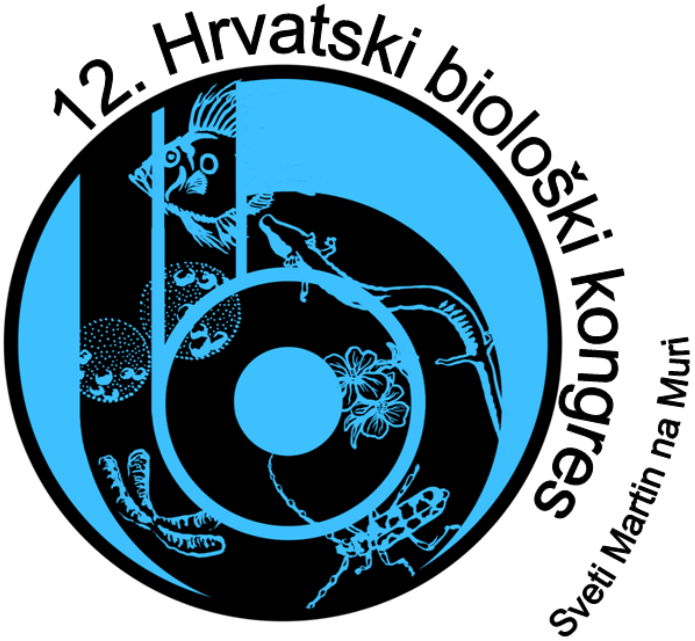
Hrvatsko biološko društvo
SOCIETAS BIOLOGORUM CROATICA
Croatian Biological Society

12. HRVATSKI BIOLOŠKI KONGRES
s međunarodnim sudjelovanjem

12th CROATIAN BIOLOGICAL CONGRESS
with International Participation

Sveti Martin na Muri, 18. – 23. IX 2015.



ZBORNIK SAŽETAKA

BOOK OF ABSTRACTS

Hrvatsko biološko društvo
SOCIETAS BIOLOGORUM CROATICA
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18. – 23. rujna 2015.
Sveti Martin na Muri, Hrvatska

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Zagreb, 2015.

ZBORNIK SAŽETAKA
12. HRVATSKOG BIOLOŠKOG KONGRESA

BOOK OF ABSTRACTS
OF THE 12th CROATIAN BIOLOGICAL CONGRESS

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Cijenjene kolegice i kolege, dragi biolozi!

Za one rijetke koji iz nekog samo njima poznatog razloga čitaju predgovore u knjigama sažetaka želim napomenuti da su vremena sve teža za organiziranje kongresa a poglavito kongresa koji su ovako sveobuhvatni kao što je to Hrvatski biološki kongres. U ovom 12-om po redu Kongresu smanjili smo broj tema kako bi objedinili sudionike kongresa sa sličnim znanstvenim interesima te smo povećali broj simpozija koji se održavaju u sklopu kongresa. Također, odlučili smo se za Sveti Martin na Muri sa željom da istaknemo prirodne vrijednosti kontinentalne Hrvatske a posebice Međimurja. Pretpostavljam da je to imalo određeni utjecaj na vašu odluku o sudjelovanju na 12. Hrvatskom biološkom kongresu jer ovaj Zbornik sažetaka sadrži 8 plenarnih i 5 pozvanih predavanja te 125 usmenih i 143 posterska priopćenja. Želim stoga zahvaliti svim sudionicima ovog Kongresa, a posebice onima koji čitaju ove predgovore, na odluci da odvoje vrijeme i određena financijska sredstva kako bi sudjelovali na ovom već tradicionalnom kongresu koji, siguran sam, ima posebno mjesto u našim srcima.

U ime Organizacijskog i programskog odbora
Göran Klobučar

Esteemed colleagues, dear biologists!

For those of you who, for some unknown reasons, read forewords in Books of Abstracts I wish to emphasize that times are not favourable for organising congresses and most of all those that are so comprehensive as Croatian Biological Congress. In this twelfth CBC we reduced the number of congress topics in order to bring together biologists with similar scientific interests and we increased the number of satellite Symposia. Also, we decided to hold this Congress at Sveti Martin na Muri in order to accentuate values of continental Croatia and especially Međimurje region for us biologists. I suppose that these changes might have had some influence on your decision to attend 12th Croatian Biological Congress as this Book of Abstracts contain 8 plenary and 5 invited lectures, 125 oral and 143 poster presentation. Therefore, I wish to thank all the participants of this Congress, especially those who took time to read this foreword, for the decision to invest their time and certain funds to participate in this already traditional Congress which has, I am sure, a special place in our hearts.

On behalf of the Organising and Programme Committee
Göran Klobučar

Poštovane kolegice i kolege, sudionici 12. Hrvatskog biološkog kongresa!

Biologija 21. stoljeća suočava se sa brojnim izazovima - razvoj i primjena novih tehnologija mijenjaju mnoga saznanja o živom svijetu na svim razinama organizacije, a otvaraju se i nova područja istraživanja. Premda oduvijek vrlo dinamična znanost, suvremena biologija danas možda brže i više nego ikada dolazi do vrijednih spoznaja, koje preispituju i pokreću nova istraživanja čak i u okvirima "klasičnih" bioloških disciplina. Biolozi su i dalje vrlo važan i nezaobilazan dio složenih istraživačkih timova i u različitim srodnim znanostima. Povećana količina novih znanja i činjenica stavlja izazove i pred biologe koji se bave obrazovanjem, počevši od osnovnih škola pa do visokoškolskih ustanova, koji moraju naći nova rješenja i putove kojima će ta nova znanja što bolje prenijeti na današnje generacije učenika i studenata.
Jedna od najvažnijih pretpostavki na kojima se zasniva suvremena znanost jest potreba za što bržim i dinamičnijim protokom informacija. Znanstveni skupovi stoga postaju vrlo važna mjesta susreta stručnjaka i istraživača koji aktivno komuniciraju rezultate svojih istraživanja i imaju mogućnost međusobnog upoznavanja te boljeg povezivanja radi što smislenije i svrsishodnije organizacije nekih budućih istraživačkih projekata i suradnja.
Hrvatsko biološko društvo kroz organizaciju svojeg dvanaestog kongresa uspješno nastavlja dugogodišnju tradiciju okupljanja domaćih, ali i inozemnih znanstvenika i stručnjaka koji aktivno djeluju u području biologije i srodnih znanstvenih grana. Nadamo se da će, svojim bogatim znanstvenim programom, ovogodišnji kongres ispuniti očekivanja sudionika jer su zastupljena sva područja od interesa naših biologa, a kroz specijalizirane simpozije moći će se dobiti dobar uvid u najnovija istraživanja i suvremena kretanja u pojedinim biološkim disciplinama.
Izborom plenarnih predavanja također smo nastojali istaknuti neka od trenutačno najvažnijih i vrlo aktivnih područja u okviru biologije, čiji vrijedni rezultati dovode do novih i ključnih spoznaja u funkcioniranju živog svijeta i nude smjerove prema nalaženjima odgovora na mnoge, još uvijek neistražene, biološke fenomene.
Zadovoljni smo što je 12. Hrvatski biološki kongres okupio veliki broj biologa svih dobnih skupina i što su se studenti biologije kroz prijavu rezultata svojih istraživanja već sada aktivno uključili u istraživački prostor jer je to najbolji put za daljnji napredak biologije u okvirima hrvatske, ali i svjetske znanosti te dobar početak za njihov osobni profesionalni razvoj. Također moramo istaknuti da ovogodišnji kongres ima i naglašen međunarodni karakter kroz veliki broj prijavljenih sudionika iz inozemstva, što upućuje na činjenicu da će naši skupovi ubuduće vjerojatno prerasti nacionalne okvire. To će zasigurno utjecati i na organizaciju kongresa biologa u budućnosti, dajući im novu kvalitetu kako u znanstvenom, tako i stručnom smislu.
Ova knjiga sadrži sve u roku pristigle sažetke usmenih i posterskih priopćenja prijavljenih za 12. Hrvatski biološki kongres. Znanstveni odbor proveo je recenzijski postupak i sažetke tehnički uredio i ujednačio prema zadanom predlošku. Nadamo se da će čitatelji u ovoj knjizi sažetaka naći korisne informacije i poticaj za svoj znanstveni i stručni rad, a svim sudionicima kongresa želimo uspješan rad i što bolju prezentaciju rezultata svojih istraživanja na 12. Hrvatskom biološkom kongresu.

U ime Znanstvenog odbora 12. Hrvatskog biološkog kongresa
Nevenka Kopjar, Predsjednica Znanstvenog odbora

Esteemed colleagues, dear participants of the 12th Croatian Biological Congress!

The field of biology in the 21st century is faced with many challenges – the development and application of new technologies has greatly affected our knowledge regarding the living world at all levels of organization and uncovered many new and interesting research areas. Although always a very dynamic field of study, modern biology is probably more efficient and faster in acquiring valuable information now than ever. This leads to critical re-examinations and initiations of new research, even within more "classical" biological disciplines. Biologists are still a very important and vital part of complex research teams in different related scientific fields. The increased amount of new knowledge also brings challenges for biologists involved in education, starting from primary school to university level. They now have to find new solutions and ways to present this new knowledge to their students.

One of the most important assumptions that underlie modern science is the need for a faster and more dynamic flow of information. Conferences have therefore become an important place for meeting researchers and experts who communicate the results of their research and engage in fruitful discussions, leading to joint research projects.

By organizing its 12th congress, the Croatian Biological Society successfully continues the longstanding tradition of joining scientists and experts active in the field of biology and related scientific fields. We hope that the enticing scientific program of this year's congress will meet the expectations of the participants because it was designed to cover almost all areas of interest. Through specialized symposia, the participants will be able to get good insight into the state-of-the-art research trends in different biological disciplines.

Through our selection of plenary lectures, we tried to stress some of the most important and highly active areas within biology today. The valuable results they have provided new directions towards finding answers to the many still unexplored biological phenomena.

We are pleased that the 12th Croatian Biological Congress brought together a large number of biologists of all ages, especially so many biology students. By presenting the results of their research, they will commence their own active involvement in the world of true research. This year's congress also boasts a more international character with many international participants, which points to the fact that our future meetings will probably outgrow only Croatia as their venue. This will certainly affect the organization of the biological congress in the future, giving them a new quality both scientifically and professionally.

This Book of Abstracts contains all abstracts submitted for the 12th Croatian Biological Congress. The Scientific Committee tried, within the best of its capability, to edit the received abstracts and conducted a peer review process. We hope that our readers will find useful information in this Book and deem it helpful in their scientific and professional work. We would also like to wish all of the participants of the 12th Croatian Biological Congress a successful participation and presentation of their findings.

On behalf of the Scientific Committee of the 12th Croatian Biological Congress
Nevenka Kopjar, President of the Scientific Committee

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Genetika, stanična i molekularna biologija
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PLENARNA PREDAVANJA

PLENARY LECTURES

Plenarna predavanja
Plenary lectures

PL-1
PROTEIN INTERACTION NETWORKS IN HEALTH & DISEASE

I. Štagljar
Departments of Biochemistry and Molecular Genetics, Donnelly Centre, University of Toronto, Canada

In the past decade, numerous comprehensive proteomics studies have allowed researchers to generate detailed protein-protein interaction ("interactome") maps, providing an unparalleled global view of the interplay between the different systems within the cell, and greatly increasing our understanding of the physiological mechanisms involved in both normal and disease states. My lab is focused specifically on understanding how the interactions of membrane proteins contribute to cellular disease states at a systems level. Despite extensive proteomics research in the past decade, there is a lack of in-depth understanding of protein networks associated with integral membrane proteins because of their unique biochemical features, enormous complexity and multiplicity. This is a major obstacle to understanding the biology of deregulation of integral membrane proteins which leads to numerous human diseases, and consequently hinders our development of improved and more targeted therapies to help treat these diseases. To address this problem, my lab has developed two unique technologies specifically suited for the study of full-length integral membrane proteins in their natural cellular context; the classic Membrane Yeast Two-Hybrid (MYTH) 1-5 and the newly created Mammalian Membrane TwoHybrid(MaMTH)6. Our ultimate goal is to uncover a wealth of information about protein interactions for the majority of "druggable" human membrane proteins, which should in turn greatly facilitate the discovery of new truths about diseases like cancer, schizophrenia, cystic fibrosis, hypertension and Parkinson's disease. During my talk, I will discuss our recent findings indicating that the application of MaMTH to the human Epidermal Growth Factor Receptor (EGFR) resulted in the identification of Crk II protein as a novel interactor of oncogenic EGFR (L858R), and showed that CRKII promotes persistent activation of aberrant signaling in non-small cell lung cancer (NSCLC) cells. I will also illustrate how MaMTH is a powerful tool for investigating dynamic interactomes of human integral membrane proteins and why it promises significant contributions to therapeutic research.

PL-2
THE RISE AND FALL OF GENOME COMPLEXITY AND OTHER MACROEVOLUTIONARY STORIES

R. Bakarić, T. Domazet-Lošo
Ruđer Bošković Institute, Zagreb, Croatia

Recent advances in understanding of evolutionary life cycle of genes reveal that gain and loss of gene families is major factor in the evolution of genome composition. However, it is unclear how gene family birth-and-death rates fluctuate at the macroevoutionary scale. Consequently it is obscure how genome complexity changed during evolutionary time in different lineages. Here, using phylostratigraphic approach and newly developed bioinformatic toolkit, we measured gene family birth-and-death rates at the phylogenetic tree comprising more than 600 eukaryotic genomes. We found that, in terms of time,

genome reduction is predominant mode of evolution sporadically interrupted by the rapid bursts of gene family gain. These episodes of gene family gain correlate with the major macroevolutionary transitions.

PL-3
DIVERGENCE AND CONSTRAINT IN THE ORIGIN OF NEW SPECIES

P. Nosil
Royal Society of London, University of Sheffield, United Kingdom

The origin of new species creates biological diversity and understanding species formation is thus a key goal in biology. In this talk, I will tackle the issue of why some populations that begin the speciation process diverge further than others, a phenomenon central to understanding diversification. Using a combination of theoretical modeling and empirical studies of plant-feeding insects I will show how adaptation to different ecological environments generally promotes speciation. However, this process can be constrained or counteracted by numerous factors. Specifically, speciation can stall 'partway' before completion due to: an insufficient number of genetic differences underlying adaptive divergence, ecological shifts that are too modest to drive strong divergence, and selective processes that increase genetic mixing between populations. The origin of new diversity thus reflects a balance between these factors driving and constraining evolutionary divergence.

PL-4
SOCIO-SCIENTIFIC ISSUES: A CHALLENGE FOR BIOLOGY TEACHERS

A. Šorgo
Faculty for Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia

In many contemporary documents is addressed need for different kind of education, where outcome of such approach is a citizen who not only possess solid knowledge foundation but also is able to use achieved knowledge to solve real-world practical or intellectual problems even after finished formal education. In documents underpinning education emphasis is given to development of skills like critical thinking, creativity, ability to solve problems and soft skills like ability to learn, to activate tacit knowledge or to work in groups. Real and yet unsolved challenge is how to organize instructions to achieve at least a part of listed goals balancing between well-tested practices what worked in the past and enthusiasm of untested novelties. Because we cannot treat instructions and school activities as from the society-isolated and value-free systems, Biology teachers cannot avoid teaching and follow up classroom discussions of sensitive themes. Beside already "traditional" themes like environmental education is nowadays in the middle of public interest biotechnology with themes like genetic engineering, genetically modified organisms (GMOs), food from GMOs, therapeutic and reproductive cloning, substitute maternity, cloning of human, impact of GMOs on human health, health of animals, and environment, etc. To cover the social and scientific aspects of such the term "socio-scientific issue" was coined. From the socio-scientific view, such issues are frequently controversial and, especially in cases when they become the subject of public interest debates, are often backed up with limited knowledge and strong attitudes. In educational practice, such public debates are reflected in the classroom, where teachers are challenged by complex issues, where proficiency in one

discipline is in most cases insufficient to answer students' curiosity. When such a theme is assigned to a Biology teacher, probably the most important obstacle is that teachers often lack appropriate knowledge about the social side of the issue.

PL-5
PLANT RESPONSE TO ABIOTIC STRESS

D. Bar-Zvi
Ben-Gurion University, Beer-Sheva, Israel

Being sessile organisms that cannot relocate, plants are continuously exposed to the ever-changing environment. To accommodate to these changes, plants evolved intensive molecular, biochemical and physiological mechanisms to respond to hostile environments. These changes include a global change in the transcriptome and proteome. I will discuss three molecular examples for plant response to drought and salt stress: (a) Salt stress impairs chloroplast structure and affects this organelle's gene expression and genome copy number. (b) Transcription factors are key players in reaching new steady states in transcript levels. The stress hormone, abscisic acid, regulated ABI4 transcription factor plays central role in root biology under stress and non-stress conditions. ABI4 modulates the activity of the Na^+ transporter HKT1;1 and controls lateral root formation. (c) The Ubiquitin-Proteasome System (UPS) is a major pathway for controlled protein degradation in all eukaryotes, including plants. Proteins descended for degradation are covalently marked by a short ubiquitin chain, by a three step pathway. The third step, is catalyzed by a large family of substrate specific ubiquitin-ligases, is believed to be the modulated step in the decision of protein fate. We have identified new ubiquitin-ligases involved in plant response to abiotic stress, and study their role in plant response to the environment.

PL-6
ANALYSES OF MORPHOLOGY, PHYSIOLOGY, AND MICROBIAL DIVERSITY AFTER A RECENT DIETARY SWITCH IN A LIZARD

A. Herrel
UMR 7179 C.N.R.S/M.N.H.N., Département d'Ecologie et de Gestion de la Biodiversité, Paris, France

Although evolution is commonly considered a slow process, recent evidence has shown that organisms can show dramatic and measurable phenotypic responses after introductions to novel environments in relatively short time spans. We have previously shown how lizards (*Podarcis sicula*) have rapidly evolved differences in head morphology, bite strength, and digestive tract structure after experimental introduction onto a small island in the Adriatic Sea, Croatia. Despite the short time scale (36 years) since this introduction, the introduced lizards became omnivores and evolved caecal valves in the hindgut, a structure rarely observed in lizards. These changes in morphology and performance parallel those typically documented among species and even families of lizards in both the type and extent of their specialization. Here, we present novel data on 1) the morphology of the cranium and its muscles using µCT scanning and 3D geometric morphometric approaches, 2) the digestive physiology of the two populations, and 3) the diversity of the microbiomes in the hindgut using metagenomic sequencing approaches.

PL-7

HOW MUCH ROOM IS LEFT FOR CONVENTIONAL BIOLOGY IN MODERN NANOSCALE AND OMICS RESEARCH

D. Drobne
Department of Biology, Biotechnical faculty, Ljubljana, Slovenia

Traditional biologists were trained to work at a specific level of biological complexity, and/or to investigate a specific group organisms. But now, many biologists call themselves integrative biologists. Also academic units and study programs have changed their names to Integrative biology. What is "Integrative Biology"? Integrative biology has different definitions. It could be defined as multidisciplinary/cross-disciplinary research of biological systems. Multidisciplinarity means bringing together researchers from different, areas of expertise or using a diversity of techniques and approaches to address particular questions (Wake 2003; Joyce and Palsson 2006). Integrative approaches seek both diversity and incorporation. These approaches deal with integrations across all levels of biological organization, from molecules to the biosphere, and with diversity across taxa, from viruses to plants and animals. Many of the questions now being addressed by biologists require both reductionistic and incorporative elements to contribute to an answer to a larger problem. Biologists are coming to the realization that our ability to deal with questions of biological complexity would benefit from high throughput systems biology, referred to as "-omics" technology. Integrated "omics" approaches have created exciting opportunities for biological researches. High-throughput studies of biological systems are rapidly accumulating a wealth of 'omics'-scale data. However, this abundance of information also presents many obstacle. The main one includes extraction of discernable biological meaning from multiple omics data sets. In the presentation, two examples on successful integration of conventional biological study, nanotoxicity data and omic approach in understanding the effects of engineered nanomaterials on model test organisms (*Dapnia magna* and *Tetrachymena termophyla*) are presented in detail. At present, one can conclude that, the omics technologies are likely to continue its expansion for all fields in biological but when integrating omic-data with the traditional approaches the difficult problems on previously unprecedented scales and could be tackled. Apparently, it is all about integrating, in biology. Integrative biology.

PL-8

GLYCANS AS INTEGRATORS OF GENES AND ENVIRONMENT – AN OFTEN-IGNORED LAYER OF BIOLOGICAL COMPLEXITY

G. Lauc
University of Zagreb Faculty of Pharmacy and Biochemistry

Glycosylation is an essential posttranslational modification generated by a complex biosynthetic pathway comprising hundreds of glycosyltransferases, glycosidases, transcriptional factors, ion channels and other proteins. This process results in the creation of branched oligosaccharide chains, called glycans, which become integral part of proteins and significantly contribute to their structure and function. Nearly all proteins invented after the appearance of multicellular life are glycosylated and glycans are glycans are functionally relevant for nearly all processes at the multicellular level. Since glycans are created without the genetic template, alternative glycosylation creates an additional layer of structural complexity by combining genetic variability with past and present environmental factors.

Individual variability in glycome composition is very large, but glycosylation of an individual protein seems to be under strong genetic influence, with the heritability of the (for example) IgG glycome being up to 80%. Structural details of the attached glycans are of great physiological significance and many pathological conditions are associated with various types of glycan changes. Since the onset of genome wide association studies (GWAS), thousands of genetic loci have been associated with different diseases and traits. However, in the last few years, and particularly after recent publication of the results from the ENCODE project, it is becoming increasingly clear that GWAS studies are only a beginning of the understanding of complex human diseases. Hypotheses generated in these studies have to be put in the context of complex biology of life and a more elaborate approach that combines different 'omics phenotypes is needed to understand disease mechanisms and perform patient stratification that transcends genomics. Glycomics, as by far the most complex epiproteomic modification, has an immense potential in this respect, which is only beginning to be investigated.

USMENA PRIOPĆENJA

ORAL PRESENTATIONS

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

O-1
VRIJEDNOST FAUNISTIČKIH ISTRAŽIVANJA KAO TEMELJA ZA PLANIRANJE AKTIVNE ZAŠTITE PRIRODE - PROJEKT INTEGRACIJE U EU NATURA 2000 (NIP)

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Projekt integracije u EU Natura 2000 (NIP projekt) je stručni projekt sektora zaštite prirode kroz koji se prikupljaju postojeći te novi podaci o rasprostranjenju vrsta iz odabranih prioritetnih taksonomskih skupina. Prvom fazom projekta predviđeno je prikupljanje literaturnih i muzejskih podataka za 13 taksonomskih skupina, a kroz drugu terensko prikupljanje recentnih podataka i laboratorijska analiza za 9 prioritetnih taksonomskih skupina. U okviru projekta izrađuje se i detaljna karta prirodnih i polu-prirodnih ne-šumskih staništa Hrvatske. Primjer skupine Plecoptera, prikazuju važnost faunističkih projekata za planiranje aktivne zaštite prirode. Prema Crvenom popisu obalčara (2007) u Hrvatskoj je bilo poznato 28 vrsta obalčara, a Državni zavod za zaštitu prirode je raspolagao s 152 nalaza obalčara. Obradom dostupnih literaturnih referenci i muzejskih nalaza, te terenskih istraživanja u 2014. i 2015. godini ukupno su prikupljeni podaci o 3979 nalaza za 110 vrsta.

Ključne riječi: aktivna zaštita prirode, faunistička istraživanja, NIP projekt

FAUNISTIC RESEARCH VALUES AS THE BASELINE FOR PLANNING ACTIVE NATURE CONSERVATION - EU NATURA 2000 INTEGRATION PROJECT (NIP PROJECT)

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EU Natura 2000 Integration Project (NIP project) is a professional project of nature protection sector, through it will be gathered existing and new data about distribution of species of the selected priority taxonomic groups. The first phase of the project foretell gathering of literature and museum data for 13 taxonomic groups, and throughout the second gathering recent data and laboratory analysis for 9 priority taxonomic groups. As a part of the project it will be also made a detailed map of natural and semi-natural non-forest habitats of Croatia. Example on taxonomic group Plecoptera is showing the importance of faunistic projects in planning active nature protection. According to the Red list of stoneflies (2007) in Croatia it was known 28 stoneflie species, and State Institute for Nature Protection disposed with 152 findings of stoneflies. Analysing the available literature references and museum findings, and also field research findings in 2014 and 2015, a total of 3979 findings for 110 species were gathered.

Key words: active nature protection, faunistic research, NIP project

O-2
ASSESSING PROTECTED AREA VALUES AND BENEFITS IN THE DINARIC ARC

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WWF recently completed a protected areas benefit assessment in 58 parks, representing over 50% of all protected territory in the Dinaric Arc. The Protected Area Benefits Assessment Tool (PA-BAT) assesses the full range of legal benefits arising from protected areas, using a structured questionnaire in a participatory way. The PA-BAT looks at issues ranging from ecosystem services such as clean water, local products, sustainable fisheries, to tourism and less quantifiable values. In many of the national and nature parks the workshop has been the first opportunity for local communities, water and energy companies, tourism experts and fisher folk to meet park staff and discuss what the protected area means to them. In many cases, managers and other workshop participants have been unaware of the benefits that are important to other stakeholders. The PA-BAT has been appli ed in about 60 PAs in eight countries, from Slovenia to Albania; certainly the largest assessment of protected area benefits in the world to date. The results will inform protected area management plans, rural development, interpretation and education programmes to help influence day to day management decisions. We will present an update of the PA-BAT methodology and consolidated results, discuss lessons learned about its use at this scale to help other users; outline how the PA-BAT results can feed into PA management and policy; and look at strategic implications for the Dinaric Arc.

Key words: Protected Area Benefits Assessment Tool (PA-BAT), Dinaric Arc Parks, WWF

O-3
PRVA PRIMJENA RADARSKE ORNITOLOGIJE NA PODRUČJU BALKANSKE REGIJE

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Na temelju podataka prikupljenih uz pomoć radarskog sustava i terenskih ornitoloških istraživanja, moguće je donijeti preciznu procjenu o karakteristikama migracije, posebno o smjeru, visini i intenzitetu. Istraživanje je provedeno u Bosni i Hercegovini, na planini Velež. Osnovni cilj istraživanja je prikupljanje podataka o aktivnosti i kretanju ptica i otkrivanje mogućeg migracijskog koridora na istraživanom području uz pomoć radarskog sustava. U istraživanju su istovremeno korištena 2 morska radara u X frekvencijskoj zoni koji su radili u različitim modovima (vodoravni i uspravni). Podaci su sakupljani u periodu 24.8.-26.9.2014. Radarski podaci (signali na radarskom zaslonu) snimljeni su uz pomoć "frame grabber" kartice na računalo. Sakupljeni podaci sastojali su se od nekoliko mjera koje uključuju udaljenost signala i horizontalnu udaljenost od radarske antene, kao i visinu signala. Uz pomoć softvera *Distance* izračunat je korekcijski faktor kako bi se u izračun uključila različita vjerojatnost detekcije signala s obzirom na njegovu udaljenost od antene radara. Rezultati pokazuju razliku u dnevnom i sezonskom intenzitetu migracije. Upotreba radarskog sustava pridonosi boljem

poznavanju migracije s obzirom na vertikalnu distribuciju i smjer migracije. Kako bi se dobili kompletni podaci o migraciji na određenom području, potrebno je kombinirati radarski sustav i standardne ornitološke metode, a idealno bi bilo potkrijepiti ih s lokalnim i regionalnim meteorološkim podacima.

Ključne riječi: radar, migracija ptica, radarska ornitologija, vertikalna distribucija leta, softver *Distance*

THE FIRST APPLICATION OF THE RADAR ORNITHOLOGY IN THE BALKAN REGION

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Based on data collected from both radar system and a field ornithologist's surveys, a precise approximation of the bird migration patterns can be made, especially on data such as birds' flight direction, altitude and intensity. The survey was conducted in Bosna and Herzegovina, on Velež mountain. The main goal was to collect bird activity and movements' data with radar system in order to investigate possible migration corridor over the area.For collection of migration data two X-band marine radars were used simultaneously, working in two different modes (horizontal and vertical). Data was collected in the period from 24th August to 26th September 2014. The radar data (signals on the radar screen) were captured using a "frame grabber" card and recorded to a computer. Collected data consisted of several distance measurements in relation to radar antennas including Euclidean distance, altitude and land distance. To count a distance-dependant probability of radar signals, a correction factor was calculated using Distance software. Results showed seasonal and diurnal difference of migration intensity. Use of radar system greatly contributes to better understanding of migration patterns especially vertical distribution of migration and migration direction. In order to acquire complete data on bird migration, it is necessary to integrate the radar system with standard ornithological research methods. Ideally, this would be substantiated with local and regional meteorological data.

Key words: radar, bird migration, radar ornithology, migration pattern, flight attitude, Distance software

O-4
MEĐUNARODNO VAŽNA PODZEMNA SKLONIŠTA ZA ŠIŠMIŠE HRVATSKOJ - PRIMJENA U OČUVANJU I ZAŠTITI

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U Hrvatskoj je zabilježeno 55 međunarodno važnih skloništa za šišmiše predano UNEP/EUROBATS Sporazumu. U podzemna skloništa uvrštene su špilje, jame, rudnici, željeznički tuneli i tuneli za odvodnju. Svako sklonište sadrži sljedeće podatke: koordinate, ime objekta, tip objekta, primarno korištenje (porodiljska kolonija, hibernacija, migracije, cjelogodišnje korištenje), ime i kod POVS područja, biogeografsku regiju u kojoj se nalazi, broj

vrsta šišmiša koji je u skloništu zabilježen i zaštita skloništa (težak pristup, zatvoreno vratima, otvoreno za turističke potrebe). Za svaku vrstu u skloništu naveden je godina u kojoj je zabilježen najveći broj jedinki od 1994. godine te način korištenja skloništa. Ukupno je u ovim podzemnim skloništima zabilježeno 25 vrsta šišmiša od 34 prisutne u Hrvatskoj. U pravilu najveće hibernacijske kolonije nalaze se u kontinentalnoj biogeografskoj regiji, dok su u mediteranskoj najveće porodiljne kolonije većine vrsta. U ekološkoj mreži Natura 2000 nalaze se svih 55 skloništa te je za sve zahvate, planove i programe potrebno sagledati utjecaj na ciljne vrste šišmiša. Tada se provode predviđeni zakonski mehanizmi koji na razini strateškog planiranja, prostornog plana ili samog zahvata uzimaju u obzir tip skloništa, način korištenja skloništa te ekologije ciljne vrste šišmiša. Predstavljamo kriterije i primjere ocjene utjecaja na razini strateške ocjene županijskog prostornog plana te nekoliko ocjena zahvata vjetroelektrana.

Ključne riječi: šišmiši, podzemna skloništa, zakonodavstvo, očuvanje, glavna ocjena utjecaja

INTERNATIONALLY IMPORTANT UNDERGROUND SITES FOR BATS - CONSERVATION AND PROTECTION APPLICATION

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There are 55 Internationally important underground roosts for bats submitted to UNEP/EUROBATS Agreement. Underground sites in Croatia refer to caves, pits, mines, railway tunnels and waterway tunnels. Each site has following data: coordinates, name, type, primary usage (all year, hibernation maternity, transient/migratory), name and code of pSCI, biogeographical region, number of bat species recorded and protection (gates, grilles, difficult access, open for public). For each bat species per site the year in which the biggest number of individuals recorded since 1994 is given together with the type of usage. In all underground sites listed, altogether 25 bat species out of 34 present in Croatia are recorded. As a general rule for the most species the most numerous hibernacula are in the continental biogeographical region, whereas in the mediterranean biogeographical region the biggest maternities are present. All 55 underground sites are a part of the Natura 2000 Ecological Network which means that for every project, plan and programme an impact to the Natura site conservation objectives should be assessed. Legal mechanisms required at the strategic planning and physical planning level or project level are presented taking into account roost type, usage type and ecology of bat species. Provided are examples of criteria and evaluation of strategic impact assessment of the county physical plan and Appropriate assessment on the project level of several windfarms.

Key words: bats, underground roosts, legislation, conservation, appropriate assessment

O-5
SUSTAV ZA MULTISPEKTRALNU ANALIZU VEGETACIJE ZA BIOLOŠKA I EKOLOŠKA ISTRAŽIVANJA

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Istražena je mogućnost uporabe autonomnog letećeg uređaja (ALU) za proučavanje vegetacije iz zraka za potrebe bioloških i ekoloških istraživanja. Prilikom istraživanja razvijen je jednostavni i mobilni sustav za daljinsko očitavanje vegetacijskih indeksa (NDVI, EVI, SAVI/MSAVI). Ovakav

sustav omogućava efektivno i brzo prikupljanje podataka o vegetaciji iz zraka. Sama tehnika detektiranja vegetacijskog indeksa temelji se na dvjema jednakim komercijalnim HD kamerama. Jedna od kamera je bila modificirana za detekciju valnih duljina u blisko infracrvenom području (*near-infrared*, NIR) dok je druga snimala valne duljine vidljive svjetlosti. Slike dobivene kamerama su se pohranjivale i obrađivale tijekom leta čime je omogućeno neposredno korištenje rezultata u daljnjim znanstvenim istraživanjima. Preliminarna analiza dobivenih podataka pokazuje veliki potencijal za korištenje ovakvog sustava u biološkim i ekološkim istraživanjima.

Ključne riječi: vegetacijski indeksi, NIR, ALU, kamera, daljinsko očitavanje

MULTISPECTRAL VEGETATION ANALYSIS SYSTEM FOR BIOLOGICAL AND ECOLOGICAL RESEARCH

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Possibility of usage of unmanned aerial vehicle (UAV) for airborne vegetation observation for demand of biological and ecological studies has been researched. Throughout the study a simple and mobile system for remote sensing of vegetation indices (NDVI, EVI, SAVI /MSAVI) has been developed. This kind of system facilitates effective and rapid accumulation of aerial vegetation data. The aforementioned technique is based on two identical consumer-grade HD cameras. One of these cameras has been modified to detect near-infrared wavelengths (NIR), while the additional camera recorded visible spectrum wavelengths. Images given by the cameras were being stored and processed during the flight, this enabled point-blank usage in further scientific research. Preliminary analysis of given data shows promising potential for use of this kind of system in biological and ecological research.

Key words: Vegetation indices, NIR, UAV, Camera, Remote sensing

O-6
ENDEMIC AND RELICTS SPECIES IN SPECIAL NATURE RESERVE ZASAVICA

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The tertiary relics of the flora of the reserve are: *Stratiotes aloides*, *Trapa natans* agg., *Butomus umbellatus*, *Erithronium dens-canis*, *Humulus lupulus*, *Hidrocharis morsus-ranae*, *Isopirum thalictroides*, *Loranthus europaeus*, *Tamus communis*, *Viscum album* and *Aldrovanda vesiculosa*. The Special Nature Reserve ''Zasavica'' is the only habitat in Serbia for the plant *Aldrovanda vesiculosa*, which was long thought to be extinct in Serbia. The three species of flora of the reserve are glacial relicts *Urtica kioviensis*, *Scirpus triqueter*, *Thelypteris palustris*, and *Achillea asplenifolia* is the Pannonian subendemic. The fauna of the reserve has recorded four endemic and relicts species of invertebrates and vertebrates. From invertebrates there is found a rare endemic branchiopods shrimp *Chirocephalus brevipalpis* and a relic living fossil *Lepidurus apus*. The entomofauna of the reserve has two endemic species *Stenopterus similatus* and *Zeuneriana amplipennis* which is found again after more than thirty years. From the class of Gastropoda there was identified a Balkan endemic species of *Tandonia kusceri*. A

special rarity of the reserve is the globally endangered relict species *Umbra krameri* for which the flow Zasavica is one of the three remaining habitats in Serbia, and this subpopulation is one of the most endangered in the whole complex in Europe. The fauna of amphibians and reptiles present three Balkan endemics *Triturus dobrogicus*, *Lacerta agilis* ssp.*bosnica* and *Vipera berus bosniensis* (black melanistic form in the reserve) which in Posavina has a relict character.

O-7

LIŠAJEVI KAO BIOINDIKATORI KVALITETE ZRAKA NA PODRUČJU MOTOVUNSKE ŠUME

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Lišajevi su među najpoznatijim biološkim indikatorima za procjenu kvalitete zraka jer svu vodu potrebnu za život u cijelosti primaju iz zraka. Obzirom na navedeno, mogu se koristiti za procjenu stanja okoliša uzimajući u obzir više izvora zagađenja i različite efekte (eng. *multi-pollutant/multi-effect*). Određivanjem vrsta i brojnosti lišajeva, moguće je mapirati učinke dugoročnog (kumulativnog) onečišćenja zraka na velikim područjima. Korištenjem lišajeva kao bioindikatora određena je kvaliteta zraka na području Motovunske šume u sklopu projekta "Plan restauracije starog korita Mirne - Biološko-ekološka istraživanja i podloge". Na 18 ploha je izvršeno uzorkovanje lišajeva te su određene prisutne vrste i njihova učestalost. Prema prisutnim vrstama i njihovoj abundaciji, zaključeno je kako se gotovo cijelo područje odlikuje iznimno visokom kvalitetom zraka kao i visokom količinom vlage u zraku. Istraživanjem je utvrđeno kako u Motovunskoj šumi, u kojoj su debla većinom prekrivena mahovinom, nije moguće odrediti kvalitetu zraka prema standardiziranoj metodi za određivanje kvalitete zraka pomoću lišajeva u Europi. Stoga se za vlažne šume slične Motovunskoj šumi predlaže korištenje metode koja se trenutno primjenjuje u SAD-u, a na koju ne utječe količina mahovina. Rezultati potvrđuju primijenjivost novih ekoloških indeksa za slična istraživanja kvalitete zraka i općeg stanja istraživanog područja.

Ključne riječi: lišajevi, onečišćenje zraka, kvaliteta zraka, bioindikatori, vlažne šume

LICHENS AS BIOINDICATORS OF AIR QUALITY IN THE MOTOVUN FOREST AREA

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Lichens are among the best known biological indicators for assessing the air quality, due to their ability to fully receive all water necessary for life directly from the air. Hence, they are important because they can be used to estimate the multi-pollutant/multi-effect environment condition. By determining the species and the number of lichens, it is possible

to map the effects of long-term (cumulative) air pollution in large areas. By using lichens as bioindicators the air quality in the Motovun forest area was determined as part of the "Plan for the restoration of the Mirna river old bed - Biological and ecological research and background". On the 18 plots lichens were sampled and present species and their frequency was determined. It was concluded that almost the entire area is characterized by extremely high air quality, as well as a high amount of air moisture. The survey identified shortcomings in the existing standardized method for determining air quality using lichens in Europe, when working in a humid forest with high moss abundancy on the tree trunk. When working in the woods with humidity levels comparable to this area, it is proposed to use the method currently used in the USA that is not biased with the moss on the tree trunk. The results confirm the applicability of new environmental indices for similar studies of air quality and general condition of the study area.

Key words: lichens, air pollution, air quality, bioindicators, humid forest

O-8

UTJECAJ METEOROLOŠKIH PARAMETARA NA VARIJACIJE DVOSATNIH KONCENTRACIJA PELUDNIH ZRNACA ČEMPRESA (CUPRESSACEAE), GRAD ZADAR, HRVATSKA

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Pelud iz porodice čempresa (Cupressaceae) najbrojnija je pelud u atmosferi grada Zadra s udjelom od gotovo 50% u ukupnom broju peludnih zrnaca u zraku. Smatra se da peludna zrnca iz porodice čempresa imaju umjeren do visok alergeni potencijal te oni spadaju u glavne alergene na mediteranskom području. Svrha istraživanja je utvrditi dvosatne fluktuacije peludnih zrnaca iz porodice čempresa u danima sa zabilježenom visokom koncentracijom (> 90 pz/m^3) te utvrditi utjecaj nekih meteoroloških parametara na dvosatne varijacije peludnih zrnaca čempresa. Istraživanje je provedeno tijekom 2007.- 2009. na temelju preporuka EAN / REA. Aerobiološki uzorci su prikupljeni pomoću automatskog sedmodnevnog volumetrijskog uzorkivača tipa Hirst. Dobiveni rezultati pokazuju da je pelud iz porodice čempresa (Cupressaceae) prisutna u atmosferi grada Zadra tijekom cijele godine, ali većina peludnih zrnaca čempresa zabilježena su tijekom zimskih mjeseci (od veljače do travnja). Na varijacije dvosatnih koncentracija peludnih zrnaca čempresa statistički značajan i pozitivan utjecaj imaju temperatura, brzina vjetra i broj sunčanih sati, dok je utjecaj relativne vlage zraka statistički značajan i negativan.

Ključne riječi: aerobiologija, Cupressaceae, dvosatne koncentracije, Hirst, Zadar

METEOROLOGICAL INFLUENCE ON DIURNAL CUPRESSACEAE POLLEN VARIATION (ZADAR, CROATIA)

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Cupressaceae pollen is the most abundant pollen in the atmosphere of Zadar with a share of almost 50% in total pollen count. The allergenic potential of Cupressaceae pollen is moderate to high and it represents the major airborne allergen in Medierranean area. The purpose of this study was to establish two-hour interval daily fluctuations of Cupressaceae pollen during days with marked high concentration (>90 pg/m^3) and to determine influence of some meterological parameters on diurnal Cupressaceae pollen variation. The study was carried during the 2007-2009 using the Spanish Aerobiological Network (EAN/ REA) recommendation. Aerobiological samples were collected via Hirst-type, seven- day volumetric sampler. The obtained results showed that the Cupressaceae pollen is present in the Zadar atmosphere during the whole year, but the majority of Cupressaceae pollen is marked during the winter months (from February till April). Correlations between Cupressaceae pollen diurnal concentration and meteorological parameters were statistically significant: influence of temperature, wind speed and hours of sunshine were positive and influence of relative humidity were negative.

Key words: aerobiology, Cupressaceae, diurnal concentration, Hirst, Zadar

O-9

MLADICA (*Hucho hucho*) NA BALKANU: RASPROSTRANJENOST I BUDUĆI UTJECAJI HIDROENERGETSKIH PLANOVA

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Mladica je jedna od najzagonetnijih slatkovodnih riba Europe. Povijesno, ova je vrsta bila široko rasprostranjena čitavim dunavskim slijevom. Od druge polovine 19. stoljeća, ukupna se brojnost mladice smanjila za dvije trećine pa su preostale populacije jako ugrožene, posebice razvojem hidroelektrana. U ovom je radu pregledano stvarno područje rasprostranjenosti mladice na Balkanu. Utvrđena su 1842 km rijeka u kojima postoji samoodrživa populacija mladice, čime je ovo područje postalo žarište za ovu vrstu. Ove se populacije nalaze u 43 različite rijeke ili odvojena riječna područja u Sloveniji, Hrvatskoj, Bosni i Hercegovini, Srbiji i Crnoj Gori. Oko 65% svih rijeka koje nastanjuje mladica nalaze se u ovim državama, što naglašava važnost balkanskih rijeka za preživljavanje ove vrste. Najveća je prijetnja ovim populacijama plan gradnje hidroelektrana. Ukupno su identificirana 93 projekta za brane izravno na rijekama u kojima živi mladica i veliki broj dodatnih projekata na pritocima ili

vodama uzvodno od sadašnjeg staništa mladice, koji će nedvojbeno degradirati stanišne uvjete nizvodno. Ako se ovi planovi izvedu, predviđamo da će najmanje 60-70% balkanskih populacija i 35-40% svjetske populacije mladice biti izgubljeno. Nadalje, preostale će populacije biti male i fragmentirane, što će im onemogućiti dugoročno preživljavanje.

Ključne riječi: mladica, Balkan, hidroenergija

THE HUCHEN (*Hucho hucho*) IN THE BALKAN REGION: DISTRIBUTION AND FUTURE IMPACTS BY HYDROPOWER DEVELOPMENT

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The Huchen is one of the most enigmatic species of Europe's freshwater fauna. Historically, the species was wide-spread across the entire Danube basin. Since the late 19th century, however, Huchen populations declined by two thirds and the remaining populations are now highly endangered by hydropower development. In this study, we review the actual occurrence of Huchen in the Balkan region. A total of 1842 river km supporting self-sustaining populations of Huchen in the region have been identified, making it the global hot spot for the species. These populations are found in 43 rivers or distinct river reaches in Slovenia, Croatia, BIH, Serbia and Montenegro. About 65% of all Huchen rivers globally are located in these countries, highlighting the importance of Balkan Rivers for the survival of the species. The major threat to these populations is a massive hydropower development plan. A total of 93 dam projects were identified directly in river reaches supporting Huchen and a large number of additional projects are located in tributaries or headwater reaches upstream of Huchen habitat that will invariably degrade environmental conditions downstream. If these plans are carried out, we predict that at least 60-70% of the Balkan population and about 35-40% of the global population of Huchen would be lost with the remaining populations being small and severely fragmented and eventually no longer able to survive in the long term.

Key words: Huchen, Balkan, hydropower

O-10
PROCJENA USLUGA EKOSUSTAVA NA PODRUČJU DRAVA-MOLVE PREMA TRI POTENCIJALNA SCENARIJA

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Poplavne nizine Save, Drave i Dunava u Hrvatskoj pod utjecajem su različitih ljudskih aktivnosti, uključujući izgradnju naselja, poljoprivredu, promet i proizvodnju energije. One također imaju velik značaj u pružanju pitke vode, zaštiti od poplava, kao staništa brojnih vrsta, u produkciji drvne mase, ribe i divljači. Stoga informacije o funkcijama i uslugama ekosustava tog područja valja uzeti u obzir prilikom izrada prostornih planova, različitih strategija te donošenja odluka u javnom i privatnom sektoru. Istraživanje je obuhvatilo 38 km riječnog toka Drave, kod općine Molve, s poplavnom nizinom ukupne površine 201 km^2. Cilj je bio dodatno argumentirati potrebu za očuvanjem riječnih ekosustava kroz promoviranje prirode kao osnove za razvoj. Analizirane su različite opskrbne, regulacijske, podržavajuće i kulturološke funkcije. Njihove vrijednosti procijenjene su prema tri potencijalna scenarija korištenja ovog područja u budućnosti: A održavanje postojećeg stanja, B provedba planiranih projekata (izgradnja HE Molve 1 i 2) i C razvoj kroz održivo korištenje prostora što uključuje proširenje poplavne zone. Korištene metode ovisile su o vrsti usluge i o ulaznim podacima. Temelj za analizu u scenariju B bili su utjecaji i promjene na uslugama ekosustava uzrokovane postojećim HE na Dravi u Hrvatskoj. Scenarij C pokazuje najvišu vrijednost za tri ključne usluge ekosustava: proizvodnji drva, osiguravanju staništa i ublažavanju poplava, dok je utjecaj scenarija B pretežno negativan.

Ključne riječi: Drava, usluge ekosustava, procjena, HE Molve

ASSESSMENT OF ECOSYSTEM SERVICES FOR THE AREA DRAVA-MOLVE ACCORDING THREE POTENTIAL SCENARIOS

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Floodplains of Sava, Drava and Danube Rivers in Croatia are a target of a variety of human activities, such as settlements, agriculture and transport or energy production. Also, they have potential to provide drinking water, flood protection, habitats for numerous species, timber production as well as fish and game animals. Information about ecosystem functions and services of this area should be taken into consideration during preparation of physical plans, strategies and by public and private sector decision making. In research is included 38 km of the Drava River, in municipality Molve and its floodplain, altogether 201 km^2. The main goal was to get additional argumentations for purpose of riverine ecosystem conservation through promotion of nature as a basis for development. Regulating, supporting, provisioning and cultural functions were analyzed. Their values w ere evaluated according three possible future scenarios: A the status as it is, B the business as usual (with HP Molve 1 and 2) and C sustainable development characterized by extending of active floodplain zone. Used methods depend on type of services and data integrity. For the scenario B were analyzed impacts and changes on ecosystem services as consequences of the three existing HP on the Drava River in Croatia. The scenario C provides the highest value for the three key services: wood production, habitats provision and flood mitigation. On the other hand the impact of the scenario B is mostly negative.

Key words: Drava, ecosystem services, assessment, HP Molve

O-11

MODELIRANJE POPULACIJE VRSTE SLABO ISTRAŽENIH KARAKTERISTIKA – PRIMJER POPOVSKE GAOVICE (*Delminichthys ghetaldii*) NA PODRUČJU OMBLE

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Matematički modeli su sastavni dio svakog suvremenog rješavanja problema u okolišu jer osim pronalaženja matematičkih zakonitosti problema modeli mogu u većoj ili manjoj mjeri sadržavati predikcijsku snagu iskoristivu za donošenje odluka te planiranje i monitoring mjera zaštite prirode i okoliša. Popovska gaovica (*Delminichthys ghetaldii*) je ugrožena vrsta šaranki koja obitava na širem području hercegovačkog i južnodalmatinskog krša. Za potrebe rješavanja jednog od okolišnih problema zahvata HE Ombla, bilo je potrebno ustanoviti da li primjerci ove vrste, koji obitavaju na području izvorišta rijeke Omble, čine samostalnu populaciju ili su samo dio veće populacijske cjeline (metapopulacije). Posebna teškoća u rješavanju ovog problema činila je činjenica da je popovska gaovica relativno slabo istražena i da je vrlo malo dostupnih podataka. Na temelju postojećih podataka, osobnih iskustava istraživača i njihovog mišljenja te teorije, načinjeno je nekoliko hipoteza. Primjenom diskretnog matričnog modeliranja u sprezi s metodama opetovanog podatkovnog uzorkovanja, utvrdile su se vjerojatnosti svake od pretpostavljenih hipoteza. Pokazano je da je popovska gaovica prisutna na području izvorišta rijeke Omble dio puno veće populacije te da bi zahvat HE Ombla, barem što se tiče opstanka ove vrste na tom području, imao pozitivan učinak.

Ključne riječi: dinamika populacija, popovska gaovica, Ombla

MODELING POPULATION OF A POORLY INVESTIGATED SPECIES - EXAMPLE ON *Delminichthys ghetaldii* IN THE RIVER OMBLA

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Mathematical models are part of every modern environmental problem solving process. Besides finding mathematical principles models can have a strong prediction power that can be used for planning and monitoring purposes and can be useful for policy makers. *Delminichthys ghetaldii* is endangered species of cyprinid family that can be found on wide Herzegovinian and the southern Dalmatian karst area. In order to solve one of the environmental problems of the project HPP Ombla, it was necessary to determine whether specimens of this species that inhabit the spring area of the Ombla, constitute an independent population or are only part of a larger population (metapopulation). Special difficulties in solving this problem accounted for the fact that the *Delminichthys ghetaldii* is relatively poorly investigated species and that there was very little information available. Based on existing data, personal experiences of researchers and their opinions and theories, we made several hypotheses. By using discrete matrix modeling in conjunction with the methods of resampling, we determined the probability of each of the proposed hypothesis. It has been shown that the *Delminichthys ghetaldii* population that is present in the Ombla spring area is part of the larger population and that a project HPP Ombla, at least concerning the survival of this species, could have a positive effect.

Key words: population dynamics, *Delminichthys*, Ombla

O-12

U POTRAZI ZA OČUVANIM RIJEKAMA: IDENTIFIKACIJA NAJZNAČAJNIJIH RIJEKA ZA ZAŠTITU PRIRODE U HRVATSKOJ

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Ovaj rad je prvi pokušaj da se sveobuhvatno ocijeni ekološko stanje i konzervacijska važnost hrvatskih rijeka. Cilj predstavljene metodologije je određivanje najznačajnijih rijeka za očuvanje cjelokupne slatkovodne bioraznolikosti u Hrvatskoj. Upotrijebili smo kombinaciju ekoregionalnog pristupa s glavnim ciljem predstavljanja svih sastavnica bioraznolikosti pojedine ekoregije u kombinaciji s određivanjem ekološkog stanja rijeka. Nakon utvrđivanja tipologije određene su glavne jedinice analize: Ocjenjivani Riječni Segmenti (ORS). Ekološko stanje svakog ORS-a određeno je kombinacijom pet kriterija: hidrologija, morfologija korita, kvaliteta vode, riparijska zona i korištenje zemljišta. Ekološko stanje svakog ORS-a dobiveno je zbrajanjem ocjena svih kriterija (maks. vrijednost 50). Podaci o rasprostranjenosti ugroženih i endemskih riba i prisutnosti zaštićenih područja korišteni su za prioritizaciju ORS-a u odličnom ili dobrom stanju. Ukupno smo analizirali 82 rijeke, ukupne dužine toka 4522 km u tri ekoregije: panonskoj, dinaridsko-kontinentalnoj i dinaridsko-mediteranskoj. Ukupna dužina toka odabranih rijeka od najveće važnosti iznosi 1607 km (22%), a dodatni ORS-i, koji obuhvaćaju ukupno 998 km toka odabrani su kako bi se osigurala reprezentativnost svih riječnih tipova u svakoj ekoregiji. Odabrani ORS-i predstavljaju najznačajnije riječne segmente, čija će zaštita osigurati dugoročno očuvanje najznačajnijih sastavnica slatkovodne bioraznolikosti u Hrvatskoj.

Ključne riječi: slatkovodna bioraznolikost, ekoregionalni pristup, tipologija, ekološko stanje, rijeke

LOOKING FOR PRISTINE RIVERS: IDENTIFICATION OF THE MOST OUTSTANDING RIVERS OF HIGH CONSERVATION VALUE IN CROATIA

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Environmental status and conservation values of Croatian rivers have never been comprehensively assessed. We present a methodology for the identification of the most pristine rivers of high conservation value which are important for the protection of freshwater biodiversity in Croatia. The methodology is a combination of ecoregional conservation approach where representation of all distinct biodiversity features is a key goal, and assessment of river reach condition. Main units of analysis, the Evaluated River Reaches (ERRs),

were identified after all river types were distinguished. The condition of each ERR was assessed using a combination of five criteria: hydrology, channel morphology, riparian vegetation, water quality and land use. The final condition of ERR was scored (max. 50) by summing scores for the five criteria. The distribution of endangered freshwater f ish species and protected areas was used for prioritizing the ERRs in high and good condition. Altogether 82 rivers and 4522 km of river length were analyzed in three ecoregions: Pannonian, Dinaric-Continental and Dinaric-Mediterranean. The total length of very high priority ERRs was 1607 km (35%), while additional ERRs comprising 998 km (22%) of river length were included to assure representation of all river types in each ecoregion. The selected ERRs represent priority river reaches whose protection should ensure the long term persistence of the most important features of freshwater biodiversity in Croatia.

Key words: freshwater biodiversity, ecoregional conservation, typology, condition, rivers

O-13

PROTOKOL PROCJENE RIZIKA OD INVAZIVNOSTI STRANIH VRSTA KORNJAČA U HRVATSKOJ

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Trgovina kućnim ljubimcima jedan je od putova unosa stranih vrsta u prirodu. Među najpoznatijim primjerima invazivne strane vrste koja je na taj način proširena svijetom je crvenouha kornjača (*Trachemys scripta elegans*). Nakon što je trgovina njome u Hrvatskoj zabranjena, došlo do značajnog smanjenja broja kornjača na tržištu te je nastala potreba za iznalaženjem drugih pogodnih vrsta. Da bi se dozvolilo stavljanje na tržište novih vrsta potrebno je isključiti postojanje ekološkog rizika u slučaju da vrsta dospije u prirodu. Budući da ne postoji standardna procedura za procjenu rizika od invazivnosti za kornjače, na temelju analiza kriterija iz literature i specifičnosti u Hrvatskoj, za tu je svrhu izrađen protokol. Prema protokolu, kriteriji za procjenu rizika mogu se podijeliti u četiri grupe koje obuhvaćaju podatke o rasprostranjenosti vrste u prirodi i područjima u kojima je vrsta kao strana uspostavila populaciju, o biološkim i ekološkim obilježjima vrste, podatke o povijesti invazivnosti te podatke o dostupnosti vrste na tržištu EU. Analiza rizika uključuje i procjenu klimatske pogodnosti staništa za što je izrađen model s kartografskim prikazom. Pitanja su zatvorenog tipa te su im pridružene vrijednosti od 0 do 3. Što je vrijednost niža to je potencijalni rizik manji. Od analiziranih vrsta one s najmanjim rizikom invazivnosti su *Graptemys versa*, *Sternotherus carinatus* i *Ocadia sinensis* dok su one s najvećim rizikom *Apalone ferox*, *A. spinifera* i *Pelodiscus sinensis*.

Ključne riječi: kornjače, strane vrste, procjena rizika, invazivnost

PROTOCOL FOR THE INVASIVENESS RISK OF THE ALIEN TURTLE SPECIES IN CROATIA

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The trade in pets is one of the main paths for introduction of alien species in nature. The most widely known example of the invasive species that has spread worldwide in this way is *Trachemys scripta elegans*. After the trade with this species was banned in Croatia the number of turtles on the market has significantly decreased and the need to find suitable species for the market has occurred. To permit the trade with a new species it is necessary to exclude the ecological risks in the case if it enters nature. As there is no standard procedure to assess invasiveness risk for turtles a protocol was created for this purpose, based on the criteria from literature and the specifics of Croatia. In accordance with the protocol criteria for risk assessment can be divided in four groups that include the data about distribution of the species in nature and in the areas in wh ich it has introduced and established a population, data about biological and ecological characteristics of the species, data about history of invasiveness and data about the availability of the species on the EU market. Risk analysis includes assessment of climate suitability. The questions are of the closed type with attributed values from 1-3. The lower the attributed value the potential risk is lower. From analysed species, the species with the least invasiveness risk are *Graptemys versa*, *Sternotherus carinatus* i *Ocadia sinensis* and with the highest risk are *Apalone ferox*, *A. spinifera* and *Pelodiscus sinensis*.

Key words: turtle, alien species, risk assessment, invasiveness

O-14

KAKO SE BORITI PROTIV INVAZIVNE VRSTE? BIOLOŠKO SUZBIJANJE KESTENOVE OSE ŠIŠKARICE UNESENOM VRSTOM PARAZITOIDA

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Kestenova osa šiškarica (*Dryocosmus kuriphilus*) je invazivna vrsta kukca u cijelom svijetu i izuzetno brzo se širi u novom staništu. Smatra se jednim od najznačajnijih štetnika na pitomom kestenu (*Castanea sativa*). Autohtone vrste parazitoida koje su trofički vezane uz hrastove ose šiškarice (*Cynipidae*) su se prilagodile ovom novom invazivnom domaćinu, ali ne mogu učinkovito utjecati na smanjenje gustoće njegove populacije. Biološko suzbijanje koristeći unesenu vrstu parazitoida *Torymus sinensis* se pokazalo kao jedina učinkovita metoda kontrole populacije kestenove ose šiškarice, usporavajući njezino širenje i smanjujući štete na podnošljivu razinu. Visoka specifičnost parazitoida *T. sinensis* upućuje na ograničeni i nepostojeći potencijal iskorištavanja autohtonih domaćina. U Hrvatskoj se u 4 godine od pojave kestenove ose šiškarice na o vog novog domaćina prilagodilo 15 vrsta autohtonih parazitoida, ali oni nemaju nikakvog utjecaja na smanjenje gustoće njezine populacije. Zbog toga je u prirodne sastojine pitomog kestena u Hrvatskog ispušten uneseni parazitoid *T. sinensis* kao dokazano učinkovito sredstvo biološkog suzbijanja. U izlaganje se prikazuju rezultati uzgoja i ispuštanja parazitoida *T. sinensis* u Hrvatskoj.

Ključne riječi: *Dryocosmus kuriphilus*, *Torymus sinensis*, autohtoni parazitoidi, invazivna vrsta kukca

HOW TO CONTROL INVASIVE SPECIES? CLASSICAL BIOLOGICAL CONTROL OF *Dryocosmus kuriphilus* WITH INTRODUCED PARASITOID SPECIES

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Dryocosmus kuriphilus is a globally invasive insect pest, spreading very quickly in new habitats and making seriously damage to sweet chestnut (*Castanea sativa*) forests in Croatia. Indigenous parasitoid species trophically associated with oak gallwasps (*Cynipidae*) have adapted to this new invasive host but cannot effectively regulate its population density. Classical biological control using introduced parasitoid *Torymus sinensis* has been proven to be the only effective method of controlling the populations of *D. kuriphilus*. *T. sinensis* can successfully control the population density of *D. kuriphilus*, slowing down the spread and mitigating negative impact of this invasive chestnut pest and keeping the damage of *D. kuriphilus* at acceptable level. High specificity of *T. sinensis* suggests that it has limited potential of exploiting native hosts. In Croatia, during 4 years 15 species of native parasitoids have adapted to new invasive host but have no impact on lowering the population density. *T. sinensis* has been released in sweet chestnut forests in Croatia as promising biological control agent. Results on laboratory rearing and release of *T. sinensis* in Croatia are presented.

Key words: *Dryocosmus kuriphilus*, *Torymus sinensis*, native parasitoids, invasive insect species

O-15
ŠTETE I KORISTI INVAZIJA UNEŠENIH RIBA U SLIVU NERETVE

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Recentna istraživanja ukazuju da brojnost većine autohtonih vrsta opada kao rezultat aktivnosti čovjeka ("gubitnici"), a zamjenjuje ih značajno manji broj invazivnih vrsta u promijenjenim ekosustavima ("dobitnici"). Ovo je posebice vidljivo u slatkovodnim ekosustavima Mediterana s visokom razinom endemizma riba, obilježenoga uskim arealom većine vrsta. Jedno od važnih područja mediteranskoga endemizma riba je sliv Neretve s opisanih dvadeset stenoendemskh vrsta. Neretva je najdulja rijeka istočno-jadranske obale (230 km), koja izvire u Bosni i Hercegovini, a utječe širokom močvarnom deltom u Hrvatskoj. Poslije 1960. godine rijeka je regulirana izgradnjom pet brana i formiranjem pet umjetnih akumulacija. Donji tok je melioriran, te je većina močvara i laguna pretvorena u poljoprivredno zemljište. Sve ove promjene dovele su do formiranja ekosustava novih značajka u odnosu na prirodne, te su se autohtone vrste riba našle pred izazovima prilagodba ili nestajanja. Dodatno su novonastali ekosustavi poribljavani nizom alohtonih vrsta, te je do danas zabilježeno dvadeset pet unešenih vrsta riba. Novija istraživanja su pokazala značajno povećanje brojnosti unešenih i smanjivanje brojnosti autohtonih vrsta riba. Ovo predavanje opisati današnju strukturu populacija riba u nekim ekosustavima, dati listu pobjednika i gubitnika, šteta i koristi u ekosustavima i ribarstvu i predloziti mjere za

ublažavanje najznačajnijih prijetnja.

Ključne riječi: invazije riba, koristi i štete, sliv Neretve

DAMAGES AND BENEFITS OF INTRODUCED FISH INVASIONS IN THE NERETVA RIVER WATERSHED

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Recent evidences show that most species are declining as a result of human activities ("losers") and are replacing by a much smaller number of expanding species that thrive in man altered environments ("winners"). This is the case in freshwater ecosystems of the Mediterranean region, where a high level of endemism makes native fish extinctions much more likely because of the small natural ranges. One of these hotspots of Mediterranean endemism is the Neretva River watershed, with 20 stenoendemic species recently described there. The Neretva River is the longest river of the Eastern Adriatic watershed, 230 km long, originating in Bosnia and Herzegovina. Only the most downstream 20 km, which are wetlands and the estuary, are in Croatia. Since the 1960s the river and its flow is regulated due to the construction of five hydroelectric power dams leading to the formation of five reservoirs. The lower part was reclaimed and significant areas or wetlands and shallow lagoons were converted to agricultural land. This provided a completely new ecological situation, creating problems for the survival of the native fish fauna. The recent list of introduced fish in the Neretva River includes 25 species. Recent studies showed significant emerging of introduced fish populations and significant decrease of the native species. This presentation will discuss new fish community structure, present list of the winners and losers and describe measures to mitigate most significant threats.

Key words: fish invasions, costs and benefits, Neretva watershed

O-16
PROTOKOL ZA DOJAVU I DJELOVANJE U SLUČAJU PRONALASKA UGINULIH, BOLESNIH ILI OZLIJEĐENIH STROGO ZAŠTIĆENIH MORSKIH ŽIVOTINJA

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U Jadranskom moru obitava nekoliko vrsta kitova, dupina i morskih kornjača. Utjecaj ljudskih aktivnosti poput ribarstva i turizma čine te vrste osobito ranjivima. Uzimajući u obzir migracijsku prirodu ovih vrsta te zajedničku odgovornost država na Jadranu, suradnja je od velike važnosti za planiranje učinkovite i dugoročne strategije zaštite te je zbog navedenog pokrenut međunarodni projekt IPA Adriatic NETCET. Državni zavod za zaštitu prirode je u okviru razvoja nacionalnog Sustava za dojavu i djelovanje još 2010. godine započeo u suradnji s Državnom upravom za zaštitu i spašavanje (112) rad na uspostavi Protokola za dojavu i djelovanje u slučaju pronalaska uginulih, bolesnih ili ozlijeđenih strogo zaštićenih morskih životinja. U rad protokola do danas su uključeni obalne i otočne veterinarske ambulante, MOC Pula, Hrvatski veterinarski institut, Veterinarski fakultet u Zagrebu, te odjeli

i službe u županijama odgovorni za Eco brodice. Vrste za koje se najčešće primaju dojave su dobri dupin (*Tursiops truncatus*) i glavata želva (*Caretta caretta*). U proteklih pet godina zaprimljene su i dojave za plavobijelog dupina (*Stenella coeruleoalba*), krupnozubog dupina (*Ziphius cavirostris*) i kornjaču sedmoprugu usminjaču (*Dermochelys coriacea*). Svi podaci skupljeni putem postojećeg Protokola upisuju se u bazu i analiziraju, dio su monitoringa vrsta, daju uvid u prijetnje i pomažu u planiranju učinkovitije zaštite.

