijskih čimbenika vode na zabilježene vrste.

Ključne riječi: Dinarski krš, krški izvori, vodencvjetovi, zajednice, mikrostaništa

MAYFLY (EPHEMEROPTERA) ASSEMBLAGES OF KARST SPRINGS

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Springs are unique habitats of high conservation value being characterized with high level of biodiversity, high number of endemic and rare organisms. Nevertheless, they represent an important source of drinking water. These habitats and their assemblages are still rather neglected in ecological research. Mayfly fauna is relatively poorly known in Dinaric karst springs. Therefore, we studied composition and abundance of their assemblages in 13 big springs in the Dinaric karst of Croatia. The samples were collected using a benthos net according to AQEM procedure at all major microhabitats during four different seasons. With 24 recorded species (30% of Croatian mayfly fauna), this study represents an important contribution to our knowledge of karst spring mayflies. Krčić and Krupa springs were characterized with high mayfly abundance in contrast to Čikola and Gacka springs, where they were rather scarce. In NMDS analysis, perennial and temporary springs generally separated based on mayfly assemblages. Temporary springs had lower species richness compared to perennial ones. Mayflies are widely used as bioindicators of freshwater

habitats' quality. Our study confirmed mayfly indicator potential in spring habitats. Although springs are generally not mayfly biodiversity hotspots, their fauna showed to be very interesting and should be investigated in more detail. Further step in the project will include analysis of physico-chemical water properties on recorded mayfly species.

Keywords: Dinaric karst, karst springs, mayflies, assemblages, microhabitats

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SEXUAL DIMORPHISM IN SOME MORPHOLOGICAL TRAITS IN THREE EUROPEAN MILLIPEDES (DIPLOPODA, JULIDA, JULIDAE)

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Sexual dimorphism (SD) in morphological traits is ubiquitous in nature. In terms of body size, it is known that males are smaller than females in most millipede species. However, data on intersexual differences in size and especially in shape of certain traits are lacking in majority of diplopods. In this study, we investigated SD in selected morphological traits (body length, antennal length, walking leg length, trunk height, trunk width, body mass) in three European millipedes, *Pachyiulus hungaricus* (Karsch, 1881), *Megaphyllum unilineatum* (C. L. Koch, 1838), and *M. bosniense* (Verhoeff, 1897). Further, centroid size (CS) and shape of the antennae and legs were scored. Measurements of the tested traits had significantly lower values in males of all species. Sexual dimorphism exists in almost all linear measurements, except in the length of the walking legs in *M. bosniense*. In all analyzed species, gender-based differences were obtained in leg shape, but not in the CS of the legs. Intersexual differences in antennal shape were detected in *M. bosniense* and *P. hungaricus*, while differences in antennal CS were observed in *M. unilineatum* and *P. hungaricus*. Our study showed that intersexual differences in millipede morphology are expressed in species-specific pattern whereby shape of analyzed traits varies more than CS. These data contributed to the knowledge on the presence of SD in the members of the order Julida.

Keywords: Diplopoda, linear measurements, shape, centroid size, morphology

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ODGOVOR EPT SVOJTI (EPHEMEROPTERA, PLECOPTERA I TRICHOPTERA) NA KONCENTRACIJE METALA U HRVATSKIM RIJEKAMA

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U ovom je radu istraživan odnos između triju bioloških metrika korištenih u procjeni kakvoće površinskih kopnenih voda i koncentracije metala u vodi. Korištene biološke metrike se najčešće vežu uz dobro stanje voda, obzirom da uključuju osjetljive skupine bentoskih makrobeskralješnjaka (Ephemeroptera, Plecoptera, Trichoptera), a radi se o broju utvrđenih EPT svojti (EPT-S) i njihovom udjelu u prikupljenom uzorku (%EPT). Također je korištena i metrika broj utvrđenih porodica (BP). Svi podaci su prikupljeni u sklopu programa sustavnog praćenja stanja površinskih tijekom 7 godina (2010-2016). Ukupno su analizirana 294 uzorka bentoskih makrobeskralješnjaka sa 172 postaje uzorkovanja, a svi uzorci prikupljeni su koristeći AQEM metodu uzorkovanja. Koncentracije metala (Cu, Zn, Cd, Cr, Ni, Pb, Hg, As, Mn, Fe, Al) mjerile su se prema standardiziranim analitičkim metodama za analizu kakvoće površinskih voda (ISO norme), u godini kada je uzorak bentoskih makrobeskralješnjaka prikupljen. Statističke analize su pokazale značajnu negativnu korelaciju EPT metrika s arsenom (%EPT = -0,51, $p<0,05$, EPT-S = -0,57, $p<0,05$, BP = -0,45, $p<0,05$, N = 179) i aluminijem (%EPT = -0,57, p = 0,042, EPT-S = -0,66, p = 0,013, BP = -0,74, p = 0,0041, N = 13).

Ključne riječi: bentoski makrobeskralješnjaci, metali, biomonitoring, arsen, aluminij

THE RESPONSE OF THE EPT TAXA (EPHEMEROPTERA, PLECOPTERA AND TRICHOPTERA) TO THE CONCENTRATIONS OF METALS IN CROATIAN RIVERS

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In this work we investigated the relationship between three biological metrics used in water quality assessment and the concentrations of metals in the surface waters. Applied biological metrics are usually connected with good water quality since they include sensitive macroinvertebrate taxa (Ephereroptera, Plecoptera and Trichoptera): the number of EPT taxa (EPT-S) and the proportion of EPT taxa (%EPT), as well as the number of recorded families (BP). Data used in this study originate from the national monitoring program of surface water quality within the period of 7 years (2010-2016). In total, 294 samples of macroinvertebrates were analysed from 172 sampling stations. All samples were collected according to AQEM sampling protocol. The concentrations of metals (Cu, Zn, Cd, Cr, Ni, Pb, Hg, As, Mn, Fe, Al) were analysed according to standard analytical methods for assessment of surface water quality (ISO norms), in the year when samples of macroinvertebrates were collected. Statistical analysis showed significant negative correlation of EPT metrics with arsenic (%EPT = -0.51, $p<0.05$, EPT-S = -0.57, $p<0.05$, BP = -0.45, $p<0.05$, N = 179) and aluminium (%EPT = -0.57, p = 0.042, EPT-S = -0.66, p = 0.013, BP = -0.74, p = 0.0041, N = 13).

Keywords: macroinvertebrate assemblages, metals, biomonitoring, arsenic, aluminium

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PALEOEKOLOŠKE KARAKTERISTIKE JEZGRE SEDIMENTA HOLOCENA BAJ7 IZ JEZERA CRNIŠEVO, BAĆINSKA JEZERA

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Alge kremenjašice (Bacillariophyta) sačuvane u sedimentu jezera izvor su mnogih informacija o paleoekološkim promjenama. Pokazatelji su kvalitete vode jezera obzirom da su njihova produktivnost i sastav zajednice osjetljivi na promjene pH, alkaliniteta, količine hranjivih tvari, temperature te saliniteta. Istraživanje je provedeno na jezeru Crniševo (Baćinska jezera) na jezgri sedimenta BAJ 7 dugoj 850 cm. Jezero Crniševo je mezotrofno, polimiktično jezero, slatkovodno do slabo bočato. U istraživanju je analizirano 30 uzoraka i to taksoni i brojnosti algi kremnjenašica te koncentracije N, C i P. U uzorcima je ukupno determinirano 158 taksona koji pripadaju 51 rodu uz dominaciju rodova *Cymbella* i *Navicula*. Prema relativnoj zastupljenosti algi kremenjašica i analiziranim parametrima definirano je 13 zona. Najveća zona proteže se od 570 do 780 cm dubine te je ujedno i najdublja zona, dok se najmanja zona proteže od 245 do 257 cm dubine. Određen je OEK (Omjer ekološke kakvoće) uz pomoć TIDHR indeksa i prema kojem jedan uzorak ukazuje na vrlo dobro stanje (površinski uzorak), 13 uzoraka na dobro i 16 uzoraka na umjereno stanje.

Ključne riječi: paleoekologija, alge kremenjašice, jezero Crniševo

PALEOECOLOGICAL CHARACTERISTICS OF LAKE CRNIŠEVO HOLOCENE SEDIMENT CORE BAJ7, BAĆINA LAKES

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Diatoms (Bacillariophyta) preserved in lake sediment provide accurate information of paleoenvironmental changes. Diatom communities reflect water quality, since their floristic composition and productivity are sensitive to variations in pH, alkalinity, nutrient status, temperature and salinity. Investigation was conducted in 850 cm long sediment core BAJ7 on Lake Crniševo (Baćina Lakes), that is a mesotrophic, polymictic freshwater to slightly brakish lake. 30 sediment samples were analyzed due to the diatom composition, N, C and P concentrations. It was found 158 diatom taxa, and within 51 detected genera Cymbella and Navicula were dominated. According to relative proportion of diatoms and analized parameters, 13 diatom zones have been defined. The biggest zone is the deepest one from 570 to 780 cm and the smallest zone was from the 245 to 257 cm. Due to Ecological Quality Ratio based on TIDHR (Trophic Diatim Indeks HR) one sample indicates high status while 13 samples indicate good and 16 samples moderate status. High status was obtained in surface sample.

Keywords: paleoecology, diatoms, Lake Crniševo

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EFFECTS OF TOMATO AND APPLE DIETS ON MORPHOLOGICAL AND BEHAVIORAL TRAITS IN *Drosophila melanogaster*

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The development and growth of *Drosophila* larvae are greatly influenced by nutritional variations. Differences in developmental processes in various nutritive environments can be further detected in adults, at morphological, physiological and behavioural levels, including sexual interactions. In this study, we questioned if there is sexual selection favouring larger *D. melanogaster* males and particular wing shape in relation to nutrition. Wing characteristics were used, since they are involved in male courtship, during phases termed as orientation and "singing". For experimental purpose, flies cultured on tomato and apple diets for years were used. We have previously observed that tomato diet contains a significantly higher amount of proteins and lower C/N ratio than the apple diet that was reflected on different fitness traits. The effects of two diets on morphological and behavioural traits were scored in female choice tests, in which males from both "nutritional strains" were offered to females of "tomato strain" and to females of "apple strain". Statistics obtained in MorphoJ program determined that "tomato" males possessed larger and narrower wings than the "apple" males. Males developed on tomato diet were also more attractive to females. Our results emphasized that the strength and shape of sexual selection acting on wing traits vary with the nutritive environment influencing male mating success.

Keywords: *Drosophila melanogaster*, diet, wings, mating success

BIOLOGIJA MORA
MARINE BIOLOGY

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UVID U BIORAZNOLIKOST REBRAŠA SJEVERNOG JADRANA METODOM METABARKODIRANJA

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Rebraši (Ctenophora) su skupina morskih želatinoznih organizama koji su sastavni dio zooplanktona. Nedavno su se istraživanja usmjerila na *Mnemiopsis leidyi*, invazivnu vrstu rebraša. Od 2016. godine je tijekom ljetnih mjeseci zabilježena prekomjerna brojnost *M. leidyi* u Jadranu. U ovom radu istražena je bioraznolikost rebraša sjevernog Jadrana u zimskom periodu s ciljem boljeg razumijevanja uzroka, tijeka i potencijala ljetnih prekomjernih brojnosti. Raznolikost rebraša u sjevernom Jadranu analizirana je koristeći metabarkodiranje. Uzorkovano je na dugogodišnjim istraživačkim postajama transekta Rovinj-rijeka Po. Izolirana je genomska DNA planktonske zajednice, V4-18S barkodovi umnoženi te sekvencirani koristeći sekvenciranje sljedeće generacije. V4-18S set podataka potvrdio je prisutnost dobro poznatih predstavnika obalnih rebraša (red Cydippida, Lobata i Beroida). Iako rebraši imaju na razini 18S rDNA najmanju varijabilnost sekvenci od svih Metazoa, vrlo strogi koraci obrade podataka (osobito taksonomija svakog pojedinog OTU) omogućili su identifikaciju pojedinih taksonomskih skupina sve do razine vrsta, uključujući i *Mnemiopsis*. Opisom zimske raznolikosti rebraša ostvareno je važno znanje za razumijevanje ljetnih prekomjernih brojnosti. Također, podacima metabarkoda po prvi put su molekularno okarakterizirane populacije rebraša u Jadranu. Bolja baza podataka rebraša omogućit će u budućim istraživanjima detaljniju identifikaciju vrsta ove skupine životinja.

Ključne riječi: Jadransko more, metabarkodiranje, *Mnemiopsis leidyi*, rebraši, 18S rDNA

FIRST INSIGHT OF THE NORTHERN ADRIATIC PHYLUM CTENOPHORA DIVERSITY USING METABARCODING

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Ctenophores are a group of marine gelatinous organisms that are a constituent part of zooplankton. Recently, studies focused on *Mnemiopsis leidyi* because of its' invasive potential. Since 2016, Mnemiopsis abundance reached bloom proportions in the Adriatic Sea every summer. In this study, winter Ctenophore diversity of the northern Adriatic was investigated in order to better understand origins, initiation and potential of summer blooms. Using metabarcoding Ctenophore diversity of the northern Adriatic in the winter period was analysed. Sampling included monitoring stations of the transect Rovinj- Po river. Plankton community genomic DNA was isolated, 18S-V4 barcodes amplified and sequenced using High-Throughput Sequencing (HTS). V4-18S dataset confirmed the presence of the well known coastal Ctenophore representatives (order Cydippida, Lobata and Beroida). Even though Ctenophores have the lowest sequence variability at the level of the 18S rDNA gene in any metazoan phylum, very stringent pipeline steps (especially taxonomic affiliation to each OUT) managed to confirm some taxa at the species level, *Mnemiopsis* included. Describing the winter Ctenophore diversity situation gathered useful knowledge to understand blooms in the summer. Also, metabarcoding data for the first time enabled molecular characterisation of the Ctenophore populations in the Adriatic. In the future, better Ctenophore database coverage would help species identification using metabarcoding.

Keywords: Adriatic Sea, Ctenophores, metabarcoding, *Mnemiopsis leidyi*, 18S rDNA

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TEMELJNA STUDIJA MAKROFAUNE MEKOG DNA NA PLINSKOM POLJU SJEVERNI JADRAN, PLINSKA PLATFORMA ANA

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Istraživanje je provedeno s ciljem: 1) utvrđivanja potencijalnog utjecaja pripremnog bušenja na sjevernojadranskom plinskim polju (44°41.7'N - 13°16.1'E; dubina 44 m) na zajednice makrofaune mekog dna, te 2) određivanja referentnih uvjeta za procjenu utjecaja eksploatacije plina na bentos. Strukturne i funkcionalne karakteristike makrobentosa su analizirane na osam postaja, zvjezdasto raspoređenih oko pripremne bušotine za platformu ANA, na udaljenosti od 100 m u smjeru S, J, I i Z, odnosno 300 m u smjeru JZ, SZ, SI, JI. Uzorci su uzeti Van Veen grabilom zahvatne površine 0,1 m^2. Na svakoj postaji su uzeta četiri replikatna uzorka za kvalitativne i kvantitativne analize makrofaune, te uzorci za analizu sedimenta. Struktura makrofaune je analizirana korištenjem univarijatnih, multivarijatnih i distribucijskih metoda. Funkcionalna raznolikost je procijenjena na temelju klasifikacije vrsta prema osjetljivosti (Grall and Glémarec, 1997). Ekološki status je određen izračunom AMBI i M-AMBI biotičkih indeksa. Rezultati istraživanja su ukazali na razlike u strukturi makrofaune s obzirom na udaljenost od bušotine (100 i 300 m) i vrijeme uzorkovanja (prije i poslije instalacije plinske platforme). Sve postaje su rangirane u kategoriju najvišeg ekološkog statusa. Istraživanjem je postavljena objektivna osnova za procjenu utjecaja plinskih platformi na zajednice slabo sortiranih pijesaka u otvorenim vodama sjevernog Jadrana, kao i referentni uvjeti za praćenje ekološkog statusa.

Ključne riječi: bentos, makrofauna, karakterizacija zajednica, plinske platforme, procjena utjecaja na okoliš

A BASELINE STUDY OF THE SOFT-BOTTOM MACROFAUNA FROM THE NORTH ADRIATIC GAS FIELD, GAS PLATFORM ANA

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The present study aimed to; 1) assess the potential impact of drilling on the soft-bottom benthic community in the North Adriatic gas field (44°41.7'N - 13°16.1'E; 44 m depth) and 2) to provide a baseline for assessment of environmental impact related to the future gas exploitation. The structural and functional characteristics of the macrobenthos were studied at eight sampling sites arranged in a star shaped formation around a drill hole at 100 m distance in N, S, E, W directions, and at 300 m distance in SW, NW, NE, SE directions. Samples were taken with the Van Veen grab (sampling area of 0.1 m^2). At each site, we obtained four replicate samples for qualitative and quantitative analysis of macrofauna, and samples for sediment analyses. Macrobenthic community structure was analysed using univariate, multivariate and distributional techniques; functional diversity following classification by Grall and Glémarec (1997) and ecological status was assessed using AMBI and M-AMBI biotic indices. Results indicated no differences in macrobenthic structure among sampling sites, both related to distance from the drill hole (100 and 300 m) and sampling time (before and after installation of gas platform). All study sites can be ranked into the class of high ecological

status. This study should provide objective benchmarks for potential environmental impact on the poorly sorted sand community in the Northern Adriatic offshore waters, as well as for ecological status assessment.

Keywords: benthos, macrofauna, communities' characterization, gast platforms, environmental impact assessment

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TEMELJNA STUDIJA MAKROFAUNE U SJEVERNOJADRANSKIM LUKAMA RAŠA, RIJEKA, BAKAR I OMIŠALJ

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Luke su recipijenti niza organskih i anorganskih onečišćivala, koja se posredno ili neposredno ispuštaju u more. U novije vrijeme, pomorski transport i popratne lučke aktivnosti su prepoznati kao rastuća opasnost za morski okoliš, a balastne vode kao jedan od glavnih vektora prijenosa alohtonih vrsta. Cilj ovog istraživanja je karakterizacija bentoskih zajednica na čvrstim i mekim dnima četiriju sjevernojadranskih luka, te analiza prisutnosti alohtonih vrsta makrofaune na istraživanom području. Strategija i metodologija uzorkovanja su se temeljile na modificiranom CRIM Protokolu (Hewitt and Martin, 2001). Na čvrstoj podlozi, utvrđena prisutnost ukupno 17 konstitutivnih jedinica (više svojte) i identificirano je 75 vrsta makrofaune, dok je fauna mekog dna bila znatno siromašnija (10 viših svojti, 36 vrsta). Rezultati univarijatnih analiza ukazali su na siromaštvo zajednica na mekim dima, te relativno bogatstvo faune na čvrstoj podlozi. Multivarijatnim analizama utvrđene su značajne razlike u sastavu makrofaune među uspoređivanim lukama. Na istraživanom području zabilježena je prisutnost sedam alohtonih vrsta koje pripadaju skupini mnogočetinaša. Vrsta *Platynereis nadiae* je po prvi puta zabilježena u Jadranskom moru, a pronađena je u lukama Bakar, Omišalj i Rijeka. U Raši je pronađeno 6 alohtonih vrsta: *Hydroides dianthus, H. elegans, H. stoichadon, Leiochrides australis, Protolaeospira striata* i *Pileolaria berkelyana*, posljednja među njima je prvi nalaz za Jadran.

Ključne riječi: bentos, luke, sjeverni Jadran, karakterizacija zajednica, alohtone vrste

A BASELINE STUDY OF MACROFAUNA FROM THE NORTH ADRIATIC SEAPORTS RAŠA, RIJEKA, BAKAR AND OMIŠALJ

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Seaports are recipients of myriad organic and inorganic pollutants that directly or indirectly released into marine environment. Recently, maritime transport and associated port activities are recognized as increasing source of environmental threats, with ballast water as one of main vectors for initial transport of non-indigenous species (NIS). The goal of this study was to characterize benthic communities associated with hard- and soft-bottom habitats from the four North Adriatic seaports and to check the presence of non-indigenous species. The sampling strategy and methodology followed modified CRIM Protocol (Hewitt and Martin,

2001). Total of 17 higher macrobenthic taxa, and 75 species were identified from the hard substrata, and much less (10 higher taxa, 36 species) from the soft bottom. The results of univariate analyses suggested occurrence of modified and impoverished soft-bottom macrofauna and rather diverse hard bottom fauna. Multivariate analysis indicated significant differences of macrofauna communities among surveyed ports. In total, seven NIS were detected, all belonging to polychaetes. One of them, *Platynereis nadiae* was recorded for the first time from the Adriatic Sea and it was found in ports of Bakar, Omišalj and Rijeka. Six NIS were reported from the port of Raša: *Hydroides dianthus, H. elegans, H. stoichadon, Leiochrides australis, Protolaeospira striata* and *Pileolaria berkelyana*, and the last one was recorded for the first time in the Adriatic Sea.

Keywords: benthos, ports, North Adriatic, communities' characterization, non-indigenous species

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PROSTORNA RASPODJELA MNOGOČETINAŠA U ALGALNIM ZAJEDNICAMA NA PODRUČJU KAŠTELANSKOG ZALJEVA

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Ovo istraživanje donosi preliminarne rezultate o rasprostranjenosti zajednica mnogočetinaša čvrstih podloga, i njihovoj povezanosti s zajednicama algi na području Kaštelanskog zaljeva (srednji Jadran) u dubinskom rasponu od 0.5 do 10 metara. 13 uzoraka je ne-replikatno prikupljeno autonomnim ronjenjem na 7 postaja, u veljači i ožujku 2017 godine. Mnogočetinaši i i makroalge određeni su do najniže moguće taksonomske razine. Ukupno je prikupljeno 952 jedinke mnogočetinaša koje čini 79 vrsta; 26 vrsta makroalgi koje prekrivaju uzorkovanu površinu je identificirano. Svi dostupni podaci o osjetljivosti vrsta na okolišne stresore pridruženi su vrstama mnogočetinaša. Statistička analiza pokazala je kako se zajednice mnogočetinaša na svakoj postaji razlikuju neznatno, s iznimkom dviju postaja, vjerojatno zbog najmanje kompleksnog algalnog pokrova. Također, ove su dvije postaje imale velik broj jedinki s malim brojem vrsta što je tipičan pokazatelj narušenog ekološkog stanja okoliša. Najveća brojnost i raznolikost pronađena je na uzorcima sa postaja koje su imale kompleksan algalni pokrov, i bile najudaljenije od okolišnih stresora. Mogući uzroci ovakve raspodjele pokušali su se objasniti.

Ključne riječi: mnogočetinaši, algalni pokrov, Kaštelanski zaljev, okolišni stresori

SPATIAL DISTRIBUTION OF POLYCHAETE ASSEMBLAGES IN ALGAL COMMUNITES IN THE AREA OF KAŠTELA BAY

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This study presents preliminary results on the distribution of hard-bottom polychaete assemblages and their relation to the various algal communities in the area of Kaštela Bay (central Adriatic Sea) in the depth range of 0.5 to 10 meters. 13 samples were non-replicately taken by scuba diving at 7 sampling stations in February and March 2017. Macroalgae and polychaete specimens were identified to the lowest possible taxonomic level. A total of 952 polychaete specimens, belonging to 79 taxa were collected; and 26 macroalgal taxa covering the sample surface were identified. All available data on species sensitivity to environmental disturbance were applied to each polychaete taxa. Statistical analysis suggested that polychaete assemblages at each station differed slightly, except at two stations, plausible due to the least complex algal cover. In addition, these two stations had a low number of species but a high abundance, which is a typical response to degraded habitats. The greatest diversity and abundance was found at stations with the complex algal substrate and the greatest distance from environmental stressors. Potential causes of the observed differences are hypothesized and discussed.

Keywords: polychaeta, algal cover, Kaštela bay, environmental stressors

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SEZONSKA PREHRANA MAČINCA CRVENOG, *Cepola macrophthalma* (LINNAEUS, 1758) NA PODRUČJU JUŽNOG JADRANA

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Mačinac crveni *Cepola macrophthalma* (Linnaeus, 1758) je predstavnik porodice Cepolidae u Jadranu. Rasprostranjen je duž cijele obale do dubine od 230 m. Cilj istraživanja bio je ustanoviti sezonske promjene prehrane unutar populacije analizama sastava i količine plijena u probavilima. Uzorci su prikupljani na području južnog Jadrana pridnenom povlačnom mrežom – kočom tijekom jednogodišnjeg razdoblja. Jedinkama je mjerena ukupna duljina tijela (cm), ukupna masa (g) te određivan spol. Za analizu prehrane nasumično je odabrano 30 jedinki tijekom svake sezone te su određivani sljedeći hranidbeni indeksi: postotak učestalosti pojavljivanja plijena %F = n/N x 100; postotak brojnosti plijena %N = np/Np x 100 i koeficijent praznosti probavila % V = E/N x 100. Glavninu prehrane mačinca crvenog čine zooplanktonski organizmi, među kojim su izrazito dominirali kopepodi iz podreda Poecilostomatoida i reda Calanoida. Od ostalih skupina zooplanktona brojne su ličinke eufauzida i dekapoda te hiperidi i mizidi. Sezonska raspodjela učestalosti i brojnosti ovih zooplanktonskih skupina u probavilima u skladu je s najvećim gustoćama njihovih populacija u južnom Jadranu: ličinke dekapoda u proljeće, furcilije eufauzida u ljeto. Udio praznih probavila tijekom cijele godine iznosio je 14,78 %. Utjecaj abiotičkih čimbenika na sezonsku prehranu nije bio značajan budući da su jedinke prikupljane na dubinama većim od 100 m te intenzitet prehrane najviše ovisi o količini raspoloživog plijena.

Ključne riječi: *C. macrophthalma*, analiza prehrane, južni Jadran

SEASONAL DIET OF RED BANDFISH *Cepola macrophthalma* (LINNAEUS, 1758) IN THE SOUTHERN ADRIATIC

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Red bandfish Cepola macrophthalma (Linnaeus, 1758) is a representative of the family Cepolidae in the Adriatic Sea distributed up to depths of 230 m. The objective of this study was to determine seasonal changes in the diet by analysing stomach content. Samples were collected in the south Adriatic using bottom trawl net - during the one-year period. For each fish, total length (cm) and weight (g) were measured and sex was determined. For diet analysis stomachs of 30 individuals, randomly selected during each season, were used to calculate following indexes: frequency of occurrence of the prey %F = (n/N) x 100; prey abundance %N= (np/Np) x 100 and vacuity index % V = (E/N) x 100. Main food of red bandfish was zooplanktonic organisms with domination of copepods from suborder Poecilostomatoida and order Calanoida. Other prey was composed of euphausids and decapods larvae and hyperiids and mysids. Seasonal distribution of frequency of occurrence and abundance of this prey is in accordance with its highest abundance in south Adriatic: decapods larvae in spring and euphausids furciliae in summer. Percentage of empty stomachs was 14.78% during the whole year. Influence of abiotic factors on seasonal diet was not significant since the individuals were collected at depths deeper than 100 m and feeding intensity depends mostly on quantity of available prey.

Keywords: *C. macrophthalma*, diet analysis, south Adratic

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PREDVIDLJIVOST UTJECAJA PLOVILA NA PROMJENE PONAŠANJA DOBRIH DUPINA (*Tursiops truncatus*) U CRESKO-LOŠINJSKOM AKVATORIJU

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Cresko-lošinjski akvatorij izložen je naglom porastu broja rekreativnih plovila tijekom ljetnih mjeseci što može imati negativan utjecaj na ponašanje rezidentnih jedinki dobih dupina koje to područje koriste za hranjenje i podizanje mladunaca. Ciljanim pretraživanjem terena istražili smo utjecaj prisutnosti plovila na dugoročne promjene ponašanja dupina. Tijekom opažanja, sakupljali smo podatke o ponašanju dupina u 3-minutnim intervalima prema metodi "focal group follow" te bilježili broj i tipove plovila prisutnih unutar 500 m od skupine. S ciljem utvrđivanja utjecaja prisutnosti plovila na nastupanje promjena u ponašanju dupina koristili smo „Markov chain" analizu. Rezultati ukazuju da je u prisustvu plovila značajno smanjena vjerojatnost da dupini ostanu pri ponašanju "ronjenje-putovanje" dok se istovremeno povećava vjerojatnost tranzicije iz ponašanja "ronjenje-putovanje" u "putovanje". Utvrdili smo da je utjecaj plovila na zastupljenost kategorija ponašanja značajan te se očituje kroz smanjenje vremenskog udjela kojeg dupini provede primjenjujući "ronjenje-putovanje" dok se udio vremena kojeg dupini provode putujući povećava u prisutnosti plovila. Vrijeme provedeno u

svakoj kategoriji ponašanja smanjuje se kad su plovila u blizini skupine. Ipak, rezultati pokazuju da procijenjena razina dnevne izloženosti plovilima (42%) nije dovoljna za uzrokovanje značajnih promjena u sveukupnom budžetu ponašanja rezidentnih dupina.

Ključne riječi: *Tursiops truncatus*, plovila, tranzicija ponašanja, zastupljenost kategorija ponašanja

PREDICTABILITY OF VESSEL EFFECTS ON BEHAVIOURAL CHANGES OF COMMON BOTTLENOSE DOLPHINS (*Tursiops truncatus*) IN LOŠINJ ARCHIPELAGO

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This study was undertaken within the Cres-Lošinj waters, an important habitat for a resident population of common bottlenose dolphins. This area is subject to a substantial increase in the number of leisure boats over the summer months. Dedicated boat- based surveys were conducted in order to assess the effects of boat presence on the long-term behavioural changes of resident bottlenose dolphins. During dolphin encounters, data on group behaviour was sampled every 3 minutes based on the focal group follow method. Data on boat presence along with their type and number was noted when boats were within 500 m from the group. Markov chain analysis was used to assess the effects of boats on the transition probability between dolphin behavioural states. Results indicate that in the presence of boats, transition probability of dolphins to remain in the 'dive-travel' behaviour decreased, behavioural change concurrently increased in probability with a switch from 'dive-travel' to 'travel' behaviour. The effect of boats on the behavioural budget was found to be significant, reducing the proportion of time dolphins spent in 'dive-travel'. The proportion of 'travel' time increased when boats were present. The time spent in each behavioural state (bout length) decreased for all three states in the presence of boats. However, the estimated level of diurnal boat exposure (42%) was not found to be sufficiently large to affect the cumulative behavioural budget of resident bottlenose dolphins.

Keywords: *Tursiops truncatus*, boat traffic, behavioural transition, behavioural budget

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HARMFUL AND POTENTIALLY HARMFUL PLANKTON ORGANISMS IN MARINA PORTO MONTENEGRO (BOKA KOTORSKA BAY)

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The aim of this study was to estimate phytoplankton and zooplankton abundance, with emphasis on harmful and potentially harmful species in the marina "Porto Montenegro"- Boka Kotorska Bay. Porto Montenegro is a marina for luxury yacht situated in the outer part of Boka Kotorska Bay- Tivat Bay (Montenegro). Sampling was performed monthly from March 2015 to February 2016. Samples were taken at three positions (P0, P1, P2) in marina "Porto

Montenegro". The highest abundance of phytoplankton was noticed in June 2016, on 5m depth (9.55 x 105 cells/l). Diatoms contributed to majority of phytoplankton abundance, with dominance of potentially toxic species from genus Pseudo-nitzschia (9.42 x 105 cells/l). From dinoflagellates, toxic and potentially toxic species were noticed from genus *Dinophysis, Prorocentrum, Phalacroma, Lingulodinium, Gonyaulax*. Dinoflagellate *Prorocentrum cordatum* reached value up to 105cells/l. This occurrence indicates on possibility of excessive growth of harmful species. There is possibility of introducing of harmful organisms through ballast water in this area which can cause negative consequences for ecosystem and human health. Zooplankton assemblage was characterized by numerous protozoa *Noctiluca scintillans* in spring which contributed 47% in total zooplankton. Gelatinous organisms generally did not have a significant share in total zooplankton except the appearance of hydromedusa *Obelia* spp. in January when it reached maximum value of 70ind/m^3.

Keywords: harmful and potentially harmful diatoms, dinoflagellates, protozoa

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TRAJNOST PRIRODNIH OZNAKA DOBROG DUPINA U JADRANU

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Kod dobrog dupina (*Tursiops truncatus*) prirodne oznake čine leđnu peraju jedinstvenom što omogućava raspoznavanje jedinki foto-identifikacijom. To je široko primjenjiva i neinvazivna metoda koja se koristi u brojnim istraživanjima, ali njena pouzdanost ovisi o trajnosti prirodnih oznaka. S ciljem unaprjeđenja foto-identifikacije u istraživanjima dobrog dupina u Jadranu, istražili smo pojavnost, vrste i trajnost prirodnih oznaka u ovisnosti o spolu i starosti. Istraživanje je provedeno na uzorku od 79 jedinki redovno fotografiranih u sjevernom Jadranu u razdoblju od 2005. do 2015. godine. Prirodne oznake kategorizirane su prema veličini, obliku i relativnoj poziciji na leđnoj peraji te su utvrđena trajanja između promjena oznaka. Rezultati pokazuju da se većina promjena (85%) događa na stražnjem rubu leđne peraje. Prosječno trajanje oznaka iznosi 868 dana (SE=92,6), a jedinke su u promatranom razdoblju prošle kroz prosječno 2,6 promjena (SE=0,25). Promjene su bile značajno češće kod mužjaka nego ženki (p=0.000014). Utvrđena je niska negativna korelacija između starosti jedinki i učestalosti promjena (r=-0.25), a prosječna starost kada jedinke zadobivaju pouzdano prepoznatljivu peraju iznosi 8 godina (SE=0.97). Rezultati ukazuju na to da je za pouzdanost foto-identifikacije važna kontinuiranost terenskog rada. Također, preporučuje se izrada kataloga sekundarnih oznaka kao nadopuna primarnim oznakama radi poboljšanja pouzdanosti foto-identifikacije.

Ključne riječi: dobri dupin, *Tursiops truncatus*, foto-identifikacija, prirodne oznake

LONGEVITY OF COMMON BOTTLENOSE DOLPHIN (*Tursiops truncatus*) DORSAL FIN MARKINGS IN THE ADRIATIC SEA

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Common bottlenose dolphins (*Tursiops truncatus*) bear natural markings which make their dorsal fins unique. This enables individual recognition through photo-identification – a powerful and non-invasive method, utilized in a myriad of studies, but sensitive to changes of natural markings through time. To provide supporting information for photo-identification studies, we analysed the occurrence, types and longevity of natural markings in relation to gender and age. The study was conducted on a sample of 79 individuals, regularly photographed in the northern Adriatic between 2005 and 2015. Natural markings were categorized according to size, shape and relative position on the dorsal fin, and durations between their changes were calculated. Results indicate that the majority of changes (85%) occur on the dorsal fin's trailing edge. The average duration between changes was 868 days (SE=92.6) with an average of 2.6 changes (SE=0.25) per individual during the study period. Males exhibited significantly higher frequency of changes than females (p=0.000014). The correlation between age and frequency of changes was low (r=-0.25) and the average age when reliably identifiable dorsal fin markings occur is 8 years (SE=0.97). Our results indicate the importance of continuous photo-identification field work and suggest the development of secondary markings catalogues to support primary markings based identifications and enhance the accuracy of photo-identification.

Keywords: bottlenose dolphin, *Tursiops truncatus*, photo-identification, natural markings

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UPOTREBA KRIOMIKROTOMSKIH PRESJEKA U PROMATRANJU PARAZITA I ENDOBIONTSIH GLJIVA KOD DAGNJE *Mytilus galloprovincialis* U SJEVERNOM JADRANU

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Histologija pruža informacije o općem zdravlju morskih organizama, no omogućava identifikaciju simbionata, parazita i patogena u njima. Dijagnoza parazita primarno je postignuta koristeći histološke metode u tkivima uklopljenim u parafin. Međutim, ovaj postupak zahtijeva određeno vrijeme za pripremu uzoraka. Cilj ovog rada je opisati promatranje parazita i endobionata mediteranske dagnje *Mytilus galloprovincialis* Lamarck, 1819 u kriomikrotomskim presjecima probavnih žlijezda. Dagnje su uzorkovane u priobalnom području Rovinja, u sjevernom Jadranu, u Hrvatskoj kroz jednu godinu. Nakon uzorkovanja dagnji, uzorci tkiva su fiksirani u ohlađenom n-heksanu, a potom uklopljeni u polimerni medij O.C.T. i izrezani kriomikrotomom. Presjeci su obojeni standardnim bojanjem hematoksilina i eozina. Protozoi roda Nematopsis Schneider, 1892 (Apicomplexa, Gregarina) otkriveni su u probavnim kanalićima, dok je u vezivnom tkivu na rubu probavne žlijezde pronađen turbulencija (Urastomidae) Urastoma cyprinae von Graff, 1913. Gljiva *Alternaria* sp. (Gljive, Ascomycota) otkrivena je u epitelnim stanicama probavnog tubula u kriomikrotomskim prerezima. Iako je upotreba kriomikrotomskih presjeka u ekotoksikološkim istraživanjima česta, ovim istraživanjem prikazana je mogućnost za praćenje parazita i endobiontskih gljiva u morskim organizmima.