Ključne riječi: Jadran, protokol, zaštićene morske vrste, kitovi, morske kornjače

PROTOCOL FOR ALERTING AND MONITORING OF DEAD, SICK OR INJURED STRICTLY PROTECTED MARINE SPECIES

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Several species of cetaceanas, dolphins and sea turtles are recorded for the Adriatic Sea. Impact of human activities such as fishing and tourism makes those species highly vulnerable. Due to the migratory nature of those species cross-border collaboration and shared management responsibility between Adriatic states is crucial in order to plan effective long-term conservation strategies. For that purpose, an international IPA Adriatic project NETCET is launched. Since 2010, in the frame of National Alerting and Monitoring System, State Institute for Nature Protection started cooperation with National Protection and Rescue Directorate (112) on development of Protocol for Alerting and Monitoring of dead, sick or injured strictly protected marine species. At the moment, Protocol involves veterinary ambulances on the coast and islands, MEC Pula, Croatian Veterinary Insti tute, Faculty of Veterinary Medicine and County offices responsible for eco-vessels. The most reported species are Bottlenose dolphin (*Tursiops truncatus*) and Loggerhead turtle (*Caretta caretta*). In the last five years there have also been reports on Striped dolphin (*Stenella coeruleoalba*), Cuvier's beaked whale (*Ziphius cavirostris*) and Leatherback turtles (*Dermochelys coriacea*). All data collected through the existing Protocol are registered in the database and analysed, they are the part of the monitoring of species, and they give an insight into threats and help in the planning of more effective protection.

Key words: Adriatic, Protocol, protected marine species, cetaceans, sea turtles

O-17
KADA I DA LI LJUDI I ŠIŠMIŠI MOGU DIJELITI ISTO SKLONIŠTE?

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Ovaj rad naglašava važnost suradnje na civilnoj, stručnoj, zakonodavstvenoj i regionalnoj razini u očuvanju i zaštiti strogo zaštićenih vrsta šišmiša. Zbog gubitka skloništa primarno u šumskim tipovima staništa, neke vrste šišmiša prilagodile su se na suživot s ljudima. Rezolucija 5.7. UNEP/EUROBATS Sporazuma daje Smjernice za zaštitu šišmiša u nadzemnim

skloništima s posenim osvrtom na kulturnu baštinu. Manje vrste šišmiša roda *Pipistrellus* obitavaju u čitavom nizu skloništa vezanim uz ljudske nastambe uz uvjet da je otvor kroz koji mogu proći širi od 5 mm. Za razliku od njih vrste roda *Rhinolophus* biraju napuštene objekte ili tavanske prostore koji se ne koriste ili se koriste rijetko. Od 2009. godine provedeni su uviđaji na temelju dojave građana većim dijelom u središnjoj Hrvatskoj. Zabilježeni su tipovi, veličina i starost stambenih objekata, način korištenja i vrste šišmiša ukoliko je bilo moguće prema predviđenom Protokolu. Na temelju različitih tipova zahtjeva stanara u smislu obnove objekta ili problema zbog suživota predstavljena su moguća rješenja u vidu postavljanja kućica za šišmiše, potrebna zakonska procedura te uspješnost pozitivnih rješenja. Predstavljeni su i mehanizmi očuvanja i zaštite šišmiša u Starom gradu Bosiljevo kroz praćenje stanja i ograničavanje konzervatorskih radova na restauraciji dvorca.

Ključne riječi: šišmiši, nadzemna skloništa, sinantropija, EUROBATS, mjere ublažavanja

WHEN AND IF HUMANS AND BATS MAY SHARE THE SAME ROOST

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In this work, importance of cooperation on citizen, expert, legislative and regional level in conservation and protection of strictly protected bat species is highlighted. Certain bat species adapted to the cohabitation with humans due to roost loss primarily in forests. Resolution 5.7 of UNEP/EUROBATS Agreement gives Guidelines for the Protection of Overground Roosts, with Particular Reference to Roosts in Buildings of Cultural Heritage. Smaller bat species of the genus *Pipistrellus* may find roosts that has opening more than 5 mm. Some other species of the genus *Rhinolophus* prefer abandoned houses or attics that are rarely used. Investigation based on the citizen calls were conducted since 2009 mostly in central part of Croatia. According to the proposed Protocol object type, age and usage together with bat species were recorded. Taking into account different demands of building owners like renovation or cohabitation conflict, different solutions in terms of bat box installation, the legislation procedure needed and success of positive solutions are presented. Conservation and protection mechanisms during restauration of Old Town Bosiljevo through monitoring and limitation of renovation works are given as an example.

Key words: bats, overground roosts, sinantropy, EUROBATS, mitigation

2. Simpozij edukacije biologije
2nd Biology Education Symposium

O-18
SIMPLIFIED IDENTIFICATION KEYS FOR ORGANISMS

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Simplified identification keys for organisms are important for many reasons. Using such keys and observing the details, we could learn how to find similarities and differences between organisms. We thus improve our powers of observation, which is nowadays quite often replaced by slipshod neglect. Furthermore, we can familiarise ourselves with the exceptional diversity of species and actually learn a few names. We also learn the basic structure of a key. Professional identification keys for plants and animals, used by biologists, are very complex and practically useless for laymen. They include numerous technical terms and too much information – they demand a great deal of prior knowledge from the reader. Using simplified identification keys, we can learn to classify plants and animals, which is one of the basic activities of natural sciences. And this encourages our thirst for knowledge, since we often want to find out more interesting facts about the various organisms, which we could look up later in other books or on the internet. If we find the right solution – the name of the animal or plant – we're usually delighted and encouraged to research further. The knowledge obtained by observation with a simplified key thus becomes more permanent. However, we must keep in mind that this simple key will not help us identify all organisms but only a limited number of them. Using simplified key, we learn how keys are structured and how to properly read them, while we familiarise ourselves with characteristics of animals and plants and their adaptations to the environment in which they live.

Key words: primary science education, biology education, identification keys for organisms, field work in nature

O-19
AFINITET UČENIKA ZA IZBOR ZANIMANJA U PODRUČJU BIOLOGIJE

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Istraživana je povezanost učeničkog opredeljenja za zanimanja u znanstvenom području biologije, s iskazanim interesima i životnim iskustvom. Istraživanje transverzalnog tipa provedeno je tijekom 2010. godine na uzorku 2114 učenika od 10 do 18 godina starosti. Afirmativni učenički odgovori za tematsko područje Razvoj i dosezi znanosti, sa srednjom vrijednošću ≥2,8 uzeti su kao pokazatelj istraživačke znatiželje. Utvrđeno je kako se učenici, s interesom za znanost u različitim dobnim skupinama odnose prema 8 faktora interesa, relevantnih za izbor zanimanja u području biologije ili u srodnim znanostima. Najveće su razlike interesa u korist biološki zainteresiranih učenika za faktore Uvjetovanost nasljeđem i okolišem te za Reakcije na fizikalne čimbenike. Biološki zainteresirani učenici su selektivniji od ukupnog uzorka ispitanika, a i kod njih interes za predmet biologiju opada s godinama. Izrazita je povezanost izbora profesije u znanstvenom području biologije i afiniteta za predmet Priroda/Biologija (r = 0,87), koju prati i vrlo visoka povezanost variranja iskaza učenika (c = 0,89). Učenici koji se žele baviti nekim oblikom znanstvenog rada u biologiji visoko vrednuju Korisnost predmeta (r = 0,56; c = 0,45) i potvrđuju u svojem iskustvu češće neposredne kontakte s prirodom (r = 0,44; c = 0,39).

Ključne riječi: učenički interesi, predmet biologija, izbor zanimanja, biološka znanost

STUDENTS' AFFINITIES FOR PROFESSIONAL CARRIER IN THE FIELD OF BIOLOGY

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We investigated the correlation of students' opting for occupations in the scientific field of biology, with the interest shown and their life experiences. Transversal study was conducted during 2010 on a sample of 2,114 students from 10 to 18 years of age. Affirmative students' responses to the thematic area of Development and achievements of science, with a mean ≥2.8 were taken as an indicator of research curiosity. Compared were the answers given by the different age groups of students interested in science concerning 8 factors, relevant to occupational choice in the field of biology or related sciences. The biggest differences in favour of the interested students were shown for topics dealing with the heritage and the environment, as well as for biological reactions to physical factors. Biologically interested students were more selective in their answers than the total sample. Biologically interested students also show the drop of interest for school subject biology as they are older. Distinct connection between professional choice for scientific work in the field of biology and a distinctive affinity for the subject Biology (r = 0.87) were found. Students who wish to engage in some form of scientific research in biology highly value the usefulness of biology as school subjects (r = 0.56, c = 0.45), and confirmed more frequent direct contacts with nature (r = 0.44, c = 0.39).

Key words: students' interests, Biology as school subject, carrier choice, biological scientific area

O-20
UTJECAJ AKTIVNOSTI UPOZNAVANJA BILJAKA NA MOTIVACIJU I ZNANJE UČENIKA

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U istraživanju se pošlo od pretpostavke da će mogućnost izbora i izražavanja kreativnosti u aktivnosti upoznavanja biljaka kroz izradu radova o biljkama utjecati na motivaciju učenika, a time i na promjenu njihovih stavova o njima te pridonijeti trajnijem usvajanju znanja. U istraživanju je sudjelovalo ukupno po 120 učenika 2., 3. i 4. razreda Gimnazije Josipa Slavenskog Čakovec u šk. god. 2014./15. Učenici 2. razreda ispitivani su prije provedbe zadatka, a učenici 3. i 4. razreda nakon što su u 2. razredu sudjelovali u izradi mape biljaka. Učenici su tijekom ljetnih praznika imali obvezu izraditi mapu biljaka prema vlastitom izboru i mogli su koristiti različite tehnike ili njihovu kombinaciju npr. fotoherbarij, crteže biljaka, prešane biljke, informatičke prikaze i sl. Učenici su uz svaku biljku u mapi prikazivali i dodatne podatke o biljci, također prema svom izboru. Analizirani su radovi učenika, ispitana su njihova znanja te su anketiranjem ispitana mišljenja učenika o provedenoj aktivnosti i vlastitom napretku kroz njih. Učenici 3. i 4. razreda pokazuju statistički značajno bolje rezultate od učenika 1. i 2. razreda. Čak 43% učenika bolje je motivirano za rad zbog mogućnosti izbora načina na koji će obraditi biljke. Preko 66% učenika nakon odrađenog zadaka prepoznaje obrađene biljke u svojoj okolini. Rezultati pokazuje da unatoč početnom nezadovoljstvu zadaćom učenici uočavaju svoj napredak i da su nakon zadatka više opažali biljke u okolišu te govore u prilog postavljene hipoteze o potrebi korištenja različitih oblika rada u nastavi koji potiču aktivnost i motivaciju učenika za trajnije usvajanje znanja.

Ključne riječi: aktivno učenje, izbor, mapa biljaka, motivacija

THE IMPACT OF ACTIVITIES OF FAMILIARIZING STUDENTS WITH PLANTS ON THE MOTIVATION AND KNOWLEDGE OF STUDENTS

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The study starts from the hypothesis that a possibility of choice and expression of creativity in the activities of familiarizing students with plants through creating a herbarium will affect the motivation of students, and their attitudes about plants and contribute to a more lasting acquisition of knowledge. The study included 120 students of 2nd, 3rd and 4th grade of high school, during the 2014/2015 The students 2th grade were surveyed prior to the implementation of the task, and others after having created herbaria of plants. The students had to complete a herbarium according to their choice during their summer holidays, and could use different techniques or their combinations, such as photo-herbarium, drawings of plants, pressed plants, etc. and added additional information with every plant in the herbarium. We analyzed the students herbaria, tested their knowled ge and surveyed their opinions on the performed activities and their own progress through them. Students grade 3 and 4 show significantly better results than students 1st and 2nd grade. Even 43% of students are better motivated to work because of the choice of a way of process plants. Over 66% of students after completing the task recognizes the plants in their environment. Results showed that despite the initial displeasure task students observed their progress and that after the task was seen more plants in the environment and in favor of the hypotheses about the need to use different forms of work in teaching that encourage activity and student motivation for permanent learning. The results collected through interviews show that despite initial displeasure with the assigned task, the students recognize their progress and that after the task has been carried out they notice more plants in their environment, which supports the hypothesis about the need to use different forms of work in teaching which encourage activity and motivation of students for permanent acquisition of knowledge.

Key words: active learning, choice, herbarium, motivation

O-21
MOTIVACIJA UČENIKA SEDMIH RAZREDA ZA STJECANJE ZNANJA IZ NASTAVNOG PREDMETA BIOLOGIJA

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Cilj istraživanja bio je korištenjem "Upitnika o motiviranosti za učenje biologije" utvrditi jesu li učenici sedmih razreda motivirani za učenje biologije. Uzorak su činili učenici sedmih razreda jedne gradske osnovne škole. Temeljni instrument bio je anketni upitnik koji sadrži trideset čestica zatvorenog tipa na koja su odgovori mogući unutar 5-stupanjske skale Likertovog tipa. Šest je elemenata unutar kojih su raspoređena pitanja upitnika: intrinzična motivacija, ekstrinzična motivacija, osobna procjena važnosti učenja biologije, lokus unutarnje kontrole, procjena vlastite uspješnosti, anksioznost pri provjeri znanja iz biologije. Ispitanici su ponekad do često motivirani za učenje biologije. Ekstrinzično su motivirani: važna im je dobra ocjena iz biologije no do takve ocjene žele doći uz povremeno učenje. Ne uočavaju povezanost između

sadržaja biologije i svakodnevnog života. Učenicima je stalo do postizanja uspjeha, ali su istovremeno obuzeti snažnim strahom od neuspjeha. Uzrok svog uspjeha i/ili neuspjeha učenici percipiraju kao više ili manje podložan njihovoj kontroli.

Ključne riječi: motivacija, biologija, ekstrinzična motivacija, intrinzična motivacija, osjećaj uspješnosti, strah od neuspjeha

SEVENTH GRADERS' MOTIVATION FOR KNOWLEDGE ACQUISITION OF ELEMENTARY SCHOOL BIOLOGY

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The aim of this research is to determine whether eight grade students are motivated to learn biology by using the "Motivation to Learn Biology Questionnaire". The sample consists of seventh-grade students from an urban elementary school. The main research instrument is a questionnaire containing 30 close-ended items with a five-point Likert-type scale. Items are distributed between six different components: intrinsic motivation, extrinsic motivation, personal relevance of learning biology, self-determination, personal success assessment, and anxiety about biology assessment. The majority of participants stated they are "sometimes to often" motivated to learn biology. They do not consider it a challenge. They feel successful when they understand the content of the subject. They are extrinsically motivated: a good grade in biology is important to them but they want to achieve it by learning occasionally. They do not see the connection between the content of biology as a school subject and everyday life. Students care about being successful, but at the same time, they dread failure. Students perceive the cause of their success and/or failure as being more or less under their control.

Key words: motivation, biology, extrinsic motivation, intrinsic motivation, feeling of success, fear of failure

O-22
E-BIOLOGIJA NA ZDRAVSTVENOM VELEUČILIŠTU

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Kako bismo studentima omogućili kvalitetnije savladavanje gradiva i olakšali nastavni proces, na Zdravstvenom veleučilištu osmislili smo jedan hibridni nastavni proces koji uz klasične načine poučavanja uključuje i napredne tehnološke istupke. Tako smo predavanja i seminare smjestili na platformu za e-učenje Moodle te na studentsku platformu za razmjenu datoteka baziranu na Microsoft sharepoint tehnologiji. Studentima smo omogućili konstantan pristup nastavnim materijalima, jednostavniju predaju seminarskih radova te ostvarili jednostavniju i bržu suradnju sa studentima. U vježbovnoj nastavi korištenje samo jednog tipa nastavnog pomagala često ograničava mogućnost izravnog tumačenja određenog gradiva, naročito kada je riječ o staničnoj biologiji. Svjetlosnim mikroskopom, kao osnovnim pomagalom u vježbovnoj nastavi stanične biologije, ne možemo vidjeti stanične organele u citoplazmi eukariotskih organizama. U tom

slučaju, osim izrade mikroskopskih preparata, koristimo i aplikacije na tabletima, poput "Cell World", "Cell and cell structure", "Cytogenetics" i "Gene Screen", koje kao i u slučaju mikroskopa, svaki student dobiva na korištenje za vrijeme nastave. Također, jedan od mikroskopa je opremljen kamerom i povezan sa svim tablet računalima.Treba naglasiti da niti jedno od navedenih pomagala nije zamjena za ono drugo, već isključivo nadopuna i tek njihovom kombinacijom, odnosno zajedničkom primjenom, dobivamo cjeloviti pristup u procesu poučavanja.

Ključne riječi: nastavni proces, biologija, mikroskop, tablet, aplikacije

E-BIOLOGY AT THE UNIVERSITY OF APPLIED HEALTH SCIENCES

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In order to enable the students to master the subject matter with more quality and in order to ease the teaching process, we have conceived a hybrid teaching process, which in addition to classic ways of teaching also includes advanced technological approaches. So we installed the teaching materials on an e-study platform Moodle and on the student platform for file exchange, based on Microsoft sharepoint technology. We have enabled the students to have constant access to teaching material, simpler hand over of seminar papers and we have accomplished a simpler and faster collaboration with students. In the practical lessons, the use of only one teaching tool often limits the possibility of direct interpretation of a certain subject topic, especially in cellular biology. By using the light microscope, as a basic tool in practical lessons, we cannot see cellular organelles in the cytoplasm of eukaryotic organisms. In that case, besides making microscopic preparations, we also use applications on tablets, such as "Cell World", "Cell and cell structure", "Cytogenetics" and "Gene Screen", which are given to each student during classes, the same as in the case of microscopes. Also, one of the microscopes is equipped with a camera and connected to all tablets. It should be emphasized that none of the mentioned tools is a replacement for the other, but exclusively a supplement and we attain a complete approach to the teaching process only through their mutual application.

Keywords: teaching process, biology, microscope, tablet, applications

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UPORABA DIGITALNIH NASTAVNIH SADRŽAJA U NASTAVI BIOLOGIJE

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Cilj istraživanja je analiza utjecaja informacijsko-komunikacijske tehnologije (IKT) na nastavu biologije i prirode u hrvatskim osnovnim školama, s naglaskom na korištenje digitalnih nastavnih sadržaja. Uporabom online upitnika dobiveni su odgovori ukupno 60 učitelja biologije. Dobiveni podaci potvrđuju značajan utjecaj IKT na suvremene nastavne metode u nastavi biologije. Projekti kojima se financijski podupiru škole u nabavci informatičke opreme imaju utječu na uvjete rada 70% ispitanika. Gotovo polovica ispitanika sudjelovala je u nekom od školskih projekata koji su uključivali korištenje IKT. Međutim čak polovica ispitanika tvrdi da o ovoj tematici uči samostalno te ističe nedostatak formalne edukacije. 65% učitelja izjavljuje da koristi digitalni nastavni sadržaj u svakodnevnom radu, ali od toga je veći broj onih koji

koriste gotove materija le izdavačkih kuća, a nešto manji broj onih koji materijale nalaze na Internetu ili izrađuju sami. Skup informacija i sadržaja dostupan putem Interneta potencijalno značajno mijenja način rada učitelja u današnje vrijeme. Način njihova korištenja i prilagodba predviđenom nastavnim sadržajima je zahtjevan, a posljedično prespor zadatak za širu zajednicu nastavnika biologije. U svrhu bolje efikasnosti, a i ubrzanja ovog procesa, potrebno je raditi na razmjeni iskustava, dijeljenju digitalnih sadržaja i formalnoj edukaciji učitelja u okviru ideje o cjeloživotnom učenju.

Ključne riječi: digitalni nastavni sadržaj, biologija, osnovna škola

THE USE OF DIGITAL EDUCATIONAL CONTENT IN TEACHING BIOLOGY

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The aim of the research is to analyze the impact of Information and Communication Technologies (ICT) in teaching Biology in Croatian elementary schools, with an emphasis on the use of digital educational content. Online questionnaire was used and responses of a total of 60 teachers of biology were obtained. The obtained data confirm the significant impact of ICT on the modern teaching methods. Projects which provide financial support for the school in the procurement of IT equipment have affected labor conditions 70% of respondents. Nearly half of respondents participated in some of the school projects involving the use of ICT. On this subject teachers learn independently and they emphasize the lack of formal education. 65% of teachers state that they use digital educational content in their daily work, but there is more of those who use the ready-made materials, and a slightly smaller number of those who search digital content on the Internet or develop it by themselves. The set of information and content available on the Internet potentially significantly changes nowadays teachers methods. The way of their use and adaptation of the planned teaching content is demanding, and consequently slow task for the wider community of teachers. To improve the efficiency and acceleration of this process, it is necessary to work on the exchange of experiences, sharing of digital content and the formal education of teachers in the framework of the idea of lifelong learning.

Key words: digital educational content, biology, primary school

O-24
PROJEKTNA TERENSKA NASTAVA NA OBRONCIMA MEDVEDNICE

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Terenska nastava kao oblik učioničke nastave pedagoški je učinkovita i zanimljiva praksa. Nastavni sadržaji predočeni kao primarni izvor znanja postaju vidljivi i opipljivi, a samim time i zanimljiviji, što uzrokuje njihovo lakše pamćenje. Okoliš Medvednice pruža mnogobrojne mogućnosti za otkrivanje i razumijevanje svijeta u kojem jesmo. Cilj je projekta motivirati i osvijestiti učenike o važnosti očuvanja prirodne baštine. Projekt je zamišljen kao višegodišnji (započet u svibnju 2014. godine) kroz koji se učenicima ukazuje na promjene u vodenom svijetu nastale djelovanjem prirode i čovjeka. Učenici su nosioci svih aktivnosti u sve tri etape terenske

nastave pri čemu je naglasak stavljen na praktičan rad na terenu – određivanje različitih parametara vode metodama i načinima prilagođenim kognitivnim i psihomotoričkim sposobnostima dane učeničke dobi. Sve se etape izvode metodom grupnog rada polustrukturiranim zadacima koji obuhvaćaju kemijsku analizu vode potoka, odnosno određivanje kloridnih, fosfatnih, nitratnih, nitritnih te amonijevih iona u vodi, tvrdoće, pH i temperature vode, slobodnog i vezanog CO_2, brzine protoka te YTT-test. Projektna terenska nastava kao složeni oblik praktičnog, misaonog i neposrednog stjecanja znanja potiče kod učenika intelektualnu radoznalost, što utječe na međuvršnjačku (znanstvenu) komunikaciju te stvaranje kvalitetnih odnosa među samim učenicima te između nastavnika i učenika.

Ključne riječi: projektna nastava, terenska nastava, motivacija, zaštita prirode

PROJECT FIELD CLASSES ON THE SLOPES OF MEDVEDNICA

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Field classes as a form of teaching process are an educationally effective and interesting practice. The curricula content presented as a primary knowledge source become visible and tangible, and in turn more interesting, which makes them easily memorable.The natural resources of Medvednica Hill offer a variety of possibilities for discovering and understanding the world we live in. The objective of the project is to motivate students and to make them aware of the importance of conservation of natural heritage. The project started in May of 2014 and it is envisioned as a several-year long productive project through which students discover the changes in the aquatic world that have occurred due to natural and man-caused effects. Students are the main contributors in the activities and the stress is on practical work, i.e. determining various properties and parameters of creek water methods tailored to the students' age in the cognitive and psychomotor sense. All the work stages are implemented by means of group work on semi-structured tasks comprising a chemical and physical analysis of the creek water. The project field classes, as a complex practical, cognitive and direct knowledge acquisition process, arouse the intellectual curiosity in students, which in turn influences not only the quality of inter-coeval (scientific) communication, but also enhances relationships and camaraderie among the students themselves.

Keywords: project strategies, field classes, motivation, environmental protection

O-25
ANALIZA STAVOVA I INTERESA UČENIKA SREDNJIH ŠKOLA O PRIRODNIM VRIJEDNOSTIMA MEĐIMURSKE ŽUPANIJE

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Zadaća ekološke edukacije je prenijeti poruku o značaju i nužnosti očuvanja prirodnih i drugih vrijednosti, a najmoćnijim sredstvom ekološke edukacije smatra se interpretacija. Već je otac interpretacije Freeman Tilden istaknuo: "Interpretacijom do razumijevanja, razumijevanjem do

poštovanja, poštovanjem do zaštite". Korištenjem izravnih i neizravnih oblika interpretacije, od strane djelatnika Međimurske prirode-JU za zaštitu prirode i dvoje nastavnika osmišljen je edukativni program Mladi čuvari prirode. Predmetni odgojno-obrazovni program polazilo je četerdesetak učenika šestih razreda osnovnih škola Međimurske županije. Program se realizirao kroz predavanja, radionice i terenski rad tijekom proljeća 2009. godine s ciljem ekološke edukacije učenika o prirodnim vrijednostima Međimurske županije i povećanjem interesa za prirodoslovlje. Cilj nam je u ovom radu utvrditi utjecaj programa Mladi čuvari prirode na razvoj pozitivnijih stavova o zaštiti prirode na području Međimurske županije te povećanju interesa za prirodoslovlje. Stavovi učenika ispitani su online anketom. U anketiranju su sudjelovali polaznici programa Mladi čuvari prirode 2009. godine, a kao kontrolna skupina njihovi vršnjaci koji nisu sudjelovali u programu. Za analizu ankete primijenjene su neparametrijske statističke metode budući da je riječ o malom uzorku. U anketi se, osim stavova, ispituju i interesi iz područja prirodoslovlja s posebnim naglaskom na zaštićene dijelove prirode.

Ključne riječi: prirodoslovlje, prirodne vrijednosti, čuvari prirode, stavovi

MIDDLE SCHOOL STUDENTS´ ATTITUDES AND INTERES ANALYSIS ABOUT THEIR NATURAL VALUES IN THE MEĐIMURJE COUNTY

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The task of ecological education is to convey a message about the importance and necessity of preserving natural and other values, and the most powerful tool for ecological education is considered to be the interpretation. The father of interpretation, Freeman Tilden said: „Through interpretation, understanding; through understanding, appreciation; through appreciation, protection.“ Employees of Međimurska priroda (Međimurje nature), and two teachers worked out, using direct and indirect forms of interpretation, an educational program called Young guardians of nature. Fourty pupils from the sixth grade from all primary schools of the Međimurje county attended the educational program. The program was realised through a series of lectures, workshops and field work which took place during the spring of 2009 with a goal of ecological education and increasing publi c interest for natural sciences. The goal in this paper is to determine the influence of the program Mladi čuvari prirode on the development of a more positive attitude on the protection of nature in the Međimurje county aswell as increasing the public interest for natural sciences. Students took an online survey and gave their opinion. Participants of the Mladi čuvari prirode 2009 took the survey, while their peers were the control group, who didn't participate in the mentioned program. Except viewpoints, the survey tested interests for natural sciences with special emphasis on protected parts of nature.

Key words: natural science, natural values, Young guardians of nature, attitudes

O-26
RAZVOJ JEZIČNIH, DRUŠTVENIH I UPRAVLJAČKIH KOMPETENCIJA U UČENIČKIM ISTRAŽIVAČKIM PROJEKTIMA U PROGRAMU GLOBE I BIOLOGIJI

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Srednja škola Prelog je 12 godina uključena u GLOBE program i u okviru GLOBE-a i izborne nastave biologije odrađeni su brojni učenički istraživački projekti vezani uz prirodne ekosustave u okruženju (jezero, rijeka, travnjak, šuma) koje učenici rade u paru ili grupi od 3 učenika. Za njihovu su izradu potrebne različite komunikacijske, socijalne i druge kompetencije. Učenici pri proučavanju uočenog problema i odabiru metoda za rješavanje istraživačkog pitanja koriste literaturu na engleskom jeziku. Cilj istraživanja je pronaći odgovarajuće alate za mjerenje jezičnih i upravljačkih vještina prije i nakon izrade projekta te odabranim alatima mjeriti društvene kompetencije u različitim fazama izrade projekta ispitivane skupine učenika (koji rade istraživačke radove) u odnosu na kontrolnu skupinu (koji ne rade istraživačke radove). Pretpos tavlja se da će ispitivana skupina mjerenjem navedenih kompetencija tijekom istraživanja pokazati bolje rezultate. Za mjerenje jezičnih kompetencija su korištene CEFR tablice te upitnici za ispitivanje društvenih i upravljačkih kompetencija. Rezultati istraživanja su sljedeći: učenici uključeni u projekte su pokazali izniman napredak unutar CEFR tablice, društvene vještine su uvelike poboljšane, učenici su naučili dijeliti zaduženja te su razvili brojne upravljačke vještine, kao što su prezentacija, javno govorenje, donošenje odluka i pregovaranje.

Ključne riječi: jezične, društvene i upravljačke kompetencije

DEVELOPMENT OF LINGUISTIC, SOCIAL AND MANAGEMENT COMPETENCIES IN GLOBE AND BIOLOGY STUDENT RESEARCH PROJECTS

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Secondary school Prelog has been involved in GLOBE for 12 years and a number of student research projects connected to nearby natural ecosystems have been done by students in pairs or groups of three. In order to work on the project, students need various communicative, social and other competencies. While researching the noticed problem and deciding on the methods to solve the research question, students search through the English literature. The goal of the research is to find adequate tools to measure linguistic and management skills before and after the project, as well as to measure social competencies by using chosen tools in different stages of the project of the questioned group of students (working on projects) compared to the contolled group (not working on projects). Iti is assumed that the questioned group will show better results of the measured competen cies during the research. CEFR grids were used to measure linguistic competencies, and questionaires for measuring social and management competencies. The results of the research are: students involved in projects have shown amazing progress within CEFR grid, their social skills were also enhanced and they have learned to share responsibilities and have also developed various management skills, such as presentation, public speaking, decision making and negotiating.

Key words: linguistic, social and menagement skills

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PREDSTAVLJANJE REZULTATA POTPROJEKATA ISTRAŽIVANJE I REKREACIJA U PRIRODI I NASTAVNE METODE U BIOLOGIJI I KEMIJI U OKVIRU PROJEKTA AFIRMATIVNA NASTAVA I INOVATIVNO UČENJE I POUČAVANJE U GIMNAZIJAMA U OKVIRU HRVATSKOG KVALIFIKACIJSKOG OKVIRA - IPAQ PETA

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V. gimnazija je bila nositelj projekta IPAQ Peta, koji su osmislili njeni profesori, a proveden je u suradnji s partnerima: PMF-om, školama iz Vukovara, Metkovića, Pakraca i Knina te suradnikom, AZOO. Kroz sedam potprojekata, namijenjenih nastavnicima, željeli smo razviti i modernizirati obrazovni sustav i kvalifikacije u gimnazijama; modernizirati postojeće i razviti napredne kurikule za gimnazije temeljene na ishodima učenja i razviti nastavne kapacitete za provedbu inovativnog učenja i poučavanja. Potprojektom Istraživanje i rekreacija u prirodi izradili smo kurikul koji sadržajima obuhvaća biologiju, kemiju, geologiju i tjelesnu i zdravstvenu kulturu. Nekoliko znanstvenih metoda istraživanja u prirodi prilagođeno je učenicima i nastavnicima te je osmišljena kvalitetna rekreacija u prirodi. Potprojektom Nastavne metode u biologiji i kemiji izradili smo kurikul koji se dijelom nadovezuje na terensku nastavu, a dijelom već poznate metode usmjerava na učenika i nastoji ostvariti pozitivan i odgovoran stav prema zdravlju, živom svijetu, prirodi i znanosti. Najvažniji cilj ovih potprojekata je promijeniti položaj učenika i položaj nastavnika u nastavnom procesu. Nastavnikovu ulogu predavača želimo promijeniti u ulogu organizatora, menadžera, mentora, instruktora i suradnika, a učenike želimo iz pasivnih promatrača promijeniti u angažirane sudionike nastave, osamostaliti ih u učenju, dodatno ih motivirati i poticati na suradnju i preuzimanje određene odgovornosti.

Ključne riječi: kurikul, ishodi učenja, Hrvatski kvalifikacijski okvir

PRESENTING THE RESULTS OF SUBPROJECTS RESEARCH AND RECREATION IN NATURE AND TEACHING METHODS IN BIOLOGY AND CHEMISTRY IN THE FRAMEWORK OF AFFIRMATIVE AND INNOVATIVE LEARNING AND TEACHING IN GYMNASIUMS WITHIN THE CROATIAN QUALIFICATIONS FRAMEWORK - IPAQ PETA

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Fifth Gymnasium was the project leader of IPAQ Peta, which was conceived by its professors and implemented in cooperation with the Faculty of Science, four schools from Vukovar, Metković, Pakrac and Knin and AZOO (Education and Teacher Training Agency). In our project, consisting of 7 subprojects, we wanted to develop and modernize the teaching system and advance qualifications in grammar schools; modernize the existing and develop advanced curricula for grammar schools based on the proper use of learning outcomes, and develop teaching capacities for the implementation of student–centred learning. Within the subproject Research and recreation in nature, we have created a curriculum that includes the fields of biology, chemistry, geology and physical education. Several scientific research methods in nature are adapted to students and teachers and it also contains quality recreation in nature. Within the subproject Teaching methods in biology and chemistry, we have created a curriculum consisting of well-known methods directed at students and aiming to achieve a positive and responsible attitude towards health, the living world, nature and science. The most important objective of this subproject is to change the position of the students and the role of teachers in the teaching process. We want to transform the teachers' role of trainers into the role of organizers, managers, mentors, instructors and assistants, and the students' role of passive observers into engaged participants, teaching them to become independent learners and further motivate and encourage them to cooperate and take some responsibility.

Keywords: curriculum, learning outcomes, Croatian Qualifications Framework

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PRIMJER INTEGRIRANJA RAZLIČITIH NASTAVNIH PREDMETA NA TEMU ZAŠTITE LOKALITETA BEDEKOVIĆEVE GRABE

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Na osnovi rezultata istraživanja bioloških osobitosti leptira iz roda *Maculinea* na lokalitetu Bedekovićeve grabe u Međimurju osmišljene su i provedene višegodišnje edukativne aktivnosti radi očuvanja i zaštite staništa velikog livadnog plavca *Maculinea teleius* Brgstr. i zagasitog livadnog plavca *Maculinea nausithous* Brgstr. Cilj rada je aktivnim uključivanjem u interdisciplinarne projekte senzibilizirati učenike različitog uzrasta i područja obrazovanja za očuvanje ugroženih i zaštićenih livadnih plavaca u Međimurju. Aktivnosti se provode uz potporu Županijskog ogranka HPKZ-a Međimurske županije, Međužupanijskog stručnog vijeća nastavnika biologije, i udruge "Ekološka škola". Ostvareno je integriranje različitih nastavnih predmeta u osnovnoj školi (1.-8. razreda) i projekata učenika srednjih škola ovisno o njihovom području interesa; ekologija, ekonomija, trgovina, turizam i ugostiteljstvo. Uključeni su učenici OŠ I. G. Kovačića Sv. Juraj na Bregu, Ekonomske i trgovačke škole Čakovec, Gimnazije Josipa Slavenskog Čakovec, SŠ Prelog, Gospodarske škole Čakovec i njihovi nastavnici u skladu s obrazovnom razinom i sektorom. U drugom djelu rada se, na temelju provedbe i rezultata navedenih aktivnosti u školama anketiranjem izabranog uzorka učenika, istražuju njihovi stavovi i kompetencije, posebice poduzetničke i prirodoznanstvene.

Ključne riječi: leptiri roda *Maculinea*, očuvanje staništa, interdisciplinarna i međugeneracijska edukacija, stavovi

AN EXAMPLE OF INTEGRATING DIFFERENT SCHOOL SUBJECTS FOCUSING ON PROTECTING THE SITE "BEDEKOVIĆEVE GRABE"

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Based on the results of the research into the biological features of the butterflies of the species *Maculinea* at the site "Bedekovićeve grabe" in Međimurje many long lasting educational activities have been devised and implemented with the aim of preservation and protection of the habitat of the scarce large blue butterfly *Maculinea teleius* Brgstr. and the dusky large blue butterfly *Maculinea nausithous* Brgst. The aim of the activities was to make students of different age and educational sector more aware of the need to preserve endangered and protected large blue butterflies in Međimurje by their active involvement in interdisciplinary projects. The results achieved were the integration of various school subjects in primary school (grade 1 to grade 8) and secondary school students' projects which depended on their area of interest: ecology, economics, trade, tourism and hotel industry. The participants involved were students and teachers from Primary School Ivan Goran Kovačić, Sv. Juraj na Bregu; Secondary School of Economics and Trade, Čakovec; Secondary School Prelog; Grammar School J. Slavenskog Čakovec and Vocational School Čakovec. Using the results of the implemented

activities, the second part of the study focuses on conducting the sample survey into the students' attitudes and competence, particularly entrepreneurial and scientific competence.

Keywords: butterflies of the species *Maculinea*, protection of habitat, interdisciplinary and intergenerational education, attitudes

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ISTRAŽIVANJE STAVOVA UČENIKA O PREHRANI

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Osnovni cilj ovoga rada bio je saznati prehrambene i druge navike, stavove i aktivnosti učenika, te utvrditi postoje li razlike među učenicima ovisno o spolu, dobi i sredini u kojoj žive. Istraživanje je provedeno putem upitnika konstruiranog za potrebe ovog istraživanja. U istraživanju je sudjelovao 651 učenik. Najveći broj učenika je normalne tjelesne mase, 481 (73,88%). Uhranjenost dječaka i djevojčica se razlikuje. Učenici ruralne sredine doručkuju redovitije (p=0,010), češće jedu perad (p=0,008) te domaće suhomesnate proizvode (p=0,003), dok učenici urbane sredine češće jedu ribu (p=0,044) i cjelovite žitarice (p<0,001). Odrastanjem učenika povećava se neredovitost doručka (p=0,022), konzumacija slastica (p=0,009) te gaziranih sokova (p=0,027). Briga učenika o tjelesnom izgledu povećava se odrastanjem (p=0,048), što je više izraženo kod djevojčica (p<0,001). Konzumacija alkohola (p=0,009) i cigareta (p=0,045) češća je kod učenika ruralne sredine. Dječaci su tjelesno aktivniji od djevojčica (p<0,001). Učenici viših razreda skloniji su sedentarnim aktivnostima (p<0,001). Ispitani učenici su uglavnom normalne tjelesne mase za svoju dob, a udio pothranjenih i pretilih učenika veći je kod dječaka. Prehrambene navike razlikuju se između učenika iz urbane i ruralne sredine i ne razlikuju se značajno između dječaka i djevojčica, a porastom dobi postaju lošije. Briga učenika o tjelesnom izgledu povećava se odrastanjem, što je više izraženo kod djevojčica.

Ključne riječi: učenici, stav učenika, zdrav način života, adolescencija, upitnik

PUPIL'S DIET-RELATED ATTITUDES TO HEALTHY LIFESTYLE

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The main purpose of this research was to find out more about eating habits along with other habits, attitudes and activities of elementary school pupils, as well as to determine possible differences between pupils, depending on their sex, age and environment. The research was conducted via questionnaire constructed for the needs of this specific research. 651 pupils took part in this questionnaire. Most of the pupils had normal body mass, 481 (73.88%). The nutritional status of boys and girls differ. Pupils from the rural environment are more keen on having breakfast (p=0.010), they more often eat poultry meat (p=0.008) as well as smoked meat (p=0.003). On the other hand, pupils from the urban environment more often eat fish (p=0.044) and whole grains (p<0.001). During childhood, breakfast irregularities increase (p=0.022) along with the consumption of sweets (p=0.009) and soft drinks (p=0.027). Pupils' concern about body

appearance increases as they grow up (p=0.048), which is more expressed among girls (p<0.001). The consumption of alcohol (p=0.009) and cigarettes (p=0.045) is more common among the pupils from the rural environment. Most of the interviewed pupils have normal body mass according to their age. The share of underweight and overweight pupils is bigger among the boys. Nutritional habits differ between pupils from the urban and rural areas, but they do not differ as much between boys and girls. In addition, their nutritional habits become worse as they grow up.

Key words: pupils, diet-related attitude, healthy lifestyle, adolescence, questionnaire

O-30

ANATOMY AND PHYSIOLOGY OF HUMAN BODY TEACHING WITH NATURAL MATERIALS

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We analyzed teachers´ usage of natural materials – fresh mammalian organs at subject of anatomy and physiology of human body at the lower secondary school. The reason why students should learn with fresh mammalian organs is experience learning, so teachers should include that kind of teaching. Study was done with students garde 8 and 9 (13-14 years old students) of five different lower secondary schools who already learned about anatomy and physiology of human body. According to results of 189 students, Slovenian teachers in rarely use fresh mammalian organs in biology classes (except bones) but students would like to examine, touch or dissected fresh mammalian organs. Paired Samples Test were done the comparison between the results of really conditions in the class (which organs students examined, touched or made dissection already) and the results where stud ents noted if and which mammalian organs they would like to examine, touch or make dissection at the biology classes. Differences between genders for several pairs were statistically significant ($p < 0.01$). In the article were analyzed 15 students´ statements about learning with fresh mammalian organs. Differences in students´ attitudes about statements were analyzed by gender but just in four statements the significant differences were found.

Key words: biology class, experience learning, fresh mammalian organs

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ISTRAŽIVANJE UČENIČKOG RAZUMIJEVANJA OSNOVNOG BIOLOŠKOG KONCEPTA MENSTRUACIJSKOG CIKLUSA

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U radu je istražena usvojenost i razumijevanje koncepta menstruacijskog ciklusa, kao jednog od najsavršenijih i najkompleksnijih bioloških sustava. U istraživanju je sudjelovalo 220 učenika trećih razreda splitskih gimnazija (148 djevojčica i 56 dječaka te 16 neopredijeljenih). Istraživanje je provedeno putom 4 kombinacije testova, od toga tri testa s grafičkim prikazom različite dužine menstruacijskog ciklusa i jedan test bez grafa. Svaki test je imao 6 pitanja. Ukupna riješenost određivanja datuma ovulacije iznosila je 67,39% i ako se usporedi rezultat

prema spolu utvrđeno je da nema signifikantnih razlika u rezultatima dječaka i djevojčica. Istraživanjem dokaza kako je došlo do ovulacije riješenost je bila vrlo niska i iznosila je 14,49%, što ukazuje na slučajnost odabira datuma ovulacije. Analizom faze s najvećom razinom ženskih spolnih hormona, ukupno je 34,78% učenika točno odgovorilo i iščitalo iz grafa te vrijednosti. Analizom najniže razine ženskih spolnih hormona riješilo je ukupno 56,16% učenika. Poveznicu između niske razine ženskih hormona i ponovne menstruacije prepoznalo je 68,12% učenika. Iz svih istraživanja utvrđena je loša usvojenost i razumijevanje koncepta menstruacijski ciklus, iz čega je vidljivo da predavačku nastavu i pasivni pristup učenika treba zamijeniti suvremenijim kurikularnim pristupima u biologiji.

Ključne riječi: menstruacijski ciklus, koncept, učenik, ovulacija, biologija

RESARCH OF STUDENT UNDERSTANDING OF BASIC BIOLOGICAL CONCEPT OF THE MENSTRUAL CYCLE

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The paper investigates the process of learning and understanding of the concept of menstrual cycle, as one of the most perfected and complex biological systems. The research was conducted on a sample of 220 participants, 3rd grade students of grammar schools in Split (148 girls, 56 boys and 16 unspecified). The research was carried out using the combination of 4 tests, out of which three tests included the graphical representation with different lengths of the menstrual cycle, and one test did not include graphical representation. Each test consisted of 6 questions. Total success rate in defining the date of ovulation was 67.39% and when a comparison was made between the results based on gender, there was no significant difference between the results achieved by the girls and those achieved by the boys. Success rate in completing the answers to further questions regarding the proof for the selected date of ovulation was quite low - as low as 14.49%, which indicates that the selected date of ovulation was random. In the section related to the analysis of the phase with the highest level of female sex hormones, 34.78% of students answered correctly and found the values in the graph. The section related to the lowest level of female sex hormones was correctly answered by 56.16% of students. The link between the low level of female sex hormones and new occurrence of menstruation was identified by 68.12% of students. The summarised results of the research show low levels of comprehension and understanding of the concept of menstrual cycle, which indicate that the lecture-based teaching model and passive role of the students should be replaced by more modern curricular approaches in teaching biology.

Key words: menstrual cycle, concept, student, ovulation, biology

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ANALIZA ZASTUPLJENOSTI RAZLIČITIH TIPOVA ZADATAKA I NJIHOVIH KOGNITIVNIH RAZINA U TESTOVIMA ŽUPANIJSKIH NATJECANJA BIOLOGIJE ZA OSME RAZREDE OD 2009. – 2014. GODINE

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U organizaciji Ministarstva znanosti, obrazovanja i sporta, Agencije za odgoj i obrazovanje te Hrvatskog biološkog društva godinama se provodi natjecanje u znanju iz biologije. Cilj istraživanja je analizirati pisane zadaće županijskih natjecanja iz biologije u osmom razredu na temelju tipova zadataka, njihove zastupljenosti te kognitivne razine pitanja (tri razine prema pojednostavljenoj Bloom-ovoj taksonomiji; Crooks). Analiza tipova zadataka i njihove zastupljenosti provedena na bazi šest pisanih zadaća od 2009 do 2014 godine (ukupno 183 zadatka) pokazala je da se najviše koriste zadaci zatvorenog tipa. Analiza također pokazuje da su najzastupljeniji zadaci višestrukog izbora, dok se zadaci otvorenog tipa iznimno rijetko koriste. Kategorizacija zadataka prema kognitivnim razinama pokazuje visoku zastupljenost zadataka prve razine, uz tendenciju povećanja uporabe zadataka druge i treće kognitivne razine tijekom istraživanog razdoblja. Temeljem dobivenih podataka ukazuje se na potrebu sustavnije i detaljnije analize pisanih zadaća na svim razinama provjeravanja (školska, županijska, državna), većeg korištenja zadataka otvorenog tipa u pisanim zadaćama, te povećanju zastupljenosti zadataka druge i treće kognitivne razine.

Ključne riječi: natjecanja, tipovi zadataka, kognitivne razine

ANALYSIS OF THE REPRESENTATION OF DIFFERENT TYPES OF TASKS AND THEIR COGNITIVE LEVELS IN COUNTY-LEVEL COMPETITION TESTS IN BIOLOGY FOR EIGHTH GRADES FROM 2009 TO 2014

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Organized by the Ministry of Science, Education and Sports, Agency for Education and the Croatian Biological Society, competitions in biology knowledge have been conducted for years. The aim of the research is to analyze written assignments in county-level biology competitions in the eighth grade based on the type of tasks, their representation and the cognitive level of questions (three levels, according to the simplified Bloom's taxonomy; Crooks).The analysis of the type of tasks and their representation conducted based on six written assignments from 2009 to 2014 (183 tasks in total) has shown that the most frequently used type of tasks is the closed type. The analysis has also shown that the most represented ones are the multiple-choice tasks, while the open tasks are used extremely rarely. The categorization of tasks according to cognitive levels shows a high representation of first level tasks, with a tendency to increase the use of second and third cognitive level tasks during the period researched. The obtained data indicates that there is a need to analyze the written assignments more systematically and in greater detail at all levels of testing (school, county, state), to use more open tasks in written assignments and to increase the representation of second and third cognitive level tasks.

Key words: competitions, task types, cognitive levels

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PROCJENA KVALITETE PITANJA IZ PRIRODE I DRUŠTVA ZA 4. RAZRED OŠ

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U nastavi prirode i društva za 4. razred osnovne škole postoji u uporabi više odobrenih udžbenika i radnih bilježnica. U radnim se bilježnicama nude zadatci različitog tipa. Autori istraživanja su proveli akcijsko istraživanje koje je temeljeno na procjeni dvoje nastavnika praktičara. Prema rezultatima akcijskog istraživanja u radnim bilježnicama dominiraju pitanja niže kognitivne razine. Namjera je bila nastaviti istraživanje s više procjenjivača raspodjeljenih u dvije grupe ispitanika: (I.) učitelji razredne nastave i (II.) nastavnici prirode i biologije u višim razredima osnovnih škola Međimurske županije. Cilj je istraživanja usporediti procjene kvalitete pitanja navedenih ispitanika. Iz svih radnih bilježnica zastupljenih na tržištu su odabrana 22 pitanja za istraživanje. Ispitanici procjenjuju kvalitetu pitanja u sljedećim kategorijama: (1.) važnost pitanja za nastavak obrazovanja, (2.) primjena postignuća u sadašnjem i budućem životu te (3.) kognitivne razine postignuća. Ispitanici procjenjuju svako pitanje Likertovom skalom od pet (1. i 2.) stupnjeva. Kognitivne razine se procjenjuju u tri stupnja. Analizom rezultata dvije skupine ispitanika ustanovljena je statistički značajna razlika u ocijenjivanju (1.) važnosti pitanja za nastavak obrazovanja i (2.) važnosti pitanja za život. Većina ispitanika iz obje skupine smatraju da najviše prevladavaju pitanja uglavnom 1. i 2. kognitivne razine. Pitanja 3. razine nisu zastupljena u radnim bilježnicama.

Ključne riječi: akcijsko istraživanje, kognitivna razina, Likertova skala

ESTIMATING THE QUALITY OF TEST QUESTIONS FROM NATURE AND SOCIETY IN THE FOURTH GRADE

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There are a couple of approved books and workbooks for teaching nature and society in the fourth grade of primary scholol. Workbooks offer various type tasks. The authors of the research have conducted action research based on the estimate of two practising teachers. According to the results of action research, there are more lower cognitive level tasks (questions) in workbooks. The aim was to continue with the research with more participants divided into two groups: (I.) first to fourth grade teachers and (II.) Biology and Chemistry teachers working in primary scholls, fifth to eighth grade, in Međimurje County. The aim of the research is to compare the estimates of the quality of questions from various participants. 22 questions have been selected for the research from all workbooks available on the market. Participants estimate the quality of questions in the next cathegories: (1.) the importance of the question for further education, (2.) application of accomplishment now and in the future and (3.) cognitive level of accomplishment. Participants estimate each question using Likert scale with 5 levels (1. i 2.). Cognitive levels are estimated in three levels. By analysing the results of two target groups of users,a statistically relevant difference was found in grading, the importance og questions for continuing education and the importance of questions for life. Most users from both target groups think that most questions belong to the first and second cognitive level. Questions from the third cognitive level are not present in workbooks.

Key words: action research, cognitive level, Likert scale

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UTJECAJ KVALITETE ZNANJA UČENIKA NA RJEŠAVANJE ZADATAKA VIŠIH KOGNITIVNIH RAZINA

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Cilj istraživanja bio je ispitati konceptualno razumijevanje nastavnih sadržaja biologije na uzorku pisanih zadaća učenika 7. razreda sa Županijske razine natjecanja. Uzorak je sadržavao najbolje riješene pisane zadaće po županijama, odnosno zadaće u kojima je točno riješeno 75% zadataka. Pisana zadaća sadržavala je zadatke oblikovane u skladu s preporukama za pripremu pisanih provjera iz biologije. Analizom bodova za vrednovanje pojedinih zadataka, udio II. kognitivne razine je 60% (raspoređenih u 14 zadataka), a III. kognitivne razine 18% (raspoređenih u 2 zadatka). Rezultati su obrađeni (SPSS 17) u odnosu na definirane ishode učenja u skladu s makrokonceptualnim okvirom biologije, procjenu kvalitete zadataka (od strane autora i 10 neovisnih osoba iz struke), prilagođenost tipa zadatka ispitivanju određenog koncepta, utjecaj kvalitete zadataka na stupanj diskriminacije, usklađenost s nastavnim sadržajima biologije 7. razreda osnovne škole propisanih nastavnim planom i programom, povezanost sa svakodnevnim životom, općom kulturom i nastavnim sadržajima drugih prirodoslovnih predmeta. Zadatci otvorenog tipa uglavnom bolje diskriminiraju uspješnije učenike. Učenici koji na cjelokupnoj pisanoj zadaći postižu veći broj bodova bolje rješavaju zadatke koji zahtijevaju cjeloviti pristup rješavanju problema u odnosu na zadatke koji ispituju detalje.

Ključne riječi: kognitivne razine, kvaliteta pitanja, nastavni sadržaji biologije, međupredmetni sadržaji prirodoslovlja

INFLUENCE OF STUDENTS' KNOWLEDGE ON PERFORMANCE IN SOLVING COMPLEX COGNITIVE TASKS

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The aim of this paper was to determine the conceptual understanding of biology curriculum content on a sample of tests written by 7th grade students in the County competition. The sample was comprised of best solved tests in the counties, that is, test scores of 75%. The tasks in the test were consistent with the recommendations for designing written tests in biology. By analysing points for task evaluation, 60% of the test was aligned for the tasks of the second cognitive level (14 tasks) and 18% was aligned for the tasks of the third cognitive level (2 tasks). The results (SPSS 17) were evaluated in view of the defined learning outcomes based on the macro-conceptual biology framework, the quality of the tasks (evaluated by authors and 10 independent experts), the suitability of the types of tasks and certain concepts, the influence of task quality on the degree of discrimination, the compliance with the 7th grade curriculum, the correlation with everyday life, general knowledge and the science curriculum in general. In general, the open-ended tasks more reliably discriminated successful students. Students with

better overall score show better results in solving tasks that require a holistic approach than solving tasks oriented to particular type of information.

Key words: cognitive levels, quality of the question, 7th grade biology curriculum, science curriculum

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USPJEŠNOST UČENIKA 8. RAZREDA U RJEŠAVANJU PISANIH ZADATAKA IZ BIOLOGIJE

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Cilj istraživanja je analizirati uspješnost učenika 8. r. u rješavanju zadataka različitih kognitivnih razina. Uzorak na kojem je provedeno istraživanje sastoji se od 463 učenika 8. r. koji su pristupili Županijskom natjecanju iz biologije u kategoriji znanja, koje se ispituje pisanim zadaćama, u šk. god. 2014./2015. Ukupan broj bodova pisane zadaće iznosio je 50. Maksimalan broj postignutih bodova je 47, a najniži broj postignutih bodova je 11. Analizom rezultata natjecanja po županijama utvrđeno je da 0,9% učenika ima 90% točno riješenog testa ili više. Također je utvrđeno da je 10% najuspješnijih učenika na natjecanju postiglo od 38,5 do 47 bodova, tj. od 77% do 94% točne riješenosti testa. Analiziranjem testova učenika potvrđena je naša pretpostavka da će učenici koji su riješili test s uspjehom većim od 90% uspješno riješiti veći postotak zadataka II. i III. kognitivne razine u odnosu na 10% najuspješnijih učenika na natjecanju.

Ključne riječi: kognitivne razine, uspješnost rješavanja zadataka

EFFICIENCY OF THE 8TH GRADE PUPILS IN SOLVING WRITTEN ASSIGNMENTS IN BIOLOGY

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The aim of this research is to analyse the efficiency of the 8th grade pupils in solving assignments of different cognitive levels. The research sample consists of 463 pupils who participated in the County Biology Competition in the category which examines knowledge with written tests, in the 2014-2015 school year. The total number of points in written test was 50. The maximum number of points achieved was 47, while the minimum achieved was 11. The analyses of the competition's results showed that the 0.01% of pupils solved 90% or more of the test accurately. The 10% of the most successful pupils that participated in the competition achieved from 38.5 to 47 points, i. e. from 77% to 94% of the test was accurately solved. The analyses of the pupil's tests confirmed our assumption that the pupils who solved 90% or more of the test accurately would solve the 2nd and 3rd cognitive level assignments in higher percentage than the 10% of the most successful pupils that participated in the competition.

Key words: cognitive levels, the efficiency of assignment solving

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

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EVOLUCIJA U DINARIDIMA – ANALIZA FILOGEOGRAFSKE I POPULACIJSKE GENETSKE STRUKTURE RIBA RODA *Delminichthys*

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Slatkovodni sustavi na području dinarskog krša nastanjeni su osobito bogatom ribljom zajednicom, s velikim udjelom endemskih vrsta. S ciljem objašnjavanja nastanka tako velike raznolikosti, predloženo je nekoliko događaja i pojava: geografska izolacija rijeka i njihova povezanost kroz podzemlje, geološka povijest, refugiji tijekom ledenih doba, specifične stope mutacije. Za razliku od većine rodova slatkovodnih riba, rod *Delminichthys* nastanjuje malo područje i obuhvaća svega četiri vrste. Kako bismo otkrili populacijsko-genetičku strukturu i demografsku povijest vrsta ovoga roda, te tako pridonijeli razjašnjavanju evolucije u slatkovodnim sustavima Dinarida, proveli smo filogeografska i populacijsko-genetička istraživanja svih vrsta roda *Delminichthys*. Na temelju mitohondrijskih i jezgrinih biljega sekvenci analizirali smo filogenetske odnose, razinu genetičke raznolikosti unutar populacija i vrsta te između njih, kao i unutarvrsnu genetičku strukturu. Najznačajniji rezultati uključuju značajan mito-jezgreni nesklad, umjeren do visok stupanj genetičke raznolikosti većine populacija te izraženu genetičku strukturiranost. U svjetlu novih rezultata također ćemo diskutirati mogućnost podzemnog protoka gena između pojedinih populacija.

Ključne riječi: Dinarski krš, slatkovodni sustavi, populacijska struktura, genetska raznolikost, protok gena

EVOLUTION IN DINARIDS – INTEGRATIVE ANALYSIS OF PHYLOGEOGRAPHY AND POPULATION GENETIC STRUCTURE OF THE FISH GENUS *Delminichthys*

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The freshwater systems of the Dinaric karst region maintain exceptionally rich fish community with great portion of endemic species. As a possible explanation of such diversity several events and phenomena have been proposed: geographic isolation of rivers and their underground connections, geological history, ice-age refuges, specific mutation rates. Contrary to the majority of the fish genera, genus Delminichthys inhabits geographically very restricted area and comprises only four species. In order to reveal population genetic structure and demographic history of each *Delminichthys* species as a contribution to explanation of evolution in Dinaric water systems, this investigation was designed as a combination of phylogeographic and population genetic analyses of all *Delminichthys* species. Based on mitochondrial and nuclear DNA, we have investigated phylogenetic rela tionships, genetic diversity and differentiation, as well as intraspecific genetic structure of four *Delminichthys*

species. The most important results include pronounced mito-nuclear discordance, moderate to high genetic diversity inside the majority of populations and significant genetic structuring. Previously reported data on the underground gene flow will be discussed in the light of the newly obtained results.

Key words: Dinaric karst, freshwater systems, population structure, genetic diversity, gene flow

O-37

FILOGENIJA I FILOGEOGRAFIJA BJELONOGOG RAKA (*Austropotamobius pallipes* KOMPLEKS) U RIJEKAMA JADRANSKOG BAZENA

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Sistematika bjelonogog raka predmet je stalne rasprave i stoga predstavlja prepreku učinkovitoj konzervaciji. Kako bi se poboljšalo znanje o ovom ugroženom kompleksu vrsta, filogenetski odnosi i filogeografija vrste u jadranskom bazenu su revidirani upotrebom rezultata analiza mitohondrijske DNA (mtDNA), kodominantnih jezgrinih DNA biljega (mikrosateliti) i morfometrijskih značajki. Analize mtDNA rezultirale su opisom novih linija unutar kompleksa. Rasprostranjenost mtDNA linija ima pravilan geografski uzorak i upućuje na vezu s geološkom poviješću jadranske regije od Mesinske krize saliniteta do pleistocenskih regresija mora. Analiza mikrosatelita otkrila je introgresiju i izmiješanost jezgrinog genoma između različitih mtDNA linija. Rezultati morfometrijskih analiza se podudaraju s genetskom strukturom dobivenom analizom mikrosatelita. Dobiveni rezultati su omogućili definiranje "Evolucijski značajnih jedinica" i "Jedinica upravljanja", korisnih u budućoj konzervaciji i upravljanju ugroženim populacijama bjelonogog raka u jadranskom slijevu. Također, rezultati potvrđuju neophodnost korištenja različitih analiza (molekularnih i morfologijskih) u pripremama planova upravljanja ugroženim vrstama.

Ključne riječi: mitohondrijski biljezi, mikrosateliti, morfometrija, preko-jadranski putovi širenja

PHYLOGENY AND PHYLOGEOGRAPHY OF THE WHITE-CLAWED CRAYFISH (*Austropotamobius pallipes* COMPLEX) IN THE ADRIATIC BASIN RIVERS

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Systematics of the white-clawed crayfish is continuously debated and as such caused problems for effective conservation and population management. To improve the knowledge on this endangered species complex, its phylogenetic relationships and species phylogeography within the Adriatic Basin were revised using results from analyses of mitochondrial DNA (mtDNA), codominant nuclear DNA markers (microsatellites), and morphometrics. Analyses on mtDNA resulted with description of new lineages. Distribution of mtDNA lineages has regular geographic pattern and can be related to geological history of the Adriatic region dated from Messinian salinity crisis until Pleistocene marine regressions. Microsatellites analyses revealed introgression and admixture of nuclear genome between distinct mtDNA lineages. Results of morphometrical analyses are in line with the genetic structur e obtained from microsatellites analyses. The obtained results enabled definition of Evolutionary Significant Units and Management Units, useful in future conservation and management of endangered white-clawed crayfish populations in the Adriatic Sea basins. Also, it confirms a necessity of multiple analyses application (molecular and morphological) in managing endangered species populations.

Key words: mitochondrial markers, microsatellites, morphometrics, trans-Adriatic dispersal routes

O-38

PRVI REZULTATI INVENTARIZACIJE VRSTA ŽOHARA, BOGOMOLJKI I RAVNOKRILACA (INSECTA: *Polyneoptera*) ŠIREG PODRUČJA PLANINE DINARE

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Fauna kukaca iz skupine *Polyneoptera* na području planine Dinare u prošlosti je razmjerno slabo istraživana, s tek vrlo malim brojem objavljenih podataka. Tijekom istraživanja provedenog 2012. i 2013. godine posjećeno je ukupno 19 podrobnije istraženih lokaliteta, a 68 vrsta žohara, bogomoljki i ravnokrilaca zabilježeno je po prvi put za ovo područje. Za jednu vrstu ovo je prvi nalaz na prostoru Hrvatske, a pronađeno je i nekoliko svojti s još nerazješnjenim taksonomskim položajem. Naši su rezultati objedinjeni sa svim dostupnim literaturnim i muzejskim podacima za ovo područje, otkrivajući ukupno čak 98 svojti, s ciljem dobivanja što potpunije slike o fauni kukaca iz skupine Polyneoptera na prostoru planine Dinare i njezine okolice.

Ključne riječi: faunistika, redovi *Polyneoptera*, Hrvatska, Dinaridi

FIRST RESULTS OF INVENTARISATION OF *Blattodea, Mantodea* AND *Orthoptera* (INSECTA: *Polyneoptera*) OF THE DINARA MOUNTAIN AREA

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The polyneopteran insect fauna of Dinara Mountain area was poorly studied in the past, with only scarce data published on the topic. During a research, conducted in 2012 and 2013, a total of 19 better investigated localities were visited and 68 species of *Blattodea*, *Mantodea* and *Orthoptera* were recorded for the first time in the investigated area. One species was recorded for the first time for Croatia, while a couple of findings include taxa with presently unresolved taxonomic status. Our results were combined with all available museum and literature data for this area, revealing as much as 98 taxa altogether, in order to complete the picture of polyneopteran insect fauna inhabiting Dinara Mt. and its surroundings.

Key words: faunistics, Polyneopteran orders, Croatia, Dinaric Alps

O-39
MOLEKULARNO-FILOGENETIČKA I FILOGEOGRAFSKA ANALIZA POPULACIJA VRSTE *Holandriana holandrii* (C. PFEIFFER, 1828) (MOLLUSCA: GASTROPODA) U HRVATSKOJ

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Slatkovodna vrsta puža *Holandriana holandrii* (Pfeiffer, 1828) široko je rasprostranjena na području jugoistočne Europe. Spada u porodicu Melanopsidae koja je vrstama i brojnošću dominirala Panonskim jezerom tijekom kasnog miocena i pliocena. Cilj ovog istraživanja bio je ustanoviti filogenetske i filogeografske odnose populacija ove vrste na temelju analize dva genska biljega, mitohondrijskih gena za COI i 16S rRNA. Filogenetski odnosi rekonstruirani su upotrebom različitih filogenetičkih metoda za svaku gensku regiju zasebno, kao i na konkateniranim setovima sekvenci. Molekularno-filogenetičke analize ustanovile su postojanje tri geografski i genetski odijeljene grupe i dvije podgrupe. Dobivene vrijednosti genetskih udaljenosti ukazuju na relativno mlade evolucijske linije koje su se razdvojile tijekom pleistocena.

Ključne riječi: Melanopsidae, slatkovodni puževi, filogenija, filogeografija, COI i 16S rRNA

MOLECULAR PHYLOGENETIC AND PHYLOGEOGRAPHIC ANALYSIS OF POPULATIONS OF *Holandriana holandrii* (C. PFEIFFER, 1828) (MOLLUSCA: GASTROPODA) IN CROATIA

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Holandriana holandrii (Pfeiffer, 1828) is a freshwater snail, widespread in south-eastern Europe. It belongs to the family Melanopsidae which dominated the Lake Pannon by the number of species

and their abundance during the late Miocene and Pliocene. The aim of this research was to determine phylogenetic and phylogeographic relationships of populations of this species based on the analysis of two genetic markers, mitochondrial COI and 16S rRNA genes. Phylogenetic relationships were reconstructed using different phylogenetic methods for each gene region individually as well as for concatenated sets of sequences. Molecular phylogenetic analyses established the existance of three geographically and genetically separated groups and two subgroups. The obtained values of genetic distances indicate relatively young evolutionary lineages which have diverged during the Pleistocene.

Key words: Melanopsidae, freshwater snails, phylogeny, phylogeography, COI, 16S rRNA

O-40

UTJECAJ OBALNOG ONEČIŠĆENJA NA EPIGENETIKU I REPRODUKTIVNI FITNES VOLKA *Hexaplex trunculus*

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Poznato je da već i vrlo niske koncentracije tributil kositra (TBT), aktivnog sastojka protuobraštajnih premaza brodova, mogu značajno smanjiti reproduktivni fitnes volka (*Hexaplex trunculus*, Gastropoda). TBT ima jaki ksenoandrogeni utjecaj na puževe i dovodi do razvitka muških spolnih organa u ženkama (imposeks), čime utječe na fitnes cijelih populacija. TBT ujedno uzrokuje i demetilaciju DNA. Cilj ovog istraživanja bio je otkriti odnos između bioakumulacije TBT-a, reproduktivnog fitnesa te epigenetske i genetske strukture populacija volka *H. trunculus*. U tu svrhu uzorkovali smo 7 populacija volka u priobalnom dijelu istočnog Jadrana: četiri populacije s postaja onečišćenih morskim prometom te aktivnostima održavanja brodova, te tri populacije s postaja niskog antropogenog onečišćenja. Epigenetske analize metodom MSAP (engl. *methylation sensitive amplification polymorphism*) ukazale su na postojanje epigenetske diferencijacije između populacija te nisku razinu genetičke strukturiranosti populacija. Veća učestalost imposeksa zabilježena je u populacijama s onečišćenih u odnosu na čiste postaje. Razina DNA metilacije značajno je korelirala sa stupnjem imposeksa, što ukazuje na epigenetsku pozadinu razvoja imposeksa.

Ključne riječi: evolucija, populacijska epigenetika, fitnes, onečišćenje

IMPACT OF COASTAL POLLUTION ON EPIGENETICS AND REPRODUCTIVE FITNESS OF MARINE SNAIL *Hexaplex trunculus*

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Hexaplex trunculus is a marine gastropod whose reproductive fitness can be severely affected by very low concentration of antifouling compound trybutil-tin (TBT). TBT has strong

xenoandrogen impact in snails, causing development of imposex, e.g. superimposition of male sexual characteristic in females, thereby affecting the fitness of entire populations. TBT is also known as a DNA demethylating agent. The aim of this study was to unravel the interactions between TBT bioaccumulation, reproductive fitness, epigenetic and genetic endpoints in murex snail *H. trunculus*. Four populations inhabiting polluted sites with intense marine traffic and boat maintenance activity and three inhabiting sites with low anthropogenic impact were sampled in the coastal eastern Adriatic. Higher incidence of imposex was observed in snails inhabiting polluted than the reference sites. Analysis of methylation sensitive amplification polymorphism (MSAP) revealed existence of epigenetic differentiation among populations and lack of genetic population structure. Level of DNA methylation significantly correlated with the imposex level, suggesting epigenetic background of imposex development.

Key words: evolution, population epigenetics, fitness, pollution

O-41
BRZA EVOLUCIJA DAGNJE POD UTJECAJEM ONEČIŠĆENJA - EKSPERIMENTALNA GENOMIKA

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Antropogeno uvjetovane promjene okoliša mogu predstavljati značajne adaptivne izazove za organizme koji ga nastanjuju. U sklopu ovog istraživanja proučavali smo evolucijski odgovor na onečišćenje u populacijama dagnje *Mytilus galloprovincialis*. Uzorkovali smo 15 prirodnih populacija dagnje koje nastanjuju postaje različitog stupnja onečišćenja, a dvije populacije koristili smo i u eksperimentu transplantacije između čistog i onečišćenog okoliša u prirodnim uvjetima *in situ* te u mezokozmosu. Rezultati su pokazali različito preživljavanje uvjetovano aklimatizacijom u *in situ* izlaganju te interakcijama populacije i okoliša u mezokozmos ekperimentu. Za preko 1800 jedinki sakupili smo genomske podatke genotipizirajući jedinke metodom sekvenciranja nove generacije. Analizom mnogobrojnih SNP-ova u genomu dobiven je uvid u genetičku strukturu prirodnih populacija dagnje te genomsku arhitekturu preživljavanja (fitnesa) u eksperimentima. Kombinirajući podatke dobivene iz prirodnih populacija s podacima iz dva tipa transplantacijskih eksperimenata utvrdili smo interakciju između okoliša, genotipa i fitnesa, te dobili novi uvid u evoluciju dagnje pod utjecajem onečišćenja i učinak adaptivnih procesa na genom dagnje.

Ključne riječi: adaptacija, fitnes, genomska arhitektura, genomsko-okolišne interakcije

EXPERIMENTAL GENOMIC TESTS FOR POLLUTION DRIVEN RAPID EVOLUTION IN THE MEDITERRANEAN MUSSEL

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Anthropogenic disturbance can rapidly and substantially alter the environment and exposed organisms are challenged to quickly adapt to these changes. We studied evolutionary responses to pollution in marine environments using the Mediterranean mussel *Mytilus galloprovincialis*. We sampled 15 natural populations along a pollution gradient and used a subset of these to perform transplant experiments between clean and polluted environments in both the wild and in lab mesocosms. The results of the experiments showed consistent differential survival between treatments and source populations driven by an acclimatization effect in the wild transplant and by population by environment interactions in the mesocosm experiment. For over 1800 mussels used in these studies we collected genome wide SNP data using a genotype-by-sequencing approach. These SNPs were analysed to test for genetic structure in natural populations and the genomic architecture of survival (i.e., fitness) in the experiments. By combining data obtained from the native populations and two types of transplant experiments we identified interactions between environment, genotype, and fitness, thus providing new insight into pollution driven evolution and the effect of adaptive processes on the mussel genome.

Key words: adaptation, fitness, genomic architecture, genotype-environment interaction

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GENETIČKA ARHITEKTURA MORFOLOŠKIH OBILJEŽJA DAGNJE *Mytilus galloprovincialis*

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Genetička arhitektura fenotipskih obilježja školjkaša, odnosno njihova nasljednost, broj uključenih lokusa i njihov učinak vrlo su slabo istraženi. Mijenjajući uvjete okoliša, antropogeno onečišćenje može putem inducirajućeg ili genotipskog učinka utjecati na morfologiju dagnje (*Mytilus galloprovincialis*). Da bismo procijenili genetičku arhitekturu morfologije dagnje mjerili smo 15 morfoloških obilježja na 1700 jedinki iz dviju populacija koje nastanjuju područja različitog stupnja onečišćenja. Koristeći sekvenciranje nove generacije sakupili smo mnogobrojne genomske podatke, te analizirali genetičku arhitekturu morfoloških obilježja pomoću metode genomske asocijacije GWAS (eng. *genome-wide association study*). Odredili smo i genetičku arhitekturu istih obilježja te procijenili fitnes za po 20 jedinki iz dodatnih 15 populacija s postaja različitog stupnja onečišćenja. Rezultati ovog istraživanja opisuju unutar i među-populacijsku varijabilnost u morfologiji dagnje te omogućuju uvid u interakciju između genotipa, fenotipa i okoliša.

Ključne riječi: GWAS, fenotipska obilježja, fitnes, onečišćenje

GENETIC ARCHITECTURE OF MORPHOLOGICAL TRAITS IN THE MEDITERRANEAN MUSSEL *Mytilus galloprovincialis*

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The genetic architecture of phenotypic traits, including their heritability, numbers of loci involved, and effect sizes, are not well understood in bivalves. Anthropogenic pollution may create environmental differences that can alter mussel (*Mytilus galloprovincialis*) morphology through either genotypic or induced environmental effects. To assess the genetic architecture of morphology we measured 15 morphometric traits in 1700 mussels from two populations inhabiting sites differing in pollution pressure. We used a genotype by sequencing approach to generate genomic data that allowed us to then describe the genetic architecture of these traits in a genome wide association study. Additionally, we estimated the genetic architecture of the same traits as well as proxies for fitness in an additional sample of 15 populations distributed across a pollution gradient using 20 mussels per population. The results thus characterise variation within and among populations in mussel morphology and assess the association between genotype, phenotype and environment.

Key words: GWAS, phenotypic traits, fitness, pollution

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ANALIZA RAZNOLIKOSTI KLOROPLASTNE DNA I FILOGEOGRAFIJA LJEKOVITE KADULJE (*Salvia officinalis* L.)

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Poznavanje genetičke raznolikosti i filogeografije *Salvia officinalis* L. preduvjet su pouzdanog utvrđivanja podrijetla ove vrste, rekonstrukcije njezina areala kroz prošlost te uvođenja efikasnih mjera zaštite. U radu smo analizirali raznolikost sekvenci dvije kloroplastne regije DNA 86 jedinki iz ukupno 21 populacije uzduž jadranske obale i otoka. Nakon sravnjenja, upotrebom računalnog programa TCS, načinjena je parsimonijska haplotipska mreža. Ukupno smo utvrdili 9 kloroplastnih haplotipova. Četiri haplotipa pokazala su se specifičnima isključivo za južne populacije. Imajući u vidu da južne populacije, uz vlastite, imaju i sve haplotipove zastupljene u sjevernom Jadranu, zaključeno je da one predstavljaju genetički izvor širenju vrste. Vrsta se nakon oledbe postupno širila prema sjeveru pri čemu su zbog efekta osnivača sjeverne populacije danas genetički siromašnije. Za genetički sastav nekolicine populacija utvrdili smo širenje pod utjecajem čovjeka. Populacije iz južnog Jadrana su, zbog svoga genetičkog bogatstva, osobito važne za buduće programe očuvanja i uzgoja.

Ključne riječi: ljekovita kadulja, filogeografija, kloroplastna DNA

ANALYSIS OF CHLOROPLAST DNA DIVERSITY AND PHYLOGEOGRAPHY OF COMMON SAGE (*Salvia officinalis* L.)

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Understanding the genetic diversity and the phylogeography of *Salvia officinalis* L is the prerequisite for identification of the origin of this species, as well as for reconstructing its areal through the past. We analysed the sequence diversity of two chloroplast DNA regions of 86 individuals from 21 populations along the Adriatic coast and islands. After aligning the sequences, a parsimony haplotype network was made using the TCS computer program. Overall we found 9 chloroplast haplotypes. Four haplotypes were specific for southern populations. Since the southern populations in addition to specific also have all the other haplotypes present in north Adriatic, we concluded that they represent the genetic source for the propagation of this species as it spread gradually after the deglaciation in the northwestern direction, which left the north populations today genetically poorer because of the founder effect. Populations from the south Adriatic, because of their genetic richness, re present particularly valuable material for future breeding programs.

Key words: common sage, phylogeography, chloroplast DNA

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GENETSKA RAZNOLIKOST PRIRODNIH I KULTIVIRANIH POPULACIJA DALMATINSKE KADULJE (*Salvia officinalis* L., LAMIACEAE)

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Salvia officinalis L., dalmatinska kadulja, stranooplodna je grmolika ljekovita i aromatična trajnica. Prirodni areal rasprostranjenosti S.officinalis je obalno područje zapadnog

balkanskog i južnog apeninskog poluotoka, ali zbog ljekovitih, začinskih i hortikulturnih svojstava široko se uzgaja još od antičkoga doba. Cilj ovoga rada bio je istražiti genetsku raznolikost i populacijsku strukturu prirodnih, naturaliziranih i kultiviranih populacija *S. officinalis* na Balkanskom poluotoku. U tu svrhu upotrjebljeno je osam mikrosatelitnih biljega na 709 jedinki iz 30 populacija. Očekivano, naturalizirane i kultivirane populacije imale su značajno niže alelno bogatstvo i genetsku raznolikost nego prirodne populacije. Na neighbour-joining stablu većina populacija pokazala je grupiranje prema geografskoj rasprostranjenosti, od Slovenije na sjeverozapadu areala do Grčke na jugoistoku, dok se sedam naturaliziranih i kultiviranih populacija grupiralo zajedno, ukazujući na zajedničko porijeklo kultiviranog materijala. Bayesovska analiza strukture populacija pokazala je dvije glavne genetičke grupe, odvojivši prirodne od naturaliziranih i kultiviranih populacija. Prirodne populacije formirale su tri podgrupe pokazujući geografsku strukturu.

Ključne riječi: *Salvia*, genetska raznolikost, mikrosateliti

PATTERNS OF GENETIC DIVERSITY IN WILD AND CULTIVATED POPULATIONS OF DALMATIAN SAGE (*Salvia officinalis* L., LAMIACEAE)

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Salvia officinalis L., Dalmatian sage, is an outcrossing, subshrubby medicinal and aromatic perennial. The natural distribution area of *S. officinalis* is coastal region of the western Balkan and southern Apennine Peninsulas but due to its medicinal, culinary and ornamental properties it has been widely cultivated since ancient times. The aim of this study was to investigate the genetic diversity and population structure of the wild, cultivated and naturalized populations of *S. officinalis* across the Balkan Peninsula. We used eight microsatellite markers to genotype 709 samples from 30 populations. As expected, non-native populations exhibited significantly lower allelic richness as well as gene diversity in comparison to native populations. The neighbour-joining tree showed that the most of the populations grouped together in accordance to geographical position of th eir collecting sites, from Slovenia in the North-West of the region to Greece in the South-East, with the exception of seven non-native populations that grouped separately from the rest, suggesting the common origin of the cultivated plant material. A

Bayesian analysis of the population structure identified two dominant genetic clusters clearly separating indigenous and cultivated/naturalized populations. The indigenous populations further formed three subclusters indicating geographical structuring.

Key words: *Salvia*, genetic diversity, microsatellites

O-45

KLONALNA STRUKTURA ENDEMIČNE I UGROŽENE KRATKOZUPČASTE KADULJE *Salvia brachyodon* (LAMIACEAE) UTVRĐENA MIKROSATELITNIM BILJEZIMA: ZNAČENJE ZA UPRAVLJANJE I ZAŠTITU

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Salvia brachyodon Vandas ili kratkozupčasta kadulja je klonalna, usko endemična i ugrožena biljna vrsta s tek dvije poznate populacije: na poluotoku Pelješcu (Hrvatska), te na planini Orjen (Bosna i Hercegovina). S ciljem utvrđivanja razine genetičke raznolikosti, na 120 jedinki iz populacije s Pelješca je provedena genetička analiza upotrebom osam mikrosatelitnih biljega. S namjerom utvrđivanja detaljne prostorno-genetičke strukture, svakoj jedinci su pridružene pripadajuće geografske koordinate. Dobiveni rezultati potvrđuju klonalno ustrojstvo, te je utvrđena prisutnost velikih i gustih sklopova genetički identičnih jedinki što upućuje na širenje ove vrste stolonima. Za tako male i izolirane populacije, ovakva strategija razmnožavanja možda i predstavlja kratkoročnu prednost, ali imajući na umu da se kao moguće posljedice klonalnosti mogu po javiti izraženo križanje u bliskom srodstvu te smanjena reproduktivnost, također predstavlja i veliku prijetnju. Nadalje, utvrđena je i prisutnost populacijskog uskog grla čime je dodatno naglašena ugroženost ove populacije. Ekološka sukcesija i nestanak odgovarajućeg staništa otvorenog tipa su vjerojatni razlozi tako izraženog smanjenja ove populacija, dok ju je požar, koji je prije 12 godina opustošio ovaj lokalitet, vjerojatno spasio od daljnjeg propadanja. S ciljem njezine zaštite, agro-pastoralni bi se sustav koji je u prošlosti na tom području bio prisutan, trebao poticati i ponovno uspostaviti.

Ključne riječi: *Salvia brachyodon*, klonalnost, zaštita, mikrosateliti

MICROSATELLITE EVIDENCE FOR HIGH CLONALITY IN NARROW ENDEMIC AND ENDANGERED *Salvia brachyodon* (LAMIACEAE): IMPLICATIONS FOR MANAGMENT AND CONSERVATION

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Salvia brachyodon Vandas or short-tooth sage is a clonal, narrow endemic and endangered plant species with only two known populations: on Pelješac peninsula (Croatia) and Mt. Orjen (Bosnia and Herzegovina). In order to assess the levels of its genetic diversity, 120 individuals from Pelješac peninsula population were genetically analysed using eight

microsatellite markers. With intention of reaching fine-scale intra-population spatial genetic structure, geographic coordinates for each plant sample were recorded. Given results confirmed the highly clonal structure of this species. Spatial genetic analysis revealed large and tightly packed stands of ramets suggesting the propagation by underground stolones. For small and isolated population, such reproductive strategy is maybe an advantage in the short-term scale, but bearing in mind that clonality can have development of inbred population with low fecundity for a consequence, it also presents a great threat. In addition, population bottleneck was also detected, confirming the high vulnerability of this population. Ecological succession and disappearance of suitable open-type habitat are recognized as the likely causes of such dramatic reduction in population size. In contrast, large scale wild fire that has overrun this location 12 years ago is presumably population saviour. In order to protect this population, agro-pastoral system that was once present in this location, should be encouraged and re-established.

Key words: *Salvia brachyodon*, clonality, conservation, microsatellites

O-46

EVOLUCIJA, RAZNOLIKOST I FILOGENIJA 5S rDNA JEDINICA PONAVLJANJA U DIPLOIDNIM I POLIPLOIDNIM VRSTAMA IZ TRIBUSA *Anemoninae* (Ranunculaceae)

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Tribus *Anemoninae* broji oko 200 vrsta koje su na temelju osnovnog broja kromosoma podijeljene u dva podroda: *Anemone* x=8 i *Anemonidium* x=7. U ovom radu istražena je evolucijia, raznolikost i filogenija 5S rDNA u diploidima i poliploidima. Dobiveni rezultati pokazali su da je razdvajanje vrsta u dva podroda praćeno promjenama u 5S rDNA koje su dovele do razlika u dužini jedinica ponavljanja, sadržaja GC baza i strukturi sekvenca. Prisutnost 5S rDNA jedinica ponavljanja kraćih od tipičnih za vrste iz roda *Anemone* i dužih od tipičnih za vrste iz roda *Hepatica* upućuju da je 5S rDNA prošla kroz dvije runde evolucije: skraćivanje i produljivanje. Prisutnost MITE-like transpozonskih elemenata u vrstama iz roda *Hepatica* ukazuje na transpozonima posredovanu amplifikaciju 5S rDNA. Razmjerno visoka razina unutarvrsne raznolikosti, prisutnost varijanti različite duž ine i pojava pseudogena ukazuje na djelovanje slabih homogenizacijskih sila na oblikovanje 5S rDNA. Filogenetska istraživanja bazirana na 5S rDNA podupiru hipotezu da je *A. parviflora* donor D subgenoma u alopoliploidnim vrstama *A. multifida* i *A. baldensis*. Proces diploidizacije u oba alopoliploida odvijao se u smjeru strukturnih promjena 5S rDNA kao što je gubitak 5S rDNA iz D subgenoma i zamjena s 5S rDNA iz B subgenoma. Filogenija vrsta iz roda *Hepatica* potvrdila je alopoliploidno porijeklo vrsta *H. transsilvanica* i *H. yamatutai* ukazujući na vrste *H. nobilis* i *H. insularis* kao potencijalne roditelje ovih alopoliploida.

Ključne riječi: 5S rDNA, *Anemone*, *Pulsatilla*, *Hepatica*, alopoliploidi, usklađena evolucija

EVOLUTION, DIVERSITY AND PHYLOGENY OF 5S RDNA UNIT ARRAYS IN DIPLOID AND POLYPLOID MEMBERS OF THE TRIBE *Anemoninae* (Ranunculaceae)

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The tribe *Anemoninae* is composed of ~200 species divided into two subgenera according to the basic chromosome number: *Anemone* x=8 and *Anemonidium* x=7. We have performed extensive study of evolution, diversity and phylogeny of 5S rDNA in diploid and polyploid species of the tribe *Anemoninae*. Our results show that diversification of *Anemoninae* into two subgenera was accompanied with major changes in 5S rDNA that led to different 5S unit array length, GC content and 5S rDNA sequence structure. Existence of 5S shorter than typical unit size variants in *Anemone* and longer than typical in *Hepatica* indicates that 5S rDNA has undergone two rounds of evolution: contraction and extension. The presence of MITE-like transposable elements in *Hepatica* suggests that amplification of 5S rDNA is transposon-mediated. 5S rDNA of the *Anemoninae* tribe was shaped by week homogenization fo rces as indicated by high intra-individual nucleotide diversity, presence of 5S unit array length variants and pseudogenes. Phylogenetic study supports the hypothesis that *A. parviflora* could be the donor of the D subgenome to allopolyploids *A. multifida* and *A. baldensis*. Both allopolyploids undergone diploidization process including loss of 5S rDNA from the D subgenome and replacement with those of the B subgenome. Phylogeny of *Hepatica* confirmed allopolyploid origin of *H. transsilvanica* and *H. yamatutai* and suggests that *H. nobilis* and *H. insularis* could be considered as their putative parental species.

Key words: 5S rDNA, *Anemone*, *Pulsatilla*, *Hepatica*, allopolyploidy, concerted evolution

O-47
EVOLUCIJA MAHOVNJAKA REKOLONIZACIJOM SLATKOVODNIH EKOSUSTAVA

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Mahovnjaci su vodeni beskralježnjaci koji nespolnim pupanjem čine velike kolonije. Svaka jedinka unutar kolonije funkcionira kao zasebni zooid te je genetički identična svakoj drugoj jedinci unutar iste kolonije. Većina slatkovodnih vrsta mahovnjaka pripada razredu Phylactolaemata, dok par vrsta koje podnose bočatu vodu pripadaju razredu Gymnolaemata. Mahovnjaci su uzorkovani u rijekama jadranskog i dunavskog sliva te u močvarnim područjima kontinentalnog dijela Hrvatske. Slatkovodne i bočate vrste mahovnjaka genetički su analizirane s ciljem utvrđivanja filogenetskih odnosa između slatkovodnih i bočatih vrsta unutar razreda Phylactolaemata i Gymnolaemata te određivanja uloge bočatih vrsta u kolonizaciji slatkovodnih i morskih ekosustava. Filogenetski odnosi dobiveni analizom gena za 18S rRNA, 28S rRNA, COI i regije ITS2 potvrdili su Phylactolaemata kao bazalnu grupu unutar skupine mahovnjaka. Komparativna analiza postojećih saznanja o filogeniji mahovnjaka proširena evolucijskom hipotezom predlaže model naseljavanja morskih i slatkovodnih ekosustava od strane skupine mahovnjaka tijekom njihove evolucijske prošlosti. Bočate svojte mahovnjaka predstavljaju poveznicu za ovu ekološko-filogenetsku hipotezu. Usporedba bočatih vrsta mahovnjaka

Lophopus crystallinus i *Conopeum seurati* potvrdila je dvostruku kolonizaciju slatkovodnih ekosustava tijekom evolucije ove skupine životinja.

Ključne riječi: COI, Gymnolaemata, ITS2, Phylactolaemata, rRNA geni

EVOLUTION OF BRYOZOANS VIA RECOLONISATION OF FRESHWATER ECOSYSTEMS

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Bryozoans are aquatic invertebrates that form large colonies by asexual budding. Each individual within a colony works as a separate zooid and is genetically identical to each other individual within the same colony. Most freshwater species of bryozoans belong to the *Phylactolaemata* class, while several species that tolerate brackish water belong to the *Gymnolaemata* class. Bryozoan samples were collected in the rivers of Adriatic and Danube basin and in the wetland areas in the continental part of Croatia. Freshwater and brackish taxons of bryozoans were genetically analyzed for the purpose of creating phylogenetic relationships between freshwater and brackish taxons of the *Phylactolaemata* and *Gymnolaemata* classes and determining the role of brackish species in colonizing freshwater and marine ecosystems. Phylogenetic relationships inferred on the genes for 18S rRNA, 28S rRNA, COI and ITS2 region confirmed *Phylactolaemata* bryozoans as radix bryozoan group. Comparative analysis of existing knowledge about the phylogeny of bryozoans and the expansion of known evolutionary hypotheses is proposed with the model of settlement of marine and freshwater ecosystems by the bryozoans group during their evolutionary past. Brackish bryozoan taxons represent a link for ecological-phylogenetic hypothesis. Comparison of brackish bryozoan species *Lophopus crystallinus* and *Conopeum seurati* confirmed a dual colonization of freshwater ecosystems throughout the evolution of this group of animals.

Key words: COI, *Gymnolaemata*, ITS2, *Phylactolaemata*, rRNA genes

O-48
ROOM TO MANEUVER: PHYSICAL ENVIRONMENT CONSTRAINTS THE PATTERN AND RATE OF CARAPACE SHAPE EVOLUTION IN CHELONIANS

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The physical environment plays an important role in driving the rate and pattern of morphological evolution by constraining developmental pathways in particular directions at ontogenetic and historical time scales. Therefore, understanding morphological evolution requires consideration of developmental, biomechanical and phylogenetic approaches. Here we investigated the rate and pattern of carapace shape evolution (phylogenetic approach) in relation to aquatic and terrestrial media (biomechanical approach) in two Chelonian suborders

(Pleurodira and Cryptodyra). Shell shape was quantified by 3D geometric morphometrics, and rates of shape evolution were compared using a time-calibrated molecular phylogeny. Patterns of shape evolution were examined by comparing evolutionary rates for upper- (vertebral and costal plates) and lower-carapace (marginal plates) relative to the physical environment and taxonomic affinity. We show that the rate of carapace evolution is greater in Cryptodira compared to Pleurodira. The first phylogenetic principal component showed a gradient from terrestrial to aquatic turtles highlighting the correlated evolution of morphology with the physical environment. Terrestrial turtles exhibited greater rates for both carapace parts compared to aquatic turtles. However, aquatic turtles exhibited a greater evolutionary rate for the upper carapace compared to lower part, while the opposite pattern was observed for terrestrial turtles.

Key words: morphological evolutionary rate, carapace, physical environment, Chelonians

O-49

A RECOGNITION OF SPECIES TAXON IS A TAIL OF MICROEVOLUTIONARY PROCESSES IN *Testudo marginata*: AN EVIDENCE OF A SEX-SPECIFIC ENVIRONMENTALLY-INDUCED VARIATION OF PLASTRON MORPHOLOGY

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The Marginated tortoise is the largest European tortoise with challenging taxonomic status. For instance, dwarf individuals from Messinia region in Peloponnese have been previously proposed as a separate species (*Testudo weissingeri*). Studies that utilized molecular markers partly confirmed validity of *T. weissingeri*, but differently explained evolutionary significance of dwarfism in this taxon. Here, we conducted a multilevel study combining three mitochondrial genes (12s rRNA, cyt b, COI), comprehensively-quantify plastron morphology and environmental data (temperature and precipitation) in order to assess: 1) taxonomic validity of *T. weissingeri*, 2) influence of environmental predictors on dwarfism. Plastron morphology (size and shape) of adult tortoises was quantified by linear and 2D geometric morphometrics. From taxonomic perspective, dwarf form cannot be separated from nominal one, although male-specific size and size-related shape divergence was observed. Moreover, five males out of 36 individuals defined as dwarf form showed diagnostic pattern of mitochondrial divergence. Plastron size of sexes was influenced by different and independent environmental sources. Likewise, in contrast to females, association between plastron shape and environmental predictors was observed in males. Therefore, dwarfism is a consequence of sex-specific and environmental-specific factors. These findings are discussed from taxonomic, microevolutionary and developmental perspective.

Key words: multilevel study, mitochondrial genes, plastron, sex-specific factors, environmental-specific factors

1. Balkanski herpetološki simpozij u suorganizaciji s Hrvatskim herpetološkim društvom

1st Balkan Herpetological Symposium in co-organization with Croatian Herpetological Society

IO-1

KOMPARATIVNA FILOGEOGRAFIJA TRI VRSTE ZAPADNOBALKANSKIH GUŠTERICA (REPTILIA, LACERTIDAE) PREKLAPAJUĆIH AREALA

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Areali endemičnih gušterica zapadnog Balkana, petrofilnih vrsta *Dalmatolacerta oxycephala* i *Dinarolacerta mosorensis* te terestrijalne vrste *Podarcis melisellensis*, u velikoj se mjeri preklapaju. Komparativna filogeografska studija temeljena na dva mitohondrijska biljega pokazala je nepodudarajuće filogeografske uzorke s obzirom na broj, starost i geografsku rasprostranjenost genetskih linija. Filogeografski uzorci sve tri vrste impliciraju postojanje nekoliko mikro-pribježišta pri čemu su pleistocenske klimatske ekstreme vrste *P. melisellensis* i *D. oxycephala* preživjele u glacijalnim, a *D. mosorensis* vjerojatnije u interglacijalnim pribježištima. Uočene razlike mogu se objasniti u svjetlu različitog odgovora na pleistocenske klimatske oscilacije zbog specifičnih ekoloških zahtjeva vrsta i sklonosti različitim tipovima staništa kao i utjecaja povijesnih geotektonskih događaja te naseljavanja djelovanjem čovjeka. Unutar trenutno monotipične vrste *D. oxycephala* pronađene su dvije duboko odijeljene grupe koje su divergirale prije otprilike 5 milijuna godina. Genetske udaljenosti među njima su usporedive ili čak premašuju one nađene između nekih srodnih vrsta porodice Lacertidae. Uočena kriptična genetska varijabilnost ukazuje na potrebu revizije taksonomskog i konzervacijskog statusa vrste. Preliminarne analize dva jezgrina biljega potvrdile su postojanje dvije grupe unutar vrste *D. oxycephala*, ali su također ukazale na ograničenu hibridizaciju u zoni sekundarnog kontakta.

Ključne riječi: Komparativna filogeografija, zapadni Balkan, *Podarcis melisellensis*, *Dalmatolacerta oxycephala*, *Dinarolacerta mosorensis*

COMPARATIVE PHYLOGEOGRAPHY OF THE THREE WIDELY CODISTRIBUTED ENDEMIC WESTERN BALKANS LACERTID LIZARDS (REPTILIA, LACERTIDAE)

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The rock climbing lizards *Dalmatolacerta oxycephala* and *Dinarolacerta mosorensis* and the ground-dwelling lizard *Podarcis melisellensis* are Western Balkans endemics having largely overlapping distribution range. Comparative phylogeographic study of those species based on two mitochondrial genes revealed highly non-concordant phylogeographic patterns regarding the number, age and geographic distribution of lineages. The existence of several possible microrefugia is implicated for all three species. While *P. melisellensis* and *D.*

oxycephala survived the unfavourable Pleistocene climatic conditions in glacial refugia, for *D. mosorensis*, interglacial microrefugia appear more likely. Observed differences can be explained in the light of different responses to Pleistocene climatic oscillations due to specific ecological and habitat preferences as well as the impact of past geotectonic events but also the anthropogenic translocations. Within *D. oxycephala*, presently monotypic species, two deeply separated clades that diverged some 5 my ago were found. The genetic distances between them are comparable or even exceed those found between some closely related Lacertid lizards. Observed cryptic genetic diversity within *D. oxycephala* prompts a revision of its taxonomic and conservation status. Preliminary analyses of two nuclear molecular markers confirmed the existence of two clades within *D. oxycephala*, but also indicated the isolated hybridization events in the secondary contact zone.

Key words: comparative phylogeography, Western Balkan, *Podarcis melisellensis*, *Dalmatolacerta oxycephala*, *Dinarolacerta mosorensis*

IO-2

ZARAZNE BOLESTI VODOZEMACA – ŠTO SE VEĆ ZNA, ŠTO JE NOVO, A ŠTO TREBA IMATI NA UMU KAKO BI SE SPRIJEČILO NJIHOVO IZUMIRANJE

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Vodozemci su danas jedna od najugroženijih životinjskih vrsta na svijetu. Osim gubitka staništa, promjene klime i zagađenja, jedan od bitnih uzroka izumiranja vodozemaca su i zarazne bolesti. Kako ljudi imaju veliku ulogu u širenju i prenošenju zaraznih bolesti s jednog mjesta na drugo, vrlo je važno znati koje bolesti uzrokuju ugibanja vodozemaca, kako ih dijagnosticirati, te kako spriječiti njihovo širenje. U predavanju će se iznijeti dosadašnja saznanja o gljivičnim i virusnim uzročnicima bolesti vodozemaca, ponajprije o hitrid gljivama *Batrachochytrium dendrobatidis* (BD), poznatom uzročniku masovnih ugibanja vodozemaca u cijelom svijetu, i o nedavno opisanom *Batrachocytrium salamandrivorans* (BS) koji, prema dosadašnjim saznanjima, uzrokuje bolest i uginuća samo repaša (urodela), te o ranavirusu, koji može izazvati oboljevanja vodozemaca, gmazova i riba. Iznijet će se i rezultati preliminarnih istraživanja infekcija BD-om, BS-om i ranavirusom u čovječje ribice (*Proteus anguinus*) provedenih u sklopu projekta zaštite čovječje ribice u Hrvatskoj, kao i rezultati testiranja ostalih vodozemaca s različitih lokacija u Hrvatskoj. Svrha predavanja je stručnjake koji se bave vodozemcima što detaljnije upoznati sa zaraznim i pogubnim bolestima vodozemaca, kako bi u svom svakodnevnom radu, osobito na terenu, znali prepoznati bolest, prikupiti potrebne uzorke i spriječiti širenje bolesti s jednog područja na drugo.

Ključne riječi: vodozemci, smrtonosne bolesti, *Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans*, ranavirus

EMERGING AMPHIBIAN DISEASES – WHAT IS KNOWN, WHAT IS NEW AND WHAT SHOULD BE BORNE IN MIND TO PREVENT THEIR EXTINCTION

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Amphibians currently belong to a group of most endangered animal species in the world. In addition to the loss of habitats, climate changes and pollution, contagious diseases are also one of significant causes of amphibian extinction. Since the role of humans in disease spreading and transfer from one area to another is significant, it is very important to learn more about emerging diseases in amphibians, how to diagnose those diseases and how to prevent their spread. In this lecture current knowledge regarding the following fungal and viral causes of emerging amphibian diseases will be presented, specifically about the: (i) chytrid fungus *Batrachochytrium dendrobatidis* (BD), a well known cause of mass amphibian mortality worldwide; (ii) another recently described chytrid fungus *Batrachochytrium salamandrivorans* (BS) which is, to our current knowledge, the cause of disease and mortality in Caudata (Urodela) only; and (iii) Ranavirus which can cause disease in amphibians, reptiles and fishes. The results of preliminary investigation of BD, BS and Ranavirus infections in the olms (*Proteus anguinus*), and of other amphibians from Croatia will also be presented. The aim of this lecture is to provide the herpetologists with more information about infectious and deleterious amphibian diseases, to help them in their everyday activities, particularly in the field, to recognize disease, collect adequate samples and prevent disease spread from one site to another.

Key words: Amphibians, Emerging diseases, *Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans*, Ranavirus

O-50

DISTRIBUTION, ABUNDANCE AND DETECTION PROBABILITY OF LACERTIDAE IN FOUR CROATIAN ISLANDS

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The evaluation of real abundance of species like reptiles may be some time problematic because those species may be very sensitive to environmental and weather conditions. Consequently the possibility to evaluate the detection probability may be an important result (McDiarmid et al., 2012). Furthermore, in Mediterranean islands, the distributions of reptiles is still incomplete (Ficetola et al., 2014). During the spring of 2014 we carried out an extensive survey in four island of Croatia: Losinj, Unije, Susak, Ilovik. The aims of this field campaign were:1. to assess the abundance of more common species of Lacertidae; 2. to evaluate the usefulness of detection probability measured by means of repeated transects; 3. to describe the microhabitat elements that may have an important role in species abundance.We covered the islands with 60 transect (36 Losinj, 10 Unije, 6 Susak, 8 Ilovik), each transect was repeated two or three times. By means of R package and of AIC approach we built models for three species (*Algyroides nigropuntaus*, *Podarcis melliselensis* and *Podarcis siculus*) we were able to get models of abundance. The most abundant is P.m. clearly linked to microhabitat such as stone walls. The repetition of transects may increase a lot the available information, altough if in large areas may require a significative amount of time. But after a repetition the possibility to casual detection decrease a lot and the general result is reliable.

Key words: Detection probability, Herpetofauna, Island biogeography, Species abundance

O-51

UČESTALOST ŽIVORODNE GUŠTERICE, *Zootoca vivipara* Lichtenstein 1823 NA PODRUČJU ZAPADNOG BALKANA

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Živorodna gušterica, *Zootoca vivipara* Lichtenstein 1823, je mali gušter iz porodice Lacertidae poznat po tome što ima dva načina razmnožavanja. Živorodna gušterica ima najveće područje geografske rasprostranjenosti od svih guštera. Ona naseljava veći dio Europe i sjeverni dio Azije. Njen areal uključuje i Hrvatsku te druge zemlje zapadnog Balkana. Geografska rasprostranjenost živorodne gušterice na zapadnom Balkanu predstavljena u ovom radu određena je na temelju objavljenih podataka uz dodatak neobjavljenih terenskih podataka sakupljenih za Hrvatsku i Bosnu i Hercegovinu. Korištene su četiri WorldClim varijable kako bi se opisali ekološki parametri unutar ovog područja. Očekivali smo da će dobiveni podaci upućivati na povezanost hrvatskih i susjednih populacija (kako je u prethodnim publikacijama dokazana filogenetska povezanost hrvatske populacije sa slovenskom i talijanskom populacijom). Značajne razlike su pronađene u ekološkim parametrima koje odabiru pojedine podpopulacije. Živorodna gušterica može živjeti na različitom tipu staništa, od nizina do visokih planina. Rezultati pokazuju da postoje tri odvojene podpopulacije u proučavanom području i one odgovaraju poznatim podvrstama živorodne gušterice - *Zootoca vivipara vivipara* Von Jacquin, 1787, *Zootoca vivipara carniolica* Mayer, Böhme, Tiedemann i Bischoff, 2000 i *Zootoca vivipara pannonica* Lác i Kluch, 1968.

Ključne riječi: rasprostranjenost, *Zootoca vivipara*, Hrvatska, Zapadni Balkan

HOW COMMON IS THE COMMON LIZARD, *Zootoca vivipara* Lichtenstein 1823 IN WEST BALKAN AREA?

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The common lizard, *Zootoca vivipara* Lichtenstein 1823, also called the viviparous lizard, is a small lacertid lizard well known for its reproductive bimodality. It has the largest geographical distribution of all lizards. It inhabits almost all of Europe and northern part of Asia. Its range also includes Croatia and other Western Balkan countries. The geographic distribution of the common lizard in the Western Balkan presented in this talk was determined according to published data with addition of unpublished field data from Croatia and Bosnia and Herzegovina. We used four basic WorldClim variables to describe the preferred ecological parameters within this range. We expected that these data would prove connectivity of Croatian populations with populations of neighbouring countries (as its phylogenetic relationship was shown between Croatian population and Slovenian and Italian populations in previous publications). Significant differences were found in ecological parameter preference between all three subpopulations. The common lizard can inhabit various types of habitats, from lowlands to high mountains. Results show that three separate subpopulations, corresponding to three subspecies of common lizard, *Zootoca vivipara vivipara* Von Jacquin, 1787, *Zootoca vivipara carniolica* Mayer, Böhme, Tiedemann i Bischoff, 2000 and *Zootoca vivipara pannonica* Lác i Kluch, 1968, are present in the researched area.

Key words: distribution, *Zootoca vivipara*, Croatia, Western Balkan

O-52

DISTRIBUTION (HORIZONTAL AND VERTICAL) AND CONSERVATION STATUS OF HERPETOFAUNA OF SARAJEVO CANTON AND ITS WIDER AREA (BOSNIA AND HERZEGOVINA)

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This paper presents data about the findings of amphibian and reptile species from the wider area of Sarajevo Canton in Bosnia and Herzegovina (B&H). As the capital of B&H, Sarajevo is proclaimed as the most polluted city in the country and sometimes even Europe. Collection of data was mostly made through field investigations and additionally by analyzes of literature data so as the private herpetological collection "Lelo" and collection of the National History Museum of B&H. Data show that the area is inhabited by 13 amphibian and 18 reptile species (61% of B&H herpetofauna). However, there are few suspicious and unspecific findings. Some species (*Rana temporaria*, *Podarcis melisellensis, Dalmatolacerta oxycephala*) encounter their distribution borders in this area. It is assumed that the majority of recorded populations of herpetofauna have an intensive decreasing trend with some populations already extincted (eg. *Bombina variegata, Vipera berus*) due to strong anthropogenic impact, habitat changing and niche shifting. In addition, one population of *Trachemys scripta* was found which represents the first data of this invasive alochtone species for B&H. Paper also provides new data on *erythronotus* mutation in *Lacerta agilis bosnica*. Conservation status (local and regional) as well as suggested conservation measure that can be introduced to improve the current situation are also discussed.

Key words: Biodiversity, Amphibia, Reptilia, threats, protection

O-53

REZULTATI PRVE GODINE SUSTAVNIH ISTRAŽIVANJA SASTAVA, DISTRIBUCIJE I BROJNOSTI HERPETOFAUNE NP "PLITVIČKA JEZERA"

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Nacionalni park "Plitvička jezera" pripada najstarijim zaštićenim dijelovima prirode u Republici Hrvatskoj, no unatoč tomu istraživanja brojnih životinjskih skupina koje ga nastanjuju nalaze se na samim početcima. Takvo je stanje i s istraživanjem vodozemaca i gmazova. Sporadičnih bilješki o herpetofauni ovoga područja imamo još iz vremena austrougarskog imperija, ali njena potpuna inventarizacija i sustavno istraživanje nije nikada provedeno. Sa stajališta herpetofaune, NP "Plitvička jezera" smješten je na zoogeografski izuzetno zanimljivom prostoru - graničnom području rasprostranjenosti brojnih vrsta vodozemaca i gmazova. Osim toga u sastav parka, pogotovo nakon proširenja njegovih granica, ulaze brojna staništa, koja omogućavaju život vodozemaca i gmazova ekološki potpuno različitih potreba. Dosadašnjim je istraživanjima zabilježeno 12 vrsta vodozemaca i 12 vrsta gmazova. Od 2014. do 2017. god. provode se prva sustavna istraživanja sastava i distribucije faune vodozemaca i gmazova te njihove brojnosti na pet različitih staništa na području NP "Plitvička jezera". U ovom radu iznose se rezultati prve godine istraživanja koji su dobiveni pregledom herpetoloških zbirki nekoliko prirodoslovnih muzeja, osmatranjem, lovom i putem postavljenih trajnih ploha sa živolovkama.

Ključne riječi: vodozemci, gmazovi, sastav herpetofaune, distribucija herpetofaune, NP "Plitvička jezera"

COMPOSITION, DISTRIBUTION, AND ABUNDANCE OF AMPHIBIANS AND REPTILES OF THE PLITVICE LAKES NATIONAL PARK DURING THE FIRST YEAR OF SYSTEMATIC RESEARCH

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Although Plitvice Lakes National Park is among oldest protected areas of nature in Croatia, research of numerous animal groups that inhabit the national park is just at the beginning. That implies to amphibians and reptiles as well. Sporadic notes about herpetofauna of this area exist since the era of Austro-Hungarian Empire, but complete species list does not exist and systematic research has never been done. From the zoogeographic point of view, National park is located in an interesting bordering area of distribution for numerous species of amphibians and reptiles. With expand of its borders, the National park now includes many different habitats which support amphibian and reptile species with very diverse ecological demands. So far, we have evidence of 12 amphibian and 12 reptilian species. From the year 2014 to 2017 the first systematic research of composition, distribution and abundance of amphibians and reptiles have been in progress, especially on five different habitats of Plitvice Lakes National Park area. This paper presents results for the first year of research. Results are based on several museum herpetological collections reviews, field research, and on data collected by live trapping in permanently established and controlled plots.

Key words: amphibians, reptiles, composition of herpetofauna, distribution of herpetofauna, Plitvice Lakes National Park

O-54

ECOLOGICAL LIMITATIONS AND PAST EVENTS AFFECT SPECIES DISTRIBUTION: ASSESSING THE QUALITY OF SPATIAL DISTRIBUTION MODELS FOR TWO REPTILE SPECIES

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Species distribution models (SDMs) are increasingly important for conservation and already used for i.e. reserve planning and evaluating the effectiveness of protected areas (PA's). However, how reliable are these models? SDMs estimate a species' fundamental niche while the realized niche, which is the niche range that a species actually occupies, is generally smaller than the fundamental niche due to human influences, biotic interactions and geographic barriers. This article used two case studies (*D. caspius and D. mosorensis*) to show the impact of these factors in modeling reptile species distribution in Croatia. MaxEnt estimates presence of *D. caspius* along the coast of Croatia and on lots of the smaller islands, however, this species is only found in the east of Croatia and on the islands Olib and Lastovo. Possible explanations of this distant distribution are residual populations, exotic species and distribution by floating on debris. *D. mosorensis* is a specialist, occupying vertical habitats on limestone rocks and cliffs on high elevation mountaintops. The populations predicted by

MaxEnt are fragmented due to elevation and horizontal habitats. MaxEnt might thus predict presence in different areas but it is questionable whether this species was able to occupy these areas due to its adaptations and poor dispersal abilities. It is thus very important to take into account species' ecological limitations and past events when assessing the quality of a SDM.

Key words: Protected areas, species distribution modelling, MaxEnt, cross-national conservation

O-55

PRELIMINARY DATA ON THE ASSESSMENT OF THE EFFECTIVENESS OF PROTECTED AREAS FOR TWO REPTILE SPECIES USING SPATIAL DISTRIBUTION MODELING

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Protected areas (PA's) should ensure the persistence of reptile species. However, several studies already confirmed that the establishment of PA's do often not correlate with conservation priorities. This study used a species distribution model (MaxEnt) to assess the effectiveness of PA's in Croatia for two data deficient reptile species (*Z. situla* & *T. fallax*). PA's were divided in Species PA's and Landscape PA's using the IUCN Protected Areas Categories System. The results show that the best suitable areas are not covered by species PA's confirming that reptiles are not taken in account during policymaking and the establishment of PA's. Using the criterion of the IUCN Red List Categories and Criteria 3.1, suggesting a minimum area requirement larger than 2.000 km^2 (excluding subcategories), we could also say that Croatia alone cannot ensure the pers istence of these species. This indicates the need for cross-national conservation to sustain viable populations.

Key words: Protected areas, species distribution modelling, MaxEnt, cross-national conservation

O-56

PROCJENA OSJETLJIVOSTI FAUNE VODOZEMACA I GMAZOVA U HRVATSKOJ I BOSNI I HERCEGOVINI UZ DEFINIRANJE VAŽNIH HERPETOLOŠKIH PODRUČJA

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U Hrvatskoj i Bosni i Hercegovini živi 60 vrsta vodozemaca i gmazova (bez morskih vrsta). Potrebno je procijeniti osjetljivost svih vrsta jer procjene ugroženosti u Crvenoj knjizi ne uzimaju u obzir ekološke parametre već samo geografsku raspodjelu. U radu je izdvojen određen broj varijabli koje uključuju ekološke, biološke, geografske i antropološke čimbenike. Kod pojedine vrste za svaku varijablu upisana je jedna od četiri kategorije, od 0 – najmanja, do 3 – najveća osjetljivost. Podaci za svaku vrstu dobiveni su pregledavanjem literature, te iz osobnih iskustava stručnjaka. Indeks osjetljivosti svake vrste izračunat je kao zbroj vrijednosti svih varijabli i podijeljen s brojem varijabli. Provedena je i korelacija dobivenih indeksa osjeljivosti i nacionalnog

statusa ugroženosti po Crvenoj knjizi, te su definirana važna herpetološka područja po moću tri karte. Rezultati su pokazali da su gmazovi osjetljiviji od vodozemaca. Također, dobiveni su indeksi osjetljivosti korelirani s procjenama u Crvenoj knjizi, uz nekoliko iznimki. Vrste *Testudo hermanni*, *Zamenis situla* i *Iberolacerta horvathi* imaju veliku osjetljivost, a mali status ugroženosti koji se treba preispitati. Važna herpetološka područja prostiru se po većini Hrvatske obale, te većim dijelom Dinarida. Zaključak je da se procjenom osjetljivosti dolazi do kvalitetnije procjene stanja ugroženosti herpetofaune nego procjenama nacionalnih statusa u Crvenoj knjizi.

Ključne riječi: indeksi osjetljivosti, herpetofauna, Hrvatska, Bosna i Hercegovina, nacionalni status ugroženosti

SENSITIVITY ASSESSMENT OF FAUNA OF AMPHIBIANS AND REPTILES OF CROATIA AND BOSNIA AND HERZEGOVINA WITH DETECTION OF IMPORTANT HERPETOLOGICAL AREAS

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There are 60 species of amphibians and reptiles in Croatia and Bosnia and Herzegovina (non-marine species), so there is a need to assess their sensitivity, since the endangerment assessments in the Red List take into account only the geographical distribution, and not the ecological parameters. In this paper a number of variables is singled out, which include ecological, biological, geographical, and anthropological factors. For each species and variable one of four categories was assigned: from 0 - the lowest, to 3 - the highest sensitivity. Data for each type is obtained from examining the literature, and also from experts' personal experiences. The sensitivity index of each species was calculated as the sum of values of all variables and divided by the number of variables. A correlation of the sensitivity index and the national status of endangerment in the Red List was made, and important herpetological areas were defined using three maps. The results show that reptiles are more sensitive than amphibians. Also, the resulting sensitivity indices correlate well with the assessments in the Red List, with few exceptions. Species *Testudo hermanni*, *Zamenis situla* and *Iberolacerta horvathi* are highly sensitive, so their low endangerment status should be reviewed. Important herpetological areas spread across most of the Croatian coast, and a large part of the Dinarides. In conclusion, the assessment of sensitivity gives better endangerment assessment than IUCN criteria.

Key words: sensitivity index, herpetofauna, Croatia, Bosnia and Herzegovina, national conservation status

O-57

HABITAT REQUIREMENTS AND ABUNDANCE OF *Dinarolacerta mosorensis* AND *D. montenegrina* IN THE MOUNTAINS OF MONTENEGRO

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We analysed habitat requirements and abundance of *Dinarolacerta mosorensis* and *D. montenegrina*, two closely related lacertid rock lizards endemic to the Dinaric mountains of the Balkan Peninsula. A total of four sites on three mountains in Montenegro were surveyed. At each study site, a number of visual transects were carried out in order to determine lizards abundance and eleven microhabitat variables describing habitat structure, type of vegetation and refuge availability. Data were examined by Analysis of Variance, Variance Components Analysis and Principal Component Analysis. We found significant differences in preferred habitat between species and among localities, but not between adults and non-mature individuals. Intraspecific variability in habitat use was greater than between the two species. There were significant differences in lizard abundances among loca lities. The lowest abundance was recorded in the southernmost population of *D. mosorensis*. A greater number of small rocks and larger areas of bare rocks without vegetation negatively affected lizard abundance, despite the greater number of refuges at these places. Our study underlines the needs for establishing a regime of more intensive monitoring of forest exploitation and implementation of concrete measures aimed at combating and reducing the number of fires in the study areas.

Key words: lacertid rock lizards, habitat selection, Dinaric mountains, forest fires, deforestation

O-58
BIOLOGIJA POSKOKA U KAMENOLOMU BIZEK

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Poskok, *Vipera ammodytes* (Linnaeus, 1758) najveća je zmija otrovnica jugoistočne Europe. Međutim, neka područja njegove biologije i dalje su nepoznata. Ovdje predstavljamo rezultate prvog istraživanja biologije suburbanih poskoka u Hrvatskoj, od 2008. do 2014. godine, u zatvorenom kamenolomu Bizek. Cilj istraživanja bio je utvrditi postojanje razlika među spolovima u veličini, odrediti korištenje mikrostaništa, područja kretanjate meteorološke prilike pri kojima su najaktivniji. Također je cilj bio utvrditi prehranu, procijeniti brojnost populacije i istaknuti posljedice nekih ljudskih aktivnosti na području kamenoloma. Ukupno je analizirano 140 jedinki poskoka. Utvrđeno je da postoje razlike u veličini među spolovima, a oba spola pokazuju vrlo slične sklonosti prema vrsti mikrostaništa i vremenskim uvjetima, osim u vlazi zraka. Među spolovima nema razlike u dnevnoj aktivnosti, ali ima u sezonskoj. Nema ni međuspolnih razlika u prehrani. Odrasli mužjaci se kreću najvećim područjem. Čišćenje kamenoloma ima jasan negativni utjecaj na populaciju, ako se izvodi na krivi način i u krivo doba godine. Rezultati ovog istraživanja daju uvid u neke dijelove biologije poskoka u kamenolomu te mogu koristiti zaposlenicima Parka prirode Medvednica u upravljanju tim prostorom.

Ključne riječi: *Vipera ammodytes*, suburbana populacija

BIOLOGY OF THE NOSE-HORNED VIPER IN THE BIZEK QUARRY

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The Nose-horned Viper, *Vipera ammodytes* (Linnaeus, 1758) is the largest venomous snake in the western Balkans. However, there's paucity of research in some aspects of its biology. Here we present the results of the first study of the biology of suburban *V. ammodytes* in Croatia, from 2008 till 2014, in the inactive Bizek quarry. Our goals were to determine the existence of sexual size dimorphism, microhabitat use, home range size, weather conditions during peak activity, food composition and population size. We also wanted raise awareness about the influence of some human activities in the quarry on the viper population. A total of 140 individuals were analyzed. Sexual size dimorphism was confirmed, and both sexes show very similar preferences in microhabitat use and weather conditions, except in relative humidity. There is no difference between the sexes in daily act ivity, but there are differences in seasonal activity. There are no differences in the diet composition between the sexes. Adult males have the largest home ranges. The cleaning activities in the quarry have a clear negative impact on the population, if done incorrectly and in the wrong time. The results of this research give a new view on some aspects of the biology of Nose-horned vipers in the Bizek quarry and can be of use to the Nature Park Medvednica staff in management of the area.

Key words: *Vipera ammodytes*, suburban population

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NEW DATA AND DISTRIBUTION OF COMMON SPADEFOOT TOAD *Pelobates fuscus* (Laurenti, 1768) IN WESTERN BALKANS

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Based on known literature data and data collected during field investigations in 2014 and 2015, we present updated distribution map of Common spadefoot toad (*Pelobates fuscus*) in Western Balkans (Bosnia and Herzegovina (B-H) and Croatia). *Pelobates fuscus* is listed as LC on the IUCN Red List and DD in Croatian Red Book, and the species is in constant population decline. Until year 2014 this species was only suspected to inhabit B-H. Today we have several positive localities in Posavina region presented with precise locality, elevation and coordinates. In B-H new populations are found near towns of Srbac, Brod, Modriča and Šamac. Distribution map includes east part of Lijevče polje and whole Posavina and there is a big chance that species range extends also through Semberija. In this paper we also present first findings of tadpoles, more precisely, the reproductive sites of Common spadefoot toad in the B-H. In Croatia this species is found along the rivers Mura, Drava and Sava, including most lowland areas up to 300 m above sea level. New localities in Croatia are confirmed in Ivanovo selo and Bjelovar, connecting the Drava and Sava River populations.

Keywords: conservation, decline, Posavina, Pannonian Plain

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KOMPARATIVNA MORFOLOGIJA, EKOLOGIJA I ANALIZA STRUKTURE HRVATSKE POPULACIJE *Pelophylax esculentus* KOMPLEKSA

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Hibridogenetski kompleks *Pelophylax esculentus* uključuje vrste *Pelophylax ridibundus* (R), *P. lessonae* (L), i *P*. kl. *esculentus* (E) jedan je od najsloženijih i evolucijski vrlo interesantnih životinjskih hemiklonskih sustava. Dosadašnja istraživanja analizirala su strukturu populacija, prehranu, reproduktivne tipove i distribuciju vrsta ovog kompleksa no podaci koji uključuju analizu populacije REL tipa su rijetki. Cilj ovog istraživanja je proučiti morfologiju i prehranu jedinki te strukturu populacije Pelophylax esculentus kompleksa u kontinentalnom dijelu Hrvatske. Morfološke karakteristike i analiza alozima rabljene su za identifikaciju vrste ukupno 93 nasumično prikupljene jedinke. Sadržaj želuca tih jedinki je analiziran kako bi se usporedio profil prehrane pojedine vrste dok je metodom skeletokronologije procjenjen broj linija zaustavljenog rasta na preparatima kosti femura za usporedbu starosti i indeksa rasta jedinki pojedine vrste. Rezultati su otkrili prisutnost populacijskog tipa REL pri čemu u uzorku najmanji broj jedinki pripada vrsti *P. ridibundus*. Unatoč velikoj morfološkoj varijabilnosti među vrstama, opažena je izraženost rijetkih i specifičnih karakteristika jedinki vrste *P. ridibundus* i *P*. kl. *esculentus*. Primjećene su i razlike u prehrani i indeksu rasta između *P. ridibundus* i jedinki ostalih vrsta proučavane populacije REL tipa što upućuje na postojanje mogućih evolucijski bitnih jedinstvenih interakcija među vrstama ove populacije.

Ključne riječi: *Pelophylax esculentus* kompleks, morfologija, analiza alozima, prehrana, skeletokronologija

COMPARATIVE MORPHOLOGY, ECOLOGY AND STRUCTURE ANALYSIS OF THE *Pelophylax esculentus* COMPLEX POPULATION IN CROATIA

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One of the most complicated and evolutionary intriguing animal hemiclonal systems is the *Pelophylax esculentus* hybridogenetic complex which includes *Pelophylax ridibundus* (R), *P. lessonae* (L), and *P*. kl. *esculentus* (E) species. Previous studies have analyzed population structure, diet, reproductive modes and distribution of *Pelophylax esculentus* complex species but similar data on the rarely observed REL populations are still scarce. This study aims to reveal population structure, morphology and diet of the *P. esculentus* complex population located in the continental part of Croatia. We used morphological traits and allozyme analysis for species identification of randomly collected 93 green frog individuals. Also, we compared diet, age and growth index profiles of species present in this population by respectively analyzing their stomach content and estimating the number of lines of arrested growth visible in the cross-sections of femur bones using skeletochronology. Results revealed the presence of REL population with *P. ridibundus* representing the lowest number of individuals. Despite great morphological interspecific variability, we observed some *P. ridibundus* and *P*. kl. *esculentus* individuals expressing rare and unique coloration features. Diet and annual growth rate of *P. ridibundus* individuals differed from other species indicating possibly evolutionary important specific species-species interactions in the studied REL population type.

Key words: *Pelophylax esculentus* complex, morphology, allozyme analysis, food diet, skeletochronology

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MOLEKULARNA ISTRAŽIVANJA ZELENIH ŽABA (ROD *Pelophylax*) U HRVATSKOJ – PRELIMINARNI REZULTATI

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Prema literaturnim podacima, na području Hrvatske južno od rijeke Save obitava vrsta *Pelophylax ridibundus*, dok se sjevernije od Save mogu pronaći vrste *P. ridibundus*, *Pelophylax lessonae* te njihov hibridni kompleks (*Pelophylax* kl. *esculentus*). Budući da je morfološko određivanje vrsta i hibrida zelenih žaba prilično nepouzdano, cilj ovog istraživanja bio je odrediti taksonomski status zelenih žaba u Hrvatskoj upotrebom molekularnih biljega (analiza varijacije duljine serum albumin gen intronske 1 regije i analiza mitohondrijskog gena za NADH dehidrogenaznu podjedinicu 3). Istraživanje je provedeno na populacijama zelenih žaba iz različitih dijelova Hrvatske (ribnjaci Crna Mlaka, Lika, Istra i Dalmacija). Rezultati su potvrdili prisutnost vrsta *P. ridibundus*, *P. lessonae* i s njima povezanog kleptona *P.* kl. *esculentus*, ali su pokazali neočekivanu prisutnost vrste *Pelophylax kurtmuelleri* te novih neopisanih hibridnih kompleksa. Prisutnost vrste *P. kurtmuelleri* u Lici i na ribnjacima Crna Mlaka, te nalaz vrste *P. lessonae* na planini Poštak (Dalmacija), ukazuju na nužnost revizije podataka o rasprostranjenosti zelenih žaba u Hrvatskoj.

Ključne riječi: zapadni Balkan, *P. kurtmuelleri*, serum albumin intron-1 regija, mitohondrijska regija ND3

MOLECULAR STUDY ON WATER FROGS (GENUS *Pelophylax*) IN CROATIA - PRELIMINARY RESULTS

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According to literature data on the distribution of water frogs in Croatia, the area south of the Sava River is inhabited by *Pelophylax ridibundus*, while *P. ridibundus*, *Pelophylax lessonae* and their hybridogenetic complex (*Pelophylax* kl. *esculentus*) can be observed in the area north of the Sava River. Since the morphological determination of species and hybrids of water frogs is not reliable, the aim of this study was to determine the taxonomical status of water frogs in Croatia using molecular markers (length variation of serum albumin gene intron-1 and the mitochondrial gene for NADH dehydrogenase subunit 3). The study was performed on water frog populations from northern part and Adriatic coast of Croatia (Crna Mlaka fish ponds, Lika, Istra, Dalmatia,). Results confirm the presence of species *P. ridibundus*, *P. lessonae* and their associated klepton *P.* kl. *esculentus*, and show an unexpected presence of species *Pelophylax kurtmuelleri* as well as new hybrid complexes. The presence of *P. kurtmuelleri* in the Lika region and Crna Mlaka fish ponds, and the discovery of *P. lessonae* in the Poštak Mountain (Dalmatia), point to the importance of revising the distribution data of water frog populations in Croatia.

Key words: Western Balkans, *P. kurtmuelleri*, serum albumin gene intron-1, mitochondrial region ND3

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PROCJENA STAROSTI JEDINKI ZELENIH ŽABA RODA *Pelophylax* (FITZINGER, 1843) IZ POPULACIJE SA SKADARSKOG JEZERA, CRNA GORA

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Na području Skadarskog jezera, pretpostavljeno je da tri različita taksona roda *Pelophylax* čine jedinstvenu populaciju sličnu već opisanim populacijama kompleksa *Pelophylax esculentus*. Budući da roditeljske vrste ove populacije pripadaju vrstama *P. kurtmuelleri* i *P. shqipericus*, ovo je, prema našem saznanju, prvi izvještaj novog, još neopisanog kompleksa zelenih žaba. Cilj rada je otkriti dobnu strukturu populacije zelenih žaba sa Skadarskog jezera (Crna Gora) procijenjujući i uspoređujući prosječnu starost i brzinu rasta svih prisutnih taksona. Iz spomenute populacije, ukupno 83 jedinke zelenih žaba su ručno ulovljene tijekom dvogodišnjeg perioda (2011. i 2012. godine). Nakon pravilne eutanazije, iz svih ulovljenih jedinki izdvojene su bedrene kosti. Metodom skeletokronologije kosti su potom pripremljene i histološki analizirane kako bi se prepoznava njem linija usporenog rasta mogla procijeniti dob

pojedine jedinke. Prema procjeni, najmlađa jedinka je imala jednu, a najstarija 6 godina. Prosječna procijenjena dob roditeljske vrste *P. kurtmuelleri* (4 god.) bitno se razlikuje od one procjenjene za *P. shqipericus* (1,5 god.) i hibridnu vrstu (1,8 god.). Zanimljivo, pokazano je da se brzina rasta razlikuje samo između *P. shqipericus* i hibridne vrste. Otkrivene razlike u procijenjenoj prosječnoj dobi i brzini rasta spomenutih vrsta mogle bi odražavati postojanje specifičnih interakcija među vrstama koje posljedično uvjetuju strukturu ove zanimljive populacije kompleksa *Pelophylax*.

Ključne riječi: populacija kompleksa *Pelophylax*, dobna struktura, skeletokronologija, histologija, brzina rasta

AGE ESTIMATION OF GREEN FROG INDIVIDUALS BELONGING TO THE *Pelophylax* GENUS (FITZINGER, 1843) FROM THE LAKE SKADAR POPULATION, MONTENEGRO

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At the Lake Skadar, it is assumed that three taxa of green frogs form a single *Pelophylax* genus population similar to already known *Pelophylax esculentus* complex populations. Since the parent species belong to *P. kurtmuelleri* and *P. shqipericus*, this is to our knowledge the first report of a new and yet undescribed green frog complex. The aim the study was to reveal the population age structure by estimating and comparing the average age and growth rates of all taxa present in this new *Pelophylax* complex population. In total, 83 green frog individuals were collected by hand during the two-year period (2011 and 2012) at the site of Lake Skadar (Montenegro). After proper euthanasia, femur bones were isolated from all collected animals. Using the skeletochronology method the bones were prepared and histologically analyzed for the existence of lines of arrested growth w hich allowed the age estimation of each individual. The youngest animal was estimated to have one whereas the oldest had 6 year of age. The estimated average age of parental species *P. kurtmuelleri* (4 years) was significantly higher than that of *P. shqipericus* (1.5 years) and their hybrid (1.8 years) species. Interestingly, the growth rate differed only between *P. shqipericus* and the hybrid species. In conclusion, detected differences in the average age and growth rates of these species could mirror the specific interspecies interactions shaping the structure of this interesting *Pelophylax* complex population.