Ključne riječi: kriomikropresjeci, paraziti, gljive, dagnje

USEFULNESS OF CRYOSECTIONS IN PARASITES AND ENDOBIONTS OBSERVING OF MUSSEL *Mytilus galloprovincialis* FROM THE NORTHERN ADRIATIC

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Histology provides a large amount of information not only of the general health of a marine organism but also the detection of a wide range of symbionts, parasites and pathogens. Traditionally, the diagnosis of diseases has primarily been achieved using histological methods in paraffin embedded tissues. However, this procedure requires a time to prepare the samples. The purpose of this study is to describe the experience of observing parasites and endobionts of Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 in cryosections of digestive glands. Mussels were sampled in the Rovinj coastal area, the Northern Adriatic, Croatia across annual cycle. Dissected mussel tissues were fixed with cooled n-hexane, embedded in O.C.T.TM compound and cut with a cryostat. Sections were stained with haematoxylin and eosin. The protozoan of the genus Nematopsis Schneider, 1892 (Apicomplexa, Gregarina) was detected in digestive tubules, while the turbellarian (Urastomidae) Urastoma cyprinae von Graff, 1913 was found in the connective tissue at the edge of the digestive gland. The filamentous fungus *Alternaria* sp. (Fungi, Ascomycota) was detected in epithelial cells of the digestive tubule in cryosections. Although up to now cryosections were used in ecotoxicological studies of monitoring pollutant exposures, it provides a useful and affordable means for monitoring parasites and endobiotic fungi.

Keywords: cryosections, parasites, fungi, mussel

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PRELIMINARNA PROCJENA FREKVENCIJE MRIJESTA I 'BATCH' FEKUNDITETA INĆUNA *Engraulis encrasicolus* (Linnaeus, 1758) U JADRANSKOM MORU

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Inćun *Engraulis encrasicolus* (Linnaeus, 1758) je mala pelagična riba rasprostranjena uz istočnu obalu Atlantskog oceana i na čitavom Sredozemlju. Mrijesti se više puta godišnje u blizini obalnih područja u razdoblju od ranog proljeća do kasnog ljeta, dok je u Jadranu mrijest najintenzivniji od svibnja do srpnja. Usprkos činjenici da se o razmnožavanju ove vrste u Jadranu zna dosta, ipak nedostaju informacije vezane uz neke od bitnih reproduktivnih parametara kao što su frekvencija mrijesta i 'batch' fekunditet. Stoga su tijekom srpnju 2016. i 2017.g. za vrijeme trajanja znanstvenog istraživanja MEDITS prikupljeni uzorci inćuna. Napravljeni su histološki preparati zrelih ovarija kako bi se odredila prisutnost postovulatornih folikula (POF-ovi) i hidratiziranih jajnih stanica. S obzirom na strukturu POF-a utvrdila se starost istih ('day-0, day-1 ili day-2') te je na temelju učestalosti pojavljivanja POF-ova starosti 1 dan dobivena frekvencija mriješćenja. Za procjenu 'batch' fekunditeta (broj hidratiziranih jajnih stanica po mrijestu) u obzir su uzete samo jedinke kod kojih nije uočena prisutnost POF-ova već je većina jajnih stanica bila hidratizirana. Dobiveni preliminarni rezultati uvelike upotpunjuju dosadašnje znanstvene spoznaje o ovoj ekološki i ekonomski važnoj vrsti. Također,

definiranjem ovdje proučavanih parametara omogućiti će se uvođenje direktne metode procjene - DEPM metode, čime bismo došli do boljih podataka vezanih uz biomasu spolnog dijela stoka inćuna.

Ključne riječi: inćun, mrijest, fekunditet, POF

PRELIMINARY ESTIMATE OF ANCHOVY *Engraulis encrasicolus* (Linnaeus, 1758) SPAWNING FREQUENCY AND BATCH FECUNDITY IN THE ADRIATIC SEA

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Histology provides a large amount of information not only of the general health of a marine organism but also the detection of a wide range of symbionts, parasites and pathogens. Traditionally, the diagnosis of diseases has primarily been achieved using histological methods in paraffin embedded tissues. However, this procedure requires a time to prepare the samples. The purpose of this study is to describe the experience of observing parasites and endobionts of Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 in cryosections of digestive glands. Mussels were sampled in the Rovinj coastal area, the Northern Adriatic, Croatia across annual cycle. Dissected mussel tissues were fixed with cooled n-hexane, embedded in O.C.T.TM compound and cut with a cryostat. Sections were stained with haematoxylin and eosin. The protozoan of the genus Nematopsis Schneider, 1892 (Apicomplexa, Gregarina) was detected in digestive tubules, while the turbellarian (Urastomidae) Urastoma cyprinae von Graff, 1913 was found in the connective tissue at the edge of the digestive gland. The filamentous fungus *Alternaria* sp. (Fungi, Ascomycota) was detected in epithelial cells of the digestive tubule in cryosections. Although up to now cryosections were used in ecotoxicological studies of monitoring pollutant exposures, it provides a useful and affordable means for monitoring parasites and endobiotic fungi.

Keywords: The European anchovy, spawning frequency , batch fecundity, POFs

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PRILOG POZNAVANJU GASTROPODA U NACIONALNOM PARKU KORNATI

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Istraživanje razreda Gastropoda napravljena su 2016. i 2017. godine na pet postaja unutar Nacionalnog parka „Kornati“, a provodila su se upotrebom autonomne ronilačke opreme, metodom vizualnog cenzusa i uz upotrebu podvodnih kamera na dubinama od 1 – 60 m. Na istraživačkim postajama uzimani su uzorci sedimenta, analizirane kolonije roda *Alcyonacea* i *Zoantharia*, ali i uzimali dijelovi zaostalih ribolovnih alata (mreže, parangali, konopci, vrše,…) s ciljem nalaženja jedinki Gastropoda. Tijekom tih istraživanja evidentirano je ukupno 157 vrsta iz razreda Gastropoda od čega je njih 94 po prvi put evidentirano za područje Nacionalnog parka Kornati. Uključivanjem novih vrsta, popis Gastropoda danas broji 250 vrsta, a pretpostavka da

je konačan broj vrsta znatno veći što ukazuje i na potrebu daljnjih istraživanja. Dodatna vrijednost ovom istraživanju jest činjenica da su vrste sakupljanje i determinirane od strane malakologa amatera (uz potvrdu domaćih i stranih stručnjaka).

Ključne riječi: Nacionalni park Kornati, Gastropoda, vizualni cenzus

A CONTRIBUTION TO KNOWLEDGE OF THE CLASS GASTROPODA IN THE KORNATI NATIONAL PARK

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The studies on the class Gastropoda were conducted in 2016 and 2017 at five stations within the Kornati National Park using autonomous diving equipment, visual census method and underwater cameras at depths of 1 to 60 m. Sediment samples were taken from the research stations, colonies of *Alcyonacea* and *Zoantharia* were analysed, and parts of the fishing gear (nets, longlines, ropes, fishing pots,...) collected with the aim of finding specimens of gastropods. During these studies, a total of 157 species of Gastropoda were recorded, of which 94 were recorded for the first time in the area of the Kornati National Park. By including new species, the list of gastropods nowadays counts 250 species, and the assumption is that the finite number of species is considerably higher, indicating the need for further research. The added value of this research is that species are collected and determined by amateur malacologists (with the confirmation of domestic and foreign experts).

Keywords: Kornati National Park, Gastropoda, visual census

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RAZNOLIKOST MAKROFAUNE UNUTAR ALGE *Corallina officinalis* NA PODRUČJU JUŽNE ISTRE

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Crvena inkrustirajuća alga *Corallina officinalis* pruža zaklon mnogim makrofaunalnim vrstama u području mediolitorala od djelovanja valova, predacije i isušivanja. Fizička struktura i složenost staništa te antropogeni utjecaj imaju veliki utjecaj na bioraznolikost ove zajednice. Cilj ovog rada bio je istražiti raznolikost naselja beskralješnjaka unutar naselja crvene alge *C. officinalis*. Odabiru lokacija za uzorkovanje prethodilo je kartiranje alge *C. officinalis* na području južne Istre. Kartiranje prisutnosti i abundancije ove alge u zoni mediolitorala provedeno je tijekom oseke metodom vizualnog opažanja. Tri lokacije uzorkovanja odabrane su na području gdje je pokrivenost algom bila veća od 75%. Na svakoj lokaciji uzorkovanje je provedeno kvantitativno u 3 replikata, struganjem unutar kvadrata veličine 20 x 20 cm. Ukupno je izolirano 30.786 jedinki, a dominantne skupine bile su amfipodni rakovi, mnogočetinaši, školjkaši i puževi. Ovi taksoni su činili ukupno 86% svih makrofaunalnih skupina povezanih s algom. Najbrojnija skupina su amfipodni rakovi koji su činili 41% ukupnih izoliranih beskralježnjaka. U naselju alge

C. officinalis makrofauna svojom abundancijom i raznolikošću dominira. U budućim istraživanjima ispitat će se djelovanje antropogenog utjecaja na sastav i strukturu makrobentoskih zajednica u naseljima ove crvene alge.

Ključne riječi: makrofauna, *Corallina officinalis*, kartiranje, bioraznolikost, obala Istre

DIVERSITY OF MACROFAUNA ASSOCIATED WITH *Corallina officinalis* IN SOUTHERN ISTRIAN PENINSULA

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Red coralligenous algae *Corallina officinalis* provides shelter to many macrofaunal species from wave actions, predation and desiccation stress in the intertidal area. Physical structure and complexity of the habitat as well as anthropogenic influence have a major influence on biodiversity of this community. The aim of the study was to examine the diversity of macrofaunal assemblages inside the red algae *C. officinalis* turf. Prior to choosing sampling locations, mapping of *C. officinalis* along the southern Istrian coast was done. Mapping was performed during low tides by visual observation of the intertidal area where the presence and abundance of the algae were marked. Three sampling locations were chosen in the areas with algal cover range above 75%. On each location sampling was done quantitatively by scraping off within 3 replicate quadrats 20 x 20 cm in size. A total of 30.786 specimens were isolated from all sampling locations. The prevalent groups were amphipod crustaceans, polychaetes, bivalves and gastropods that made a total of 86% of all macrofaunal groups associated with algal turfs. The most abundant group were amphipods that made 41% of the total separated individual invertebrates. Future studies will examine how the anthropogenic influence is affecting macrobenthic communities associated with *C. officinalis* as it is a very important habitat with high abundance and diversity of macrofaunal assemblages.

Keywords: acrofauna, *Corallina officinalis*, mapping, biodiversity, Istrian coast

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RAZVOJ EDUKATIVNIH AKTIVNOSTI U NACIONALNOM PARKU BRIJUNI

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Javna ustanova Nacionalni park Brijuni prepoznala je važnost edukativnih aktivnosti kao učinkovitog alata u podizanju razine svijesti javnosti o važnosti zaštite prirode. Projekt razvoja edukativnih aktivnosti uključuje razvoj edukativnih programa za osnovne škole i vrtiće, edukativnih programa za hotelske goste, volonterskih programa, stručnih vodstva, razvoj edukativnih izložbi te raznih obilježavanja važnih datuma u zaštiti prirode. Projekt traje već duži niz godina, ali se od 2013. godine sustavno bilježi struktura i broj sudionika. Cilj projekta je uključiti lokalnu zajednicu te lokalne i regionalne škole, vrtiće i fakultete u aktivnosti nacionalnog parka i osvijestiti sudionike o važnosti i dobrobiti zaštićenog područja, bogatstvu

prirodnih i kulturnih vrijednosti te približavanje nastavnih programa učenicima kroz interakciju, igru i učenje na terenu. Edukativni programi pokrivaju širok spektar tema, od biologije, ekologije, paleontologije pa sve do arheologije i povijesti. Edukativne aktivnosti vrlo su dobro prihvaćeni od strane učitelja, profesora, odgajatelja, djece i ostalih sudionika što je zabilježeno i s postotkom sudionika koji se po drugi put vraćaju sudjelovati na ponuđene programe. Lokalne i regionalne škole odlaze izvan Istarske županije kako bi provodili „školu u prirodi“ tako da je ovakav oblik edukacije definitivno potreban u regiji, ali i šire.

Ključne riječi: edukativni programi, biologija, zaštita prirode, Nacionalni park Brijuni

DEVELOPMENT OF EDUCATIONAL ACTIVITIES IN BRIJUNI NATIONAL PARK

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Public Institution Brijuni National Park recognized the importance of educational activities as an effective tool for raising public awareness about the importance of nature protection. Development of educational activities is a project that includes development of educational programs for elementary schools and kindergartens, educational programs for hotel guests, volunteering programs, expert guidance, development of educational exhibitions and various celebrations of important dates in nature protection. This project has been going on for several years, but since 2013 the structure and number of participants are systematically recorded. The aim of the project is to involve local community, local and regional schools, kindergartens and universities in national park’s activities and to raise awareness about the importance and benefits of protected areas, the richness of natural and cultural values, and bringing the school curriculum closer to students through interaction, play and field learning. Educational programs cover a wide range of topics from biology, ecology, paleontology all the way to archaeology and history. Educational activities are very well accepted by teachers, professors, educators, children and other participants, that is also recorded with a percentage of participants returning for the second time. Local and regional schools spend their “school in nature” outside the Istrian County, so this type of education is definitely needed in the region, and beyond.

Keywords: educational programs, biology, nature protection, Brijuni National Park

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ZBIRKA BODLJIKAŠA PRIRODOSLOVNOG MUZEJA U SPLITU

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Prirodoslovni muzej i zoološki vrt u Splitu posjeduje relativno malu zbirku bodljikaša (Echinodermata) koja ima povijesnu vrijednost. Između ostalih, sakupljali su je i bivši ravnatelji muzeja: Umberto Girometta, Antun Cvitanić, Vladimir Golubić, a prvi primjerci su sakupljeni početkom 20. stoljeća. Zbirka se još nadopunjava, a čine je primjerci iz Jadranskog mora te jedan darovani primjerak sakupljen iz Tihog oceana (Kalifornijski zaljev). Zbirka se sastoji od 142

primjerka raspoređenih u 72 inventarna broja, svrstanih u 21 porodicu i 26 rodova. Najzastupljenije porodice su Toxopneustidae, Astropectenidae, Echinasteridae, Parechenidae i Loveniidae. Svi su primjerci determinirani, fotodigitalizirani, inventarizirani i uneseni u bazu podataka.

Ključne riječi: Umberto Girometta, Vladimir Golubić, Antun Cvitanić, zbirka bodljikaša, Prirodoslovni muzej

ECHINODERMATA COLLECTION OF THE SPLIT NATURAL HISTORY MUSEUM

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Natural history museum and zoo in Split holds relatively small historically important Echinodermata collection. Former museum principles such as Umberto Girometta, Antun Cvitanić and Vladimir Golubić, are some of its collectors and the first specimens were collected in the beginning of the 20th century. Collection is formed of specimens from the Adriatic sea and one gifted specimen from the California Bay from Pacifik. New specimens are still collected. Collection consists of 142 specimens organised in 72 inventory number, presenting 21 family and 26 genus. The most represented family are Toxopneustidae, Astropectenidae, Echinasteridae, Parechenidae and Loveniidae. All the specimens are determined, photodigitalized, inventoried and entered in a database.

Keywords: Umberto Girometta, Vladimir Golubić, Antun Cvitanić, Echinodermata collection, Natural History Museum

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MREŽNI ZOOPLANKTON ŽUPSKOG ZALJEVA (JI JADRANSKO MORE) TIJEKOM ZIMSKO-LJETNOG RAZDOBLJA

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Istraživanje mrežnog zooplanktona obavljeno je u Župskom zaljevu (JI Jadransko more) od siječnja do svibnja 2017. Zooplanktonski uzorci prikupljeni su vertikalnim potezima mrežom gustoće tkanja 125 µm na tri postaje. Najveća gustoća ukupne populacije zooplanktona zabilježena je u siječnju na postaji P2 (2436 jed. m^{-3}). Kopepodi su bili najbrojnija i dominantna skupina u svim uzorcima tijekom istraživanog razdoblja s ukupnim udjelom od 73% u u ukupnoj populaciji zooplanktona. Najbrojniji red kopepoda bili su kalanoidi. Njihov udio u ukupnoj populaciji kopepoda iznosio je 59%. Od ostalih zooplanktonskih skupina, isticale su se ličinke školjkaša i mnogočetinaša. Najveća količina ličinki školjkaša zabilježena je u svibnju, a ličinki mnogočetinaša u siječnju. Holoplankton je dominirao u svim uzorcima tijekom istraživanog razdoblja, s udjelom od 78% u ukupnoj populaciji zooplanktona. Meroplanktonti su bili manje brojnija skupina, a njihova brojnost se povećala tijekom proljeća i to s povećanjem broja ličinki školjkaša, mnogočetinaša i bodljikaša.

Ključne riječi: Župski zaljev, mrežni zooplankton, meroplankton, holoplankton

NET ZOOPLANKTON OF THE ŽUPA DUBROVAČKA BAY (SE ADRIATIC SEA) DURING WINTER-SPRING PERIOD

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Net zooplankton was investigated in the Župa dubrovačka Bay (SE Adriatic Sea) from January to May 2017. Zooplankton was sampled with 125-µm mesh size planktonic net by vertical hauls at three stations. The highest total zooplankton population densities were recorded in January at the station P2 (2436 ind. m^{-3}). Copepods were the most abundant and dominant group in all samples during research period, with the contribution of 73% in the total zooplankton population. Calanoids, the most numerous order of copepods, participated with 59% in the total abundance of all copepods. Among the other groups of zooplankton, higher values of the population densities of polychaete larve were recorded in January, while bivalve larvae were recorded in higher quantities in May 2017. Holoplankton was dominant in all samples during research period with the contribution of 78% in the total zooplankton population. The meroplankton density values were higher during spring period as the result of increase of the abundance of bivalve, polychaete and echinoderm larvae.

Keywords: Župa dubrovačka Bay, net zooplankton, holoplankton, meroplankton

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ESTIMATING THE HOME RANGE OF RESIDENT COMMON BOTTLENOSE DOLPHINS (*Tursiops truncatus*) IN VIS ARCHIPELAGO

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The home range size of selected individuals from the common bottlenose dolphin community in Vis archipelago (CE Adriatic Sea, Croatia) was estimated using sighting location and photo-identification data collected as part of a long-term seasonal boat based study in the period 2007-2017. Based on mark-recapture abundance estimates, the research area is inhabited by about 300 dolphins while a subset of individuals shows high site fidelity, with sightings recorded in all years. Two methods were employed – the minimum convex polygon method (MCP) and fixed kernel density estimation (KDE). Input data was selected using a range of cut-off criteria in order to investigate variability arising from differences in minimum number of sightings and sample period longevity. Depending on chosen criteria, the resulting mean home range sizes vary between 154.72 km^2 (SD ± 94.66) and 491.69 km^2 (SD ± 415.75) (MCP), 319.22 km^2 (SD ± 343.63) and 560.55 km^2 (SD ± 518.89) (50% KDE) and 1306.20 km^2 (SD ± 1446.38) and 2380.72 km^2 (SD ± 2100.56) (95% KDE). There is a marked difference in the mean home range sizes of females and individuals of unknown gender when a 11-year sampling period is used (Mann-Whitney U test, MCP: U=817.00, P=0.013; 50% KDE: U=843.00, P=0.021; 95% KDE: U=874.00, P=0.036). However, no significant gender related differences were detected when

more relaxed data selection criteria were applied. These results are comparable to reports from other research areas within the basin.

Keywords: minimum convex polygon, kernel density, Adriatic Sea

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SEZONSKE PROMJENE U SASTAVU DEKAPODNE FAUNE (CRUSTACEA, DECAPODA) SEDIMENTNOG DNA PLITKOG INFRALITORALA U ZALJEVU MELINE, OTOK KRK

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Istraživane su vrste dekapoda (Crustacea, Decapoda) na sedimentnom dnu gornje infralitoralne zone (od 0 do 1 m dubine), u zaljevu Meline, na otoku Krku (sjeverni Jadran). Staništa tog tipa su vrlo rijetka na sjevernom Jadranu, ali i cijeloj istočnoj obali Jadrana. Istraživanje je provedeno u razdoblju od lipnja 2002. do travnja 2003. godine. Dekapodi su prikupljeni pomoću povlačne mreže, četiri puta tijekom ispitivanog razdoblja. Sakupljanje je obavljano dnevnim i noćnim potegom u svakoj sezoni. Sezonskim prikupljanjem pokušali smo utvrditi moguće godišnje vertikalne migracije decapoda. Dodatno uzimanje uzoraka noću uklonilo je nedostatke isključivo dnevnog uzorkovanja zbog ukopavanja ili neaktivnosti tijekom dana. Prikupljeno je više od 600 primjeraka dekapoda unutar 7 vrsta. U ulovu su dominirale dvije vrste: *Carcinus aestuarii* i *Crangon crangon*. Dominantnost ovih vrsta izraženija je u toplijim godišnjim dobima (proljeće i ljeto) nego tijekom ostatka godine. Sastav vrsta, njihova brojnost i dominantnost određena je za svaku sezonu. Provedena su i komparativna istraživanja te multivarijantna analiza podataka (CANOCO).

Ključne riječi: Decapoda, gornji infralitoral, sedimentno dno, povlačna mreža, sjeverni Jadran

SEASONAL VARIATIONS IN A DECAPOD FAUNA (CRUSTACEA, DECAPODA) OF THE SHALLOW SEDIMENTARY INFRALITORAL ZONE AT THE MELINE BAY, ISLAND OF KRK

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The decapod species (Crustacea, Decapoda) living on the sedimentary bottom of the upper infralitoral zone (from 0-1 meter depth), at the Meline Bay, Island of Krk (North Adriatic), were investigated. Habitats of that type are very rear in the North Adriatic, but also on the whole eastern Adriatic coast. The study was performed between June 2002 and April 2003. The decapods were collected by means of beach seine, four times during the investigated period. Each time the collecting was performed once in the day time and once in the night. With drawing the seine in every season we tried to determine possible decapod annual vertical migrations. Additional night time sampling eliminated the shortcomings of the daily sampling resulting from certain species being covered up or inactive at daytime. More than 600 specimens belonging to 7 species were collected. Two species were dominant in the catch: *Carcinus aestuarii* and *Crangon crangon*. Dominancy of these species is more evident during

the warm seasons (spring and summer) than during the rest of the year. The species composition, their abundance and dominancy are given for each season, upon which a comparative study and multivariate analysis (CANOCO) has been conducted.

Keywords: Decapoda, shallow sedimentary infralitoral, beach seine, North Adriatic, Canoco

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PROMJENE BIORAZNOLIKOSTI JADRANA - INDIKATOR KLIMATSKOH PROMJENA?

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Postoji mnogo podataka kako klimatske promjene smanjuju bioraznolikost svjetskih oceana i mora i kako bi te promjene u budućnosti mogle biti i mnogo gore. Promjena klime je već odgovorna za promjene u rasprostranjenosti vrsta, masovnu smrtnost, nestanak vrsta i pojavu invazivnih vrsta. Učinci globalnih klimatskih promjena posebno su vidljivi u područjima kao što je Jadransko more, jedno od hladnijih dijelova Sredozemnog mora. Relativno malo podataka je dostupno o temperaturi mora (većinom površinske temperature) Jadrana od 1950-tih godina. Godišnje vrijednosti temperature mora u zadnjih 40 godina pokazuju povećanje do dubine od 45 metara. Ljetni uvjeti postaju sve duži zadnjih desetak godina što uzrokuju povećanje prisutnosti vrsta (pogotovo invazivnih) koji žive u toplijem moru. Prisutnost vrsta toplijeg mora u Jadranu (širenje areala prema srednjem i sjevernom dijelu) znatno se povećava i te populacije postaju prilično stabilne i udomaćene. Ribe koje su nekad bile ograničene samo za južni dio Jadrana, a danas se šire prema sjeveru su papigača *Sparisoma cretense* i vladika arbanaški *Thalassoma pavo*. Ove dvije vrste postale su udomaćene u srednjem dijelu Jadrana od 2012. godine. S druge strane, vrste poput crvene gorgonije *Paramuricea clavata* ili kolonijalnog kamenog koralja *Cladocora caespitosa* izložene su različitim kombinacijama promjene temperature i manjka hrane što uzrokuje masovnu smrtnost populacija ovih vrsta. Zbog sve veće prisutnosti vrsta toplijeg mora, te povećanje pojavnosti masovne smrtnosti pojedinih autohtonih vrsta, cijelo Sredozemno more je pod procesom „tropikalizacije“.

Ključne riječi: bioraznolikost, klimatske promjene, Jadransko more

CHANGES IN THE ADRIATIC SEA BIODIVERSITY - AN INDICATOR OF CLIMATE CHANGE?

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There are lots of proofs that climate change is already reducing marine biodiversity in world oceans and seas and this may worsen in the future. Climate warming has already triggered responses such as shifts in species distributions, mass mortalities, extinctions and invasions. The effects of global climate change are particularly serious in areas where range shifts of species are physically constrained such as the Adriatic Sea, which is one of the coldest parts of the Mediterranean. Some scarce historical information on water temperature (mostly the sea

surface temperature) from the Adriatic Sea dates back to the 1950s. Annual variation in sea temperature in the last 40 years increased over the entire period for the water layer down to 45 m. Summer conditions have become much more prolonged in recent decades causing that warm-water native and alien species richness increased during the warming summer periods. The presence of warm-water species in the Adriatic Sea (northward expansions of the geographical range of warm-water species, especially in the northern and central part) greatly increased in the last decade, suggesting establishment there of quite stable populations. A fish species once restricted to the southern Adriatic but now moving towards the north are the parrotfish *Sparisoma cretense* and the ornate wrasse *Thalassoma pavo*. These two fish species have now become established in the central part of the Adriatic Sea since summer 2012. On the other hand, some species, like well-studied gorgonian *Paramuricea clavata* or colonial coral *Cladocora caespitosa* were exposed to different combinations of temperature and food concentration that cause mass mortalities of their populations. Because of the increased occurrence of warm-water biota and present mass mortalities of certains species, the whole Mediterranean Sea is under a process of, like some scientists like to say, "tropicalization".

Keywords: biodiversity, climatic changes, Adriatic Sea

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PREDATORI KORALJA U JADRANSKOM MORU

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Poznato je da predatori koralja imaju različit utjecaj na stanje koralja i koraljnih zajednica, strukturu koraljnih zajednica i funkcioniranje ekosustava. Predatori koralja imaju različite strategije hranjenja polipima koralja i mogu biti striktni (obligatni) ili uvjetni (fakultativni). Oštećenja polipa kolonija mogu biti mala ili letalna, ali postoje brojni dokazi kako i mala oštećenja mogu imati za posljedicu smanjeni rast kolonije koralja. Pregled literature pokazuje širok raspon vrste koje se hrane koraljima, ali je utvrđeni i kako se te vrste hrane sa relativno malim brojem rodova koralja. Nedavno je utvrđeno hranjenje papigače *Sparisoma cretense* (Linnaeus, 1758) na kamenim koraljima (kao površinske lezije) u Jadranskom moru. Mnogočetinaš vatreni crv *Hermodice carunculata* (Pallas, 1766) hrani se polipima gorgonija. Puž *Pseudosimnia carnea* (Poiret, 1789) hrani se polipima crvenog koralja, a puž *Simnia spelta* (Linnaeus, 1758) polipima gorgonija rodova *Eunicella, Paramuricea* i *Lophogorgia*. Puž *Coralliophila meyendorffi* (Calcara, 1845) poznati je predator polipa kolonijalnog kamenog koralja *Cladocora caespitosa*, posebno u istočnom dijelu Jadranskog mora gdje eksplozija populacija ovog puža može drastično reducirati rasprostranjenost koralja. Jedinke puža *C. meyendorffi* hrani se isključivo sa polipima skidajući ih sa skeleta koralja. Velike populacije puža *C. meyendorffi* napravile su značajnu štetu na grebenu koralja *C. caespitosa* u Velikom Jezeru u Nacionalnom parku Mljet.

Ključne riječi: koralji, predatori, Jadransko more

CORAL PREDATORS IN THE ADRIATIC SEA

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It is well known that corallivores have numerous and diverse impacts on coral fitness, community structure and ecosystem function. Consumers of live coral tissue employ a wide variety of feeding strategies and can be obligate or facultative coral feeders. Damage by corallivores ranges from minor to lethal, but there is a growing body of evidence to support that even limited removal of tissue or skeletal structures has growth and fitness consequences for a scleractinian coral colony. Literature review reveals a complex array of corallivores across the globe, although these corallivores span a wide taxonomic range, we found that they have been reported to feed on relatively few genera of corals. Recent frequent predation on scleractinian corals by parrotfish *Sparisoma cretense* (Linnaeus, 1758), leaving superficial lesions, was noticed for the first time in the Adriatic Sea. The bearded fireworm *Hermodice carunculata* (Pallas, 1766) was found feeding on gorgonian corals. The corallivorous gastropod *Pseudosimnia carnea* (Poiret, 1789) fed on red coral (*Corallium rubrum*) polyps, while *Simnia spelta* (Linnaeus, 1758) fed on other gorgonian species from the genus *Eunicella, Paramuricea* and *Lophogorgia*. Gastropod *Coralliophila meyendorffi* (Calcara, 1845) is a well-known predator of the coral *Cladocora caespitosa*, particularly in the eastern Adriatic Sea, where population outbreaks can drastically reduce coral cover. Specimens of *C. meyendorffi* feed exclusively on live coral tissue, stripping it from the calcium carbonate skeleton. Recent outbreaks of the gastropod *C. meyendorffi* have caused considerable damage on a *C. caespitosa* bank in Veliko Jezero (the Mljet National Park, eastern Adriatic Sea).

Keywords: corals, predators, Adriatic Sea

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MALAKOLOŠKA ZBIRKA VLADIMIRA GOLUBIĆA

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Prirodoslovni muzej i zoološki vrt u Splitu posjeduje 7 malakoloških zbirki od kojih je jedna u stalnom postavu (Malakološka zbirka obitelji Bakotić) te 6 deponiranih: zbirke P. Novaka, A. Cvitanića, M. Kolander, J. Save, V. Golubića i don B. Cvitanovića. Malakološka zbirka Vladimira Golubića je prikupljana od 1984. do 1998. godine i čine je predstavnici puževa, školjkaša, mnogoljušturaša i koponožaca Jadranskog mora. Primjerci su sakupljeni na području Splita i srednjodalmatinskih otoka (Brač, Šolta, Čiovo, Drvenik Veli, Sveta Fumija) i to u infralitoralnom području do 5 m dubine. Zbirka se sastoji od 7.675 primjerka raspoređenih u 698 inventarna broja, svrstanih u 61 porodicu. Najzastupljenije porodice su Trochidae, Veneridae, Patellidae, Nassariidae, Donacidae, Columbellidae i Cerithiidae. Svi su primjerci determinirani, inventarizirani i uneseni u bazu podataka. Zbirka se priprema za prijavu stjecanja svojstva kulturnog dobra RH.

Ključne riječi: Vladimir Golubić, malakološka zbirka, Prirodoslovni muzej

MALACOLOGICAL COLLECTION OF VLADIMIR GOLUBIĆ

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The Natural History Museum and ZOO in Split holds 7 malacological collections of which one is permanently exhibited (Malacological collection Bakotić) and the other ones are deposited: collections of P. Novak, A. Cvitanić, M. Kolander, J. Savo, V. Golubić and B. Cvitanović. The Malachological Collection of Vladimir Golubic has been collected from 1984 to 1998 and consists of snails, bivalves, polyplacophorans and scaphopods of the Adriatic Sea. The specimens were collected in the Split area, on middle dalmatian islands (Brač, Šolta, Čiovo, Drvenik Veli, Sveta Fumija) mostly in the infralittoral area depths up to 5 meters. The collection consists of 7.675 specimens organised in 698 inventory number, presenting 61 family. The most represented family were Trochidae, Veneridae, Patellidae, Nassariidae, Donacidae, Columbellidae and Cerithiidae. All the specimens are determined, inventoried and stored in a database. The collection is being prepared for becoming a part of cultural goods of the Republic of Croatia.

Keywords: Vladimir Golubić, malacological collection, Natural History Museum

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ODREĐIVANJE SASTAVA OTPADA IZ MORA NAPLAVLJENOG NA PLAŽI PREMA METODOLOGIJI PROJEKTA DEFISHGEAR

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Zabrinjavajuće količine otpada u moru jedna su od najbrže rastućih prijetnji okolišu. Otpad u morski okoliš dospijeva isključivo uslijed ljudskih aktivnosti, odnosno nedostataka u sustavu gospodarenja otpadom. Velike količine otpada iz mora akumuliraju se na plažama uslijed djelovanja snažnog vjetra i morskih struja. U ovom su radu prikazani rezultati kvalitativne i kvantitativne analize otpada iz mora prikupljenog monitoringom plaže u Pupnatskoj Luci na otoku Korčuli tijekom 2016. godine. Ukupno je zabilježeno i određeno 24524 komada otpada ukupne težine 420,6 kg. Sakupljeni otpad razvrstan je u osam glavnih kategorija: umjetni polimerni materijali – plastika, tekstil, staklo/keramika, metal, papir, guma, obrađeno drvo i ostalo. Plastika je utvrđena kao prevladavajuća vrsta otpada iz mora s udjelom od 91,1%, nakon čega slijede tekstil (2,9%), staklo/keramika (2,4%), metal (1,5%), guma (0,5%), papir (0,5%), obrađeno drvo (0,2%) i ostalo (0,1%). Prosječna gustoća otpada iznosi 6,2 kom/m². Izvori prikupljenog otpada mogu se povezati sa lošim gospodarenjem odlagalištima komunalnog otpada, kanalizacijom, turizmom i rekreacijskim aktivnostima, ribarstvom i marikulturom, te brodskim prometom. Analizom podrijetla otpada utvrđeno je da najveća količina dolazi iz susjednih zemalja, prvenstveno Albanije. Zbog značajnih okolišnih, ekonomskih, sigurnosnih, zdravstvenih i kulturnih implikacija, nužna su efektivna i dugoročna rješenja ovog problema.

Ključne riječi: otpad iz mora, plaže, plastika, izvori zagađenja, prekogranični utjecaj.

QUANTIFICATION OF MARINE LITTER ON THE BEACH USING METHODOLOGY PROPOSED BY THE DEFISHGEAR PROJECT

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Huge amounts of litter present in the marine environment is one of the fastest growing environmental threats today. It's discharged in the sea due to the human activities and lack of adequate waste management in the coastal areas. Great quantities of litter stranded on the shoreline due to strong winds and prevailing sea currents. This work presents the results of qualitative and quantitative analysis of litter collected seasonally in 2016 during beach monitoring in Pupnatska Luka, Island Korčula. A total of 24,524 items were collected and classified, with a total weight of 420.6 kg. Litter found falls into eight major categories: artificial polymer materials - plastics, textiles, glass/ceramics, metal, paper, rubber, processed wood and other. The majority of litter items were made of plastics accounting for 91.1% , followed by textile (2.9%), glass/ceramics (2.4%), metal (1.5%), rubber (0.5%), paper (0.5%), processed wood (0.2%) and other (0.1%), with the average of 6.2 items/m². Collected litter could be attributed to the following sources: shoreline, poor waste management practices, tourism and recreational activities; fisheries and aquaculture; sanitary and sewage-related; shipping; medical related. Analysis of the litter sources showed that the significant amount originated from the neighbouring countries, mostly Albania. Due to its significant environmental, economic, safety, health and cultural implications, effective and long-term measures and solutions are required.