Key words: *Pelophylax* complex population, age structure, skeletochronology, histology, growth rate

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ODREĐIVANJE VRSTA I HIBRIDA ZELENIH ŽABA (ROD *Pelophylax*) SA SKADARSKOG JEZERA (CRNA GORA) ANALIZOM MOLEKULARNIH BILJEGA

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Zapadno-palearktičke zelene žabe (rod *Pelophylax)* je iznimno složen rod u kojem je trenutno opisano dvadesetak vrsta i tri hibridna kompleksa. Danas se zelene žabe intenzivno istražuju, ponajprije zbog postojanja hibridnih kompleksa s hemiklonalnim načinom razmnožavanja. Iako prema literaturnim podacima na Balkanskom poluotoku nije dokazano postojanje hibridnih kompleksa, preliminarna istraživanja morfoloških osobina zelenih žaba sa Skadarskog jezera (Crna Gora) ukazuju da oni ipak postoje. Budući da određivanja vrsta i hibrida zelenih žaba na temelju morfoloških osobina nije pouzdano ono je u ovom istraživanju provedeno korištenjem molekularnih biljega. Istraživanje je provedeno na 100 uzoraka zelenih žaba sa Skadarskog jezera, koji su analizirani upotrebom jezgrinog biljega (serum albumin intron-1 regija) i mitohondrijskog biljega (gen za NADH dehidrogenaza podjedinicu 3). Rezultati su pokazali prisutnost vrsta *Pelophylax kurtmuelleri* i *Pelophylax shqipericus*, te u najvećem broju, novog, do sad neopisanog hibridnog kompleksa. U ovom istraživanju nisu zabilježene vrste *Pelophylax ridibundus*, *Pelophylax lessonae* i njihov hibrid *Pelophylax* kl. *esculentus*, iako je njihova prisutnost bila moguća prema literaturnim navodima.

Ključne riječi: Balkanski poluotok, *P. kurtmuelleri*, *P. shqipericus*, serum albumin intron-1 regija, mitohondrijska regija ND3

DETERMINATION OF SPECIES AND HYBRIDS OF WATER FROGS (GENUS *Pelophylax*) FROM THE SKADAR LAKE (MONTENEGRO) USING MOLECULAR MARKERS

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Western Palearctic water frogs (genus *Pelophylax*) is extremely complex genus with approximately twelve species and three hybrid complexes. Today, water frogs are being intensively studied, primarily because of the presence of hybrid complexes with hemiclonal strategy of reproduction. Although, according to literature, the existence of hybrid complexes has not been proven on the Balkan Peninsula, preliminary research into the morphological characteristics of water frogs from the Skadar Lake (Montenegro) indicate that they do exist. Since the determination of species and hybrids of water frogs on the basis of morphological features is not reliable, the aim of this study was to analyse the water frogs from the Skadar Lake using molecular markers. The study was conducted on 100 samples of water frogs, which were analysed using nuclear marker (serum albumin intron-1) and mitochondrial marker (gene NADH dehydrogenase subunit 3). The results showed the presence of species *Pelophylax kurtmuelleri* and *Pelophylax shqipericus*, and mostly, a new, up to now undescribed hybrid complex. In this study, species *Pelophylax ridibundus*, *Pelophylax lessonae* and their hybrid *Pelophylax* kl. *esculentus* were not recorded, although their presence was possible according to the literature.

Key words: Balkan Peninsula, *P. kurtmuelleri*, *P. shqipericus*, serum albumin intron-1, mitochondrial region ND3

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THREATENING LEVELS AND EXTINCTION RISKS BASED ON DISTRIBUTIONAL, ECOLOGICAL AND LIFE-HISTORY DATASETS (DELH) VERSUS IUCN CRITERIA – EXAMPLE OF SERBIAN REPTILES

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Recent studies on the world reptiles' extinction risks pointed out that about 20% of species are threatened, with another 20% are data-deficient following IUCN criteria. IUCN set up criteria for assessment of extinction risk at global and regional scales, relying on distribution data, population parameters and quantitative analyses. Only 12% of reptilian species were listed as globally threatened upon population data. Long-term population studies are time-consuming, thus it would be practical to use some other parameters for estimations of extinction risks. Threatening level (and extinction risks) of Serbian reptiles have never been investigated. We aimed to scrutinize species threatening level (thus set up conservation priorities) on the basis of distributional, ecological and life-history datasets (DELH). We estimated which traits are the most important for the conservation of species. Separately, we used IUCN criteria for regional level to assess extinction risk and compared them with the results obtained by analyses of DELH datasets. The two types of criteria gave similar estimates for the chelonian species. In snakes, two species exhibited higher conservation scores by DELH than by IUCN criteria, the opposite being true for two other species. In lizards, only two species had higher extinction risks by IUCN whereas five lizards were more threatened according to DELH. Our intention was not discredit the values of IUCN criteria for setting regional (or national) extinction.

Key words: range, natural history, redlisting, threatened categories

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HEMIPENIAL MORPHOLOGY AS A USEFUL COMPLEMENTARY TOOL SUPPORTING THE FULL SPECIES STATUS OF CLOSELY RELATED SCINCID LIZARDS (SCINCIDAE, SAURIA, SQUAMATA)

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Despite the great species diversity of the scincid, few useful determinative morphological characteristics have been evaluated, related mainly to pholidotic and osteological characters. Hemipenial morphology has been widely used in squamate interspecific reptiles'taxonomy. The currently available data for about 3% of the total scincid diversity renders this topic widely unexplored. Here we present the first description of hemipenial

morphology of the closely related in phylogenetic sense *Ablepharus kitaibelii* and *A. budaki*. We adopted techniques developed for eversion and staining of endophallus of large moths instead of the classical techniques. The shape of the everted organs closely resembled the sphenomorphine skinks, being deeply bifurcated with relatively long branches. In both taxa terminal awls were observed and no calcified but minute keratin microscopic structures were present with varying density from the top to the base of the organ. Besides its generally more elongated shape and branches, unique leaf-shaped folds named auriculi were also observed on both sides of the body in *A. budaki*. The differences between the hemipenes of both taxawere substantial and revealed that they differed by more essential specific morphological characters, than those mentioned in already published sources. Results could serve as a complementary tool in support to their full species status.

Key words: *Ablepharus*, hemipenis, interspecific differentiation, folds

O-66

EX-SITU DRŽANJE AUTOHTONIH VODOZEMACA I GMAZOVA U ZOOLOŠKOM VRTU GRADA ZAGREBA U SVRHU EDUKACIJE, ZAŠTITE I ISTRAŽIVANJA

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Ex-situ držanje životinja omogućava prikupljanje velikog broja podataka koje je vrlo teško prikupiti u prirodnom staništu. Zoološki vrtovi imaju veliko iskustvo u držanju i brizi velikog broja raznovrsnih vrsta životinja. Također, često imaju raspoloživ prostor, dostupnost raznovrsne prehrane potrebne životinjama te stalan nadzor i skrb za životinje. Držane životinje mogu se koristititi za brojna biološka i veterinarska istraživanja, te za edukaciju javnosti direktnim putem ili implementacijom u edukativne programe za djecu. Zoološki vrt grada Zagreba tijekom posljednjih godina sudjelovao je u držanju i istraživanju velikog broja autohtonih vodozemaca i gmazova kao što su planinski žutokrug (*Vipera ursinii macrops*), riđovka (*Vipera berus*), riječna kornjača (*Mauremys rivulata*), čovječja ribica (*Proteus anguinus*), ivanjski rovaš (*Ablepharus kitaibelii*) i mnogi drugi. Spomenuta istraživanja odnosila su se primarno na životni ciklus, reproduktivnu biologiju, inkubaciju jaja, morfometriju mladunaca i njihovu prehranu kao i na parazitološka i mikrobiološka istraživanja. Prikupljanjem podataka o životnom ciklusu, prehrani i razmnožavanju autohtonih vodozemaca i gmazova moguće je poboljšati mjere zaštite u prirodi te pritom educirati veliki broj javnosti. U slučaju potrebe, pomoću prikupljenih saznanja, moguće je osnovati i populacije kritično ugroženih vrsta za reintrodukciju u budućnosti.

Ključne riječi: vodozemci, gmazovi, zaštita, edukacija, istraživanje

EX-SITU KEEPING OF NATIVE AMPHIBIANS AND REPTILES IN ZAGREB ZOO FOR EDUCATION, CONSERVATION AND RESEARCH PURPOSES

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Ex-situ animal keeping allows collecting high number of data that is difficult to collect in nature. Zoological gardens have a lot of knowledge in keeping variety of animal species and their husbandry. Also, they have space available, proper food and constant surveillance of animals. Animals in captivity can be used for different kind of biological and veterinary research, and also for education of public directly, or by implementing certain species in education programs for kids. During last years, Zagreb Zoo has been taking part in keeping and research of many native amphibian and reptile species, like Karst viper (*Vipera ursinii macrops*), Adder (*Vipera berus*), Olm (*Proteus anguinus*), Balkan Terrapin (*Mauremys rivulata*), Snake-eyed skink (*Ablepharus kitaibelii*) and many other species. Research is focused mostly on life cycle, reproductive biology, egg incubation, morphometry of young animals and their nutrition, but also on parasitology and microbiology research. Collecting data about life cycle, nutrition and reproduction of native amphibian and reptile species allows more efficient conservation measurements as well as raising of public awareness. If needed, gained knowledge might be used for establishing populations of critically endangered native species for future reintroduction.

Key words: amphibians, reptiles, conservation, education, research

O-67

"I WAS A NINJA AT YOUR AGE" SAID AN ELDERLY TORTOISE TO A HATCHLING – ONTOGENY SHIFT IN AGILITY

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Tortoise's rigid shells protect soft body parts, but also disable flexion of torso, thus negatively affect their agility. For example, tortoises have problems overcoming inclined obstacles, and self-righting when overturned on their dorsum. Juvenile tortoises are surrounded with relatively larger obstacles than adults from the same habitat, thus are more challenged during everyday movements. To explore change in agility during ontogeny I did two tests on wild running Hermann's tortoises (*Testudo hermanni*). First experiment (149 examinees) explored how fast tortoises can overcome 50 cm high negative steep step. I compared juveniles and adults from two localities. Although adults needed longer time to start moving, they crossed the obstacle far quicker than juveniles at both localities. Second experiment (338 examinees) tested differences in self-righting behavior between juveniles and adults from three populations. Again juveniles started moving after shorter period of time, but also overturned quicker and were more successful to finish the task. Both experiments demonstrated fast reaction in juvenile tortoises. This could be result of predator avoidance response. Soft, barely ossified shells of juveniles do not offer efficient protection from wide range of predators. Secretive lifestyle could be their main anti-predator response, thus prompt reaction and efficient moving from conspicuous place could have a vital importance for young tortoises.

Key words: Tortoise, Agility, Behaviour, Ontogeny

O-68

COMPARATIVE MORPHOLOGY OF *Salamandra atra prenjensis* (BOSNIA AND HERZEGOVINA) WITH ITS NOMINOTYPIC SUBSPECIES *Salamandra atra atra* (AUSTRIA)

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Mikšić (1969) described the subspecies *Salamandra atra prenjensis*, endemic to Dinarides. The subspecies status has been debated. Because of discontinuous distribution from nominal subspecies (*Salamandra atra atra*) and different ecosystems they inhabit, this study aimed to detect developmental changes in form and covariance between ecological factors and shape, by using two subpopulations in Austria (Austrian population) and two subpopulations in Bosnia and Herzegovina (B&H population). 14 quantitative characteristics were measured for 60 adult individuals from Austria and 195 adult individuals from B&H. In comparison Austrian *vs* B&H population, significant difference between all characteristics besides Body mass, TL, SL and Distance between legs is registered. Same results appear when comparing intrasex variation between populations from different countries, where Head length differed only between females and body mass only between males. Most significant intersex difference in general was found in length of front and hind legs, which is closely related to the copulation act phases. Austrian population expresses significant intersex differences in all characteristics besides Nb. of costal grooves, TL, SL, Tail length, Head and Jaw length. Females within Bosnian population significantly differ from males in characteristics related to size of feet, hands and limbs. Analyzes were performed using ANOVA. Results contributed in deducing conclusions on function and evolutionary relationships between these two subspecies.

Key words: Comparative evolution, morphometrics, amphibians

O-69

APPLICATION OF MICRO CT SCANNING AND 3D GEOMETRIC MORPHOMETRICS IN EXPLORING EVOLUTION OF SKULL SHAPE IN *Triturus* NEWTS (CAUDATA: SALAMANDRIDAE)

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Application of Micro CT scanning and 3D geometric morphometrics in exploring evolution of skull shape in *Triturus* newts (Caudata: Salamandridae). The development of geometric morphometrics, CT scanning technique and reconstruction of 3D models of an organ or entire organism open a new arena for the studies of evolution of morphological structures. Here, we apply micro CT scanning to obtain detailed information on skull shape and to explore evolutionary changes in skull shape of eight species of *Triturus* newts. Their well-studied phylogenetic relationships and the marked difference in ecological preferences among five species groups makes this genus an excellent model system for the study of morphological evolution. The reconstruction and visualization of character changes during evolution and the

distribution of the species in the morphological space indicate that evolutionary changes in skull shape were related to evolutionary changes in skull size (evolutionary allometry). Also, in this group, changes in skull shape appear to be highly correlated to the body elongation, and changes in the number of vertebrae.

Key words: microtomography, geometric morphometrics, cranium, allometry

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DATA ABOUT STRIPED-NECKED TERRAPIN, *Mauremys rivulata* IN VLORA BAY, ALBANIA

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This study aims to provide information about morphometrics, population structure and biology of the Striped-necked Terrapin, *Mauremys rivulata* in the area of Vlora Bay, Albania. Since the studies and publications in Albania are very scarce, this study has a highly importance in understanding the bioecology of this species. Two main different habitats, freshwater channles (several channels in different areas) and ponds or swamps (two ponds in different areas) were monitored from April 2013 to May 2015. A high number of *Mauremys rivulata* were observed in the water or out of the water (basking) in all different habitats, where 27 were captured for the first time and 1 was captured for the second time (recapture). The largest number of individuals of *M. rivulata* were observed in Zvernec pond [40^{0}30'39.19''N 19^{0}24'27.43''E] and in freshwater channel of Panaja [40^{0}34'46.86''N 19^{0}28'20.40''E], while very few individuals were observed in Orikumi pond [40^{0}19'26.87''N 19^{0}27'09.56''E]. All captured individuals were studied using different methodologies. Curved Carapace Length (CCL), Curved Carapace Width (CCW), Plastron Length (PL), Plastron width (PW) and body size were measured for each individual of *Mauremys rivulata* and were statistically analyzed. Each adult individual of *Mauremys rivulata* was marked by notching the marginal scutes of carapace. Sex was determined by secondary sexual characteristics (e.g., plastral concavity, length of tail) and resulted that 14.8% were males, 33.4% females and 51.8% juveniles. The presence of *Mauremys rivulata*in the area of Vlora Bay is an important finding, because it is considered as a rare species in Albania.

Key words: Albania, female, juvenile, habitat, male, *Mauremys rivulata*, Vlora Bay

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MODELING THE POTENTIAL DISTRIBUTION OF THE SPUR-THIGHED TORTOISE (*Testudo graeca*) AND THE HERMANN'S TORTOISE (*T. hermanni*) AT THE NORTHERN LIMIT OF THEIR RANGE IN ROMANIA

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The two species of tortoises reach the limit of their northernmost range in Romania and we tested if their ranges were restricted by geographic or climatic barriers. Our study focused on the entire range of the two tortoises in Romania. We ran the Maximum Entropy algorithm (MaxEnt) to model the current potential distribution using presence-only occurrence records (1321 records for *T. graeca* and 767 for *T. hermanni*) and a selection of high-resolution climatic surfaces derived from the national network of weather stations in Romania. *T. graeca* is widespread in Dobrogea region except from the Danube Delta due either to the barrier represented by the river or to the high mortality induced by the periodic floods. *T. hermanni* has a narrow range limited to the southernmost areas of Romania and seems to be mostly affected by precipitation of the driest month and minimum temperatures of coldest month. The Spur-thighed tortoise is clearly limited geographically by the Danube river since there is no occurrence outside Dobrogea region, and the Hermann's tortoise range seems limited by extreme temperature values during the winter.

Key words: *Testudo*, *graeca*, *hermanni*, MaxEnt, species distribution modeling

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DETECTION OF *Proteus* eDNA IN KARST GROUNDWATER

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Due to inaccessible subterranean habitat of *Proteus anguinus*, its presence can only rarely be confirmed by classical survey methods such as trapping and visual encounters. To overcome this problem, we employed environmental DNA (eDNA) method using a SYBR chemistry-based real-time PCR assay with two sets of specific primers to amplify short mitochondrial DNA sequences in the 16S rDNA gene (153 bp) and in the control region (106 bp). The specificity of the assay was previously tested on trout, crested newt and human DNA. In controlled conditions at the Tular Cave Laboratory the minimum density of *Proteus* at which its DNA could still be detected corresponded to one animal per 256 m^3 of standing water, when sampling 20 L of water. The method, tested at three Slovenian field test sites occupied by different lineages of *Proteus* (the springs of Vir pri Stični and Mahniči, and the cave Kompoljska jama), was 100% effective. Subsequently, a pilot survey of *Proteus* distribution was conducted along the southern limit of its known range in Herzegovina and Montenegro. Using DNA-based identification we unequivocally established the presence of *Proteus* at four sites, and found its likely traces at additional eight sites – all new localities for this species. By applying eDNA method to *Proteus*, we developed innovative non-invasive approach for reliable and efficient monitoring of the species and possibly for other aquatic cave-dwelling organisms.

Key words: environmental DNA, real-time PCR, distribution, non-invasive sampling, *Proteus*

O-73
ZNAČAJKE POPULACIJE RIJEČNE KORNJAČE, *Mauremys rivulata* IZ KONAVOSKOG POLJA

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Na području Hrvatske obitavaju dvije vrste slatkovodnih kornjača, riječna kornjača (*Mauremys rivulata*) i barska kornjača (*Emys orbicularis*). Za razliku od barske kornjače koja naseljava cijelu državu, riječna kornjača je prisutna samo na krajnjem jugu Hrvatske, što predstavlja rubni dio areala ove vrste. U Hrvatskoj dolazi samo u tri male, geografski izolirane populacije te spada u najugroženije vrste gmazova u Hrvatskoj. U svrhu prikupljanja osnovnih podataka o ekologiji i populacijskoj biologiji riječne kornjače, tijekom 2013. godine započeta su istraživanja na populaciji riječne kornjače u Konavoskome polju. Istraživanja su se provodila na poligonu veličine 2000x500 m metodom ulovi/označi/ponovno ulovi. Po prvi puta je ocjenjena veličina, gustoća te dobna i spolna struktura konavoske populacije. Na nekoliko dijelova kanala koje ova vrsta naseljava, opaženi su u potpunosti betonirani i kanalizirani dijelovi vodotoka, što predstavlja značajnu prijetnju riječnoj kornjači, ali i drugim vrstama vodozemaca i gmazova koji kanale koriste kao privremeno ili stalno stanište. Prikupljeni podatci o populacijskoj biologiji i ekologiji značajno će pridonijeti poznavanju riječne kornjače u Hrvatskoj te dugoročno osigurati njen opstanak i zaštitu.

Ključne riječi: Konavle, populacijska istraživanja, slatkovodna kornjača

CHARACTERISTICS OF THE BALKAN TERRAPIN POPULATION FROM KONAVLE FIELD

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The territory of Croatia is inhabited by two species of freshwater turtles: the Balkan Terrapin (*Mauremys rivulata*) and the European Pond Turtle (*Emys orbicularis*). Unlike the European Pond Turtle, which is widespread throughout the whole country, the Balkan Terrapin is present only in the far south which also represents the southwestern edge of the species range. In Croatia it occurs in three, small geographically isolated populations and is one of the most endangered reptile species in the country. During 2013 we started the population studies on the Balkan Terrapin in the area of Konavle field in order to collect basic information about ecology and population biology of the Balkan Terrapin. Studies were carried out on a 2000 by 500 m polygon field using capture/mark/recapture method. Population size and density, age and sex structure of the population from the Konavle field was estimated for the first time. Some parts of the water channel, used by the Balkan Terrapin, were recently concreted, and thus represents significant threat not just to the Balkan Terrapin but also to all other amphibian and reptile species which are using channels as permanent or temporary habitats. Data collected on population biology and ecology will significantly contribute to the knowledge of the Balkan Terrapin in Croatia and will ensure its long-term existence and protection.

Keywords: Konavle, population studies, freshwater turtle

Biologija mora
Marine Biology

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TRENUTNI STATUS BIORAZNOLIKOSTI IHTIOFAUNE JADRANSKOGA I SREDOZEMNOGA MORA

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Bioraznolikost morskih organizama u Sredozemnom moru danas je suočena s značajnim strukturnim promjenama u flori i fauni. Iste promjene su zabilježene i u Jadranskom moru. Tijekom posljednjih nekoliko desetljeća, različiti čimbenici, uključujući klimatske promjene, antropogeno djelovanje i lesepsijske migracije su promijenili sastav jadranske ihtiofaune. Te promjene se ogledaju i u broju vrsta navedenih u popisima jadranskih riba, od 407 vrsta u 1996. do 440 u 2010. godini. Od 2010., 12 novih vrsta riba je zabilježeno za Jadransko more, podižući sada broj na 452 vrsta i 139 obitelji. Od 14 lesepsijskih migranata koji su zabilježeni Jadranu, *Fistularia commersonii* i *Siganus luridus* su se pokazali uspješnim u uspostavljanju svojih populacija i to posebice u južnom dijelu Jadranskoga mora. Prema različitim popisima broj vrsta riba zabilježenih za Sredozemno more u posljednjih 14 godina kretao se od 648 do 664. Do 2014. godine ukupno je zabilježeno 151 alohtona vrsta za cijelo Sredozemno more (u posljednjih 5 godina čak 34 vrste, od 2000. godine najmanje 36 vrsta samo iz Crvenog mora). Značajan porast lesepsijskih migranata se jednim dijelom povezuje sa proširenjem i produbljenjem Sueskog kanala. Neke od njih su vrlo brzo uspostavile svoje populacije u Sredozemnom moru. Neke vrste imaju i izražen pozitivan gospodarski značaj dok druge vrste pokazuju negativan učinak na morsko ribarstvo i bioraznolikost.

Ključne riječi: Sredozemno more, Jadransko more, promjene, ribe, bioraznolikost

THE CURRENT STATUS OF THE ADRIATIC AND MEDITERRANEAN FISH BIODIVERSITY

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The marine biodiversity of the Mediterranean Sea is nowadays facing to substantial structural changes in flora and fauna. Such changes were recorded in the Adriatic Sea, as well. During the last few decades, various factors including climate change, anthropogenic activity and lessepsian migration have altered the composition of Adriatic ichthyofauna. These changes are reflected in the number of species quoted in the checklist of Adriatic fishes, from 407 in 1996 to 440 in 2010. Since 2010, 12 new species were recorded, which raised the number to 452 species and 139 families. Of the 14 Lessepsian migrants that were recorded in the Adriatic, *Fistularia commersonii* and *Siganus luridus* proved to be successful invaders for its southern part. Several checklists for the Mediterranean Sea in the last 14 years quoted the number of Mediterranean fish species from 648 to 664 species. Until to 2014, 151 of non-indigenous fish species were observed in the Mediterranean Sea (34 in the last 5 years, at least 36 species only from the Red Sea since 2000). A significant increase of Lessepsian migrants is partly associated with the expansion and deepening of the Suez Canal. Some of them established populations very quickly in the Mediterranean and some have positive economic significance and some species exhibit negative impacts on marine fisheries and biodiversity.

Key words: Mediterranean Sea, Adriatic Sea, changes, fish, biodiversity

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MIKROZOOPLANKTON BOKOKOTORSKOG ZALJEVA I OBALNOG PODRUČJA JUŽNOG JADRANA

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Mikrozooplankton je važna komponenta "mikrobnog kruga" i ukupne hranidbene mreže mora. Sastav, horizontalna i vertikalna raspodjela brojnosti mikrozooplanktona istraživani su Niskinovim crpcem (5 L) tijekom proljeća 2013. godine na tri postaje unutar Bokokotorskog zaljeva (Crna Gora) te na postaji Molunat (Hrvatska). Nelorikatni trepetljikaši su bili dominantna skupina mikrozooplanktona (37%) s najvišim vrijednostima u površinskom sloju gdje je utjecaj slatke vode bio jače izražen. Ukupna brojnost tintinida bila je niska i slična onim zabilježenim na postajama otvorenog Jadrana. Velike raznolikosti vrsta tintinida nađene su na svim postajama s najvećim indeksom raznolikosti u Tivatskom zaljevu zbog jakog utjecaja slatke vode u površinskom sloju te voda otvorenog mora u pridnenom sloju. Ukupno je zabilježeno 46 vrsta tintinida od kojih je 20 estuarijsko-obalnih te 26 vrsta otvorenog mora. U odnosu na malobrojna prethodna istraživanja 23 vrste tintinida po prvi put su zabilježene za Bokokotorski zaljev. Vrsta *Stenosemella nivalis* je dominirala na svim istraživanim postajama. Naupliji veslonožaca bili su brojna skupina mikrozooplanktona s udjelom od 32%, a znatan udio imali su zajedno kopepoditi i mali odrasli veslonošci (17%). Međutim, brojnosti populacija nauplija u Bokokotorskom zaljevu su bile manje od glavnine poznatih za zaljeve istočne obale u proljeće. Ovi rezultati mogu se posljedično povezati sa zabilježenim klimatskim promjenama utvrđenim za Jadran od 1990.-tih godina gdje već u zimskom razdoblju dolazi do povećane primarne produkcije, a izostaje proljetni maksimum.

Ključne riječi: nelorikatni trepetljikaši, tintinidi, naupliji, kopepoditi, južni Jadran

MICROZOOPLANKTON IN THE BOKA KOTORSKA BAY AND IN THE COASTAL REGION OF THE SOUTH ADRIATIC SEA

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Microzooplankton is important component of "microbial loop" and total marine food web. The horizontal and vertical distributions of microzooplankton were studied during spring 2013 by Niskin 5-L bottle at three stations in Boka Kotorska Bay (Montenegro) and a single station off Molunat Bay (Croatia). Non-loricate ciliates were the most numerous microzooplankton (37%), with the highest values at the surface layer that was strongly influenced by the fresh water. Tintinnids abundance were low, and similar to those recorded in the open Adriatic Sea. Tintinnids species diversity was considerably high. The highest diversity was at the station in the Tivat arm of the Boka Kotorska Bay system due to specific environmental conditions caused by the strong influence of fresh water in the surface and the open sea water in the bottom layer. In total, 46 tintinnids were identified, among which 20 were estuarine-neritic and 26 were characteristic for the open sea. Compared to few previous researches, 23 species were recorded for the first time

in Boka Kotorska Bay. *Stenosemella nivalis* was the dominant tintinnid at all stations. Copepod nauplii were numerous and contributed with 32% in total microzooplankton abundance, followed by copepodites and small adult copepods (17%). However, nauplii densities were lower than those known from the majority of coastal Adriatic region. These results can be linked with the observed climate changes identified in the Adriatic Sea from the 1990s, where already in the winter period occurred increase of primary production with the lack of a spring peak.

Key words: non-loricate ciliates, tintinnids, nauplii, copepodites, Southern Adriatic

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TAKSONOMIJA I PROSTORNA RASPODJELA FITOPLANKTONA U JUŽNOM JADRANU; ZIMSKI ASPEKT

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U svrhu istraživanja specifičnih obrazaca zimske termohaline cirkulacije i raspodjele planktona u južnom Jadranu u sklopu HRZZ projekta BIOTA, provedeno je oceanografsko istraživanje u razdoblju od 28. veljače do 3. ožujka 2015. Istraživanje je obuhvatilo 15 postaja smještenih duž dva transekta: (i) od Dubrovnika do izobate od 1000 m (u smjeru 210º) (P100 – P1000), te (ii) od izobate od 1000 m do otoka Lastova u Južnojadranskoj kotlini (M100 – M1000). Pomoću inverznog svjetlosnog mikroskopa ukupno je analizirano 117 uzoraka i određeni su sastav i brojnost fitoplanktonskih vrsta. Maksimalna brojnost mikrofitoplanktona (1,97 x 10^4 stanica/L) te nanofitoplanktona (4.40 x 10^4 stanica/L) zabilježena je u površinskim uzorcima na postaji P100 i P120. Mikrofitoplanktonom su dominirale dijatomeje, a njihova neobično velika brojnost (<2 x 10^4 stanica/L) zabilježena je i u dubokomorskim uzorcima (>300 m dubine). Taksonomskom analizom određeno je ukupno 146 fitoplanktonskih svojti, od kojih dominiraju dijatomeje sa 110 morfotipova. Dominantne vrste dijatomeja bile su *Asterionelopsis glacialis*, *Pseudo-nitzschia* sp., *Bacteriastrum furcatum* te vrste roda *Chaetoceros*. Nadalje, preliminarnim analizama određen je veliki broj neidentificiranih planktonskih penatnih dijatomeja (34 morfotipa) za čiju su precizniju taksonomsku identifikaciju potrebne dodatne analize transmisijskim i skenirajućim elektronskim mikroskopom.

Ključne riječi: južni Jadran, fitoplankton, dijatomeje

TAXONOMY AND SPATIAL DISTRIBUTION OF PHYTOPLANKTON IN THE SOUTH ADRIATIC; WINTER ASPECT

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A total of 117 water samples were analysed and phytoplankton composition and abundance was determined using an inverted light microscope. Maximal abundance of microphytoplankton (1.97 x 10^4 cells/L) and nanophytoplankton (4.40 x 10^4 cells/L) was detected in surface samples at P100 and P120 stations, respectively. Diatoms were the dominant microphytoplankton group, with peculiarly large abundances (< 2 x 10^4 cells/L)

detected in deep water samples (> 300 m). Via taxonomic analysis 146 phytoplankton taxa were determined, out of which diatoms dominated with 110 morphotypes. The dominant diatoms were *Asterionelopsis glacialis*, *Pseudo-nitzschia* sp., *Bacteriastrum furcatum* and species belonging to the genus *Chaetocero*s. Further, preliminary analyses showed large number of unidentified planktonic pennate diatoms (34 morphotypes) and for their more accurate taxonomic iden tification additional analyses with transmission and scanning electron microscope are essential.

Key words: southern Adriatic, phytoplankton, diatoms

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USKLAĐENO RAZMNOŽAVANJE ALGI RODA *Ulva*

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Zelene alge roda *Ulva* s preko 130 danas poznatih svojti, jedne su od najuobičajenijih algi plitkog morskoga dna. O njihovoj biologiji i ekologiji uključujući i razmnožavanje, objavljeni su brojni radovi. Većina istraživanja razmnožavanja obavljena je u laboratorijskim uvjetima. Izuzev malog broj usputnih terenskih opažanja, ne postoje radovi o sustavnom istraživanju fenologije spolnog razmnožavanja koje je provedeno u prirodi. Tijekom proljetnih razdoblja od 2012. do 2015., na području Splita obavljena su terenska istraživanja razmnožavanja algi roda *Ulva*. Utvrđeno je kako se ispuštanje gameta uklađuje s izlaskom sunca. Unutar nekoliko minuta sve fertilne alge ispuštaju gamete pri čemu se razvija lako uočljiv gametni oblak. Gametni oblak može biti toliko intenzivan da smanji proziranost mora gotovo na nulu. Ovisno o gustoći naselja algi i uvjetima u moru, gametni oblak ostaje vidljiv i duže od jednog sata. Sporofiti s druge strane nemaju usklađeno ispuštanje spora. Zoospore se vjerojatno ispuštaju polako tijekom cijeloga jutra. Naglo ispuštanje zoospora može biti potaknuto značajnim promjenama ekoloških uvjeta kao što je uronjavanje alge nakon isušivanja za vrijeme oseke. Usklađeno ispuštanje gameta kod sesilnih morskih organizama potrebno je da bi se osigurala uspješna oplodnja. Premda je bila očekivano i kod morskih salata, masovnost i vidljivost događaja više je nego iznenađujuća i pokazuje koliko je naše znanje o uobičajnim vrstama još uvijek nepotpuno.

Ključne riječi: *Ulva*, alge, razmnožavanje

SYNCHRONIZED REPRODUCTION OF *Ulva* SPECIES

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The green algae of genus *Ulva*, with about 130 accepted taxa, are among the most common algal groups of shallow marine bottom. There are many articles published on their biology and ecology, including reproduction. The majority of the research on reproduction were conducted in laboratory but there are only a few publications on phenology of reproduction in the field. During spring time in the area of Split from 2012 till 2015, we have found that population of alge *Ulva* released gametes just within the sunrise. All fertile algae released gametes within a few minutes. That leads to development of the visible green dense gamete cloud can which reduce the visibility in the sea. Depending on the algal density and sea state condition, gamete

cloud can persist even over an hour. On the other hand, release of zoospores is not synchronized. They are probably released slowly during entire morning. Rapid release of zoospores can be provoked by significant changes in environmental conditions, like immersing after desiccation due to low tide. Synchronized release of gametes in sessile species is essential to ensure successful fertilization. Although the synchronized release was also predicted in sea lettuces, quantity and visibility of the events is far more than expected. That also indicates how our knowledge on the common species is still incomplete.

Key words: *Ulva*, algae, reproduction

O-78
NOVONASTALE PROMJENE FAUNE HIDROMEDUZA SJEVERNOG JADRANA

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Hidromeduze su među prvim planktonskim vrstama opisanim u sjevernom Jadranu početkom 20. stoljeća. Ekološke promjene (eutrofikacija) i smanjenje otopljenog kisika uz dno od 1950.-tih pa nadalje, uspostavili su nove ekološke uvjete u tom akvatoriju. Te promjene uzrokovale su znatno smanjenje biološke raznolikosti bentosa, uključivši meroplanktonske meduze. U ovom radu prikazani su rezultati istraživanja sastava i brojnosti faune hidromeduza sjevernog Jadrana, imajući u vidu njihovu moguću repopulaciju zadnjih godina. Uzorci planktona su sakupljeni mjesečno vertikalnim potezima od dna do površine s WP2 Nansenovom mrežom na četiri postaje. Predstavljeni rezultati obuhvatila su tri vremenska razdoblja: 1999.-2002., 2003.-2007. i 2009.-2013. Rezultati jasno ukazuju na porast broja vrsta i njihove brojnosti od prvog razdoblja istraživanja prema zadnjem (2009.-2013.). Pretpostavljamo da su neke od tih vrsta unesene ulaznim strujama u sjeverni Jadran, međutim, njihova brojnost je višestruko veća u usporedbi s prijašnjim vrijednostima. Zabilježene visoke prosječne i maksimalne vrijednosti za pojedine metagenetske vrste ukazuju ponovnu mogućnost uspostave stabilnih populacija. Ove promjene su u skladu s novonastalim stanjem sastava i brojnosti cjelokupnog planktona sjevernog Jadrana uzrokovanih klimatskim promjenama i posljedičnom oligotrofikacijom istraživanog područja.

Ključne riječi: biodiverzitet, planktonski žarnjaci, klimatske promjene, sjeverni Jadran

RECENT CHANGES IN THE NORTHERN ADRIATIC HYDROMEDUSAN FAUNA

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Hydromedusae were among the first described plankton species of the northern Adriatic at the beginning of 20th century. Ecological changes (eutrofication) and low oxygen saturation near bottom in this area demonstrated new established trend from 1950s to forward. Such conditions consequently caused a significant reduction of biodiversity of benthic organisms, including meroplanktonic hydrozoans. In this study we present the comparative results of the composition and abundance of hydromedusan fauna of northern Adriatic, pointing to the possibility of species repopulating recent years. Plankton was sampled monthly by bottom-to-

surface vertical tows with WP2 Nansen mesh plankton net at four stations of the northern Adriatic. Presented data are results of investigations during three study periods: 1999-2002, 2003-2007 and 2009-2013. Our results clearly showed that the n umber of species and their abundance considerable increased from the first to the third investigated period. We assume that some species were introduced by input currents in the northern Adriatic, but their abundances were several times higher than in previous records. Our high mean and maximum values of this metagenetic species could indicate the possibility of establishing stable populations in the area. These changes are consistent with the recent changes in the plankton composition and abundance, probably caused by a climatic forcing and recognized oligotrophication of the entire northern Adriatic.

Key words: biodiversity, planktonic cnidaria, climatic change, North Adriatic Sea

O-79

VREMENSKA DINAMIKA ZAJEDNICA KALANOIDNIH KOPEPODA U IZOLIRANOM MORSKOM JEZERU (VELIKO JEZERO, JUŽNI JADRAN): VEZE S KLIMATSKIM PROMJENAMA NA VEĆOJ SKALI

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Dugoročne podatke zajednica kalanoidnih kopepoda (1952.-2009.) u poluzatvorenom morskom jezeru "Veliko jezero" na otoku Mljetu (Južni Jadran) usporedili smo s varijacijama u okolišu. Promjene u hidrografskim svojstvima pod utjecajem su produbljivanja i proširenja kanala između Velikog jezera i otvorenog mora ranih 1960-ih, što se najviše odrazilo na povećanje saliniteta. Gustoća kalanoida odlikuje se visokom unutar- i među- godišnjom fluktuacijom. Kalanoide u jezeru sačinjavaju 22 obalne i estuarijske vrste, od kojih je njih pet bilo prisutno tijekom cijelog razdoblja istraživanja: *Calanus helgolandicus*, *Paracalanus parvus*, *Centropages kroyeri*, *Isias clavipes*, *Acartia* (*Acartiura*) *clausi*. Uočene promjene kao što su niske gustoće ranih 1980-ih, pomak u dominantnim vrstama, smanjenje i, konačno potpuni nestanak vrste *Pseudocalanus elongatus*, povećanje gustoće vrsta *Paracalanus parvus* i *Diaixis pygmaea*, mogu biti povezane sa sinkroniziranim promjenama kroz trofičke razine koje su se dogodile u drugim europskim morima. Naši rezultati naglašavaju važnost dugoročnih praćenja promjena u poluzatvorenim morskim jezerima i lagunama, kao sustava koji su izrazito osjetljivi na globalne klimatske promjene.

Ključne riječi: kalanoidni kopepodi, dugoročna dinamika, Jadransko more, morsko jezero

TEMPORAL PATTERNS OF THE CALANOID COPEPOD COMMUNITY IN VELIKO JEZERO, AN ISOLATED MARINE LAKE (SOUTH ADRIATIC SEA): LINKS TO A LARGER-SCALE CLIMATE CHANGES

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We complied and analysed a long-term calanoid community data set (1952-2009) with respect to environmental variations in nearly closed seawater lake Veliko jezero (South Adriatic Sea). Changes in hydrographic properties were influenced by deepening and broadening the channel between Veliko Jezero and the sea in the early 1960s, which was in particular reflected by

increasing salinity. Calanoid abundance displayed high intra- and inter-annual fluctuations. Calanoids in the lake comprised 22 coastal and estuarine species, with five of them present over the entire study period (*Calanus helgolandicus*, *Paracalanus parvus*, *Centropages kroyeri*, *Isias clavipes*, *Acartia* (*Acartiura*) *clausi*). Observed changes - low densities in early 1980s; a shift in the dominant species; reduction and, finally, disappearance of *Pseudocalanus elongatus*; increases in *Paracalanus parvus* and *Diaixis pygmaea* - can be associated with synchronous changes across trophic levels that have occurred in other European seas. The results presented here underline the importance of long-term studies of semi-closed marine lakes and lagoons, as these are particularly sensitive to global climatic changes.

Key words: calanoid copepods, long-term dynamics, Adriatic sea, seawater lake

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UTJECAJ STRATEGIJE HRANJENJA NA DRUŠTVENU STRUKTURU DOBRIH DUPINA (*Tursiops truncatus*) U ISTOČNOM JADRANU, HRVATSKA

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Ranije studije opisuju utjecaj koćarenja ili marikulture na veličinu i sastav skupina dobrog dupina (*Tursiops truncatus*), no do sada nije promatran kombinirani utjecaj. Tijekom 2013. i 2014. provedeno je istraživanje u tri područja Jadrana: cresko-lošinjski arhipelag, sjeverna Dalmacija (Zadarska i Šibensko-kninska županija) te viški arhipelag. Cilj istraživanja bio je utvrditi kombinirani utjecaj koćarenja i marikulture na veličinu, društveni i dobni sastav skupina u tri područja koja se razlikuju u intenzitetu koćarenja i marikulture. Ukupno je zabilježeno 176 opažanja grupa koje se hrane na otvorenom moru (OM), 59 grupa pri lovu iza koćarice (K) te 8 grupa pri lovu na ribljim farmama (RF). Prosječna veličina skupina bila je (5±0,56), najviša u cresko-lošinjskom (5,81±0,43), najniža u viškom arhipelagu (3,6 ±0.73), no nije se znatno razlikovala među OM, RF i K skupinama (K=0,138, df=2, p>0,05). Ukupan stupanj asocijacije jedinki bio je nizak (0,1±0,1), no viši za RF (0,07 ±0,03) i K (0,07±0,03), nego OM skupine (0,02±0,01). Hijerarhijska cluster analiza pokazala je visoko strukturiranu ukupnu populaciju (CCC=0,91), a prisutnost koćarica i ribljih farmi ima veći utjecaj na sastav skupina u sjevernoj Dalmaciji i viškom arhipelagu nego cresko-lošinjskom arhipelagu. Analiza dobnog sastava skupina pokazuje veću zastupljenost odraslih jedinki u T i RF, nego u OM skupinama (χ^2 = 9,21, p<0,001). Ovi rezultati pružaju temelj za daljnja istraživanja i osmišljavanje mjera zaštite.

Ključne riječi: dobri dupin, *Tursiops truncatus*, socijalna ekologija, ribarstvo, zaštita

INFLUENCE OF FORAGING STRATEGIES ON SOCIAL STRUCTURE OF BOTTLENOSE DOLPHINS (*Tursiops truncatus*) IN EASTERN ADRIATIC SEA, CROATIA

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Previous studies have shown that either bottom trawlers or fish farms influence bottlenose dolphin (*Tursiops truncatus*) group size and structure, however no study described combined effect. In 2013 and 2014 a photo-ID based study was conducted in three regions of the Adriatic sea: Cres-Lošinj, North Dalmatia and Vis. The aim was to examine combined effect of bottom trawlers and fish farms upon group size, social and age-class structure of bottlenose dolphin communities in the three regions that differ in intensity of bottom trawler and aquaculture fisheries. Research effort resulted in sightings of 176 groups engaged in "open water foraging" (OWF), 59 in "bottom-trawler foraging" (BTF) and 8 in "foraging at fish farm" (FFF). Overall group size was (5±0.56), being highest in Cres-Lošinj (5.81±0.43) and lowest in Vis region (3.6±0.73), but it did not vary significantly between the foraging strategies (K=0.138, df=2, p>0.05). Simple-ratio association index was overall low (0.1±0.1), but higher for FFF (0.07 ±0.03) and BTF (0.04 ±0.02) than OWF groups (0.02±0.01). Hierarchial cluster analyses showed overall highly structured population (CCC=0.91), structure being more affected by presence of bottom trawlers and fish farms in North Dalmatia and Vis than in Cres-Lošinj region. Age composition of groups varied, with more adults in BTF and FFF than in OWF groups (χ^2=9.21, p<0.001). These results provide baseline information for further research and conservation plans.

Key words: bottlenose dolphin, *Tursiops truncatus*, social ecology, fisheries, conservation

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PRILOG POZNAVANJU EKOLOGIJE SPUŽVE *Suberites domuncula* (Olivi, 1872): PREKO MORFOLOGIJE DO ANALIZE POPULACIJE

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Vrsta *Suberites domuncula* (Olivi, 1792) (Porifera, Demospongiae, Tetractinomorpha, Hadromerida, Suberitidae) izvorno je opisana u Jadranu, a kasnije je zabilježena širom svijeta. Smatralo se da je njena globalna raširenost artefakt, te da je *S. domuncula* u stvari kompleks sličnih, ali različitih vrsta spužava. Primjerci *S. domuncula* sakupljeni su na dvije odvojene postaje: 2 km zapadno od Rovinja i 7 km zapadno od Umaga, sjeverni Jadran, Hrvatska. Zabilježili smo jedinke različitih boja i uzoraka koje žive zajedno na istoj postaji u isto vrijeme. Cilj ovog istraživanje bio je analiza pigmenata spužvi, te taksonomska identifikacija i populacijska analiza uzorkovanih jedinki upotrebom genetskog markera mitohondrijskog gena za citokrom oksidazu - podjedinica 1 (COI). Topivom ekstrakcijom razdvojili smo crvenu i plavu boju. Spektrometrijskom analizom (usporedba spektra 300-700 nm) je crvena kloroformska frakcija identificirana kao *β*-karoten, a plava boja je izdvojena acetonskom precipitacijom i SDS PAGE elektroforezom. Analiza proteina i sekvenciranje (MALDI) je u tijeku. Sekvenciranje gena za COI i analiza sekvenci korištenjem BLAST servisa (NCBI GenBank baza podataka) potvrdilo je da i crvene i plave jedinke spužvi pripadaju vrsti *Suberites domuncula* sa manjim brojem haplotipova na obje postaje. Potrebno je opsežnije istraživanje kako bi se mogla utvrditi moguća veza različite obojenosti i haplotipova sa fizikalno kemijskim značajkama okoliša.

Ključne riječi: *Suberites domuncula*, Jadran, pigmenti, citokrom oksidaza, barkodiranje

CONTRIBUTION TO THE ECOLOGY OF THE SPONGE *Suberites domuncula* (Olivi, 1872): FROM MORPHOLOGY TO POPULATION ANALYSES

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Suberites domuncula (Olivi, 1792) (Porifera, Demospongiae, Tetractinomorpha, Hadromerida, Suberitidae) was originally described from the Adriatic Sea, but subsequently was widely recorded from all over the world. It was suggested that this widespread occurrence is an artefact and, consequently, worldwide known *S. domuncula* should be a complex of similar but different sponge species. Specimens of *S. domuncula* were collected at two distinct areas: 2 km off Rovinj and 7 km off Umag, northern Adriatic, Croatia. Among collected material we observed specimens of different colour patterns living at the same time and site together. The present research was directed towards the study of sponge colour source and pigments origin, followed by specimen identification and barcoding population analysis using mitochondrial cox gene. By solvent extraction we separate blue and red colour. The red chloroform fraction was identified as β-carotene by spectrometry analyses comparing spectra (300-700 nm), and blue colour was separated by acetone precipitation, and SDS PAGE. The protein analyses and sequencing (MALDI) are underway. The population analysis of cox1 gene was applied as tool for sponge specimens' identification (barcoding) and determination of possible haplotypes abundance. The red and blue *S. domuncula* morphs were proved as single species by sequencing and DNA analyses (blastn, NCBI), with few haplotypes at both sampling sites.

Key words: *Suberites domuncula*, Adriatic, pigments, cytochrome oxydase, barcoding

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EKOLOŠKI STATUS LIVADA MORSKE CVJETNICE *Posidonia oceanica* UZDUŽ HRVATSKE OBALE

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Morska cvjetnica *Posidonia oceanica* i njene livade su od 2012. godine uvrštene u program praćenja za potrebe Okvirne direktive o vodama. Da bi se odredio ekološki status livada, koristi se modificirani multivarijatni indeks za *Posidonia oceanica* (POMI9). Ovdje donosimo ekološki status za 45 livada koje se nalaze uzduž čitave hrvatske obale. Trinaest livada obrađeno je tijekom 2014. godine, 14 livada tijekom 2012. i 2013., te 18 livada tijekom implementacijske faze Direktive 2007. godine. Za 39 livada je zabilježen dobar i vrlo dobar ekološki status, za 3 livade je zabilježen umjereni ekološki status, za dvije livade loš, te za jednu livadu vrlo loš ekološki status. Livade za koje nije zabilježen dobar i vrlo dobar status se nalaze u neposrednoj blizini luka, uzgajališta i sidrišta. Analize potvrđuju da su livade morske cvjetnice *P. oceanica* uzduž hrvatske obale u zadovoljavajućem (dobrom i vrlo dobrom) ekološkom statusu.

Ključne riječi: ekološki status, *Posidonia oceanica*

ECOLOGICAL STATUS OF *Posidonia oceanica* MEADOWS ALONG THE CROATIAN COAST

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Seagrass *Posidonia oceanica* and its meadows in Croatian coastal area of the Adriatic Sea have been monitoring for the purpose of Water Frame Directive since 2012. To assess the ecological

status of P. oceanica meadows, modified *Posidonia oceanica* multivariate index (POMI9) has been used. Here we present the ecological status of 45 *Posidonia* meadows located along the Croatian coast. Thirteen meadows were examined during the monitoring program in 2014, 14 meadows were examined in the period 2012 - 2013 and 18 meadows during the implementation phase of the Directive in 2007. As many as 39 meadows had good and very good ecological status, three had moderate ecological status, two had bad and one had very bad ecological status. Meadows with very bad, bad and moderate ecological status were in vicinity of ports, mariculture facilities or in anchoring sites. According to analyses meadows along the Croatian coast are in satisfying (good and very good) ecological status.

Key words: ecological status, *Posidonia oceanica*

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RANI RAZVOJNI STADIJI DVA TROPSKA PUŽA (OPISTHOBRANCHIA) U KLIMATSKIM PROMJENAMA

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Istraženi su sinergistički učinci zagrijavanja i zakiseljavanja oceana na oplodnju, razvoj i preživljavanje nedoraslih jedinki dva tropska puža golaća *Berghia stephanieae* i *Elysia clarki* (Opisthobranchia). Ove vrste se često koriste kao biološki modeli u ekološkoj biokemiji i istraživanjima fotosimbioze. Četiri pokusna tretmana su testirana u svim kombinacijama (varirajući od 26 ºC do 30 ºC i pH od 8 do 7,5), te uspoređena s kontrolom (26 ºC, pH 8). Ovo istraživanje pokazalo je da su zagrijavanje i zakiseljavanje imali velikog utjecaja na *E. clarki* i *B. stephanieae*. Za razliku od zagrijavanja, zakiseljavanje je smanjilo broj oplođenih jaja po jedinki i usporilo razvoj kod obiju vrsta. Za *B. stephanieae* kombinirani utjecaji su rezultirali prestankom razvoja embrija nakon šestog dana nakon oplodnje. Također, primjećene su ozbiljne deformacije veligera (tijelo, ljuštura i usni aparat) kod obiju vrsta kod zagrijavanja i zakiseljavanja (93% deformacija kod *B. stephanieae* i 60% deformacija kod *E. clarki*). Pošto je preživljavanje juvenilnih stadija poprilično smanjeno, budućnost ovih vrsta je u vrlo upitnom i nesigurnom stanju s obzirom na predikcije IPCCa. Naše istraživanje ukazuje na potrebu za više eksperimentalnih istraživanja globalnog zagrijavanja i zakiseljavanja oceana istodobno i pojedinačno kako bi se bolje opisale posljedice koje budućnost nosi za Opisthobranchia.

Ključne riječi: Opisthobranchia, klimatske promjene, rani razvojni stadiji

ONTOGENIC DEVELOPMENT OF TWO TROPICAL OPISTHOBRANCH MOLLUSKS IN A CHANGING OCEAN

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This study investigated the interactive effects of warming and CO_2-driven acidification on fertilization, development and juvenile survival of two tropical Opisthobranchs, *Berghia stephanieae* and *Elysia clarki*. These marine tropical sea slugs are commonly used as biological tools for scientific research particularly to study their chemical ecology and photosymbiotic associations.Four experimental treatments (varying from 26 ºC to 30 ºC and pH from 8 to 7.5) were tested in all combinations and compared with the control scenario (26 ºC, pH 8). E. clarki and *B. stephanieae* marine sea slugs were severely impacted by the effects of warming and acidification. Contrary to global warming, ocean acidification reduced the number of egg masses per individual and development in both species. For *B. stephanieae* the combined effects of warming and acidification were so outstanding that no egg masses evolved after the 6 day of deposition. Also, severe veliger deformities (body, shell and oral apparatus) were observed for both species at ocean warming and acidification treatment (93 and 60% deformed veligers in *Berghia* and *Elysia*, respectively). Concomitantly, for these sea slugs the future scenario is not promising, since juvenile's survival was negatively impacted. Our study emphasizes the need for experiments that address ocean warming and acidification on Opisthobranchs concurrently and place single stressor studies in context.

Key words: Opisthobranchia, climate change, early stages

O-84
MORFOLOGIJA I STRUKTURA KUĆICE PUŽEVA STRAŽNJOŠKRŽNJAKA *Aplysia* spp.

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Stražnjoškržnjaci srodnih vrsta *Aplysia punctata* i *A. fasciata* (Gastropoda, Opisthobranchia, Anaspidea) pojavljuju se istovremeno na opsežnim infralitoralnim dnima obalnih područja Sredozemlja i Jadrana. Tijekom rasta i razvoja ličinke spiralna kućica potpuno obuhvaća i štiti meko tijelo. Nakon preobrazbe veligera vanjska kućica postaje unutarnja i reducira se do jednostavnog konkavnog štita iznad meke utrobe. Skenirajuća elektronska mikroskopija (SEM), infracrvena spektroskopija s Fourierovom transformacijom (FTIR) i difrakcija X-zraka (XRD) su metode korištene za određivanje morfoloških, fizikalnih, strukturnih i kristalografskih značajki "odraslih" kućica obiju vrsta. Istovremeno, u uzorcima je mjerena aktivnost ugljikove anhidraze (CA), te su određivane molekularne značajke primjenom proteomiksa i imunohistološke lokalizacije nizom specifičnih protutijela. Odrasla kućica vrste *A. punctata* ima dva organska sloja, između kojih se nalazi kalcificirani sloj sa dominantnim aragonitom (71 M %) i feldšparom (19 M %), dok se kućica srodne vrste *A. fasciata* sastoji gotovo isključivo od kristala aragonita (oko 90 M%). Kristali halita su prisutni vanjskoj i unutarnoj strani kućice kod obje vrste, ali se razlikuju po obliku, orijentaciji i pravcu rasta kristala.

Ključne riječi: *Aplysia fasciata*, *Aplysia punctata*, veliger, unutarnja kućica, biominerali

MORPHOLOGY AND STRUCTURE OF THE OPISTHOBRANCH GASTROPOD SHELL *Aplysia* spp.

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Related sea slug species *Aplysia punctata* and *A. fasciata* (Gastropoda, Opisthobranchia, Anaspidea) occur simultaneously in the Mediterranean and Adriatic infralittoral coastal zone. During larval growth and development spiral shell completely encloses and protects the specimen's soft body. After veliger metamorphosis, the external shell becomes internal and reduced to a simply concave shield over soft viscera. Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) were used to determine the morphology, physical, structural and crystallographic characteristics of the adult shells of both species. Concomitantly, a comparative study via different molecular characterizations was carried out by measuring enzymatic activities of carbonic anhydrase (CA), and by performing proteomics and immunohistological localization by a set of antibodies. Adult *A. punctata* shells consist of two organic layers between which is inserted a calcified layer with dominant aragonite (71 M %) and feldspar (19 M %) crystals. The adult internal shells of the related species *A. fasciata* were found to consist almost only of aragonite crystals (about 90 M %). The halite crystals are present on the internal and outside surfaces of the shell. They are characteristic of both species, but differ in shape, orientation and direction of the crystal growth.

Key words: *Aplysia fasciata*, *Aplysia punctata*, veliger, internal shell, biominerals

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ŽIVOTNI CIKLUS PSA MEKUŠA (*Mustelus punctulatus*) U SJEVERNOM I SREDNJEM JADRANSKOM MORU

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Pas mekuš (*Mustelus punctulatus* Risso, 1827) je učestala vrsta pridnenog morskog psa koja se komercijalno izlovljava, a prema IUCN-ovom Crvenom popisu svrstana je u kategoriju nedovoljno poznate svojte. Uporabom histoloških i morfometrijskih metoda na spolnim organima i kralješcima istražili smo demografske i reproduktivne karakteristike psa mekuša. Uzorci su prikupljeni od 220 jedinki (108 ženki i 112 mužjaka) ulovljenih pridnenim koćama od 2005. do 2007. godine u sjevernom i srednjem Jadranu. Ulov je bio ravnomjerno raspoređen po sezonama, osim tijekom ljeta kada je prikupljeno samo 7 životinja. Ženke su u prosjeku imale veće dužine tijela (DT) (srednja vrijednost DT: 77,1 ± 22,1 cm) od mužjaka (76,9 ± 20,4 cm), i bile starije (srednja vrijednost: 7,2 ± 4,6 godina) od mužjaka (6,7 ± 4,0 godina). Najstarija procijenjena dob za ženke iznosila je 22 godine, dok je dob najstarijeg mužjaka procijenjena na 16 godina. U uzorku su prevladavale jedinke (74%) starosti između 2+ i 9+ godina. Procijenjena starost najmanje trudne ženke (DT: 114,9 cm) bila je 12 godina, dok je najmanji mužjak (DT: 74,3 cm) s razvijenim sjemenicima bio star 7 godina. Dobiveni rezultati ukazuju da je pas mekuš k selektivna vrsta, koja ima spori somatski rast, kasno spolno sazrijevanje i dugi životni ciklus čime je posebice osjetljiva

na izlov. Stoga je za očuvanje populacije u Jadranskom moru ključno održivo upravljanje i izrada akcijskog plana za zaštitu ove vrste.

Ključne riječi: brzina somatskog rasta, spolna zrelost, morski pas, *k* selektivna vrsta

LIFE–HISTORY TRAITS OF A BLACKSPOTTED SMOOTH-HOUND (*Mustelus punctulatus*) IN THE NORTHERN AND CENTRAL ADRIATIC SEA

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Blackspotted smooth-hound (*Mustelus punctulatus* Risso, 1827) is frequently recorded and commercially exploited demersal shark, classified by IUCN Red List as Data Deficient. We used histological and morphometrical techniques on reproductive organs and vertebrae to analyse demographic and reproductive traits of blackspotted smooth-hound. We sampled 220 specimens (108 females and 112 males) caught by demersal trawls between years 2005 and 2007 in the northern and central Adriatic Sea. Catches were equally distributed throughout the year, except during summer season when only 7 animals were sampled. In average, females had larger body lengths (BL) (average BL: 77.1±22.1 cm) than males (76.9±20.4 cm) and were older (average: 7.2±4.6 years) than males (6.7±4.0 years). Estimated age of the oldest female was 22 years, while the oldest male was aged 16. Majority of analysed sample (74 %) were specimens aged between 2+ and 9+ years. Estimated age of the smallest pregnant female (BL: 114.9 cm) was 12 years, while the smallest male (BL: 74.3 cm) with developed testes was 7 years old. These results suggest that blackspotted smooth-hound is a k-selected species, with slow growth rate, late age at sexual maturity and long life cycle, therefore highly vulnerable to exploitation. Hence, sustainable management and providing action plans for protection of the species will be crucial for maintaining populations in the Adriatic Sea.

Key words: somatic growth rate, sexual maturity, sharks, *k*-selected species

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NASELJA KORALJA NA KORALIGENU PUČINSKIH PLIČINA U ISTOČNOM DIJELU JADRANSKOG MORA

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U ovom istraživanju opisuje se koraligen pučinskih pličina na dubinama od 25 do 100 m, na četiri lokaliteta pokraj Dugog otoka i otoka Kornat (istočni dio Jadrana). Istraživanja su napravljena metodom ronjenja i snimanja ROV ronilicom. Pregledana su naselja gorgonija *Paramuricea clavata* i *Eunicella cavolini*, zajedno sa nešto rjeđom vrstom *Savalia savaglia*. Naselja ovih vrsta

rasprostranjena su na dva različita staništa pličine: više izdignuta stijena slična tornju ("sika") na kojoj prevladava žuta gorgonija i pličina sa laganim padom (miješanje stijene i ljušturnog pijeska), gdje prevladavaju ostala dva koralja. Istraživane postaje su pod velikim utjecajem ribarenja, koje uzrokuje oštećenja na mnogim kolonijama. Oštećenja na gorgonijama kasnije prerastaju epibionti (poput crvenih alga i obrubnjaka). Ove pučinske pličine popularne su malim i profesionalnim ribarima zbog bogate faune riba. Velik problem pučinskog koraligena su premala istraženost i volja za utvrđivanjem utjecaja ribarstva na ove bentoske zajednice. Upravljanje tim područjima je također problem, jer nedostaje učinkovitih rješenja. Ovo izvješće predstavlja preliminarne podatke istraživanja i doprinosi poznavanju dubokog koraligena istočnog dijela Jadranskog mora.

Ključne riječi: koraligen, koralji, Jadransko more

THE CORAL ASSEMBLAGES OF AN OFF-SHORE CORALLIGENE BANKS IN THE EASTERN ADRIATIC SEA

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In this study we characterized coral assemblages of an off-shore coralligene banks dwelling at 50-100 m depth on a four locations near Islands of Dugi otok and Kornati (eastern Adriatic Sea). Surveys were made by scuba diving and ROV-imaging. Assemblages of suspension feeders, dominated by the gorgonians *Paramuricea clavata* and *Eunicella cavolini* together with *Savalia savaglia*, were examined. This three surveyed species were distributed into two areas corresponding to different habitat preferences: a more elevated hard ground ("steeple") hosting yellow gorgonian and a gently sloping, silted rocky bottom ("pyramid") hosting the other two coral species. The study area is subjected to a heavy pressure from the professional fishery, resulting in the mechanical damage of numerous colonies, some of which are then overgrown by various epibionts (mostly red algae and hydrozoans). These off-shore rocky banks are widely known among recreational and professional fishermen due to their rich fish fauna. The main problem about off-shore coralligene bank is the lack of knowledge and also effort to characterizing the extent of the impact and consequences on these benthic communities. The management of these areas is big issue, still lacking of an effective solution in the Adriatic Sea. This report represents a preliminary data of the whole study and contributes to the knowledge of deep coralligene banks in the eastern Adriatic Sea.

Key words: Coralligenous, Corals, Adriatic Sea

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PROSTORNA RASPODJELA BIOOPTIČKIH I TERMOHALINIH SVOJSTAVA U SJEVEROZAPADNOM DIJELU JUŽNOJADRANSKE KOTLINE

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Cirkulacija značajno utječe na biogeokemijske procese u Jadranskom moru. U cilju istraživanja specifičnih obrazaca zimske cirkulacije i distribucije fitoplanktona u južnom Jadranu provedeno je oceanografsko krstarenje u razdoblju od 28. veljače do 03. ožujka 2015. godine. Istraživana su dva transekta: (i) Dubrovnik do izobate od 1000 m (u smjeru 210º), i (ii) izobata od 1000 m do otoka Lastova u Južno-jadranskoj kotlini. Istraživano područje je pod utjecajem Levantinske intermedijarne vode (eng. *Levantine Intermediate Water*, LIW) i Istočno jadranske struje (eng. *East Adriatic Current*, EAC). Topla i slana LIW je dio EAC-a te ulazi u Jadransko more iz Jonskog mora, šireći se sjeverno u intemedijarnom sloju, većinom na dubini između 100 i 600 m, sa jezgrom između 200 i 400 m. Napravljeno je 45 CTD mjerenja, te su te su dobiveni podaci temperature, saliniteta, a provedena su i bioptička mjerenja fluorescencije klorofila (Chl F), optičkog raspršenja i oslabljenja zraka. Izmjerena je heterogena distibucija Chl F u ovisnosti o cirkulaciji. Značajan Chl F signal je zabilježen do 450 m dubine što upućuje ili na aktivnu primarnu produkciju u dubokim slojevima ili nedavnu subdukciju površinske vode. Jezgra LIW-a nije bila izražena kao u prethodnim godinama. Ipak, više vrijednosti saliniteta i temperature, zajedno s minimumom otopljenog kisika, su zabilježene na oko 450 m dubine.

Ključne riječi: južni Jadran, zimska dinamika vodenih masa, distribucija fitoplanktona, bio-optička mjerenja

SPATIAL DISTRIBUTION OF BIOOPTICAL AND THERMOHALINE CHARACTERISTICS IN NW PART OF SOUTHERN ADRATIC PIT

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Biogeochemical processes in the Adriatic Sea are highly influenced by circulation patterns and water mass dynamics. In order to investigate specific winter circulation and distribution of phytoplankton, an oceanographic cruise was conducted from February 28th till March 3rd 2015 in the southern Adriatic. Two major transects were investigated: (i) Dubrovnik to 1000 m isobath (in direction 210º), and (ii) 1000 m isobath to Lastovo island. The surveyed area is greatly influenced by the Levantine Intermediate Water (LIW) and East Adriatic Current (EAC). The warm and saline LIW represents a part of the EAC and enters the Adriatic from the Ionian Sea spreading northwards at the intermediate layer, usually at depths between 100 and 600 m, with the core from 200 to 400 m. Total of 45 CTD casts were performed and concurrent measurements of temperature, salinity and bio-optical parameters; bio-optical measurements of chlorophyll fluorescence (Chl F), optical particulate backscattering and beam attenuation were collected. The surveyed area demonstrated circulation dependent high patchiness in distribution of Chl F. The significant Chl F signal was detected up to 450 m depth, indicating either active primary production in deep layers or recent subduction of the surface water. The core of the LIW was not as pronounced as in previous years. However, higher salinity and temperature values, together with dissolved oxygen minimum were observed around 450 m.

Key words: southern Adriatic, winter water mass dynamics, phytoplankton distribution, bio-optical parameters

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KORALJI PODMORSKIH ŠPILJA JADRANSKOG MORA

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Morske špilje su poznate po svojoj posebnoj bioraznolikosti unutar obalnog područja Sredozemnog mora. Zbog svog krškog porijekla, potopljene i polupotopljene špilje su uobičajene duž istočne obale Jadranskog mora. Osnovni problem kod istraživanja morskih špilja (biocenoza polutamnih špilja) u Jadranskom moru je što nema dovoljno podataka o ovim staništima. Kvalitativno uzorkovanje napravljeno je u 15 morskih špilja uzduž istočne Jadranske obale. Utvrđeno je ukupno 22 vrste koralja. Najčešće vrste su moruzgve *Cerianthus membranaceus* (Spallanzani, 1784), *Halcampoides purpureus* (Studer, 1879) i *Parazoanthus axinellae* (Schmidt, 1862), kameni koralji *Ceratotrochus magnaghii* Cecchini, 1914, *Leptopsammia pruvoti* Lacaze-Duthiers, 1897, *Hoplangia durotrix* Gosse, 1860, *Phyllangia americana mouchezii* (Lacaze-Duthiers, 1897), *Caryophyllia* (Caryophyllia) *inorna* ta (Duncan, 1878), *Polycyathus muellerae* (Abel, 1959), *Madracis pharensis* (Heller, 1868) i *Parazoanthus axinellae* (Schmidt, 1862), kao i vrlo ugrožena vrsta *Corallium rubrum* (Linnaeus, 1758). Sve koraljne vrste utvrđene su na zidovima i stropu špilja, dok je moruzgva *C. membranaceus* česta na sedimentnom (mulj ili ljušturni pijesak) dnu. Dobiveni rezultati istraživanja ukazuju kako raznolika topografija, dubina i geografski položaj, kao i strujanja mora, količina svjetlosti i dostupnost hrane određuju rasprostranjenost koralja unutar morskih špilja.

Ključne riječi: koralji, morske špilje, Jadransko more

CORAL FAUNA OF SUBMERGED MARINE CAVES IN THE ADRIATIC SEA

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Marine caves are widely acknowledged for their unique biodiversity and constitute a typical feature of the Mediterranean coastline. Because of karstic origin, submerged or partially submerged sea caves are common along eastern Adriatic Sea. The main problem about sea caves (the biocoenosis of semidark caves) in the Adriatic Sea is that these habitats are insufficiently studied. Qualitative sampling was performed in fifteen marine caves along the Croatian coast. A total of 22 anthozoan species were recorded in the researched marine caves. The most common anthozoans were anemones *Cerianthus membranaceus* (Spallanzani, 1784), *Halcampoides purpureus* (Studer, 1879) and *Parazoanthus axinellae* (Schmidt, 1862), scleractinian corals *Ceratotrochus magnaghii* Cecchini, 1914, *Leptopsammia pruvoti* Lacaze-Duthiers, 1897, *Hoplangia durotrix* Gosse, 1860, *Phyllangia americana mouchezii* (Lacaze-Duthiers, 1897), *Caryophyllia* (Caryophyllia) *inornata* (Duncan, 1878), *Polycyathus muellerae* (Abel, 1959), *Madracis pharensis* (Heller, 1868) and *Parazoanthus axinellae* (Schmidt, 1862), as well as the endangered gorgonian species *Corallium rubrum*

(Linnaeus, 1758). All these coral species could be found on the cave walls, while the anemone *C. membranaceus* is quite abundant on sandy (detritic) or muddy substrate at the bottom of the caves. Results indicate that the different topography, depth and geographic location of submerged caves, determine coral distribution along the marine caves.

Key words: corals, marine caves, Adriatic Sea

6. Simpozij Hrvatskog Društva za biljnu biologiju
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PROTEIN TaMAB2 SUDJELUJE U REMODELIRANJU KROMATINA I METABOLIZMU RNA U ZIGOTI I 2-STANIČNOM PROEMBRIJU PŠENICE

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Proteinska porodica MATH-BTB zajednička je i za životinje i biljke. Zanimljiva je činjenica da genomi uročnjaka i čovjeka kodiraju samo nekoliko MATH-BTB proteina (šest, odnosno dva) dok se u nekim biljnim i životinjskim organizmima ova porodica proteina proširila na više desetaka članova. Između nekoliko desetaka MATH-BTB gena prisutnih u genomu pšenice identificirali smo dva (*TaMAB1-2*) eksprimirana isključivo u jajnoj stanici (*TaMAB1*) ili proembriju (*TaMAB2*). Gen *TaMAB2* kodira protein koji u interfaznoj stanici lokalizira s mikrotubulima, na asimetričan način u području jezgrine ovojnice i unutar interfazne jezgre, dok tijekom mitoze protein slijedi pozicije formiranja diobenog vretena i fragmoblasta. Štoviše, tijekom diobe zigote *TaMAB2* uvijek se naslijeđuje u bazalnu stanicu proembrija. Asimetrična lokalizacija i nasljeđivanje ukazuju na moguću uključenost proteina TaMAB2 pri uspostavi asimetrične stanične diobe i stanične specifikacije. Analizama interakcija proteina s proteinom TaMAB2 pokazali smo da uspostavlja izravnu interakciju s culinom 3 (podjedinica ligaze E3) što ukazuje na njegovo sudjelovanje u ubikvitinskom putu razgradnje proteina. Spektrometrijom mase identificirali smo veći broj proteina koji ukazuju na moguće uloge TaMAB2 pri modeliranju kromatina i regulaciji metabolizma RNA.

Ključne riječi: MATH-BTB, *Triticum aestivum*, stanična lokalizacija, asimetrična dioba, TAP tag

WHEAT MATH-BTB PROTEIN TaMAB2 PARTICIPATES IN CHROMATIN REMODELLING AND RNA METABOLISM IN ZYGOTE AND 2-CELLED PROEMBRYO

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The MATH-BTB protein family is common to both animals and plants. It is a phenomenon that *Arabidopsis* and human genomes encode only few members (six and two, respectively) of the MATH-BTB protein family but in some plant and animal organisms, the same protein family has expanded more than 10-fold. Among tens of MATH-BTB genes in wheat genome, we have identified two MATH-BTB genes expressed exclusively in egg cell (*TaMAB1*) or in two celled

proembryo (*TaMAB2*). The zygotic induced gene *TaMAB2* encodes a protein that asymmetrically co-localises with microtubuli around the nuclear envelope and within an interphase nucleus, while during mitosis protein follows spindle and phragmoblast formation. Moreover, zygote deposited *TaMAB2* is always inherited to the large basal cell after first asymmetric zygotic division. The asymmetrically inheritance indicate that the protein might be involved not only into establishment of asymmetry but also into the cell specification in two-celled embryo. By interaction studies we showed that TaMAB2 directly interact with Cullin 3-based E3 ligases and are involved in ubiquitin-dependent degradation pathway. By mass spectrometry we have identified a set of novel interacting proteins that indicate the possible roles of TaMAB2 in chromatin remodelling and RNA metabolism.

Key words: MATH-BTB, *Triticum aestivum*, intracellular localization, asymmetric division, TAP tag

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OPORAVAK KLIJANACA JEČMA REHIDRACIJOM NAKON STRESA IZAZVANOG SUŠOM

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Tolerancija na sušu jedna je od najčešće istraživanih značajki s obzirom na globalni problem nedostatka vode. U ovom radu cilj je bio istražiti oporavak dehidriranih i slabo vijabilnih klijanaca ječma nakon ponovnog zalijevanja. Ječam (*Hordeum vulgare* L., kultivar Bravo) je uzgajan deset dana u uzgojnoj komori (16 sati svjetla, ~65 µmol m^{-2} s^{-1}, 22±1 ^{o}C; 8 sati tame, 19 ^{o}C) i potom izložen suši prestankom zalijevanja. Nakon osam dana, kada je relativni sadržaj vode u listu (RWC) iznosio oko 20% klijanci su ponovno svakodnevno zalijevani kao i kontrolne biljke. Za potrebe svih analiza uzorkovani su prvi potpuno razvijeni listovi prije stresa (0. dan), 8. dan suše i svaka 24 sata tijekom perioda rehidracije koji je trajao četiri dana. Povećana vrijednost indeksa fotosintetske učinkovitosti (PIABS), parametra fluorescencije klorofila a, i imunodetekcija proteina Rubisco LSU pokazali su učinkovit fotosintetski aparat na kraju perioda rehidracije. Rastuće vrijednosti RWC-a, smanjenje razine lipidne peroksidacije te razgradnja nakupljenog prolina ukazali su na visoku tendenciju ka ponovnom uspostavljanju homeostaze, prethodno narušene sušom. Zaključili smo da su klijanci unatoč niskom RWC-u i smanjenoj fotosintezi tijekom snažne suše uspjeli na kraju rehidracijskog perioda u potpunosti oporaviti ukupnu fotosintetsku učinkovitost (PIABS). Dobiveni rezultati mogli bi biti korisni oplemenjivačima ječma tijekom selekcije kultivara u svrhu kreiranja genotipova tolerantnih na sušu.

Ključne riječi: *Hordeum vulgare*, suša, oporavak, relativni sadržaj vode u listu, fotosinteza

RECOVERY OF DROUGHT STRESSED BARLEY SEEDLINGS BY RE-WATERING

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Drought tolerance is one of the most studied features considering the global problem of water deficit. Here we investigated recovery of dehydrated and poorly viable barley seedlings upon

re-watering. Barley seedlings (*Hordeum vulgare* L. cv. Bravo) were cultivated in the growth chamber (16 h day, ~65 µmol m^{-2} s^{-1}, 22±1 ^{o}C; 8 h night, 19 ^{o}C) for ten days and then subjected to drought induced by withholding water. After 8 days, when leaf relative water content (RWC) was about 20% seedlings were watered daily with the same amount of water as controls. For all measurements, first fully developed leaves were sampled before stress (0th day), 8th day of drought and every 24 h during rehydration period of four days. Increased performance index (PIABS), the parameter of chlorophyll a fluorescence called index vitality, and immunodetection of Rubisco LSU revealed efficient photosynthetic apparatus after re-watering period. Increasing of RWC, decreasing the lipid peroxidation level, and degradation of accumulated proline indicated high tendency for the establishment of homeostasis previously disrupted by drought. We can conclude that despite the low RWC and downregulated photosynthesis under severe drought, seedlings were able to recover their overall photosynthetic efficiency (PIABS) at the end of rehydration period. Obtained results might be helpful to barley breeders during selection of cultivars in order to create a new generation of genotypes better adapted to water deficit.

Key words: *Hordeum vulgare*, drought stress, recovery, leaf relative water content, photosynthesis

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FOTOSINTETSKI ODGOVOR MLADIH I RAZVIJENIH LISTOVA SMOKVE NA VISOKI INTENZITET SVJETLOSTI

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Visoki intenzitet svjetlosti često uzrokuje fotoinhibiciju zbog ograničenog transporta elektrona i smanjenog kvantnog prinosa fotosinteze. Cilj je bio istražiti utjecaj visokog intenziteta svjetlosti na fotosintetsku učinkovitost mladih (YL) i razvijenih (ML) listova smokve (*Ficus carica* L.) izloženih povišenoj temperaturi. Oba tipa listova aklimatizirani su 12 h u mraku na sobnoj temperaturi te nakon toga izloženi temperaturi od 35±1 °C u kombinaciji s niskim (LI, ~50 µmol m^{-2} s^{-1}) ili visokim (HI, ~800 µmol m^{-2} s^{-1}) intenzitetom svjetlosti tijekom 4 h. Za određivanje fotosintetske učinkovitosti mjerena je fluorescencija klorofila a metodom saturacijskog pulsa. Visoka vrijednost maksimalnog kvantnog prinosa fotosustava II izmjerena na LI tretmanu ukazuje da su oba tipa listova potpuno funkcionalna. Tretman HI izazvao je kod ML značajan pad Fv/Fm, smanjenu stopu elektronskog transporta i nisku razinu nefotokemijskog gašenja fluorescencije klorofila a. Povećanje kvantnog prinosa ne-reguliranog rasipanja energije (Y(NO)) u kombinaciji s padom optimalnog kvantnog prinosa (Y(PSII)) i kvantnog prinosa rasipanja energije ovisnom o pH gradijentu (Y(NPQ)) ukazuju na smanjeni kapacitet zaštite ML. YL nisu pokazali razlike između mjerenih parametara nakon izlaganja LI i HI tretmanima što ukazuje na stabilnu fotosintetsku učinkovitost. Može se zaključiti da su nakon izlaganja HI tretmanu YL zadržali učinkovite mehanizme zaštite, dok je pojačana osjetljivost ML rezultirala fotoinhibicijom.

Ključne riječi: *Ficus carica* L., fotosinteza, svjetlosni stres, fluorescencija klorofila a

PHOTOSYNTHETIC RESPONSE OF YOUNG AND MATURE FIG LEAVES TO HIGH IRRADIATION

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High irradiation often causes photoinhibition due to limited electron transport and reduced photosynthetic quantum conversion. We aimed to investigate influence of high irradiation on photosynthetic performance of young (YL) and mature (ML) fig leaves (*Ficus carica* L.) exposed to elevated temperature. Detached YL and ML were acclimated at room temperature in darkness for 12 h and then exposed to 35±1 °C combined with low (LI, ~50 µmol m^{-2} s^{-1}) or high irradiation (HI, ~800 µmol m^{-2} s^{-1}) for 4 h. To evaluate primary photochemistry of photosystem II (PSII), modulated chlorophyll a fluorescence was measured. High maximum quantum yield of PSII photochemistry (Fv/Fm) indicated full functionality of both leaf types exposed to LI. Exposure of ML to HI induced significant reduction of Fv/Fm, followed with decreased electron transport rate (ETR) and non-photochemical quenching (NPQ). Increase in the quantum yield of non-regulated energy dissipation (Y(NO)), accompanied with decline in both, the effective quantum yield of photochemical energy conversion in PSII (Y(PSII)) and the quantum yield of pH-dependant energy dissipation (Y(NPQ)), suggested reduced capacity of photoprotective reactions. YL exposed to HI showed no significant difference in all measured parameters compared to LI, thus indicating stable photosynthetic performance. In conclusion, YL maintained efficient photoprotective mechanisms at HI, while increased susceptibility of ML to HI resulted with severe photoinhibition.

Key words: *Ficus carica* L., photosynthesis, light stress, chlorophyll a fluorescence

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ODREĐIVANJE IZOFORMI PEROKSIDAZA SKUPINE III I LAKAZA TIJEKOM RAZVOJA STABLJIKE JEČMA (*Hordeum vulgare* L.)

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Enzimi peroksidaze skupine III sudjeluju u brojnim fiziološkim procesima u biljkama. Zajedno s enzimima lakazama uključene su u oksidaciju monolignola kao završnog koraka u sintezi lignina. Lignin je prirodni polimer koji izgrađuje staničnu stijenku i daje čvrstoću stabljici. U ovom istraživanju cilj je bio utvrditi karakteristike pojedinih izoformi peroksidaza i lakaza u internodijima tijekom četiri razvojne faze stabljike ječma: elongacija, klasanje, cvatnja i nalijevanje zrna. Izolirane su ukupne peroksidaze (tPOD) i lakaze te peroksidaze kovalentno vezane za staničnu stijenku (cPOD). Izoenzimske forme razdvojene su izolelektričnim fokusiranjem na poliakrilamidnom gelu. Za utvrđivanje peroksidazne aktivnosti korišten je gvajakol, a za lakaze siringaldazin. Analiza je pokazala prisutnost 8 različitih izoformi tPOD s izoelektričnom točkom (pI) 8-10 i jedne izoforme s pI 2,5-5. Najveća aktivnost uočena je u prvom internodiju u svim razvojnim fazama. U staničnoj stijenci cPOD prisutne su u jednoj izoformi, s profilom aktivnosti sličnom kao tPOD. Lakaze su zastupljene s dvije izoforme (pI 8-10 i pI 2,5-5) bez razlike u jačini aktivnosti u pojedinom internodiju i razvojnoj fazi. Dobiveni

rezultati ukazuju na prisutnost i karakteristike različitih izoformi peroksidaza i lakaza uključenih u razvojne procese u stabljici ječma. Buduća istraživanja imaju za cilj razjasniti ulogu pojedinih izoformi u procesu biosinteze lignina.

Ključne riječi: internodij, stanična stijenka, lignin, izoforme, bakar vezane oksidaze

DETERMINATION OF CLASS III PEROXIDASE AND LACCASE ISOFORMS DURING DEVELOPMENT OF BARLEY (*Hordeum vulgare* L.) STEM

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Class III peroxidases are involved in many physiological processes in plants. Together with laccases they are involved in oxidation of monolignols as the final step in the synthesis of lignin. Lignin is a natural polymer that builds the cell wall and provides mechanical strength to the stem. In the present study, objective was to determine the characteristics of individual isoforms of peroxidase and laccase in internodes of barley during the four developmental stages: elongation, heading, anthesis and grain filling. Total peroxidase (tPOD), laccase and peroxidase covalently bound to the cell wall (cPOD) were extracted and separated using isolelectric focusing on polyacrylamide gels. Guaiacol was used for detection of peroxidase activity and syringaldazine for laccase activity. The analysis showed the presence of eight different isoforms of tPOD with isoelectric point (pI) 8-10 and one isoform with pI 2.5-5. Highest activity was recorded in the first internode in all developmental stages. cPOD were present in a single isoform with activity profile similar to tPOD. Laccase were represented with two isoforms (pI 8-10 and pI 2.5-5) with no difference in activity in a particular internode and developmental stage. The results show the presence and characteristics of the various isoforms of peroxidase and laccase involved in development of barley stem. Future studies aim to clarify the role of these enzymes in the biosynthesis of lignin.

Key words: internode, cell wall, lignin, isoformes, multicopper oxidase

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USPOREDBA GASTROINTESTINALNE STABILNOSTI HRVATSKIH DIVLJIH JESTIVIH BILJAKA: ANALIZA FENOLNIH SPOJEVA

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Kvalitativne i kvantitativne analize fenolnih spojeva hrvatskih divljih jestivih biljaka oštrolisne šparoge (*Asparagus acutifolius* L.), bodljikave veprine (*Ruscus aculeatus* L.) i običnog bljušta (*Tamus communis* L.) provedene su metodom tekućinske kromatografije visoke moći razlučivanja u sustavu obrnutih faza. Najveća količina fenolnih spojeva zabilježena je u bljuštu, a najmanja u veprini. Glikozidi flavonola najzastupljeniji su fenoli u bljuštu i šparogi, a fenolne kiseline u veprini. Vodeni ekstrakti podvrgnuti su dvofaznoj (želučana i duodenalna) *in vitro* digestiji s ljudskim enzimima s ciljem usporedbe gastrointestinalne stabilnosti glavnih fenolnih spojeva odabranih biljnih ekstrakata. Fenolne kiseline svih testiranih vodenih ekstrakata bile su potpuno probavljene već nakon *in vitro* želučane faze probave. Od flavonoida, nakon duodenalne probave najbolje su bila očuvana dva glavna glikozida kempferola iz vodenog ekstrakta bljušta (50% svaki). U vodenom ekstraktu šparoge otprilike 20% kvercetin-3-O-rutinozida i isoramnetin-3-O-rutinozida je ostalo stabilno, dok u ekstraktu veprine nakon duodenalne faze flavonoidi nisu zabilježeni. S obzirom da bljušt sadrži najveću količinu fenolnih spojeva i najveći postotak njegovih fenola je očuvan nakon *in vitro* gastrointestinalne probave, zaključujemo da je za ljudsku prehranu među istraženim biljkama bljušt najbolji izvor prirodnih antioksidativnih tvari.

Ključne riječi: RP-HPLC, *in vitro* digestija, kempferol, kvercetin-3-O-rutinozid, izoramnetin-3-O-rutinozid

COMPARISON OF GASTROINTESTINAL STABILITY BETWEEN CROATIAN WILD EDIBLE PLANTS: CASE OF PHENOLICS

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Qualitative and quantitative reversed-phase high performance liquid chromatography analyses of phenolics in water and ethanolic extracts of Croatian wild edible plants asparagus (*Asparagus acutifolius* L.), butcher's broom (*Ruscus aculeatus* L.) and black bryony (*Tamus communis* L.) were conducted. Black bryony contained the highest amount of phenolics, followed by wild asparagus and butcher`s broom. Flavonol glycosides were the most abundant phenolics in black bryony and wild asparagus, in butcher`s broom phenolic acids were prevalent. Water extracts were subjected to two-phase (gastric and duodenal) *in vitro* digestion with human enzymes in order to compare the gastrointestinal stability of major phenolics in the selected plants extracts. Phenolic acids in all the tested water extracts were totally digested already after *in vitro* gastric digestion phase. Among flavon oids, two main kaempferol-glycosides from black bryony extract were best preserved (50% of each) after duodenal digestion. In wild asparagus extract approximately 20% of quercetin-3-O-rutinoside and isorhamnetin-3-O-rutinoside remained stable, and in the butcher`s broom extract no flavonoids could be detected upon duodenal digestion. Accordingly, among the tested plants black bryony would be the best source of natural antioxidants both because it contains the highest amount of flavonol glycosides, and because the highest percentage of this plant phenolics was preserved after *in vitro* gastrointestinal digestion.

Key words: RP-HPLC, *in vitro* digestion, kaempferol, quercetin-3-O-rutinoside, isorhamnetin-3-O-rutinoside

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ASR SLIČNI PROTEINI OTKRIVENI U *IN VITRO* KULTURI KAKTUSA *Mammillaria gracilis*

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Apscizinskom kiselinom, stresom i dozrijevanjem inducirani (ASR) proteini su specifični za biljke, male su molekulske mase, otporni na toplinu te jako hidrofilni. ASR su po svojoj ulozi transkripcijski faktori ali i šaperonu slični proteini. Uključeni su u otpornost biljke na sušu i salinitet, regulaciju šećera, razgranatih amino kiselina i u metabolizam stanične stijenke. Porodica ASR proteina je jako rasprostranjena u biljnom carstvu. Međutim, uročnjak kao i druge vrste iz porodice krstašica nemaju ASR gene, što navodi na to da ASR nije prisutan u svim biljkama. Do sada nije poznato da li porodica kaktusa, koja je poznata po svojoj visokoj otpornosti na vodni stres, kodira ASR protein. Stoga smo istražili tkiva (biljka, kalus, tumor) vrste *M.gracilis*, koja su rasla u kulturi *in vitro*, kako bi otkrili da li se ASR slični proteini nalaze u vrstama porodice kaktusa. ASR se može učinkovito pročistiti pomoću Ni-NTA agaroze, jer ima autentičnu pentahistidinsku sekvencu blizu N-kraja. Stoga, kako bi pročistili ASR slične proteine iz tkiva kaktusa, koristili smo afinitetnu kromatografiju. Eluirani proteini s Ni-agaroze su analizirani u denaturirajućim i nativnim uvjetima, prijenosom proteina na membranu i detekcijom specifičnim protutijelom i spektrometrijom masa. Ove analize pokazale su, po prvi puta, da kaktusi kodiraju ABA i dozrijevanjem inducirane proteine. Nadalje, otkrili smo i neke moguće interakcijske partnere s ASR sličnim proteinima kaktusa koje ćemo dalje analizirati.

Ključne riječi: ASR proteini, kaktus, kultura biljnog tkiva, spektrometrija masa

ASR-LIKE PROTEINS DETECTED IN *IN VITRO*-GROWN CACTUS *Mammillaria gracilis*

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Abscisic acid-, Stress-, and Ripening-induced (ASR) proteins are plant specific, low molecular weight, heat-stable proteins that have high hydrophilicity. ASR proteins were shown to possess transcription factor and chaperon-like activities. They are involved in plant tolerance to drought and salinity, in regulation of sugar, branched amino acids and cell wall metabolism. The ASR protein family is widespread in the plant kingdom. However, the *Arabidopsis* as well as other Brassicaceae family species lack ASR genes, suggesting that ASR proteins are not ubiquitous to all plants. There is no knowledge if plants from the Cactaceae family, known for its highly tolerance to water stress, encode ASR proteins. We thus used *in vitro*-grown *M. gracilis* tissues (plant, callus

and tumor) in order to reveal if the ASR-like proteins can be found in a member of the Cactaceae family. A SR proteins can be effectively purified to homogeneity on Ni-NTA-agarose, since they contain an authentic pentahistidine sequence close to their N-termini. We thus applied ion chelating chromatography to purify ASR-like protein from cactus tissues. Eluted proteins from Ni-agarose were analyzed by means of SDS-PAGE, Native-PAGE, western blot with anti-ASR1 antibody and mass spectrometry. These analyses demonstrated, for the first time, that cacti encode ABA- and ripening-induced proteins. Moreover, we revealed some possible interacting partners with cactus ASR-like proteins which need to be further analyzed.

Key words: ASR proteins, cactus, plant tissue culture, mass spectrometry

3. Simpozij Hrvatskog entomološkog društva
3rd Symposium of the Croatian Entomology Society

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VRETENCA (ODONATA) OTOKA KRKA – UGROŽENOST I STATUS

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Istraživanja faune vretenaca otoka Krka, obavljana su od travnja do rujna 2013 godine na 45 lokaliteta. Korištene su slijedeće metode: prikupljanje entomološkim mrežicama, obilaženje i promatranje (metoda linijskog transekta). Kartiranje i digitalna obrada podataka načinjeni su pomoću GIS tehnologije upotrebom ArcView 9.0 programa. Razlika u kvalitativnom i kvantitativnom sastavu podataka odabranih parametara (brojnost jedinki, vrsta, lokaliteta) izvršena je pomoću klaster analize te ordinacijske metode multidimenzionalnog skaliranja pomoću programa PRIMER 5.0. Korištena je matrica sličnosti dobivena izračunavanjem Bray-Curtis indeksa sličnosti. Za klaster analizu korištena je metoda povezivanja po prosjeku grupa, a ordinacijska MDS metoda provedena je ponavljanjem 100 puta. Ukupno je obrađeno 1043 podataka (jedinki) različitih životnih stadija (ličinke, odrasle jedinke i svlakovi) te je utvrđeno 40 vrsta, 21 rod, 8 porodica i 2 podreda reda Odonata. Prvi puta je utvrđeno ukupno 2 nova nalaza ugroženih i 1 nalaz rizičnih vrsta za faunu istraživanog područja. Podaci o rasprostranjenosti pojedinih vrsta vretenaca i promjenama veličina njihovih populacija, kao i rezultati ekoloških istraživanja potvrđuju da su one vrlo osjetljive, te da je opstanak stabilnih populacija izravno ovisan o čovjekovom djelovanju. Utvrđeni kvalitativni sastav faune vretenaca na području otoka Krka predstavlja 55% ukupne faune vretenaca u Republici Hrvatskoj, što dokazuje značaj ovog područja u zaštiti.

Ključne riječi: Vretenca, Krk, ugroženost, zaštita

DRAGONFLIES (ODONATA) OF THE KRK ISLAND – ENDANGERMENT AND STATUS

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Systematic studies of dragonflies on the Island Krk were carried out from April to September 2013

at 45 localities. The following methods were used: the method of collecting by entomological nets and the linear transect method by strolling and observing. Mapping and digital analyses of the data were done by GIS technology, and the ArcView 9.0 program was used. The difference within the composition of the qualitative and quantitative data structure (the total number of specimens, species, and locality) was done by cluster analysis and the ordination method of the multidimensional scaling of the statistical programme PRIMER 5.0. It was used the Bray-Curtis index of similarity. For the cluster analysis the group average method was used. On the basis of the 1043 collected individuals at different stages of life, a total of 40 species, 21 genera, 8 families and 2 suborder of the order Odonata were established. Two endangered, and one vulnerable species are making an appearance for the first time in the investigated area. Data relating to the distribution and fluctuation of species and change in their populations as well as the results of some eco-research show that the dragonflies are very sensitive species and that the survival of stabile population directly depends on the human activity. The established dragonfly species assemblage on surveyed area represents 55% of the total number of species in Croatia which proves this area as the significant in terms of protection.

Key words: Dragonflies, Krk, endangerment, protection

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SEZONSKA DINAMIKA KOMARACA NA PODRUČJU ŽUPANJSKE POSAVINE NAKON POPLAVE 2014. GODINE

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Nakon katastrofalne poplave u svibnju 2014. godine, područje županjske Posavine postalo je izuzetno povoljno stanište za razvoj velikog broja komaraca budući da se voda nakon povlačenja i dalje zadržala u inundacijama uz Savu, kanalima, depresijama tla, septičkim jamama i mnogim drugim umjetnim leglima. Za potrebe provođenja javnozdravstvenih mjera na navedenom području s krajnjim ciljem smanjenja rizika od pojave i širenja vektorskih bolesti, trebalo je utvrditi kvantitativni i kvalitativni sastav faune komaraca i sezonsku dinamiku dominantnih vrsta s naglaskom na vrstu *Culex pipiens* kao potencijalnog prijenosnika virusa Zapadnog Nila. Istraživanje je provedeno u razdoblju od svibnja do rujna 2014. godine na 16 postaja. Za uzorkovanje odraslih jedinki komaraca korištena je metoda CDC–klopki uz suhi led kao atraktant. Ukupno je uhvaćeno 319 037 jedinki komaraca, a utvrđeno je 17 vrsta od kojih su tri dominantne: *Aedes vexans*, *Ochlerotatus sticticus* i *Cx. pipiens*. Brojnost komaraca značajno je počela rasti tri tjedna nakon poplave i svoj vrhunac dosegla u drugoj polovici mjeseca lipnja. Tijekom kolovoza pojavila se druga generacija, po brojnosti nešto slabijeg intenziteta. Na pojedinim je postajama broj komaraca bio vrlo velik kroz duže vremensko razdoblje, što ukazuje na činjenicu da su komarci dugo uznemiravali stanovnike županjske Posavine. Rezultati dobiveni ovim istraživanjem uvelike su doprinijeli uspješnom suzbijanju komaraca na poplavom ugroženim područjima.

Ključne riječi: Komarci, županjska Posavina, poplava, virus Zapadnog Nila

SEASONAL DYNAMICS OF MOSQUITOES ON THE TERRITORY OF POSAVINA NEAR ŽUPANJA AFTER THE 2014 FLOOD

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After the disastrous flood in May 2014, the territory of Posavina near Županja became an ideal mosquito breeding site since water after retreating still retained in the inundations near the river Sava, in the canals, ground depressions, septic tanks and many other artificial breeding sites. In order to implement public health measures in that area with the aim of lowering the risk of occurrence and spread of vector diseases, it was necessary to determine the quantitative and qualitative composition of mosquito fauna and seasonal dynamics of dominant species, with the emphasis on the *Culex pipiens* species as the potential vector of West Nile virus. The research was conducted from May to September 2014 at sixteen stations. The CDC trap method with dry ice as an attractant was used for sampling of adult specimens of mosquitoes. A total of 319 037 mosquitoes were caught, and 17 species were recognized, with three dominant ones including *Aedes vexans*, *Ochlerotatus sticticus* and *Cx. pipiens*. The mosquito population has significantly increased three weeks after the flood and reached its peak in the second half of June. The second generation emerged during August, but with somewhat lower numbers. At some stations the mosquito population remained high for a longer time period, which shows that nuisance mosquitoes have bothered the inhabitants of Posavina near Županja for a long time. The results of this study have contributed to successful mosquito control in flood affected areas.

Key words: Mosquitoes, Posavina near Županja, flood, West Nile virus

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Epitrix hirtipennis (Melsheimer, 1847) DUHANOV BUHAČ, NOVI ČLAN ENTOMOFAUNE HRVATSKE

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Proizvođači duhana imaju sve većih problema u proizvodnji duhana. Prvi vidljivi simptomi uočeni su na listovima duhana ili u korijenju mladih biljaka. Utvrđeno je kako se radi o duhanovom buhaču *Epitrix hirtipennis* (Melsheimer, 1847). Kukac veličine oko 1,5 mm, kreće se skakanjem. Ličinke su veličine do 4,2 mm, bjelkaste s tamnom glavom. Imago pravi štete u vidu karakterističnih okruglih rupica na listu, a ličinke prave hodnike na korijenu u biljci domaćinu. Kukac prezimi u stadiju imaga u tlu ili u ostacima bilja. Javlja se rano u proljeće za vrijeme toplih dana. Dobar je letač te lako pronalazi biljku domaćina. Štete pravi u klijalištu kao i u polju. *E. hirtipennis* se smatra štetnikom duhana, ali može se hraniti mnogim biljkama iz porodice Solanaceae. Porijeklom je iz Srednje i Sjeverne Amerike, a u Europi prvi put je otkriven 1983 u Italiji. U Hrvatskoj je utvrđen na površinama Hrvatskih duhana P.C. Kutjevo te kod njihovih kooperanata. U polju je hvatan entomološkom mrežom, a u proizvodnji presadnica postavljene su žute ljepljive ploče. Mladu biljku potrebno je zaštititi u najranijoj fazi razvoja zato se sadnice prije sadnje potapaju se u 1% otopinu insekticida. Prema literaturi kritični brojevi za *E. hirtipennis* su 5 imaga/biljci rano u vegetaciji ili 5-10% uništeno lisne mase. Osim na duhanu bio je prisutan i na krumpiru, ali ne smatra se ozbiljno štetnik krumpira u europskim zemljama kao neke druge vrste iz roda *Epitrix* koje su karantenske za našu zemlju.
Ključne riječi: duhan, duhanov buhač, *Epitrix hirtipennis*, novi član entomofaune RH

Epitrix hirtipennis (Melsheimer, 1847) TOBACCO FLEA BEETLE - NEW MEMBER OF CROATIAN ENTOMOFAUNA

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Tobacco producers face major problems in the production of tobacco. The first visible symptoms were observed in tobacco leaves or the roots of young plants. It was determined that it was tobacco flea beetle *Epitrix hirtipennis* (Melsheimer, 1847). Insect size is about 1.5 mm, and it moves by jumping. The larvae are up to 4.2 mm, white, with a dark head. Adult insect made damages by doing holes in the leaf known as "shotholing", and the larvae make the corridors at the root of the plant host. It overwinters in the stage of imago in soil or in plant residues. Damage can be made in plant bed as well as in the field. *E. hirtipennis* is considered a pest of tobacco, but it can feed many plants of the family Solanaceae. Its origin is from Central and North America, and it was found for the first time in Europe, in 1983 in Italy. In Croatia, pest is established on fields of Croatian tobacco PC Kutjevo and on the field of their subcontractors. The pest was caught by entomological net and in the production of seedlings by yellow sticky traps. Young plant should be protected at an early stage of development. Before planting seedlings are immersed in 1% solution of the insecticide. According to the literature, critical numbers are 5 image/plant early in growth or 5-10% destroyed pant leaves. In addition to tobacco, pest was also present on the potato, but is not considered a serious pest of potatoes in European countries while some other species of the genus *Epitrix* are quarantine for our country.

Key words: tobacco, tobacco flea beetle, *Epitrix hirtipennis*, a new member Croatian entomofauna

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RAZNOLIKOST NOĆNIH LEPTIRA STRAHINJŠČICE I IVANŠČICE

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Tijekom 2014. i 2015. godine istraživana je fauna noćnih leptira na Natura2000 područjima Strahinjščica i Ivanščica, Krapinsko-zagorska županija. Na 13 posjećenih lokaliteta zabilježeno je preko 700 vrste noćnih leptira. Najveći broj vrsta zabilježen je na lokacijama Strahinje Radobojsko (435 vrsta), Radoboj, Plat (352 vrste) i vrh Ivanščice (269 vrsta). Vrste *Chersotis rectangula*, *Nemophora ochsenheimerella*, *Catoptria pyramidellus*, *Udea inquinatalis* i *Udea cyanalis* predstavljaju prve nalaze za faunu Hrvatske. S obzirom da su povijesni podatci o noćnim leptirima ovog područja gotovo nepostojeći, radi čega ovo istraživanje možemo smatrati prvim sustavnim istraživanjem faune noćnih leptira Strahinjščice i Ivanščice. Vrlo veliki broj zabilježenih vrsta leptira svrstava ovo područje među najbolje istražena područja u Hrvatskoj, te ukazuje na veliko bogatstvo i značaj ovih planina kao centara bioraznolikosti. Buduća istraživanja istih, ali i novih staništa i lokaliteta, svakako će povećati broj poznatih vrsta leptira Krapinsko-zagorske županije.

Ključne riječi: Natura 2000, novi nalazi, rijetke vrste

DIVERSITY OF MOTH FAUNA OF STRAHINJŠČICA AND IVANŠČICA

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During the years 2014 and 2015 moth fauna was surveyed in the Natura 2000 areas Strahinjščica and Ivanščica, Krapinsko-zagorska county. At the 13 visited localities, more than 700 moth species were recorded. The highest number of species was recorded at the localities Strahinje Radobojsko (435 species), Radoboj, Plat (352 species) and the peak of Ivanščica (269 species). The species *Chersotis rectangula*, *Nemophora ochsenheimerella*, *Catoptria pyramidellus*, *Udea inquinatalis* and *Udea cyanalis* represent first records for the fauna of Croatia. As the historical data about the moth fauna of the area are virtually non-existent, this survey represents the first systematic research of moth fauna of Strahinjščica and Ivanščica. A very large number of recorded species of moths makes this area one of the best researched areas in Croatia, and points to the great wealth and importance of these mountains as centres of biodiversity. Future studies of the same, as well as new habitats and localities will definitely increase the number of known species of butterflies of Krapinsko-zagorska county.

Key words: Natura 2000, new records, rare species

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STRUKTURA SAPROKSILNIH KORNJAŠA (COLEOPTERA) NIZINSKIH ŠUMA

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Saproksilni kornjaši predstavljaju kukce usko vezane uz mrtvo drvo. Neodrživo gospodarenje šumama doprinijelo je sve većoj ugroženosti saproksilnih kornjaša te se zbog toga nalaze na crvenim popisima ugroženih vrsta. Republika Hrvatska označena je kao područje s velikim brojem ugroženih vrsta od kojih su najčešće iz porodica strizibuba (Cerambycidae), klišnjaka (Elateridae) i jelenaka (Lucanidae). Cilj ovog rada je utvrditi brojnost i raznolikost saproksilnih kornjaša unutar različitih šumskih zajednica Kopačkog rita te pružiti osvrt na ugrožene vrste. Utvrdit će se učinkovitost različitih metoda uzorkovanja koje se koriste za praćenje saproksilnih kornjaša: metoda prekinutog leta (dizajn pleksiglas i mreža), metoda lovnih klopki i metoda zračne klopke s atraktantom. Terensko istraživanje bilo je provedeno od travnja do kolovoza 2014. godine, a ukupno je postavljeno 918 klopki tijekom istraživanja. Uzorkovano je 1088 jedinki i 64 vrste iz 14 porodica saproksilnih kornjaša. Najbrojnije porodice bile su Cerambycidae, Cetoniidae i Lucanide te vrste *Cetonia aurata*, *Dorcus parallelipipedus* i *Valgus hemipterus*. Najbrojnija i najraznolikija fauna saproksilnih kornjaša bila je u šumi hrasta lužnjaka. Od ukupnog broja na europskoj crvenoj listi se nalazi 20 vrsta, od čega su tri vrste gotovo ugrožene (*Cucujus cinnaberinus*, *Gnorimus variabilis* i *Lucanus cervus*) dok su tri nedovoljno poznate od kojih je zadnja i ugrožena vrsta (*Protaetia affinis*, *Omoglymmius germari*, *Rhysodes sulcatus*). Najučinkovitija metoda uzorkovanja saproksilnih kornjaša bila je metoda prekinutog leta.

Ključne riječi: Kornjaši, ugrožene vrste, metoda prekinutog leta, šumski ekosustav

SAPROXYLIC BEETLE (COLEOPTERA) ASSEMBLAGES IN LOWLAND FOREST

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Saproxylic Coleoptera are dead wood associated beetles. They have been particularly affected by forest management and make up one of the largest groups of red-listed species in many countries. The Republic of Croatia is designated as an area with a large number of endangered species, mostly from family Cerambycidae, Elateridae and Lucanidae. The aim of this study is to determine the abundance and diversity of saproxylic beetles within the different forest communities in Kopački rit and to give an overview of endangered species. Likewise, to compare the efficiency of sampling methods that are primarily used in monitoring studies: window-flight trap (cross-vanes and single-plane), pitfall trap and air trap with attractant. The field research was conducted from April to August 2014 whereas 918 traps were set during research. A total of 1088 individuals and 64 species from 14 families of saproxylic beetles were caught in this study. The most abundant families were Cerambycidae, Cetoniidae and Lucanidae with the most common species *Cetonia aurata*, *Dorcus parallelipipedus* and *Valgus hemipterus*. The most abundant and diverse assemblages of saproxylic beetles were found in oak wood forest. Of total, 20 species were on European red list, three were near threatened (*Cucujus cinnaberinus*, *Lucanus cervus*) and three were data deficient species of which last one is also endangered species (*Protaetia affinis*, *Omoglymmius germari*, *Rhysodes sulcatus*). The most efficient sampling method was cross-vanes window flight trap.

Key words: Coleoptera, endangered species, flight intercept method, forest ecosystem

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VEKTOR *Aedes albopictus* – AZIJSKI TIGRASTI KOMARAC U OSIJEKU, HRVATSKA

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Azijski tigrasti komarac, *Aedes albopictus* biološki je i zdravstveno značajna vrsta komarca čije se širenje u svijetu prati posljednjih četrdeset godina. Ova vrlo agresivna i tijekom dana aktivna vrsta sposobna je prenijeti različite arboviruse i parazite. Od 2004. godine kada je prvi put zabilježena u Hrvatskoj, invazivno se širi Hrvatskom do danas. Nakon Zagreba, Istre i Dalmacije vrsta je započela svoje širenje i na istočni dio Hrvatske. Tijekom 2013. godine, u mjesecu kolovozu zabilježen je prvi i jedini nalaz ove vrste na području prigradskog naselja Tenja kod Osijeka, što je bio dovoljan razlog za intenzivnija istraživanja. U 2014., u razdoblju od kolovoza do studenog, obavljena su uzorkovanja jaja ove vrste metodom ovipozicijskih klopki. Klopke su postavljane na 10 lokaliteta u gradu Osijeku i bližoj okolici. Dobiveni rezultati ukazuju da je tigrasti komarac prisutan i širi se u istočnom dijelu Hrvatske.

Ključne riječi: Azijski tigrasti komarac, *Aedes albopictus*, ovipozicijske klopke, širenje, Osijek
VECTOR *Aedes albopictus* - ASIAN TIGER MOSQUITO IN OSIJEK, CROATIA

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Aedes albopictus, asian tiger mosquito is biologically and medically important species of mosquito, whose distribution has been followed for the last forty years. This species is very aggressive and active during the day and is able to transmit different arboviruses and parasites. Since 2004, when it was first recorded in Croatia, it continues to spread invasively over Croatia. After Zagreb, Istria and Dalmatia asian tiger mosquito began its expansion to the eastern part of the Croatia. In August 2013, *A. albopictus* was observed for the first time in the suburban area of Tenja near Osijek. This was reason enough for more intensive research. In 2014, during the period from August to November, sampling of eggs was carried out with the oviposition traps. Trapping was conducted at 10 sites in the city of Osijek and its surroundings. The results indicate that the tiger mosquito is present and spreading in the eastern part of the Croatia.

Key words Asian tiger mosquito, *Aedes albopictus*, oviposition trap, expansion, Osijek

Biologija kopnenih voda i kopna
Biology of freshwater and terrestrial ecosystems

IO-3

BRYOPHYTE CONSERVATION BIOLOGY IN EUROPE - INSIGHTS INTO CONTEMPORARY ACHIEVMENTS, PROBLEMS, SOLUTIONS AND MANAGEMENT

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Bryophytes are the second largest group of terrestrial plants with 20000 species world-wide (2097 in Europe) and highly neglected in conservation issues. Many species remain in just a few patches across Europe and were not studied previously. The phylogeography and molecular data are needed for *in vitro* propagation, cryopreservation, proper *ex situ* and reintroduction i.e. long-term sustainable conservation. Survival can depend as much on genetic factors such as inbreeding and gene flow as on factors such as population growth rate and distribution. However, what is a population/individual in bryophytes? The phenomena such as bottlenecks, inbreeding depression, hybridization, population subdivision, genetic restoration, invasiveness, reintroductions, fragmentation, mutational meltdown are poorly documented in bryophytes. Thus, bryophyte conservationists have quite few data at start points and face significant challenges. Many new insights comes out during work on specific taxa rescue from extinction. Once the stable production and propagation in *ex situ*/captivity condition have been achieved the legislative and management problems come out. Bryophyte Biology Group in Belgrade (BBGB) has a collection of 131 bryophytes among which 52% represent red-listed species in European, regional or national level, Bern Convention and EU habitat directive. Case studies on active bryophyte conservation, achievements, problems, solutions, further activities will be further presented.

Key words: bryophytes, conservation, Europe, *ex situ*, reintroduction

O-101

PROMJENE SASTAVA IHTIOFAUNE RIJEKE SAVE U HRVATSKOJ TIJEKOM POSLJEDNJEG DESETLJEĆA (2003.-2013.)

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Tijekom redovitog monitoringa rijeke Save na pojedinim lokacijama primijećeno je znatno organsko opterećenje, kao i uzvodno širenje Ponto kaspijskih glavoča (P-C glavoča). Navedeno upućuje na pretpostavku da su ovi elementi mogli utjecati na sastav ihtiofaune rijeke Save. Stoga je napravljena analiza ihtiofaune od 2003. do 2013. za gornji tok rijeke i 2010. do 2013. za donji tok rijeke Save. Korišten je nCPUE (broj jedinki ulova po jedinici napora) 41 vrste prikupljenih riba za analize. Posebno je testirana lokacija Medsave jer su primijećene antropogene promjene u svrhu obrane od poplave. Korištena je RDA i CCA multivarijatna analiza ovisno o duljini testiranog gradijenta. Kvaliteta vode rijeke Save nema utjecaj na sastav ribljih vrsta, čak niti na lokaciji najvećeg organskog opterećenja (Ivanja Reka). Sastav vrsta gornjeg toka (Medsave i Zagreb) se znatno razlikuju od nizvodnih lokacija. Širenje P-C glavoča utječe na smanjenje raznolikosti vrsta, a pretpostavka je da utječu i na ugrožene riblje vrste kao što su *Sabanejewia balcanica* i *Zingel streber*. U posljednje dvije godine u cijelom toku rijeke Save uočen je manji diverzitet ihtiofaune nego ranije. Na lokaciji Medsave se u posljednjih 10-tak godina promijenio sastav vrsta i smanjio diverzitet. Nastavak monitoringa rijeke se preporuča što bi moglo doprinijeti rasvjetljavanju ekoloških interakcija koje određuju uspješnost invazije P-C glavoča u različitim vodenim sustavima te njihovom utjecaju na nativne riblje populacije.

Ključne riječi: monitoring, Ponto kaspijski glavoči, nCPUE, multivarijatna analiza, diverzitet

ICHTHYOFAUNAL CHANGES OF THE RIVER SAVA, CROATIA OVER LAST DECADE (2003-2013)

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During the regular monitoring of the River Sava, organic pollution at particular locations as well as invasive Ponto Caspian gobies (P-C gobies) upstream expansion was noted. It is assumed that these elements could affect the species composition of the Sava. Therefore, an analysis of the ichthyofauna from 2003 to 2013 for upstream and 2010 to 2013 for the downstream locations was performed. nCPUE (catch per unit effort) of 41 fish species caught was used for analysis. Medsave was tested separately because anthropogenic changes of the river bank against flood protection were observed. Depending on the gradient length, RDA and CCA multivariate data analysis was used. The water quality of the Sava has no influence on the fish species composition, even on the location with the largest organic pollution. Species composition of the upper rhitron (Medsave and Zagreb) is dif ferent from the downstream locations. The spread of P-C gobies has effect on species diversity and impact on endangered species such as *Sabanejewia balcanica* and *Zingel streber* is assumed. In the last two years lower ichthyofaunal diversity of the Sava was observed. At the location Medsave the species composition has been changed, and lower diversity was observed as well. Continued monitoring of the river is recommended which could contribute to elucidation of the ecological interactions that determine the successfulness of P-C gobies invasion in different aquatic environments and their influence on native fish populations.

Key words: monitoring, Ponto Caspian gobies, nCPUE, multivariate analysis, diversity

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ELEKTRIČNA POLJA - URBANI PRITISAK NA ORGANIZME U KOPNENIM VODAMA

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Od mnoštva pritisaka kojima čovjek djeluje na svoj okoliš, s biološkog stajališta je relativno najslabije istražen utjecaj elektromagnetskih polja stvorenih elektroenergetskim sustavima. Vjerojatno jer je taj utjecaj razmjerno nedavno došao u žarište pažnje urbanizacijom i posljedično izrazitim rasprostiranjem. Zahvati u okolišu vezani uz elektroenergetske sustave su među najbrže rastućim urbanim pritiscima na prirodu u Europi 21. stoljeća. Na razini Europske unije, koja je već među najurbaniziranijim područjima na svijetu, planirano je približno 20 postotno proširenje elektroenergetske mreže u sljedećih 6 godina. Budući da je relativno poznat štetan utjecaj elektromagnetskih polja na ljude, osnovni poticaj za istraživanje potencijalnog utjecaja na ekosustave je izgradnja trajnih izvora elektromagnetskih polja u prostoru u kojem ih do tada nije bilo. Do sada su vrlo rijetka bila istraživanja utjecaja elektromagnetskih polja na vodne organizme, a u njima nije razlučen utjecaj različitih komponenti tih polja. Cilj ovog istraživanja bio je u laboratorijskim uvjetima istražiti i utvrditi razinu utjecaja električnog polja na vodne organizme. Kao ogledne organizime koristili smo protiste koje smo izlagali električnim poljima pločastih kondenzatora. Utvrdili smo promjene u fiziološkom odgovoru odnosno antioksidativnog stresnog enzima superoksid dismutaze te smanjenje populacije već pri kratkotrajnom izlaganju električnom polju.

Ključne riječi: antropogeni stres, superoksid dismutaza, *Euglena*, *Paramecium*, Hrvatska

ELECTRIC FIELDS - THE URBAN PRESSURE ON FRESHWATER ORGANISMS

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Human pressures on its environment are numerous, and from the biological point of view among the least researched is the impact of electromagnetic fields created by power systems. It came into the focus of attention relatively recently due to increased urbanization and power system propagation. Construction of electric power systems are among the fastest growing environmental pressures in 21st century Europe. At the EU level, which is already among the most urbanized areas in the world, about 20 percent expansion of the electrical network is planned in the next 6 years. Since the adverse effects of electromagnetic fields on humans are known, construction of permanent sources of electromagnetic fields in the areas where they had not been before are the main motivation for the research of potential impact on ecosystems. To date, research of the effects of electromagnet ic fields on aquatic life was rare and they did not distinguish the impact of the field components. The aim of this study was to study and determine the level of influence of the electric field on aquatic organisms in the laboratory. We used protists as model organmisms. We exposed them to electric fields made by plate capacitors. We have identified changes in the physiological response i.e. enzymatic activity of superoxide dismutase and reduction of populations after short-term exposure to an electric field.

Key words: anthropogenic stress, superoxide dismutase, *Euglena*, *Paramecium*, Croatia

O-103
UTJECAJ HIDROLOŠKOG STRESA NA OBRAŠTAJ U NP KRKA

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U genezu sedrenih barijera uključena je i obraštajna zajednica, što je čini jednom od biomedijatora osedravanja. Razvoj obraštaja praćen je na umjetnim podlogama (predmetna stakalca) od rujna 2013. godine do lipnja 2014. godine na sedrenim barijerama Roški slap (RS) i Skradinski buk (SB) u NP Krka u dva reotopa (brzoj i sporoj struji vode). Ukupno je zabilježeno 98 svojti heterotrofnih organizama u obraštaju, od tog 64 svojte trepetljikaša koji su s prosjekom od 80% dominirali i u abundanciji. Usprkos gotovo identičnim fizikalno-kemijskim čimbenicima vode na dvije barijere, primarna produkcija bila je veća na postaji RS. Suprotno tome, heterotrofni organizmi su imali 5 do 18 puta veću abundanciju na postaji SB u odnosu na isti reotop na postaji RS, kao i veću raznolikost zajednice. Skupine Peritrichia i Suctoria dominirale su među trepetljikašima na postaji SB, dok su na postaji RS dominirale pokretne forme, ukazujući na utjecaj uzvodnog jezera Visovac na strukturu zajednice sedrene barijere SB. Vršni protoci zabilježeni tijekom zime i proljeća doveli su manjih promjena u abundanciji, ali velikih promjena u strukturi perifitona. Dok su pokretni oblici i Peritrichia relativno dobro podnijeli hidrološki stres, došlo je do znatnog smanjenja abundancije Suctoria. Negativan utjecaj vršnih protoka zabilježen je i za ameboidne praživotinje, kao i za većinu meiofaune.

Ključne riječi: sedrene barijere, umjetne podloge, trepetljikaši, primarna produkcija

INFLUENCE OF HYDROLOGICAL STRESS ON PERIPHYTON IN KRKA NATIONAL PARK

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Periphyton is involved in genesis of tufa barriers, and thus represents one of biomediators of tufa deposition. Periphyton development was observed using artificial substrate (glass slides) from September 2013 to June 2014 on tufa barriers Roški slap (RS) and Skradinski buk (SB) in Krka National park in two rheotopes (fast and slow current). Overall 98 taxa of heterotrophs were observed, out of which 64 taxa of ciliates. With an average of 80% ciliates also dominated in abundance. Contrary to almost identical physical-chemical water parameters measured on two barriers, praimary production was higher on RS site. Opposite to that, heterotrophic organisms had 5 to 18 times higher abundance on SB site than the same rheotopes in RS site, and the same trend was observed in diversity. Peritrichia and Suctoria dominated between ciliates on SB site, while vagile forms of ciliates dominated periphyton in RS site, indicating the influence of upstream Visovac Lake on community structure in tufa barrier SB. Peak discharges during winter and spring led to minor changes in abundance, but caused major changes in periphyton structure. While vagile forms and Peritirichia coped well with hydrological stress, there was significant decrease in abundance of Suctoria. Negative influence of peak discharges was also observed for ameboid protozoa and most of meiofaunal groups.

Key words: tufa barriers, artificial substrate, ciliates, primary production

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ZAKRIVENOST VEGETACIJOM KAO GLAVNI POKRETAČ STRUKTURE ZAJEDNICE IZVORSKIH DVOKRILACA

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Izvori, a osobito krški izvori, su stabilna staništa sa stalnim abiotičkim faktorima i stoga se mogu koristiti u brojnim ekološkim eksperimentima. Istraživali smo utjecaj zakrivenosti izvora krošnjama na brojnost, raznolikost i hranidbeni sastav zajednice dvokrilaca (Diptera) dvaju bliskih reokrenih krških izvora. Izvori su se prvenstveno razlikovali u pokrovnosti okolne vegetacije, dok su ostale fizikalno-kemijske karakteristike vode bile iste ili slične. Šest emergencijskih klopki je bilo postavljeno na svaki od izvora kroz godinu dana na sva prisutna mikrostaništa. Glavna hipoteza našega rada je da će zakrivenost imati najveći utjecaj na brojnost i sastav vrsta dvokrilaca. Sličnost između izvora je bila samo 37,5%, sa 24 zajedničke vrste/svojte od 73 vrste/svojte koje su nađene. Brojnost dvokrilaca je bila 8,5x veća na slabo zakrivenom izvoru, dok su raznolikost i broj vrsta/svojti bili veći na jako zakrivenom izvoru. Većina vrsta su bili detritivori, a nakon toga sakupljači i nije bilo prevelike razlike između izvora. Zaključak ovog istraživanja je da zakrivenost vegetacijom može biti glavni pokretač promjena u strukturi zajednice dvokrilaca, s brzinom strujanja kao dodatnim faktorom, dok su supstrat i ostali fizikalno-kemijski čimbenici zanemarivi.

Ključne riječi: osvjetljenje, raznolikost vrsta, organska tvar, emergencija, Chironomidae

CANOPY COVERAGE AS A MAIN DRIVER IN STRUCTURING SPRING DIPTERA ASSEMBLAGES

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Springs are stable environments with constant abiotic factors and therefore are of use in variety of ecological experiments. We investigated the influence of canopy coverage on abundance, diversity, and feeding guilds among Diptera assemblages at two rheocrene karst springs located near each other. The springs differed by canopy coverage while other physicochemical characteristics of the water were similar. We set six emergence traps for one year at each spring covering all available microhabitats. We hypothesized that canopy coverage will have a strong effect on assemblage composition of Diptera as well as on diversity; abundance and feeding guilds between sites and that it will have a stronger effect than microhabitat characteristics. Similarity among springs was only 37.5%, with 24 common species/taxa out of 73 species/taxa. Abundance of Diptera was 8.5x higher at open canopy spring, while diversity and number of species/taxa was higher at closed canopy spring. Majority of species were detritus feeders followed by collectors and there was no substantial difference among sites. We conclude that at springs with similar water characteristics, canopy coverage is the main driver of Diptera assemblage structure, with water velocity as a complementary factor. Substrate and other physicochemical factors are unimportant.

Key words: Illuminance, species diversity, organic matter, emergence, Chironomidae

O-105

RAZNOLIKOST STANIŠTA LITORALNE ZONE KAO ODREDNICA SASTAVA ZAJEDNICE PUŽEVA U PLITKOM EUTROFNOM JEZERU

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Glavni cilj ovog rada bio je utvrditi utjecaj makrofitskih sastojina na raznolikost, brojnost, uzrasnu (veličinsku) i trofičku strukturu puževa u rukavcu rijeke Sutle. Rukavac se sastoji od dva bazena, jednog veće prozirnosti s dobro razvijenim submerznim makrofitima kompleksnog habitusa, dok je drugi bazen bez makrofita i veće mutnoće. Istraživanje je provedeno tijekom dviju vegetacijskih sezona. Tijekom istraživanja determinirano je 10 vrsta puževa, od kojih su četiri vrste zajedničke za oba bazena. U litoralnoj zoni bazena bez makrofitskih sastojina puževi su zabilježeni sporadično, s vrlo malom brojnošću. Veća brojnost jedinki utvrđena je u bazenu s razvijenim makrofitima, a vrsta koja je dominirala tijekom istraživanog razdoblja je *Gyraulus laevis*. Analiza uzrasne strukture puževa ukazuje da su u kasno proljeće i ljeto dominirale juvenilne jedinke, a udio odraslih jedinki bio je značajniji tek u jesenskom razdoblju. Od funkcionalnih hranidbenih skupina puževa najveći udio imali su strugači koji su izvore hrane nalazili u obraštaju (perifitonu), a zatim usitnjivači, koji su se hranili sitnim dijelovima makrofita. Rezultati ovog rada ukazuju na važnost raznolikosti mikrostaništa u sastojinama submerznih makrofita koja su puževima predstavljala raznolik i bogat izvor hrane, sigurno mjesto za mrijest te sklonište od predatora.

Ključne riječi: mekušci, makrofiti, brojnost jedinki, uzrasna struktura, funkcionalne hranidbene skupine

HABITATS HETEROGENEITY OF THE LITTORAL ZONE IN THE DETERMINATION OF SNAIL ASSEMBLAGES IN THE SHALLOW EUTROPHIC LAKE

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The main objective of this study was to determine the effect of macrophyte stands on diversity, abundance, age (size) and trophic structure of snails in the backwater of the Sutla River. The backwater consists of two pools, one with transparent and well-developed submerged macrophytes with complex habitus, while the other pool is turbid and without submersed macrophytes. The study was conducted throughout two vegetation seasons. During the study 10 species of snails were determined, of which four species were common to both pools. In the littoral zone of the pool without macrophytes, snails have been recorded sporadically and in very low abundance. Increased abundance of snails was found in a pool with macrophyte stands, with species *Gyraulus laevis* being dominant. Snails age structure analyses indicate that juveniles dominate in the late spring and summer, and the s hare of adults was significant in the fall. From snails functional feeding groups the largest share had scrapers, feeding on periphyton, then grazers, feeding on small parts of macrophytes. Results of this study indicate the importance of the microhabitats diversity in the stands of submerged macrophytes for snails, because those habitats provide diverse and rich source of food, safe place for spawning and shelter from predators.

Key words: molluscs, macrophytes, abundance, age structure, functional feeding guilds

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ISTRAŽIVAČKA AKTIVNOST DINARSKOG VOLUHARA (*Dinaromys bogdanovi* Martino, 1922)

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Kompleksnost staništa ima značajan utjecaj na korištenje staništa, migraciju i osvajanje novog teritorija. Populacije dinarskog voluhara (DV) su fragmentirane i kompleksnost staništa mogla bi značajno utjecati na povezanost između populacija. Četrnaest jedinki DV promatrano je kako bi se proučile njihove bihevioralne i kognitivne karakteristike. Provedena su dva oblika istraživanja za proučavanje njihove istraživačke aktivnosti. Postav s novim teritorijem osmišljen je za promatranje ponašanja u potpuno novom okolišu. Čine ga tri varijante različite kompleksnosti: otvoreni teritorij, teritorij s nekoliko prepreka i teritorij s mnogo prepreka. Postav s mirisima osmišljen je za promatranje ponašanja u prividno zauzetom teritoriju. Čine ga četiri varijante: u svakoj varijanti teritorij je označen jednim mirisom, i to mirisom mužjaka ili ženke iste vrste, mirisom mužjaka druge vrste (laboratorijski miš) i mirisom predatora (poskok, *Vipera ammodytes*). Sva istraživanja su snimana u istom, prethodno određenom razdoblju dana, u trajanju od jednog sata. Analizirano je 25 elemenata ponašanja. Uočene su razlike u ponašanju ovisno o kompleksnosti varijante u postavu s novim teritorijem te ovisno o spolu u postavu s mirisima. Rezultati ovog istraživanja mogu poslužiti za procjenu mobilnosti DV u prirodnom staništu i ugroženosti izoliranih populacija.

Ključne riječi: ponašanje, kompleksnost staništa, teritorijalnost, međuvrsni i unutarvrsni odnosi, mobilnost

EXPLORATORY ACTIVITY OF MARTINO'S SNOW VOLE (*Dinaromys bogdanovi* Martino, 1922)

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Habitat complexity has a great influence on habitat utilization, migration and new territory invasion. Martino's snow vole (MSV) occurs in fragmented populations and habitat complexity could significantly affect interpopulation connectivity. Fourteen specimens of MSV were observed to study their behavioral and cognitive characteristics. Two types of experiments were performed to study their exploratory activity. New Territory Setup was designed to monitor behavior in a completely new environment. It consisted of three trials of varying complexity; open field, field with a few obstacles, and field with many obstacles. Scent Setup was designed to monitor behavior in a seemingly occupied territory. It consisted of four trials; territory was marked with scent of a male or female conspecific, a heterospecific male (laboratory mouse), and a predator (*Vipera ammodytes*), respectively. Each trial lasted one

hour and was recorded during the same predefined period of day. Twenty-five behavioral elements were analyzed. Differences in behavior were observed depending on setup complexity in the New Territory Setup, and depending on sex in the Scent Setup. Results from this study could be used to assess mobility of MSV in natural habitat and to estimate endangerment of isolated populations.

Key words: behavior, habitat complexity, territoriality, conspecific and heterospecific interactions, animal mobility

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PRVI PODACI O AKUSTIČNOM REPERTOARU DINARSKOG VOLUHARA (*Dinaromys bogdanovi*) U ZATOČENIŠTVU

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Glasanje glodavaca bitna je komponenta njihovog ponašanja, a u slučaju dinarskog voluhara (*Dinaromys bogdanovi*) potpuno neistraženo područje. Istraživanje akustičnog repertoara tog endemičnog glodavca provedeno je na 15 odraslih jedinki u prostorijama Zoološkog vrta grada Zagreba. Cilj je bio odrediti glasanje jedinki te utvrditi različitosti u glasanju u ovisnosti o okolišnim uvjetima. S tom svrhom smo životinje testirali u različitim postavima uključujući novi teritorij, prisutnost mirisa te prisutnost drugih jedinki iste vrste ili drugih vrsta. Glasanja smo snimali u realnom vremenu mikrofonom UltraMic 200K. Sonografskom analizom akustičnih parametara utvrđeni su frekvencijski modulirani jednostavni signali s uzlaznom, silaznom, uzlazno-silaznom tendencijom; signali konstantne frekvencije te kompleksni signali sastavljeni od više komponenata koji uključuju harmonike, frekvencijske korake i skokove. Ustanovljeno je da prilikom socijalnog kontakta jedinki dolazi do glasanja u zvučnom i ultrazvučnom spektru s velikom varijacijom tipova ultrazvučnih signala dok je prilikom osvajanja prostora ili stimulacije mirisom druge jedinke količina i učestalost glasanja smanjena na minimum. Ovim istraživanjem dobiveni su prvi podaci o vokalizaciji kod dinarskog voluhara, varijabilnosti i mogućoj važnosti glasanja kod ove vrste endemskog glodavca.

Ključne riječi: ultrazvučno glasanje, međuvrsna i unutarvrsna komunikacija, Rodentia

FIRST DATA ON THE ACOUSTIC REPERTOIRE OF BALKAN SNOW VOLE (*Dinaromys bogdanovi*) IN CAPTIVITY

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Rodents' vocalisation is an important component of their behaviour, and in the case of the Balkan snow vole (*Dinaromys bogdanovi*) it is completely unknown. The study on vocalisation of this endemic rodent was conducted on 15 adult specimens housed at the Zagreb Zoo. The aim of the research was to identify the acoustic repertoire of this species and to determinate differences in acoustic signals in array of environmental situations. The animals were tested in various settings,

including new territory, presence of scent or presence of conspecific or heterospecific. The vocalisation was recorded in real time with a UltraMIc 200K microphone. The sonographic analysis of acoustic parameters has determined simple signals with frequency modulation, including high, low, and high-low tendencies; signals of a constant frequency and complex signals based from multiple components including harmonics, frequency steps and leaps. Our results showed that vocalisation in the sonic and ultrasonic spectra with a high variance of ultrasonic signals could be found during a specimen's social contact, while during spatial exploration or scent simulation of another species, the variability and frequency of the calls were reduced to a minimum. This study is the first report of the vocal repertoire of *D. bogdanovi*, variability and possible importance of vocalisation for this highly endemic rodent species.