Keywords: marine litter, beach, plastics, pollution sources, cross-border impact

EVOLUCIJA, SISTEMATIKA, FILOGENIJA I BIOGEOGRAFIJA
EVOLUTION, SYSTEMATICS, PHYLOGENY AND BIOGEOGRAPHY

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FLORA OTOKA DRVENIK MALI

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Flora otoka Drvenik Mali, istraživana je tijekom 2017 i 2018. godine, pokazuje veliku raznolikost, obuhvaća 430 vrsta i podvrsta iz 75 porodica. Najzastupljenije porodice su Fabaceae (12,10%), Poaceae (11,53%), Asteraceae (6,92%), Lamiaceae (4,90%) i Liliaceae (3,75%). Najzastupljeniji životni oblici su terofiti (46,97%), fanerofiti (19,02%) te hemikriptofiti (17%). Na istraživanom području najveći broj vrsta i podvrsta pripada mediteranskom flornom elementu (51,59%). Zabilježeno je 15 endemičnih biljka te sedam ugroženih svojti koje se nalaze u Crvenoj knjizi vaskularne flore Hrvatske. U flori otoka je 30 zaštićenih i 24 strogo zaštićenih svojti.

Ključne riječi: flora, Drvenik Mali, endemi

FLORA OF ISLAND DRVENIK MALI

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The research of the flora of the island Drvenik Mali was explored during 2017 and 2018. showing a great variety, covering 430 taxa from 75 families. The most abundant families are Fabaceae (12,10%), Poaceae (11,53 %), Asteraceae (6,92 %), Lamiaceae (4,90%) and Liliaceae (3,75%). The most abundant life-forms in the flora are therophytes (46,97%), phanerophytes (19,02%) and hemicryptophytes (17%). In the flora of the researched island the most dominant element is Mediterranean chorological element (51,59 %). The research recorded 15 endemic taxa, and seven endangered taxa noted in the Red Book of Vascular Flora of Croatia. Thirty protected taxa and 24 strictly protected taxa were recorded in the flora of the island Drvenik Mali.

Keywords: flora, Drvenik Mali, endems

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OBSERVATION AND DISTRIBUTION OF THE DIATOM GENUS *Surirella* TURPIN IN ANCIENT LAKES OHRID AND PRESPA

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The genus *Surirella* Turpin is polyphyletic and one of the oldest diatom genera described; the name appears for the first time as “Surirelle” in Turpin (1828). The genus is highly diverse, comprises more than 500 taxa described worldwide, and is characterized by a circumferential marginal raphe system, raised or not raised within a keel canal. Taxa from this genus are found in marine, brackish and freshwater environments. In the past few decades several studies have focused on the re-examination, typification and description of *Surirella* taxa from various regions remarkable for their high diversity and endemism, such as lakes Malawi, Tanganyika, Baikal and the Malili lakes. The oldest lakes in Europe, Ohrid and Prespa, have been the focus of intensive diatom research since the early 1900’s. While the genus *Surirella* has been extensively studied in the contemporary flora of Lake Ohrid, there was less and/or no information about its diversity in Lake Prespa, and the fossil record of the lakes. In this study, *Surirella* taxa were observed by LM and SEM in a number of recent and fossil samples from both lakes, and 13 taxa were identified as potentially new to science. Whereas the species richness appears higher in Lake Prespa; with 28 recent and fossil taxa; Lake Ohrid has more endemic taxa. From a total number of 23 *Surirella* taxa recorded in Lake Ohrid, eight were found exclusively in the lake, while an additional five taxa are considered as endemic for this“sister” lake system.

Keywords: Lake Ohrid, Lake Prespa, diatoms, *Surirella*

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TAKSONOMSKI PREGLED RODA *Holwaya* (ASCOMYCOTA, FUNGI)

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Do nedavno se smatralo da rod *Holwaya* sadrži samo jednu vrstu s brojnim sinonimima. U tom je rodu opisano, odnosno kombinirano sedam vrsta: *H. gigantea*, *H. leptosperma*, *H. mucida* (uključujući subsp. *nipponica*), *H. ophiobolus*, *H. pusilla*, *H. salicis* i *H. tiliacea*. Danas se većina ovih taksona smatra sinonimima vrste *H. mucida*, osim *H. pusilla* koja je premještena u rod *Claussenomyces* i *H. salicis* koja pripada rodu *Durandiella*. U razdoblju od prve detaljne molekularno filogenetske analize reda Helotiales Wanga i sur. (2006) pa do Baralove (2016) revizije dogodile su se tek male promjene u klasifikaciji. Rod *Holwaya* smatran je članom porodice Bulgariaceae (Lumbsch & Huhndorf 2010), ali su ga Crous i sur. (2014) isključili iz te porodice smatrajući Bulgariaceae sinonimom porodice Phacidiaceae. Premda *Holwaya* nije nikada bila uključena u porodicu Phacidiaceae, Baral (2016) proširuje koncept reda Phacidiales dodajući mu druge porodice te svrstavajući rod *Holwaya* u porodicu Tympanidaceae. Cilj ovoga rada je prikazati taksonomski pregled roda *Holwaya* na temelju podataka koje su sakupili autori tijekom nekoliko zadnjih desetljeća. To uključuje nova mikroskopska obilježja otkrivena istraživanjima uzoraka u živom stanju te nove spoznaje u taksonomiji i sistematici, kao što je korektan generički položaj vrste *Claussenomyces tympanoides* (*C. pusillus*) potvrđen istraživanjem tipskog materijala.

Ključne riječi: integrativna taksonomija, gljive reda Phacidiales, reklasifikacija, Tympanidaceae

TAXONOMIC REVIEW OF THE GENUS *Holwaya* (ASCOMYCOTA, FUNGI)

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The genus *Holwaya* was considered to be monospecific until recently because of extensive species synonymy. Seven species have been described or combined in the genus: *H. gigantea, H. leptosperma, H. mucida* (with subsp. *nipponica*), *H. ophiobolus, H. pusilla, H. salicis*, and *H. tiliacea*. Nowadays most of these taxa are considered to be synonyms of *H. mucida*, except *H. pusilla* that was transferred to the genus *Claussenomyces*, and *H. salicis* to *Durandiella*. Since the first more thorough molecular phylogeny of Helotiales (Wang et al. 2006), there have been few changes in the classification until that proposed by Baral (2016). Holwaya was previously considered a member of the family Bulgariaceae (Lumbsch & Huhndorf 2010), but it was removed from this family by Crous et al. (2014) who regarded Bulgariaceae as a synonym of Phacidiaceae. Although *Holwaya* was never included in Phacidiaceae, Baral (2016) expanded the concept of Phacidiales by adding other families and placing the genus *Holwaya* in *Tympanidaceae*. The aim of this work is to provide a taxonomic review of the genus *Holwaya* based on data gathered by the authors through the last few decades. This includes new microscopical features revealed by studies of the material in living state and new taxonomic and systematic information, such as the correct generic affiliation of *Claussenomyces tympanoides* (*C. pusillus*) based on type studies.

Keywords: integrative taxonomy, phacidialean fungi, reclassification, Tympanidaceae

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NEW AND INTERESTING *Gomphonema* EHRENBERG (BACILLARIPHYCEAE) SPECIES FROM MACEDONIA

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The genus *Gomphonema* in R. Macedonia was recently studied in detail. More than 125 species have been observed and around 30 species were described as new for science. Highest diversity was observed in Lake Ohrid and Mt. Shar Planina. Further observations of the samples from Lake Ohrid reveal existence of five new species in a single sample. One of the species resembles *G. irroratum* Hustedt, a species with very specific combination of features (biseriate striae composed by round unoccluded areolae and simple stigma) that makes it closer to the genus *Gomphoneis* Cleve sensu stricto. These two species probably have intermediate position between *Gomphonema* and *Gomphoneis*. The second species is closely related to *G. perolivaceoides* Levkov, having four stigmata in the central area, but it has longer and wider valves with lower stria density. Two of potentially new species are related to *G. balcanicum* Levkov & Krstic, but differences can be noticed in the valve shape, axial area and stria density. The fifth species belongs to the group of *G. pumilum* (Grunow) Reichardt & Lange-Bertalot and differs from members of this species complex by the valve shape, size and stria and areola density. Three other species have been observed from extreme habitats such as halomorphic soils and mineral springs. Their morphological features are observed both by LM and SEM and compared with similar species.

Keywords: diatoms, *Gomphonema*, new species, Macedonia

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SELECTED *Cyclotella* sensu lato (BACILLARIOPHYTA) FROM LAKE OHRID FOSSIL RECORD

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Lake Ohrid is known as biodiversity hotspot, with more than 150 endemic extant diatom taxa. Most taxa belong to the group of pennates, while the diversity of centric diatoms is relatively limited. Over the last few years, substantial research has been focused on the fossil record of the lake. The ~580m (>1.3 Ma) long sediment sequence recovered within the project Scientific Collaboration on Past Speciation Conditions in Lake Ohrid in 2013 is unique by the continuous diatom preservation and provides wealth of data to study fossil centric diatoms.
More than 400 samples were observed with light microscope to document the diversity of the genus *Cyclotella* sensu lato. LM and SEM analyses reveal presence of high *Cyclotella* species richness and here we present six morphologically distinct taxa. High morphological diversity was observed in *Cyclotella cavitata* and *C. sollevata*, including heterovalval frustules. At least two *Cyclotella* sensu lato species have unique combination of characters and will be described in

forthcoming studies. Apart from the endemic taxa, two widespread taxa, e.g. *Pantocsekiella ocellata* and *P. delicatula* are common in parts of the fossil record.

Keywords: Lake Ohrid, *Cyclotella*, fossil, core

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RASPROSTRANJENOST SLATKOVODNIH PUŽEVA IZ NADPORODICE RISSOOIDEA U SPELEOLOŠKIM OBJEKTIMA I IZVORIMA OGULINA I OKOLICE

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Ogulin i okolica s brojnim speleološkim objektima, izvorima, ponorima, rijekama i krškim poljima čine kompleksnu hidrogeološku mrežu. Podzemni vodeni puževi iz nadporodice Rissooidea predstavljaju zanimljivu, ali nedovoljno istraženu skupinu beskralješnjaka s velikim potencijalom pronalaska novih vrsta za znanost. Osim taksonomske vrijednosti, zbog svoje iznimno ograničene sposobnosti kretanja, endemizma i rasprostiranja uglavnom vodom, predstavljaju potencijalan alat za rekonstrukciju recentnih i prošlih hidrogeoloških mreža. Ovim istraživanjem određena je rasprostranjenost, prikazane su konhološke karakteristike i ustvrđena je korelacija obrazaca rasprostranjenosti s hidrogeološkom povezanošću staništa svojti ove nadporodice. Obrađeni su podaci s terenskih istraživanja kao i literaturni podaci za 22 speleološka objekta u kojima je pronađeno 18 svojti od kojih je 16 određeno do razine vrste. Osim što je prošireno područje rasprostranjenosti 4 roda i 5 vrsti podzemnih slatkovodnih puževa koji do sada nisu pronađeni na istraživanom području, predložena je i upotreba obrazaca rasprostranjenosti kao metodološkog alata u određivanju sadašnjih i prošlih hidrogeoloških veza podzemnih voda.

Ključne riječi: podzemni slatkovodni puževi, Rissooidea, Ogulin, rasprostranjenost, hidrogeologija, krš, endemi

DISTRIBUTION OF FRESHWATER SNAILS FROM THE SUPERFAMILY RISSOOIDEA IN CAVES AND SPRINGS OF OGULIN AND THE SURROUNDING AREA

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Ogulin and it's surrounding with it's caves, springs, ponors, rivers and karst poljes form a complex hydrogeological web. Subterranean freshwater snails from the superfamily Rissooidea represent an interesting but insufficiently examined invertebrate group with a great potential for finding

new species. Apart from their taxonomic value, because of their extremely limited moving capability, endemism and distribution mechanism which include mostly water, they show a potential as a tool for reconstructing recent and past hydrogeology connections. Through this research distribution, conchological characteristics and correlation between hydrogeological connectivity and distribution patterns of this superfamily were defined. The study included data from field surveys and literature data from 22 localities in which 18 taxa were found. 16 of them were determined to the species level. Apart from widening the distribution area of 4 genera and 5 species which haven't been found in this area until now a proposition has been made for using distributional patterns of these taxa as a methodological tool in reconstructing recent and past hydrogeological connections of underground waters.

Keywords: subterranean freshwater snails, Rissooidea, hydrogeology, karst, endemic species

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DNA BARKODIRANJE FAUNE HRVATSKE S OSVRTOM NA RAZNOLIKOST I DNA BARKODIRANJE TULARA (INSECTA, TRICHOPTERA)

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Trichoptera s drugim skupinama vodenih kukaca čine jedan od najraznolikijih dijelova vodene faune te nastanjuju različite tipove vodenih staništa: izvore, potoke, rijeke, jezera, močvare. Sistematska istraživanja bioraznolikosti tulara u Hrvatskoj započele su prije 20 godina. Do sada je zabilježeno oko 215 vrsta Trichoptera u fauni Hrvatske. Metoda DNA barkodiranja predložena je 2003. godine kao univerzalni sistem za determinaciju biološkog materijala u smislu identifikacije vrste, a također i kao metoda za otkrivanje novih, još neopisanih vrsta koje se ne mogu međusobno razlikovati na temelju morfoloških obilježja (tzv. kriptičnih vrsta). Temelji se na određivanju slijeda nukleotida standardiziranog fragmenta mitohondrijskog gena za podjedinicu I citokrom okidaze (COI) duljine oko 650 pb. Godine 2017. započeo je znanstveni projekt "DNK obilježavanje bioraznolikosti hrvatske faune" kojeg financira Hrvatska zaklada za znanost. Projekt uključuje analizu 15 životinjskih skupina (oko 1450 vrsta) uključujući i Trichoptera. Primarni cilj projekta je istražiti rasprostranjenost i genetsku raznolikosti odabranih skupina hrvatske faune primjenom metode DNA barkodiranja. DNA barkodiranje vrlo je značajna metoda u procesu određivanja vrsta. Posebno je značajna u određivanju vrlo sličnih vrsta. Do sada smo DNA barkodirali oko 150 vrsta Trichoptera zabilježenih za faunu Hrvatske. Tijekom istraživanja utvrđeno je nekoliko novih vrsta u fauni Hrvatske i vjerojatno neke nove - kriptičke vrste za znanost.

Ključne riječi: DNA barkodiranje, Trichoptera, Hrvatska

DNA BARCODING OF FAUNA OF CROATIA WITH NOTICE ON BIODIVERSITY AND DNA BARCODING DATA OF CADDISFLIES (INSECTA, TRICHOPTERA)

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Caddisflies, along with other groups of aquatic insects, comprise one of the most abundant segments of aquatic fauna inhabiting all types of aquatic habitats: springs, streams, rivers, lakes, marshes. Systematic studies of the biodiversity of caddisflies in the Croatia have started 20 years ago. So far we recorded about 215 species of Trichoptera in fauna of Croatia. DNA barcoding was proposed in 2003 as a universal system for assignment of specimens to a particular species but also as a tool for recognition of yet undescribed morphologically indistinguishable cryptic species. The method is based on the sequencing of standardized, ~650 bp long fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (COI). In 2017, started a scientific project, "DNA barcoding of Croatian faunal biodiversity" financed by the Croatian Science Foundation. The project included 15 animal groups (about 1450 species) including Trichoptera. The primary goal of the project is to investigate the amount and geographic distribution of the genetic biodiversity of selected groups of Croatian fauna by using DNA barcoding methodology. DNA barcoding provides valuable tool in the laborious process of species identification. It helps for determinations of morphologically very similar species. So far we DNA barcoded about 150 species of Trichoptera recorded for Croatian fauna. In this investigation we found several new species in fauna of Croatia and probably some new - cryptic species for science.

Keywords: DNA barcoding, Trichoptera, Croatia

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PRILOG INVENTARIZACIJI FLORE MEĐIMURJA

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Istraživana je flora odabranih lokacija kraj naselja Praporčan, Prelog, Otok, Kotoriba i Sveta Marija na prostoru Međimurske županije. Terenska istraživanja su obavljena tijekom 2016. i 2017. godine. Tijekom terenskih istraživanja determinirane su 186 biljne vrste. Brojem vrsta najzastupljenije su Asteraceae (10,32%), Rosaceae (5,43%), Ranunculaceae (5,43%), Fabaceae (4,89%) i Brassicaceae (4,34%). Invazivnih vrsta ima 13 (7,06%). Zabilježen je utjecaj mediteranskih i submediteranskih biljaka (5,9%). Analizom životnih oblika ustanovljena je najviša zastupljenost hemikriptofita (28,26%), geofita (22,28%), terofita (12,09%) i fanerofita (10,98 %). Determinirane vrste su unesene na mrežne stranice Flora Croatica database te Flora i fauna Međimurja.

Ključne riječi: Međimurje, florni elementi, životni oblici biljaka

ANNEX TO THE INVENTARISATION OF FLORA IN MEĐIMURJE

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The research was carried on flora on selected locations next to Praporčan, Prelog, Otok, Kotoriba

and Sveta Marija in Međimurje county. Field research was done during the years of 2016 and 2017. During the research, 184 plant species were determined. With the number of species, the most common are Asteraceae (10,32%), Rosaceae (5,43%), Ranunculaceae (5,43%), Fabaceae (4,89%) i Brassicaceae (4,34%). There are 13 invasive species (7,06%). An influence of mediterranean and submediterranean plants has been noted (5,9%). By analysing life forms, we have determined that the most present are hemicryptophyte (28,26%), geofite (22,28%), terofite (12,09%) i fanerofite (10,98 %). Determined species have been entered to Flora Croatica database and Flora i fauna Međimurja web pages.

Keywords: County Međimurje, plants life forms, floral elements

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FILOGEOGRAFSKA STRUKTURA UGROŽENOG POTOČNOG RAKA - NOVE SPOZNAJE

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Potočni rak *Austropotamobius torrentium* (Schrank, 1803) jedan je od pet nativnih Europskih vrsta rakova koje nastanjuju manje vodotokove na većim nadmorskim visinama središnje i jugoistočne Europe. U proteklim desetljećima uočeno je smanjenje populacija potočnih rakova uzrokovano antropogenskim pritiskom na njihova staništa, širenjem stranih vrsta rakova i klimatskim promjenama. Stoga se potočni rak smatra ugroženom vrstom, zaštićenom međunarodnim i nacionalnim zakonima. Poseban naglasak potrebno je staviti na razvoj konzervacijskih planova te upotpuniti saznanja o samoj vrsti, kako bi se razvila učinkovita zaštita. To uključuje i nedavna molekularno filogenetička istraživanja vrste *A. torrentium* koja su pokazala da unutar vrste postoji namanje 7 različitih monofiletskih haplogrupa s najvećom raznolikosti u sjevernim i središnjim Dinaridima. Dosad neistražene populacije potočnog raka iz Hrvatske, Slovenije i Makedonije uključene su u ovo istraživanje uz uzorke s više od 190 lokaliteta s ciljem da se pomoću mitohondrijskih (16S rRNA i COI) i jezgrinih (ITS2 regija) DNA biljega odrede filogenetski odnosi unutar vrste. Zabilježeno je ukupno 48 16S rRNA i 147 COI haplotipova te veći broj ITS2 alela. Filogenetska rekonstrukcija napravljena na sekvencama sva tri DNA biljega, pokazala je kongruentne topologije te nove haplogrupe nisu pronađene. Raspravljena je primjena dobivenih rezultata u budućim planovima očuvanja vrste *A. torrentium*.

Ključne riječi: *Austropotamobius torrentium*, jugoistočna Europa, konzervacija, bioraznolikost

PHYLOGEOGRAPHIC STRUCTURE OF THREATENED STONE CRAYFISH – AN UPDATE

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The stone crayfish *Austropotamobius torrentium* (Schrank, 1803) is one of five native European crayfish species inhabiting smaller waterbodies at higher altitudes of central and south-eastern Europe. In the last decades severe declines in numbers of stone crayfish populations have been recorded, due to anthropogenic pressure on their habitats, spread of non-native crayfish species and climate change. Hence stone crayfish is considered threatened species, protected by international and national laws. The sound knowledge of species, including information on genetics, is a necessity to develop efficient strategies for its protection. Recent molecular-phylogenetic research of *A. torrentium* revealed that within the species at least 7 distinct monophyletic haplogroups exist, with highest genetic diversity in the north-central Dinaric region. This research aimed to update previous results through the inclusion of previously unstudied populations of stone crayfish from Croatia, Slovenia and Macedonia. Samples from more than 190 localities were analysed either for mitochondrial (16S rRNA and COI) or nuclear (ITS2 region) DNA markers. A total of 48 16S rRNA and 147 COI haplotypes, and several ITS2 alleles were obtained. Phylogenetic relationships were reconstructed using all three datasets with different methods of phylogenetic inference showing congruent topologies and no new haplogroups. Applications of acquired results in the future *A. torrentium* conservation programs are discussed.

Keywords: *Austropotamobius torrentium*, south-eastern Europe, conservation, biodiversity

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LAKE OHRID DURING MARINE ISOTOPE STAGE 15: A DIATOM DERIVED PALEOENVIRONMENTAL INTERPRETATION

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A long sediment sequence (584 m; ~1.9 Ma) was retrieved from the oldest lake in Europe, Lake Ohrid, within the scope of the project Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO). By analysing the fossil diatom record, in comparison to the (bio)geochemical data, here, we interpret the lakes' limnology, environment and climate between 625–557 ka. Dominance of the (oligo-)mesotrophic *Aulacoseira subarctica* and high potassium intensities reflect increased clastic input, probably derived through enhanced catchment erosion during the late glacial warming at the MIS16/15 transition. MIS15 (621–563 ka) is being regarded as relatively weak interglacial, with pronounced climatic instability. Shifts in dominance between *Cyclotella cavitata* var. *ambigua* and *C. fottii*, and the epilimnetic taxon, *Cribrionella ohridana*, correspond to changes in biogenic silica, total organic and inorganic carbon, and K content. These variations are reflecting the changes in water temperature, water mixis and lake productivity during MIS15. High abundance of *C. ohridana* corresponds to high K intensities between 573–557 ka indicates low temperatures and light availability, as well as increased catchment erosion, probably due to glacial cooling towards the end of MIS15 and the early

MIS14. Results show that the diatom response during this period was driven by global and regional change in temperature and by local environment, primarily, nutrient availability and water mixing.

Keywords: Lake Ohrid, diatoms, MIS15, paleoecology

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DIJALEKTI POPULACIJE ŽUTE STRNADICE *Emberiza citrinella* L. U HRVATSKOJ

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Proučavanjem dijalekata ptičjeg pjeva mogu se objasniti brojni aspekti života ptica poput ponašanja, adaptacije, migracija i distribucije. Žuta strnadica (*Emberiza citrinella* L.) je mala ptica pjevica idealna za proučavanje uloge dijalekata u komunikaciji ptica. Mužjaci su vrlo uporni pjevači, pjevaju dugo u sezoni, od ranog proljeća do kasnog ljeta. Pjev je jednostavan, sastoji se od dva dijela i završava frazom od dva do tri tona koja imaju specifičnu kombinaciju u svakom dijalektu. Dobro je dokumentirano više dijalekata sa širokom distribucijom u Europi, ali mozaičnim rasporedom. Većina ptica na jednom lokalitetu pjeva samo jedan dijalekt što je stabilno tijekom godina. U ovom istraživanju proučavani su dijalekti populacije žute strnadice u Hrvatskoj kako bi se ustanovila raznolikost i geografska distribucija pojedinih dijalekata. Snimke su prikupljene mikrofonom za snimanje na daljinu u kombinaciji s digitalnim snimačem, pametnim telefonom ili samo digitalnim snimačem. Za utvrđivanje dijalekta analiziran je zadnji dio pjeva po sluhu i programom za analizu zvuka Raven Pro 1.5. Rezultati pokazuju zastupljenost minimalno dvaju dijalekata u ispitivanoj populaciji od kojih je češći dijalekt BE. Istraživanjem je dobro pokriven samo manji dio teritorija Republike Hrvatske, stoga ga je potrebno provesti i na širem području kako bi se obuhvatilo cijelo područje obitavanja žute strnadice u Hrvatskoj.

Ključne riječi: žuta strnadica, dijalekti

SONG DIALECTS IN A POPULATION OF YELLOWHAMMERS *Emberiza citrinella* L. IN CROATIA

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Studying birdsong dialects and their geographical distribution can easily explain many aspects in bird life such as behaviour, adaptation, migrations and distribution. The yellowhammer (*Emberiza citrinella* L.) is a small passerine ideal for studying the function of dialects in bird vocal communication. Males sing very persistently long throughout the season, from early spring to late summer. Its song is simple, consisting of two parts and ending in a phrase with two or three notes with a specific combination for each dialect. Several dialects have been well documented, broadly distributed in Europe but overlapping in a mosaic fashion. Most birds at one locality sing only one dialect, which seems stable over years. In this study dialects of yellowhammers in a population in Croatia were recorded and analysed to establish the variety as well as the local geographical prevalence of a specific dialect. The recordings were collected using a shotgun microphone coupled with a digital voice recorder, a smartphone application or only a digital voice recorder. To determine the dialect only the last phrase of the song was analysed by ear and using

Raven Pro 1.5 interactive sound analysis software. The results show that in this yellowhammer population there are at least two dialects, the most common being BE. However, only a part of Croatian territory was well covered. Therefore, further effort is going to be necessary to obtain more data and get the full geographical distribution.

Keywords: yellowhammer, dialects

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TAKSONOMSKI STATUS MISTERIOZNOGA "ZELENOGA HRASTA" IZ HRVATSKE TEMELJEN NA FILOGENETSKOJ ANALIZI

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„Zeleni hrast" je već dugo poznato i specifično stablo hrasta nepoznatoga podrijetla koje raste u blizini Zadra u Hrvatskoj. Ovisno o autorima, zeleni hrast opisan je kao križanac cera (*Quercus cerris* L.) i hrasta plutnjaka (*Q. suber* L.) ili alternativno kao križanac cera i hrasta crnike (*Q. ilex* L.) opisan kao novi takson *Quercus × viridis* Trinajstić, hybr. nov. =?. Ovaj hrast bio je dosad predmet mnogih istraživanja, kako taksonomskih tako i ekoloških, botaničkih, anatomskih i morfoloških. Međutim, do danas taksonomski status zelenoga hrasta i njegovo podrijetlo ostaju neizvjesni. Kako bismo konačno utvrdili taksonomski status zelenoga hrasta provedena je analiza filogenetskih odnosa zelenoga hrasta temeljem molekularnih biljega u odnosu na ostale blisko srodne vrste hrasta koje rastu u njegovoj široj regiji, uključujući sve potencijalne roditeljske vrste. Ukupno je analizirano devet taksona i 16 jedinki roda *Quercus* L. koristeći kloroplastne (trnK-matK i trnH-psbA) i jezgrine (5.8S + ITS2) DNA biljege. Rezultati filogenetskih odnosa između zelenoga hrasta i ostalih zastupljenih taksona ne podržavaju teoriju da je *Q. ilex* jedna od njegovih roditeljskih vrsta. Umjesto toga, rezultati nedvojbeno dokazuju kako je zeleni hrast takson poznat pod prihvaćenim nazivom *Q. crenata*, te potvrđuje alternativnu hipotezu kako se radi o križancu vrsta *Q. cerris* i *Q. suber*. Na osnovi naših rezultata zaključujemo da je vrsta *Q. crenata* prisutna u hrvatskoj flori.

Ključne riječi: zeleni hrast, *Quercus*, *Quercus crenata*, filogenija, taksonomija

RESOLVING THE TAXONOMIC STATUS OF THE MYSTERIOUS „GREEN OAK" FROM CROATIA BASED ON PHYLOGENETIC ANALYSIS

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The "green oak" is a well-known specific individual oak tree of unknown origin growing near Zadar in Croatia. Depending on the authors, it was described either as a hybrid taxon between *Quercus cerris* L. and *Q. ilex* L. (named *Q. × viridis* Trinajstić) or alternatively as a presumed hybrid between *Q. cerris* and *Q. suber* L. To finally resolve the origin of this taxon, we performed

molecular analyses and investigated the phylogenetic relationships between the "green oak" and other closely related oak taxa from the surrounding area, including all putative parental species. A total of 16 individuals representing nine *Quercus* L. taxa were investigated based on both plastid (trnK-matK and trnH-psbA) and nuclear (5.8S + ITS2) DNA sequence variation. Placement of the green oak in the phylogenetic relationships between the studied oak taxa does not support *Q. ilex* as one of its parental species but rather indicates that this taxon is in fact *Q. crenata* Lam., reaffirming previous alternative hypothesis that the green oak is a hybrid between *Q. cerris* and *Q. suber*. We therefore confirm the presence of *Q. crenata* in the Croatian flora and based on historical literature survey, we explore and discuss the implication of its occurrence and possible hybridogenic origin in the Croatian territory.

Keywords: Green oak, *Quercus*, *Quercus crenata*, phylogeny, taxonomy

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VARIJABILNOST GENA ZA CITOKROM OKSIDAZU I U JADRANSKOJ POPULACIJI DAGNJE *Mytilus galloprovincialis*

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Nukleotidni slijed mitohondrijskog gena za podjedinicu 1 citokrom oksidaze (COI ili COXI) jedan je od najčešće korištenih markera za identifikaciju vrsta i studije populacijske genetike. Cilj ovog preliminarnog istraživanja bilo je utvrđivanje razlika u distribuciji COI haplotipova u dagnje *Mytilus galloprovincialis* duž istočnog dijela Jadrana. Na različitim lokacijama očekivana je različita raspodjela haplotipova. Po pet dagnji uzorkovano je na šest različitih lokacija. Ukupna genomska DNA izolirana je iz tkiva škrga. Fragmenti COI DNA umnoženi su lančanom reakcijom polimerazom (PCR). Amplificiranim uzorcima mtDNA određeni su nukleotidni slijedovi. Dobiveni su slijedovi COI haplotipova dužine 602 nukleotida. Analizom je ustanovljeno 8 različitih haplotipova, različitih učestalosti i rasprostranjenosti. Najčešći haplotipovi HapA3 (33,33% svih sekvenci) i HapA4 (26,67%) prisutni su na svim postajama. Drugi, manje česti haplotipovi detektirani su kako slijedi: HapA1 i A5, s 10%-tnom učestalošću prisutni su na po 2 postaje, HapA2 (10%) na 3 postaje i HapA6, A7 i A8 prisutni su s po jednom kopijom (3.33%) samo na po jednoj postaji. Ova preliminarna studija, iako vrlo ograničena, otkrila je veliku varijabilnost i nejednaku raspodjelu haplotipova duž istočnog dijela Jadrana. Distribucija haplotipova na različitim postajama mogla bi biti povezana s različitim fizikalno-kemijskim svojstvima okoliša, ali priroda takvih veza može se odrediti tek nekom većom i detaljnijom studijom.

Ključne riječi: mediteranska dagnja, COI, COXI, haplotip, distribucija, Jadran

CYTOCHROME OXYDASE I GENE VARIABILITY IN ADRIATIC MUSSEL *Mytilus galloprovincialis*

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Mytochondrial Cytochrome oxidase subunit I (COI or COXI) gene sequence is often used as a marker in species determination and population genetic studies. This preliminary study aimed to determine putative differences in COI haplotype distribution in blue mussel *Mytilus galloprovincialis* along the eastern Adriatic. Different distribution of COI haplotypes were expected at different locations. Six different locations were selected as sampling sites. Five mussels were sampled at each location. Total genomic DNA was isolated from gills tissue. Mytochondrial DNA fragments were PCR amplified and sequenced. The obtained sequences were 602 nucleotides long, excluding primers. Their comparison and analysis showed 8 different haplotypes, differing in their frequency and distribution. The most frequent haplotypes HapA3 (33.3% of all sequences) and HapA4 (26,7%) were present on all sampled locations. Other, less frequent haplotypes were present as follows: HapA1 and A5, each with 10% and present on 2 locations; HapA2 (10%) on 3 locations and HapA6, A7 and A8 present with one copy (3.3%) at one location only. This preliminary study, although very limited, has detected a high variability and unequal distribution of haplotypes along the eastern Adriatic. Distribution of haplotypes at different locations could be connected to different physico-chemical properties of the environment, but the nature of such connections can be determined only after a larger and more elaborate study.

Keywords: Blue mussel , COI, COXI, haplotype, distribution, Adriatic

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DIVERSITY AND TAXONOMY OF THE GENUS *Odontidium* KÜTZING (BACILLARIOPHYCEAE) IN MACEDONIA

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The genus *Odontidum* Kützing is considered as relatively small with around 10 species. Although described in the middle of 19th century, long time it was considered as part of Diatoma Bory sensu lato. More recently, the genus was re-established comprising species with heavily silicified internal transapical ribs. It was initially distinguished from Diatoma by the shape of the colonies, (short ribbons in *Odontidium*). *Odontidium* species generally, occur in cold oligotrophic waters. Detailed observations of this genus in Macedonia reveal existence of 12 taxa, belonging to two different complexes: *Odontidium hyemale* and *O. mesodon* sensu auct. The complex of *O. hyemale* comprises six taxa, which differ in the valve outline and the shape of the apices. Some of these taxa were recently (re-)described. The second complex, *O. mesodon* also has six members, but four of them have morphological features different than known taxa and should be described as new species.

Keywords: *Odontidium*, diatoms, new species, taxonomy, diversity

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RAZNOLIKOST VRSTA I RASPROSTRANJENOST TVRDIH KRPELJA (ACARI: IXODIDAE) NA

PODRUČJU ISTOČNOHRVATSKE RAVNICE

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Na području Istočnohrvatske ravnice istraživana je raznolikost faune tvrdih krpelja u različitim šumskim zajednicama od veljače do sredine prosinca 2016. godine. Tvrdi krpelji uzorkovani su krpeljnom zategom na 37 lokaliteta. Skupljeno je 1054 krpelja svrstanih u sedam vrsta. Najbrojnija vrsta je *Ixodes ricinus* sa 777 jedinki, iznoseći 73.7% svih skupljenih krpelja, slijede vrste *Haemaphysalis inermis* sa 17%, *Dermacentor reticulatus* s 6.7%, *D. marginatus* s 0.9%, *H. concinna* s 0.8%, *H. parva* s 0.7%, i *I. canisuga* s 0.1%. Vrsta *I. ricinus* zabilježena je na 34 lokaliteta, slijede vrste *D. reticulatus* na 16 lokaliteta i *H. inermis* na 8 lokaliteta, dok su vrste *H. concinna, H. parva, D. marginatus* i *I. canisuga* zabilježene na manjem broju lokaliteta. Najviše (72.44%) skupljenih krpelja je u odraslom stadiju, s prevladavanjem ženki u skupljenom uzorku. Odsječci gena za podjedinicu I citokrom oksidaze i 16S rDNA u genomu mitohondrija su sekvencirani iz 96 krpelja s ciljem molekularne potvrde i usporedbe vrsta krpelja. Utvrđen je prilično je visok stupanj sličnosti između faune tvrdih krpelja istraživanih šumskih zajednica (75% i 67%).

Ključne riječi: krpelji, Ixodidae, molekularni markeri, Hrvatska

SPECIES DIVERSITY AND DISTRIBUTION OF HARD TICKS (ACARI: IXODIDAE) ON THE AREA OF EASTERN CROATIAN PLAIN

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On the area of Eastern Croatian Plain hard tick fauna diversity was studied in different forest communities from the beginning of February to mid-December, 2016. Hard ticks were collected on 37 localities using the dragging-flagging method. A total of 1054 ticks were collected and classified in seven species. The most abundant species was *Ixodes ricinus* with 777 specimens, comprising 73.7% of the collected ticks, followed by *Haemaphysalis inermis* with 17%, *Dermacentor reticulatus* with 6.7%, *D. marginatus* with 0.9%, *H. concinna* with 0.8%, *H. parva* with 0.7%, and *I. canisuga* with 0.1%. *Ixodes ricinus* was recorded in 34 localities followed by *D. reticulatus* in 16 localities and *H. inermis* in 8 localities, while species *H. concinna, H. parva, D. marginatus* and *I. canisuga* were recorded on fewer localities. The highest ratio (72.44%) of collected ticks was in the adult stage, with the predominance of females in the collected sample. Gene fragments for cytochrome oxidase I and mitochondrial 16S rDNA were sequenced from 96 ticks for molecular confirmation and comparison of tick species. A rather high degree of similarity between tick faunas of studied forest communities was established (75% and 66.7%).