Key words: ultrasonic calls, interspecies and intraspecis communication, Rodentia

Toksikologija i ekotoksikologija
Toxicology and ecotoxicology

IO-4
KVALITATIVNI PRISTUP EKOTOKSIKOLOŠKIM ISTRAŽIVANJIMA – FORMALNA POTREBA ILI NUŽNOST

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Kvalitativna istraživanja su primarna znanstvena istraživanja koja, nakon detekcije problema ili fenomena, kao rezultat donose hipoteze ili objašnjenja njihovih uzroka, mehanizama njihovog nastanka, te deskripcije njihovih posljedica. Kvantitativna istraživanja nalaze svoje hipoteze u rezultatima kvalitativnih istraživanja no njihov cilj je kvantifikacija problema ili fenomena u smislu generiranja podataka dostatnih za statističku analizu i, u najboljem slučaju, za matematički opis fenomena ili problema. Potreba postojanja kvalitetnih kvantitativnih podataka koji zadovoljavaju potrebe suvisle statističke analize vrlo često se u praksi smatraju formalnom potrebom pa se na taj način statističke metode i koriste. Zbog toga vrlo često među dobivenim rezultatima ostaju skriveni brojni implicitni podaci, fenomeni ostaju opisani na razini deskripcije, a problemi vrlo često bivaju rješavani na krivi način ili uz bespotrebnu štetu. U okviru ovog predavanja biti će najprije prikazana suština korištenja statističkih metoda i matematičkih modela kao korisnog i nužnog alata u ekotoksikološkim istraživanjima izvan okvira formalnih potreba. Nadalje, na nekoliko konkretnih primjera iz prakse, biti će prikazan razvoj ekotoksikoloških pokusa pomoću kvantitativnih metoda i premošćivanje jaza između tzv. teoretičara i eksperimentalista.

Ključne riječi: ekotoksikologija, kvalitativna istraživanja, kvantitativna istraživanja, matematičko modeliranje, statistika

THE QUALITATIVE APPROACH IN ECOTOXICOLOGICAL STUDIES – A FORMAL NEED OR A NECESSITY

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Qualitative research is a basic scientific research which, after the detection of a problem or a phenomenon, as a result brings a hypothesis or an explanation of their causes, a mechanisms of their formation, and descriptions of their consequences. Quantitative research find their hypothesis in the results of qualitative research but their goal is a quantification of problems and phenomena in the sense of generating sufficient data for a statistical analysis and, at best, for a mathematical description of a phenomenon or a problem. The need for quality quantitative data that meet the needs of a meaningful statistical analysis in practice is very often being considered a formal need and in such manner the statistical methods are being used. Therefore, very often among the obtained results numerous implicit data remain hidden, phenomena remain at the level of mere de scription and the problems are very often being resolved in a wrong way or with an unnecessary damage. Within this lecture the essence of usage of statistical methods and mathematical models as a useful and necessary tool in ecotoxicological studies outside the framework of a formal requirements will be shown. Furthermore, in a number of practical examples, the development of ecotoxicological experiments using quantitative methods and bridging the gap between so called theoreticians and experimentalists will be shown.

Key words: ecotoxicology, qualitative research, quantitative research, mathematical modelling, statistics

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UTJECAJ PESTICIDA NA ŽABLJA JAJA

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Vodozemci su izuzetno osjetljivi na promjene u okolišu. Već i najmanja promjena okolišnih čimbenika za posljedicu ima mjerljive promjene u dinamici njihovih populacija. Okolišni stres, kao što je stres uzrokovan pesticidima, stoga bez sumnje za posljedicu ima promjene, najčešće negativne, u osobinama populacija vodozemaca (smanjenje abundance do potpune ekstinkcije). Iako je općepoznato da pesticidi mogu negativno djelovati na slatkovodnu faunu, vrlo malo se zna o njihovom djelovanju na jaja žaba, pošto se smatra da su ona dovoljno zaštićena želatinoznim ovojem koji obavija sam embrij. Stoga je svrha ovog rada istražiti djelovanje tri pesticida, dimetoata, glifosata i pirimifos-metila, čija je upotreba vrlo učestala, na jaja smeđe šumske žabe, *Rana dalmatina*. Istražili smo utjecaj jednokratnog izlaganja jaja šumske smeđe žabe navedenim pestic idima u okolišno relevantnim koncentracijama te smo pratili brzinu i uspješnost njihovog izlijeganja i mortalitet. Sva tri pesticida su korištena u dvije serije po 200 jaja. Osim toga, mjerili smo i veličinu izleglih punoglavaca tijekom razvoja te ih uspoređivali s kontrolnom skupinom. Naši pokusi su pokazali da postoji statistički značajna razlika između kontrolne skupine i skupina izloženih djelovanju pesticida, kao i razlika između različitih pesticida. Najniža izležljivost je zabilježena kod skupine izložene djelovanju herbicida glifosata, dok je najmanji utjecaj zabilježen kod skupine izložene djelovanju insekticida pirimifos-metila.

Ključne riječi: Amphibia, Anura, *Rana dalmatina*, pesticidi

EFFECTS OF PESTICIDES ON FROGS' EGGS

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Amphibians are very susceptible to various threats posed by the environment. Sometimes, even the slightest change can cause measurable shifts in population dynamics. Environmental stress, such as stress caused by pesticides, can result in changes in population characteristics (mostly negative; decrease of abundance to extinction). Although it is known that pesticides can negatively affect freshwater fauna, little is known on the effects of pesticides on frogs' eggs since they are generally considered to be well protected by gelatinous substance surrounding the embryos. Thus, the purpose of our research was to examine the influence of three commonly used pesticides, dimethoate, glyphosate and pirimiphos-methyl on eggs of agile frog, *Rana dalmatina*. We tested the influence of a one-time exposure, at the beginning of the experiment, of environmentally relevant concent rations of the aforementioned pesticides on the agile frogs' eggs, and we measured time to hatching and hatching rate/mortality. Each pesticide was used in two replicates, with 200 tadpoles in each tank. We also monitored the survival rate and body size of hatched tadpoles in comparison to the control group. The number of tadpoles hatched differed significantly between the pesticides and the control group, but also between different pesticides. The lowest hatching and survival rate was found with herbicide glyphosate, while insecticide pirimiphos-methyl had the lowest impact of all the examined pesticides.

Key words: Amphibia, Anura, *Rana dalmatina*, pesticides

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SPERMIOTOXICITY AND EMBRYOTOXICITY OF ESBIOTHRIN IN SEA URCHIN *Paracentrotus lividus* USING SHORT-TERM MARINE TOXICITY LARVAL DEVELOPMENT METHODS

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Ecotoxicological and early life stage embryotoxicity data lacks for widely used synthetic pyrethroids. Comprehensive risk assessment necessitates larval development studies with marine organisms. In this study we evaluated esbiothrin effects on sperm toxicity and embryo development in model organism the purple sea urchin (*Paracentrotus lividus*). Six esbiothrin exposure concentrations on sperm toxicity and plutei development were tested: 10, 20, 40, 65, 100 and 300 µg/L. Sperm were exposed for 30 min, then allowed to fertilize ova during 30 min for the spermiotoxicity bioassays; evaluation based on fertilization rate as percent fertilization determined on a random sample of 100 eggs. Embryotoxicity bioassay: determined by comparing the frequency of normal development and malformations (skeletal deformation and developmental retardation) after 48-72 h exposure to esbiothrin throughout their development (pluteus stage) and 100 larvae/exposure concentration were evaluated for skeletal deformations and undeveloped; compared with the normal. Esbiothrin inhibited fertilization success in a concentration dependent manner, with IC_{25} and IC_{50} values of 17.7825 (CL = 13.6959–47.1007) and 60.5234 (CL = 54.8970–67.2627) µg/L, respectively. As normal pluteus frequency decreased; an increase in malformations as skeletal deformities were recorded, esbiothrin is highly toxic to embryos than to sperm, and may present a potential risk for the sea urchin in contaminated marine environments.

Key words: Spermiotoxicity, marine bioassay, embryotoxicity, sea urchin, esbiothrin, *Paracentrotus lividus*

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DINAMIKA RASTA ALGI NA MEDIJIMA S DODATKOM CRVENOG MULJA

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Optimalno suvremeno rješavanje okolišnih problema podrazumijeva multidisciplinaran pristup i po mogućnosti obuhvaćanje više problema u jednom rješenju. Nezaobilazan nusprodukt proizvodnje aluminija je crveni mulj. Produkcija aluminija raste, a tako i količina crvenog mulja koji se smatra toksičnim otpadom, te se većinom odlaže u umjetne ili prirodne depresije. Dobivanje energije iz obnovljivih izvora je civilizacijski prioritet. Jedan od smjerova razvoja novih energetskih tehnologija je i dobivanje energenata iz algi. Dosadašnja istraživanja su pokazala da bi se upotrebom crvenog mulja u proizvodnji algi, s jedne strane mogao smanjivati potencijal toksičnosti crvenog mulja, a s druge strane povećati proizvodnja algi koje bi mogle biti korištene za dobivanje energenata. U ovom radu korišten je crveni mulj iz Mađarske (Ajka rezervoar). Mjerene su razlike u brzini i dinamici rasta algi u ovisnosti o količini upotrijebljenog crvenog mulja, kao i promjene toksičnog potencijala crvenog mulja izmjerene setom biokemijskih markera na crvenoj kompoštarki (*Eisenia fetida*). Rezultati su pokazali statistički značajnu i mjerljivu razliku u toksičnom potencijalu smjese crvenog mulja i medija za uzgoj alga, prije i nakon uzgoja u trajanju od 21 dan. Usporedba dinamike rasta alga u mediju bez i sa sadržajem crvenog mulja pokazala je primjenjivost crvenog mulja u postupku uzgoja algi.

Ključne riječi: crveni mulj, uzgoj algi, dinamika rasta, toksični potencijal, *Eisenia fetida*

ALGAE GROWTH DYNAMICS ON MEDIUM SUPLEMENTED WITH RED MUD

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Optimal modern solving of environmental issues implies a multidisciplinary approach and possibly including multiple problems in one solution. An unavoidable by-product of aluminum production is red mud. Production of aluminum increases, and thus the amount of red mud, which is considered hazardous waste and is mostly deposited in artificial or natural depressions. Obtaining energy from renewable sources is a civilization priority. One of development directions of new energy technologies is getting energy from algae. Previous research has shown that the use of red mud in algae production could on the one hand reduce potential toxicity of red mud and on the other hand increase the production of algae that could be used to produce energy. Red mud used in this study is from reservoirs of alumina plant in Ajka, Hungary. Differences in speed and dynamics of algae growth

depending on the amount of used red mud were measured, as well as changes in toxicity potential of the mud measured with biochemical markers on earthworm *Eisenia fetida*. The results showed statistically significant and measurable difference in toxicity potential of the mixture of red mud and medium for algae growth, before and after the cultivation period of 21 days. A comparison of algae growth dynamics in the medium with and without the content of red mud showed applicability of red mud in the process of algae cultivation.

Key words: red mud, algae cultivation, growth dynamics, toxicity potential, *Eisenia fetida*

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ELECTROMAGNETIC FIELDS AT THE MOBILE PHONE FREQUENCY (900 MHz) TRIGGER THE ONSET OF THE GENERAL STRESS RESPONSE ALONG WITH DNA MODIFICATIONS IN *Eisenia fetida* EARTHWORMS

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Eisenia fetida earthworms were exposed to electromagnetic field (EMF) at the mobile phone frequency (900 MHz), and at field levels ranging from 10 to 120 V m^{-1} for a period of 2 h. Potential effects of longer exposure (4 h), field modulation, and recovery period of 24 h after 2 h of exposure were addressed at the field level of 23 V m^{-1}. All exposure treatments induced significant DNA modifications as assessed by a quantitative random amplified polymorphic DNA-PCR. Even after 24 h of recovery following a 2 h exposure, the number of probe hybridization sites displayed a significant 2-fold decrease as compared to untreated control earthworms, implying a loss of hybridization sites. Expression of genes involved in the response to general stress (*HSP70* and *MEKK1*, the latter involved in signal transduction), oxidative stress (*CAT*, encoding catalase), chemical and immune defence (*LYS*, encoding lysenin, and *MYD*, encoding a myeloid differentiation factor) were up regulated after exposure to 10 and modulated 23 V m^{-1} field levels. Western blots showing an increased quantity of HSP70 and MTCO1 proteins confirmed this stress response (MTCO1 is the subunit 1 of cytochrome c oxidase, indicating a mitochondrial impact). *HSP70* and *LYS* genes were found up regulated after 24 h of recovery following a 2 h exposure, meaning that the effect of EMF exposure lasted for hours.

Key words: DNA modifications, earthworm, exposure to electromagnetic field, stress, Western blotting

O-112

ANALIZA PAH METABOLITA U ŽUČI ODABRANIH KOMERCIJALNIH RIBA DUŽ ISTOČNE JADRANSKE OBALE

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Kao biomarkeri izloženosti nafti i kao mogući pokazatelj kvalitete morskog okoliša analizirani su metaboliti policikličkih aromatskih ugljikovodika (PAH) u žuči komercijalnih vrsta riba *Mugil cephalus*, *Sarpa salpa* i *Mullus barbatus* sa postaja: Ploče (F2), Split (F4), Šibenik (F5), Rijeka (F8) i Rovinj (F9). Ribe su kupljene svježe na lokalnim ribarnicama s šireg istraživanog područja jer ih je bilo nemoguće uloviti na lokacijama koje su ispitivane u okviru pilot projekta Jadran II. Vrste riba su odabrane prema hranidbenoj poziciji: *M. cephalus* (vodeni stupac, svejed), *S. salpa* (vodeni stupac, biljojed) i *M. barbatus* (dno, detritofag). Osim morfoloških pokazatelja za identifikaciju jedinki primijenjena je i analiza cox1 gena (barcoding). PAH metaboliti su analizirani pomoću fluorimetra i fluorescencije fiksnih valnih duljina (FF): 290/335, 341/383 i 380/430 nm (pobuda/emisija). FF 290/335, pretežno mjeri naftalenske vrste PAH metabolita, benzo[a]piren vrste metabolita najbolje su detektirane na FF380/430, a FF341/383 uglavnom mjeri metabolite pirena. Rezultati pokazuju pretežno izloženost riba naftalen-vrsti metabolita FF290/335 (304±91), s vrijednostima cca. 10-30 puta višim za od ostale dvije vrste metabolita FF341/383 (24±6) i FF380/430 (14±4). Nadalje, ukupne vrijednosti PAH metabolita svih triju vrsta riba rangirale su istraživana područja obzirom na izloženost riba nafti, odnosno policikličkim aromatskim ugljikovodicima kako slijedi: F5>F9>F4>F2>F8.

Ključne riječi: PAH, ribe, Jadransko more, nafta, onečišćenje

BILE PAH METABOLITES CONTENT IN SEVERAL COMMERCIAL FISHES ALONG EASTERN ADRIATIC COAST

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Bile PAH metabolite content in selected commercial species *Mugil cephalus*, *Sarpa salpa* and *Mullus barbatus* purchased from local fish markets: Ploče (F2), Split (F4), Šibenik (F5), Rijeka (F8) and Rovinj (F9) were analyzed as a biomarker of exposure to oil and possible indicator of marine environment quality. Local fish markets were used because it was not possible to cath chosen species at sites investigated in the frame of pilot Project Adriatic II. Fishes were selected according food web positions: *M. cephalus* (water column, omnivore), *S. salpa* (water column, herbivore) and *M. barbatus* (benthic, detritofag). The analysis of cox1 gene was applied as tool for fish specimens' identification (barcoding). PAH metabolites were analyzed in bile by means of fixed wavelength fluorescence (FF) measurement using fluorimeter excitation/emission wavelength pairs 290/335, 341/383, and 380/ 430 nm. By FF290/335, mainly naphthalene type of metabolites, typically associated with petroleum products are detected, benzo[a]pyrene type of metabolites are best detected by FF380/430 and by FF341/383 mainly pyrene-derived metabolites. Results indicate mostly naphthalene-type fish exposure cca. 10-30 times higher values for FF290/335 (304±91), than two other types FF341/383 (24±6) and FF380/430 (14±4). Further, total sum values of all three fish species ranked fish contaminant exposure at investigated areas as follows: F5>F9>F4>F2>F8.

Key words: PAH metabolites, fish, Adriatic Sea, oil, pollution

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ALERGENI GRINJA, ŽOHARA I PLIJESNI U SEDIMENTIRANOJ PRAŠINI U KUĆANSTVU U HRVATSKOJ

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Svrha rada bila je ustanoviti razine alergena unutarnjih prostora koji su povezani sa zdravstvenim rizicima. U sedimentiranoj prašini odredili smo koncentracije alergena: grinja *Dermatophagoides pteronyssinus* (Der p1) i *Dermatophagoides farinae* (Der f 1), žohara *Blatella germanica* (Bla g 1), te plijesni *Alternaria alternata* (Alt a 1) i *Aspergillus fumigatus* (Asp f 1). Razina alergena grinja Der p 1 i Der f 1 veća od 2 μg/g prašine povezana je s alergijskom senzibilizacijom, a razina veća od 10 μg/g s kliničkim respiratornim simptomima. Sakupljeno je 30 uzoraka sedimentirane prašine uzorkovane u kućanstvima sa zagrebačkog područja. Nakon ekstrakcije, alergeni su određeni standardnom enzim-imunokemijskom metodom (ELISA) (Indoor Biotechnologies Ltd, Cardiff, UK). Alergeni grinje ustanovljeni su u 75% kućanstava. Koncentracija Der p 1 bila je u rasponu od 0,1-12,5 μg/g, a Der f 1 od 0,10-3,2 μg/g. Koncentracija Der p 1 + Der f 1 > 2 μg/g, povezana s povećanim rizikom za senzibilizaciju, ustanovljena je u 27% kućanstva, a Bla g 1 u manje od 3% kućanstva. Alergeni plijesni Alt a 1 i Asp f 1 nisu ustanovljeni niti u jednom uzorku kućne prašine (ispod granice detekcije metode). Zaključuje se da su alergeni grinja najčešći alergeni u kućanstvima zagrebačkog područja.

Ključne riječi: alergeni unutarnjih prostora, grinje kućanske prašine, žohari, plijesni, Der p 1

ALLERGENS OF MITES, COCKROACHES AND MOULDS IN SETTLED DUST IN HOUSHOLDS IN CROATIA

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We evaluated the level of main allergens of indoor environment associated with health effects in man. We have determined the level of dust mites allergens Der p 1 (*Dermatophagoides pteronyssinus*) and Der f 1 (*Dermatophagoides farinae*), cockroach allergen Bla g 1 (*Blatella germanica*), and mould allergens Alt a 1 (*Alternaria alternata*) and Asp f 1 (*Aspergillus fumigatus*). For mite allergens Der p 1 and Der f 1 a threshold levels of 2 and 10 μg/g of dust have been proposed for development of allergic sensitization and respiratory symptoms, respectively. A total of 30 dust samples were collected in urban homes in Zagreb. After extraction, all allergens were detected by using standard enzyme immunoassays (ELISA) (Indoor Biotechnologies Ltd, Cardiff, UK). Mite allerges were detected in 75% of households. The concentration of Der p 1 and Der f 1 were in the range 0.1-12.5 μg/g and 0.10-3.2 μg/g, respectively. While the levels of Der p 1 + Der f 1 >2 μg/g associated with increased risk of sensitization were found in 27% of homes, this risk was found in less than 3% of dust samples for cockroach allergen Bla g 1. Mold allergens Alt a 1 and Asp f 1 were not detected in any dust samples (lower than limit detection of method). The results suggest that mite allergens are the most common indoor allergens in households in Zagreb area.

Key words: indoor allergens, house dust mites, cockroaches, moulds, Der p 1

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SINERGISTIČKI UČINCI VAZODILATACIJSKIH I ANTIKOAGULACIJSKIH LIJEKOVA NA OKSIDATIVNI STRES U MOZGU

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Prethodna toksikološka istraživanja ukazuju da su krvno moždana barijera i krvotok unutar središnjeg živčanog sustava vrlo osjetljivi na učinke lijekova s vazodilatacijskim ili antikoagulativnim svojstvima. Dobro su opisani štetni učinci oksidativnog stresa na staničnoj razini uzrokovanog lijekovima uobičajenima u domaćinstvu, koji se uzimaju bez recepta (acetilsalicilna kiselina) te biljnih lijekova i tableta (npr. ginko) ili lijekova na recept s antikoagulativnim svojstvima (varfarin). Mnogo manje se zna o međusobnim interakcijama navedenih tvari na razine oksidativnog stresa, osobito u mozgu. U okviru ove studije Y59 štakori tretirani su dnevno s odgovarajućim preporučenim dozama i kombinacijama acetilsalicilne kiseline, ginka i varfarina, tijekom 15 dana. Razina oksidativnog stresa procijenjena je mjerenjem četiri markera oksidativnog stresa; superoksid dismutaze (SOD), katalaze (CAT), glutationa (GSH) i malondialdehida (MDA) u različitim regijama mozga. Rezultati su pokazali promjenjene razine oksidacijskog stresa u prefrontalnom korteksu, kortikalnoj regiji i malom mozgu izloženih životinja. Ove promjene ukazuju na sinergistički toksični potencijal izazvan simultanim uzimanjem navedenih lijekova.

Ključne riječi: mozak, oksidativni stres, antikoagulativni i vazodilatacijski lijekovi

SYNERGISTIC EFFECTS OF VASODILATATIONAL AND ANTICOAGULATIVE DRUGS ON BRAIN OXIDATIVE STRESS

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Previous toxicological research showed that blood brain barrier and brain vascular system are highly susceptible to the effects of pharmaceuticals with vasodilatation or anticoagulative properties. The adverse effects on cellular oxidative stress caused by over the counter (OTC) of household drugs (acetylsalicylic acid), herbal remedies and pills (for example gingko) or prescription drugs (warfarin) are well described. Much less is known about mutual interaction between these substances on oxidative stress levels especially in the brain. Within this study Y59 rats were treated daily with corresponding recommended doses and combinations of acetylsalicylic acid, ginkgo and warfarine during 15 days. Four markers of oxidative stress were assessed; superoxide dismutase (SOD), catalase (CAT), gluthathione (GSH) and malondialdehyde (MDA) in various brain regions. The results have shown changes of oxidative stress levels in prefrontal cortex, cortical regions and cerebellum of exposed animals. These changes implicate synergistic and potentiated toxic potential of combinations of named drugs.

Key words: brain, oxidative stress, vazodilatational and anticoagulative drugs

Mikrobiologija
Microbiology

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KOMPARATIVNA ANALIZA GENOMA FITOPLAZMI: OD NESTABILNOSTI GENOMA DO PRILAGODBE NA DOMAĆINE

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Fitopatogene bakterije roda '*Candidatus* Phytoplasma' malih su genoma koji su kroz dinamičnu koevoluciju s biljnim domaćinima i kukcima-vektorima doživjeli specifične gubitke i dobitke gena. Još uvijek ih nije moguće uzgojiti u čistoj kulturi *in vitro*. Unatoč tome, u potpunosti su sekvencirana i dostupna 4 fitoplazmatska genoma uz nekolicinu nepotpunih. Iako reducirani, genomi fitoplazmi bogati su ponavljajućim sljedovima. Ponavljajući sljedovi nazvani „potencijalne pokretne jedinice“ (*potential mobile units*; PMUs) nalik su replikativnim transpozonima i mogu postojati kao kružni ekstrakromosomski elementi s povećanom ekspresijom u kukcima u odnosu na biljke. PMUs doprinose varijacijama u veličini te visokoj plastičnosti genoma i prilagodbi na različite domaćine. Jedan od ponavljajućih gena jest i gen ssb, Samo jedna kopija gena ssb u genomu im a drugačije evolucijsko porijeklo, nalazi se u specifičnom genomskom okruženju te se smatra izvornom kopijom. Izvorna kopija gena ssb izolata vrsta '*Ca*. P. asteris' i '*Ca*. P. solani' umnožena je i sekvencirana. Filogenetskom analizom pokazana je očuvanost izvorne kopije gena ssb, uz izuzetak vrste '*Ca*. P. mali' što je u skladu s posebnom organizacijom i stabilnošću genoma ove fitoplazme. Metodom Southern blot, u genomu '*Ca*. P. solani' dokazana je prisutnost još jedne kopije tog gena, što ukazuje na mogućnost postojanja ekstrakromosomskog elementa karakterističnog za ovu vrstu čiji je genom najveći i najnestabilniji među fitoplazmama.

Ključne riječi: fitoplazma, genomika, filogenija, genom, biljni patogen

COMPARATIVE ANALYSIS OF PHYTOPLASMA GENOMES: FROM GENOME INSTABILITY TO HOST ADAPTATION

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Insect-transmitted bacteria from the genus '*Candidatus* Phytoplasma' have small genomes that experienced specific gene losses and gains through their dynamic co-evolution with plant and insect hosts. Axenic cultivation of these bacteria is still challenging. However, a number of genome drafts and 4 fully annotated genomes are available. In spite of being reduced, phytoplasma genomes are repeat-rich and contain multicopy genes. Repeats termed potential mobile units (PMUs) have characteristics of replicative transposons and may exist as circular extrachromosomal elements with enhanced expression in insects compared with plant hosts. PMUs contribute to phytoplasma genome size variations and high plasticity as well as to adaptation to diverse hosts. One of the multicopy genes is the ssb gene with only one copy located in a specific genomic neighbourhood having a diffe rent evolutionary origin. In this study, ssb gene was amplified and sequenced from a number of '*Ca*. P. asteris' and '*Ca.* P. solani'

isolates. Phylogenetic analyses have shown that all “original” ssb genes from different phytoplasmas clustered together. The exception was the '*Ca*. P. mali' single ssb gene, which is in accordance with its' different genome organization and stability. Southern blot analyses have revealed another copy of the ssb gene in '*Ca*. P. solani', suggesting the presence of an extrachromosomal element characteristic of the species having the largest and the most unstable genome among phytoplasmas.

Key words: phytoplasma, plant pathogen, genomics, genome, phylogeny

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MOLEKULARNA TIPIZACIJA UZROČNIKA FILODIJE ULJANE REPICE

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Filodija uljane repice (*Brassica napus* ssp. *oleifera* (DC.) Metzg.) je bolest koja izaziva velike gubitke i do sada je bila povezivana sa zarazom fitoplazmama (rod '*Candidatus* Phytoplasma'). Biljke prikupljene na području Zagreba imale su, tipično za sindrom filodije, virescenciju, male deformirane mahune i reduciran broj sjemenki. Također su zabilježene nekroze stabljika i listova, te kloroze listova tipične za infekciju virusom mozaika repe (*Turnip mosaic virus*; TuMV). Kako bi se otkrio uzročnik bolesti, provedena je molekularna tipizacija fitoplazmi i virusa. Biološka i molekularna istraživanja otkrila su infekciju TuMV-om, a izolat CRO184A klasificiran je u skupinu „world-B“. Filogenija nije pokazala tragove rekombinacija. PCR-RFLP analizom i sekvenciranjem 16S rRNA očekivano je potvrđena prisutnost fitoplazme "aster yellows" (AY, '*Ca*. P. asteris'), soja iz ribosomske podskupine 16SrI-B. Multigenska analiza konzerviranih gena *tufB*, *secY*, *groEL* i ribosomskih proteinskih gena te varijabilnog gena *amp* potvrdila je klasifikaciju fitoplazme. Gen *amp* kodira AY-specifični imunodominantni membranski protein, a promjene u njemu ukazuju na drukčiju prenosivost kukcima-vektorima tj. prilagodbe novom tipu vektora. Molekularnom tipizacijom po prvi puta je detaljno opisana fitoplazma-uzročnik filodije uljane repice, a također je prvi put dokazana koinfekcija repice fitoplazmom i virusom. Ova studija predstavlja osnovu za daljnja istraživanja njihovih sinergističkih učinaka.

Ključne riječi: '*Candidatus* Phytoplasma asteris', genom, MLST, virus mozaika repe

MOLECULAR TYPING IN DECIPHERING ETIOLOGY OF RAPE PHYLLODY DISEASE

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Rape phyllody disease associated with phytoplasmas can decimate the oilseed rape (*Brassica napus* ssp. *oleifera* (DC.) Metzg.) production. Plants sampled in Zagreb area had typical rape phyllody syndrome including virescence, small deformed siliques and seed reduction. Also, atypical stem necrosis, leaf chlorotic spots and necrotic changes reminiscent of a Turnip mosaic virus (TuMV) infection were recorded. Molecular typing was performed aimed at characterizing agents involved in this rape phyllody outbreak. Biological and molecular investigations revealed the infection with TuMV and the isolate CRO184A was classified into the world-B phylogenetic

lineage. No evidence for interlineage recombination was obtained. PCR-RFLP and 16S rRNA gene sequencing identified an aster yellows phytoplasma (AY, '*Candidatus* Phytoplasma asteris') strain of the 16SrI-B subgroup. Multig ene sequence characterization of house-keeping tufB, secY, groEL and ribosomal protein genes plus a highly variable amp gene confirmed its affiliation. The AY specific amp gene encoding an immunodominant membrane protein showed significant variability suggesting altered insect vector specificity and transmissibility. Molecular typing provided the first detailed characterization of '*Ca*. *P. asteris*' strain associated with the rape phyllody disease. Also, this was the first recorded '*Ca*. *P. asteris*' and TuMV co-infection in oilseed rape. This research lays foundation for investigating their possible synergistic effects.

Key words: '*Candidatus* Phytoplasma asteris', MLST, Turnip mosaic virus, genome

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RAZNOLIKOST GLJIVE *Cryphonectria parasitica* U ZARASTAJUĆIM KALUSIMA KESTENA

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Cryphonectria parasitica je patogena gljiva koja uzrokuje rak kestenove kore. Zaraženi pitomi kesteni mogu se oporaviti prirodnim putem ukoliko se gljiva koja je uzrokovala rak zarazi hipovirusom *Cryphonectria hypovirus 1* (CHV1). Time dolazi do konverzije gljive u hipovirulentnu, što omogućuje biološku kontrolu bolesti. CHV1 kao genom ima dsRNA, čija prisutnost u miceliju reducira virulenciju i reproduktivni kapacitet gljive. Ukoliko se CHV1 unese u aktivni rak uzrokovan virulentnim sojem gljive *C. parasitica*, prestaje širenje raka te se formira kalus – zarastajući rak. Zbog toga je važno da hipovirulentni sojevi opstaju u populaciji gljive *C. parasitica*. Cilj ovog istraživanja bio je ustanoviti raznolikost sojeva gljive *C. parasitica* u zarastajućim kalusima kestena u populacijama iz Ozlja i Markuševca u Zagrebu. Uzorci kore prikupljeni su s 24 zarastajuća raka kestena i to iz kalusnog tkiva i tkiva na granici kalusa i debla. Iz uzoraka kore izolirana je čista kultura gljive *C. parasitica*. Prisutnost CHV1 provjerena je izolacijom hipovirusne dsRNA i RT-PCR-om. Zanimljivo je da smo često nalazili virulentne sojeve gljive *C. parasitica* u zarastajućim rakovima, iako hipovirulentni sojevi potiču zarastanje. Pretpostavljamo da se to moglo dogoditi ili zbog toga što se soj koji je uzrokovao zarastanje, nakon nekog vremena spontano oslobodio od virusa, ili zbog naseljavanja novih, virulentnih, sojeva gljive koji bi ponovno mogli izazvati bolest na već zaraslim rakovima.

Ključne riječi: *Cryphonectria parasitica*, rak kestenove kore, *Cryphonectria hypovirus 1* (CHV1), zarastajući rak

DIVERSITY OF *Cryphonectria parasitica* IN HEALED CHESTNUT CANKERS

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Cryphonectria parasitica is a pathogenic fungus that causes chestnut blight. Diseased chestnuts recover naturally if the fungus which caused the canker is infected with *Cryphonectria hypovirus 1* (CHV1). This virus causes the conversion of fungus from virulent into hypovirulent and thus enables biological control of the disease. CHV1 is dsRNA virus without a capsid. The presence of this dsRNA in the mycelium reduces virulence and reproductive capacities of the fungus. If CHV1 is introduced into an active canker caused by virulent *C. parasitica* strain, canker expansion ceases and callus (healing canker) is formed. Therefore, it is important that hypovirulent strains persist in *C. parasitica* populations. The aim of this research was to evaluate the diversity of *C. parasitica* populations in healed cankers in Ozalj and Markuševac in Zagreb. Bark samples were collected from 24 heal ed cankers; from callus tissue and callus-wood interface pure *C. parasitica* cultures were isolated. The presence of CHV1 was confirmed by dsRNA isolation and RT-PCR. It is interesting that virulent *C. parasitica* strains were found quite frequently in healed cankers, although hypovirulent strains are usually associated with healing. We suspect that this is the result of either spontaneous loss of the virus from hypovirulent fungal strain, which previously caused healing or colonization of the callus by new, virulent, fungal strains which could reinitiate the disease on the healed cankers.

Key words: *Cryphonectria parasitica*, chestnut blight, *Cryphonectria hypovirus 1* (CHV1), healed canker

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OPSTOJNOST VIRUSA *Cryphonectria hypovirus 1* MOŽE OVISITI O NJEGOVOM PRIJENOSU IZMEĐU RAZLIČITIH TIPOVA VEGETATIVNE KOMPATIBILNOSTI GLJIVE *Cryphonectria parasitica*

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Cryphonectria parasitica, fitopatogena gljiva koja uzrokuje rak kestenove kore značajno je uništila sastojine pitomoga kestena u Europi. Gljiva je domaćin virusa *Cryphonectria hypovirus 1* koji omogućuje prirodnu biokontrolu ovog patogena. Učestali način prijenosa virusa između različitih sojeva gljive je putem anastomoze hifa. Mogućnost anastomoze regulirana je sustavom vegetativne kompatibilnosti: prijenos virusa najuspješniji je između micelija istog vegetativnog (vc) tipa, dok je između micelija različitih vc tipova otežan. U laboratorijskim uvjetima prenijeli smo različite sojeve virusa u izolate gljive različitih vc tipova. Zamijetili smo da je opstojnost virusa u miceliju akceptoru ovisila o tome pripadaju li donorski i akceptorski micelij istom ili različitom vc tipu. Pretpostavljamo da do gubitka virusa iz micelija dolazi zbog nedovoljne prilagodbe na novog domaćina koji je drugačijeg vc tipa. Rezultati dobiveni *in vitro* ne moraju nužno implicirati isti proces u prirodi, ali čini se da se ovi rezultati podudaraju s rezultatima terenskih istraživanja u kojima je zamijećeno da u kalusima, rak-ranama zaraslima pod utjecajem gljive zaražene virusom, često nakon nekog vremena više ne nalazimo virus.

Ključne riječi: biološka kontrola, invazivna vrsta, virulentnost

PERSISTENCE OF *Cryphonectria hypovirus 1* IN *Cryphonectria parasitica* MIGHT BE AFFECTED BY THE TRANSFER BETWEEN THE DIFFERENT VEGETATIVE COMPATIBILITY TYPES OF THE HOST

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Phytopathogenic fungus *Cryphonectria parasitica*, causing agent of chestnut blight, severely devastated sweet chestnut forests in Europe. This fungus is a host of the virus *Cryphonectria hypovirus 1*, which is a natural biocontrol agent of this pathogen. Hyphal anastomosis is a frequent way of virus transmission between fungal strains. The persistence of anastomosis is regulated by vegetative compatibility (vc) system of the fungus. The most efficient virus transmission occurs between mycelia of the same vc type, but it becomes difficult between mycelia that belong to different vc types. In laboratory conditions, we transferred various virus strains into various fungal isolates belonging to different vc types. The persistence of the virus in the acceptor mycelia was determined by vc types of both, the virus-donor and virus-acceptor mycelia in cases when they belonged to different vc types. We hypothesize that the loss of the virus is caused by unsuccessful adaptation of the virus to the new host of different vc type. These *in vitro* results do not necessarily implicate similar scenario in the natural conditions, but they support observation in nature, where it was found that calli (healed cankers formed under the influence of hypovirulent fungus) often lose viruses with time.

Key words: biological control, invasive species, virulence

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

IO-5
PREDVIĐANJE ISHODIŠNIH STANICA RAKA NA TEMELJU ORGANIZACIJE KROMATINA

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Rak je bolest potencirana somatskim mutacijama, koje nisu jednoliko distribuirane duž genoma stanice raka, već se pojedine genomske regije razlikuju u lokalnoj gustoći somatskih mutacija. Pokazano je da je organizacija kromatina jedna od glavnih odrednica genomske raspodjele somatskih mutacija u stanicama raka. Kako su i somatske mutacije i organizacija kromatina tkivno specifične pojave, koristeći metode računalne biologije usporedili smo raspodjelu mutacija u više neovisnih tipova raka s brojnim epigenetskim podacima mjerenim u različitim

normalnim tkivima. Pokazali smo da epigenetska svojstva i vrijeme replikacije objašnjavaju do 86% varijance u raspodjeli gustoće mutacija duž genoma različitih tipova raka. Najbolja točnost predviđanja lokalne gustoće somatskih mutacija dobivena je upotrebom epigenetskih svojstava stanica u kojima je proučavani tip raka najvjerojatnije nastao. Uz to, otkrili smo da je moguće točnije predvidjeti raspodjelu mutacija u raku na temelju kromatinske strukture stanica u kojima je rak nastao nego na temelju kromatinske strukture odgovarajućih staničnih linija. Nadalje, pokazali smo da se u skoro 90% slučajeva tip stanica iz kojih je rak nastao može precizno odrediti na temelju raspodjele mutacija uz njegovu genomu. Dakle, naši rezultati pokazuju da genom stanica raka obuhvaća mnoštvo informacija o identitetu i epigenetskim obilježjima stanica u kojima je pojedini rak nastao. Taj podatak, pak, može biti izuzetno važan u dijagnozi i tretmanu malignih bolesti.

Ključne riječi: epigenetska obilježja, genom, rak, somatske mutacije

USING CHROMATIN ORGANIZATION TO PREDICT CANCER CELL-OF-ORIGIN

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Cancer is a disease potentiated by mutations in somatic cells, which are not distributed uniformly along the human genome. Instead, different human genomic regions vary by up to fivefold in the local density of cancer somatic mutations. Epigenomic organization has been proposed as a major determinant of the cancer mutational landscape. However, both somatic mutagenesis and epigenomic features are highly cell-type-specific. We investigated the distribution of mutations in multiple independent samples of diverse cancer types and compared them to cell-type-specific epigenomic features. Here we show that chromatin accessibility and modification, together with replication timing, explain up to 86% of the variance in mutation rates along cancer genomes. The best predictors of local somatic mutation density are epigenomic features derived from the most likely cell type of origin of the corresponding malignancy. Moreover, we find that cell-of-origin chromatin features are much stronger determinants of cancer mutation profiles than chromatin features of matched cancer cell lines. Furthermore, we show that the cell type of origin of a cancer can be accurately determined based on the distribution of mutations along its genome. Thus, the DNA sequence of a cancer genome encompasses a wealth of information about the identity and epigenomic features of its cell of origin. This information can be important for the accurate diagnosis and treatment of malignant diseases.

Key words: cancer, epigenomic features, genome, somatic mutation

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REPLIKACIJA U B-STANIČNIM LIMFOMIMA

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Mutacije pojedinih gena smatraju se ključnim koracima tumorigeneze, no to je složen proces koji uključuje i regulaciju transkripcije i adaptaciju tumorskih stanica na signale iz mikrookoliša. Iako tumorske stanice pojedinih vrsta B-limfoma potječu od B-stanica istog stadija diferencijacije, njihova prilagodba na mikrookoliš dovodi do promjene ravnotežnog stanja proteina uključenih u proces replikacije. Replikacija tako postaje manje pouzdana pa dolazi do nakupljanja dodatnih pogrešaka koje uzrokuju bržu progresiju tumora. U ovome je istraživanju analizirana količina transkripata onih gena koji kodiraju ključne proteine prereplikacijskog/replikacijskog kompleksa (PCNA, MCM2, MCM7, GMNN i CDT1) te proteine koji osiguravaju ispravnu uspostavu kromatina nakon prepisivanja DNA (p300, DNMT1 i EZH2). Istraživanje je provedeno na uzorcima B-limfoma različitih stupnjeva agresivnosti. Iz tumorskog tkiva laserskom mikrodisekcijom izdvojene su tumorske stanice i okolno netumorsko tkivo, a razina ekspresije navedenih gena određena je lančanom reakcijom polimerazom u stvarnom vremenu. Kao kontrola služile su tonzile. Rezultati su pokazali značajno povećanu ekspresiju gena *DNMT1, MCM2, CDT1* i *p300* te smanjenju ekspresiju geminina u tumorima veće agresivnosti. Ova studija ukazuje na važnost promjene ekspresije gena uključenih u replikaciju i ponovnu uspostavu kromatina za progresiju malignih tumora.

Ključne riječi: B-stanični limfomi, replikacija, kromatin

REPLICATION IN B-CELL LYMPHOMA

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Mutations of specific genes are considered as key steps in tumorigenesis, but this process equally includes transcription regulation and tumor cell adaptation to the signals from microenvironment. Although tumor cells of specific B-cell lymphoma have origin in B-cells from the same differentiation stage, their adaptation to the microenvironment changes equilibrium of proteins involved in replication process and makes them different from each other. Replication then becomes less accurate leading to the accumulation of aberrations in DNA. Additional changes contribute to tumor progression. In this study we analyzed expression level of prereplication/replication complex proteins (PCNA, MCM2, MCM7, GMNN and CDT1) and proteins included in reassembly of chromatin after the replication (p300, DNMT1 and EZH2). Study was conducted on tissue samples with the diagnosis of diffe rent B-lymphomas. From the tissue samples, using laser microdissection technique, tumor cells and non-tumor parts were isolated. Expression levels were analyzed using real-time quantitative PCR. As a control, tonsils were used. Results showed significant DNMT1, MCM2, CDT1 and p300 overexpression as well as geminin underexpression during B-lymphoma progression. This study suggests importance of changes in expression levels of genes involved in replication and chromatin reassembly for malignant tumor progression.

Key words: B-cell lymphoma, replication, chromatin

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RAZVOJ TESTOVA ZA ANALIZU METILACIJE DNA REGULATORNIH REGIJA GENA GMNN I EZH2 I NJIHOVA PRIMJENA U ISTRAŽIVANJU PODTIPOVA DIFUZNOG B-VELIKOSTANIČNOGA LIMFOMA

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Istraživanja malignih tumora ukazala su na važnost gena uključenih u replikaciju i ponovnu uspostavu kromatina u onkogenezi. Njihova poremećena funkcija može biti povezana s epigenetičkim promjenama. Cilj ovog istraživanja bio je analizirati stupanj metilacije DNA regulatornih regija gena GMNN i EZH2 kod podtipova difuznog B-velikostaničnog limfoma. U tu su svrhu razvijeni testovi za procjenu stupnja metilacije pojedinih CpG mjesta unutar regulatornih regija tih gena. Odabrani su parovi početnica koji lančanom reakcijom polimerazom umnažaju fragmente bisulfitno konvertirane DNA unutar regulatornih regija gena od interesa. Razvijena su tri testa za regulatorne regije gena GMNN i četiri testa za regulatorne regije gena EZH2. Nakon toga određeni su optimalni uvjeti reakcija pirosekvenciranja. Završno je razvijen po jedan test za svaki gen. Ti su testovi primijenjeni u analizi podtipova difuznog B-velikostaničnog limfoma. Analizirani dijelovi regulatornih regija gena GMNN i EZH2 nisu pokazali značajne razlike između podtipova. Budući da su ovim istraživanjem obuhvaćeni relativno mali dijelovi regulatornih regija oba gena, analizu bi trebalo proširiti na dodatna CpG mjesta te uključiti veći broj uzoraka.

Ključne riječi: GMNN, EZH2, DLBCL, pirosekvenciranje, metilacija DNA

ASSAY DEVELOPMENT FOR DNA METHYLATION ANALYSIS OF REGULATORY REGIONS OF GENES GMNN AND EZH2 AND THEIR APPLICATION IN EVALUATION OF DIFFUSE LARGE B-CELL LYMPHOMA SUBTYPES

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Cancer research pointed to the importance of genes involved in replication and reestablishment of chromatin in oncogenesis. Gene dysfunction in tumours is often associated with epigenetic changes. The aim of this study was to analyse DNA methylation of regulatory regions of genes GMNN and EZH2 in diffuse large B-cell lymphoma subtypes. Methylation assays were developed for the analysis of those regions at single CpG site resolution. Primers for PCR-amplification of bisulfite treated DNA fragments were designed. PCR conditions were optimized for the amplification of three and four fragments of regulatory region of GMNN and EZH2, respectively. Subsequently, optimal conditions for pyrosequencing were determined. Finally, one assay per gene was created. Newly developed assays were used in the analysis of diffuse large B-cell lymphoma subtypes. The results showed no statistically significant difference between the subtypes. Since the analysis covered only a small part of the regulatory regions of GMNN and EZH2, the study should be expanded to additional CpG sites and repeated on a larger group of patients.

Key words: GMNN, EZH2, DLBCL, pyrosequencing, DNA methylation

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HISTOKOMPATIBILNOST I IMUNOGENETIKA U TRANSPLANTACIJI SOLIDNIH ORGANA U HRVATSKOJ

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Program transplantacije solidnih organa odvija se u Hrvatskoj od 2007. g. u okviru međunarodne organizacije Eurotransplant. Pristupanje je uz ispunjavanje mnogobrojnih zakonskih i organizacijskih uvjeta, zahtijevalo i posjedovanje laboratorija za tipizaciju tkiva s važećom akreditacijom Europske federacije za imunogenetiku (EFI) kako bi se osigurala standardizirana kvaliteta rada na području histokompatibilnosti i imunogenetike. Ovaj preduvjet Hrvatska je ispunila akreditiranjem Zavoda za tipizaciju tkiva KBC Zagreb (ZZTT) 2007. g., koji je time postao prvi medicinski laboratorij u Hrvatskoj s europskom akreditacijom. U ZZTT danas se provodi imunogenetska obrada bolesnika za prijavu na listu čekanja za 4 transplantacijska centra (KBC Zagreb, KB Merkur, KB Dubrava i KBC Osijek) te imunogenetska obrada kadaveričnih davatelja organa 15 donorskih bolnica. U razdoblju 2007.-2015. godine imunogenetska obrada za listu čekanja napravljena je za 1842 primatelja bubrega, 364 primatelja srca, 1074 primatelja jetre i 92 primatelja gušterače. U istom razdoblju provedeni su imunogenetski testovi za 1423 transplantacije bubrega, 230 transplantacija srca, 774 transplantacija jetre i 75 transplantacija gušterače. Ovako intenzivan program učinio je Hrvatsku jednom od vodećih zemalja u svijetu na području transplantacije organa, čemu Zavod za tipizaciju tkiva KBC Zagreb daje svoj stručan i znanstveni doprinos kao europski akreditiran imunogenetski centar izvrsnosti.

Ključne riječi: imunogenetika, tipizacija tkiva, transplantacija, solidni organi

HISTOCOMPATIBILITY AND IMMUNOGENETICS IN SOLID ORGAN TRANSPLANTATION IN CROATIA

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Transplantation program of solid organs in Croatia is the integral part of Eurotransplant since 2007, when Croatia become a full member of this biggest European non-profit organization for optimal use and cross-border sharing of cadaveric donor organs. Joining Eurotransplant requests fulfilling numerous organizational and legislative prerequisites having an EFI accredited tissue typing laboratory being the one of them. Croatia fulfilled these criteria when Tissue Typing Centre UHC Zagreb obtained the EFI accreditation in 2007, becoming the first Croatian medical laboratory with European accreditation. Since then, Tissue Typing Center Zagreb is responsible for immunogenetic testing of patients from 4 transplantation centers (UHC Zagreb, CH Merkur, CH Dubrava and UHC Osijek) and for donor typing from 15 donor hospitals. In the period 2007-2015 immunogenetic testing were conducted for 1842 kidney recipients, 364 heart recipients, 1074 liver recipients and 92 pancreas recipients. In the same period, testings were done for supporting 1423 kidney transplantations, 230 hear transplantation, 774 liver transplantations and 75 pancreas transplantations. Such an intensive donor and transplantation program put Croatia among the world most successful countries with Tissue Typing Center giving its professional and scientific impact as a European accredited immunogenetic center of excellence.

Key words: immunogenetics, tissue typing, transplantation, solid organs

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HISTOKOMPATIBILNOST I IMUNOGENETIKA U ALOGENOJ TRANSPLANTACIJI KRVOTVORNIH MATIČNIH STANICA U HRVATSKOJ

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Program transplantacije krvotvornih matičnih stanica (TKMS) započeo je u Hrvatskoj 1983. godine prvom alogenom transplantacijom od srodnog davatelja. Odabir davatelja prema imunogenetskim kriterijima osnova je i od presudnog značaja za uspješan ishod TKMS, a provodi se u Zavodu za tipizaciju tkiva KBC Zagreb. U programu srodne TKMS prosječno se godišnje obradi 150 bolesnika i 700 članova obitelji, s uspješnošću pronalaska HLA genotipski identičnog davatelja u oko 30% obitelji. Bolesnici kojima davatelj nije pronađen u obitelji, uključuju se u program TKMS s HLA fenotipski podudarnog nesrodnog davatelja. Program se temelji na postojanju nacionalnih registara uključenih u svjetski registar (Bone Marrow Donors Worldwide - BMDW) dobrovoljnih davatelja KMS. Hrvatski registar dobrovoljnih davatelja KMS (RDDKMS) danas ima upisanih, i u Zavodu za tipizaciju tkiva imunogenetski obrađenih, više od 40 000 dobrovoljnih davatelja, dok BMDW broji 26 milijuna davatelja. Odabir nesrodnog davatelja uključuje složene algoritme pretraživanja hrvatskog i svjetskog registra, tipizaciju visokog razlučivanja lokusa HLA (A, B, C, DRB1 i DQB1) bolesnika i odabranih mogućih davatelja te završni odabir s potvrdnim testiranjem primatelja i davatelja. Godišnje se ovaj postupak provodi za oko 100 bolesnika, a uključuje obradu i 400 mogućih nesrodnih davatelja. TKMS je u Hrvatskoj opsežan i uspješan program liječenja u kojem Zavod za tipizaciju tkiva KBC Zagreb ima značajnu i nezaobilaznu ulogu.

Ključne riječi: imunogenetika, tipizacija tkiva, transplantacija, krvotvorne matične stanice

HISTOCOMPATIBILITY AND IMMUNOGENETICS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CROATIA

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Hematopoietic stem cell transplantation (HSCT) program in Croatia started in 1983 when first allogeneic transplantation from related donor was performed. Recruitment of the donor following immunogenetic criteria is a crucial step in HSCT which is done exclusively in Tissue Typing Center UHC Zagreb. Related HSCT program includes testings of around 150 patients and 700 family members annually, resulting in successful search of HLA genotipically identical donor in 30% of families. Patients for whom the donor was not found within family are included in HSCT program from HLA phenotipically identical unrelated donor. This program relies on national registries of unrelated volunteer HSCT donors which are all included in the world registry Bone marrow donors worldwide (BMDW). Croatian bone marrow donor registry (CBMDR) has more than 40 000 registered donors, all of them being HLA typed in Tissue Typing Center Zagreb, while BMDW reached the number of 26 million donors. Protocols for finding unrelated donor includes sophisticated search algorithms of CBMDR and BMDW, high resolution HLA typing at five loci (A, B, C, DRB1, DQB1) and final confirmatory testings of the recipient and the most phenotipically compatible donor. Around 100 patients are included in this program annually together with more than 400 potential unrelated donors. HSCT is a large and successful program in Croatia with a Tissue Typing Center UHC Zagreb being its integral part with a crucial role.

Key words: immunogenetics, tissue typing, transplantation, hematopoietic stem cells

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AKUTNI UTJECAJ KOMBINIRANOG TRETMANA DIJAFRAGMALNOG DISANJA I PROGRESIVNE MIŠIĆNE RELAKSACIJE NA ANTIOKSIDACIJSKU UČINKOVITOST SLINE U MLADIH ODRASLIH LJUDI POVEZAN S NJIHOVIM CRTAMA OSOBNOSTI

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Stres je postao sastavni dio modernog stila života današnjice, te sukladno tome i predmet znanstvenih istraživanja u različitim disciplinama. Brojni dokazi biokemijskih mehanizama patogeneze velikog broja bolesti i poremećaja povezanih sa stresom pokazali su poveznicu sa stvaranjem i djelovanjem reaktivnih kisikovih jedinki (ROS). Kad su prisutne u suvišku, ROS uzrokuju oksidacijska oštećenja najvažnijih biomolekula te na taj način dovode do poremećaja u funkcioniranju stanica. Veliki broj molekula pokazuje antioksidacijska svojstva što omogućuje učinkovitu zaštitu od oksidacijskog stresa. U ovom istraživanju određena je antioksidacijska učinkovitost sline (SAC) mladih odraslih ljudi prije i neposredno nakon jednog kombiniranog tretmana (u trajanju od oko 35 min.) koji se sastojao od dijafragmalnog disanja (DB) i progresivne mišićne relaksacije (PMR). I DB i PMR su ne-medikamentozni tretmani koji se rutinski koriste u kliničkoj psihologiji i psihijatriji. Primjenom jednog tretmana došlo je do značajnog povećanja vrijednosti SAC. Individualne razlike u porastu SAC su, međutim, bile u rasponu od 2-3% do 60%. Pokazalo sa da je takav veliki rasap vrijednosti u značajnoj negativnoj korelaciji s neuroticizmom, jednom od "velikih pet" crta osobnosti. Biološki učinci koji su se pokazali u ovom istraživanju potvrđuju potrebu upotrebe DB i PMR kao vrlo moćnih tehnika ne samo u kliničkoj praksi već i u smislu svakodnevne mentalne higijene kod zdrave populacije mladih odraslih ljudi.

ACUTE RESPONSES OF SALIVA ANTIOXIDANT CAPACITY TO COMBINED DIAPHRAGMATIC BREATHING AND PROGRESSIVE MUSCLE RELAXATION TREATMENTS IN YOUNG ADULTS RELATED TO THEIR PERSONALITY TRAITS

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Presently, stress became integral part of the modern life style and thus a scientific topic in different disciplines. Biochemical mechanisms of stress-related pathogenesis are well documented in a broad spectrum of illnesses and disorders and were shown to comprise the formation and action of different reactive oxygen species (ROS). When present in excess, ROS induce oxidative damage to essential biomolecules and in turn disturb normal cell functioning. A plethora of molecules reveals antioxidative properties facilitating efficient

protection against oxidative stress. In this investigation saliva antioxidant capacity (SAC) was measured in young adults before and immediately after a single combined treatment (duration of app. 35 min.) consisting of diaphragmatic breathing (DB) and progressive muscle relaxation (PMR). Both, DB and PMR are routinely used as non medicamentous treatments in clinical psychology and psychiatry. After a single treatment applied the significant increase in SAC was revealed. However, the individual differences in SAC increase appeared to be dissipated from 2-3 to 60%. Such great dissipation was further confirmed to be significantly negatively correlated with the neuroticism, the one of the "big five" personality traits. Biological effects revealed in our study confirmed the requirement of DB and PMR application as powerful anti-stress tools in both clinical praxis and especially as daily mental hygiene in healthy young adult population.

Key words: stress, saliva, diaphragmatic breathing, progressive muscle relaxation, non medicamentous anti-stress treatments

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MONKEYS WITH PERSONALITY: CONSISTENT INTER-INDIVIDUAL DIFFERENCES IN COMMON MARMOSETS (*Callithrix jacchus*)

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The first ideas of personality came from human psychology, nowadays is this research question studied in a wide range of study species, from insects to non-human primates. Consistent individual differences (animal personalities) are defined as groups of correlated behaviours, that are temporally and contextually repeatable within an individual, and are usually divided in three non-social ("Boldness-Shyness", "Exploration-Avoidance" and "Activity") and two social ("Aggressiveness" and "Sociability") behavioural traits. In this study, we tested 21 individuals from three family groups of common marmosets (*Callithrix jacchus*) in five different experiments (general activity, novel food, novel object, predator and foraging under risk) in a solitary setting. Our results indicated that monkeys behaved consistently not only throughout time, but also across different contexts. These behaviours formed a construct of four major non-social personality components: "Boldness-Shyness in Foraging", "Boldness-Shyness in Predation", "Stress-Activity" and "Exploration-Avoidance". The models revealed no sex or age differences in the components, but they revealed a difference between groups in "Exploration-Avoidance". As common marmosets are highly gregarious animals, we will discuss the presence of the group-specific personality trait "Exploration-Avoidance" with regards to the possible influence of social environment on behaviour of individuals, even outside the immediate social context.

Key words: personality, consistent inter-individual differences, exploration-avoidance, common marmosets

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USPOREDBA MASNOKISELINSKOG SASTAVA POTKOŽNOG MASNOG TKIVA SMEĐEG MEDVJEDA (*Ursus arctos*) I SIVOG VUKA (*Canis lupus*)

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Analiza masnokiselinskog sastava (MS) masnog tkiva koristi se za utvrđivanje prehrambenih navika slobodno živućih životinja. Ovisno o vrsti, mnoge masne kiseline (MK) masnog tkiva potječu direktno iz hrane. Cilj ovog rada bio je bolje razumijevanje prehrambenih navika i fiziološkog statusa slobodno živućih jedinki smeđeg medvjeda (SM) i sivog vuka (SV), što je ključno za strategije gospodarenja i zaštite populacija divljih životinja. Istraživanje je provedeno na 91 jedinki SM (ženke: n=25, 4,66±2,65 g.; mužjaci: n=66, 5,30±2,72 g.) i 20 jedinki SV (ženke: n=11, 1,95±1,86 g.; mužjaci: n=9, 2,59±1,59 g.). Uzorci su skupljeni tijekom sezone lova (uzorci SM 2013. i uzorci SV 2009.-2011.). Nakon odmrzavanja uzoraka, homogenizacije i ekstrakcije lipida, MS određen je plinskom kromatografijom. U MS potkožnog masnog tkiva obje vrste dominiraju MK s jednom dvostrukom vezom (48% u ženki i 41% u mužjaka SM *vs.* 46% u ženki i 46% u mužjaka SV). Zasićene MK čine 26% u ženki i 38% u mužjaka SM *vs.* 42% u ženki i 44% u mužjaka SV. Višestrukonezasićene MK (PUFA) čine 26% u ženki i 21% u mužjaka SM. U ženki SV PUFA su bile zastupljene u 12% dok su u mužjaka činile 10%. U MS potkožnog masnog tkiva SM i SV utvrđene su značajne razlike s obzirom na dob, tjelesnu masu, sezonu i lokaciju uzorkovanja. Sličnosti MS potkožnog masnog tkiva u SM i SV proistječu iz filogeneze zvijeri, dok su razlike rezultat različitog fiziološkog statusa i prehrambenih navika jedinki.

Ključne riječi: smeđi medvjed, sivi vuk, masnokiselinski sastav, potkožno masno tkivo

COMPARISON OF FATTY ACID COMPOSITION OF SUBCUTANEOUS ADIPOSE TISSUE OF BROWN BEAR (*Ursus arctos*) AND GRAY WOLF (*Canis lupus*)

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Analysis of fatty acid (FA) composition is a technique used to examine the foraging habits of free-ranging animals. Depending on the species, many of the FAs contained in adipose tissue are derived directly from diet. The objective of this study was better understanding of foraging patterns and physiological status of free ranging brown bears (BB) and gray wolves (GW), which is essential for management and conservation strategies of wildlife populations. The study was conducted on 91 BB (female: n=25, 4.66±2.65 yr.; male: n=66, 5.30±2.72 yr.) and 20 GW (female: n=11, 1.95±1.86 yr.; male: n=9, 2.59±1.59 yr.). Samples were collected during the legal hunting

season (2013 for BB and 2009-2011 for GW). After thawing, homogenization and lipid extraction, the FA composition was determined by gas chromatography. The subcutaneous adipose tissue (SAT) of both species is do minated by FAs with one double bond (48% in female and 41% in male BB *vs.* 46% in female and 46% in male GW). Saturated fatty acids constituted of 26% in female and 38% in male BB *vs.* 42% in female and 44% in male GW. Polyunsaturated fatty acids were found in 26% in female and 21% in male BB. In females GW PUFA was in 12% while in males it was 10%. The FAs of SAT of BB and GW showed age-, body mass-, season- and location related significant differences. Similarities in FAs of BB and GW are derived from phylogeny of carnivores, while diferences are the result of different physiological status and foraging patterns.

Key words: brown bear, gray wolf, fatty acid composition, subcutaneous adipose tissue

POSTERSKE PREZENTACIJE

POSTERS

KONZERVACIJSKA BIOLOGIJA, ZAŠTITA PRIRODE I OKOLIŠA
CONSERVATIONAL BIOLOGY, NATURE AND ENVIRONMENT PROTECTION

P-1

KOCKAVICA (*Fritillaria meleagris* L., LILIACEAE) – 10 GODINA AKCIJE PRAĆENJA STANJA ZA OSNOVNE I SREDNJE ŠKOLE

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Kockavica (*Fritillaria meleagris* L., Liliaceae) je proljetnica vlažnih livada i svijetlih, otvorenih vlažnih šikara i šuma. Rasprostranjena je u kontinentalnoj Hrvatskoj, s nekoliko izoliranih nalazišta s područja Velebita. Prema Crvenoj knjizi vaskularne flore Hrvatske smatra se osjetljivom vrstom (VU).
Akcija praćenja stanja kockavice započela je 2005. godine u suradnji sa školama Međimurske i Krapinsko-zagorske županije. Do danas se akcija proširila, te se provodi u suradnji s javnim ustanovama za upravljanje zaštićenim prirodnih vrijednosti ukupno devet županija, Međimurske, Krapinsko-zagorske, Koprivničko-križevačke, Varaždinske, Bjelovarsko-bilogorske, Sisačko-moslavačke, Zagrebačke, Karlovačke i Požeško-slavonske županije, te osnovnim i srednjim školama ovih županija. Akcijom su prikupljeni značajni podaci o rasprostranjenosti kockavice u Hrvatskoj koji su prikazani u ovom priopćenju. Isto tako, akcijom je utvrđeno kako je kockavica s pojedinih nalazišta nestala, uglavnom zbog melioracija, širenja građevinskih područja, izgradnje cesta te pretvaranja livada u obradive površine. Temeljem svih dostupnih podataka, koji uključuju literaturne navode, vlastita terenska opažanja te podatke prikupljene akcijom praćenja stanja kockavice provedena je revizija kategorije ugroženosti kockavice u Hrvatskoj.

Ključne riječi: prava kockavica, prebrojavanje, kategorija ugroženosti, prijetnje

SNAKE'S HEAD FRITILLARY (*Fritillaria meleagris* L., LILIACEAE) – 10 YEARS OF MONITORING CAMPAIGN FOR PRIMARY AND SECONDARY SCHOOLS

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Snake's head fritillary (*Fritillaria meleagris* L., Liliaceae) is a spring flower of wet grasslands and light, open wet thickets and forests. It is widespread in continental Croatia, with a few isolated localities from Velebit area. According to the Red Book of Vascular Flora of Croatia it is considered a vulnerable species (VU). Snake's head fritillary monitoring campaign began in 2005 in cooperation with schools of Međimurje and Krapina-Zagorje County. To date, the action spread, and is being conducted in collaboration with public institutions for management of protected natural values of the nine counties, Međimurje, Krapina-Zagorje, Koprivnica-Križevci, Varaždin, Bjelovar-Bilogora, Sisak-Moslavina, Zagreb, Karlovac and Požega-Slavonia County, and primary and secondary schools of these counties. During the action important data on the distribution of Snake's head fritillary in Croatia was collected, as presented in this presentation. Also, it was found that the Snake's head fritillary has disappeared from several localities, mainly due to land ameliorations, expansion of commercial or residential areas, road construction and the conversion of grassland to arable land. Based on all available data,

including the literature data, our own field observations and data collected through the monitoring campaign the threat category of Snake's head fritillary was reassessed.

Key words: Fritillary, counting, threat category, threats

P-2

DEFINIRANJE EKOLOŠKIH CILJEVA U SKLADU S EKOLOŠKOM MREŽOM NATURA 2000 ZA POTREBE REVITALIZACIJE ERDUTSKO-DALJSKOG DUNAVCA

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Područje Erdutsko-daljskog dunavca je sustav od tri riječna rukavca u desnoj inundaciji Dunava. Dio je ekološke mreže Natura 2000 (područje očuvanja značajno za ptice "HR1000016 Podunavlje i donje Podravlje", područje očuvanja značajno za vrste i staništa "HR2000372 Dunav-Vukovar"). Dosad izvedeni građevinski zahvati (željeznički i cestovni most, hidrotehnički objekti za potrebe održavanja plovnog puta), uz prirodno zapunjavanje riječnim materijalom, smanjenje dubine i eutrofikaciju, doprinose ubrzanju sukcesijskih procesa, zbog čega dolazi do promjene strukture i funkcije dunavca. Cilj projekta bio je izraditi idejno rješenje revitalizacije kojim bi se dugoročno osiguralo izmjenu i osvježavanje dunavca vodom iz Dunava, kako bi se postojeći nepovoljni procesi usporili te očuvalo ili poboljšalo stanje vodenih i močvarnih staništa, uz istovremeno osiguranje održivog korištenja područja. U sklopu projekta revitalizacije utvrđeno je postojeće stanje vodenih i močvarnih staništa te značaj područja za očuvanje faune, s naglaskom na ciljne vrste riba i ptica. Analizom stanja ciljnih vrsta i staništa te ključnih ekoloških uvjeta potrebnih za njihovo očuvanje, utvrđeni su specifični (ekološki) ciljevi. Svrha ekoloških ciljeva revitalizacije bila je dati smjernice i uvjete za projektiranje idejnog rješenja revitalizacije i planiranje korištenja prostora u svrhu rekreativnog ribolova i drugih turističkih aktivnosti.