Keywords: Ticks, Ixodidae, molecular markers, Croatia

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PALEONTOLOŠKA ZBIRKA BESKRALJEŽNJAKA GASPERINI-GIROMETTA

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Paleontološka zbirka beskralježnjaka Gasperini-Girometta jedna je od najstarijih zbirki Prirodoslovnog muzeja Split. Broji 382 inventarna broja sa 2726 primjeraka. Tri inventarna broja: 1756., 1757. i 1758. sa sedam primjeraka subrecentnih neolitičkih mekušaca prikupio je tijekom istraživanja Grapčeve špilje na otoku Hvaru 1920. i 1921. godine, Umberto Girometta. Sve ostale primjerke prikupio je Riccardo Gasperini širom Dalmacije, od Velebita do Metkovića, od otoka Palagruže do Knina i Imotskoga. Pojedine primjerke prikupio je u Bosni i Hercegovini, Crnoj Gori i Srbiji (ukupno 43 primjerka). Najviše primjeraka fosila prikupljeno je na području Splita, Sinja i Lišana Ostrovičkih (ukupno 1976 primjeraka). Najviše inventarnih brojeva odnosi se na fosilni materijal za koji nije poznato mjesto nalaza, odnosno lokalitet (94 inv.br. sa 148 primjeraka), a veliki broj inventarnih brojeva odnosi se na područje planine Promine, ali s manje primjeraka (68 inv.br. sa 108 primjeraka). Navedena determinacija primjeraka izvršena je u vrijeme stvaranja zbirke te je planirano obaviti taksonomsku reviziju građe, uključujući provjeru validnosti prikupljenih rodova i vrsta kao i nomenklaturnu klasifikaciju.

Ključne riječi: Paleontološka zbirka beskralježnjaka Gasperini-Girometta, Prirodoslovni muzej Split, Hrvatska

PALEONTOLOGICAL COLLECTION OF INVERTEBRATES GASPERINI-GIROMETTA

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Paleontological collection of invertebrates Gasperini-Girometta is one of the oldest collections of the Natural History Museum in Split. It contains 382 inventory numbers with 2726 specimens. Three inventory numbers – 1756., 1757. 1758. – with seven specimens of subrecent neolithic molluscans, were collected by Umberto Girometta, during researches of the Grapčeva cave on the Hvar island in 1920 and 1921. The rest of the specimens were collected by Riccardo Gasperini throughout Dalmatia, from Velebit to Metković, from Palagruža island to Knin and Imotski. Some specimens were collected in Bosnia and Herzegovina, Montenegro and Serbia (total of 43 specimens). The most fossil specimens were collected in the areas of Split, Sinj and Lišane Ostrovičke (total of 1976 specimens). The most inventory numbers refer to fossil material with unknown location of findings or locality (94 inventory numbers with 148 specimens), and a great number of inventory numbers refer to the Promina mountain area, but with less specimens (68 inv. numbers with 108 specimens). The determination of specimens mentioned above was made during the period of creation of the collection, and the taxonomic revision of the material, including checking of the validity of genera and species, as well as the classification of the nomenclature, are planned to be done.

Keywords: Paleontological collection of invertebrates Gasperini-Girometta, Natural History Museum Split, Croatia

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PRELIMINARNI REZULTATI DNA BARKODIRANJA TVRDOKRILACA RODA *Elmis* (INSECTA: COLEOPTERA: ELMIDAE) HRVATSKE

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Slapoljubi (Insecta: Coleoptera: Elmidae) je porodica vodenih tvrdokrilaca čiji su predstavnici česti stanovnici tekućica. Oni se danas sve više koriste kao pokazatelji kvalitete staništa. Za razliku od određivanja do razine roda, određivanje do razine vrste na osnovu morfoloških značajki je u mnogim slučajevima zahtjevno pa je neophodna upotreba integrativne taksonomije. DNA barkodiranje je uobičajena metoda određivanja vrsta, osobito u slučajevima blisko srodnih svojti. U Hrvatskoj su do sada zabilježene 23 vrste slapoljuba unutar osam rodova. U ovom izlaganju predstavljamo preliminarne rezultate DNA barkodiranja vrsta roda *Elmis* Hrvatske. Naši rezultati predstavljaju osnovu za buduća istraživanja procesa specijacije vodenih tvrdokrilaca i doprinose poznavanju biološke raznolikosti slatkovodnih staništa Hrvatske. Nadalje, rezultati ovog istraživanja ukazuju na postojanje rijetkih, endemskih i/ili ugroženih vrsta vodenih tvrdokrilaca što će doprinijeti budućim istraživanjima s aspekta konzervacije slatkovodnih vrsta i staništa.

Ključne riječi: bioindikatorske vrste, integrativna taksonomija, slatkovodna staništa, slapoljubi

PRELIMINARY DATA ON DNA BARCODING OF GENUS *Elmis* (INSECTA: COLEOPTERA: ELMIDAE) IN CROATIA

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Riffle beetles (Insecta: Coleoptera: Elmidae) are usually the dominant beetle family in fast running waters, and are frequently used as bioindicators for assessing water quality and monitoring of their habitats. Many species of elmids are difficult to identify morphologically so integrative taxonomy is required. DNA barcoding is nowadays commonly used in species identifications especially in cases of closely related species. In Croatia 23 species of Elmidae are recorded representing eight genera so far. Here we present preliminary results of DNA barcoding of genus *Elmis* in Croatia. Our results represent the base for future investigations on the processes of the speciation of water beetles and contribute to the knowledge of freshwater biodiversity in Croatia. Moreover, our results indicate the existence of potentially rare, endemic and/or endangered species of water beetles, being essential in establishing projects on conservation of aquatic species and aquatic biotopes.

Keywords: bioindicators, integrative taxonomy, freshwater habitats, riffle beetles

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DIVERSITY AND TAXONOMY OF *Neidium* PFITZER (BACILLARIOPHYTA) ON MT. SHAR PLANINA, REPUBLIC OF MACEDONIA

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Neidium Pfitzer is considered a large genus with more than 300 species distributed in freshwater habitats worldwide. *Neidium* is typically represented in natural environments with a small number of species in low abundance. Recently, a more detailed study of *Neidium* in Macedonia has been initiated. Observations of more than 2500 samples from Macedonia reveal the existence of ca. 40 different taxa. Most of the taxa were observed from cold, oligotrophic, circumneutral water habitats. Such habitats are quite common on Shar Planina, the largest mountain in Macedonia. The observations of *Neidium* on Shar Planina reveal the existence of more than 25 different taxa. Some of them such as, *N. bergii* (Cleve-Euler) Krammer, *N. bobmarshallensis* Bahls, *N. decoratum* Brun, *N. distinctepunctatum* Hustedt, N. *fogedii* Bahls, and *N. kozlowii* var. *parva* Mereschkowsky are considered extremely rare and were observed in a single locality. At least 10 taxa possess unique morphological features that distinguish them from the existing species. Their morphological features are examined with LM and SEM and compared with other known taxa.

Keywords: diatoms, *Neidium*, taxonomy, Shar Planina

GENETIKA, STANIČNA I MOLEKULARNA BIOLOGIJA
GENETICS, CELLULAR AND MOLECULAR BIOLOGY

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CITOTOKSIČNI UČINAK ASKORBINSKE KISELINE NA MATIČNE STANICE OSTEOSARKOMA

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Osteosarkom (OS) je tumor kostiju visoke malignosti kod djece i mladih s lošom stopom preživljavanja. Uzrok leži u postojanju male populacije stanica otpornih na kemoterapiju, tzv. matične stanice osteosarkoma (OS-SC). Iako askorbinska kiselina (AA) ima dugu i kontroverznu povijest kao antikancerogen in vitro i in vivo, mehanizam djelovanja je ostao nedostižan, posebno u kontekstu matičnih stanica raka. Stoga je cilj studije bio analizirati AA kao potencijalno terapijsko sredstvo za selektivno ciljanje OS-CS. Tumorsko tkivo se enzimski razgradilo kako bi se izolirale sve tumorske stanice, a potom testom za sarkosfere izolirale samo OS-CS. Stanične linije osteosarkoma U2OS, hMSC i HEK 293 korištene su kao kontrolni stanični tipovi. Test MTT je korišten za procjenu citotoksičnog učinka AA (2,5 - 55 ug/ml) tijekom 72 sata, kao i za određivanje odnosa između broja stanica i koncentracije AA. Dok AA nije imao nikakav učinak na hMSC, U2OS i HEK 293, AA je učinkovito smanjio vijabilnost OS-CS ovisno o dozi. IC50 vrijednosti AA rastu s

brojem nasađenih OS-CS. Učinak AA na sposobnost formiranja sarkosfera analiziran je tijekom 28 dana, a pokazuje uspješno smanjenje stvaranja sarkosfera nakon 6. dana. Tip stanične smrti je određen bojanjem Annexin V/PI koje pokazuje da je apoptoza glavni tip stanične smrti sa 70% Annexin V-pozitivnih stanica. Na temelju dobivenih rezultata može se zaključiti da AA ima citotoksični utjecaj na OS-CS.

Ključne riječi: askorbinska kiselina, matične stanice osteosarkoma, citotoksičnost, apoptoza

CYTOTOXIC EFFECT OF ASCORBIC ACID ON OSTEOSARCOMA STEM CELLS

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Osteosarcoma (OS) is a highly malignant bone tumor of children and young adults with poor survival rate. The cause lies in small population of chemotherapy-resistant cells, i.e. osteosarcoma stem cells (OS-SCs). Although ascorbic acid (AA) has long and controversial history of anti-cancer properties in vitro and in vivo, the mechanism of action has remained elusive especially in context of cancer stem cells. Thus, the aim of the study was to analyze AA as potential therapeutic for selective targeting of OS-CSs. Tumor tissue was enzymatically digested to isolate tumor cells, followed by sarcosphere assay to isolate OS-CSs. Osteosarcoma U2OS cell line, hMSCs and HEK 293 were used as control cell types. MTT assay was used to evaluate cytotoxic effect of AA (2.5 - 55 µg/ml) during 72h as well as relationship between cell number and AA concentration. While AA did not have any effect on hMSCs, U2OS and HEK 293, AA efficiently induced dose-dependent viability reduction of OS-CSs. IC50 values of AA increased with the number of seeded OS-CSs. Effect of AA on sarcosphere-forming ability was analyzed during 28 days showing successful reduction of sarcosphere formation after 6th day. AA-induced cell death type was determined by Annexin V/PI staining showing that apoptosis was prevalent death mode with 70% of Annexin V-positive cells. Based on the obtained results, it can be concluded that AA has cytotoxic impact on OS-CSc.

Keywords: ascorbic acid, osteosarcoma stem cells, cytotoxicity, apoptosis

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POPULATION GENETIC STRUCTURE OF EUROPEAN ROE DEER IN THE MODEL HUNTING GROUND OF CENTRAL SLOVENIA

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Roe deer (*Capreolus capreolus*) is the most widespread wild ungulate in Europe and has been actively managed by hunting throughout its range for decades. Effective management of this species should be based on the knowledge of population dynamics and structure; however, reliable information on population parameters are rather lacking. We tried to determine

genetic makeup of model population with a high intensity of sampling, i.e. on >70% of all registered yearly mortality of roe deer in a typical, 2,400 ha large hunting ground of central Slovenia. We used microsatellite markers to analyse the genetic variation and structure of populations. Genetic assignment of samples was performed using methods implemented in two software programmes, i.e. STRUCTURE and GENELAND. We successfully genotyped 69 individual samples (out of 94 registered mortality cases in 2017). The number of alleles/locus ranged from 7 to 20 with a mean of 11.9. A similar pattern was also observed for heterozygosity, ranging between 0.91 and 0.30, and expected heterozygosity, ranging between 0.74 and 0.15 on each locus. The obtained results suggest separation of population into two clusters. However, individuals of both clusters were continuously presented throughout the hunting ground, and both clusters mixed on several locations. This indicates that there is no isolation within the study area, and that dispersion of closely related genes is much larger as are home ranges of this territorial species.

Keywords: Roe deer, genetic variation, structure of populations

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NISKO ZASTUPLJENE SATELITNE DNA KESTENJASTOG BRAŠNARA TRIBOLIUM CASTANEUM OTKRIVENE SEKVENCIRANJEM NOVE GENERACIJE

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Satelitne DNA su uzastopno ponavljajuće sekvence raširene u (peri)centromernim kromosomskim regijama, a karakterizira ih još uvijek nedovoljno rasvijetljena funkcija. Kukac kestenjasti brašnar *Tribolium castaneum* je izvrstan modelni organizam koji se desetljećima koristi u molekularnim istraživanjima. Genom vrste T. castaneum sekvenciran je prije deset godina pristupom „shotgun“, no u asembliranom dijelu genoma nedostaje 20% ukupne DNA primarno sastavljene od satelitnih sekvenca. U svrhu proučavanja centromernih DNA, sekvencirali smo genom vrste *T. castaneum* tehnologijom nove generacije sekvenciranja (NGS). U analizi satelitoma, odnosno sveukupnog sadržaj satelita u genomu, koristili smo bioinformatički alat TAREAN koji omogućava detekciju potencijalnih satelitnih sekvenca na temelju kratkih, neposloženih NGS sljedova. Bioinformatičkom analizom utvrdili smo tri moguća satelita, koji nisu bili detektirani u prethodnim istraživanjima. Premda ova tri satelita čine mali udio u genomu (<0.1%), uspješno smo dokazali njihovu uzastopno ponovljenu organizaciju metodom Southern blot. Metodom fluorescencijske hibridizacije in situ također smo odredili njihovu kromosomsku lokalizaciju. Kombinirajući bioinformatičke alate i eksperimentalne metode, ovim smo istraživanjem potvrdili primjenjivost kratkih, neposloženih NGS sljedova u otkrivanju nisko zastupljenih satelita, koji pak mogu biti značajni za razumijevanje strukture i funkcije genoma.

Ključne riječi: satelitna DNA, *Tribolium castaneum*, satelitom, sekvenciranje nove generacije

LOW-COPY SATELLITE DNAs OF THE FLUOR BEETLE TRIBOLIUM CASTANEUM REVEALED BY NEXT-GENERATION SEQUENCING

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Satellite DNAs (satDNAs) are tandemly repeated sequences prevalent in (peri)centromeric chromosomal regions that represent one of the most enigmatic parts of eukaryotic genomes. The red flour beetle *Tribolium castaneum* is an excellent model organism used in molecular studies for decades. Ten years ago its genome was sequenced by a shotgun approach; however, 20% of the genome is omitted from the current assembly due to the repetitive set-up based primarily on satDNAs. In course of the study of centromeric DNA sequences in *T. castaneum*, we re-sequenced its genome by Illumina Next-Generation Sequencing (NGS). The satellitome, representing the complete set of satDNAs in the genome, was analyzed by a computational pipeline TAREAN, which enables detection of putative satellite sequences from unassembled short reads. We identified three novel *T. castaneum* satDNAs that have not been detected in the previous studies. In spite of low contribution to the genome (<0.1%), we successfully proved tandem organisation of these repeats by Southern blot method. Fluorescence in situ hybridization also revealed their localization on different chromosomal subsets. By combining bioinformatics tools and experimental procedures we affirmed the applicability of NGS unassembled reads in detection and characterization of low-copy satDNAs, which might be relevant to understanding genome structure and function.

Keywords: satellite DNA, *Tribolium castaneum*, satellitome, Next-Generation Sequencing

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POVEZANOST SUSTAVA UROKINAZE I SIGNALNOG PUTA TGFβ U STANIČNOJ LINIJI GLIOBLASTOMA

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Put transformirajućeg faktora rasta β (TGFβ) jedan je od glavnih signalnih putova procesa diferencijacije i reprogramiranja stanica. Primarna je uloga TGFβ zaustavljanje proliferacije i tumorska supresija, međutim, TGFβ može regulirati migraciju, angiogenezu i invazivnost stanica kroz procese epitelno-mezenhimske tranzicije. Tim procesom reprogramiranja stanice epitelnog poprimaju karakteristike mezenhimskog fenotipa, uključujući proizvodnju komponenata ekstracelularnog matriksa, kao i lučenje ekstracelularnih proteaza. Jedan od ekstracelularnih proteaznih sustava je sustav urokinazne plazminogenske aktivacije. Urokinaza cijepa plazminogen u plazmin, proteazu koja degradira i remodelira izvanstanični matriks. Regulacija plazminogenske aktivacije odvija se na razini transkripcije gena urokinaze, njegovih inhibitora PAI1 i PAI2 te sinteze i degradacije njihovih proteina. Cilj ovog istraživanja je

analizirati utjecaj TGFβ na urokinazni sustav plazminogenske aktivacije kod stanične linije glioblastoma. Povećana je ekspresija faktora rasta unosom plazmida sa sekvencijom TGFβ. Uzgojene stanice pokazale su povećanu aktivnost urokinaze. Analizirane su promjene u ekspresiji seta gena koji reguliraju urokinaznu aktivnost, gena uključenih u put TGFβ kao i krovnih gena epitelno-mezenhimske tranzicije, promjene u ekspresiji urokinaze i njenog inhibitora na razini proteina te stanična migracija i proliferacija. Zaključeno je da TGFβ modulira sustav plazminogenske aktivacije.

Ključne riječi: TGFβ, urokinazni plazminogenski aktivator, epitelno-mezenhimska tranzicija, glioblastom

CROSSTALK BETWEEN UROKINASE SYSTEM AND TGFβ PATHWAY IN HUMAN GLIOBLASTOMA CELL LINE

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins involved in regulation of many cellular pathways. Poly(ADP-ribose) (PAR) consists of chains of repeating ADP-ribose nucleotide units and is synthesized by the family of enzymes called poly(ADP-ribose) polymerases (PARPs). This modification can be removed by the hydrolytic action of poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3). Hydrolytic activity of macrodomain proteins (MacroD1, MacroD2 and TARG1) is responsible for the removal of terminal ADP-ribose unit and for complete reversion of protein ADP-ribosylation. Poly(ADP-ribosyl)ation is widely utilized in eukaryotes and PARPs are present in representatives from all six major eukaryotic supergroups, with only a small number of eukaryotic species that do not possess PARP genes. The last common ancestor of all eukaryotes possessed at least five types of PARP proteins that include both mono and poly(ADP-ribosyl) transferases. Distribution of PARGs strictly follows the distribution of PARP proteins in eukaryotic species. At least one of the macrodomain proteins that hydrolyse terminal ADP-ribose is also always present. Therefore, we can presume that the last common ancestor of all eukaryotes possessed a fully functional and reversible PAR metabolism and that PAR signalling provided the conditions essential for survival of the ancestral eukaryote in its ancient environment.

Keywords: TGFβ, urokinase plasminogen activator, epithelial-mesenchymal transition, glioblastoma

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METILACIJA PROMOTORA GENA MGAT3 I BACH2 KORELIRA S GLIKOMOM IMUNOGLOBULINA G U UPALNIM BOLESTIMA CRIJEVA

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Upalne bolesti crijeva (IBD) predstavljaju skupinu autoimunih bolesti koje se pojavljuju zbog poremećenog imunosnog odgovora na crijevnu mikrofloru u osoba s genetičkom predispozicijom. Cijelo-genomskim i -epigenomskim studijama povezanosti identificirane su značajne promjene na razini genoma i epigenoma u IBD-u. Genetičke varijante unutar gena BACH2 i MGAT3 povezane su s IBD-om i glikozilacijom imunoglobulina G (IgG). U ovom istraživanju analizirana je CpG-metilacija u promotorima ovih gena iz periferne krvi IBD-pacijenata i zdravih ljudi dviju neovisnih kohorti, upotrebom bisulfitnog pirosekvenciranja, te su pronađene značajne razlike. Isti uzorak promjena metilacije gena MGAT3 i BACH2 utvrđen je i u CD19+ B-limfocitima iz manje skupine pacijenata jedne velike kohorte i zdravih ljudi. Korelirani su podatci metilacije gena MGAT3 i BACH2 i glikozilacije IgG-a analizirani u krvi istih osoba. Metilacija gena MGAT3 značajno je korelirala s galaktozilacijom, sijalinizacijom i N-acetilglukozaminom na IgG-u, što ukazuje na moguću promjenu aktivnosti enzima kodiranog ovim genom u IBD-u. Rezultati ovog istraživanja ukazuju na epigenetičku deregulaciju gena MGAT3 kao potencijalni mehanizam uključen u promjene glikozilacije IgG-a u krvi IBD-pacijenata, kojim se povećava proupalni karaker ovog antitijela, karakterističan za upalne bolesti crijeva.

Ključne riječi: metilacija DNA, BACH2, MGAT3, imunoglobulin G, IBD

MGAT3 AND BACH2 PROMOTER METHYLATION CORRELATES WITH THE IMMUNOGLOBULIN G GLYCOME IN INFLAMMATORY BOWEL DISEASES

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Inflammatory bowel diseases (IBD) represent a group of autoimmune diseases that occur due to aberrant immune response to intestinal microbiome in genetically susceptible individuals. Recent genome and epigenome-wide association studies have identified significant genetic and epigenetic changes in IBD. Genetic variants in the BACH2 and MGAT3 loci have been associated with both IBD and immunoglobulin G (IgG) glycosylation. We analysed CpG methylation in promoter regions of these genes from peripheral blood of IBD patients and healthy controls (HC) from two independent cohorts, using bisulfite pyrosequencing. We found significant differences in the methylation levels in BACH2 and MGAT3 between IBD patients and HC. The same pattern of methylation changes was also identified in CD19+ B cells from a subset of the IBD patients and HC. We also performed a correlation analysis between methylation and individual IgG glycans, measured in the same individuals. MGAT3 methylation correlated significantly with galactosylation, sialylation and bisecting GlcNAc on IgG, suggesting that activity of the enzyme encoded by this gene might be altered in IBD. Our results suggest that

epigenetic deregulation of the MGAT3 gene might lead to an increase in proinflammatory properties of IgG in IBD through changes in IgG glycosylation

Keywords: DNA methylation, BACH2, MGAT3, Immunoglobulin G, IBD

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STRUKTURNE PROMJENE PROTEINA CAS3 U *Escherichia coli* OVISNE O TEMPERATURI

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Sustav CRISPR-Cas je važan mehanizam adaptivne imunosti u bakterija i arheja za obranu od virusa i plazmida. Sastoji se od ponavljajućih sekvenci DNA odvojenih razmaknicama stranog porijekla (lokus CRISPR), te gena cas koji imaju ulogu u različitim koracima obrane. U *E. coli*, protein Cas3 sudjeluje u cijepanju strane DNA kao zadnjem koraku obrane. Nedavna istraživanja su pokazala da bi protein Cas3 mogao imati ključnu ulogu u regulaciji CRISPR-Cas imunosti zbog njegovog neobičnog ponašanja – gubitka aktivnosti na 37°C inkubiranja, osim ako je protein prisutan u suvišku. U ovom radu željeli smo istražiti je li gubitak aktivnosti proteina Cas3 uzrokovan promjenom u njegovoj strukturi koja ovisi o temperaturi. Strukturne promjene u pročišćenom proteinu pratili smo mjerenjem promjene eliptičnosti metodom cirkularni dikroizam i mjerenjem intrinzične fluorescencije triptofana metodom fluorescentne spektrometrije. Obje metode su dale isti rezultat, na 35°C dolazi do blage konformacijske promjene u helikoidalnoj regiji proteina što je u skladu s promijenjenom aktivnošću proteina in vivo. Ovo je vjerojatno prvi eksperimentalni nalaz da je aktivnost proteina Cas3 iz *E. coli* regulirana temperaturom putem promjene konformacije proteina. Slična promjena strukture uočena je i u arhealnom proteinu Cas3 što sugerira da je ova osobina sačuvana i u drugim vrstama. Rezultati istraživanja doprinijet će razumijevanju regulacije aktivnosti proteina Cas3, a time i samog sustava CRISPR-Cas.

Ključne riječi: CRISPR-Cas, Cas3, cirkularni dikroizam, intrinzična fluorescencija triptofana, *E. coli*

TEMPERATURE-DEPENDENT STRUCTURAL CHANGES IN CAS3 PROTEIN IN *Escherichia coli*

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The CRSISPR-Cas system is a significant mechanism of bacteria and archea that provide adaptive immunity against viruses and plasmids. It consists of DNA repeats separated by spacers of foreign origin (CRISPR locus), and cas genes responsible for various stages of defense. In *E. coli*, Cas3 protein is involved in a degradation of invader DNA as a last stage of defense. Recent studies

showed that Cas3 could be the limiting factor for regulation of the CRISPR-Cas immunity due to its unusual property – loss of activity at 37°C, unless the protein is present in abundance. In this work we wanted to investigate if the loss of Cas3 activity is caused by structural change of protein which is temperature-dependent. We monitored structural changes in the purified protein by measuring a change of ellipticity using circular dichroism and by measuring intrinsic tryptophan fluorescence using fluorescence spectrometry. Both methods gave the same result, a subtle conformational change in helical region at 35°C which is in agreement with the protein activity change in vivo. This is probably the first experimental evidence that Cas3 activity from *E. coli* is temperature-dependent due to the change in protein conformation. Also, similar structural change was observed in archaeal Cas3 suggesting that this trait is preserved in other species as well. The results of this research will contribute to better understanding of regulation of Cas3 activity as well as to the progress of the CRISPR-Cas field.

Keywords: CRISPR-Cas, Cas3, circular dichroism, intrinsic tryptophan fluorescence, *E. coli*

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POVEZANOST CRISPR ADAPTACIJE, REKOMBINACIJE I NUKLEAZA U BAKTERIJI *Escherichia coli*

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Sustav CRISPR-Cas je jedan od obrambenih mehanizama prokariota protiv stranih genetičkih elemenata. Obrana se zasniva na ugradnji malih dijelova (razmaknica) strane molekule DNA u lokus CRISPR. Proces ugradnje prve razmaknice se naziva naivna adaptacija, a za njega je neophodan proteinski kompleks Cas1-Cas2. Smatra se da u bakteriji *E. coli* enzim RecBCD pomaže u naivnoj adaptaciji stvarajući fragmente jednolančane molekule DNA (jlDNA) koji se zatim spajaju u dvolančane fragmente i dalje obrađuju kompleksom Cas1-Cas2 te se kao razmaknica ugrađuju u lokus CRISPR. Cilj ovog rada bio je detaljnije istražiti ulogu enzima RecBCD i drugih egzonukleaza bakterije *E. coli* u pripremi supstrata DNA za ugradnju u lokus CRISPR. Rezultati naše genetičke analize pokazuju da nukleazna aktivnost enzima RecBCD nije potrebna za adaptaciju te da ju na DNA vezani protein RecA sprječava, vjerojatno jer onemogućuje obradu krajeva molekule DNA i potiče homolognu rekombinaciju. Ipak, za adaptaciju je neophodna helikazna aktivnost enzima RecBC(D) i aktivnost egzonukleaza bakterije *E. coli* koje cijepaju jlDNA u smjeru 3'-5' jer na taj način povremeno stvaraju supstrate prikladne za vezanje kompleksa Cas1-Cas2. Naši in vitro rezultati pokazuju da Cas1-Cas2 stvara stabilan kompleks sa supstratima DNA koji imaju 5' stršeće krajeve i mogu ih cijepati. Sumarno, naši rezultati sugeriraju da su za adaptaciju važni 5' stršeći krajevi kao supstrati za proteinski kompleks Cas1-Cas2.

Ključne riječi: CRISPR-Cas, RecBCD, Cas1-2, *E. coli*, nucleases

INTERPLAY OF CRISPR ADAPTATION, RECOMBINATION AND HOST NUCLEASES IN *Escherichia coli*

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CRISPR-Cas system is a prokaryotic adaptive immune system against foreign genetic elements. Immunity is acquired through insertion of small fragments (spacers) of invader DNA into a CRISPR array. This process is called adaptation, and can be mediated solely by the protein complex Cas1-Cas2 ("naïve adaptation"). In *E. coli*, RecBCD is thought to aid naïve adaptation by generating single-stranded DNA intermediates that are reannealed and further processed by Cas1-Cas2, and then integrated into the CRISPR array. In this work, we wanted to better understand the role of RecBCD and other host exonucleases in the process of prespacer preparation. Our genetic analysis shows that nuclease activity of RecBCD enzyme is not required for spacer preparation and that RecA inhibits adaptation probably because it prevents DNA processing and stimulates homologous recombination. However, helicase activity of RecBC(D) is required and is helped by 3'-5' host ssDNA exonucleases to occasionally generate appropriate DNA substrates for Cas1-Cas2 binding. Our in vitro analysis implies that Cas1-Cas2 forms a stable complex on DNA substrates with 5' overhangs and catalyses their cutting. Overall, our data suggest that 5' overhangs are important as substrates for adaptation and that these may be bound and processed by Cas1-Cas2.

Keywords: CRISPR-Cas, RecBCD, Cas1-2, *E. coli*, nucleases

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PRVI PODACI O RAZNOLIKOSTI MITOHONDRIJSKE DNK VELIKOG VEČERNJAKA (*Nyctalus lasiopterus*) UZORKOVANOG U HRVATSKOJ

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Veliki večernjak (*Nyctalus lasiopterus*) je najveća i jedna od najrjeđih Europskih vrsta šišmiša. Puno je nepoznanica o biologiji ove vrste, dok je iz područja populacijske genetike do sada objavljeno jedino istraživanje kolonija iz Španjolske. Prisutnost velikog večernjaka u Hrvatskoj potvrđena je u literaturi tek nekoliko puta, prvi puta krajem 19. stoljeća, a u novije vrijeme je vrsta zabilježena na otocima Mljet, Kornat i Veliki Brijun. Članovi Udruge za zaštitu šišmiša Tragus su 2016. godine na otoku Veliki Brijun u mreže uhvatili 23 jedinke velikog večernjaka te prikupili uzorke letnica. Nakon izolacije DNK, analizirano je pet regija mitohondrijske DNK s različitim evolucijskim stopama - ND1, Cyt b, COI, HVI i HVII, sveukupne duljine 2943 parova baza. Kao što je i očekivano, najviša razina haplotipske (Hd) i nukleoitidne raznolikosti (h) utvrđena je za HVI i HVII regiju (Hd=0.06; h=0.008 za obje), dok su COI (Hd=0,298, h=0,001) i ND1 (Hd=0.522, h=0.001) imali najnižu razinu raznolikosti. Usporedbom HVI I HVII sljedova iz našeg istraživanja i onih pohranjenih u GenBank bazi nismo našli podudaranja, dok se sljedovi Ctyb iz Hrvatske poklapaju sa sljedovima iz Španjolske, a sljedovi ND1 poklapaju sa sljedovima iz Grčke i Mađarske. Tumačenje ovih usporedba je ograničeno zbog niskog broj sljedova iz

drugih populacija dostupnih u GenBank bazi, dok su naši sljedovi COI gena prvi opis za ovu vrstu u literaturi.

Ključne riječi: ND1, Cyt b, COI, HVI, HVII

FIRST DATA ON MITOCHONDRIAL DNA DIVERSITY OF GIANT NOCTULE (*Nyctalus lasiopterus*) SAMPLED IN CROATIA

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The giant noctule (*Nyctalus lasiopterus*) is the largest and one of the rarest bat species in Europe. Many aspects of biology of this species are still unknown and population genetics has been researched only on several colonies from Spain. The presence of *N. lasiopterus* in Croatia was confirmed in the literature only several times, for the first time in late 19th century, and recently the species was found on islands Mljet, Kornat and Veliki Brijun. In 2016 members of Association for Bat Conservation Tragus captured and took patagium samples from 23 individuals of N. lasiopterus. After DNA isolation, 5 mitochondrial regions with different evolutionary rates were analysed - ND1, Cyt b, COI, HVI and HVII, in a total length of 2943 base pairs. As expected, the highest levels of haplotype (Hd) and nucleotide (h) diversity were found for HVI and HVII (Hd=0.06; h=0.008 for both), while COI (Hd=0,298, h=0,001) and ND1 (Hd=0.522, h=0.001) were the least variable among the analysed regions. No matches were found among HVI and HVII sequences from this study and sequences deposited in the GeneBank. Ctyb sequences from this study matched sequences from Spain, while ND1 matched sequences from Greece and Hungary. Interpretation of this comparison is limited due to the low number of sequences from other populations available in the GenBank, while our description of COI gene sequences are the first ones for this species in the literature.

Keywords: ND1, Cyt b, COI, HVI, HVII

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MORPHOGENESIS OF DIGESTIVE GLANDS DURING EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT OF CRUSTACEAN PORCELLIO SCABER - INTEGRATION OF ORGAN, TISSUE AND CELL LEVELS

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Differentiation of epithelia is an integrative part of organogenesis during embryonic development. Elucidation of the cell differentiation and tissue formation principles is one of the key issues in developmental biology. Different model systems need to be investigated to advance knowledge on epithelial morphogenesis and on different aspects of epithelial functional specialisations during development. We are currently focusing on the differentiation of epidermal and gut epithelium in an invertebrate model system that enables us to obtain ''in

situ'' insight into the ultrastructural aspects of cell differentiation and tissue formation. Here we report on digestive glands morphogenesis during embryonic and postembryonic development. Digestive glands are endodermal in origin and in adult animals consist of four blind ending tubules that are composed of one-layered epithelium of two cell types (B and S cells). In the mid-stage embryo a pair of digestive glands primordia is visible and egg yolk is accumulated in their lumen. In late embryo the outgrowths of the second pair of gland tubules are observed, foregut and hindgut are fused and digestive glands are connected to the stomach. In the postembryonic stage named marsupial manca, all four gland tubules are formed and yolk content in the lumen is reduced. We expect that major ultrastructural changes in digestive glands' epithelium during development coincide with embryo hatching and with release of the manca from the brood pouch.

Keywords: development, epithelium, electron microscopy, ultrastructure

KOMPARATIVNA FIZIOLOGIJA, IMUNOBIOLOGIJA I BIOLOGIJA ČOVJEKA
COMPARATIVE PHYSIOLOGY, IMMUNOBIOLOGY AND HUMAN BIOLOGY

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OKSIDATIVNI STRES I ANTIOKSIDATIVNA OBRANA U TKIVIMA DAGNJE (*Mytilus galloprovincialis)* TIJEKOM EKSTREMNE IZLOŽENOSTI VAN MORA

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Fiziologija i metabolizam organizama u zoni oseke adaptirani su ekstremnim promjenama kisika (u suvišku tijekom plime i smanjenjem tijekom oseke). Smjenu plime i oseke prate promjene mitohondrijske aktivnosti i izmjene aerobnog metabolizma i fermentacije. U školjke *Mytilus galloprovincialis*, za oseke razgrađuju se masne i amino kiseline. Akumuliraju se krajnji proizvodi mitohondrijske β-oksidacije- i metaboliti konjugiranog karnitina. Kako je mitohondrijska aktivnost povezana je s oksidacijskim stresom (OXS), željeli smo istražili kako se tijekom oseke mijenja OXS, lipidau peroksidacija (LPO), karbonilacija proteina (PC) i antioksidativna obranu (AOD: SOD, GST, Katalaza) u hepatopancereasu, gonadama, mišićima i škrgama dagnji. Parametri su izmjereni nakon 24, 48, 72 i 96 sati boravka izvan mora. LPO i PC povećali su se nakon 24 sata u hepatopankreasu (najosjetljivije tkivo), dok su se u škrgama i gonadama oštećenja molekula javlja tek od 72 do 96 sata. Tijekom eksperimenta vitalnost / morbiditet dagnji praćen je elektrokardiogramom (EKG). Razine AOD-a promijenile su se različito u svakom organu ovisno o vremenu a tijekom produljenog boravka izvan mora svako tkivo se drugačije adaptira na razinu oštećenja lipida i proteina. Rezultati također imaju praktičnu važnost u znanostima o hrani i biotehnologiji namirnica ili u ekotoksikološkim istraživanjima kada se promjene nastale osekom trebaju razmotriti prilikom odabira biomarkera i uzorkovanja organizama.

Ključne riječi: oksidacijski stress, dagnja, fiziologija, plima, oseka

OXIDATIVE STRESS AND ANTIOXIDATIVE DEFENCE IN TISSUES OF MUSSELS (*Mytilus galloprovincialis*) DURING PROLONGED AERIAL EXPOSURE

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Physiology and metabolism of inhabitants of the intertidal zone are adapted to extreme changes of oxygen bursts during submergence and restriction during sea emergence. Aerobic metabolism and fermentation alternate and follow intertidal cycles with changes in mitochondrial activity. In *Mytilus galloprovincialis* at low tide fatty acids and branched amino acids are degraded. Mitochondrial β-oxidation-end-products and carnitine-conjugates accumulate. Mitochondrial activity is connected to oxidative stress (OXS). We studied how extreme 96 h of aerial exposure influence OXS, lipid peroxidation (LPO), protein carbonylation (PC) and antioxidative defence (AOD: SOD, GST, Catalase) in hepatopancereas, gonads, muscles and gills of *M. galloprovincialis* at 24, 48, 72 and 96 hours out of sea. LPO and PC increased after 24h in hepatopancreas (most sensitive tissue), while in gills and gonads damage occurred at 72-96h. During experiment vividness/morbidity of mussels was monitored by electrocardiogram (ECG). Levels of AOD were changed differently in each organ in time dependent manner. Each tissue has preferred AOD system during prolonged dwelling out of sea and individual capability to acclimate the enzymatic AOD to the degree of LPO and PC at prolonged period of emergence. Results also have practical importance in food sciences and alimentary biotechnology or in ecotoxicological studies when choosing biomarker and sampling the organisms should be considered in between tides.

Keywords: oxidative stress, mussels, intertidal physiology

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PROCJENA UTJECAJA RESPIRATORNE I POSTURALNE MOBILNOSTI DIJAFRAGME U PREVENCIJI PNEUMONIJE

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Totalna laringektomija (TL) dovodi do razdvajanja gornjeg i donjeg dišnog sustava, što rezultira odsustvom zagrijavanja, vlaženja i filtriranja zraka s nepovoljnim učinkom na niže dišne putove i kapacitet pluća, te dovodi do razvoja pneumonije (PM). Cilj istraživanja bio je procjena utjecaja respiratorne i posturalne mobilnosti dijafragme u prevenciji PM u poslijeoperativnom razdoblju kod bolesnika kojima je učinjena TL. U radu je korištena baza podataka pacijenata operiranih (TL) u KBC-u Zagreb, Zavod za tumore glave i vrata. U uzorku od 17 bolesnika praćena je: respiratorna i posturalna mobilnost dijafragme; učinak respiratornih vježbi (RE) na perifernu zasićenost kisikom (POS); frekvenciju disanja (BF); krvni tlak (BP) i srčani puls (CP) prije i poslije RE; upalni parametri (WBC, CRP, tjelesna temperatura); promjene u plućima (RTG, auskultacija). Podaci su obrađeni ANOVA testom. Postoji statistički značajna razlika u smanjenju broja leukocita (WBC) u 5. i 10. poslijeoperativnom danu (p=0,02; p=0,000005) i BF (p=0,0034), te u povećanju POS (p=0,00018) nakon respiratornih vježbi. Dva (11,46%) bolesnika su razvila PM kao komplikaciju infekcije s *Pseudomonas aeruginosa*. Respiratorne vježbe imaju

pozitiva n utjecaj na respiratornu i posturalnu mobilnost dijafragme, što je vidljivo iz POS i BF rezultata. Pojavnost PM (11,46%) je posljedica nozokomijalne infekcije.

Ključne riječi: posturalna mobilnost dijafragme, totalna laringektomija, pneumonija

IMPACT ASSESSMENT OF RESPIRATORY AND POSTURAL MOBILITY OF DIAPHRAGM IN PREVENTION OF PNEUMONIA

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Total laryngectomy (TL) leads to a separation of the upper and lower airways, which result with absent warming, humidifying and filtering air.This have unfavourable effects on the lower airways, lung capacity and leads to the development of pneumonia (PM). The aim of this study was impact assessment of respiratory and postural diaphragm mobility in prevention of PM in the postoperative period in patients who have had TL. This study used database of the patients who have had TL surgery at University Hospital Center Zagreb, Department of head and neck tumors. A sample of 17 patients was observed: respiratory and postural diaphragm mobility; effect of respiratory exercises (RE) on peripheral oxygen saturation (POS), breathing frequency (BF), blood pressure (BP) and cardio pulse (cp) before and after respiratory exercises; inflammatory parameters (WBC , CRP, body temperature); changes in the lungs (RTG, auscultation). Statistical analysis was made by ANOVA. There is a statistically significant difference in decrease of WBC 5th and 10th postoperative day (p=0,02; p=0,000005) and BF (p=0,0034), and increase of POS (p=0,00018) after respiratory exercises. Two (11.46%) patients developed PM as a complication of *Pseudomonas aeruginosa* infection. Respiratory exercises had a positive influence on respiratory and postural diaphragm mobility, as evidenced by POS and BF results. The incidence of PM (11.46%) is consequence of nosocomial infections.

Keywords: postural diaphragm mobility , total laryngectomy, pneumonia

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USPOREDBA RAZINE MONOAMINA U MOZGU GUŠTERICA *Podarcis siculus* I *Podarcis melisellensis* METODOM TEKUĆINSKE KROMATOGRAFIJE VISOKE UČINKOVITOSTI

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Uz veličinu i prehranu, uzrok kompetitivnog isključivanja krške gušterice (*Podarcis melisellensis*) od strane primorske gušterice (*Podarcis siculus*) moglo bi biti i afektivno ponašanje, koje je regulirano monoaminskim neurotransmiterima: serotoninom (5HT), dopaminom (DA),

noradrenalinom (NA) i adrenalinom (A). U ovom istraživanju usporedili smo razinu monoamina u mozgu spomenutih vrsta tekućinskom kromatografijom visoke djelotvornosti (HPLC) s UV/VIS detekcijom. Homogenati 36 mozgova su analizirani HPLC-om reverzne faze, uz kolonu Kromasil 100-5-C18 kao stacionarnu te mješavinu mravlje kiseline (pH 2,8) i acetonitrila kao mobilnu fazu. Signali su detektirani pri valnoj duljini od 280 nm. Koncentracije monoamina u uzorcima izračunate su pomoću kalibracijske krivulje monoaminskih standarada i podataka o površini signala te su izraženi kao pg neurotransmitera po gramu tkiva. Ženke i mužjaci vrste *P. siculus* pokazali su značajno više vrijednosti prosječne razine DA (9.62 pg/g i 13.39 pg/g) nego ženke i mužjaci vrste *P. melisellensis* (6.26 pg/g i 7.46 pg/g). Razine 5HT i NA+A nisu se statistički značajno razlikovale između ove dvije vrste. Budući da je porast koncentracije DA povezan s povećanjem lokomotorne aktivnosti, bržim učenjem i agresivnijim ponašanjem, statistički značajna razlika u razini DA između ove dvije vrste guštera mogla bi stajati u podlozi njihovog različitog afektivnog ponašanja i prilagodljivosti.

Ključne riječi: monoamini, 5HT, katekolamini, ponašanje, gušteri

COMPARISON OF MONOAMINE LEVELS IN THE BRAIN OF *Podarcis siculus* AND *Podarcis melisellensis* USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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In addition to the size and diet, the cause of competitive exclusion of Dalmatian wall lizard (*Podarcis melisellensis*) by the Italian wall lizard (*Podarcis siculus*) could be affective behavior regulated by monoamine neurotransmitters: serotonin (5HT), dopamine (DA), noradrenaline (NA) and adrenaline (A). In this study, we compared the levels of monoamines in the brain of mentioned species by high performance liquid chromatography (HPLC) with UV/VIS detection. Homogenates of 36 brains were analyzed by reverse phase HPLC with Kromasil 100-5-C18 column as a stationary and formic acid (pH 2.8) and acetonitrile mixture as a mobile phase. Peaks were detected at a wavelength of 280 nm. Monoamine concentrations in samples were calculated using the calibration curves of the monoamine standards and peak surface data, and expressed as pg of neurotransmitter per gram of tissue. *P. siculus* females and males showed significantly higher average levels of DA (9.62 pg/g and 13.39 pg/g) than *P. melisellensis* (6.26 pg/g and 7.46 pg/g, respectively). There wasn't any statistically significant difference in the levels of 5HT and NA+A between these two species. Since the increase in DA concentration is associated with increased locomotor activity, faster learning, and more aggressive behavior, a significant difference in DA level between these two species of lizard may stand in the background of their different affective behavior and adaptability.

Keywords: monoamines, 5HT, catecholamines, behavior, lizards

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UČINAK IKARINA NA BIOMARKERE OSTEOPOROZE

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Prehrana kao glavni čimbenik utječe na metabolizam kostiju, endokrini i/ili parakrini sustav i homeostazu mineralnih elemenata kostiju. Istražen je antiosteoporotski učinak ikarina i alendronata (ALN) na gubitak koštane mase na modelu osteoporoze izazvane retinoičnom kiselinom (13cRA) u štakora. Ikarin ima dobar učinak na zdravlje kostiju; pospješuje težinu kostiju, duljinu i promjer bedrene kosti, količinu kalcija i fosfora u kostima i sadržaja koštanog pepela, gustoću minerala kostiju (BMD) i biokemijske biljege koštanog preokreta te smanjuje atrofiju maternice induciranu s 13cRA. Svi rezultati sugeriraju da antioksidativni i estrogenski učinak ikarina može spriječiti nastanak osteoporoze izazvane s 13cRA u štakora stimulirajući formiranje kostiju te smanjujući resorpciju kostiju bez toksičnih učinaka zapaženih kod obrade s ALN.

Ključne riječi: ikarin, model osteoporoze-izazvan s 13 c-retinoičnom kiselinom, štakor, biljezi osteoporoze

THE EFFECTIVENESS OF ICARIIN ON BIOMARKERS FOR OSTEOPOROSIS

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Nutrition is important factor that influence bone metabolism, the endocrine and/or paracrine system, and bone-active mineral elements homeostasis. We studied antiosteoporetic effects of icariin and alendronate (ALN) in retinoic acid-induced (13cRA) bone loss in rats. Icariin has beneficial effects on bone health; it improved the decrease in bone weight, the length and the diameter of the bone, calcium, and phosphorus content in bone ash, bone mineral density (BMD) and the biochemical markers of bone turnover and uterus atrophy induced by 13cRA. All result suggests that icariin reverses osteoporosis in 13cRA rats by stimulating bone formation or regulating bone resorption by its antioxidative and estrogenic-like activity without toxic-side effect observed in ALN treatment.

Keywords: Icariin, retinoic acid-induced bone loss, rats, bone markers

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IN VIVO INHIBITORNI I ANTI-ANGIOGENI UČINAK GALNE KISELINE NA UZNAPREDOVALI EHRLICHOV ASCITESNI TUMOR U MIŠA

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Novačenje makrofaga te produkcija citokina/kemokina, čimbenika rasta i proteolitičkih enzima je zajednička karakteristika upale, angiogeneze i raka, te važan cilj novog pristupa liječenja raka. Galna kiselina kao prirodna fitokemikalija i snažan antioksidans pokazuje kemopreventivna i terapijska svojstva u liječenju raka. Istražili smo učinak galne kiseline na rast tumora, angiogenezu, polarizaciju makrofaga i oksidacijski stres na modelu angiogeneze prouzročene ip unosom EAT stanica (2,5x106) u Swiss albino miša. Rezultati ukazuju da galna kiselina pokazuje imunostimulatorni, protuupalni, antiangiogeni i protutumorski učinak. Protutumorski učinak galne kiseline temelji se na citotoksičnoj djelotvornosti M1 makrofaga, izravnoj inhibiciji rasta EAT stanica, inhibiciji čimbenika rasta krvožilnog endotela (VEGF) te rasta krvožilja u peritonealnoj šupljini miševa nositelja EAT. Galna kiselina kroz inhibiciju produkcije proangiogenih čimbenika, posebice VEGf čimbenika, metaloproteinaza 2 i 9 (MMP-2 i MMP-9) te aktivnosti ciklooksigenaze 2 (COX-2), inhibira proces angiogeneze, rast tumora i povećava preživljenje miševa nositelja EAT stanica. Antioksidativna sposobnost galne kiseline blokira pojavnost M2 tumoru pridruženih makrofaga (TAMs) i značajno inhibira tumorogenezu u mišjem modelu. Temeljem navedenog, inhibicija TAMs može biti učinkovita u liječenju tumora i drugih kroničnih bolesti povezanih s procesom angiogeneze.

Ključne riječi: angiogeneza, galna kiselina, polarizacija makrofaga, proangiogeni čimbenici, TAMs

IN VIVO GROWTH INHIBITORY AND ANTI-ANGIOGENIC EFFECTS OF GALLIC ACID ON ADVANCED EHRLICH ASCITES TUMOR IN MOUSE

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Macrophage recruitment and cytokine/chemokine production, growth factors and proteolytic enzymes is a common feature of inflammation, angiogenesis and cancer, and an important goal of a new approach to cancer treatment. Gallic acid as natural phytochemical and strong antioxidant shows chemopreventive and therapeutic properties in cancer treatment. We investigated the effect of gallic acid on tumor growth, angiogenesis, macrophage polarization and oxidative stress on the angiogenic model caused by ip inoculation of EAT cells (2.5x106) in Swiss albino mouse. The results show that gallic acid exhibit immunostimulatory, anti-inflammatory, antiangiogenic and antitumor effect. The antitumor effect of gallic acid is based on the cytotoxic efficacy of M1 macrophage, direct inhibition of EAT cell growth, inhibition of vascular endothelial growth factors (VEGF) and growth of blood vessels in the peritoneal cavity of EAT-bearing mice. Gallic acid through the inhibition of proangiogenic factors, particularly VEGF, metalloproteinases 2 and 9 (MMP-2 and MMP-9) and cyclooxygenase 2 activity (COX-2), inhibit process of angiogenesis, tumor growth and increase survival of EAT-bearing mice. The antioxidant ability of gallic acid blocks the occurrence of M2 tumor associated macrophages (TAMs) and significantly inhibits tumorigenesis in mouse model. Based on the above, TAMs inhibition may be effective in the cancer treatment and other chronic diseases associated with process of angiogenesis.

Keywords: angiogenesis, gallic acid, macrophage polarization, proangiogenic factors, TAMs

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ANTIOKSIDATIVNI UČINAK KAFEINSKE KISELINE: MOGUĆNOST PRIMJENE U DIJABETESU

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Dijabetes mellitus je bolest s visokom stopom rasta. Cilj ovog istraživanja bio je proučiti moguće zaštitne učinke kafeinske kiseline na nastala oštećenja u miševa s dijabetesom prouzročenim aloksanom. Tip 1 dijabetes induciran je u miševa davanjem aloksana u dozi od 75 mg kg^{-1} iv. Nakon 9. dana od iniciranja aloksanom dolazi do gubitka tjelesne mase te povećanja glukoze, triglicerida i ukupnog kolesterola u serumu u odnosu na normalnu kontrolnu skupinu. Kafeinska kiselina (50 mg kg^{-1}) injicirana miševima s dijabetesom tijekom sedam dana bila je učinkovita u održavanju normalne tjelesne mase životinja, smanjenju % hemolize eritrocita, koncentracije triglicerida i kolesterola u krvi oboljelih miševa, te povećanju funkcionalne aktivnosti makrofaga peritonealne šupljine, ali nije pokazala učinak na preživljenje miševa. Rezultati komet testa ukazuju da je kafeinska kiselina smanjila oštećenja DNA u limfocitima periferne krvi uzrokovana oksidativnim stresom te da kafeinska kiselina ima obećavajući učinak na smanjenje negativnih posljedica razvoja dijabetesa, čemu doprinosi njen antioksidativni potencijal.

ključne riječi: dijabetes, oksidativni stres, aloksan, kafeinska kiselina, komet test

ANTIOXIDATIVE ACTIVITY OF CAFEIC ACID: APPLICABILITY IN DIABETES

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Diabetes mellitus is a disease with high growth rate. The present study was designed to investigate the possible antidiabetic and protective effect of caffeic acid against alloxan-induced oxidative damage in diabetic mice. Type 1 diabetes was induced in mice by injection alloxan in dose of 75 mg kg^{-1} bwt iv. After 9 days of alloxan injection, there was an apparent reduction in the animal body weight and significant increase in serum glucose, triglyceride and total cholesterol levels as compared to the control healty mice. Treatment of diabetic mice with caffeic acid in dose of 50 mg kg^{-1} for seven days reduced the decline in body mass, % hemolysis of erythrocytes, blood triglyceride and cholesterol levels and increased functional activity of macrophages peritoneal cavity but did not increase the survival of mice. Additionally comet assay results show that treatment of diabetic animals with caffeic acid reduces the alloxan-induced DNA damage to lymphocytes. The results indicate that caffeic acid has promising effects in reducing negative consequences of developing diabetes, which contributes to its antioxidant potential.

Keywords: diabetes, oxidative stress, aloxan, caffeic acid, comet assay

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PROTUTUMORSKA UČINKOVITOST KURKUMINA, BLEOMICINA I HIPERTERMIJE NA STANICE EHRLICHOVOG ASCITESNOG TUMORA

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Primjenom kemoterapije sprječava se proliferacija i širenje tumorskih stanica, te potiče proces apoptoze. Kemoterapiju osim apoptoze često prati proces nekroze koja prouzrokuje oštećenje i okolnoga zdravoga tkiva. Budući da su flavonoidi u drugim istraživanjima već pokazali učinkovitost protiv tumorskih stanica i mogućnost smanjivanja razine oštećenja u zdravim stanicama, u ovom istraživanju proučavan je in vitro učinak kurkumina na proliferaciju stanica Ehrlichovog ascitesnog tumora (EAT), te njegov utjecaj na pokretanje apoptoze/nekroze kod stanica EAT i njegov učinak u kombinaciji s bleomicinom i hipertermijom. Stanice EAT su rasle u Swiss albino miševima iz kojih su izvađene nakon 7 dana, te su kultivirane u hranjivom mediju, obrađene kurkuminom, bleomicinom i hipertermijom. Antiproliferativni učinak test komponenti potvrđen je testom kolorimetrije neposredno nakon kratkoročne inkubacije bleomicinom tijekom 2 h te kurkuminom slijedeća 2 h pri fiziološkim ili hipertermalnim (43˚C) uvjetima i nakon obrade stanica tijekom 24 sata. Analiziran je postotak tumorskih stanica u apoptozi i nekrozi na protočnom citometru te istražen genotoksični učinak komet testom. Kurkumin je pokazao da može zaustaviti proliferaciju stanica EAT u uvjetima in vitro, te da slabo povećava postotak stanica u apoptozi. Kombinacija s citostatikom bleomicinom i hipertermijom daje bolji antiproliferativni učinak koji se temelji na visokom postotku indukcije nekroze te potpunoj lizi stanica.

Ključne riječi: Ehrlichov ascitesni tumor, bleomicin, kurkumin, hipertermija

ANTITUMOR EFFICACY OF CURCUMIN, BLEOMYCIN AND HYPERTHERMIA ON EHRLICH ASCITES TUMOR CELLS

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Chemotherapy is used to stop proliferation and spreading of cancer cells, as well as to initiate apoptosis in cancer cells. Due to its necrotic nature, chemotherapy usually causes damage to nearby healthy tissue. Flavonoids have shown their capability to fight cancer cells and to prevent some damage to healthy cells. In this research curcumin was tested in vitro for its effect on Ehrlich ascites tumor (EAT) cells, their proliferation and ability to initiate apoptosis/necrosis in the cells, and curcumins effect in interaction with bleomycin and hyperthermia. EAT was grown in Swiss albino mice, the cells were isolated on the 7th day, and were incubated in culture medium, and treated with curcumin, bleomycin and hyperthermia. The antiproliferative effect of the test components was confirmed by a colorimetry assay after a short incubation directly with bleomycin for 2 h and curcumin for

the next 2 h at physiological or hyperthermal (43°C) conditions and after the treatment of cells for 24 hrs. The percentage of tumor cells in apoptosis and necrosis on the flow cytometer was analyzed and the genotoxic effect of the comet test was investigated. Curcumin demonstrated the potential to inhibit the proliferation of EAT cells in vitro, and to slightly increase the percentage of cells in apoptosis. The combination with cytostatic bleomycin and hyperthermia provides a better anti-proliferative effect which is based on the high percentage induction of necrosis and complete cell lysis.

Keywords: Ehrlich ascites tumor, bleomycin, curcumin, hyperthermia

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UTJECAJ SEVOFLURANA NA OKSIDACIJSKI STRES I UPALU IZAZVANU ALUMINIJEVIM KLORIDOM U MOZGU ŠTAKORA

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Temeljni cilj ovog rada bio je istražiti mogući antioksidativni/prooksidativni učinak sevoflurana na AlCl3 izazvani oksidacijski stres praćenjem: promjena u oksidoredukcijskom statusu mozga mjenjenjem razine glutationa (GSH) i malondialdehida (MDA, krajnjeg produkta lipidne peroksidacije), te aktivnosti katalaze (CAT) i superoksid dismutaze (SOD) u tkivu mozga; procjenom razine neuroupale, mjerenjem relativne težine mozga u odnosu na zdravu kontrolnu skupinu; analizom promjene hematoloških pokazatelja krvi te osmotsku fragilnost eritrocita kao pokazatelja toksičnosti $AlCl_3$, sevoflurana ili njihove kombinacije. Rezultati pokazuju da $AlCl_3$ uzrokuje povećanu razinu oksidacijskog stresa; značajno povećava razinu MDA i snižava razinu GSH u mozgu te u manjoj mjeri mjenja aktivnosti antioksidativnih enzima u odnosu na sevofluran. Sevofluran u kombinaciji s $AlCl_3$ pokazuje jači toksični učinak; razina MDA je značajno povećana kao i aktivnost antioksidacijskih enzima SOD i CAT. Povećana neuroupala vidljiva je u skupini obrađenoj s $AlCl_3$, dok sevofluran u kombinaciji s $AlCl_3$ pokazuje propadanje moždanih stanica te značajne toksične učinke na hematološkim odrednicama. Temeljem dobivenih rezultata možemo zaključiti da značajno povećana razina lipidne peroksidacije u mozgu i neuroupale ukazuje na neurotoksičnost $AlCl_3$ dok sevofluran u kombinaciji s $AlCl_3$ pokazuje jači toksični učinak i značajno propadanje moždanih stanica.

Ključne riječi: sevofluran, aluminijum klorid, antioksidativni sustav, neuroupala

THE EFFECT OF SEVOFLURANE ON OXIDATIVE STRESS AND INFLAMMATION INDUCED BY ALUMINIUM CHLORIDE IN THE RAT BRAIN

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The aim of this study was to investigate possible antioxidant/prooxidative effect of sevoflurane on $AlCl_3$-induced oxidative stress by monitoring:change in oxidoreduction status of the brain by changing the level of glutathione (GSH) and malondialdehyde (MDA); activity of catalase (CAT) and superoxide dismutase (SOD) in brain tissue; evaluation of the neuro-inflammation, by measuring the relative weight of the brain compared to a healthy control group; analyzing changes in haematological parameters and osmotic fragility of erythrocytes as indicators of the toxicity of $AlCl_3$, sevoflurane and/or these combination. Results show that $AlCl_3$ causes an increased level of oxidative stress; significantly increases MDA levels and decreased GSH levels in the brain and changes to a lesser extent the activity of antioxidant enzymes compared to sevoflurane. Sevoflurane in combination with $AlCl_3$ exhibits a stronger toxic effect; MDA level was significantly increased as well as activity of antioxidant enzymes SOD and CAT. Increased neuro-inflammation is visible in group treated with $AlCl_3$, while sevoflurane + $AlCl_3$ cause decaying of brain cells and significant toxic effects on haematological parameters. Based on obtained results we can conclude that significantly increased level of lipid peroxidation in the brain and neuro-inflammation indicates neurotoxicity of $AlCl_3$, while sevoflurane in combination with $AlCl_3$ exhibits a higher toxic effect and a significant degradation of brain cells.

Keywords: sevoflurane, aluminium chloride, antioxidative system, neuroinflammatory

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SEASONAL HEMATOLOGICAL STATUS OF OHRID ROACH (*Rutilus ohridanus,* Karaman 1924) FROM LAKE OHRID

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This study was conducted with the aim to investigate the possible seasonal changes in the hematological status of Ohrid Roach (*Rutilus ohridanus*, Karaman 1924) from Lake Ohrid. Hematological parameters (RBC, Hb, Hct, MCH, MCHC, MCV, WBC) were measured in blood samples from fish collected in three different seasons (December, May and September). The statistical analysis confirmed significant difference regarding the season in all of the investigated parameters. Highest values for all red blood cell indices except RBC where found in the winter season, whereas the WBC count was highest in the spring. These variations may play an important protecitve role for the survival of the fish ,and as an addaptible mechanism in the regulation of the fish physiology.

Keywords: Ohrid Roach, season, hematological status

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INVAZIVNE STRANE VRSTE U HRVATSKOJ – ZBOG ČEGA JE EUROPA ZABRINUTA?

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Uredba (EU) br. 1143/2014 o sprječavanju i upravljanju unošenja i širenja invazivnih stranih vrsta, koja je na snagu stupila 1. siječnja 2015. godine, sustavno obrađuje problem invazivnih stranih vrsta na razini Europske unije. Ona uspostavlja pravila za sprječavanje, smanjivanje na najmanju moguću mjeru i ublažavanje štetnih učinaka namjernog i slučajnog unosa i širenja invazivnih stranih vrsta na bioraznolikost. Okvir za provedbu odredbi Uredbe u hrvatskom zakonodavstvu utvrđen je Zakonom o sprječavanju unošenja i širenja stranih te invazivnih stranih vrsta i upravljanju njima (Narodne novine 15/18). Uredba se odnosi na popis invazivnih stranih vrsta koje izazivaju zabrinutost u Uniji ("Unijin popis"), koji se donosi putem provedbenih akata. Trenutno je na popisu ukupno 49 vrsta, od čega je 17 vrsta prisutno u Hrvatskoj. One uključuju pet biljaka (Plantae), tri raka (Crustacea), dvije slatkovodne ribe (Actinopterygii), jednog gmaza (Reptilia), jednu pticu (Aves) i pet sisavaca (Mammalia). U ovom radu na temelju literaturnih podataka i dostupnih baza podataka predstavljamo rasprostranjenost svih 17 vrsta koje izazivaju zabrinutost u Uniji, a prisutne su u Hrvatskoj. Navodimo njihov utjecaj na bioraznolikost i moguće mjere kontrole i upravljanja koje su potrebne da bi se spriječilo njihovo daljnje širenje.

Ključne riječi: Uredba EU o IAS, Unijin popis, rasprostranjenost, utjecaj

INVASIVE ALIEN SPECIES IN CROATIA - WHAT IS EUROPE CONCERNED ABOUT?

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Regulation (EU) No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species, which entered into force on 1 January 2015, systematically deals with the problem of invasive alien species at European Union level. It sets out rules to prevent, minimize and mitigate the adverse impacts on biodiversity of intentional and unintentional introduction and spread of invasive alien species. Framework for its implementation in Croatian legislation is set by the new Act on Prevention and Management of the Introduction and Spread of Alien and Invasive Alien Species (Official Gazette 15/18). The Regulation refers to the list of invasive alien species of the Union concern ("Union List"), which is delivered through implementing acts. Currently, there are 49 invasive alien species on the Union List, out of which 17 species are present in Croatia. They include five plants (Plantae), three crustaceans (Crustacea), two freshwater fishes (Actinopterygii), one reptile (Reptilia), one bird (Aves), and five mammals (Mammalia). In this work we reviewed literature and available databases for all 17 species of Union concern that occur in Croatia, and we report their current distribution. We address their impact on biodiversity, and possible measures of control and management needed to prevent further spread.

Keywords: EU regulation on IAS, Union list, distribution, impact

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ANALIZA SASTAVA OTPADA NA MORSKOM DNU U PLITKOM (<20 M) PRIOBALJU METODOM AUTONOMNOG RONJENJA

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Otpad iz mora značajni je okolišni problem koji uzrokuje povećanu zabrinutost na nacionalnoj i međunarodnoj razini. Takav tip zagađenja složeni je i rastući okolišni problem koji utječe ne samo na morski okoliš, nego i na ljudske obalne zajednice i sve gospodarske sektore vezane uz more. Otpad na morskom dnu ima tendenciju gomilanja u područjima niske cirkulacije vode i visoke akumulacije sedimenta. U ovom radu istraživan je kvalitativno-kvantitativni sastav, prostorna raspodjela i brojnost otpada u plitkom priobalnom području (<20m) na jugoistočnoj strani otoka Korčule. Istraživanje je provedeno sezonski tijekom 2016. godine metodom korištenom u projektu DeFishgear; vizualno prebrojavanje uz primjenu autonomne ronilačke opreme. Navedeno predstavlja prvu takvu procjenu otpada iz mora u Hrvatskoj, provedenom prema protokolu kompatibilnom Okvirnoj direktivi o morskoj strategiji. Glavne kategorije prikupljenog otpada čine plastika, metal, tekstil, staklo, guma, papir i ostalo. Među navedenim, prevladavajuća kategorija je plastika s udjelom od 74,3%, zatim tekstil (10%), metal (8.6%) te staklo i keramika (4,3%). Ostale kategorije su manje zastupljene i čine preostalih 3%. Zabilježena je gustoća otpada od 0.01 to 0.6, s prosjekom od 0.2 kom/m2. Dobiveni rezultati naglašavaju utjecaj brojnih lokalnih čimbenika na količinu i akumulaciju otpada, poput izvora otpada i oceanografskih uvjeta, čime se utvrđuje potreba za direktnim i aktivnim djelovanjima.

Ključne riječi: otpad iz mora, morsko dno, vizualno istraživanje, zagađenje, monitoring protokol.

QUANTIFICATION OF MARINE LITTER ON THE SEAFLOOR IN SHALLOW (<20 M) AREA USING A SCUBA DIVING

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Marine litter is an environmental problem that gained increasing concern both nationally and internationally. This kind of pollution is a complex and growing issue, affecting not only the marine environment, but also human coastal communities and the economic sectors related to the sea. Benthic litter tends to become trapped in areas of low water circulation, high sediment accumulation and convenient seafloor morphology. In the present work qualitative composition, spatial distribution and abundance of seafloor litter were investigated in the shallow coastal area (<20 m) of southeast Korčula Island (Croatia). Investigation was performed seasonally in 2016 using the visual surveys by SCUBA diving monitoring protocol proposed by

DeFishGear project. This represents the first assesment of this kind of litter in Croatia carried out by protocol compatible to MFSD. The main categories of litter collected appear into the various types of plastic, metal, glass, rubber, paper and other. Among them, significantly predominant were plastics with 74.3**%**, followed by the textile (10%), metal (8.6%), and glass/ceramic (4.3%). Other categories were quantitatively irrelevant, making the remaining 3% of the total amount. The density was ranging from 0.01 to 0.6 with an average of 0.2 item/m^2. The data analysis highlighted the influence of many local factors, like the sources and oceanographic conditions, on the litter quantity and accumulation establishing the urgent need for specific actions.

Keywords: marine litter, sea floor, visual survey, pollution, monitoring protocol.

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SUŽIVOT LJUDI I PRIRODE NA PRIMJERU NACIONALNOG PARKA PLITVIČKA JEZERA – JE LI ISTO MOGUĆE?

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Cilj provedenog istraživanja bio je ispitati kakve su mogućnosti suživota stanovništva zaštićenog područja s prirodom te jesu li u skladu sa zakonskom regulativom. U svrhu istraživanja provedeno je anketiranje među lokalnim stanovništvom. Na temelju rezultata ankete te direktnog uvida u način života lokalnog stanovništva u sklopu NP Plitvička jezera može se zaključiti da lokalno stanovništvo u najvećoj mjeri i dalje živi od turizma koji je vezan uz postojanje Nacionalnog parka, no ne u tolikoj mjeri kao nekad. Kao najveći problem života u ovom kraju stanovnici ističu da je školovanje mladih, u svrhu daljnjeg zapošljavanja u parku skoro u potpunosti nestalo, kako lokalne poljoprivrede povezane s opskrbom ugostiteljsko-turističkih sadržaja u parku više nema, a sezonske poslove rade ljudi iz svih krajeva Hrvatske, bez prednosti lokalnog stanovništva. Smatraju kako bi Uprava parka, kao i općine, zaštićenom području trebali pridati mnogo veću pozornost te bi samim time poboljšali suživot prirode i lokalnog stanovništva. Na samom kraju nameće se zaključak kako je suživot prirode i stanovništva moguć ukoliko se postigne ravnoteža između poštivanja zakonskih odredbi o zaštiti prirode te ukoliko iste ne onemogućuju radnje nužne za normalan život.

Ključne riječi: Nacionalni park, Plitvička jezera, lokalno stanovništvo, suživot, priroda

COEXISTENCE OF LOCAL POPULATION AND NATURE IN THE EXAMPLE OF THE PLITVICE LAKES NATIONAL PARK – IS IT POSSIBLE?

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The goal of research was to examine what are the possibilities of coexistence of the population in the protected area with the nature and whether they are in compliance with the statutory regulations. For the purpose of research, an opinion poll was conducted among the local population. Based on the results of the poll and direct inspection of the manner of life of the locals within the Plitvice Lakes National Park, it may be concluded that the local population for

the most part still lives of tourism, which is related to the National Park, but not in the proportions as it once did. As the major problem in their life, the locals point out that the schooling of the young for the purpose of further employment in the park has almost completely disappeared, that there is no longer local agriculture linked to the supply of the catering-tourist facilities in the park, and the locals no longer have privileges in seasonal jobs which are nowadays also available to other people from all parts of Croatia. They think that the park's Management, as well as the municipalities, should devote much greater attention to the protected area, which would simultaneously improve coexistence of the nature and local population. At the end, the conclusion is that the coexistence of nature and locals is only possible if the balance is achieved between the compliance of statutory provisions on protection of nature and if such provisions do not hinder activities required for normal life.

Keywords: National Park, Plitvice Lakes, local population, coexistence, nature

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ZAŠTITA OSJETLJIVIH RIBLJIH STANIŠTA U JABUČKOJ KOTLINI USPOSTAVOM ZAŠTIĆENOG RIBOLOVNOG PODRUČJA

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Jabučka kotlina predstavlja najvažnije ribolovno područje u Jadranskom moru za hrvatsku i talijansku ribolovnu flotu s udjelom u ukupnom koćarskom ulovu od oko 30%. Istovremeno Jabučka kotlina predstavlja rastilište i mrijsetilište za najvažnije pridnene gospodarske vrste oslića i škampa. Prema posljednjim procjenama GFCMa i STECFa obje ove vrste se već nekoliko godina nalaze u stanju prelova te je potrebno pristupiti hitnim restriktivnim mjerama smanjenja ribolovne ribolovnog napora i zaštite resursa. Bilateralnim dogovorom Hrvatske i Italije 2015 godine u području Jabučke kotline uspostavljena je jednogodišnja zona zabrane koćarskog ribolova. Podatci o učincima zabrane prikazani u ovom radu prikupljeni su kroz međunarodna istraživanja koji uključuju analizu sastava pridnenih zajednica kroz ekspedicije MEDITS i UWTV, korištenjem podataka o komercijalnom ribolovu (DCF), kao i distribuciju ribolovnog napora kroz analize VMS i AIS podataka. Analiza pokazuje pozitivne učinke zabrane na stanje populacija oslića i škampa kroz povećanje indeksa biomase i intenziteta novačenja, kao i poboljšanje komercijalnih lovina. Nadalje, iz podataka je vidljivo da jednogodišnja zabrana nije dovoljna te da treba nastaviti s mjerama smanjenja ribolovnog napora i zaštite resursa. Imajući ovo u vidu, GFCM je 2017 godine na području Jabučke kotline uspostavio Zaštićeno ribolovno područje (FRA) na period od 3 godine uz obavezu stalnog znanstvenog monitoringa stanja.

Ključne riječi: pridnene zajednice, kočarski ribolov, zaštićena područja, Jabučka kotlina

FISHERIES RESTRICTED AREA AS A TOOL FOR PROTECTION OF ESSENTIAL FISH HABITATS IN JABUKA PIT

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Jabuka Pit is the most important fishing area in the Adriatic Sea for Croatian and Italian fishing fleets with a share of total catch of about 30%. At the same time, represents a spawning and nursery area for the commercially most important species, European hake and Norway lobster. According to recent estimates by GFCM and STECF, both species have been in the state of overfishing for several years and need to be tackled by urgently restrictive measures to reduce fishing effort and resource conservation. Bilateral agreement in 2015 established a one-year zone of ban on fishing in the area of Jabuka Pit. Data on the effects of banning presented in this paper have been collected through international surveys (MEDITS and UWTV) that includes the analysis of the composition of the demersal communities, monitoring of the commercial fisheries (DCF) and distribution of fishing effort through VMS and AIS data. The analysis shows the positive effects of bans on the condition of hake and Norway lobster populations by increase in the biomass index and the intensity of recruitment as well as the improvement of commercial catches. It is apparent from the data that a one-year ban is not sufficient and that measures to reduce fishing effort and resource conservation should continue. Bearing this in mind, the GFCM has established a Fisheries Restricted Area for a period of 3 years in the area of the Jabuka Pit in 2017 with the obligation of continuous scientific monitoring of the condition.