Ključne riječi: revitalizacija riječnih rukavaca, idejno rješenje, Natura 2000, očuvanje ciljnih vrsta i staništa

DEFINING ECOLOGICAL OBJECTIVES ACCORDING TO ECOLOGICAL NETWORK NATURA 2000 FOR THE PURPOSE OF REVITALIZATION OF "ERDUT-DALJ DUNAVAC"

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The area of "Erdut-Dalj dunavac" is the system of three river side arms in the right inundation of Danube River. It is part of ecological network Natura 2000 (SPA "HR1000016 Podunavlje i donje Podravlje", SCI "HR2000372 Dunav-Vukovar"). Already implemented construction works (railway and road bridge, hydrotechnical objects for the maintenance of waterway), natural filling with river sediments, lowering of depth and eutrophication, all together contribute to

acceleration of succession processes, causing changes in structure and function of river side arms. The project aim was to design concept solution of revitalization to ensure long-term water exchange and refreshment with water from Danube main channel. This would slow down the existent unfavourable processes in order to maintain or improve the state of aquatic and wetland habitats, and allow sustainable use of the area. Current state of aquatic and wetland habitats was assessed, as well as the value of the project area for conservation of fauna, with an emphasis on target fish and target bird species. Specific (ecological) objectives were defined based on the analysis of current status of target species and target habitats, and key ecological requirements needed for their conservation. The purpose of defining ecological objectives of revitalization is to give recommendations and framework for designing the concept solution and planning the use of the area for recreational fishing and other touristic activities.

Key words: river side arms revitalization, concept solution, Natura 2000, conservation of target species and target habitats

P-3

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.

Key words: National Park, Plitvice Lakes, local population, coexistence, nature

P-4

ANALIZA METODA ZA PRAĆENJE PRIJELAZA DIVLJIH ŽIVOTINJA PREKO ZELENIH MOSTOVA

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Zeleni mostovi smanjuju negativan učinak prometnica na populacije divljih životinja, a njihova učinkovitost procjenjuje se različitim metodama praćenja. Temeljem trogodišnjeg praćenja četiri zelena mosta na autocesti A1, uspoređena je učinkovitost metode praćenja tragova na kontrolnoj pješčanoj traci, metode fotozamki i metode infracrvenih (IC) senzora. Za svaku metodu je utvrđeno koliko točno detektira različite vrste divljih životinja, kao i može li se temeljem nje procijeniti stvarni broj prijelaza. Granulometrijski sastav pješčane trake utjecao je na točnost određivanja životinjskih vrsta. Najbolji rezultati dobiveni su kada je korišten materijal s većim udjelom silta i gline. Što se tiče sastava vrsta, metodom praćenja tragova na kontrolnoj traci potcijenjen je udio malih kanida, dok je metodom fotozamki potcijenjen udio srna. Nadalje, metodom fotozamki je potcijenjen ukupan broj prijelaza životinja. Suprotno tome, IC senzorima je zabilježeno od 11 do 19 puta više prijelaza nego fotozamkama. Međutim, oko 80% IC događaja bili su artefakti, odnosno nisu ih uzrokovali prijelazi životinja. Stoga je razvijen algoritam za korekciju IC podataka kako bi se dobila točnija procjena stvarnog broja prijelaza. Zaključno, prikazani rezultati predstavljaju vrijedan doprinos jer daju pregled prednosti i nedostataka različitih metoda monitoringa te će doprinijeti većoj točnosti rezultata praćenja prijelaza divljih životinja preko zelenih mostova.

Ključne riječi: zeleni mostovi, monitoring prijelaza životinja, usporedba metoda

ANALYSIS OF METHODS FOR WILDLIFE MONITORING ON GREEN BRIDGES

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Green bridges mitigate negative impact of roads/highways on wildlife populations. Their effectiveness is evaluated by various monitoring methods. Based on the 3-year monitoring period of four green bridges on A1 motorway in Croatia, the effectiveness of three monitoring methods was compared: track-pads, camera traps and active infrared (IR) trail monitoring system. The methods were evaluated in terms of their ability to detect different species and to give good estimation of the number of animal crossings. The accuracy of species detection by track-pad method was influenced by granulometric composition of the track-pad material, with the best results obtained when higher percentages of silt and clay were used. Regarding the species composition, monitoring by tracks underestimated the ratio of small canids, while camera traps underestimated the ratio of roe deer. Furt her, camera trap method underestimated the real number of animal crossings. On the contrary, active IR detectors recorded from 11 to 19 times more events than camera traps. However, circa 80% of IR events were not caused by wildllife crossings. Therefore, an algorithm for correction of the IR dataset was developed, in order to approximate the real number of crossings. In conclusion, presented results are valuable for future monitoring of wildlife crossings since they provide advantages and disadvantages of different monitoring methods.

Key words: green bridges, wildlife crossing monitoring, comparison of methods

P-5
PRELIMINARNI REZULTATI ISTRAŽIVANJA VUKOVA U PARKU PRIRODE BIOKOVO

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U okviru projekta "Istraživanje i monitoring vukova u Parku prirode Biokovo" od 2013. godine provodi se istraživanje s ciljem utvrđivanja prisutnosti, brojnosti i stabilnosti čopora vukova te utvrđivanja veličine područja obitavanja čopora. U istraživanju se primjenjuju metode fotozamki, praćenja tragova, akustična metoda i metoda GPS telemetrije te analiza strukture prehrane. Tijekom prvih 18 mjeseci istraživanja postavljene su fotozamke na 20 lokacija te su prikupljene 3922 fotografije pogodne za analizu. Vuk je snimljen na 5 različitih lokacija na 46 fotografija odnosno 1,17% slika. Na istoj fotografiji su zabilježene najviše dvije jedinke vuka. Na temelju tragova ili izmeta prisutnost vuka utvrđena je na 25 lokacija te je prikupljeno 53 izmeta pogodnih za analizu strukture prehrane. Provedbom akustične metode odaziv vukova zabilježen je na osam lokacija. Samo na jednoj lokaciji utvrđen je odaziv najmanje 5 jedinki. Provedba GPS telemetrije je u fazi hvatanja i obilježavanja jedinke vuka. Na temelju dosadašnjih rezultata još nije moguće izvesti pouzdane zaključke o broju čopora i brojnosti

jedinki vuka u Parku prirode Biokovo. Rezultati akustične metode ukazuju na najmanje pet jedinki u čoporu, dok ostale metode ukazuju na pojedinačno pojavljivanje vukova kao privremeno ili trajno dispergiranih jedinki ili ostatku "razbijenog" čopora što je moguće posljedica značajnog pritiska na vuka od strane lovaca i stočara.

Ključne riječi: sivi vuk, *Canis lupus*, Park prirode Biokovo

THE RESEARCH OF WOLVES IN THE NATURE PARK BIOKOVO – PRELIMINARY RESULTS

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From 2013, the project "Research and monitoring of wolves in the Nature Park Biokovo" is being conducted to determine the presence, abundance and stability of the wolf packs and to determine the size of their home range. Monitoring methods being applied are: digital camera traps, recording of footprints and scat, acoustic method, GPS telemetry and analysis of the diet. During the first 18 months of research, camera traps were placed at 20 locations and 3922 photos suitable for analysis were collected. Wolves were recorded in 46 photos (1.17%) at 5 different locations. No more than two wolves were recorded in the same photograph. According to observed footprints or scats, the presence of wolf was determined at 25 locations and 53 scats suitable for diet analysis were collected. Using acoustic method, howling reply was recorded at 8 locations while the reply of at least five individuals is recorded only in one location. GPS telemetry is at the stage of capturing and marking an individual. Based on the preliminary results it is not possible to draw reliable conclusions about the number of packs and number of wolf's individuals in the Nature Park Biokovo. Results of acoustic method indicate at least five individuals in a pack, while other methods point to appearance of individuals as a temporary or permanent dispersed individuals or the remainder of the "broken" pack which could be the result of a significant influence on the wolf by hunters and stockbreeders.

Key words: grey wolf, *Canis lupus*, Nature Park Biokovo

P-6

NOVA STRANA VRSTA DESETERONOŽNIH RAKOVA (MRAMORNI RAK (*Procambarus fallax* (Hagen, 1870) *f. virginalis*) U SLATKOVODNIM EKOSUSTAVIMA HRVATSKE

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Mramorni rak (*Procambarus fallax* (Hagen, 1870) *f. virginalis*) treća je alohtona vrsta slatkovodnih deseteronožnih rakova koja zabilježena u vodotocima Hrvatske. Ova partenogenetska vrsta raka zabilježena je prvi put 2013. godine u šljunčari Šoderica na sjeveru Hrvatske. Tijekom 2015. godine ispitali smo potencijalne putove unosa mramornog raka u Hrvatsku, te istražili osnovne značajke populacije zabilježene u šljunčari Šoderica (relativna

gustoća rakova, uzrasna struktura populacije, značajke godišnjeg ciklusa) i potencijal daljnjeg širenja ove vrste na području Međimurja i Podravine. Kako bi upravljanje i aktivnosti usmjerene na kontrolu širenja ove vrste bile pravovremene, neophodni su detaljni podaci o strukturi i dinamici populacije mramornog raka u Šoderici.

Ključne riječi: slatkovodne invazije, partenogenetska vrsta, populacijske značajke, invazivni areal

NEW NON-INDIGENOUS CRAYFISH (THE MARBLE CRAYFISH (*Procambarus fallax* (Hagen, 1870) *f. virginalis*) IN CROATIAN FRESHWATERS

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The marble crayfish (*Procambarus fallax* (Hagen, 1870) *f. virginalis*) is the third non-indigenous crayfish species established in Croatian freshwater habitats. This parthenogenetic species was discovered in 2013 in the Šoderica gravel pit lake, located in the north of Croatia. In this study we looked into potential introduction pathways of the marble crayfish to Croatia, examined some of its basic population characteristics (i.e. relative crayfish abundance, population size structure and year cycle characteristics) and analysed its potential for dispersal in the region. Research and monitoring programme for the Šoderica marble crayfish population is urgently required in order to immediately start with management actions targeted at its control.

Key words: freshwater invasion, parthenogenetic species, population characteristics, invasive range

P-7
SMJERNICE ZA PROCJENU UTJECAJA VJETROELEKTRANA NA VELIKE ZVIJERI

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Razvoj vjetroparkova u Hrvatskoj velikim je dijelom zbog odgovarajućeg vjetropotencijala usmjeren na Dinarsko područje koje ujedno predstavlja i vrijedno stanište velikih zvijeri (vuka, medvjeda i risa). Najveću prijetnju opstanku velikih zvijeri predstavljaju fragmentacija i gubitak staništa, a vjetroelektrane s pripadajućim pristupnim putovima značajno doprinose ovom procesu. S obzirom na potrebu da se u adekvatno ocijene utjecaji vjetroparkova u različitim postupcima sukladno propisima iz područja zaštite prirode i okoliša te EU direktivama, Zavod za biologiju Veterinarskog fakulteta u Zagrebu i Državni zavod za zaštitu prirode zajednički su pristupili izradi stručnih Smjernica za procjenu utjecaja vjetroelektrana na velike zvijeri. Dosadašnja praksa pokazala je da je za smanjivanje i izbjegavanje pojedinačnih samostalnih i kumulativnih utjecaja vjetroparkova ključan njihov odgovarajući smještaj u prostoru. Stoga Smjernice predlažu metodologiju ocjene temeljenu na karti pogodnosti staništa koja putem matematičkog modela i GIS-a objedinjava informacije o opažanjima velikih zvijeri s informacijama o osobinama staništa te pokazuje koliko je određeno područje značajno za pojedinu vrstu velikih zvijeri. Izdvojeno je devet kategorija pogodnosti staništa, te su

postavljeni postotni i apsolutni limiti daljnjeg gubitka za svaku kategoriju. Također je obrazložen način kako koristiti kartu u različitim postupcima ocjene.

Ključne riječi: vjetroelektrane, velike zvijeri, ocjena utjecaja, smjernice, GIS

GUIDELINES FOR ASSESSMENT OF WIND FARMS IMPACT ON LARGE CARNIVORES

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The Dinaric mountain range due to its wind potential is the preferred area for wind farm development in Croatia but in the same time it represents a valuable habitat for large carnivores (wolf, bear and lynx). The biggest threat to large carnivores' survival is habitat fragmentation and loss. Wind farms with access roads and associate disturbance significantly contribute to that process. To ensure adequate assessment of wind farm impacts in different environmental and nature protection procedures pertaining to national and EU legislation, Biology Department of Veterinary Faculty in Zagreb and State Institute for Nature Protection jointly developed Guidelines for assessment of wind farms impact on large carnivores. The experience shows that the best approach for reduction and avoidance of individual and cumulative impacts of wind farms is their appropriate spatial p ositioning. Therefore the Guidelines propose an assessment methodology based on the sensitivity map, which was created throughout mathematical modeling and GIS using data on large carnivores' occurrences and related habitat characteristics. The sensitivity map indicates importance of certain area for each species of large carnivores and defines nine categories of significance. Also percent and absolute limits of further loss for each category have been determined. In addition, the appropriate use of sensitivity map in as assessment procedures is described and proposed.

Key words: wind mills, large carnivores, impact assessment, guidelines, GIS

P-8
MODIFICIRANI LESLIE-LEFKOVITCH GENETIČKI ALGORITAMSKI MODELSKI SUSTAV DINAMIKE POPULACIJA KOMARACA

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Fluktuacije veličine i promjene starosne strukture populacija komaraca tijekom godine važni su parametri nužni za planiranje i provođenje mjera kontrole brojnosti komaraca na nekom području. Osim toga, dinamika populacija komaraca je i izuzetno zanimljivo akademsko pitanje zbog njenih osobina kao što su podložnost Allee-efektu i periodična kaotičnost. Upravo zbog tih osobina je dinamiku populacija komaraca teško predviđati temeljem modela načinjenih uobičajenim tehnikama. Do sada su najbolje rezultate u modeliranju dinamike populacija komaraca pokazali modificirani diskretni Leslie-Lefkovitch matrični modeli (MLL). Kako bi se poboljšala svojstva predikcije rezultata tretiranja komaraca larvicidima i adulticidima te doprinijelo izradi računalne podrške sustavu odlučivanja, u ovom radu su se postojećim modelima dodali programski moduli na temelju gen etičkih algoritama. Tako dobiveni hibridni

sustav iskoristio se za dobivanje serija predikacija koje su uspoređene s predikcijama dobivenim pomoću jednostavnog MLL-sustava. Osim u poboljšanju prediktivnosti rezultata tretiranja, hibridni sustav je pokazao i nova aplikativna svojstva modela koja znatno doprinose računalnom upravljanju kontrole komaraca.

Ključne riječi: komarci, genetički algoritam, MLL, predikcija

MODIFIED LESLIE-LEFKOVITCH-GENETIC ALGORITHM HYBRID MODELS SYSTEM OF MOSQUITO POPULATION DYNAMIC

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Fluctuations in size and changes in age structure of mosquito populations during the year are important parameters necessary for planning and implementation of mosquito control measures. Additionally, mosquito population dynamic is an exceptionally interesting academic question due to its periodic chaos system and susceptibility to Allee-effect. It is those characteristics that make mosquito population dynamic difficult to predict using the usual methods. Until now, discrete Leslie-Lefkovitch matrix models (MLL) showed the best results in mosquito population dynamic modelling. To improve result prediction properties of larvicides and adulticides, and to contribute to the development of computer support to the deciding system, program modules based on genetic algorithms were added to the existing models. This hybrid system was used to make a series of predictions whic h were compared to the predictions made by simple MLL-system. Hybrid system, aside from improving prediction of treatment results, showed new applicable model properties which significantly contribute to computer management of mosquito control.

Key words: mosquitoes, genetic algorithm, MLL, predictions

P-9
ŠIŠMIŠI NA BRIJUNIMA – REZIDENCIJALNA ELITA ILI MASOVNI TURIZAM?

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Prikupljanje prvih podataka o fauni šišmiša Nacionalnog parka Brijuni započelo je preliminarnim istraživanjem u rujnu 2010. godine. Pretražen je veći broj potencijalnih skloništa i provedena pregledna istraživanja hvatanjem mrežama i snimanjem ultrazvučnim detektorom. Već prvi podaci pokazali su iznenađujuću brojnost jedinki i raznolikost vrsta. Iz tog razloga su u razdoblju od 2011. do 2014. nastavljena istraživanja na većem broju objekata te na odabranim staništima značajnim za šišmiše. Istraživanja su provedena na Velikom i Malom Brijunu, ali i na drugim manjim otocima Brijunskog arhipelaga. Hvatanjem mrežama, snimanjem ultrazvučnim detektorom i pretragom objekata utvrđeno je ukupno 14 vrsta šišmiša. Značajno je istaknuti prisutnost porodiljne kolonije vrste *Myotis nattereri*, jedinke vrste *Myotis mystacinus* u hibernaciji na otoku Mali Brijun te uhvaćenog mužjaka vrste *Nyctalus lasiopterus* s izraženim testisima i bukalnim žlijezdama, što je tek drugi nalaz žive jedinke ove vrste u Hrvatskoj nakon 89 godina. Analiza sonograma ukazuje na područja s većom brojnošću vrsta i gustoćom jedinki te na prostore koje koriste vrste od osobitog značaja. Temeljem analize rezultata istraživanja,

dani su prijedlozi mjera zaštite šišmiša i podržavanja populacija na području NP Brijuni. Obzirom na značaj dosad prikupljenih podataka, daljnja istraživanja temeljit će se na usmjerenom praćenju najvažnijih elemenata (staništa i vrsta) u razdobljima značajnim za godišnji ciklus šišmiša.

Ključne riječi: šišmiši, Nacionalni park Brijuni

BATS ON BRIJUNI – RESIDENTIAL ELITE OR MASS TOURISM?

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Initial data gathering on bat fauna in Brijuni National Park began with preliminary research in September 2010. Many potential sites were examined and screening was conducted by catching with mist nets and recording with ultrasound detector. The first data indicated a surprising number of individuals and great species diversity. For this reason, during the period between 2011 and 2014, research was extended to a greater number of sites and selected habitats important to bats. The research was conducted on Veliki and Mali Brijun, as well as on other smaller islands within the Brijuni archipelago. Altogether, 14 bat species were found by mist netting, recording with ultrasound detectors and examination of sites. It is important to emphasise the presence of *Myotis nattereri* maternity colony, hibernating *Myotis mystacinus* individuals on island Mali Brijun and captured *Nyctalus lasiopterus* male, with enlarged testes and buccal glands, which is the second finding of a live individual of this species in Croatia after 89 years. Sonogram analysis reveals areas with greater number of species and density of individuals, as well as areas used by species of importance. Based on the analysis of the results, measures to protect bats and support bat populations in Brijuni National Park were suggested. Given the importance of the collected data, further research will be focused on monitoring of the key elements (habitats and species) in the periods important for the annual cycle of bats.

Key words: bats, Brijuni National Park

P-10
RASPROSTRANJENOST I TREND GNIJEZDEĆE POPULACIJE VRANE GAČAC (*Corvus frugilegus* L. 1758) NA PODRUČJU OSIJEKA

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Iako je u nedavnoj prošlosti bila tipična stepska ptica, vrana gačac (*Corvus frugilegus*) je danas sve brojnija u urbanim područjima. Cilj istraživanja je utvrđivanje trenda gnijezdeće populacije, te utvrđivanje veličine zimujuće populacije vrane gačca na području grada Osijeka. Prebrojavanje broja gnijezdećih parova gačaca u kolonijama obavljeno je metodom brojanja sigurno aktivnih gnijezda u kolonijama tijekom proljeća 2014. i 2015. godine, a tijekom zima gačci su brojani na okupljalištima prije odlaska na spavalište. Obradom literaturnih i podataka s terenskih istraživanja uz pomoć softverskog paketa TRIM izračunat je trend populacije u zadnje 23 godine. Tijekom zima 2014. i 2015. godine, na okupljalištima se prije spavanja nalazilo prosječno 4000 gačaca. Tijekom 2014. godine na području grada Osijeka gnijezdilo je 560

parova gačaca u 13 kolonija, dok je u 2015. godini gnijezdilo 623 parova u 12 kolonija. Najveći broj kolonija (šest) pripada malim kolonijama, do 50 parova, u kojima je prosječno gnijezdilo 19 parova. Srednje velikih kolonija (50-100 parova) je bilo između jedne (2014.) i četiri (2015.), a velikih kolonija sa više od 100 parova su tijekom istraživanog perioda je bilo samo dvije. Trend gnijezdeće populacije gačaca na području grada Osijeka tijekom 23 godine je umjereno opadajući. Glavni razlozi tomu jesu stalna rastjerivanja kolonija, orezivanje stabala na kojima su kolonije izgrađene, te rušenje njihovih gnijezda.

Ključne riječi: vrana gačac, trend gnijezdeće populacije, Osijek

DISTRIBUTION AND TREND OF BREEDING POPULATION OF ROOKS (*Corvus frugilegus* L.1758) IN OSIJEK

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Despite the fact that Rook was a typical bird of the steppe habitats, their numbers are nowadays increasing in urban areas. The aim of this study is to present the trend of breeding population and size of wintering population of Rook in Osijek town. Breeding population was established by counting Apparently Occupies Nest in Rook colonies during springs. Wintering population was studied by counting Rook at their roosts during 2014 and 2015. Software package TRIM was used to calculate the breeding population trend for last 23 years. On winter roosting localities 4000 Rooks were counted in average during winters of 2014 and 2015. During 2014 560 breeding pairs were counted in 13 colonies, while 623 breeding pairs were breeding in 12 colonies during 2015. Majority of the colonies (6) were classified as small (below 50 pairs), with average of 19 nesting pairs. Number of medium-sized colonies (50-100 pairs) varied between one in 2014 and four in 2015, while two big colonies (over 100 pairs) were present in both years. In the past 23 years the breeding population trend shows moderate decline, mainly because of the harassment of the colonies, nesting sites destruction and disturbance of breeding Rooks.

Key words: Rook, breeding population trend, Osijek

P-11
OPTIMIZACIJA METODA ZA MOLEKULARNO ODREĐIVANJE SPOLA KOD ČAPLJI (ARDEIDAE)

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Genetski markeri su koristan alat za praćenje promjena u populacijskim studijama. Cilj rada je optimiziranje metode za izolaciju DNA i određivanje spola molekularnim metodama kod čaplji (Ardeidae). Genomska DNA je izolirana iz perja i ljuski jaja koristeći dva različita protokola. Za određivanje spola korištene su početnice 2550F/2781R za kromo-helikazu DNA-vezujući gen (CHD). Rezultati su pokazali da je najveći prinos DNA dobiven iz pera koja su u razvoju (prokrvljena pera) i ljuski jaja. Prinos DNA je bio manji kad se koristio komercijalni kit za izolaciju u odnosu na izolaciju s TNE puferom uz dodatak natrijevog acetata za taloženje

keratina. Ključan korak u izolaciji je koncentracija ditiotreitola (DTT) i proteinaze K u puferu za liziranje kao i količina krvi prisutna u perju.

Ključne riječi: genetski markeri, određivanje spola, čaplje

OPTIMIZATION OF METHODS FOR MOLECULAR SEX TYPING IN HERONS (ARDEIDAE)

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Genetic markers are useful tool for tracking changes in population studies. This work, is focused on optimization of method for DNA isolation and molecular sex determination in herons (Ardeidae). Genomic DNA was isolated from feathers and eggshells by using two different protocols. For sex-typing of herons we used primers 2550F/2781R for chromo-helicase–DNA binding gene 1 (CHD1). Results showed that the highest DNA yield was obtained from the blood feathers and eggshells while mature feathers had the lowest yield. DNA yield was lower when commercial kit was used for isolation compared to the isolation with TNE buffer followed by addition of sodium acetate for keratin precipitation. Critical step is concentration of dithiothreitol (DTT) and proteinase K in lysis solution as well as the amount of blood present in the feathers.

Key words: genetic markers, sex-typing, herons

P-12
BIOLOŠKA ZAŠTITA PITOMOG KESTENA GLJIVAMA ANTAGONISTIMA

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Sastojine pitomog kestena ugrožene su od dva invazivna organizma, gljive *Cryphonectria parasitica* i ose šiškarice *Drycosmus kuriphilus*. Gljiva *C. parasitica* uzročnik je raka kore, a osa šiškarica *D. kuriphilus* oštećuje pupove i listove te uzrokuje formiranje šiški u krošnji. Na šiškama, granama i plodu nalazimo gljivu *C. parasitica*, saprotrofne i druge parazitske gljive. Osim njih, nalazimo i gljive antagoniste, najčešće *Trichoderma viride*, *T. citrinoviride* i *Trichothecium roseum*. Cilj ovog istraživanja bio je utvrditi djelovanje gljiva antagonista na rast gljive *C. parasitica* u svrhu biološke zaštite pitomog kestena. Da bismo utvrdili odnos gljiva antagonista *T. viride*, *T. citrinoviride* i *T. roseum* prema gljivi *C. parasitica*, postavljen je laboratorijski pokus. *T. viride* je ostvarila kontakt 4.-5. dan, a prerastanje 6.-10. dan. *T. citrinoviride* je ostvarila kontakt 4.-6. dan, a prerastanje i djelomično prerastanje 25.-46. dan. *T. roseum* je ostvarila kontakt 9.-11. dan, a prerastanje 15.42. dan. *T. viride* ima najbrži rast i najjače antagonističko djelovanje. Gljive antagonisti *T. viride*, *T. citrinoviride* i *T. roseum* sveprisutne su u kestenovoj sastojini. Laboratorijskim pokusom dokazano je da navedene gljive antagonisti djeluju inhibicijski prema *C. parasitica*. To ukazuje na to da je u sastojinama pitomog kestena prisutna prirodna biološka zaštita gljivama antagonistima.

Ključne riječi: pitomi kesten, *Cryphonectria parasitica*, gljive antagonisti, biološka zaštita

BIOLOGICAL CONTROL OF SWEET CHESTNUT WITH ANTAGONISTIC FUNGI

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Chestnut stands are threatened by two invasive organisms, fungus *Cryphonectria parasitica* and gall wasp *Drycosmus kuriphilus*. The fungus *C. parasitica* cause cancer bark disease. Gall wasp *D. kuriphilus* cause the damage on buds and leaves and form galls in the crown. On the galls, branches and nut *C. parasitica*, saprophytic and parasitic fungi were found. Apart from them antagonistic fungi, usually *Trichoderma viride*, *Trichoderma citrinoviride* and *Trichothecium roseum* were found. The aim of this study was investigate the effect of the antagonistic fungi on *C. parasitica* growth and possibility of biological control. To determine the interaction between antagonistic fungi T. viride, *T. citrinoviride* and *T. roseum* and *C. parasitica* the laboratory test were established. *T. viride* has made contact on days 4-5, and was overgrown by *C. parasitica* within 6-10 days. *T. citrinoviride* has made the contact on days 4-6, and was overgrown or partial overgrown within 25-46 days. *T. roseum* has made contact on day 9-11, and was overgrown within 15-29-42 days. *T. viride* has the fastest grow and the most powerful antagonistic activity. Antagonistic fungi *T. viride*, *T. citrinoviride* and *T. roseum* are ubiquitous in chestnut stands. Laboratory experiment demonstrated that the antagonistic fungi were inhibited *C. parasitica*. This indicates that in the chestnut stands natural biological control with antagonistic fungi is present.

Key words: sweet chestnut, *Cryphonectria parasitica*, antagonistic fungi, biological control

P-13
SOLARNI PARK KOSORE – UTJECAJI NA OKOLIŠ I EKOLOŠKU MREŽU

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Solarni park Kosore graditi će se na području radne zone Kosore, na području grada Vrlike. Planirana je gradnja sedam različitih fotonaponskih elektrana na tlu, svaka snage 300 kW. Zahvat će omogućiti proizvodnju električne energije, koja će se distribuirati u elektroenergetsku mrežu. Obzirom da se radi o solarnom parku u kojem se prezentira sedam različitih tehnologija, može poslužiti kao ogledni prostor za optimiranje učinkovitoski tehnologije u odnosu na utjecaje na okoliš i ekološku mrežu, za potrebe budućih solarnih parkova na sličnim lokacijama u Republici Hrvatskoj.

Ključne riječi: sunčane elektrane, sedam različitih tehnologija, utjecaji, okoliš, ekološka mreža

SOLAR PARK KOSORE – ENVIRONMENTAL IMPACT AND NATURA 2000 NETWORK

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Solar park Kosore will be build within Kosore work zone, in the town of Vrlika. It is planned to build seven different photovoltaic power plants on the ground, each rated at 300 kW. The

procedure will allow production of electricity, which will then be distributed in to the electricity network. Given that this is a solar park which represents seven different technologies and it can be used as a prime area for technology effectivenes optimization in relation to the impacts on the environment and Natura 2000 network, and also for the future solar parks in similar locations in the Republic of Croatia.

Key words: solar power stations, seven different technologies, impacts, environment, Natura 2000 network

P-14

SIDRIŠTE U UVALI MEZUPORAT, OTOK BIŠEVO – KAKO SAČUVATI EKOLOŠKU MREŽU?

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Sidrište u uvali Mezuporat, na otoku Biševu je planirano za 20 brodova, na površini akvatorija od 4500 m^2, koji je dio ekološke mreže HR3000098 Biševo more. Sidrište se nalazi u blizini Modre špilje – jedinog od rijetkih zaštićenih objekta te vrste na Jadranu koji se posjećuje i dio je ekološke mreže, HR3000184 Modra špilja. Razlog za izgradnju sidrišta je pritisak posjetitelja na Modru špilju, koji raste iz godine u godinu, uslijed čega u ljetnim mjesecima cijela uvala postaje prostor sve brojnijeg "divljeg" sidrenja brodova, čime se sustavno uništava morsko dno, zaštićene biljne i životinjske vrste, kao dio ekološke mreže Republike Hrvatske.

Ključne riječi: Modra špilja, uvala Mezuporat, posjetitelji, utjecaji, ekološka mreža, sidrište

THE ANCHORAGE IN THE MEZUPORAT BAY, ISLAND OF BIŠEVO - HOW TO PRESERVE NATURA 2000 NETWORK?

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The anchorage in the Mezuporat bay, on the island of Biševo is planned for 20 ships, in the aquatorium measuring 4500 m^2 of surface of which is the part of Natura 2000 network HR3000098 Biševo more. The anchorage is located near the cave Modra - one of the fewest protected areas of this kind in the Adriatic, which is frequently visited and it is part of the Natura 2000 network HR3000184 Modra špilja.The reason for building the anchorage is the rising visitor pressure on to the Modra cave, which is growing each year, resulting in the impact of the "wild" ships anchoring through in the whole bay area in the summer months which systematically destroys the seabed, protected plant and animal species all being a part of Croatia Natura 2000 network.

Key words: Modra cave, Mezuporat bay, visitors, impacts, Natura 2000 network, anchorage

P-15

KVALITETA VODE U VODOTOCIMA POLJOPRIVREDNOG, MOČVARNOG I ŠUMSKOG PORJEČJA

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Procjena ograničavajućih čimbenika rasta algi i trofičkog stanja vodotoka i poplavnog područja, koja karakteriziraju nepravilni hidrološki uvjeti i promjene vodostaja, uključuje različite pristupe potrebne za prihvatljivo razumijevanje problema eutrofikacije. Cilj ovog rada je pružiti informacije o prostornoj i vremenskoj raspodjeli hranjivih tvari i ograničavajućih čimbenika rasta algi, kako bi se stvorila znanstvena osnova za procjenu stanja trofije kao nužnog preduvjeta definiranja mjera za smanjenje opterećenja voda hranjivim tvarima. Za postizanje tog cilja dizajniran je eksperiment u svrhu kvantitativne procjene rasta algi nakon dodatka hranjivih tvari, a kao rezultat dobivena je mjerljiva vrijednost ograničavajućeg djelovanja određene hranjive tvari. Način korištenja zemljišta kao i vrijeme uzorkovanja imali su značajan utjecaj na utvrđe nu varijabilnost fizikalno kemijskih čimbenika, kao i potencijala rasta algi. U skladu s tim, kvaliteta voda u slivu s najvišim postotkom poljoprivrednog zemljišta bila je vrlo loša s iznimno visokim koncentracijama fosfora. Samo blagi znakovi odstupanja od referentnih vrijednosti zabilježeni su u vodotocima pretežno šumskog sliva kao rezultat antropogenih aktivnosti. S druge strane, u vodotocima močvarnih slivnih područja povremeno je utvrđen i nedostatak hranjivih tvari.

Ključne riječi: eksperiment obogaćivanja nutrijentima, trofički uvjeti, N/P omjer

WATER QUALITY OF WATERCOURSES DRAINING AGRICULTURAL, WETLAND AND FORESTED CATCHMENTS

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Assessment of resources that are limiting algal growth and evaluation of the trophic status of rivers and wetlands, which present hydrological irregularities, incorporates different approaches in order to provide acceptable understanding of the problem. Our goal was to provide information on the temporal and spatial variation of resource limitation of algal growth rates to create a scientific basis for evaluating regional strategies of nutrient management in the investigated catchments. Therefore, a responsive test was designed to assess quantitatively algal growth after nutrient additions in order to obtain a biologically relevant measure of nutrient deficiency. As a result, a bioassay method was introduced for the evaluation of trophic status and limiting nutrients in the investigated watercourses. The observed variability in many of the physical and chemical const ituents, as well as algal growth potential, was attributed to the significant influence of both catchment land use and sampling time. Accordingly, the water quality of watercourses in the catchment with the highest percentage of arable land was determined to be very bad with extremely high phosphorus concentrations. Only moderate signs of distortion were observed in flowing waters of forested catchments as a result of anthropogenic activities. On the other hand, watercourses draining wetland watersheds showed even some nutrient deficiency.

Key words: nutrient enrichment experiment, trophic conditions, N/P ratio

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EKOLOŠKA MREŽA NATURA 2000 - ŠTO SE PROMIJENILO NAKON BIOGEOGRAFSKOG SEMINARA?

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Ekološka mreža Natura 2000 je mreža očuvanih područja Europe, namijenjena očuvanju više od 1000 ugroženih divljih vrsta i oko 230 stanišnih tipova. Dva su propisa temelj politike EU za postizanje očuvanja bioraznolikosti – Direktiva o staništima i Direktiva o pticama. Pristupanjem EU, Hrvatska je predložila Natura 2000 područja za više od 250 vrsta i 70 stanišnih tipova značajnih za Uniju, koji se pojavljuju u Hrvatskoj i time značajno doprinijela zaustavljanju gubitka bioraznolikosti na razini Europe. Područja mreže odabrana su na temelju stručnih kriterija za pojedinu vrstu i stanišni tip, prošla su proces konzultacija s javnošću te ih je Uredbom u 2013. (NN 124/13) donijela Vlada RH. Kako bi zadovoljila zahtjeve Direktive o staništima, Hrvatska je morala proći postupak potvrđivanja mreže, koji se provodi u vidu Biogeografskog seminara i za Hrvatsku je proveden u rujnu 2014. Na Seminaru su, osim predstavnika DZZP-a kao koordinatora inventarizacije divljih vrsta i stanišnih tipova i predstavnika Europske komisije, sudjelovali i predstavnici NVO te neovisni stručnjaci. Temeljem zaključaka EK sa Seminara, DZZP usklađuje popis Područja očuvanja značajnih za vrste i stanišne tipove te ih RH dostavlja EK. Ključne promjene u područjima ekološke mreže predstavljene su u ovom radu, a obuhvaćaju dopunu mreže područjima rijeke Korane i Save uzvodno od Zagreba te dopunu postojećih područja novim ciljnim vrstama i stanišnim tipovima.

Ključne riječi: ekološka mreža, Natura 2000, Biogeografski seminar, Direktiva o staništima

NATURA 2000 NETWORK - WHAT HAS CHANGED AFTER THE BIOGEOGRAPHICAL SEMINAR?

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Natura 2000 network is a network of conservation areas in Europe, aimed to conserve more than 1000 threatened species and about 230 habitat types. The cornerstones of EU policy for achieving biodiversity conservation are two Directives – Habitats Directive and Birds Directive. By joining EU, Croatia has proposed Natura 2000 areas for more than 250 species and 70 habitat types present in Croatia, thus significantly contributing to halting the biodiversity loss in Europe. The areas have been selected according to scientific benchmarks, undergone the process of public consultations and in 2013 promulgated with the Ordinance of Croatian Government. To meet the requirements of the Habitats Directive, Croatia went through the confirmation process, conducted in the form of Biogeographical Seminar, which was carried out in September 2014. Apart from the SINP representatives as the species and habitats inventory coordinator and the representatives of the European Commission, the representatives of NGOs as well as independent experts participated in the Seminar. On the basis of the EC conclusions from the Seminar, SINP is harmonising the Sites of Community Importance which the Republic of Croatia is submitting to the EC. The essential changes in Natura 2000 areas are presented in this paper and they include two new sites as an addendum to the network (River Korana and Upper Sava) as well as addition of new target species and habitat types to existing areas.

Key words: Natura 2000 network, Biogeographical Seminar, Habitats Directive

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ISTRAŽIVANJE RIJETKIH I UGROŽENIH SVOJTI MALAKOFAUNE ISTRE

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Prilikom projekta inventarizacije malakofaune Istre provođenog od 2012. do 2014. godine zabilježen je veći broj rijetkih i ugroženih vrsta mekušaca. Među njima je prema stupnju ugroženosti odabrano pet vrsta od posebnog značaja - slatkovodni školjkaši *Unio mancus* i *Microcondylaea compressa*, slatkovodni puž *Istriana mirnae* i kopneni puževi *Vertigo angustior* i *Vertigo moulinsiana*). Cilj istraživanja bio je detaljnije proučiti rasprostranjenost i stanje populacija odabranih vrsta na području Istre, odrediti čimbenike koji ih ugrožavaju i predložiti eventualne mjere zaštite. Prilikom terenskih istraživanja korištene su uobičajene metode prikupljanja kopnenih i slatkovodnih mekušaca. Materijal je nakon prikupljanja determiniran te su izrađene karte rasprostranjenosti za svaku vrstu. Rezultati su pokazali da su odabrane vrste na području Istre prisutne na malom broju lokaliteta, a u nekim slučajevima (*Microcondylaea compressa* i *Istriana mirnae*) radi se i o jedinom nalazištu vrste u Hrvatskoj. Čimbenici koji ugrožavaju istraživane vrste su zahvati na vodotocima, onečišćenja, melioracije i unos alohtonih vrsta mekušaca. U svrhu zaštite istraživanih vrsta potrebno je redovito provjeravati stanje njihovih populacija i neutralizirati ili smanjiti sve štetne čimbenike koji na njega djeluju.

Ključne riječi: mekušci, ugrožene vrste, *Microcondylaea compressa*, *Istriana mirnae*, Istra

RESEARCH OF RARE AND ENDANGERED TAXA OF ISTRIAN MALACOFAUNE

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During the project of molluscs inventarisation in Istria carried out between 2012 to 2014, a large number of rare and endangered species was recorded. Based on the level of threat they are facing, five of them were chosen for this study: the freshwater Bivalvia - *Unio mancus* and *Microcondylaea compressa*, the freshwater Gastropoda - *Istriana mirnae*, terrestrial Gastropoda - *Vertigo angustior* and *Vertigo moulinsiana*. The purpose of the project was to examine the prevalence and population states of the chosen species in Istria, as well as determine the factors that present a treat to them, and suggest possible measures of protection. During the field work operations, usual methods for sampling of terrestrial and freshwater molluscs were used. The samples were determined and distribution maps were made for each species. The results show that the chosen species are present in a small number of locations in Istria, and for some of them (i.e. *Microcondylaea compressa*, and *Istriana mirnae*) this was the first time their presence was confirmed in Croatia. The factors that present a threat to the investigated species are interventions on the watercourses, polution, land reclamation, and alien molluscs species. For protection purposes, state of populations of these species should be examined regulary, and all harmful factors neutralized or reduced.

Key words: molluscs, endangered species, *Microcondylaea compressa*, *Istriana mirnae*, Istria

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STRUKTURA I VIJABILNOST POPULACIJE SRNE, *Capreolus capreolus* U KONTINENTALNOJ HRVATSKOJ

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Kako bi predvidjeli buduće promjene u dinamici divljih populacija, osobito vjerojatnost izumiranja, potrebne su kvantitativne analize temeljene na podacima o ekološkim populacijskim značajkama. Ovo je istraživanje provedeno s ciljem određivanja ekoloških značajki populacije srne na lovištu u kontinentalnoj Hrvatskoj i kvantitativne procjene preživljavanja te populacije. Na temelju dobivenih rezultata zaključili smo da stanišna struktura i antropološke aktivnosti uvelike utječu na rasprostranjenost, populacijsku gustoću i preživljavanje populacije srne. Istraživana je populacija prometnicom podijeljena u dvije subpopulacije. Ukupan broj jedinki te gustoća i broj lanadi po ženki veći su u istočnoj subpopulaciji, vjerojatno kao posljedica bolje kvalitete staništa (većeg udjela šuma) na području njenog obitavanja. Usporedbom utvrđenih populacijskih parametara s literaturnim podacima dolazi se do zaključka da je kvaliteta staništa na cijelom istraživanom području visoka, a populacijska gustoća zadovoljavajuća, s obzirom da se radi o populaciji pod lovnim pritiskom. Rezultati analize vijabilnosti ukazuju na tanku liniju između stabilne populacije i izumiranja, naglašavajući važnost strogih kontrola i sprječavanja krivolova.

Ključne riječi: srna, struktura populacije, procjena vijabilnosti, lovni pritisak

STRUCTURE AND VIABILITY OF THE ROE DEER *Capreolus capreolus* POPULATION IN THE CONTINENTAL CROATIA

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In order to predict future alterations of the dynamics of wild animal populations, in particular the possibility of extinction, quantitative analyses based on the information on the ecological population parameters, are required. This investigation was conducted in order to estimate the population parameters of the Roe deer population on the hunting-ground in northern Croatia and to quantitatively explore its viability. It was concluded that the habitat structure and anthropological activities have great effect on the distribution, population density and survival of the investigated population. That population is divided into two subpopulations by a traffic road. The total number of individuals, population density and the number of fawns per female are greater in the eastern subpopulation, probably due to the better habitat quality (greater portion of forests) in its distribution range. Nevertheless, by comparing the population parameters obtained with literature data, it can be concluded that the environment quality is high in the whole investigated area and that the population density is satisfactory for populations which are under hunting pressure. Results of the viability analyses indicate that the line between balanced population density and extinction is very thin for the Roe deer populations under the anthropological influence, implying the importance of rigorous control and prevention of illegal hunting.

Key words: Roe deer, population structure, viability estimation, hunting pressure

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OCJENA PROGRAMA RADOVA ODRŽAVANJA U PODRUČJU ZAŠTITE OD ŠTETNOG DJELOVANJA VODA U HRVATSKOJ - UVJETI I MJERE ZAŠTITE PRIRODE: TEMELJI, IZAZOVI I PLANOVI ZA BUDUĆNOST

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Radovi održavanja voda utječu na hidromorfološke i biološke elemente vodotoka što može znatno narušiti njihov ekološki status. Ukupna duljina prirodnih i umjetnih vodotoka u Hrvatskoj iznosi oko 32.100 km, a njima upravljaju Hrvatske vode. Zakonom o zaštiti prirode propisano je da planovi gospodarenja prirodnim dobrima sadrže uvjete zaštite prirode, a ocjena prihvatljivosti provodi se za one koji mogu imati značajan negativan utjecaj na ciljeve očuvanja i cjelovitost područja ekološke mreže. Suradnja vodnog i sektora zaštite prirode na Programu radova održavanja započela je 2011. godine podjelom radova u 11 grupa. Za njih je DZZP temeljem niza primjera dobre prakse iz literature do danas razradio 70 tipiziranih mjera zaštite i preporuka. Ocjenom Programa izdaju se mjere koje se odnose na ukupnu bioraznolikost te ciljne vrste i staništa područja ekološke mreže. Ovisno o ekološkim potrebama ciljnih vrsta osmišljene su specifične mjere. Od 2012. godine problemi kod ocjene Programa su kasno dostavljanje zahtjeva što dovodi do slučajeva da se radovi počnu izvoditi prije izdanih uvjeta, nedostatak detaljnog opisa radova te krivo tumačenje mjera/neupućenost izvođača radova. Planira se nastavak suradnje ključnih sektora, edukacija dionika, odobravanje izvođenja samo nužnih radova, bolji sustav nadzora i izvješćivanja, monitoring učinkovitosti mjera te njihovo unapređivanje u skladu s rezultatima, daljnja razrada mjera zaštite te provođenje ocjene prihvatljivosti na strateškoj razini.

Ključne riječi: uvjeti i mjere zaštite prirode, Program radova održavanja voda, ocjena prihvatljivosti

ASSESSMENT OF THE PROGRAMME FOR MAINTENANCE WORKS FOR PROTECTION AGAINST HARMFUL EFFECTS OF WATER IN CROATIA – NATURE PROTECTION CONDITIONS AND MEASURES: FUNDAMENTALS, CHALLENGES AND PLANS FOR THE FUTURE

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Maintenance works affect the hydromorphological and biological elements of watercourses which can adversely affect their ecological status. Around 32,100 km of natural and artificial watercourses in Croatia is managed by state's water agency Hrvatske vode. The Nature Protection Act stipulates that natural resources management plans include nature protection conditions, and appropriate assessment is conducted for those which can have a significant impact on conservation objectives and integrity of the ecological network. Cooperation of water and nature protection sectors began in 2011 with dividing works into 11 groups, for which SINP has developed 70 standardised protection measures, based on a good practice examples from the literature. Issued measures refer to the overall biodiversity and conservation objectives of the ecological network. Regarding ecological needs of target species

specific measures were devised. Since 2012 problems in assessing the Programme are late submission which can lead to the beginning of works before conditions are issued, deficient works description and misinterpretation of measures/uninformed contractors. Plans for the future are further cooperation between key sectors, stakeholder education, realization of only necessary works, better system of monitoring and reporting, monitoring efficiency of measures and their improvement based on the results, further elaboration of measures and appropriate assessment at the strategic level.

Key words: nature protection conditions and measures, Programme for water maintenance works, appropriate assessment

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DRVENE OBALOUTVRDE: EKOLOŠKA ALTERNATIVA KAMENU I BETONU NA OBALAMA VODOTOKA

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Održivo upravljanje vodama u skladu s Okvirnom direktivom o vodama uključuje ekološke, hidrotehničke i upravljačke mjere i postupke koji su usmjereni prema zadržavanju ili obnovi prirodnog stanja vodotoka. Erozija obale predstavlja jedan od ključnih prirodnih procesa dinamike vodotoka kojim je u određenim slučajevima potrebno upravljati. Ovaj rad za cilj ima prikazati mogućnost uporabe drvenih obaloutvrda kao ekološki prihvatljive hidrotehničke mjere za stabilizaciju obala vodotoka u svrhu upravljanja procesom erozije. Za gradnju drvenih obaloutvrda koriste se stabla tvrdih listača koja su uzgojena u rijetkim nasadima. Sidrenjem stabala duž obale potiče se prirodno taloženje nanosa unutar grana krošanja te se usporava tok vode i smanjuje erozija obale. Nataloženi riječni nanos unutar i izvan krošanja pruža vlažnu i plodnu podlogu za razvoj vegetacije i mjesta za skrovišta divljim životinjama. S vremenom drveni dijelovi obaloutvrde trule, a na lokaciji ostaje posađena autohtona obalna vegetacija koja sprječava daljnje erodiranje obale. Za razliku od klasičnih obaloutvrda koje se grade od kamenog nabačaja i materijala, drvene obaloutvrde uz kontrolu erozije obale omogućuju i zadržavanje ili obnovu prirodi bliskog obalnog staništa. Drvene bi obaloutvrde trebale imati prednost nad kamenim i betonskim konstrukcijama u nedirnutim dijelovima vodotoka, u zahvatima revitalizacije antropogeno izmijenjenih vodotoka te posebice u upravljanju vodotocima u zaštićenim područjima prirode.

Ključne riječi: drvene obaloutvrde, upravljanje vodama, erozija obala, ekološke metode stabilizacije obala

TREE REVETMENTS: ECOLOGICAL ALTERNATIVE TO STONE AND CONCRETE AT THE RIVER BANK

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Sustainable water management in accordance with the Water Framework Directive includes environmental, hydro-technical and control measures designed to maintain or reintroducenatural features of watercourses. One of the key processes in the riverine natural dynamics, that requires active management, is erosion of river banks. In this paper we aim to demonstrate the possibility

of using wood revetmentsas anenvironmentally-friendly methodto stabilize stream banks and control erosion. Hardwood trees grown in plantations are commonly used for the construction of tree revetments. By anchoring the trees along the river banks, natural deposition of sediment within the branches of the tree crowns is encouragedwhile slowing the flow of water in the same time, thereby reducing the overall effect of erosion. Moreover, precipitated river deposit among canopies provides a moist and fertile basis for the development of vegetation and shelter for wild animals. Finally, woodrots and planted native riparianvegetation remains to prevent further erosion. In contrast to conventional revetments that are built from stone and concrete, tree revetments control erosion and allow the maintenance or revitalization of riparian habitats. Tree revetments have clear advantage over the stone and concrete in pristine natural parts of the watercourses, for the revitalization of anthropologically altered watercourses and especially in the management of watercourses in protected areas.

Key words: tree revetments, watercourse management, coastal erosion, ecological methods of river banks stabilization

2. SIMPOZIJ EDUKACIJE BIOLOGIJE
2nd BIOLOGY EDUCATION SYMPOSIUM

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PRIMJENA ONLINE TEČAJA ZA UČENJE BIOLOGIJE U SREDNJOJ ŠKOLI

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Online alati se sve više primjenjuju u nastavnom procesu. E-obrazovanje postaje tehnološki imperativ, a uvode se nove metode učenja koje će učenike pratiti kroz daljnje školovanje. Online tečajevi uključuju moderne elemente učenja i poučavanja u odnosu na tradicionalnu nastavu. Učenik uči po vlastitom tempu, u vrijeme koje mu odgovara. Pretpostavka je da primjena online tečaja doprinosi boljem razumijevanju i povećanom interesu za nastavne sadržaje iz biologije. Pomoću online tečajeva uspostavlja se bolja komunikacija među učenicima preko razmjene iskustava i ideja na forumima. Također se uspostavlja i bolja komunikacija učenika s nastavnicima kroz razmjenu pitanja i odgovora. Dodatno motivira i mogućnost vrednovanja znanja. U tečaju je omogućeno da nastavnici evaluiraju ishode učenja polaznika. Cilj istraživanja bio je usporediti rezultate učenika 1. i 3. razreda Gimnazije Vladimira Nazora iz Zadra i Srednje škole Bartula Kašića iz Paga, koji imaju podjednak uspjeh iz biologije. Učenici kontrolne skupine nisu koristili online alate, a učenici pokusne skupine bili su aktivni sudionici online tečaja. Provedena je i anketa s pitanjima zatvorenog tipa među učenicima pokusne skupine. Obrada podataka potvrdila je našu hipotezu: (1) da je povećan interes za sadržaje iz biologije i (2) da su postignuti bolji rezultati. Stoga možemo zaključiti da se korištenjem online tečaja kod učenja lakše postižu ciljevi i ishodi nastave biologije, te se unaprjeđuju kognitivna postignuća učenika.

Ključne riječi: Online tečaj, ciljevi i ishodi, kognitivna postignuća, biologija

APPLICATION OF ON-LINE SEMINAR FOR STUDYING OF BIOLOGY IN HIGH SCHOOL

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The online tools are being more frequently applied in education process. Our presumption is that the application of the online seminars contributes to a better understanding and to an increased interest for education alcontents of biology. Via on-line seminars, it is established a better communication between pupils through exchange of experiences and ideas on the forums. Also, a better communication is established between pupils and teachers through exchange of questions and answers. The aim of this investigation was to compare the results of learning between pupils of the 1st and 3rd class of the High School Vladimir Nazor, Zadar and those from the High School Bartul Kašić, Pag, which have attained almost the same success in learning biology. The pupils from the control group did not use the online tools while the pupils from the experimental group actively participated in the online seminar. We have also performed the questionnaire including of the questions of the "closed type" (blind type) among the pupils from the experimental group. Data analysis confirmed our hypothesis that the online learning resulted in: (1) an increased interest for biology contents and (2) better results of learning. According to that, we concluded that the use of the online seminar for learning may facilitate the attaining of objectives and outcomes in the education of biology and may improve the cognitive accomplishments of pupils.

Keywords: online seminar, objectives and outcomes, cognitive accomplishments, biology

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USVOJENOST OSNOVNIH KONCEPATA IZ BOTANIKE KOD UČENIKA PETOG RAZREDA OSNOVNE ŠKOLE

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Cilj istraživanja bio je utvrditi usvojenost osnovnih koncepata botanike koje su učenici usvojili na kraju prvog obrazovnog ciklusa iz nastavnog predmeta „Priroda i društvo“ u nižim razredima osnovne škole. Istraživanje je provedeno s učenicima 5. razreda osnovne škole, a prije obrade nastavne teme "Biljka cvjetnjača" nastavnog predmeta "Priroda". Instrument istraživanja sastojao se od niza zadataka objektivnog tipa. Sadržavao je ukupno 26 pitanja koja su ispitivala tri kognitivne razine znanja: reproduktivno znanje (prva razina), konceptualno razumijevanje (druga razina) te rješavanje problema (treća razina). Zadaci su bili otvorenog tipa (nadopunjavanja, dosjećanja i esejskog tipa) te zatvorenog tipa (alternativnog izbora, višestrukog izbora, povezivanja ujednačenog tipa). Utvrđena je nedovoljna usvojenost osnovnih koncepata botanike. Učenici bolje rješavaju zadatke kojima se ispituje reproduktivno znanje, a manje su uspješni u rješavanju zadataka kojima se ispituju više kognitivne razine znanja.

Ključne riječi: priroda, botanika, niži razredi osnovne škole, koncepti, kognitivne razine

ACQUISITION LEVEL OF BASIC BOTANY CONCEPTS BY ELEMENTARY SCHOOL STUDENTS IN THE FIFTH GRADE

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The aim of this research was to determine the acquisition level of basic botany concepts that students adopted by the end of the first educational cycle in the subject "Nature and Society" during the first four years of their formal education. Research was conducted among elementary school students in the fifth grade, in Nature Class, before the chapter "The Flowering Plant". The survey instrument consisted of 26 objective test items that assessed three levels of cognitive domain: recalling knowledge (first level), conceptual understanding (second level) and problem solving (third level). Tasks were open-ended (short answers, recalling facts and essays) and fixed-choice (alternative choice, multiple choice, matching). Results show an unsatisfactory permanence of knowledge of basic botany concepts. Students were more successful at solving tasks that tested knowledge, and less successful at solving tasks that required higher thinking skills.

Keywords: nature, botany, primary school, elementary school, concepts, cognitive domain

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USVOJENOST NASTAVNOG SADRŽAJA PRIMJENOM KONCEPTUALNIH MAPA KOD UČENIKA SREDNJE ŠKOLE

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Cilj istraživanja bio je usporediti nivo usvojenog znanja stečenog primjenom konceptualnih mapa s nivoom usvojenog znanja stečenog tradicionalnim (frontalnim) oblikom rada. Također se ispitao i nivo retencije znanja. U istraživanje su bili uključeni učenici drugih razreda Prirodoslovno-matematičke gimnazije u Osijeku (101 učenik). Tijekom travnja i svibnja školske godine 2013./2014. provedeni su nastavni sati s obradom nastavnih tema "Ptice" i "Sisavci". Nastavne teme u dva su razreda obrađene frontalnim oblikom rada, a u dva razreda pomoću konceptualnih mapa nakon čega je uslijedilo testiranje učenika pisanom provjerom znanja. U rujnu sljedeće školske godine (2014./2015.) proveden je test retencije znanja sa svim učenicima. Istraživanjem je utvrđeno da učenici koji su u učenju koristili konceptualnu mapu postižu statistički značajno bolje rezultate u odnosu na učenike koji su sadržaj učili frontalnim oblikom rada. Isti rezultat je dobiven i nakon provedenog testa retencije. Ovim radom prikazana je mogućnost primjene konceptualnih mapa u učenju i poučavanju sadržaja iz Biologije kao efikasne nastavne metode koja pridonosi postizanju konceptualnog razumijevanja.

Ključne riječi: konceptualna mapa, retencija znanja, biologija

ADOPTION OF TEACHING CONTENT USING CONCEPTUAL MAPS AMONG SECONDARY SCHOOL STUDENTS

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The aim of this research was to explore whether the students achieve better results during their learning process using conceptual maps or expository teaching. Also, the level of knowledge retention was tested.This research was conducted on the sample of 101 students of second grade of Natural Sciences High School and Mathematics High School in Osijek. During April and May in the school year 2013-2014 students have been teaching about two topics: "Bird" and "Mammals". In two classes students used traditional way of learning, so-called direct instruction. In other two classes student taught by themselves using conceptual maps. After teaching period was completed, all students wrote final test. Next school year (2014-2015) in September the knowledge retention test was carried out with all students. This research showed that students who used conceptual maps during learning process achieve statistical significant better results compared to students who have teach by using direct instructions. The same result is obtained after the retention test. This paper presents the good possibility for applying the conceptual maps in learning and teaching biology topics as an effective teaching method that contributes to the achievement of conceptual understanding.

Keywords: conceptual map, retention of knowledge, biology

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UTJECAJ KOGNITIVNIH RAZINA ZADATAKA NA VJERODOSTOJNOST PROCJENE USVOJENOSTI KONCEPATA GENETIKE KOD GIMNAZIJSKIH UČENIKA

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Cilj ovog rada bio je istražiti kako na vjerodostojnost provjere usvojenosti nastavnih koncepata utječe struktura zadataka postavljenih u tu svrhu. Analiziran je 141 test, što predstavlja 10% najbolje riješenih testova sa županijskog natjecanja iz biologije za IV. razred gimnazije, provedenog 2014. godine, na području cijele Hrvatske. Izdvojena su tri zadatka čija je riješenost bila najlošija, a koji su obuhvaćeni nastavnom cjelinom genetika. Utvrđeno je koje koncepte ispituju navedeni zadaci, a to su: 1) Kodiranje za aminokiseline; 2) Kretanje alela u populaciji; 3) Nastanak rekombinantnih potomaka. Zatim su sastavljeni novi testovi koje je činilo ukupno devet zadataka. Ti zadaci su postavljeni tako da se pomoću njih provjerava usvojenost utvrđenih koncepata na sve tri razine znanja (svaki od tri koncepta ispitan je s tri nova pitanja, svako na jednoj od tri razine znanja). Novi testovi su dani na riješavanje učenicima četvrtih razreda V. i XV. gimnazije u Zagrebu (ukupno 54 učenika). Iz dobivenih rezultata može se izvući zaključak da vjerodostojnost procjene usvojenosti koncepata dobrim dijelom ovisi o pristupu ispitivača u oblikovanju zadataka. Isto tako možemo zaključiti da riješenost zadataka viših kognitivnih razina ne mora biti garancija za potpunu usvojenost koncepta i ostvarenje planiranih ishoda učenja na nižim razinama. Da se ostvari što sigurnija procjena usvojenosti određenog koncepta od strane učenika, poželjno je ispitati taj koncept na sve tri razine znanja.

Ključne riječi: kognitivna razina, koncept, zadatak

IMPACT OF COGNITIVE LEVELS OF TASKS ON GENETICS CONCEPTS ASSESSMENT CREDIBILITY IN HIGH SCHOOL STUDENTS

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The aim of this study was to investigate how the structure of tasks affects concepts assessment credibility. 141 tests were analyzed, which represents 10% of the best solved tests in Croatian National Biology Competition for 12th grade (4. high school grade) students, conducted in 2014. Three worst solved tasks from genetics topic were observed. Concepts that evaluate those tasks were determined, and they are: 1) Amino acid coding; 2) Movement of alleles in a population; 3) Recombinant offspring production. New tests with nine tasks were prepared. These tasks were used to evaluate adoption of the established concepts at all three cognitive levels of knowledge (each of the three concepts was evaluated with three new tasks, each task on one of the three cognitive levels). New tests were given to 12th year students of V. Gimnazija and XV. Gimnazija in Zagreb (54 students total). It can be concluded from the results that concept assessment credibility depends on structure of tasks. We can also conclude that solving of tasks on higher cognitive levels may not be a guarantee for realization of intended learning outcomes at lower cognitive levels. It is desirable to examine the concept at all three cognitive levels of knowledge, to make sure students have adopted the concepts properly.

Keywords: cognitive level, concept, task

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KORIŠTENJE LOKALNIH RESURSA U TERENSKOJ NASTAVI BIOLOGIJE

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U Srednjoj školi Prelog, Gimnaziji Fran Galović i Srednjoj školi Tina Ujevića je u okviru izborne nastave biologije u drugim i trećim razredima gimnazije zastupljen terenski rad. Nastava ekologije, botanike i zoologije na terenu je vezana uz istraživačke učeničke projekte. Za učenička istraživanja živog svijeta korišteni su prirodni ekosustavi. Cilj rada je ispitati stavove učenika o kompetencijama koje steknu tijekom vlastitog istraživanja koristeći ekosustave svog okruženja. Kontrolnu skupinu čine učenici drugih i trećih razreda gimnazije koji nisu radili terensko istraživanje, a iz biologije su ocjenjeni ocjenom vrlo dobar ili odličan. Eksperimentalnu skupinu čine učenici drugih i trećih razreda navedenih škola koji su radili terensko istraživanje. Pretpostavka je da će učenici koji su radili terenska istraživanja cjelovitije, svrsishodnije sagledavati i objasnjavati uzročno-posljedične veze i povezivati s kognitivnim postignućima te znati povezati sa primjenom u svakodnevnom životu. Za istraživanje je korištena anketa u kojoj su ispitanici odgovarali na pitanja zatvorenog tipa brojčano prema skali po Likertu (1- uopće ne; 2- uglavnom ne; 3- nisam siguran; 4- uglavnom da; 5- u potpunosti). Obradom rezultata ankete ustanovljeno je da je eksperimentalna skupina izrazila pozitivnije statove prema uspjesima baziranih na korištenju lokalnih prirodnih resursa u učeničkim istraživačkim projektima. Nastava prirodnih znanosti trebala bi se više usredotočiti na metodu istraživačkog rada koristeći lokalne prirodne resurse. Kvalitenije usvojeni koncept održivog razvoja je temelj za aktivnije uključivanje u rješavanje problema u lokalnoj zajednici. Učenici eksperimentalne grupe smatraju da je rad na istraživačkom projektu poboljšao razvoj njihovih govorničkih vještina. Pri provjeri znanja o ekosustavima koji ne pripadaju lokalnom prirodnom okolišu učenici eksperimentalne skupine su pokazali bolje rezultate.

Ključne riječi: učenički istraživački projekt, terenski rad, kompetencije

USING LOCAL RESOURCES IN BIOLOGY FIELD WORK

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In Secondary school Prelog, Grammar school Fran Galović and Secondary school Tin Ujević field work is a part of elective Biology classes for students in the second and third grade. Teaching ecology, botany and zoology in the field is connected to student research projects. For the student research of the living world, natural ecosystems have been used. The topic of the paper is to examine student attitudes towards competencies that they acquire during their individula research while using ecosystems in their surroundings. The control group is made up of the second and third year grammar school students who have never done field research and have an A or B in Biology. The experimental group is made up of the second and third year students from above mentioned schools who have done field research. The assumpion is that the students who have done field work will look at problems more thoroughly and with more sense and will be able to explain cause and effect relationships, connect it to cognitive achievements and will know how to connect it to every-day situations. For the sake of research, a poll was used where the examinees answered closed type questions on the Likert scale (1- not at all; 2- mostly no; 3- not sure; 4- mostly yes; 5- completely). After data processing, the results showed that the experimental group has stated more positive attitudes towards accomplishments based on usage of local natural resouces in student research projects. Science classes should focus more on the method of research study, using local natural resources leads to a quality understanding of sustainable development which leads to more active involvement in the political life of a grown-up responsible citizen. Experimental group students think that working on a research project has lead to development of independence and security in presenting. Experimental group of students has shown better results during tests when examined about ecosystems which do not belong to local environment.