Keywords: demersal communities, bottom trawl fisheries, protected area, Jabuka Pit

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PROLJETNA FLORA PARKOVA SAMOBORA I VIROVITICE

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Istraživanje proljetne flore parkova Samobora i Virovitice provedeno je tijekom ožujka 2017. g. i 2018. g. na dva lokaliteta u Samoboru i jednom u Virovitici. Na istraživanim lokalitetima u Samoboru zabilježeno je 20 svojti, a u Virovitici 15 svojti. Više je dvosupnica nego jednosupnica. Prevladavaju hemikriptofiti. Vrste *Gagea lutea* (L.) Ker Gawl. i *Anemone ranunculoides* L. zabilježene su u Virovitici, a nisu zabilježene u Samoboru. U isto vrijeme vrste *Hacquetia epipactis* (Scop.) DC, *Helleborus atrorubens* Waldst. et Kit. i *Isopyrum thalictroides* L. nisu zabilježene na području Virovitice. Pet zabilježenih svojti u Virovitici i šest u Samoboru zaštićeno je Zakonom o zaštiti prirode. Porodica ljubica (Violaceae) sa 7 svojti je najbrojnija u Virovitici. Mirisna ljubica, *Viola odorata* L. s dva varijeteta različite boje cvijeta (ružičaste, ljubičaste i bijele) zabilježena je na svim lokalitetima u Virovitici. *Viola reichenbachiana* Jord. ex Boreau rjeđa je i zabilježena je na lokalitetima gdje prevladavaju grmovi i stabla. Najbrojnija porodica na području Samobora su žabnjaci (Ranunculaceae), s 5 svojti. Prema ovim i dosadašnjim istraživanjima (Kletečki i sur., 2016) proljetnice su u oba grada najviše zastupljene na travnjacima. Većina proljetnica u Virovitici dolaze na zelenim površinama budući da su prije

presađene iz prirodnih staništa, dok su proljetnice u Samoboru više zastupljene u brežuljkastom dijelu grada gdje su samonikle.

Ključne riječi: proljetna flora, parkovi, Samobor, Virovitica

SPRING FLORA OF PARKS IN SAMOBOR AND VIROVITICA

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Research of spring flora of parks in Samobor and Virovitica was conducted during March of 2017 and 2018 on two localities in Samobor and one in Virovitica. On localities in Samobor we recorded 20 taxa and in Virovitica 15 taxa. There were more dicotyledons than monocotyledons, and hemicryptophytes were dominant. Species *Gagea lutea* (L.) Ker Gawl. and *Anemone ranunculoides* L. were recorded in Virovitica but not in Samobor. At the same time species *Hacquetia epipactis* (Scop.) DC, *Helleborus atrorubens* Waldst. et Kit. and *Isopyrum thalictroides* L. were not recorded on Virovitica locality. Five recorded taxa in Virovitica and six in Samobor are protected by the Nature Protection Law. The violet family (Violaceae) with 7 taxa is the most numerous in Virovitica. Sweet violet *Viola odorata* L. with two colour varieties (pink, violet and white) was recorded on all localities in Virovitica. *Viola reichenbachiana* Jord. ex Boreau is more rare and was recorded on localities with more shrubs and trees. The most numerous family in Samobor area was the buttercup family with 5 taxa. According to this and previous research (Kletečki et al., 2016) spring flowers are in both towns most numerous on grasslands. The majority of spring flowers in Virovitica grow in green city areas because they were earlier transplanted from their natural habitats, while in Samobor spring flowers are more common in the hilly part of town where they grow in the wild.

Keywords: spring flora, parks, Samobor, Virovitica

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USPOREDBA FAUNE ŠIŠMIŠA NA PODRUČJU PARKA MAKSIMIR (2010. - 2016.)

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Park Maksimir je u središtu grada Zagreba, te usprkos njegovoj maloj površini (316 he) obiluje biljnim i životinjskim vrstama. Radi očuvane stoljetne hrastove šume i mnogih vodenih površina, Maksimir je vrlo važno stanište za faunu šišmiša. Udruga studenata biologije (BIUS), u suradnji s vanjskim stručnjacima, je kroz dugogodišnja istraživanja tokom proljeća, ljeta i jeseni (s prekidima od 2010. do 2016.) utvrdila ukupno 14 vrsta šišmiša koji koriste ovo iznimno stanište. Kroz nalaze trudnih ili laktirajućih ženki, te juvenilnih i subadultnih jedinki, možemo zaključiti da mnoge vrste potencijalno koriste ovo područje za osnivanje porodiljnih kolonija unutar parka ili u njegovoj neposrednoj blizini (*E. serotinus*, *M. bechsteinii*, *M.*

aurascens/mystacinus, *N. leisleri*, *N. noctula*, *P. nathusii*, *P. pipistrellus*, *P. pygmaeus*, *P. auritus*, *P. austriacus*, *R. ferrumequinum*). Kroz nalaz sedentarnih vrsta *P. kuhlii* (uhvaćene jedinke) i *T. teniotis* (snimkanje eholokacijskih signala) unutar parka također možemo zaključiti da potencijalno postoje kolonije ovih vrsta i u okolnom području unutar grada. Nalaz mužjaka i ženke rijetko nađene migratorne vrste *M. daubentonii* također ukazuje na veliku značajnost parka Maksimir. Potrebna su dodatna istraživanja faune šišmiša (mreže i analiza eholokacijskih signala) unutar grada Zagreba općenito kako bi se ustanovile sve potencijalne lokacije kolonija ovih strogo zaštičenih životinja.

Ključne riječi: šišmiši, Maksimir, mreže, eholokacija, monitoring

COMPARISON OF BAT FAUNA IN THE MAKSIMIR PARK AREA (2010. - 2016.)

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Maksimir park is located in the center of Zagreb, and in spite of its small surface (316 he) it has an abundance of plant and animal species. Due to the well preserved centuries-old oak forests and many fresh water areas in the park it is a very important habitat for bat fauna. Members of the Biology student association (BIUS) in cooperation with external experts have found through their research in the spring, summer and autumn periods (with interruptions from 2010. to 2016.), a total of 14 bat species that use this extraordinary habitat. Through the findings of pregnant or lactating females, juvenile and subadult individuals, we can conclude that many species potentially use this area to establish maternal colonies within or near the park (*E. serotinus*, *M. bechsteinii*, *M. aurascens/mystacinus*, *N. leisleri*, *N. noctula*, *P. nathusii*, *P. pipistrellus*, *P. pygmaeus*, *P. auritus*, *P. austriacus*, *R. ferrumequinum*). By finding sedentary species such as *P. kuhlii* (captured individuals) and *T. teniotis* (eholocation) within the park, we can also conclude that there are pottential colonies of these species in the surrounding area in the city. The findings of both male and female rarely caught migratory *M. daubentonii* species also points out the great significance of Maksimir park. Additional exploration of bat fauna (mistnetting and echolocation signal analisies) within the city of Zagreb is needed in order to pinpoint all potential locations of colonies of these highly protected animals.

Keywords: bats, Maksimir, mistnetts, eholocation, monitoring

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DNA BARKODIRANJE FAUNE HRVATSKE S OSVRTOM NA RAZNOLIKOST I DNA BARKODIRANJE TULARA (INSECTA, TRICHOPTERA)

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Trichoptera s drugim skupinama vodenih kukaca čine jedan od najraznolikijih dijelova vodene faune te nastanjuju različite tipove vodenih staništa: izvore, potoke, rijeke, jezera, močvare. Sistematska istraživanja bioraznolikosti tulara u Hrvatskoj započele su prije 20 godina. Do sada

je zabilježeno oko 215 vrsta Trichoptera u fauni Hrvatske. Metoda DNA barkodiranja predložena je 2003. godine kao univerzalni sistem za determinaciju biološkog materijala u smislu identifikacije vrste, a također i kao metoda za otkrivanje novih, još neopisanih vrsta koje se ne mogu međusobno razlikovati na temelju morfoloških obilježja (tzv. kriptičnih vrsta). Temelji se na određivanju slijeda nukleotida standardiziranog fragmenta mitohondrijskog gena za podjedinicu I citokrom okidaze (COI) duljine oko 650 pb. Godine 2017. započeo je znanstveni projekt "DNK obilježavanje bioraznolikosti hrvatske faune" kojeg financira Hrvatska zaklada za znanost. Projekt uključuje analizu 15 životinjskih skupina (oko 1450 vrsta) uključujući i Trichoptera. Primarni cilj projekta je istražiti rasprostranjenost i genetsku raznolikosti odabranih skupina hrvatske faune primjenom metode DNA barkodiranja. DNA barkodiranje vrlo je značajna metoda u procesu određivanja vrsta. Posebno je značajna u određivanju vrlo sličnih vrsta. Do sada smo DNA barkodirali oko 150 vrsta Trichoptera zabilježenih za faunu Hrvatske. Tijekom istraživanja utvrđeno je nekoliko novih vrsta u fauni Hrvatske i vjerojatno neke nove - kriptičke vrste za znanost.

Ključne riječi: DNA, barkodiranje, Trichoptera

DNA BARCODING OF FAUNA OF CROATIA WITH NOTICE ON BIODIVERSITY AND DNA BARCODING DATA OF CADDISFLIES (INSECTA, TRICHOPTERA)

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Caddisflies, along with other groups of aquatic insects, comprise one of the most abundant segments of aquatic fauna inhabiting all types of aquatic habitats: springs, streams, rivers, lakes, marshes. Systematic studies of the biodiversity of caddisflies in the Croatia have started 20 years ago. So far we recorded about 215 species of Trichoptera in fauna of Croatia. DNA barcoding was proposed in 2003 as a universal system for assignment of specimens to a particular species but also as a tool for recognition of yet undescribed morphologically indistinguishable cryptic species. The method is based on the sequencing of standardized, ~650 bp long fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (COI). In 2017, started a scientific project, “DNA barcoding of Croatian faunal biodiversity” financed by the Croatian Science Foundation. The project included 15 animal groups (about 1450 species) including Trichoptera. The primary goal of the project is to investigate the amount and geographic distribution of the genetic biodiversity of selected groups of Croatian fauna by using DNA barcoding methodology. DNA barcoding provides valuable tool in the laborious process of species identification. It helps for determinations of morphologically very similar species. So far we DNA barcoded about 150 species of Trichoptera recorded for Croatian fauna. In this investigation we found several new species in fauna of Croatia and probably some new - cryptic species for science.

Keywords: DNA, barcoding, Trichoptera

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INTERAKCIJE GUJAVICA I GLJIVA U TLU – UČINAK CELOMOCITA GUJAVICE (*Eisenia andrei*) NA RAST FITOPATOGENIH GLJIVA

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Uloga gujavica u funkcioniranju ekosustava tla vrlo je važna, kako u razgradnji organske tvari i kruženju tvari, tako i u interakcijama sa ostalim organizmima tla i njihovoj povezanosti preko hranidbenih mreža. Vrlo su bitne i interakcije između gujavica i gljiva u tlu. Poznato je da gujavice putem svoje celomske tekućine mogu direktno utjecati na rast fitopatogenih gljiva, ali točan mehanizam je još nepoznat. Primjenom elektrostimulacije, gujavica ispušta svoju celomsku tekućinu koja sadrži celomocite, imunosne stanice gujavice. U ovom istaživanju istražen je utjecaj ekstrakta celomske tekućine epigejne vrste gujavice *Eisenia andrei* na šest vrsta fitopatogenih gljiva: *Macrophomina phaseolina, Rhizoctonia solani, Scleroctinia sclerotiorum, Fusarium culmorum, Pythium irregulare* i *Chalara elegans*. Korištene koncentracije celomocita iznosile su 2000, 3500, 5000, 6000 i 7000 celomocita/mL ekstrakta. Kako je utvrđeno značajno inhibitorno djelovanje ekstrakta celomske tekućine na rast svih šest vrsta gljiva, u daljnjim istraživanjima potrebno je utvrditi mehanizam ovih interakcija koje mogu biti od iznimne važnosti u poljoprivredi i zaštiti bilja.

Ključne riječi: gujavice, fitopatogene gljive, celomska tekućina, inhibicija rasta

INTERACTIONS OF EARTHWORMS AND SOIL FUNGI – EFFECT OF EARTHWORM COELOMIC FLUID (*Eisenia andrei*) ON GROWTH OF PHYTOPATHOGENIC FUNGI

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Earthworms have great importance in the functioning of soil ecosystems, both in the degradation of organic matter and in the cycle of nutrients, as well as in interaction with other soil organisms and their connection through the food webs. The interactions between earthworms and fungi in the soil are very important. It is known that earthworms can directly affect the growth of phytopathogenic fungi through their coelomic fluid, but the exact mechanism is unknown. By applying electrostimulation earthworm releases its coelomic fluid that contains the coelomocytes, immune cells of the earthworm. In the present research, the effect of the coelomic fluid extract of epigeic species *Eisenia andrei* was investigated on six different species of phytopathogenic fungi: *Macrophomina phaseolina, Rhizoctonia solani, Scleroctinia sclerotiorum, Fusarium culmorum, Pythium irregulare* and *Chalara elegans*. Applied coelomocyte concentrations were 2000, 3500, 5000, 6000 and 7000 coelomocyte/mL extract. Since the significant inhibitory effect of coelomic fluid extract on growth of all six species of fungi has been determined, further research should determine the mechanism of these interactions that may be of great value in agriculture and plant protection.

Keywords: earthworms, phytopathogenic fungi, coelomic fluid extract, growth inhibition

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SUSTAV ZA DOJAVU I PRAĆENJE UHVAĆENIH, USMRĆENIH, OZLIJEĐENIH I BOLESNIH STROGO ZAŠTIĆENIH ŽIVOTINJA - PROTOKOL ZA MORSKE ŽIVOTINJE

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Hrvatska agencija za okoliš i prirodu vodi Sustav za dojavu i praćenje uhvaćenih, usmrćenih, ozlijeđenih i bolesnih strogo zaštićenih životinja u okviru kojeg se izrađuju procedure postupanja u slučajevima pronalaska. Sustav doprinosi praćenju pritisaka i prijetnji vrstama kao i praćenju stanja njihovih populacija. Obrazac za dojavu nalaza objavljen je na internetskoj stranici HAOP-a: http://www.haop.hr putem kojeg je svakoj osobi omogućen upis podataka o nalazu. Dio Sustava je i Protokol za dojavu i djelovanje u slučaju pronalaska uginulih, bolesnih ili ozlijeđenih strogo zaštićenih morskih životinja (morski sisavci, morske kornjače i hrskavične ribe). U razdoblju od 2015. do 2017. godine zabilježeno je ukupno 414 jedinki strogo zaštićenih morskih životinja. Od toga 208 jedinki morskih sisavca (50,24%), 202 jedinke morskih kornjača (48,79%), te četiri jedinke hrskavičnih riba (0,97%). U najvećem broju slučajeva radilo se o uginulim životinjama (57,49%), dok su se ostali slučajevi odnosili na ozlijeđene (14,73%) ili neozlijeđene životinje (27,29%). Od pojedinih vrsta, najviše su stradavali dobri dupin (*Tursiops truncatus*) i glavata želva (*Caretta caretta*), odnosno vrste koje su stalni stanovnici Jadranskog mora. Slučajni ulov u ribolovne alate najčešći je zabilježeni uzrok smrtnosti kod morskih sisavaca, prvenstveno dobrog dupina i hrskavičnih riba, a kod morskih kornjača mehaničke ozljede koje su rezultat ranjavanja propelerom i udaraca o stijene.

Ključne riječi: Sustav za dojavu, strogo zaštićene životinje, morske životinje, pritisci i prijetnje

NATIONAL ALERTING AND MONITORING SYSTEM FOR CAPTURED, DEAD, INJURED AND SICK ANIMALS OF STRICTLY PROTECTED SPECIES - PROTOCOL FOR MARINE SPECIES

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Croatian Agency for the Environment and Nature runs National Alerting and Monitoring System for captured, dead, injured and sick animals of strictly protected species. Within the System the procedures for acting are being developed. System contributes to the monitoring of pressures and threats to species, as well as for the assessing the state of their populations. The report format is published on the HAOP website: http://www.haop.hr, through which each person can enter the data on the findings. In the frame of the System, Protocol for Alerting and Monitoring of dead, sick and injured strictly protected marine species (marine mammals, sea turtles and cartilaginous fish is active. From 2015 to 2017, a total of 414 individuals of strictly protected marine species have been recorded. Thereof 208 individuals of marine mammals (50.24%), 202 individuals of sea turtles (48.79%) and four individuals of cartilaginous fish (0.97%). Most of them were dead animals (57.49%), while the rest were injured (14.73%) or healthy animals (27.29%). As for recorded species, most of them were individuals of Bottlenose dolphin (*Tursiops truncatus*) and Loggerhead turtle (*Caretta caretta*), the species that are permanent residents of the Adriatic. Accidental catch in fishery tools was the most common recorded cause of death of marine mammals, especially Bottlenose dolphin and cartilaginous fish, and for the sea turtles mechanical injuries caused by boat propeller or collision with coastal rock.

Keywords: Alerting System, strictly protected animals, marine animals, pressures and threats

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IZRAČUN INDEKSA OSJETLJIVOSTI SLATKOVODNIH RAKOVA HRVATSKE

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Slatkovodni rakovi naseljavaju brojne brzace, rijeke i jezera, a imaju izuzetno veliku važnost zbog svog aktivnog sudjelovanja u biološkoj kakvoći vode. Danas su ove vrste ugrožene zbog prekomjernog ribolova i širenja raznih bolesti. U Hrvatskoj postoje četiri vrste slatkovodnih rakova, od kojih se čak tri smatraju ugroženima. Postoji nekoliko globalnih metoda procjene ugroženosti biljaka i životinja. Svakako je daleko najpoznatija IUCN Crvena lista. Međutim, većina metoda (uključujući i IUCN) određuje ugroženost vrsta samo kada su ove vrste već u opadanju. Metoda procjene indeksa osjetljivosti temeljena na određenim ekološkim, bihevioralnim i antropogenim kriterijima pokazala je statistički značajnu korelaciju s IUCN globalnim statusom vrsta i omogućava određivanje ugroženosti vrsta prije nego što njezina brojnost počinje opadati. U ovom radu smo pokušali utvrditi indeks osjetljivosti svih vrsta slatkovodnih rakova u Hrvatskoj na temelju 10 kriterija razvrstanih u četiri kategorije: 0, 1, 2, 3 boda. Karakteristike su uvijek posložene od najmanjeg prema najvećem utjecaju na osjetljivost. Ukupni prosječni indeks svih kriterija izračunat je za sve vrste i određen je prema njihovoj poznatoj geografskoj distribuciji kako bi se definirala najvrijednija područja za očuvanje. Ovim istraživanjem danas smo odredili nova područja očuvanja u Republici Hrvatskoj.

Ključne riječi: slatkovodni rakovi, indeks osjetljivosti, ugroženost, Hrvatska

AN ASSESSMENT OF THE SENSITIVITY INDEX (SENSIN) OF FRESHWATER CRAYFISH IN CROATIA

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Freshwater crayfish are inhabitants of numerous rivers and lakes and they have an extremely great significance as they actively participate in the biological water quality. Today, these species are endangered due to overfishing and the spread of various diseases. In Croatia there are four species of freshwater crayfish of which three are considered vulnerable. There are several global methods of assessing the endangerment of plants and animals. The best known is certainly the IUCN Red listing method. However, most methods (including the IUCN) determine the endengerment of species only when these species are already declining. The method of assessing the sensitivity index based on certain ecological, behavioral and anthropogenic criteria has shown a statistically significant correlation with IUCN global status and allows to determine the type of endangerment of species before it starts to decline. Here we tried to determine the sensitivity index of all species of freshwater crayfish in Croatia based on 10 criteria broken in four categories: 0, 1, 2, 3 points. Characteristics were always ordered from lowest to highest contribution to sensitivity, overall mean index of all criteria was calculated for all species and it was plotted against their known geographical distribution to define the most valuable areas for conservation. Through these calculations we have come up

with new knowledge in the preservation of parts of Croatia.

Keywords: freshwater crayfish, sensitivity index, endangerment, Croatia

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PROMJENA OBILJA HLAPA (*Homarus gammarus*) U JADRANU KAO POSLJEDICA POVEĆANJA TEMPERATURE MORA

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Hlap (*Homarus gammarus*) je rasprostranjen u cijelom Jadranu iako je brojniji u sjevernom dijelu. Kao borealna vrsta, njegov mrijest, naseljavanje i novačenje može biti snažno pogođeno globalnim trendom povećanja površinske temperature mora i desetogodišnjom termohalinom varijabilnosti koja se javlja u Jadranskom moru, upravljana režimom jadransko-jonskog bimodalnog oscilacijskog sustava (BiOS). U ovom istraživanju uspostavili smo odnos između pridnenih temperatura mora u prošlom desetljeću s podacima o ulovu hlapa. Podaci o temperaturi su uzeti s redovito uzorkovanih oceanografskih postaja, dok su podaci o ulovu hlapa dobiveni od Uprave ribarstva za 10 ribolovnih zona u razdoblju od 2008 do 2017. Rezultati pokazuju pad ulova u južnom i srednjem Jadranu, dok se ulov povećava u sjevernom Jadranu nakon 2012. Ove se promjene podudaraju s povećanjem pridnene temperature mora nakon 2012. Kako se povećanje temperature podudara i s izmjenom negativnog i pozitivnog BiOS režima, od kojih potonji uzrokuje ulazak toplije i slanije vode u Jadran, pretpostavljamo da BiOS mehanizam snažno utječe na životni ciklus hlapa, posebice u južnom i srednjem Jadranu. Povrh toga, daljnje povećanje temperature može dovesti do istaknutijih migracija hlapa prema sjevernom Jadranu, uzrokujući pad ulova hlapa u južnom i srednjem Jadranu.

Ključne riječi: *Homarus gammarus*, obilje, BiOS, pridnena temperatura

TEMPERATURE-DRIVEN ABUNDANCE CHANGE OF THE EUROPEAN LOBSTER (*Homarus gammarus*) IN THE ADRIATIC SEA

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European lobster (*Homarus gammarus*) is distributed throughout the whole Adriatic Sea, although it is more abundant in the northern part. As it is a boreal species, H. gammarus spawning, settlement and recruitment success might be strongly affected by both global trend of increasing sea surface temperatures and decadal thermohaline variability which occurs in the Adriatic Sea, driven by the Adriatic-Ionian Bimodal Oscillating System (BiOS) regimes. We related sea bottom temperatures measured during last decade with landings data of *H. gammarus*. The temperature data were taken from regularly sampled oceanographic stations, while landings data were obtained by Directorate of Fisheries for 10 fishing zones in a period

from 2008 to 2017. The results show a decline in landings in the south and the middle Adriatic, while landings increase in the northern Adriatic after 2012. Those changes coincided with the increase of bottom sea temperature after 2012. The temperature increase coincides with the change between negative and positive BiOS regimes, of which the latter is known to advect warm and salty waters to the Adriatic, and thus we hypothesize that the BiOS mechanism has a strong effect to the lobster life cycle, particularly in the southern and middle Adriatic. The overall increase in temperatures in the future climate might result in more prominent migration of *H. gammarus* towards the northern Adriatic, causing a decline in lobster catch in south and middle Adriatic.

Keywords: *Homarus gammarus*, abundance, BiOS, sea bottom temperature

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RAZVOJ METODOLOGIJE OCJENE PROSTORA KROZ POGODNOSTI STANIŠTA ZA PTICE ZA POTREBU MONITORINGA NA PODRUČJU RIJEKE DRAVE

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Slatkovodni ekosustavi predstavljaju žarišta biološke raznolikosti, ali su nerijetko pod velikim antropogenim pritiscima. Rijeka Drava, a posebno njen hrvatski dio toka, ističe se kao utočište za brojne organizme vezane uz navedene ekosustave. Kako bi se revitalizirala vrijedna staništa, u sklopu projekta DRAVA LIFE sanirat će se ključne prirodne osobine riječnog ekosustava na sedam pilot područja. Biotički monitoring uključuje praćenje biljnih i životinjskih vrsta prije i nakon revitalizacije, što uključuje i praćenje indikatorskih vrsta ptica. U svrhu utvrđivanja nultog stanja te praćenja stanja ekosustava, provedeno je terensko istraživanje prije provedbe zahvata. Kako bi se ocijenilo stanje staništa za ptice razvijena je metodologija na temelju ekologije indikatorskih vrsta, modificirane detaljne karte staništa (izrađene u sklopu ovog projekta), literaturnih i terenskih podataka predmetnog područja. Kreirane su karte s ocjenjenim prisutnim staništima, s obzirom na njihov značaj za ornitofaunu, te zabilježenu prisutnost indikatorskih vrsta ptica. Uz ocjenu prostora, ovaj nam pristup omogućuje i uvid u pritiske na ornitofaunu koji nisu vezani uz manjak pogodnih staništa. Nakon provedbe revitalizacije istom će se metodologijom dobiti prostorni raspored i količina pogodnih staništa za ugroženu ornitofaunu te tako pokazati učinkovitost ovakvih revitalizacija na riječne ekosustave.

Ključne riječi: praćenje stanja, Natura 2000, slatkovodni ekosustavi, ornitofauna

DEVELOPMENT OF METHODOLOGY FOR SPATIAL EVALUATION USING HABITATS SUITABILITY FOR BIRDS - DRAVA RIVER MONITORING STUDY

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Freshwater ecosystems represent focal points for biodiversity, but they are often under great anthropogenic pressure. Drava River, especially the Croatian part, stands out as a shelter for numerous organisms associated with these ecosystems. In order to revitalise valuable habitats, the key natural features of the river ecosystem will be rehabilitated at seven sites as a part of

the Drava Life Project. Biotic monitoring refers to the monitoring of flora and fauna, before and after the revitalisation, and includes monitoring of indicator bird species. Fieldwork research before the revitalization was conducted, with the aim of determining the baseline state and using it in monitoring of the ecosystems. In order to estimate the habitats condition, a methodology was developed based on the ecology of indicator bird species, modified habitat map (developed as a part of this Project), literature and fieldwork data. The maps which were created contained evaluated habitats, considering its significance for birds, and also using the information about presence of indicator bird species, based on the fieldwork data. This approach, along with the spatial evaluation, also indicates the pressures on ornithofauna that are not based on the lack of the suitable habitats. After the revitalisation, the same methodology will be used to obtain spatial layout and to quantify the suitable habitats for endangered birds that will show us the effectiveness of revitalisations of the river ecosystems.

Keywords: monitoring, Natura 2000, freshwater ecosystems, ornithofauna

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VREDNOVANJE USLUGA EKOSUSTAVA – NOVI ALAT U ZAŠTITI PRIRODE

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Posljednjih nekoliko godina tema usluga ekosustava postaje sve zastupljenija na globalnoj i europskoj razini, a sam koncept važnim alatom u zaštiti prirode i okoliša. Koncept usluga ekosustava naročito dobiva na važnosti po usvajanju Strateškog plana Konvencije o biološkoj raznolikosti za razdoblje 2010-2020 (CBD, 2010), a razvijen je kako bi pomogao u vrednovanju prirodnih koristi i time ojačao argumente za očuvanje ekosustava koji ljudima donose mnoge dobrobiti. Kao takav može biti korišten u širokom spektru ciljeva i područja: zaštita i očuvanje prirode, ekonomski razvoj i smanjenje siromaštva, planiranje i procjena infrastrukture, upravljanje vodnim resursima, poljoprivreda i šumarstvo, smanjenje rizika od klimatskih promjena i nepogoda, prostorno planiranje, ribarstvo i upravljanje obalnim morem. U sklopu projekta „Tršćaci - vrednovanje usluga slatkovodnih ekosustava“ provedeno je istraživanje o razini svijesti o uslugama ekosustava i njihovom vrednovanju u Hrvatskoj kod različitih korisnika. Kroz provedene upitnike željeli smo valorizirati koliko je poznavanje ove tematike, koliki je interes za nju, koriste ili ga stručnjaci praktično u svom poslu te kroz tu analizu procijeniti je li koncept vrednovanja usluga ekosustava trenutno korisno i učinkovito sredstvo za zaštitu prirode.

Ključne riječi: usluge ekosustava, zaštita prirode, tršćaci, slatkovodni ekosustavi

ECOSYSTEM SERVICES ASSESSMENT – NEW TOOL IN NATURE PROTECTION AND CONSERVATION

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In the last decade, studies about ecosystem services are gaining more attention at the global and European level, while the concept itself is becoming an important tool in the nature protection and conservation. The importance of the concept of ecosystem services assessment has become particularly appreciated after adaptation of the Strategic Plan for Biodiversity 2011-2020 of the Convention on Biological Diversity (CBD, 2010). It was developed in order to help evaluate the natural benefits thus strengthening the arguments in favour of protection of the ecosystems. It can be used in a broad spectrum of goals and areas: nature conservation and protected areas, economic development and poverty reduction, infrastructure planning or assessment, water resources management, agriculture and forestry, climate change or disaster risk reduction, spatial planning, fisheries and coastal marine management. Within the project „Reed beds – freshwater ecosystem services assessment", study has been conducted about the level of awareness about the ecosystem services in Croatia between different groups of users. Through conducting questionnaires, we wanted to evaluate the familiarity with the theme, the interest about it, if this approach is being practically used and, through these analyses, assess if the ecosystem services assessment concept is potentially useful and efficient means in the nature protection.

Keywords: ecosystem services, nature protection, reed beds, freshwater ecosystems

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UGROŽENE, STROGO ZAŠTIĆENE I RIJETKE VASKULARNE BILJKE U HRVATSKOJ – NOVI PRILOZI S PODRUČJA GRADA SLATINE

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Vaskularna flora područja grada Slatine i okolice bila je nedovoljno poznata sve do terenskih istraživanja novijeg datuma. Stoga se od 2009. godine, kroz brojne terenske izlaske, provode detaljna opažanja koja se trenutno nalaze u svojoj završnoj fazi. Zaključno s 2016., biljna raznolikost istraženog područja dosegla je broj od 830 biljnih svojti, raspodijeljenih u 109 porodica i 420 rodova. Nakon konačne obrade podataka, procjenjuje se kako će ovaj broj premašiti 900 biljnih svojti, uključujući ornamentalne primjerke iz vrtova i parkova. Tijekom istraživanja zabilježeno je 54 važnih biljaka koje su pod statusom ugroženosti (kategorije Crvene liste CR, EN, VU) ili stroge zakonske zaštite, ili su pak u Hrvatskoj relativno rijetke i ograničenog areala, posebice u istočnim dijelovima zemlje. Kritično ugrožene svojte, konkretno *Myosurus minimus*, *Scirpus mucronatus* i *Scirpus supinus*, kao vrste s najvećim rizikom od izumiranja, isključivo su vezane za povremeno plavljene poljoprivredne površine na području Slatine. Pripadnici porodice Orchidaceae primjer su zakonom strogo zaštićenih vrsta, među koje također ubrajamo i mnoge biljke vezane za poplavna nizinska staništa. Dodatno, *Cardamine parviflora*, *Carpesium cernuum*, *Senecio sylvaticus* ili *Veronica peregrina* poznate su s malog broja lokaliteta i mogu se smatrati rijetkima ili nedovoljno istraženima. Nove spoznaje usmjerene su na poboljšanje kvalitete distribucijskih podataka vezanih za važne biljne vrste unutar granica Hrvatske.

Ključne riječi: vaskularna flora, Crvena lista, strogo zaštićene, Slatina, Hrvatska

THREATENED, STRICTLY PROTECTED AND RARE VASCULAR PLANTS IN CROATIA – NEW CONTRIBUTIONS FROM THE CITY OF SLATINA

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Vascular flora of Slatina and its neighbouring areas was poorly known prior to the recent field researches. Therefore, extensive surveys with numerous field visits have been conducted since 2009 and are currently in the final stages. At the end of 2016, plant diversity of the surveyed area has reached a total of 830 plant taxa, categorized into 109 families and 420 genera. After the final data analysis, the number is estimated to surpass 900 plant taxa, including ornamental specimens from gardens and parks. In this research, 54 important plants have been recorded as threatened (Red List categories CR, EN, VU) or under strict legal protection, or are otherwise of relatively rare and restricted occurence in Croatia, especially in the eastern parts of the country. Critically endangered taxa, namely *Myosurus minimus*, *Scirpus mucronatus* and *Scirpus supinus*, as species with the highest risk of extinction, are exclusively associated with periodically inundated agricultural land in the area of Slatina. Members of the Orchidaceae family are an example of species strictly protected by the law, further including many plants related to the flooded lowland habitats. Additionally, *Cardamine parviflora*, *Carpesium cernuum*, *Senecio sylvaticus* or *Veronica peregrina* with a small number of known sites can be considered rare or under-recorded. New observations are aimed towards the improvement of quality in distributional data regarding valuable plant species within the borders of Croatia.

Keywords: vascular flora, Red List, strictly protected, Slatina, Croatia

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VELIČINA POPULACIJE I KVALITETA MRIJESNIH STANIŠTA LOMBARDIJSKE SMEĐE ŽABE (*Rana latastei*) U ISTRI, HRVATSKA

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Lombardijska smeđa žaba (*Rana latastei*) strogo je zaštićena vrsta u Hrvatskoj, za čije je očuvanje izdvojeno sedam područja ekološke mreže Natura 2000. Ranija istraživanja nisu utvrdila najpogodnije značajke mrijesnih staništa i njihove optimalne vrijednosti, a veličina populacije nije procijenjena za područje ekološke mreže HR2001016 Kotli. Cilj ovog istraživanja bio je utvrditi veličinu populacije i kvalitetu mrijesnih staništa lombardijske smeđe žabe u svim područjima ekološke mreže u Hrvatskoj izdvojenim za ovu vrstu. Da bi se odgovorilo na ova pitanja provedeno je terensko istraživanje u ožujku 2017. godine u središnjoj i sjevernoj Istri. Na temelju prikupljenih podataka veličina populacije lombardijske smeđe žabe procijenjena je na 15 000 - 19 000 jedinki, s najvećim dijelom populacije u Motovunskoj šumi. Analiza podataka pokazuje da su sva mrijesna staništa na području istraživanja (uključujući potoke, lokve i kanale) degradirana. Broj jajašaca pokazuje pozitivnu korelaciju s nagibom i pH vrijednosti te negativnu korelaciju s koncentracijom kisika otopljenog u vodi. Nadalje, rezultati generalnog linearnog modela (GLM) potvrđuju značajan odnos između broja jajašaca i tri od devet mjerenih varijabli (nagib, količina vodenih biljaka i provodljivost vode). Na temelju rezultata predložene su dodatne mjere očuvanja, kao i dodatna istraživanja koja je potrebno uključiti u plan upravljanja za ovu vrstu.

Ključne riječi: *Rana latastei*, lombardijska smeđa žaba, veličina populacije, kvaliteta mrijesnih staništa, Hrvatska

POPULATION SIZE AND BREEDING HABITAT QUALITY OF ITALIAN AGILE FROG (*Rana latastei)* IN ISTRIA, CROATIA

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Italian agile frog (*Rana latastei*) is a strictly protected endangered species in Croatia, with seven Natura 2000 sites designated for its conservation. Previous research did not determine the most favourable breeding habitat characteristics and their optimal values, and population size was never estimated for the Natura 2000 site HR2001016 Kotli. The main objective of this research was to determine the population size and the breeding habitat quality of *Rana latastei* in all designated sites in Croatia. In order to answer these questions, the field research was conducted during March 2017 in central and northern Istria. Based on the collected data the population size in the research area is estimated to 15 000 – 19 000 individuals, with the largest part of population in the Motovun forest. The data analyses show that all breeding habitats (including streams, ponds and channels) are degraded. The number of the egg masses shows a positive correlation with slope and pH level, and negative correlation with dissolved oxygen. Also, the results of the general linear model (GLM) confirm significant relationship between the number of the egg masses and three of the nine studied variables (slope, amount of the water plants, and water conductivity). Based on the results, we propose implementation of additional conservation measures, as well as additional research which should be included in a management plan for this species.

Keywords: *Rana latastei*, Italian agile frog, population size, breeding habitat quality, Croatia

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CENTAR ZA INVAZIVNE VRSTE - KONCEPT GRAĐANI ZNANSTVENICI U MONITORINGU I UPRAVLJANJU INVAZIVNIM STRANIM VRSTAMA NA LOKALNOJ RAZINI

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Monitoring i upravljanje invazivnim stranim vrstama (ISV) koje uvelike utječu na usluge ekosustava predstavljaju veliki izazov za temeljito istraživanje ove problematike. Kontrola i smanjenje utjecaja invazivnih vrsta na zavičajne vrste spadaju među najveće probleme zaštite prirode u svijetu. Kvalitetno obrazovanje i jačanje svijesti o ovom problemu ključni su elementi za sprječavanje unosa i širenja ISV-a. Pod inicijativom znanstvenika te uz potporu lokalne samouprave i obrazovnih ustanova, pokrenut je Centar za invazivne vrste blizak građanima, turistima i školama. U suradnji s brojnim lokalnim, nacionalnim i međunarodnim dionicima, Centar pruža sustavno obrazovanje, mapiranje, praćenje i podizanje svijesti javnosti o problemu lokalnih invazivnih vrsta. Glavni fokus za učinkovito upravljanje ISV-om stavljen je na angažmanu lokalne zajednice koji prolaze kroz edukaciju te sudjeluju u prikupljanju podataka na terenu. Koncept građani znanstvenici, kao relativno nova metoda u Hrvatskoj koja pruža sudjelovanje javnosti u istraživanjima omogućava znanstvenicima lakše

prikupljanje i analizu velikih skupova podataka. Istodobno, projekti ovakvog tipa pružaju priliku javnoj zajednici da stekne znanje i podigne svijest o znanstvenoj metodi. Glavni cilj ovog rada je predstaviti primjer dobre primjene koncepta građani znanstvenici u monitoringu invazivnih vrsta u gradu Poreču.

Ključne riječi: Centar za invazivne vrste, građani znanstvenici, obrazovanje, prikupljanje podataka

INVASIVE SPECIES CENTRE - CITIZEN SCIENCE APPROACH TO INVASIVE SPECIES MONITORING AND MANAGEMENT AT A LOCAL LEVEL

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Invasive alien species (IAS) impact on ecosystem services are thoroughly studied in many ways, although their practical monitoring and management present a great challenge. The control of invasive species and the reduction of their impact on native species today is one of the greatest issues in nature protection in the world. Since education and awareness are the key elements for the prevention of IAS spread, a local Centre close to citizens, tourists and schools is an efficient tool in IAS management. Initiated by scientists, supported by the local government and embraced by teachers, the Invasive Species Centre (ISC) is providing a systematic approach to the education, mapping, monitoring and raising of public awareness of the local invasive species problem. Although the ISC collaborates with numerous local, regional, national and international entities, its main focus is on the engagement of the local community. Citizens are mainly involved in education and field data collection. Citizen science as a rather new approach in Croatia is a new method for public participation in scientific research. Citizen science provides researchers with the means to gather or analyse large datasets. At the same time, citizen science projects offer an opportunity for a public community to gain knowledge and to raise awareness about the scientific process. The main goal of this paper is to present a good example of a citizen science approach in ecology in the city of Poreč.

Keywords: Invasive Species Centre, Citizen Science, education, data collection

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SEZONSKE PROMJENE STRUKTURE FITOPLANKTONA PLITKOG JEZERA U POPLAVNOM PODRUČJU

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Cilj ovog istraživanja je utvrditi utjecaj okolišnih čimbenika na strukturu i dinamiku fitoplanktona plitkog jezera (Kopačko jezero, površina 200-250 ha, dubina 2-5 m) u poplavnom području Kopačkog rita. Istraživanje je provedeno u različitim sezonskim i hidrološkim uvjetima, u zimskom, kasnoproljetnom i ljetnom razdoblju. Koncentracije hranjivih tvari bile su visoke tijekom cijelog razdoblja istraživanja, a posebno se isticao sadržaj ukupnog fosfora (od 560 µg/L do 1804 µg/L). Utvrđeno je 129 fitoplanktonskih vrsta,

a brojnost se kretala od 8,83x106 do 87,52x106 jedinki/L. Stabilni hidrološki uvjeti i visoke koncentracije hranjivih tvari u zimskom razdoblju omogućili su razvoj vrsta iz C, D i X1 (X3, J i TB) funkcionalnih skupina fitoplanktona. Visoki vodostaj Dunava i plavljenje poplavnog područja u kasnoproljetnom razdoblju pogodovali su vrstama iz skupina J, Y i X1 (C i D), dok su tijekom stabilnih hidroloških uvjeta i pri nižim koncentracijama hranjivih tvari dobro bile razvijene cijanobakterije iz H1 i S1 te klorokokalne zelene alge iz funkcionalnih skupina J i X1. Rezultati pokazuju da je unatoč razlikama u hidrološkim uvjetima i koncentraciji dostupnih hranjivih tvari, sastav fitoplanktona u kasnoproljetnom i ljetnom razdoblju ovisio o strukturi zimske zajednice. Stoga je praćenje zimske fitoplanktonske zajednice važno u svrhu procjene i predviđanja godišnjih promjena fitoplanktona u plitkim jezerima.

Ključne riječi: sukcesije, zimski fitoplankton, funkcionalne skupine, zaštićeno područje

SEASONAL CHANGES OF PHYTOPLANKTON IN SHALLOW FLOODPLAIN LAKE

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The study was conducted to determine the influence of environmental parameters on phytoplankton structure and dynamics in the shallow lake (Lake Kopačko, 200-250 ha, depth 2-5 m) within the Kopački Rit floodplain. The investigation was carried out in different seasonal and hydrological conditions during winter, late spring and summer period characterised by high nutrient concentrations, especially total phosphorus (from 560 µg/L to 1804 µg/L). A total of 129 phytoplankton species were found, while phytoplankton abundance ranged from 8.83 x 106 to 87.52 x 106 individuals/L. Stable hydrological conditions and high nutrient concentrations during the winter period were favourable for the development of C, D and X1 (X3, J and TB) phytoplankton functional groups. High Danube water level and flooding conditions in late spring favoured the species from groups J, Y and X1 (C and D), while during the stable hydrological conditions and lower concentrations of nutrients, cyanobacteria from H1 and S1 and chlorococcal green algae from J and X1 functional groups were well developed. Results show that despite the differences in hydrological conditions and nutrient concentrations, phytoplankton structure was highly dependent on the winter community. Therefore, studies on winter phytoplankton communities are important for assessing and predicting annual changes of phytoplankton in shallow lakes.

Keywords: succession, winter phytoplankton, functional classification, protected area

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ODREĐIVANJE STUPNJA SUKCESIJE TRAVNJAČKIH POVRŠINA ODRANSKOG POLJA

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Različite vrste i frekvencije poremećaja rezultiraju različitim fizionomskim tipovima vegetacije, što se može upotrijebiti za određivanje stupanja sukcesije. Zbog izostanka adekvatnog

gospodarenja, travnjačke površine na Odranskom polju u fazi su zarastanja što ugrožava opstanak travnjačkih staništa te vrsta vezanih uz njih. Određivanjem stupnja sukcesije travnjačkih površina moguće je bolje planirati očuvanje travnjaka određivanjem potencijalnih površina za revitalizaciju i trajno održavanje travnjačkih površina. Istraživano područje obuhvaća dio travnjačkih površina Odranskog polja unutar Zagrebačke županije. Na temelju daljinskih istraživanja i terenskog rada određeni su fizionomski tipovi vegetacije prisutni na području istraživanja. Za svaki fizionomski tip definiran je referentni poligon na temelju čega je napravljena fizionomska vegetacijska karta. Kako bi odredili stupanj sukcesije istraživanog područja, fizionomska vegetacijska karta je reklasificirana prema tri stupnja sukcesije. Više od 50% površine istraživanog područja spada u klasu 'Visok stupanj sukcesije', što je uglavnom posljedica širenja šikare vrste *Amorpha fruticosa*. Istraživanje ukazuje na visok stupanj sukcesije istraživanog područja, a time i na ugroženost travnjačkih površina Odranskog polja. Na temelju dobivenih rezultata moguće je definirati program očuvanja travnjaka i odrediti površine na kojima bi obnova travnjaka mogla imati uspješne rezultate.

Ključne riječi: sukcesija, prioritizacija, fizionomska klasifikacija, Odransko polje, *Amorpha fruticosa*

ASSESSING THE SUCCESSION DEGREE OF GRASSLAND AREAS IN ODRANSKO POLJE

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Different disturbance types and frequencies result in different types of physiognomic vegetation that can be used for assessing the degree of succession. Lack of adequate management caused succession of grassland areas in Odransko polje which threatens the survival of grassland habitats and species relying on them. Assessment of the succession degree enables better planning of grassland conservation by defining areas for revitalization and long-term management. The study area encompasses some of the grasslands in Odransko polje (Zagreb County). Physiognomic vegetation types of the study area were determined by remote sensing and field assessment. For every physiognomic vegetation type a reference polygon was defined in order to create a physiognomic vegetation map. To determine the succession degree of the study site, the physiognomic vegetation map was reclassified according to the three degrees of succession. More than 50% of the study area was classified as 'a high degree of succession', which is mostly caused by encroachment of the species *Amorpha fruticosa*. This study shows a high degree of succession of grasslands of Odransko polje and indicates their vulnerability. Our results can be used to define a conservation programme and identify areas for their successful revitalisation.

Keywords: Succession, Prioritization, Physiognomic classification, Odransko polje, *Amorpha fruticosa*

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PREGLED VLAŽNIH STANIŠTA NA JADRANSKIM OTOCIMA

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Vlažna staništa jedna su od najugroženijih staništa u Mediteranskoj regiji i u posljednjih su sto godina suočena s ubrzanim nestankom ili u vidu smanjenja brojnosti ili smanjenja površine. Kako bi procjenila trenutno stanje vlažnih staništa na jadranskim otocima, Udruga Hyla je zajedno s partnerima počela provoditi dva projekta, "KARST - the Dinaric Arc biodiversity conservation programme" i "MedIsWet". U sklopu projekata prikupljeni su svi podatci o vlažnim staništima te su sva otočka vlažna staništa mapirana uz pomoć satelitskih snimki. Također su korištene topografske karte iz različitih vremenskih perioda, kako bi se uspjele zabilježiti promjene u brojnosti vlažnih staništa kroz povijest. Kao rezultat napravljen je prvi popis otočkih vlažnih staništa, koji se sastoji od 2739 vlažnih staništa na 71 otoku. Najbrojnija vlažna staništa su krške lokve (1459), od kojih se njih 1329 nalazi unutar zaštićenih i Natura 2000 područja. U sklopu projekata također se posjećuje većina jadranskih otoka te se popisuju uzroci ugroženosti i procjenjuje se status vlažnih staništa. Do sada su posjećena četiri otoka te su podatci uspoređeni s rezultatima dobivenima topografskim kartama i satelitskim snimkama i utvrđen je gubitak vlažnih staništa na tim otocima od 10% u posljednjih 50-ak godina. Glavni razlozi nestanka su prirodna sukcesija i namjerno zatrpavanje. Dobiveni rezultati temelj su za buduću zaštitu te restauracijske aktivnosti otočkih vlažnih staništa.

Ključne riječi: lokve, potoci, obalna vlažna staništa, Natura 2000

AN OVERVIEW OF WETLANDS OF THE ADRIATIC ISLANDS

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Wetlands in the Mediterranean area represent one of the most endangered habitats, which have in the last century faced significant losses in numbers and in surface coverage. In order to assess the current status of the wetlands on the Adriatic islands, Association Hyla, along with partners, started to work on two projects, "KARST - the Dinaric Arc biodiversity conservation programme" and "MedIsWet". All the available data about the wetlands were unified, and all island wetlands were mapped using satellite imagery. In order to assess the possible changes in the number of wetlands in the past, topographic maps of different time periods were also consulted. Accordingly, the first list of wetlands on the Adriatic islands was created, containing a total of 2739 wetlands on 71 islands. Most numerous wetlands are karstic ponds (1459), of which 1329 are located in the protected or Natura 2000 areas. During this projects most of the Adriatic islands will be visited and status of the wetlands, along with their threats will be assessed. Until now four islands were visited and collected data compared with the results from topographic maps and satellite imagery. Loss of 10% of wetlands in the last 50 years on that islands has been recorded. Main reason for habitat loss is ecological succession and deliberately in-filling. With all gained results a first baseline for the future conservation and restoration actions of the Island wetlands will be created.

Keywords: ponds, streams, coastal wetlands, Natura 2000

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ANALIZA SKLONOSTI DIVLJE MAČKE (*Felis silvestris silvestris* L.) TIPOVIMA STANIŠTA (PO NKS) NA PODRUČJU BILOGORE

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Do sada su u Hrvatskoj telemetrijski praćene dvije jedinke divlje mačke koje su obilježene VHF ogrlicom. Divlja mačka Bela obilježena je u ožujku 2013. na obronicima Bilogore te je praćena 173 dana. Mužjak Felix je obilježen na nizinskom području Bilogore u srpnju 2014. i praćen je 238 dana. Tijekom redovitih obilazaka praćeno je njihovo kretanje metodom triangulacije. Napravljena je analiza kretanja te indeks sklonosti divlje mačke prema pojedinom tipu staništa (po NKS) kao omjer učestalosti boravljenja divlje mačke u nekom staništu i zastupljenosti tog staništa na području kretanja. Prema indeksu sklonosti, Bela je preferirala mozaike kultiviranih površina (I.2.1., 4,04), mješovite hrastovo-grabove i čiste grabove šume (E.3.1., 1,27), a slabije se zadržavala u staništu srednjoeuropske neutrofilne do slaboacidofilne, mezofilne bukove šume (E.4.1., 0,74) te na intenzivno obrađivanim oranicama na komasiranim površinama (I.3.1., 0,55). Felix je preferirao intenzivno obrađivane oranice na komasiranim površinama (I.3.1., 1,28) i mozaike kultiviranih površina (I.2.1., 1,25) te, iako na području kretanja prevladavaju mješovite hrastovo-grabove i čiste grabove šume (E.3.1., 58,33%), indeks sklonosti ovom tipu staništa iznosio je 0,97. Rezultati ovog istraživanja doprinose učinkovitijoj zaštiti te strogo zaštićene životinje, odnosno zaštiti staništa koja su nužna za njezin opstanak.

Ključne riječi: europska divlja mačka, *Felis silvestris silvestris* L., telemetrija, sklonost staništu, Hrvatska

ANALYSIS OF WILD CAT'S (*Felis sivestris silvestris* L.) PREFERENCE TO THE HABITAT TYPES (BY NCH) IN THE BILOGORA MOUNTAIN, CROATIA

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Two individuals of wildcat have been tagged with a VHF collar and tracked by telemetry in Croatia to date. The female, Bela was tagged on the slopes of Bilogora in March 2013 and was tracked for 173 days. The male Felix was collared in the lowlands area of Bilogora in July 2014 and was monitored for 238 days. Their location was determined by a triangulation method during regular patrolling of the terrain. The movement was analysed and the index of wildcat's habitat preference (the ratio of the time wildcat spent in certain habitats and the presence of

these habitats in their home range) (by National Classification of habitats) was calculated. According to the habitat preference index, Bela preferred mosaics of cultivated areas (I.2.1, 4.04), mixed oak-hornbeam forests and hornbeam forests (E.3.1, 1.27), Central European neutrophilous to acidophilous, mezophilous beech forests (E.4.1, 0.74) and heavily cultivated arable lands (I.3.1, 0.55). Felix preferred heavily cultivated arable lands (I.3.1, 1.28) and mosaics of cultivated areas (I.2.1, 1.25). Although mixed oak-hornbeam forests and hornbeam forests (E.3.1, 58.33%) prevail in Felix's home range, its habitat preference index to this type of habitat was only 0.97. Results of this research contribute to more effective protection of this strictly protected animal, i.e. to the protection of habitats that are necessary for its survival.

Keywords: European wildcat, *Felis silvestris silvestris* L., telemetry, habitat preference, Croatia

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ANALIZA SKLONOSTI SIVOG VUKA (*Canis lupus* L.) TIPOVIMA STANIŠTA (NKS) U PP BIOKOVO

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U PP Biokovo, istraživanje i monitoring vukova kontinuirano se provodi od 2013. godine. Svrha istraživanja je procijeniti broj i stabilnost čopora te brojnost jedinki unutar čopora. Kao jedna od metoda, koristi se GPS telemetrija. Na osnovi zabilježenih koordinata napravljena je analiza kretanja te indeks sklonosti vukova prema pojedinom tipu staništa (po NKS) kao omjer učestalosti boravljenja vuka u nekom staništu i zastupljenosti tog staništa na području kretanja. Od 2015. do 2017. uhvaćene su i obilježene GPS ogrlicom 3 jedinke vuka. Analizom kretanja utvrđeno je da u PP Biokovo i kontaktnoj zoni prebivaju 2 čopora te da dvije obilježene jedinke pripadaju sjeverozapadnom (SZ) čoporu, a treća jedinka jugoistočnom (JI). JI čopor se zadržavao u centralnom dijelu Biokovskog masiva, dok se SZ najčešće zadržavao u nižim dijelovima Zabiokovlja te time i na značajno različitim staništima. Sukladno tome, razlikuje se indeks sklonosti staništa jedinki tih dvaju čopora. SZ čopor najskloniji je staništu mozaika kultiviranih površina (1,77), zatim staništu primorske, termofilne šume i šikare medunca (1,18) i staništu submediteranskih i epimediteranskih suhih travnjaka (0,60). JI čopor najskloniji je staništu tirensko-jadranskih vapnenačkih stijena (2,89), zatim staništu submediteranskih i epimediteranskih suhih travnjaka (1,53) i staništu bušika (1,16). Navedena istraživanja omogućuju stupnjevanje važnosti tipova staništa te zoniranje zaštićenih područja.

Ključne riječi: sivi vuk, *Canis lupus* L., GPS telemetrija, sklonost staništu, PP Biokovo

ANALYSIS OF GREY WOLF'S (*Canis lupus* L.) PREFERENCES TO THE HABITAT TYPES (BY NCH) IN THE NATURE PARK BIOKOVO

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Research and monitoring of wolves in the Nature Park Biokovo has been continually performed since 2013. The purpose of the research is to estimate the number and stability of wolf packs and the number of individuals within a pack. GPS telemetry is used as one of the methods. Based on the recorded coordinates, the movement was analyzed and the index of wolf's preference (the ratio of the time wolf spent in certain habitats and the presence of these habitats in their home range) to the habitat type (by National Classification of habitats) was calculated. Three wolves were GPS collared between 2015 and 2017. In NP Biokovo and the contact zone there are 2 packs. Two of the collared wolves belong to the NW pack and the third wolf is a member of the SE pack. The SE pack stayed in the central part of the Biokovo Massif, while the NW pack mostly remained in the lower parts of hinterland of Biokovo. Accordingly, the habitat preference index of these two packs differs. The NW pack mostly prefers mosaics of cultivated areas (1.77), then Submediterranean, termophilous forests and scrubs of *Quercus pubescens* (1.18), Sub-Mediterranean and Epimediterranean dry grasslands (0.60), etc. The SE pack mostly prefers Lime stone of Tyrrhenian-Adriatic region (2.89), then Sub-Mediterranean and Epimediterranean dry grasslands (1.53), Garrigues (class *Erico-Cistetea*) (1.16), etc. This study enables determining levels of importance of different habitat types and zoning of protected areas.

Keywords: Grey wolf, *Canis lupus* L., GPS telemetry, habitat preference, Nature Park Biokovo

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HISTOPATHOLOGICAL FINDING OF LIVER, KIDNEY AND GILL TISSUES OF FISH FROM LAKE OHRID

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Histopathological biomarkers can be indicators for the effects on organisms by various anthropogenic pollutants and represent reflection of the overall health of the entire population in the ecosystem. The alterations in cells and tissues in vertebrate fish are recurrently used biomarkers in many studies, but such changes occur in all vertebrates and invertebrates inhabiting the aquatic basins. Liver, kidney and gill pieces of 12 individuals of fish collected from Lake Ohrid, were excised and processed for standard histopathological analysis. The obtained results revealed pathological changes in the liver, kidney and gill tissue.

Keywords: histopathology, liver, kidney, gill, Lake Ohrid

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INVAZIVNA FLORA GRADOVA DUGO SELO I SAMOBOR

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Istraživanja invazivne flore gradova Dugog Sela i Samobora provedena su tijekom vegetacijskih sezona 2012. i 2016. god., na plohama dimenzija 250x250 m. Istražene su 54 plohe u Dugom Selu i 109 ploha u Samoboru. Komparativna analiza prostorne rasprostranjenosti u istraživanom periodu pokazala je da se u Dugom Selu broj novih nalaza invazivnih vrsta povećao za 60,35%, a u Samoboru za 90,01%. Uočene promjene povećavanja broja invazivnih vrsta više su izražene u urbanim dijelovima gradova, a manje na suburbano-ruralnih ploha. Najveće povećanje učestalosti pojavljivanja u navedenom razdoblju pokazale su vrste *Erigeron annuus* (L.) Pers. s.l., *Conyza canadensis* (L.) Cronquist i *Ambrosia artemisiifolia* L. Vrsta *E. annuus* proširila se u Samoboru s 147 lokacija (unutar 85 istraživanih ploha) na 241 lokaciju (unutar 96 ploha), a u Dugom Selu s 44 lokacije (28 ploha) na 66 lokacija (94 ploha); *C. canadensis* u Samoboru se proširila s 111 lokacija (71 ploha) na 198 lokacija (87 ploha), a u Dugom Selu s 29 lokacija (21 ploha) na 60 lokacija (54 plohe); *A. artemisiifolia* u Samoboru se proširila s 65 lokacija (48 ploha) na 134 lokacije (70 ploha), a u Dugom Selu s 26 lokacija (25 ploha) na 47 lokacija (33 plohe). Ovim istraživanjem po prvi puta se sustavno istražila i analizirala invazivna alohtona flora Dugog Sela i Samobora te je ukazano na potrebu intenzivnijeg monitoringa i kontroliranja njenog širenja u svrhu ublažavanja negativnog utjecaja u gradovima Zagrebačke županije.

Ključne riječi: invazine alohtone biljke (IAS), Zagrebačka županija, prostorna rasprostranjenost i širenje

INVASIVE FLORA OF THE CITIES OF DUGO SELO AND SAMOBOR

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Research of invasive flora of the cities of Dugo selo and Samobor was conducted during the vegetation seasons of 2012 and 2016 on plots measuring 250x250 m2. Altogether, 54 plots were surveyed in Dugo Selo and 109 in Samobor. The comparative analysis of the spatial distribution of invasive taxa during the study period showed that in Dugo Selo the number of new IAS findings has risen by 60,35%, and in Samobor by 90,01%. The increase of invasive plants was more expressed in urban parts of the cities, and less in the suburban-rural areas. The largest increase in frequency of occurrence was shown by the species *Erigeron annuus* (L.) Pers. s.l., *Conyza canadensis* (L.) Cronquist and *Ambrosia artemisiifolia* L.: *E. annuus* has spread in Samobor from 147 locations (within 85 surveyed plots) to 241 locations (within 96 plots), and in Dugo Selo from 44 (28 plots) to 66 locations (94 plots); C. canadensis in Samobor expanded from 111 sites (71 plots) to 198 (87 plots), and in Dugo Selo from 29

locations (21 plots) to 60 (54 plots); *A. artemisiifolia* in Samobor expanded from 65 (48 plots) to 134 locations (70 plots), and in Dugo Selo from 26 (25 plots) to 47 locations (33 plots). This was the first organised research of invasive flora in Dugo Selo and Samobor, which has pointed to the need for more intensive monitoring and control of IAS spread in order to mitigate their negative impact in the towns of the Zagreb County.

Keywords: invasive alien plants (IAS), Zagreb County, spatial distribution and spread

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GEOKEMIJA OTOLITA KAO ALAT ZA ODREĐIVANJE KRETANJA KOMARČE, *Sparus aurata* LINNAEUS 1758. IZMEĐU PRIJELAZNIH I PRIOBALNIH VODA

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Otoliti riba su metabolički inertne aragonitne strukture sa sastavom koji je pod utjecajem fizikalnih i kemijskih obilježja okoliša. Ontogenetski, otoliti rastu i bilježe podatke o okolišu u kojem ribe obitavaju. Jezgra otolita predstavlja nedoraslu životnu fazu, dok rub otolita pruža informaciju o okolišu u kojem je jedinka boravila neposredno pred ulov. Razlike u geokemijskom sastavu otolita komarče, *Sparus aurata* u odnosu na otvoreno more, priobalne vode i estuarijsko područje su analizirane kako bi se utvrdili obrasci kretanja. U jesen 2017. godine prikupljeno je 9 odraslih jedinki komarče na 3 lokacije u istočnom Jadranu. Uz biometrijske mjere, izvađeni su im otoliti koji su oprani, očišćeni i pohranjeni u dekontaminiranim tubama za mikrocentrifugiranje. Na poprečnim presjecima otolita, na kojima su vidljivi jezgra i zone prirasta, provedena je geokemijska analiza laserske ablacije vezane na induktivni plazmatski maseni spektrometar (LA-ICP-MS). Određene su koncentracije ukupno 12 kemijskih elemenata duž maksimalne osi rasta, ali su samo Na, Mg, Sr, Ba, Mo, Li pokazali vidljive razlike između analiziranih jedinki. Utvrđene koncentracije su uspoređene u odnosu na različita staništa, a utvrđeni pikovi su povezani sa specifičnim dijelom otolita svake jedinke. Rezultati predstavljaju prvo istraživanje geokemije otolita komarče u Jadrana i otkrivaju potencijale ove metode za interpretaciju gibanja unutar različitih staništa.

Ključne riječi: komarča, geokemija, otoliti, migracija

ASESSMENT OF OTOLITH GEOCHEMISTRY FOR IDENTIFYING GILTHEAD SEABREAM, SPARUS AURATA LINNAEUS 1758. MOVEMENT BETWEEN MARINE WATERS AND ESTUARIES

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Fish otoliths are metabolically inert aragonite structures with a composition influenced by the physical and chemical properties of the environment. Otoliths grow continuously and record information on environmental conditions experienced by fish from hatching to capture. The otolith core, represents early developmental stages while the otolith edge provides information on the environment experienced by the individuals prior to capture. Differences in otolith geochemistry of gilthead seabream, *Sparus aurata* inhabited open sea, coastal waters and estuary were determined to evaluate movement pattern. In total nine adults of gilthead seabream were collected on three locations in the eastern Adriatic during 2017. In addition to biometric measures, otoliths have been extracted, cleaned and stored in decontamintaed microcentrifuge tubes. On the predetermined sections of otoliths with visible cores and growth marks, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to quantify concentrations of 12 chemical elements along maximum growth rates. Among them, just Na, Mg, Sr, Ba, Mo, Li showed clear differences between the individuals. Obtained concentrations and peaks were related to the different habitats and certain part of the otoliths for each individiual. The results represent the first geochemical study of *S. aurata* otoliths in the Adriatic and reveal the potentials of this method for the interpretation of ontogenetic movement between different habitat

Keywords: gilthead seabream, geochemistry, otoliths, migration

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OD TERENSKIH OPAŽANJA DO ORGANIZIRANOG BARATANJA S PODACIMA U HRVATSKOJ

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Razvoj informacijske tehnologije i upravljanja bazama podataka tijekom zadnjih nekoliko desetljeća omogućili su biolozima lako sakupljanje, obrađivanje i analiziranje velikih količina podataka. Usporedno, razvoj mrežnih sustava i pojava sve jačih pametnih telefona i tableta omogućili su pristup tim podacima iz bilo kojeg kraja svijeta putem interneta. Udruga Hyla je započela raditi na bazi podataka vodozemaca i gmazova prije deset godina, za internu upotrebu, koja se širila i na vretenca, kornjaše i leptire te njihova staništa. No u njoj nedostaje način prikaza podataka i mogućnost izravnog unosa, koji bi bili jednostavniji od e-maila ili društvenih mreža Udruge kako je to do sada bilo uobičajeno. U svijetu su razvijene mnoge baze za bilježenje bioraznolikosti (npr. Fauna Europaea, GBIF i iNaturalist). Među bazama koje su usredotočene na Hrvatsku postoji samo za floru – Flora Croatica, ali za faunu se tek razvijaju javno dostupni sustavi za bilježenje vrsta (npr. Marine Partnership, CroDolphin Little i Fauna.hr/NaturaList). Jednostavna i otvorenog koda, Biologer.org platforma nedavno je napravljena za Srbiju te uključuje mogućnost jednostavnog unosa podataka putem aplikacije za pametne telefone. Sustav je usvojen i u Udruzi Hyla, uz neke prilagodbe. Iako su trenutačno odvojene baze, još uvijek su kompatibilne – moguće ih je povezati u budućnosti, kao temelj pan-balkanske faunističke baze i baze staništa.

Ključne riječi: baza podataka, prikupljanje podataka, volonteri

FROM FIELD OBSERVATION TO ORGANISED DATA MANAGEMENT IN CROATIA

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Over the last several decades, many technological advances in information technologies and database management have made it easier for biologists to collect, handle and analyse large quantities of data. In parallel, advances in networking and the advent of ever powerful smartphones and tablets have made it possible to access that data via the internet from everywhere in the world. Association Hyla started working on an internal database for herpetofauna ten years ago, which was recently expanded to cover Lepidoptera, Coleoptera, Odonata, and their habitats. But it is lacking a way to present the data to the public and the ability for direct data input, instead of piecemeal collecting through various social media platforms. Many global biodiversity databases had been developed (e.g. Fauna Europaea, GBIF, and iNaturalist). However, a database focusing on Croatia exist only for flora (Flora Croatica Database) while for fauna several platforms are currently being developed (e.g. Marine Partnership, CroDolphin Little and Fauna.hr/NaturaList). A user friendly and open source database, Biologer.org, had been created recently for Serbia along with an application for smartphones. This system, with some adaptations, has been adopted by Association Hyla. Although currently separated, the databases are compatible and can be merged to serve as a foundation for a future pan-Balkan faunistic and habitat database.

Keywords: database, gathering of data, citizen science

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BIOMONITORING NAKUPLJANJA TEŠKIH METALA IZ ZRAKA POSREDSTVOM MAHOVINA RASLIH DUŽ GRADIJENTA NADMORSKE VISINE U PODRUČJU PLANINE RISNJAK

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Planine su jedan od najosjetljivijih ekosustava kojeg klimatske promjene pogađaju brže nego ostala kopnena staništa. Planine stoga predstavljaju jedinstvena područja za procjenu učinaka klimatskih promjena, uključujući prijenos atmosferskog onečišćenja na velike udaljenosti. Mahovine služe kao učinkovit bioindikator kakvoće zraka zbog velikog omjera površine i volumena te vrlo tanke kutikule. U ovom istraživanju ispitali smo varijacije u taloženju teških metala iz atmosfere duž visinskog gradijenta na području planine Risnjak upotrebom mahovina kao bioindikatorima. Kapacitet akumulacije teških metala uspoređen je između dvije vrste *Brachythecium rutabulum* i *Hylocomium splendens* sakupljenih tijekom kišne i suhe sezone 2016. godine, na 5 mjesta uzorkovanja na visini od 350 do 1450 m. Cd,

Cr, Pb i Zn analizirani su metodom optičke emisijske spektroskopije induktivno spregnutom plazmom (HRN ISO 22036:2011), dok je Hg analizirana metodom atomske apsorpcijske spektrometrije hladnih para (HRN ISO 16772:2009). Koncentracija Cd bila je u rasponu od 0,2 do 0,58 mg kg-1, Cr od 0,6 do 27 mg kg^{-1}, Hg od 0,036 do 0,136 mg kg^{-1}, Pb od 3,0 do 8,2 mg kg^{-1} i Zn od 8,6 do 82 mg kg^{-1} suhog tkiva. Najveće koncentracije teških metala izmjerene su u vrsti *B. rutabulum*, osobito u uzorcima sakupljenim u kišnoj sezoni na većim nadmorskim visinama, što može biti povezano s intenzivnim taloženjem metala iz atmosfere uslijed obimnijih kiša u razdoblju prije uzorkovanja.

Ključne riječi: biomonitoring, mahovine, tlo, onečišćenje, metali

BIOMONITORING OF ATMOSPHERIC HEAVY METAL DEPOSITION BY MOSSES GROWING ACROSS AN ELEVATION GRADIENT IN THE MOUNTAIN RISNJAK AREA

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Mountais are among the most sensitive ecosystems to climate change and are being affected at a faster rate than other terrestrial habitats. Mountains therefore represent unique areas for the assessment of climate-related impacts, including long-distance transport of air pollutants. Mosses serve as effective bioindicators of air quality because of their high surface-to-volume ratio and a very thin cuticle. In this study, we investigated variations in atmospheric heavy metal deposition across an elevation gradient in the Risnjak mountain region using mosses as bioindicators. The capacity for heavy metal accumulation was compared between two species, *Brachythecium rutabulum* and *Hylocomium splendens* collected during rainy and dry seasons in 2016, from 5 sampling sites at 350 to 1450 m elevation. Cd, Cr, Pb and Zn were analysed by inductively coupled plasma optical emission spectroscopy (HRN ISO 22036:2011), while Hg was analysed by cold-vapor atomic absorption spectrophotometry (HRN ISO 16772:2009). The Cd concentration ranged from 0.2 to 0.58 mg kg^{-1}, Cr from 0.6 to 27 mg kg^{-1}, Hg from 0.036 to 0.136 mg kg^{-1}, Pb from 3.0 to 8.2 mg kg^{-1}, and Zn from 8.6 to 82 mg kg^{-1} of dry tissue weight. The highest concentrations of heavy metals were measured in *B. rutabulum*, especially in the samples collected during the rainy season at higher altitudes, which can be related to more intensive deposition of metals from the atmosphere due to higher precipitation rates in the period before sampling.

Keywords: biomonitoring, mosses, soil, pollution, metals

MIKROBIOLOGIJA
MICROBIOLOGY

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IMPORTANCE OF BOTTOM UP CONTROLE IN THE MONTENEGRIN PART OF THE SOUTHERN ADRIATIC SEA

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The paper presents the first findings of heterotrophic nanoflagellates (HNF) abundance and distribution along the Montenegrin part of the southern Adriatic Sea. Sampling was carried out monthly during 2009 and 2010 at six stations of three embayments in Boka Kotorska Bay and five stations at open sea. The average monthly abundance of HNF obtained as the average value from the surface to the bottom layers, ranged from 0,06 x 10^3 cells mL^{-1} to 0,8 x 103 cells mL^{-1} for the Boka Kotorska Bay, and from 0,05 x 10^3 cells mL^{-1} to 1,17 x 10^3 cells mL^{-1} for open sea. The values of the heterotrophic bacteria abundance were within 10^4 – 10^5 cells $mL^{-1.}$ To examine the mechanisms regulating bacterial abundance in the investigated area we observed the relationship between bacteria and HNF and between bacteria and chlorophyll *a*. Weak coupling between bacteria and HNF suggests that predation (top down control) is not dominant in controlling of bacterial abundance what confirms the oligotrophic status of the studied area.

Keywords: bottom up controle, heterotrophic nanoflagellates, heterotrophic bacteria

TOKSIKOLOGIJA I EKOTOKSIKOLOGIJA
TOXICOLOGY AND ECOTOXICOLOGY

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EVALUATING THE CELLULAR & HUMORAL IMMUNE RESPONSES OF THE TERRESTRIAL ISOPOD, *Porcellio scaber*, TO CERIUM NANOPARTICLES

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Cerium nanoparticles (nCeO2) are receiving more attention due to their use as catalysts and antioxidants. Terrestrial isopods, *Porcellio scaber*, are well-studied and have previously been used as models for environmental toxicity. As the immune system is an early responder to foreign matter and it is anticipated that organisms may recognise nanoparticles (NPs) as “foreign” and respond by modulating their immune system. The aim of this study is to investigate the immune response of *P. scaber* to ingested nCeO2. Studying the immune system in conjunction with traditional toxicology parameters can give more information into the possible effects these particles may have. In this study we present the effects of ingested nCeO2 on *P. scaber* after 14 days. During which, the feeding, defecation and survival rates were recorded. After 14 days, the number, viability and proportion of hemocytes were counted. The humoral side of the immune system was investigated by measuring the activity of phenoloxidase, which is associated with melanisation, and the level of nitric oxide, a toxic defence molecule. Energy levels and the amount of copper in the copper reserves, were measured as an indirect effect on the immune system. The biomarkers, glutathione S-transferase and acetylcholinesterase, were also assayed. Our results show that the immune system provides more sensitive results on the health of isopods than traditional toxicology parameters and insight into how NPs interact with organisms.

Keywords: nanoparticles, toxicity, Isopod, immunity

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AKUMULACIJA I UNUTARSTANIČNA RASPODJELA ODABRANIH METALA I METALOIDA U PROBAVNOJ ŽLIJEZDI ŠKOLJKAŠA *Anodonta exulcerata* Porro, 1838 IZ VISOVAČKOG JEZERA

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Toksični učinci metala ne ovise samo o ukupnoj koncentraciji metala nego i o njihovoj raspodjeli unutar stanica. Cilj istraživanja bio je usporediti akumulaciju i unutarstaničnu raspodjelu 9 odabranih metala i metaloida (esencijalni: Cu, Zn, Fe, Mo i Se; neesencijalni: Cd, Ag, Pb i As) u probavnoj žlijezdi školjkaša *Anodonta exulcerata* s plićih i dubljih dijelova Visovačkog jezera. Analizirani su sadržaj metala u tkivu i citosolskoj frakciji tkiva probavne žlijezde, raspodjela metala između topljive citosolske i netopljive frakcije tkiva kao i raspodjela između citosolskih biomolekula. Razdvajanje citosolskih biomolekula provedeno je pomoću HPLC-a s isključenjem molekula po veličini, a koncentracije metala određene su pomoću ICP-MS-a. Analiza sadržaja ukupnih metala ukazala je na značajne razlike u bioraspoloživosti metala s obzirom na dubinu jezera. Se, Fe i As akumulirani su značajno više na dubljem, a Cu i Zn na plićem lokalitetu. Pri povećanoj akumulaciji u probavnoj žlijezdi uočeno je povećanje udjela metala u netopljivoj frakciji kod Se, Fe, Cu, Zn, Cd i Ag, dok je kod Pb i As zadržana ista postotna zastupljenost u svakoj od frakcija. Analiza raspodjele metala među citosolskim biomolekulama omogućila je definiranje molekulskih masa biomolekula koje sudjeluju u vezanju pojedinih metala te je za većinu analiziranih elemenata potvrdila sudjelovanje istih citosolskih biomolekula pri različitim razinama akumulacije metala.

Ključne riječi: metali, unutarstanična raspodjela, toksičnost, slatkovodni školjkaši

ACCUMULATION AND INTRACELLULAR DISTRIBUTION OF SELECTED METALS AND METALOIDS IN THE DIGESTIVE GLAND OF *Anodonta exulcerata* Porro, 1838 FROM VISOVAC LAKE

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Toxic effects of metals depend not only on the total metal concentration but also on their distribution within cells. Aim of the study was to compare the accumulation and intracellular distribution of 9 selected metals and metalloids (essential: Cu, Zn, Fe, Mo and Se, non-essential: Cd, Ag, Pb and As) in the digestive gland of *Anodonta exulcerata* bivalves from shallower and deeper parts of Visovac Lake. The metal content in tissue and cytosolic

fraction of digestive gland, the distribution of metals between the soluble cytosolic and insoluble tissue fractions as well as between the cytosolic biomolecules were analysed. Separation of cytosolic biomolecules was performed by size exclusion-HPLC, and metal concentrations were determined by ICP-MS. Analysis of total metal content showed significant differences in metal bioavailability with regard to lake depth. Se, Fe and As were accumulated significantly more in the deeper locality, and Cu and Zn in the shallow locality. Increased metal accumulation in the digestive gland resulted with an increase of metal proportion in the insoluble fraction of Se, Fe, Cu, Zn, Cd and Ag, while Pb and As had similar percentages in each fraction. Analysis of the metal distribution among cytosolic biomolecules allowed the definition of molecular masses of biomolecules involved in binding of particular metals and for most of the elements confirmed the inclusion of same cytosolic biomolecules at different levels of metal accumulation.

Keywords: metals, intracellular distribution, toxicity, freshwater bivalves

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PRIMJENA LEMNA-TESTA U PROCJENI KVALITETE VODE ODVODNOG KANALA KARAŠICA U BARANJI

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Kakvoća vode Odvodnog kanala Karašica u Baranji je pod jakim antropogenim utjecajem (poljoprivreda, komunalne otpadne vode), a unos zagađivala u vodotok ovisi i o sezonskim promjenama klimatskih parametara. Visoke koncentracije enterokoka i vrste *Escherichia coli* pokazatelji su svježeg fekalnog onečišćenja otpadnim vodama, dok je prisutnost specifičnih onečišćujućih tvari (organoklorovi pesticidi, heksaklorcikloheksani te DDT i metaboliti) i metala kao zagađivala u kanalu najviše posljedica ispiranja s poljoprivrednih površina, posebno u jesen. Odabrani parametri u Lemna-testu (prirast i svježa masa biljaka, fotosintetski pigmenti, ukupni topljivi proteini i malondialdehid) dobri su pokazatelji utjecaja zagađivala prisutnih u istraživanoj vodi na vrstu *Lemna minor*. Niske razine hranjivih tvari u vodi, posebno dušika i visoke razine zagađivala uvjetovali su stres u *L. minor* indukcijom lipidne peroksidacije u gotovo svim uzorcima vode Odvodnog kanala, kao i povećanja omjera Chl*a*/*b* i smanjenja omjera Chl(*a*+*b*)/Car, smanjene koncentracije ukupnih proteina i svježe mase biljaka *L. minor*. Uporabom Lemna-testa na različitim razinama složenosti (morfološke promjene, pigmenti, proteini i lipidna peroksidacija) postignuta je preciznija procjena kakvoće vode iz okoliša.

Keywords: kakvoća vode, Odvodni kanal Karašica, Lemna-test, *Lemna minor*

APPLICATION OF LEMNA-TEST IN THE ASSESSMENT OF WATER QUALITY IN THE KARAŠICA DRAINAGE CHANNEL, BARANJA

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Water quality in the Karašica drainage channel in Baranja is under a strong influence of anthropogenic sources (agriculture and municipal wastewaters), and the input of polluted waters into this watercourse also depends on seasonal changes in climate parameters. High concentrations of enterococci and *Escherichia coli* were good indicators of recent faecal contamination with wastewaters, while the presence of specific pollutants (organochlorine pesticides, hexachlorocyclohexanes, and DDT and its metabolites), as well as metals, evidently derived from agricultural run-off, especially in the autumn. Selected parameters in the Lemna-test (growth rate and yield, photosynthetic pigments, total soluble proteins and malondialdehyde) proved to be useful indicators of the influence of present pollutants in the investigated waters on *Lemna minor*. Low levels of nutrients in the water, especially nitrogen, and high levels of specific contaminants, have caused growth inhibition of *L. minor* associated with enhanced lipid peroxidation, as well as decreased Chl*a*/*b* and Chl(*a*+*b*)/Car and reduced protein concentrations in almost all water samples of the drainage channel. A more accurate assessment of water quality was achieved by using Lemna-test with relevant structural or functional endpoints (morphological changes, pigments, proteins and lipid peroxidation) and by considering time-varying exposure of different sources of pollution.

Keywords: water quality, the Karašica drainage channel, Lemna-test, *Lemna minor*

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EKSTRAKCIJA CELOMOCITA GUJAVICA – NEINVAZIVNA METODA PRIKUPLJANJA UZORAKA ZA ODREĐIVANJE UČINAKA PESTICIDA

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Gujavice se često koriste u ekotoksikološkim istraživanjima zbog prirodnog staništa u kojem mogu biti izložene djelovanju različitih pesticida. Budući da se u istraživanjima žrtvuje veliki broj organizama za dobivanje potrebnih uzoraka za mjerenje, bilo bi korisno razviti neinvazivne metode za analizu ciljnih parametara. Stoga je svrha ovog istraživanja utvrditi može li se ekstrakt celomocita gujavica, dobiven neinvazivnom metodom, koristiti za mjerenje aktivnosti biokemijskih biomarkera te utvrditi njegovu primjenu u procjeni učinaka pesticida za koje je već poznato da imaju negativan učinak na gujavice. U istraživanju se koristila gujavica *Eisenia andrei* koja je primjenom kontaktnog filter papir testa izložena organofosfatu dimetoatu. Nakon 48 h su se priredili ekstrakti celomocita u kojima su mjerene aktivnosti enzima acetilkolin-esteraze (AChE) i karboksilesteraze (CES). Postotak inhibicije mjerenih enzima u ekstraktu celomocita uspoređen je sa inhibicijom istih enzima u uzorcima dobivenim homogenizacijom cijelog tijela gujavice. Rezultati su pokazali značajnu inhibiciju AChE u ekstraktu celomocita i homogenatu gujavica, te je jačina inhibicije bila usporediva. U slučaju CES, razina inhibicije bila je veća u homogenatima, a velika varijanca u ekstraktu celomocita onemogućila je dobivanje značajne razlike. Ovo istraživanje ukazuje na mogućnost primjene ekstrakta celomocita za mjerenje biokemijskih biomarkera i procjenu učinaka pesticida.

Ključne riječi: gujavice, celomociti, pesticidi, biokemijski biomarkeri

EARTHWORMS COELOMOCYTE EXTRACTION – NON-INVASIVE METHOD OF SAMPLE COLLECTION FOR PESTICIDE EFFECT ASSESSMENT

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Earthworms are often used in ecotoxicological research because of natural habitat where they can be exposed to various pesticides. Since a large number of organisms has to be sacrificed it would be useful to develop non-invasive methods for the analysis of target parameters. The aim of this study is to determine whether the coelomocyte extract, obtained by the non-invasive method, can be used to measure responses of biochemical biomarkers and to determine whether it can be used in assessing the effects of pesticides already known to have a negative impact on the earthworms. In the present study Eisenia andrei earthworms were exposed to organophosphate dimethoate by applying the filter paper contact test. After 48 h, coelomocyte extracts were prepared and acetylcholinesterase (AChE) and carboxylesterase (CES) activities were measured. The percentage of inhibition of the measured enzymes in the coelomocyte extract was compared with the inhibition of the same enzymes in the samples obtained from whole body homogenate. The results showed significant inhibition of AChE both in the coelomocyte extract and the whole body homogenate and the inhibition rate was comparable. In the case of CES, the level of inhibition was higher in homogenates, and the high variance in coelomocyte disabled obtaining significant differences. This study indicates the possibility of using the coelomocyte extract for measurement of biochemical biomarkers and assessment of the pesticide effects.

Keywords: earthworms, coelomocyte, pesticides, biochemical biomarkers

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UTJECAJ ALUMINIJ KLORIDA NA LIPIDNU PEROKSIDACIJU I DISTRIBUCIJU TOKSIČNIH I ESENCIJALNIH ELEMENATA/ELEMENATA U TRAGOVIMA U TKIVU JETRE I SLEZENE ŠTAKORA

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Aluminij (Al) je neesencijalni, toksični element koji se povezuje s brojnim poremećajima kod ljudi (npr. Alzheimerova bolest). Oprečno tome, hematopoetski organi (jetra i slezena) su vitalna mjesta uključena u detoksikaciju. Studija je usmjerena na istraživanje

antioksidativne i detoksikacijske sposobnosti jetre i slezene nakon primjene aluminij klorida. Dvanaest mužjaka Y59 štakora tretirano je intraperitonalno s: (a) 0,9% NaCl (kontrolna skupina) i (b) $AlCl_3$ (10 mg/kg) i D-(+)-galaktoza (60 mg/kg) dnevno tijekom 28 dana. Analiza esencijalnih i toksičnih elemenata, u tkivu jetre i slezene, provedena je metodom masene spektrometrije induktivno spregnute plazme (ICP-MS). Lipidna peroksidacija (LP) mjerena je testom tiobarbiturne kiseline u homogenatu jetre i slezene. Rezultati ukazuju na statistički značajno povećanje toksičnih metala (Al, Cd, As) u tkivima jetre i slezene, također neesencijalnih elemenata/elemenata u tragovima u tkivu jetre (Co, Sr, MoP, S, Ca, Fe, Cu, Zn, Se, Na, Mg) i slezene (Mo, Ca, Fe, Se) kod štakora tretiranih s $AlCl_3$ u usporedbi s kontrolnom skupinom. S druge strane P, S, Mn, Zn i Mg su sniženi u tkivu slezene. Razina LP je značajno povećanja u tkivu slezene kod štakora tretiranih s $AlCl_3$. Rezultati ukazuju na nepovoljne učinke $AlCl_3$ na metaboličku funkciju jetre i elemenate u tragovima, čija koncentracija nije značajno promjenjena u slezeni gdje je uočen proksidativni učinak $AlCl_3$.

Ključne riječi: aluminijev klorid, toksični elementi, esencijalni elementi, detokosikacija

EFFECTS OF ALUMINUM CHLORIDE ON LIPID PEROXIDATION AND DISTRIBUTION OF TOXIC AND ESSENTIAL/TRACE ELEMENTS IN RATS LIVER AND SPLEEN TISSUES

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Aluminum (Al) is a nonessential, toxic element which is assoiated with number of disorders in people (e.g. Alzheimer's disease). While, hematopoietic organs (liver and spleen) are the vital sites involved in detoxification. This study was aimed to explore antioxidative and detoxifying capacity of the liver and spleen after administration of aluminum chloride. Twelve male Y59 rats were intraperitoneally administered with: (a) 0,9% NaCl (control group) and (b) $AlCl_3$ (10 mg/kg) and D-(+)-galactose (60 mg/kg) per day consecutively for 28 days. The analysis of essential and toxic elements in the liver and spleen tissues was performed by mass spectrometry inductively coupled plasma (ICP-MS). Lipid peroxidation (LP) was measured by thiobarbituric acid assay in homogenates of liver and spleen. The results showed statistically significant increase of toxic metals (Al, Cd, As) in liver and spleen tissues, also essential/trace elements in liver (Co, Sr, Mo P, S, Ca, Fe, Cu, Zn, Se, Na, Mg) and spleen (Mo, Ca, Fe, Se) tissues of rats treated with AlCl3 compared to the control group. While P, S, Mn, Zn, Mg in the spleen tissues was significantly reduced. The level of LP was significantly increased in spleen tissues of $AlCl_3$-treated rats. The results indicate that $AlCl_3$ interfere with the metabolic function of the liver and leads

to disruption of trace element, while spleen has less changes in the essential elements but higher sensibility to proxidative effect of $AlCl_3$.

Keywords: aluminum chloride, toxic elements, essential elements, detoxification

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UTJECAJ IZOPROTURONA NA VODENU LEĆU, *Lemna minor* L.

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Izoproturon je jedan od najčešće primjenjivanih fenilurea herbicida u konvencionalnoj poljoprivrednoj proizvodnji te se često pojavljuje u uzorcima površinskih voda. Nekoliko čimbenika, poput različitih uvjeta izloženosti, mogu utjecati na sposobnost biljaka da odgovarajuće reagiraju na toksičnost uzrokovanu herbicidom. Stoga je cilj ovog rada bio istražiti ekotoksikološki utjecaj ponavljanih pulsnih izlaganja (tri dana tretman herbicidom nakon kojeg je uslijedio period oporavka u trajanju od četiri dana) na vrstu *Lemna minor* L. u usporedbi s kontinuiranim tretmanom u trajanju od 21 dan. Biljke su tretirane s 50, 100, 150 i 200 µg/L izoproturona u hranjivoj otopini. Izoproturon je uzrokovao inhibiciju stope umnožavanja broja frondova i smanjenu akumulaciju svježe i suhe mase tretiranih biljaka. Blagi oporavak rasta zabilježen je u pulsnom tretmanu nižim koncentracijama herbicida dok je najizraženija inhibicija rasta utvrđena u kontinuiranom izlaganju. Tretman herbicidom doveo je do značajnog smanjenja koncentracije proteina i fotosintetskih pigmenata, što je u skladu s načinom djelovanja testiranog herbicida. Aktivnosti antioksidacijskih enzima kao ekotoksikoloških biomarkera stresa, ukazale su da u tretiranim biljkama dolazi do pojave oksidacijskog stresa. Istraživanja utjecaja višestrukih pulsnih izlaganja ne-ciljnih vrsta ksenobioticima, uz standardne testove toksičnosti mogu doprinijeti točnijoj procjeni rizika utjecaja na okoliš.

Ključne riječi: *Lemna minor*, herbicidi, izoproturon, uvjeti tretmana, inhibicija prirasta

EFFECTS OF ISOPROTURON ON THE DUCKWEED *Lemna minor* L.

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Isoproturon is one of the most frequently used phenylurea herbicides in conventional agriculture production, and it is often detected in surface water samples. Several factors, such as different exposure conditions, can affect the ability of plants to respond adequately to herbicide toxicity. Consequently, the present study investigated the ecotoxicological effects of repeated pulsed isoproturon exposures (three days of herbicide treatment followed by four days of recovery) on *Lemna minor* L. relative to continuous exposure during 21 days. Common duckweed was treated with 50, 100, 150 and 200 µg/L of isoproturon added to the nutrient solution. Isoproturon inhibited frond multiplication rate and plant growth evident from decreased fresh and dry weight accumulation in treated plants. The slight recovery of growth rate was recorded in lower pulsed concentrations while continuous regime caused the most pronounced growth inhibition.

Herbicide treatment caused a significant reduction in protein content as well as the decline of photosynthetic pigment content, which is in agreement with the mode of action of the tested herbicide. Antioxidant enzymes activities as ecotoxicological stress biomarkers, indicated that isoproturon treated plants were under oxidative stress. Investigations on the effects of multiple pulse exposure of non-target species to xenobiotics, parallel with standard toxicity tests can contribute to a more accurate environmental impact assessment.

Keywords: *Lemna minor*, herbicides, isoproturon, exposure conditions, growth inhibition

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AKUTNA TOKSIČNOST 10 PESTICIDA NA GUJAVICU *Eisenia andrei* I NJIHOVI UČINCI NA AKTIVNOST EFLUKS CRPKE

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Upotreba pesticida u stalnom je porastu tijekom posljednjih desetljeća, a osim djelovanja na ciljne organizme pesticidi također mogu imati štetne učinke na različite neciljne organizme. Međutim, učinci na neciljne organizme su i dalje često nepoznati i potrebno ih je istražiti kako bi se mogli procijeniti potencijalni rizici upotrebe pesticida. Gujavice imaju važnu ulogu u ekološkim sustavima tla zbog pozitivnih utjecaja na funkcioniranje tla i predstavljaju dobre modelne organizme u ekotoksikologiji tla. Stoga je u ovom istraživanju akutna toksičnost deset pesticida istraživana na gujavici *Eisenia andrei*. Također, mjereni su i učinci pesticida na aktivnost efluks crpke. Izlaganja su provedena primjenom kontaktnog filter papir testa, te je na temelju 48 h LC50 vrijednosti jedan pesticid klasificiran kao superotoksičan (fungicid), pet kao ekstremno toksični (dva herbicida i tri fungicida), dva kao jako toksični (herbicidi) i dva kao umjereno toksični (insekticid i fungicid). Što se tiče aktivnosti efluks crpke, šest pesticida uzrokovalo je značajnu inhibiciju aktivnosti. Inhibicija aktivnosti efluks crpke može dovesti do veće toksičnosti zbog dužeg zadržavanja pesticida u stanicama. Ovo istraživanje pokazuje da pesticidi koji se koriste u poljoprivredi mogu imati značajne učinke na gujavice.

Ključne riječi: gujavice, pesticidi, akutna toksičnost, efluks crpka

ACUTE TOXICITY OF 10 PESTICIDES TO EARTHWORM *Eisenia andrei* AND THEIR EFFECTS ON THE EFFLUX PUMP ACTIVITY

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The usage of pesticides has been steadily increasing over the last decades and besides affecting the target organisms, they can also have adverse effects on different non-target organisms. However, the effects on these non-target organisms are still often unknown and need to be assessed in order to estimate the potential risks of pesticide use. Earthworms play an important role in soil ecosystems due to their positive impacts on the soil functioning and represent good model organisms in soil ecotoxicology. Therefore, in the present study the acute toxicity of ten pesticides to the earthworm *Eisenia andrei* was assessed. Additionally, effects of pesticides on the efflux pump activity were also measured. The exposures were conducted using the filter paper contact toxicity method and based on the 48h LC50 values one pesticide was classified as supertoxic (fungicide), five as extremely toxic (two herbicides and three fungicides), two as very toxic (herbicides) and two as moderately toxic (insecticide and fungicide). Regarding the efflux pump activity, six pesticides showed significant effects with a recorded inhibition of the activity. An inhibition of the efflux pump activity can lead to a higher toxicity due to longer retention of the pesticides in the cells. This study shows that earthworms could be significantly affected by pesticides commonly used in agriculture.

Keywords: earthworms, pesticides, acute toxicity, efflux pump

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SILVER NANOPARTICLES INDUCE NEUROTOXICITY IN VITRO

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Silver nanoparticles (AgNPs) are among the most extensively used nanoparticles and are found in a variety of products. Consequently, frequent and long-term exposure of humans to AgNPs is expected. However, the effects of AgNPs on neuron and astrocyte networks are still largely unknown. In this study, we used neurons and astrocytes derived from human embryonic stem cells as a cellular model to study the neurotoxicity that is induced by citrate-coated AgNPs (AgSCs). Cells were exposed to AgSC and cell morphology was evaluated using an inverted phase contrast microscopy. Expression of different proteins (GFAP, MAP2, pGSK-3, caspase 3, pAkt, Syn, PSD-95) were further investigated using immunohistochemistry staining and Western blot analysis. Our results showed that AgSCs (1.0 and 5.0 mg/mL) changed the astrocyte/neuron ratio and cell morphology, reduced neurite outgrowth, decreased the expression of postsynaptic density protein 95 and synaptophysin, and altered isoform expression and phosphorylation status of MAP2. We also found that $AgNO_3$ exposure only slightly reduced neurite outgrowth and had little effect on MAP2 expression, suggesting that AgSCs and $AgNO_3$ have different neuronal toxicity mechanisms. In addition, most of these effects were reduced when the cell culture was co-treated with AgSCs and the antioxidant ascorbic acid, suggesting that oxidative stress is the major cause of AgSC-mediated neurotoxicity and that antioxidants may have a neuroprotective effect.

Keywords: citrate-coated silver nanoparticles; neurons and astrocytes; human embryonic stem cells; neurotoxicity; nanotoxicity

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ODREĐIVANJE POTENCIJALNE TOKSIČNOSTI NENOČESTICA CERIJEVA DIOKSIDA U RAZVOJU EMBRJA: UTJECAJ NA TRI MEDITERANSKE VRSTE JEŽINACA *Arbacia lixula*, *Paracentrotus lividus* I *Sphaerechinus granularis*

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Nanočestice cerijeva dioksida (CeO_2NP) nedavno su pronašle primjenu u velikom broju aplikacija, uključujući funkcionalne premaze, elektroniku te kao dodaci dizelskom gorivu. Posljednje spomenuto može biti od velike važnosti jer se očekuje da će opterećenje okoliša koje bi moglo proizaći iz ispušnih plinova automobila biti značajno. U ovom radu ispitivana je potencijalna toksičnost CeO_2NP na tri vrste ježinaca *Arbacia lixula, Paracentrotus lividus* i *Sphaerechinus granularis* primjenom testa embrionalnog razvoja ježinaca (SUEDT). Sperma ježinaca tretirana je s CeO_2NP u koncentracijskom rasponu od 0.01-10 mg/L. Rezultati su pokazali da CeO_2NP nisu imale nikakav učinak na sposobnost oplodnje spermija ježinca, s više od 90% uspješno oplođenih jajnih stanica kod svake vrste. Na ovaj način oplođene jajne stanice uspješno su se razvile do stadija morfološki normalne larve nakon 48/72 h, osim kod 10 mg/L CeO_2NP tretmana gdje su larve *A. lixula* i *P. lividus* pokazale samo 66,8 ± 8,4 i 82,3 ± 3,4 % normalno razvijenih larvi nakon 48 sati. Nadalje, nakon izlaganja zigota CeO_2NP 30 min nakon oplodnje embrionalni razvoj nastavljen je za sve tri vrste bez negativnog učinka. Dakle, testom SUEDT, nije dokazano da su ispitivane CeO_2NP materijal zbog kojeg bi se trebalo brinuti, čak i pri vrlo visokim koncentracijama. Međutim, potrebno je više istraživanja na temelju drugih modela, posebno modela pluća, prije nego što se zabrinutost oko nanočestica cerija može potpuno isključiti.

Ključne riječi: nanočestice cerijeva dioksida, embrionalni razvoj ježinaca, *Arbacia lixula, Paracentrotus lividus, Sphaerechinus granularis*

ASSESSING TOXICITY POTENTIAL OF CERIUM OXIDE NANOPARTICLES TOWARDS DEVELOPING EMBRYOS; IMPACT ON THREE MEDITERANEAN SEA URCHIN SPECIES *Arbacia lixula*, *Paracentrotus lividus* AND *Sphaerechinus granularis*

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Cerium dioxide nanoparticles (CeO_2NP) have recently found use in a wide range of applications including functional coatings, electronics and as fuel additives. It is the latter which may be of particular concern as the environmental load deriving from automobile exhaust is expected to be significant. Herein, the potential toxicity of CeO_2NP was investigated using the sea urchin embryonal development test (SUEDT) with three sea

urchin species *Arbacia lixula, Paracentrotus lividus* and *Sphaerechinus granularis*. Sea urchin sperm were treated with CeO_2NP at concentrations ranging between 0.01-10 mg/L. Results indicated that CeO_2NP did not affect the fertilization ability of sea urchin sperm with, more than 90% of eggs for each species successfully fertilized. These eggs developed into morphologically normal larvae at all NP concentrations except for the 10 mg/L CeO_2NP treatment where larvae of *A. lixula* and *P. lividus* showed only 66.8 ± 8.4 and 82.3 ± 3.4 % normally developed larvae after 48h, respectively. Embryonal development following exposure of zygotes to CeO_2NP at 30 min post fertilisation proceeded for all three species without significant differences to the control. Thus, SUEDT, as a standardized test, did not indicate that the investigated CeO_2NP is a material of concern, even at extremely high concentrations. However, more research is required based on other models, particularly lung models, before concerns about nanoparticulate ceria may be completely alleviated.

Keywords: cerium oxide nanoparticles, sea urchin embrional development, *Arbacia lixula, Paracentrotus lividus, Sphaerechinus granularis*

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ZAŠTITINI UČINCI CISTEINA U FITOTOKSIČNOSTI NANOČESTICA SREBRA

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Nanočestice (NPs), s tri dimenzije između 1 i 100 nm, pokazuju nova svojstva u usporedbi s istim materijalom čije dimenzije nisu na nanoskali te imaju primjenu u velikom broju komercijalnih proizvoda. Zbog toga je vjerojatno da će se otpustiti u okoliš, gdje mogu imati učinak na biljke. Budući da biljke imaju važnu ulogu u akumulaciji i biodistribuciji tvari otpuštenih u okoliš, mogu djelovati kao put za prijenos i akumulaciju NPs u prehrambene lance. U ovom istraživanju ispitali smo učinak nanočestica srebra (AgNPs) stabiliziranih s dva omotača [polivinilpirolidon (AgNP-PVP) i cetiltrimetilamonijev bromid (AgNP-CTAB)] na fotosintezu i parametre oksidacijskog stresa u klijancima duhana (*Nicotiana tabacum*). Čestice AgNP-PVP i AgNP-CTAB primijenjene su u tri koncentracije (25, 50 i 100 µM). Kako bismo procijenili doprinos disociranih iona Ag+ učinku čestica AgNPs, koristili smo cistein (125, 250 i 500 µM), jaki ligand srebra koji kompleksira ione Ag+ . Rezultati mjerenja fluorescencije klorofila pokazali su da je učinkovitost fotosinteze smanjena nakon tretmana s AgNPs, što ukazuje na negativan učinak na fotosintetski sustav, koji je bio ublažen nakon dodavanja cisteina u podlogu. Nadalje, sadržaj malondialdehida i proteinskih karbonila bio je povećan u klijancima izloženim djelovanju AgNPs, dok su kombinirani tretmani s cisteinom smanjili oksidacijski stres. Dobiveni rezultati pokazuju da je toksičnost AgNPs barem djelomično posljedica disocijacije iona Ag+.

Ključne riječi: *Nicotiana tabacum*, nanočestice, fluorescencija klorofila, fotosinteza, oksidacijski stres

PROTECTIVE EFFECTS OF CYSTEINE AGAINST SILVER NANOPARTICLES-INDUCED PHYTOTOXICITY

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Nanoparticles (NPs), with three dimensions between 1 and 100 nm, exhibit new characteristics compared to the same material without nanoscale features and are applied in the wide range of commercial products. Therefore, they are likely to be released into the environment and affect plants. Since plants play a significant role in accumulation and biodistribution of environmentally released substances, they can serve as a route for NPs transportation and bioaccumulation into food chains. In this study we have examined the effects of silver nanoparticles (AgNPs) with two different coatings [polyvinylpyrrolidone (AgNP-PVP) and cetyltrimethylammonium bromide (AgNP-CTAB)] on photosynthesis and oxidative stress parameters of tobacco (*Nicotiana tabacum*) seedlings. Three AgNP-PVP and AgNP-CTAB concentrations (25, 50 and 100 µM) were tested. In order to estimate the contribution of dissolved Ag+ to the effects of AgNP, 125, 250 and 500 µM cysteine, a strong silver ligand, has been applied to complex Ag+. Results of chlorophyll fluorescence showed that photosynthesis efficiency was decreased in AgNP treatments, indicating a negative impact on photosynthetic apparatus, which was, however, alleviated when cysteine was added to the medium. Moreover, malondialdehyde and protein carbonyl content increased in AgNP-exposed seedlings, while combined treatments with cysteine mitigated oxidative stress. Obtained results suggest that AgNP toxicity at least partially originates from dissociated Ag+.

Keywords: *Nicotiana tabacum*, nanoparticles, chlorophyll fluorescence, photosynthesis, oxidative stress

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EFFECTS OF MAGNELI PHASE TiOx NANOPARTICLES TO A549 HUMAN LUNG CELLS

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The use of Magnéli phase titanium oxide particles (TiOx) is expected to increase in the near future, due to their high electrical conductivity and other unique physical and chemical properties. This dictates the need for comprehensive assessment of interactions between TiOx and biological systems in order to understand its beneficial uses and potential hazards. The aim of our work was to compare biological effects of well-studied TiO2 particles with Magnéli

phase TiOx particles. The photocatalytic activity of TiO2 and TiOx particles were assessed according to ISO 10678:2010. The toxicity of both particles was tested at different exposure concentrations (1, 10 and 50 µg/mL) on A549 human lung cells. After 24 h exposure in cell medium with or without FBS, the cytotoxicity was assessed by Resazurin assay, Neutral Red Uptake assay and by Coomassie Blue assay. The stability of lysosomes in exposed A549 cells were examined by Acridine Orange relocalization assay. Results showed that both TiO2 and TiOx were not cytotoxic in cell medium with FBS, but they both had a negative effect on lysosomal stability. TiO2 particles had higher photocatalytic activity and cytotoxicity in FBS free medium when compared to TiOx particles. In the future, more studies on TiOx particle toxicity must be conducted in order to fully explore its use and avoid unwanted effects.

Keywords: Magnéli phase particles, titanium suboxides, photoreactivity, lysosomes, toxicity

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PROCJENA ANTROPOGENOG UTJECAJA NA KAKVOĆU VODE RIJEKE KRKE PRAĆENJEM BIOLOŠKIH PROMJENA U POTOČNE PASTRVE (*Salmo trutta* Linnaeus, 1758)

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Sedreni slapovi i jezera čine rijeku Krku krškim fenomenom, koji je 1985. god. zaštićen kao Nacionalni park. Samo 2 km uzvodno od sjeverne granice parka u rijeku Krku se ispuštaju industrijske otpadne vode tvornice vijaka te komunalne otpadne vode grada Knina bez adekvatnog pročišćavanja te je praćenjem kakvoće vode, kao i koncentracija citosolskih metala i biomarkera oksidativnog stresa u potočne pastrve *(Salmo trutta* Linnaeus, 1758) procijenjen njihov utjecaj. Uzorkovanje je provedeno na izvoru rijeke Krke (kontrolna postaja) i uz grad Knin (utjecaj otpadnih voda) u travnju, rujnu i listopadu 2015. te svibnju 2016. Fizikalno-kemijski čimbenici kakvoće vode, posebno nitrati, nitriti i ortofosfati, ukazuju na pogoršanje ekološkog stanja uz grad Knin. Koncentracije većine izmjerenih otopljenih metala u vodi su povišene nizvodno od ulijevanja otpadnih voda u odnosu na izvor, statistički značajno u pojedinim razdobljima za As, Co, Cu, Fe, Mn, Mo, Rb, Sr i Zn. Isti trend je uočen za većinu metala u citosolu jetara riba, uz izuzetak Cd, Cs, Rb i Tl koji su povišeni u riba ulovljenih kod izvora. Oksidativni stres, koji mogu izazvati i metali i organska zagađivala, nije potvrđen u potočne pastrve jer nisu uočene razlike u koncentracijama malondialdehida. Pogoršanje ekološkog stanja, kao i porast koncentracija metala nizvodno od unosa otpadnih voda ukazuje na antropogeni utjecaj i važnost provođenja sustavnog monitoringa u svrhu očuvanja kakvoće vode i biote rijeke Krke.

Ključne riječi: grad Knin, otpadne vode, biomonitoring, metali, malondialdehid

EVALUATION OF THE ANTHROPOGENIC IMPACT ON THE WATER QUALITY OF THE KRKA RIVER BY MONITORING BIOLOGICAL CHANGES IN BROWN TROUT (*Salmo trutta* Linnaeus, 1758)

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Travertine waterfalls and lakes make the Krka River a karst phenomenon, which was proclaimed National Park in 1985. Only 2 km upstream from the northern border of the Park industrial and municipal wastewaters from the Town of Knin are released in the Krka River without proper purification. Their impact was assessed by measuring water quality as well as concentrations of cytosolic metals and biomarkers of oxidative stress in brown trout (*Salmo trutta* Linnaeus, 1758). Sampling was performed at the Krka source (reference location) and near Knin (wastewater influence) in Apr, Sept, Oct 2015 and May 2016. Physico-chemical water parameters, especially NO_3^-, NO_2^- and PO_4^{3-}, indicated deteriorated ecological status near Knin. Concentrations of the most metals in water were increased downstream of the wastewater outfall compared to the source, significantly in some periods for As, Co, Cu, Fe, Mn, Mo, Rb, Sr and Zn. The same trend was observed for most metals in fish hepatic cytosol, except for Cd, Cs, Rb and Tl which were elevated in fish caught near the source. Oxidative stress, which may be caused by metals and organic pollutants, was not confirmed in brown trout because differences in malondialdehyde concentrations were not observed. Deterioration of the ecological status as well as increase in metal concentrations downstream of the wastewater outfall indicated anthropogenic pressure and importance of comprehensive monitoring to preserve water quality and biota of the Krka River.

Keywords: Town of Knin, wastewaters, biomonitoring, metals, malondialdehyde

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THE CONTRIBUTING EFFECTS OF DIFFERENT SOIL TYPES TO THE BIOKINETICS OF SILVER NANOPARTICLES IN TERRESTRIAL ISOPODS

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Depending on the properties of the surrounding media (pH, ionic strength, ionic composition), silver nanoparticles (Ag NPs) can undergo a number of transformations, resulting in different bioavailability of silver ions and/or intact NPs. Most biokinetic studies in soil dwelling organisms are performed on standard soils, but in reality field soils can have significantly different properties which may affect the transformation of NPs. Currently, such data are missing. We compared the uptake and elimination kinetics of Ag

in the terrestrial isopod Porcellio scaber, exposed to three different test soils with different pH (standard Lufa 2.2 soil and two field soils (North Wales and Dorset, obtained through the NanoFase consortium)), spiked with different forms of Ag: ionic Ag, pristine AgNPs of different sizes (3-8 nm, 50 nm) and aged (sulfidized) Ag NPs (Ag_2S)) at a concentration of 10 mg Ag/kg soil. We used a classic one-compartment kinetic model to derive the uptake and elimination kinetic parameters. In contrast to our expectations, the results of the study show no significant difference between the biokinetic parameters between exposures of the same form of Ag in different soils, indicating a minor effect of soil physical and chemical parameters on the fate of the nanoparticles at the environmentally relevant concentration.

Keywords: biokinetic parameters, silver nanoparticles, isopods, standard soil, field soil

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