Key words: students' research projects, field research, competencies

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IGRA U PONAVLJANJU NASTAVNIH SADRŽAJA VEZANIH UZ ŽIVOTNE ZAJEDNICE MORA

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U nastavi prirode 6. razreda obrađuju se teme vezane uz more u nastavnoj cjelini "Životne zajednice mora i voda na kopnu". Kod ponavljanja sadržaja iz ovog područja učiteljima je teško postići maksimalnu učeničku koncentraciju i aktivnost svih učenika, pogotovo ako se sat svede na klasično propitivanje. Upravo zato se umjesto klasičnih metoda ponavljanja uvodi igra. Cilj je unaprjeđivanje kvalitete nastavnog procesa i uspostavljanje aktivnijeg sudjelovanja učenika u usvajanju nastavnih sadržaja vezanih uz more kao stanište u 6. razredu. Tijekom provođenja ponavljanja gradiva primjenjuju se igre asocijacije, križaljke i rebusi, koje učenici rješavaju u grupama. Uspoređuju se i rezultati anketiranja po 30 učenika 6. razreda iz OŠ Bogumila Tonija

Samobor i OŠ Ivane Brlić-Mažuranić Virovitica, čime se ispituje motiviranost učenika i njihovo zadovoljstvo ovakvim načinom rada. Učenici OŠ Bogumila Tonija Samobor su tijekom obrade nastavnih sadržaja vezanih uz more koristili zbirku morskih organizama i edukacijsku knjižicu "More je živo" Društva za ekološka istraživanja Paks s popratnim video materijalima dok su učenici OŠ Ivane Brlić-Mažuranić Virovitica koristili samo zbirku morskih organizama.Vrijednost primjene igre u nastavi prirode je u tome što se kod učenika, razvija osjećaj zadovoljstva usvojenim znanjem uz pozitivan natjecateljski duh kojim se potiče usmena komunikacija, tolerancija, uči slušati i jača samopuzdanje učenika. Na ovaj način učenici brže i lakše usvajaju sadržaje i uče se timskom radu.

Ključne riječi: ponavljanje, igra, osnovna škola, životne zajednice mora

THE PLAY IN REPEATING THE EDUCATIONAL CONTENTS ABOUT MARINE LIFE

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In primary schools topics about marine life are elaborated in the 6th grade nature education, in the educational unit "Marine and freshwater life communities". It is difficult for teachers to achieve maximum pupils' concentration and activity while repeating the contents of this topic, especially if the class is done in a way of classical knowledge testing. It is for this reason that the play is introduced into the learning process. The goal is to improve the educational process and to establish a more active participation of pupils in acquiring the educational contents related to marine habitats in the 6th grade. In repeating of these contents, games of association, crosswords, dice and rebus are introduced into the learning process. Results of a poll taken among 30 pupils of the 6th grade from the 'Bogumil Toni' Primary school in Samobor and 30 pupils of the 6th grade from the 'Ivana Brlić-Mažuranić' Primary school in Virovitica are compared, examining their motivation and their satisfaction with this way of learning. Pupils from the 'Bogumil Toni' Primary school in Samobor in their classes about marine life also used a collection of sea organisms and an educational booklet "The sea is alive" published by the Society for ecological research Paks with accompanying video material, while pupils from the 'Ivana Brlić-Mažuranić' Primary school in Virovitica used only a collection of sea organisms. The value of play in teaching nature contents lies in the fact that pupils develop a capability of oral communication and tolerance, learn to listen, strengthen their self-confidence, develop a sense of satisfaction with the acquired knowledge and encourage their competitive skills. In this way pupils learn faster and more easily acquire educational contents, as well as learn how to work in team.

Keywords: repeating, play, primary school, marine life

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EKOLOŠKE TEME U UDŽBENICIMA REPUBLIKE HRVATSKE I SAVEZNE REPUBLIKE NJEMAČKE

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Europska obrazovna politika usmjerena na razvoj kompetencija učenika naglašava važnost integracije ekoloških kompetencija u nacionalne okvirne kurikule. Kompetencije učenika za područje ekologije obuhvaćaju poznavanje svijeta prirode, zakonitosti održivog razvoja, kritičko poimanje znanosti i tehnologije po pitanju etike, sigurnosti i zaštite okoliša i dr. U radu su analizirani sadržaji Nacionalnih obrazovnih kurikula (NOK i NRW), npr. kompetencije učenika nižih razreda osnovne škole u svezi ekološkog odgoja, te udžbenici Prirode i društva s ciljem usporedbe zastupljenost ekoloških tema u njima u Republici Hrvatskoj i u Saveznoj Republici Njemačkoj, pokrajini Nordrhein-Westfallen. Može se zaključiti kako su u oba kurikula vrlo dobro zastupljene kompetencije učenika koje odražavaju ekološku pismenost. Rezultati kvantitativne analize govore da se u hrvatskim udžbenicima iz prirode i društva 1.- 4. razreda nalazi najviše šest ekoloških tema, a u njemačkim njih dvadeset. Utvrđene su i raspravljane razlike u pristupu pojedinim temama. Pretpostavlja se kako je holistički pristup ekološkoj tematici u Nacionalnom kurikulu Njemačke i njihovim udžbenicima jednim dijelom posljedica okolišne politike Europske Unije koja promiče ekološki standard temeljen na akcijskim programima. Uspjeh se ekološkog odgoja najbolje mjeri ponašanjem čovjeka pa je zato važno osigurati uvjete u kojima učenici svakodnevno mogu razvijati svoje ekološke kompetencije doprinoseći tako što boljoj sutrašnjici.

Ključne riječi: Nacionalni obrazovni kurikul, Hrvatska i Njemačka, udžbenici, okoliš, priroda i društvo

ENVIRONMENTAL ISSUES IN TEXTBOOKS OF CROATIAN AND FEDERAL REPUBLIC OF GERMANY

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European educational policy focused on developing competencies of students emphasizes the importance of integration of environmental competencies in national framework curricula. Competences of students in the field of environmental issues include understanding world of nature, the principles of sustainable development, a critical understanding of science and technology in terms of ethics, safety and environmental protection etc. The paper analyzes contents of the national educational curricula (NOK and NRW), e.g.: competences of lower grade primary school students on environmental issues, and analyzes textbooks for Nature and Society in Croatia and Germany (Nordrhein-Westfalen province) in order to compare presence of environmental topics in them. It can be concluded that in both curricula pupils' competencies reflecting environmental literacy are very well represented. Results of quantitative analysis show that the Croatian Nature and Society textbooks 1^{st}-4^{th} grade is up to six environmental topics, contrary to twenty in German textbooks. Some observed differences in the approach to particular topics were discussed as well. We assume that a holistic approach to environmental issues in the National Curriculum of Germany and their textbooks, are partly a consequence of the environmental policy of the EU which promotes standards based on action programs. It is important to provide conditions in which pupils can develop their daily environmental abilities.

Keywords: National education curriculum, Croatia and Germany, textbooks, environment, nature and society

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UČENIČKO POZNAVANJE ŽIVOTINJA HRVATSKE

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Cilj istraživanja bio je analizirati koliko učenici poznaju činjenice o životinjama koje žive u Hrvatskoj te je postavljena hipoteza da poznavanje životinja ovisi o nastavnom programu, pri čemu će najbolje rezultate ostvariti učenici 3. razreda. Provedeno je 2013. godine na uzorku od 127 učenika od 1. do 4. razreda V. gimnazije u Zagrebu.Učenici su rješavali anketu s 2 tipa pitanja. U prva 3 pitanja traženo je opće znanje o životinjama Hrvatske, a u sljedećih 16 učenici su trebali zaokružiti jesu li navedene tvrdnje o životinjama točne ili netočne. Pitanja su podijeljena u kategorije: a) jesu li ili nisu u nastavnom planu i programu, b) na ona koja jesu ili nisu važna za život učenika, c) pitanja vezana uz raznolikost te d) pitanja vezana uz prilagodbe životinja.Na temelju dobivenih podataka može se uočiti da su najslabije rezultate postigli učenici 1. razreda, a najbolje učenici 3. razreda. Nisu uočene razlike s obzirom na spol te se može zaključiti da djevojčice i dječaci podjednako poznaju životinje Hrvatske. Ukupna riješenost od oko 60% upućuje na zaključak da bi se u nastavi biologije trebalo više pažnje posvetiti toj tematici kako bi učenici shvatili važnost upoznavanja određenih vrsta i njihovih karakteristika što zbog životne važnosti (prepoznavanje zmija otrovnica) što zbog opće kulture (autohtone pasmine Hrvatske).

Ključne riječi: istraživanje, V. gimnazija, životinje Hrvatske, analiza anketa

STUDENT`S KNOWLEDGE OF ANIMALS THAT LIVE IN CROATIA

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The aim of this study was to analyze how much facts the students know about the animals that live in Croatia and it has been hypothesized that knowledge of animals depends on the curriculum. The best results achieved 3rd grade students. The study was made in 2013 on a sample of 127 students into 4 grades in V. High School in Zagreb. Students were solving 2 types of survey questions. In the first three questions they were asked about general knowledge of animals in Croatia, and in the following 16, students had to choose whether the following statements about animals are correct or incorrect. The questions are divided into categories: a) to those that are or are not in the curriculum, b) to those that are or are not relevant to the life of students, c) questions related to diversity and d) questions related to the adaptation of animals.Based on the results, the lowest score had 1st grade students, and the best score had students of 3rd grade. There is no essential difference between the knowledge of girls and boys. The total score of about 60% indicates that biology teachers should pay more attention to this topic so that students understand the importance of getting to know certain species and their characteristics. Because that is very important for every student (for example, students should be able to identifying poisonous snakes).

Keywords: study, V. High School, animals of Croatia, analisis of survey

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STAVOVI UČITELJA O BIOLOŠKIM NASTAVNIM SADRŽAJIMA U OSNOVNOJ ŠKOLI

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Cilj istraživanja bio je utvrditi stavove učitelja Prirode i Biologije o Nastavnom planu i programu u osnovnoj školi. 180 učitelja u razdoblju od 2004. do 2006. godine izražavali su slaganje uz 18 pitanja anketnog upitnika vezanih za nastavni program Prirode i Biologije. Nastavni plan i program itekako utječe na učenička postignuća te na njihove interese i stavove prema predmetu (citat). 84% učitelja se izjasnilo da se zadaće u Nastavnom planu i programu temelje na činjenicama.46% učitelja smatra da se novi koncepti ne izgrađuju prema usvojenim predkoncepcijama. Učitelji su se također izjasnili da učenici ne razumiju gradivo o razmnožavanju u 5 razredu te njih 67% smatra da učenici to gradivo ne mogu povezati sa stvarnim životom i razvojem stava prema vlastitom zdravlju. Većina učitelja, njih 73% smatra da bi trebalo promijeniti postojeći Nastavni plan i program. Sadašnji Nastavni plan i program nije u dovoljnoj mjeri prilagođen dobi i interesima učenika te ne podupire izgradnju novih koncepata na usvojenim predkoncepcijama. Sadržaji Nastavnog programa bi trebali podupirati usvajanje prirodoznanstvene metode i kritički pogled na svijet na principu aktivnog učenja, što veći broj profesora u anketi smatra da nije zastupljeno u sadašnjem Nastavnom programu.

Ključne riječi: kurikulum, učitelji, interesi, učenici

TEACHERS' ATTITUDES TOWARD THE BIOLOGY CURRICULUM CONTENTS IN ELEMENTARY SCHOOLS

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The aim of this study was determing attitudes of elementary school teachers toward the biology curriculum. From 2004 to 2006, 180 elementary school biology teachers took part in this study. They answered to a yes/no questionnaire, which contains 18 questions related to the biology curriculum. The curriculum has a major influence on students' achievements and their interests and attitudes toward the school subject (quote). 84% of teachers stated that the homework tasks in the curriculum are based on solely theoretical knowledge. 46% of teachers reported that students often do not use theoretical knowledge for acquisition of new knowledge. In addition, students in 5th grade do not comprehend the lessons about human reproduction, therefore they can not apply this knowledge in real life and do not see the benefits of it. 73% of teachers considered that the existing curriculum should be changed. The current curriculum is not sufficiently arranged to the age and interests of the students and does not encourage the developement of new knowledge. The content of the curriculum should support studying a natural-scientific method, have a critical view and encourage active learning. Many teachers in this survey agree with methods not being represented in the current curriculum.

Keywords: curriculum, students, biology, teachers

EVOLUCIJA, SISTEMATIKA, FILOGENIJA I BIOGEOGRAFIJA
EVOLUTION, SYSTEMATICS, PHYLOGENY AND BIOGEOGRAPHY

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THE POTENTIAL ROLE OF ECOLOGICAL ADAPTATION AND *Wolbachia* INFECTION IN THE DIVERSIFICATION OF *Rhagoletis cerasi*

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Ecological adaptation to different hosts can be an important factor in the diversification of different species. The genus *Rhagoletis* is a model for sympatric speciation (speciation with gene flow). The apple maggot, *Rhagoletis pomonella*, has proved to be a prominent model case for sympatric speciation, due to adaptation to different hosts. Host choice is positively related to mate choice which results in prezygotic isolation. Similarly, the European cherry fruit fly *Rhagoletis cerasi* infests two different hosts, *Prunus* spp and *Lonicera* spp. The phenological and behavioural adaptation to the different hosts could lead to reduction of gene flow and potentially drive speciation. A previous study documented genetic differences between the different host populations for a single allozyme locus. To perform a more thorough genome wide search for differentiation we will use ddRADSeq, to identify genetic markers of host differentiation within sympatric and allopatric populations of *R. cerasi*. Additionally, previous studies showed that *R. cerasi* is infected by *Wolbachia* that causes cytoplasmic incompatibilities between host associated populations in allopatry. Therefore our ddRADSeq approach will allow us to determine the potential role of *Wolbachia* in the diversification of *R. cerasi*.

Key words: sympatric speciation, host-induced differentiation, cytoplasmic incompatibility

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PRIMJENA SUVREMENIH TEHNIKA PROSTORNE STATISTIKE U EKOLOŠKIM ISTRAŽIVANJIMA

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Bitan dio kvantitativnog pristupa ekološkim istraživanjima je prostorna komponenta. Stoga su metode prostornog određenja, bez obzira radi li se o veličini areala vrste ili koncentraciji nutrijenata ili polutanata u nekom okolišnom kompartmentu, nezaobilazan korak u interpretaciji istraživanja. Razvojem informatičke znanosti paralelno s tehnologijom otvorile su se mogućnosti vrlo složenog simuliranja ekoloških eksperimenata što je dovelo do razvoja novih tehnika njihovog dizajniranja. Zahvaljujući suvremenim metodama prostorne statistike moguće je postići optimizaciju ekoloških istraživanja u smislu reduciranja potrebnog vremena, potrebne veličine uzoraka i veličine obuhvaćenog prostora. U ovom radu će biti prikazana primjena simulacije temeljene na prostorno statističkim modelima i simulacijama uzorkovanja faune tla. Usporedbom rezultata dobivenih simuliranim eksperimentom u laboratoriju i realnim eksperimentom u okolišu ustanovila se statistički značajna sličnost. U okviru rada donesene su hipoteze o porijeklu greške simuliranog i realnog rezultata.

Ključne riječi: prostorna statistika, ekologija, fauna tla

APPLICATION OF MODERN SPATIAL STATISTICAL TECHNIQUES IN ECOLOGICAL RESEARCH

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Spatial component is an important part of quantitative approach to ecological research. Whether they're used for areal size or nutrient (or pollutant) concentrations in the environmental compartment, methods based on spatial determination are the essential step in the interpretation of research. Development of informatics sciences alongside with technology opened a lot of possibilities for complex simulation of ecological experiments which then led to development of new design techniques. Thanks to modern methods of spatial statistics, it is possible to achieve the optimization of ecological research in the term of reducing needed time, sample size and size of the sampled area. In this paper the application of simulation based on spatial statistical models will be shown together with simulation of sampling soil fauna. Through comparison of results obtained from simulated experiment in laboratory and real experiment in environment, statistical significance was established. In the framework of this paper, hypotheses were adopted regarding the origin of error in results obtained from simulated and real experiment.

Key words: spatial statistics, ecology, soil fauna

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GENETSKA STRUKTURA BJELONOGOG RAKA (*Austropotamobius pallipes* KOMPLEKS) OTKRIVA MOGUĆU POVEZANOST IZMEĐU TRI KRŠKE RIJEKE TIJEKOM GEOLOŠKE PROŠLOSTI

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Provedeno je opsežno istraživanje populacijsko-genetičkih i morfoloških obilježja bjelonogog raka u jadranskom slijevu. Ovdje prikazujemo rezultate koji se odnose na analize triju populacija (rijeke Rječina (RJE), Reka (REK) i Glinščica (GLI)) kod kojih je zabilježen neočekivani genetski uzorak. Unatoč sadašnjoj geografskoj izoliranosti tih triju populacija, prema rezultatima genetskih analiza mitohondrijskih i mikrosatelitnih biljega, one se svrstavaju u istu grupu. Dobivena genetska struktura mogla je nastati kao posljedica prenošenja posredovanog čovjekom, ili kao posljedica prirodnog širenja. Prirodno širenje je najvjerojatniji uzrok dobivene strukture iz nekoliko razloga; Malo je vjerojatno da bi se dobivene umjerene vrijednosti očekivane heterozigotnosti (He =0,34 u GLI, =0,36 u REK, =0,44 u RJE) pojavile u novo uspostavljenim populacijama nako n prenošenja posredovanog čovjekom. Nadalje, povezanost između Reke i Rječine je već otprije utvrđena na osnovu proučavanja rasprostranjenosti vrsta roda *Niphargus*. Zaključno, prema geografskom položaju i hidrologiji istraživanih rijeka, prirodno širenje se moglo dogoditi kao posljedica povezanosti izvorišnih dijelova tijekom geološke prošlosti ili zbog širenja rakova kroz podzemne vodene sustave. Zadnju mogućnost treba uzeti u obzir jer postoje brojni nalazi bjelonogog raka, i njegove sestrinske vrste, potočnog raka, vrlo duboko unutar špiljskih sustava.

Ključne riječi: istočno-jadranski sljevovi, Hrvatska, Slovenija, mitohondrijski biljezi, mikrosatelitni biljezi

GENETIC STRUCTURE OF THE WHITE-CLAWED CRAYFISH (*Austropotamobius pallipes* COMPLEX) REVEALED POSSIBLE HISTORICAL CONNECTIONS BETWEEN THREE KARSTIC RIVERS

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The comprehensive research on population genetics and morphological characteristics of the white-clawed crayfish from Adriatic Basin was conducted. We present results that include analyses of three populations (Rječina (RJE), Reka (REK), and Glinščica (GLI) rivers), in which an unexpected genetic pattern was observed. Despite the current geographical isolation of these three populations, results of genetic analyses grouped them into the same cluster. The obtained genetic structure could be a consequence of human-mediated translocations, or a consequence of natural dispersal. Natural dispersal is the most probable cause of obtained structure for several reasons; Observed moderate values of expected heterozygosity (He =0.34 in GLI, =0.36 in REK, =0.44 in RJE) were not likely to appear in newly established populations after human-mediated translocations. Further, the connection between the Reka and Rječina rivers was recognized based on the distribution of *Niphargus* species. Finally, according to geographical position and hydrology of examined rivers, natural dispersal could appear due to historical headwater river captures or due to dispersal of crayfish through underground water systems. The last possibility should be taken into consideration because it is not unusual to find the white-clawed crayfish and its congeneric species, the stone crayfish, very deep inside cave systems.

Key words: Eastern Adriatic Basins, Croatia, Slovenia, mitochondrial markers, microsatellite markers

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FILOGENIJA I FILOGEOGRAFIJA RODA *Elmis* (COLEOPTERA, ELMIDAE) KRŠKIH TEKUĆICA JUGOISTOČNE EUROPE

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Vodeni kornjaši iz porodice Elmidae su slabo istraženi na području jugoistočne Europe pa je malo podataka i o njihovim filogenetskim i filogeografskim odnosima. Tako su za ovo istraživanje sakupljeni predstavnici navedene porodice u Hrvatskoj, Bosni i Hercegovini i Makedoniji, dok su oni iz Austrije korišteni za usporedbu. Upotrebom standardnih genetičkih markera (ITS1, COI i COII) pokazali smo da su vrste *Elmis aenea* i *E. rioloides* sestrinske te zajedno najsrodnije vrsti *E. bosnica*. Također, sve su tri navedene vrste u srodstvu sa vrstom

E. maugetii maugetii. S druge strane, filogenetski položaj vrste *E. obscura* nije još razjašnjen. Kod vrsta *E. aenea* i *E. rioloides* sakupljenih na rijeci Cetini uočene su morfološke razlike u odnosu na jedinke istih vrsta u središnjem dijelu Europe. Stoga je glavni cilj ovog istraživanja analizirati može li se uočena morfološka razlika potvrditi i na molekularnoj razini. Uzorci vrste *E. aenea* ne pokazuju značajne molekularne razlike s obzirom na uočeni morfološki dimorfizam i lokalitete. Analiza uzoraka vrste *E. rioloides* s rijeke Cetine ukazuje na odvajanje od tipičnih predstavnika srednjoeuropske faune podupirući dosadašnji status potencijalne kompleks vrste. Metodom molekularnog datiranja izračunali smo da je do razdvajanja između grupa vrste *E. rioloides* iz Srednje Europe i s područja Cetine došlo u Pleistocenu što je u skladu s pretpostavkom o važnosti pleistocenskih refugija u specijacijskim procesima na području jugoistočne Europe.

Ključne riječi: vodeni kornjaši, Elmidae, kompleks vrste, Balkan, pleistocen

PHYLOGENY AND PHYLOGEOGRAPHY OF GENUS *Elmis* (COLEOPTERA, ELMIDAE) IN KARSTIC RUNNING WATERS IN SOUTHEASTERN EUROPE

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Elmidae is a water beetles family poorly studied in southeastern Europe, so phylogenetic and phylogeographic data are very scarce. Representatives of the family were collected in various freshwater habitats in Croatia, Bosnia and Herzegovina and Macedonia, while Austrian representatives were used for needed comparisons. Using standard genetic markers (ITS1, COI and COII) we revealed sister group of *Elmis aenea* and *E. rioloides* together being related to *E. bosnica*. *E. maugetii maugetii* forms a separate branch, while position of *E. obscura* could not be affirmed with high support. In addition, *E. aenea* and *E. rioloides* collected in Cetina River showed morphological variations from Central European representatives. Therefore, the main aim of this study was to analyse whether the observed differences can be confirmed at the molecular level. *E. aenea* shows high molecular h omogeny across all sampled populations in respect to both morphological features and locations. On contraire, screening of *E. rioloides* specimens indicated the separation of population from the Cetina River from typical representatives of Central European fauna, supporting the hypothesis that this is a complex species. By molecular dating we calculated that *E. rioloides* separated from Central European representatives in the Pleistocene. This is consistent with the observation of the importance of Pleistocene refugia in speciation processes in the Southeastern Europe.

Key words: water beetles, Elmidae, complex species, Balkan, Pleistocene

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SIMBIOZA HIDRE I ALGE: KARAKTERIZACIJA ENDOSIMBIOTSKIH ZELENIH ALGI IZOLIRANIH IZ RAZLIČITIH SOJEVA ZELENIH HIDRI POMOĆU ELEKTRONSKE MIKROSKOPIJE, MORFOMETRIJSKE I ENZIMSKE ANALIZE

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Proces endosimbioze jedan je od pokretača specijacije. Zelena hidra (*Hydra viridissima* Pallas, 1766) tipičan je primjer endosimbioze, jer u svojim gastrodermalnim mioepitelnim stanicama sadrži jednostanične fotoautotrofne alge. Endosimbiotske alge izolirane iz zelenih hidri održavamo u trajnim stabilnim laboratorijskim kulturama. Sojevi zelenih hidri prikupljeni su sa šest različitih geografskih lokaliteta: s četiri različite lokacije u Hrvatskoj, te iz Izraela i iz Njemačke. Endosimbiotske zelene alge izolirane su koristeći mikrobiološke metode. Izolirane endosimbiotske alge karakterizirane su prema citološko-morfometrijskim parametrima te prema aktivnosti i sastavu enzima katalaze, peroksidaze i esteraza. Stanice endosimbiotskih algi karakterizirane su morfometrijskim mjerenjem promjera, opsega i površine. Alge HV i M9 odvojile su se od T i BV u veličini stanica. Rezultati dobiveni analizom sastava izoenzima upućuju na razliku između algalnih endosimbionata s hrvatskih lokaliteta i algalnih endosimbionata iz Europe, ukazujući na biološku raznolikost među algalnim simbiontima. PAGE elektroforezom izolirano je ukupno pet izoenzima katalaze i pet izoenzima peroksidaze u endosimbiotskim algama. Izoenzim K1 pojavio se jedino kod endosimbiotske alge M9 iz Izraela, a izoenzim K3 jedino kod T soja. Izoenzim P1 pojavio se jedino kod endosimbionta HV, izoenzim P5 kod HV i M9. Izoenzimi P3 i P4 pojavili su se jedino kod algi BV i T iz Hrvatske.

Ključne riječi: endosimbiotske alge, zelena hidra, simbioza, morfometrija, izoenzimska analiza

HYDRA – ALGAE SYMBIOSIS: CHARACTERIZATION OF ENDOSYMBIOTIC GREEN ALGAE ISOLATED FROM DIFFERENT GREEN HYDRA STRAINS USING ELECTRON MICROSCOPY, MORPHOMETRIC AND ENZYME ANALYSIS

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Process of endosymbiosis is one of promoters of speciation. The green hydra (*Hydra viridissima* Pallas, 1766) is a typical example of endosymbiosis. Its gastrodermal myoepithelial cells harbor the individuals of unicellular photoautotrophic algae. We have established prosperous laboratory cultures of endosymbiotic green algae isolated from green hydra strains. Hydra strains were from 6 different geographical localities: 4 from Croatia, 1 from Israel and 1 from Germany. Endosymbiotic green algae were successfully isolated using microbiological methods. Isolated endosymbiotic algae were characterized employing cytological morphometric parameters, enzyme activity and isoenzyme pattern analysis (catalase, peroxidase and

esterases). Cells of endosymbiotic algae were characterized by morphometric measurements: diameter, perimeter and area. Algal HV and M9 strains were separated from strains T and BV. The results obtained by isoenzyme pattern analysis suggest a difference between Croatian and European algal endosymbionts, indicating biological diversity among algal symbionts isolated from green hydras. Five isoenzymes of catalase and five isoenzymes of peroxidase were resolved by PAGE electrophoresis. Isoenzyme K1 appeared only in endosymbiotic algae M9 from Israel and isoenzyme K3 only in T strain. Isoenzyme P1 was observed only in endosymbiont HV while isoenzyme P5 in both endosymbionts HV and M9. Isoenzymes P3 and P4 were visible only in algal strains BV and T from Croatia.

Key words: endosymbiotic algae, green hydra, symbiosis, morphometry, isoenzyme analysis

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ErIK: SOFTVERSKA PLATFORMA ZA IDENTIFIKACIJU GUJAVICA HRVATSKE

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Fauna beskralješnjaka u Hrvatskoj nedovoljno je istražena. Čest razlog neistraženosti je "neaktraktivnost" svojti, ali i nedovoljna dostupnost ključeva za određivanje. Osobito su neistraženi beskralješnjaci tla, kako u Hrvatskoj tako i u Europi što se u posljednje vrijeme pokušava promijeniti s obzirom na značaj edafobionata za funkcionalnost i zdravlje ekoloških sustava. Jedna skupina edafobionata su gujavice (Lumbricidae), kojih je za faunu Hrvatske do sada zabilježeno 68 vrsta raspoređenih u 17 rodova. Osobitu faunističku značajnost čini deset endemskih vrsta. Kako bi se olakšalo njihovo određivanje razvili smo Ključ za određivanje vrsta gujavica Hrvatske - ErIK u obliku računalnog programa. Ključ je dizajniran tako da se pomoću različitih alata i slika olakša određivanje do razine vrste. Glavni prozor programa sadrži opisne tekstove (vanjska i unutrašnja građa, distribucija), kartu pronalaska vrste, crtež vrste i popis svih vrsta u Hrvatskoj. Određivanje se može provesti na dva načina - pomoću tabličnog i pomoću dihotomskog ključa. Tablični ključ omogućuje izravno upisivanje morfoloških i anatomskih parametara promatrane jedinke. Moguće je upisati ukupno 18 parametara, a nije potrebno upisati sve kako bi ključ radio. Dihotomski ključ temelji se na klasičnom ključu određivanja vrsta po principu dva izbora. Unutar programa se nalazi i priručnik za upotrebu s opisom svih morfoloških i anatomskih obilježja. Program je besplatan i dostupan za skidanje sa mrežne stranice.

Ključne riječi: Lumbricidae, tablični ključ, dihotomski ključ

ErIK: A SOFTWARE PLATFORM FOR IDENTIFICATION OF EARTHWORMS OF CROATIA

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The fauna of invertebrates in Croatia is insufficiently researched. A frequent reason lies in "unattractiveness" of taxa and insufficient availability of determination keys. Particularly unexplored are soil invertebrates both in Croatia and Europe which has recently being changed due to the importance of edaphobionts for functionality and health of ecosystems. One group of edaphobionts are earthworms (Lumbricidae). So far 68 species distributed in 17 genera have

been recorded for Croatia, of which 10 endemic species have a particular faunistic significance. In order to facilitate their determination we have developed Earthworm Identification Key - ErIK as a computer program. The key is designed so that usage of different tools and images facilitates determination to species level. The main window contains descriptions (anatomy and morphology, distribution), map with species records, drawing of the species and the list of all species in Croatia. Determination can be carried out in two ways, using tabular and dichotomous key. The tabular key enables entering morphological and anatomical parameters of observed individuals. A total of 18 parameters can be inserted, but not all for key to function. The dichotomous key is based on a classic determination key with two choices. There is a manual with description of all morphological and anatomical characteristics within the software. The software is free and available for download from a website.

Key words: Lumbricidae, tabular key, dichotomous key

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FLORA OTOČIĆA ŠOLTANSKOG ARHIPELAGA

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Istraživana je flora 7 otočića: Stipanska, Balkun, Rudula, Polebrnjak, Grmelj, Saskinja i hrid Kamik, smještenih jugozapadno od obala otoka Šolte. Utvrđena je velika raznolikost vaskularne flore, najveći broj vrsta je zastupljen na otočiću Stipanska i iznosi 141 vrstu. Najzastupljenije porodice u ukupno istraživanoj flori su Fabaceae (10,55%), Poaceae (8,69%) i Asteraceae (5,59%). Najveći broj vrsta i podvrsta flore otočića pripada mediteranskom flornom elementu (63,96%) i to opće mediteranskim biljkama (64%). U okviru mediteranskog elementa veliko značenje imaju ilirsko-mediteranske biljke (9,7%) te u sklopu njih ilirsko-jadranske endemične biljke (9,87%). Analizom životnih oblika flore otočića utvrđena je najveća zastupljenost terofita (30,43%), potom fanerofita s (24,85%) i hemikriptofita (18,63 %). U flori otočića utvrđeno je 5,6 % endemičnih biljaka. Istražene su i ugrožene svojte (2%), zaštićene (12%) i strogo zaštićene svojte (6,8%).

Ključne riječi: flora, Stipanska, Balkun, Rudula, Polebrnjak, Grmelj, Saskinja, Kamik, otočići, endemi

THE FLORA OF ŠOLTA ARCHIPELAGO ISLETS

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We investigated the flora of 7 islands: Stipanska Balkun, Polebrnjak, Grmelj, Saskinja and rock Kamik, located south-west coast of the island of Solta. There was a great diversity of vascular flora, the largest number of species is represented on the island Stipanska and is 141 species. The most abundant families in the studied flora of the Fabaceae (10.55%), Poaceae (8.69%), and the Asteraceae (5.59%). The highest number of species and subspecies of flora islands belong to the Mediterranean floral element (63.96%) and general Mediterranean plants (64%). Within the Mediterranean element of great significance have Illyrian and Mediterranean plants (9.7%) and within them Illyrian-Adriatic endemic plants (9.87%). The analysis of life forms flora islands established a major presence Therophyta

(30,43%), then Phanerophytes with (24.85%) and Hemikryptophytes (18.63%). The flora of the islands was found 5.6% of endemic plants. Have been investigated and threatened species (2%), protected (12%) and strictly protected species (6.8%).

Key words: flora, Stipanska, Balkun, Rudula, Polebrnjak, Grmelj, Saskinja, Kamik, endems

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ANALIZA DIJELA HERBARIJA CARLA STUDNICZKE (ORD. CAPRIFOLIACEEN, LONICEREEN I STELLATEEN)

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Analizirani su redovi originalno označeni kao "Lonicereen", "Caprifoliaceen" i "Stellateen" (157 herbarijskih listova). Najveći dio biljaka sakupljen je u Europi (146 listova), a najviše s područja današnje Austrije (30 listova). Prema pripadnosti pojedinim herbarijima ističu se biljke iz zbirke "Flora Dalmatiens". U odnosu na dosada obrađeni dio herbarija navode se nove zbirke: Flora d. from Brandeburg, Herb F. Tremols i Herbier de la Société d'Histoire Naturelle de Colmar. Najviše biljnog materijala sakupio je sam Studniczka (92 herbarijska lista). Najstariji herbarijski list je iz 1856. god., a najmlađi iz 1904. god. Studniczka unutar 157 herbarijskih listova navodi 15 rodova s 83 vrste u okviru kojih je zabilježeno 19 varijeteta.

Ključne riječi: Prirodoslovni muzej Split, herbarij, Studniczka

THE ANALYSIS OF PART OF CARL STUDNICZKA'S HERBARIUM (ORD. LONICEREEN, CAPRIFOLIACEEN AND STELLATEEN)

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Orders originally assigned as "Lonicereen", "Caprifoliaceen" and "Stellateen" (157 herbarium sheets) were analysed. The majority of herbarim material was collected in Europe (146 sheets) in the area of Austria (30). Most herbarium sheets belong to Flora Dalmatiens collection. In reference to the part of Studniczka's herbarium which has already been analysed, there are some new collections: Flora d. from Brandeburg, Herb F. Tremols and Herbier de la Société d'Histoire Naturelle de Colmar. Most herbarium sheets were collected by Studniczka himself (92). The oldest herbarium sheet dates from the year 1856 and the newest one is from 1904. According to Studniczka, within 157 sheets 15 genera with 83 and 19 varietets were recorded.

Key words: Studniczka's herbarium, Natural History Museum Split

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POPULACIJSKA EKOLOGIJA I SPOLNI DIMORFIZAM LIVADNE GUŠTERICE *Lacerta agilis*, LINNAEUS 1758 S DINARE U HRVATSKOJ

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Livadna gušterica, *Lacerta agilis* Linnaeus, 1758 je mali gušer koji naseljava Europu i istočni dio Azije, uključujući i veći dio Hrvatske. Početkom kolovoza 2012. godine, proveli smo istraživanje ove vrste na planini Dinara u Hrvatskoj. Tijekom tog istraživanja smo uhvatili i izmjerili 100 jedinki livadne gušterice koje smo kasnije podijelili u dvije grupe na temelju spola. Jedna grupa je sadržavala 56 odraslih ženki dok je druga sadržavala 40 odraslih mužjaka. Ulovljene su i četiri juvenilne jedinke koje su izostavljene iz statističke analize. Za svaku je jedinku zabilježen set ekoloških i stanišnih parametara po ulovu. A prije puštanja je za svaku jedinku izmjerena masa (m) i sljedeće morfometrijske karakteristike: ukupna dužina tijela ženke mjerena od vrha njuške do kraja repa (Ltot), dužina tijela ženke od vrha njuške do kloake (SVL), d užina repa (Lt), dužina od vrha njuške do kraja slušnog otvora (Lhel), dužina od vrha njuške do prednjeg uda (Lsfl), duljina od prednjeg do stražnjeg uda (Lfhl), visina glave (HH) i širina glave (HW). Sve su jedinke ulovljene na visokoplaninskom travnjaku između 1000 i 1400 mnm sa središnjom temperaturom staništa od 24,9 °C. Srednja tjelesna temperatura gušterica bila je 27,7 °C bez statistički značajne razlike između dvije grupe. Na temelju analize morfometrijskih podataka, zabilježen je spolni dimorfizam u tri kategorije: SVL, Lt i tjelesna masa. Oko 60% ulovljenih jedinki je imalo regeneriran rep što upućuje na visoki predatorski pritisak.

Ključne riječi: *Lacerta agilis*, dimorfizam, morfometrija

POPULATION ECOLOGY AND SEXUAL DIMORPHISM IN POPULATION OF THE SAND LIZARD, *Lacerta agilis*, Linnaeus 1758 ON DINARA MOUNTAIN, CROATIA

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The sand lizard, *Lacerta agilis* Linnaeus, 1758 is a small lacertid lizard distributed across Europe and eastern Asia. Sand lizard also inhabits most of Croatia and is proclaimed strictly protected by Croatian Nature Protection Act. In August 2012, we conducted a research on Dinara Mountain in Croatia. Dinara is the highest Croatian mountain stretched from Dalmatia over to Bosnia and Herzegovina. During this research we collected and measured one hundred specimens. Later on, we put them into two groups according to sex. One group consisted of 56 females and other of 40 males. Four juveniles were captured during this research but were excluded from following statistical analysis. For each individual a set of ecological and habitat parameters was taken within

minutes from capture. Before release, body mass (m) and following morphometric characters were taken: total body length (Ltot), snout-to-vent length (SVL), tail length (Lt), snout to ear - end length (Lhel), snout to forelimb length (Lsfl), forelimb to hind limb length (Lfhl), head height (HH) and head width (HW). All individuals were collected on high mountain grasslands between 1000 and 1400 m a.s.l. with mean habitat temperature of 24.9 °C. Mean body temperature was 27.7 °C with no significant difference between sexes. Morphological data indicate that there is statistically significant sexual dimorphism in SVL, Lt and body mass. Almost 60% of captured individuals had regenerated tails, indicating high predation pressure.

Key words: *Lacerta agilis*, dimorphism, morphometry

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DOPRINOS POZNAVANJU HERPETOFAUNE DINARE U HRVATSKOJ I BLIŽE OKOLICE

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Dinara je planina smještena u Dinarskom gorju na granici Hrvatske i Bosne i Hercegovine. Izgrađena je od karbonatnih stijena i duga 84 kilometra. Vrhom Dinara ili Sinjal (1831 mnm) čini najvišu planinu u Hrvatskoj. Ovo planinsko, nepristupačno područje je nedovoljno istraženo, dijelom i zbog miniranog terena. Tijekom svibnja i kolovoza 2012. godine, Udruga studenata "BIUS" provodila je istraživačko-edukacijski projekt "Dinara 2012". Cilj našeg dijela istraživanja je bio kvalitativno ispitivanje sadržaja herpetofaune Dinare. Literaturni podaci navode 20 vrsta vodozemaca i gmazova koji žive na ovim područjima. Našim istraživanjem je zabilježeno sveukupno 16 vrsta: 13 vrsta gmazova i 3 vrste vodozemaca, uključujući i vrste pronađene na putu od Kina do Dinare. Najčešće zabilježena vrsta je bila livadna gušterica, *Lacerta agilis*, a najznačajniji je bio pronalazak planinskog žutokruga, *Vipera ursinii*, koji je regionalni endem ovog područja, vrsta koja je uvrštena u Crvenu knjigu vodozemaca i gmazova Hrvatske te strogo zaštićena Zakonom o zaštiti prirode.

Ključne riječi: Dinara, vodozemci, gmazovi

CONTRIBUTION TO THE KNOWLEDGE OF HERPETOFAUNA OF CROATIAN PART OF DINARA MOUNTAIN AND ITS VICINITY

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Dinara is a mountain located in the large mountain chain Dinarides on the border between Croatia and Bosnia and Herzegovina. It is made of carbonate rocks and is 84 kilometers long. Its peak Sinjal, also called Dinara (1831 m a.s.l.), is the highest mountain peak in Croatia. Due to its limited accessibility and mined areas, knowledge of Dinara's flora and fauna is incomprehensive. Biology Students Association – BIUS carried out two field trips to mountain Dinara, one in May and one in August 2012. Main goal of our research was to make a quantitative list of herpetofauna of researched parts of Mt. Dinara. Literature data states

that 20 species of amphibians and reptiles inhabit these areas. Our inventory list counts 13 reptile and 3 amphibian species (16 in total), including species found on hiking trail to mountain Dinara from town Knin. The most frequently recorded species was the sand lizard, *Lacerta agilis*. The most important finding was Orsini's viper, *Vipera ursinii*, which is a regional endemic species of the area, a species that is listed in the Croatian Red Book of Amphibians and Reptiles and strictly protected by the Croatian Nature Protection Act.

Key words: Dinara, amphibians, reptiles

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IS POPULATION OF MOOR FROG (*Rana arvalis*) UNDER SEVERE DECLINE IN MURA RIVER BASIN IN SLOVENIA?

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Distribution of *Rana arvalis* in Slovenia is well known. Since it is a charismatic species in Slovenia, there are many known observations by enthusiast and only in few occasions data are results of systematic research. As a result population conservation status and population size and trends are in majority unknown. The only systematic survey targeted for *Rana arvalis* was made in 2011 on Ljubljansko Barje and in spring 2013 and 2014 on a large part of Mura river Basin (presented here). We performed a complete screening of Natura 2000 site Mura from Gornja Radgona to Vučja vas. An insight of population size and density in this area was obtained by egg mass counting and counting of blue adult males. Data are also available for some other major spawning sites of the species in Mura basin (from years 2013-2014, 2011 and 2008 respectively). Numbers gathered during our study were surprisingly low. There are only few known localities with high number of breeding animals (more then 100 egg mass or adult males counted) in the Mura river basin: oxbows Zaton (Petanjci), Bunčani, Krapje (Veržej), Bobri (Gornja Bistrica), Muriša (Lendava) and forest Polanski log (Velika Polana) and forest Črni log (Lendava). We noticed a severe decline of populations in the area in the last decade. The results of egg mass counts in Polanski log between years 2011-2014 showed that in 2014 only one tenth of numbers of 2011 were counted. A similar trend of decline is observed from 2008 to 2013 from Zaton oxbow. Exact causes for the decline are not known, but several natural and anthropogenic factors such as pollution, habitat disappearance and degradation are in place. Potential causes for the decline are the drainage of the oxbows due to lowering of the ground water level, afforestation of oxbow banks and shading of breeding sites and large forest clear cuts. Moor frogs namely prefer to deposit eggs in shallow water on the banks of oxbows, with higher water temperatures in springtime whereas later in summer these areas often dry out. We cannot say weather 90% decline in the numbers of moor frogs gathered in last few years are a sign of population decline heading toward local extinctions, but the results emphasize the need for further population monitoring and better identification of threats.

Key words: Moor frog, *Rana arvalis*, Slovenia, river Mura, population decline

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HERPETOFAUNA OF POSAVINA REGION IN BOSNIA AND HERZEGOVINA AND CROATIA

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Posavina region received its name from Sava River, which dominates its landscape, and it incorporates a large part of the surrounding hilly areas and lowlands of large tributary rivers. Border area is still mine polluted, especially on B&H side, and there is a serious lack of data for many species groups, including herpetofauna. During year 2014 and 2015 we collected significant new data for B&H and Croatia, which we combined with known literature data, data from museum collections and local expert personal data in order to present updated list of Posavina herpetofauna. Field work was done during day and night and all species were recorded and usually photographed. We were strictly focused on lowland areas (up to 300 m a.s.l.) with typical riparian habitats. In Posavina region we found total of 17 amphibian and 12 reptile species. Specifically for B&H we recorded all together 14 amphibian species (we highlight *Pelobates fuscus* and *Pelophylax lessonae* as species confirmed for the first time) and 11 reptile species (we highlight *Lacerta agilis argus* and *Vipera berus bosniensis* also first time confirmed). One individual of *Pelodiscus sinensis*, allochthonous species, has been caught on Bardača in 2010 and moved into captivity. Croatia had much more reliable literature data and combined with new personal data we arrive to the number of 17 amphibian and 11 reptile species. The most interesting data for Croatia are lowland populations of *Zootoca vivipara* and *Ichtyosaura alpestris*.

Key words: amphibia, reptilia, lowland, distribution, Pannonian Basin

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EFFECT OF ENVIRONMENTAL TEMPERATURE ON BODY CONDITION AND JUMPING PERFORMANCE OF TWO GEOGRAPHICALLY DISTANT *Rana temporaria* POPULATIONS

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Environmental temperatures affect all physiological processes and performance of ectotherms. Performance directly influences fitness by determining the probability of survival. Therefore, physiological adjustments to local thermal environment are extremely important for achieving optimal performance levels. *Rana temporaria* is a widely distributed European frog, present in a variety of habitats. This makes it an exceptional model for studying population-specific physiological adjustments to the local environmental conditions. We hypothesized that *R. temporaria* populations from colder (Central Europe) and warmer (Southeast Europe)

environments show population-specific variation in developmental time, morphometric traits and jumping performance, as a consequence of differences in local thermal conditions. We raised tadpoles from two populations under the same set of developmental conditions – in two constant temperature treatments, and in a semi-natural setup, in garden. After metamorphosis, we compared the influence of environmental temperatures on duration of developmental period, morphometric traits and jumping performance among froglets from different treatments, within and across populations. We detected significant effect of developmental temperatures on all measured traits. Moreover, we found significant differences in those traits between different populations, indicating that reaction mechanisms to environmental temperatures are population-specific.

Key words: frog, environmental temperature, physiological adjustment, climate change

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SPATIAL REPLACEMENT OF CLOSE RELATIVES, A PASSIONATE STORY OF A MOUNTAINOUS POPULATION OF *Vipera ammodytes*

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We studied the uppermost part of Struma valley in Western Bulgaria, where the nominate subspecies and *V. ammodytes montandoni* come into contact. Historically, till the mid-nineties only the nominate subspecies has been found regularly there. In the mid-nineties, however, multiple farms for venom went bankrupt and thus an unknown number of specimens were released outside of their native range; unconfirmed data suggests that hundreds of snakes were incidentally released close to the study area. When a single *V. a. montandoni* was found in 2008, an incidental introduction as well as a possible natural penetration in the study area was assumed. During the next years, intensive studies revealed virtually a complete exchange of the nominate subspecies by *V. a. montandoni*. In order to evaluate the degree of this process, spatial analyses with Maxent software of the existing locations were carried out together with six geographic and same number climatic variables. The analyses demonstrated a great degree of spatial niche overlap (73%) between the two taxa. The additional analysis of the locations raster values revealed statistically significant difference in two parameters, namely the potential evapo-transpiration, where the nominate ssp. was with higher values, being more related to places with higher overall humidity, and the altitude, where *V. a. montandoni* showed a more narrow altitudinal range. Likely, recent global and local climate changes contributed to the observed process that could also be a natural distribution shift.

Key words: invasion, ecological substitution, co-ocurrence

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FAUNISTIČKI NALAZI VODOZEMACA I GMAZOVA U OKOLICI NAŠICA

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U novije vrijeme nije bilo mnogo fanustičkih istraživanja herpetofaune na području Slavonije. U ovom radu prikazani su nalazi istraživanja vodozemaca i gmazova na osam lokaliteta u okolici Našica od 2010. do 2014. godine. Vodozemci su hvatani mrežama ili rukom, a gmazovi samo rukom. Jedinke se fotografiralo i pustilo neozljeđene nazad na mjesto ulova. Ukupno je zabilježeno devet vrsta vodozemaca i devet vrsta gmazova. Gotovo sve zabilježene vrste su očekivane, uz iznimku invazivne crvenouhe kornjače, *Trachemys scripta elegans* (Wied-Neuwied, 1839). Nađena je jedna odrasla jedinka u umjetnom jezeru u gradskom parku u Našicama, 2010. Vrlo vjerojatno je riječ o namjerno puštenom kućnom ljubimcu. Dvije zabilježene vrste, žuti mukač, *Bombina variegata* (Linaneus, 1758) i barska kornjača, *Emys orbicularis* (Linnaeus, 1758), koji su na Dodacima II i IV Direktive o staništima Europske komisije. Kroz ovo istraživanje prikupljeni su prvi konkretni podaci o prisutnosti vrsta vodozemaca i gmazova na tom području Hrvatske i može koristiti kao podloga za buduća istraživanja.

Ključne riječi: herpetofauna, Natura 2000, Slavonija

FAUNISTIC RECORDS OF AMPHIBIANS AND REPTILES IN THE NAŠICE AREA

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In recent times there has not been a lot of faunistic research of the herpetofauna in the area of Slavonija. Here we give the list of amphibians and reptiles found on eight localities around Našice, from 2010 till 2014. Amphibians were caught by nets or by hand, while reptiles were caught only by hand. Caught individuals were photographed and released unharmed immediately afterwards at the same place where they had been found. A total of nine amphibian and nine reptile species were found. Almost all of the species had been expected for the area, except the invasive red-eared slider, *Trachemys scripta elegans* (Wied-Neuwied, 1839). One adult individual was found in the artificial lake in the city park in Našice, in 2010. Most likely it is an intentionally introduced pet. Two of the species we found, the yellow-bellied toad, *Bombina variegata* (Linaneus, 1758) and the European pond terrapin, *Emys orbicularis* (Linnaeus, 1758) are on Annexes II and IV of the European Commision's Habitats Directive. This research yields the first exact records on the occurence of amphibians and reptiles in this part of Croatia and can be used as a basis for future work.

Key words: herpetofauna, Natura 2000, Slavonia

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MOLEKULARNA FILOGENIJA IVANJSKOG ROVAŠA (*Ablepharus kitaibelii*) NA SREDIŠNJEM I SJEVERNOM DIJELU PODRUČJA RASPROSTRANJENOSTI

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Ivanjski rovaš je termofilna vrsta guštera i spada među najmanje gmazove Europe. Rasprostranjen je od Slovačke, prema jugu Europe sve do Turske. U Hrvatskoj je nedavno potvrđena njegova prisutnost na obroncima Papuka i u gradu Iloku, što čini sjeverozapadnu granicu rasprostranjenosti. Do sada su korištenjem mitohondrijskih molekularnih biljega (16S rRNA i citokrom b) istraživani filogenetski odnosi populacija ivanjskog rovaša u južnom dijelu areala. Zaključeno je da ivanjski rovaš nije monofiletska skupina, već kompleks vrsta. Cilj ovog istraživanja je da se korištenjem mitohondrijskih biljega, istraže populacije na ostatku područja rasprostranjenosti u Europi, kako bi se upotpunili zaključci o filogeniji. Zabilježeni haplotipovi iz rumunjskih populacija, odvojili su se kao nova zasebna linija na filogenetskom stablu. Međutim, genetska varijabilnost populacija s područja Hrvatske, Srbije, Bugarske i Makedonije je izrazito niska. Na cijelom tom području, zabilježena je prisutnost samo jednog haplotipa na 16S rRNA i citokrom b biljegu. Haplotipovi su istovjetni onima iz kontinentalne Grčke. Taj rezultat ukazuje na naglo širenje područja rasprostranjenosti, najvjerojatnije iz izvorišne populacije s područja Grčke. Naša hipoteza je da se naglo širenje zbilo u najtoplijem razdoblju zadnjeg interglacijala koje odgovara razdoblju atlantika (prije 8000 do 5000 godina).

Ključne riječi: termofilna vrsta guštera, Balkanski poluotok, pleistocenska pribježišta, brzo širenje areala, holocen

MOLECULAR PHYLOGENY OF THE SNAKE-EYED SKINK *(Ablepharus kitaibelii)* ON THE CENTRAL AND NORTH PART OF ITS DISTRIBUTION RANGE

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The Snake-eyed Skink is a small thermophilous lizard. It is distributed from Slovakia, towards south of Europe all the way to Turkey. In Croatia, this species has been recently recorded in the Papuk Mountain and in the city of Ilok, forming its western distribution border. Up to now, mitochondrial molecular markers (16S rRNA and cytochrome b) have been used to assess the phylogeny of the Snake-eyed Skink populations in the southern part of its distribution range. It was concluded that the species does not form a monophyletic group; rather it is a complex of several species. The aim of our research was to examine populations from the remaining parts of distribution range in Europe, using mitochondrial markers. Obtained haplotypes from a Romanian population, form a new distinct lineage on the phylogenetic tree. However, extremely low genetic variability was observed in Croatia, Serbia, Bulgaria and Macedonia, where only a single haplotype was detected on each particular mitochondrial marker. The

same haplotypes were reported for the Central Greece. This result indicates a rapid range expansion, from ancestral population most probably situated in Greece. Being a thermophilous species, we suppose that the Snake-eyed Skink's expansion happened in the warmest periods during the Holocene, corresponding to the Atlantic period (8000-5000 years BP).

Key words: thermophilous lizard species, Balkan Peninsula, Pleistocene refugium, rapid range expansion, Holocene

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NOVI DOKAZI O POSTOJANJU TROGLOKSENIH PRILAGODBI HERPETOFAUNE DINARSKOG KRŠA

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Ulazak vodozemaca u podzemlje Dinarskog krša do sada nije detaljnije promatran. U literaturnim podacima su do sada zabilježeni slučajni ulasci vrstau podzemlje, ali njima se nije pridavala posebna pažnja.U ovom radu pružali smo više dokaza o ovom fenomenu i dali smo osnovan pregled literaturnih podataka. 14. i 15. travnja 2015. godine organizirano je speleoronilačko istraživanje Pazinske jame, s glavnim ciljem pronalaska našeg krškog endema čovječje ribice (*Proteus anguinus*). Pazinska jama je smještena u Pazinu (Istra, Hrvatska) (N 5416357; E 5011182, 185 m n.v.). Tok rijeke Pazinčice je dug 16,5 km te ponire u Pazinsku jamu. Nakon ulaza slijedi sto metara dug kanal koji se proširuje u dvoranu (80 * 20 m) s velikim podzemnim jezerom (Martelovo jezero). Nakon detaljnog pregleda jezera, sifona i kanala, čovječja ribica nije pronađena, ali su pronađene 20 jedinki lombardijske smeđe žabe (*Rana latastei*), dvije jedinke smeđe krastače (*Bufo bufo*) te jedna jedinka riječne kornjače (*Emys orbicularis*). Sve tri vrste su zamijećene u najudaljenijem suhom dijelu jame. Vrste su uhvaćene, fotografirane i potom puštene. Ovo je prvi nalaz lombardijske smeđe žabe i riječne kornjače u špilji u Hrvatskoj, i to na mjestu gdje je prisutna potpuna tama. Duž plaže Martelovog jezera zabilježene su i nakupine jaja smeđe krastače. Nije primjećeno da su vrste pothranjene. Uočeno je da je vanjska obojanost smeđe krastače bila nešto svijetlija od uobičajene. Budući da je u Pazinskoj jami zabilježen ovako veliki broj vodozemaca, te da postoje i druge indikacije o prisutnosti vodozemaca u podzemlju, naša hipoteza je da bi ove vrste mogle biti ne samo stigoksene, već možda i stigofilne vrste.

Ključne riječi: podzemlje, vodozemci, *Rana latastei*, Pazinska jama, dinarski krš

NEW EVIDENCE TO EXISTENCE OF TROGLOXENE ADAPTATIONS IN HERPETOFAUNA OF DINARIC KARST

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Entering of amphibians into the underground of Dinaric Karst has received very little attention in published literature. There is only literature data about the accidental access of amphibians into the underground, but without any special overview. Here we provide more evidence on this phenomenon and give basic overview of previous records. During the 14th and 15th of April 2015 we made speleological exploration of Pazinska cave with the main purpose of prospecting for the *Proteus anguinus*. Enterance of Pazinska cave is located in Pazin (Istria, Croatia) at about 185 m a.s.l.. Watercourse of river Pazinčica is 16.5 km long and plunges into Pazinska cave. Behind the

entrance there is a hundred meter long channel which expands into the hall (80 * 20 m) with a large cave lake (Martel Lake). After detailed research of the lake, siphons and channels, the Proteus has not been found, but we made observation of 20 individuals of Italian agile frog (*Rana latastei*), two individuals of Common toad (*Bufo bufo*) and one individual of European pond turtle (*Emys orbicularis*). All of these three species were observed in the deepest dry part of the cave. The specimens were caught, photographed and released.This is the first finding of Italian agile frog and pond turtle in Croatia inside the cave, where complete darkness is presented. Along the sandy edge of the underground lake a stripes of *Bufo bufo* eggs were also noticed. There was no indication of starvation in species. It was observed that the external coloration of the *Bufo bufo* speciments was somewhat brighter than usual. Because of such a big number of amphibians in Pazinska cave and because of the other indications about the presence of this species in the caves, our hypothesis is that these species may be not only stygoxene, but maybe also stygophile.

Key words: underground, amphibians, *Rana latastei*, Pazinska cave, Dinaric Karst

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COASTAL POPULATION OF DICE SNAKES (*Natrix tessellata*) HELPS CLARIFY THE EVOLUTION OF EURYHALINE PHYSIOLOGY IN MARINE TETRAPODS

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The widespread relationship between salt excreting structures (e.g. salt glands) and marine life strongly suggests that the ability to regulate salt balance has been crucial during the transition to marine life in tetrapods. However, lacking data from intermediate stages (species lacking salt glands but occasionally using saline environments) preclude to draw a comprehensive picture of the evolution of euryhaline physiology in these organisms. Populations of Dice snakes (*Natrix tessellata*) foraging in the marine environment along the coast of the Black sea provide a unique opportunity to study physiological adaptations to marine life in a marine snake prototype lacking salt glands. We show that coastal free-ranging Dice snakes display highly variable natremia (plasma sodium concentration) ranging from normonatremia (~145 mmol L^{-1}) up to strong hypernatremia (i.e. >195 mmol L^{-1}). Our laboratory experiments indicate that this species can sustain life in full-strength seawater (~35 g of salt per L) up to one week with no visible long-term damage, but that such treatment entailed a marked hypernatremia that can be regulated only trough access to freshwater. Taken together these results suggest that hypernatremic Dice snakes require regular access to freshwater in order to periodically restore osmotic balance. Our results further suggest that the development of a strong physiological tolerance toward deviations of the osmotic balance (e.g., increased plasma sodium) has been a critical innovation in the evolution of aeuryhaline physiology and may well have preceded the evolution of salt glands.

Key words: salinity, natremia, marine life

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A PRELIMINARY STUDY ON THE BIOACTIVITY OF THE MICROBIOTA IN THE POST-CAECAL GI TRACT OF THREE Testudo SPECIES (TESTUDINIDAE, TESTUDINES) WITH EMPHASIS ON LACTIC ACID BACTERIA

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We investigated the microbiota inhabiting the colon of *Testudo graeca*, *T. marginata* and *T. hermanni*. We sampled fragments of fresh feces obtained from specimens kept in a rescue centre. Fecal samples were stored in sterile tubes, chilled to 6 °C and transported to the laboratory in cooling bags. Samples were incubated for 24 hours at 37 °C. The delivered inoculum (100 µl) was filtered over a sterile media MRS agar with staining agent X-gal dissolved in 20 ml DMSO (dimethyl sulfoxide). Applying this specific staining, we identified *Lactobacillus* species. Out of three samples we isolated pure cultures that were then stained by the Gram method. As expected, the cultures were all Gram positive. We will perform DNA and biochemical analysis to identify the exact bacterial species. In addition, we conducted investigation on the antimicrobial and antifungal activities of the isolated bacterial strains (colonies) on eleven pathogen species. We compared the bioactivity of the colonies to those of two broad-spectrum antibiotics - Sefpotect and Chloronitromycin (250 mg/ml). Our results indicate that the investigated tortoise possess microflora which protects them from a variety of gastro enteric pathogens.

Key words: tortoise, gastro enteric bacteria, pathogens

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MORFOLOŠKE ZNAČAJKE ŠARE POLJARICE, *Hierophis gemonensis* (1768, Laurenti)

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Makar se šara poljarica smatra jednom od najčešćih vrsta hrvatskog primorja i otoka dosadašnji radovi ne obuhvaćaju njenu morfologiju i rasprostranjenost. Kako bi odredili točnu distribuciju vrste *Hierophis gemonensis* u Hrvatskoj koristili smo bazu podataka HHD Hyla sa potvrđenim GPS koordinatama. Cilj je također bio analizirati karakteristike i morfološke značajke vrste. Istraživanje je provedeno na uzorku od 50 jedinki sačuvanih u 70%-tnom etanolu i 51 živih jedinki premjerenih tijekom terenskih istraživanja. Za svaku jedinku su zabilježena 3 meristička i 14 morfometrijskih obilježja. Ženke su uglavnom manje i lakše od mužjaka. Spolni dimorfizam je potvrđen u dužini repa te broju trbušnih i podrepnih ljuski. Rezultati ukazuju na značajne razlike u morfometrijskim značajkama između kopnenih i otočnih populacija vrste *H. gemonensis*.

Ključne riječi: morfološke razlike, spolni dimorfizam, merističke značajke, Hrvatska

DISTRIBUTION AND MORPHOLOGY OF BALKAN WHIP SNAKE, *Hierophis gemonensis* (1768, Laurenti)

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Although Balkan whip snake is considered one of the most common snakesofcostal Croatia and islands, there are no studies about morphological differences and distribution of *Hierophis gemonensis* to this day. To determine exact distribution of *H. gemonensis* we used HHD Hyla database of confirmed finding sites with GPS coordinates.Our goal was also to analyse characteristics of species morphology. The study was carried out on 50 specimens preserved in 70% ethanol and 51 living specimens measured during field studies. Specimens were examined for 3 meristic and 14 morphometric characters. Females showed tendency to be smaller and lighter than males. Sexual dimorphism is confirmed in tail length, and number of ventral and subcaudal scales. Results indicate that there is significant difference in morphometric characteristics between insular and land population of Balkan whip snake.

Key words: morphological differences, sexual dimorphism, meristic, Croatia

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PRAĆENJE ZDRAVSTVENOG STANJA ČOVJEČJE RIBICE (*Proteus anguinus*) U ZATOČENIŠTVU

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U sklopu projekta zaštite čovječje ribice u Hrvatskoj obavljaju se i *in situ* istraživanja životinja u zatočeništvu. Budući da nema podataka o fiziološkoj mikroflori, ni o mikroorganizmima koji mogu narušiti zdravstveno stanje čovječje ribice, svrha je ovog istraživanja steći što detaljniji uvid u zdravstveno stanje životinja, razlučiti fiziološku mikrofloru od oportunističkih mikroorganizama i pravih patogena, te u slučaju potrebe provesti ispravnu terapiju. Obavljaju se sljedeća testiranja: (1) Standardna mikrobiologija – obrisci usne šupljine, kloake i kože; (2) Parazitološka testiranja – izmet, strugotine crijeva i organi uginulih životinja – nativni preparati i otisci; (3) Testiranje na *Batrachochytrium dendrobatidis* (BD) i na ranavirus – obrisci kože metodom Real Time PCR; (4) Testiranje na *Chlamydia* sp. – obrisci usne šupljine i kloake metodom Real time PCR; (5) Razudba i patohistološka pretraga uginulih životinja – da se dobije što više podataka o anatomiji i funkciji pojedinih organa. Dosad je nađen veći broj mikroorganizama tla, poput *Aeromonas hydrophila* i *Aspergillus flavus*, koji u nepovoljnim uvjetima mogu uzrokovati bolesti vodozemaca, no nađene su i bakterije koje sudjeluju u sprječavanju zaraza vodozemaca BD-om poput *Janchtinobacterium lividum*. Parazitološkom pretragom izmeta nađeni su samo pseudoparaziti iz vode i vlažnog tla. Sve dosad testirane životinje bile su negativne na BD, ranavirus i bakterije *Chlamydia* sp.

Ključne riječi: čovječja ribica, zdravstveno stanje, mikroorganizmi, Real time PCR

MONITORING OF HEALTH STATUS OF THE OLM (*Proteus anguinus*) IN CAPTIVITY

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Within the conservation of *Proteus anguinus* (the olm) project in Croatia, besides the field observations, *in situ* investigation of the species is also carried out.Since there is a lack of information on physiological flora and microorganisms which can compromise the olm's health, the aim of the veterinary part of the project is to learn as much as possible about the health status of these animals. In order to distinguish between physiological microflora, opportunistic and true pathogens, and to provide the right treatment, if necessary, the followings tests are performed:(1) Standard microbiology of oral cavity, cloacal and skin swabs; (2) Parasitological tests of feces, intestinal scrapings and organs of dead specimens – native preparations and prints; (3) *Batrachochytrium dendrobatidis* (BD) and Ranavirus tests ofskin swabs by the Real Time PCR method; (4) *Chlamydia* sp. test oforal cavity and cloacal swabs by the Real time PCR method; (5) Autopsy and pathohistologyof dead specimens, to get more information on various organ anatomy and function. So far, a larger number of soil microorganisms were identified, such as *Aeromonas hydrophila* and *Aspergillus flavus*, potential causes of disease in amphibians under suboptimal conditions. Bacteria species, such as *Janchtinobacterium lividum,* which could participate in prevention of BD infection in certain amphibian species were also identified. Parasitological tests of fecal samples revealed the presence of pseudoparasites from water. All the animals analyzed so far have been negative for BD, Ranavirus and *Chlamydia* sp.

Key words: Olm, health status, microorganisms, Real time PCR

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ISOENZYME PROFILES OF SUPEROXIDE DISMUTASE AND CATALASE IN LIVER AND MUSCLE SELECTED ANURAN SPECIES

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Reactive oxygen species (ROS) may cause cell damage and contribute to changes in cellular function. Superoxide dismutase (SOD) and catalase (CAT) represent key enzymes of cell antioxidative metabolism. This study focuses on first comparative analysis of CAT and SOD isoenzyme profiles found in liver and muscle of following anuran species: *Rana graeca, Bombina variegata* and *Pelophylax* kl. *esculentus*. Animals were collected at several localities in Bosnia and Herzegovina. SOD and CAT isoforms were identified using native polyacrylamide electrophoresis (PAGE). Native electrophoresis resolved the presence of one CAT isoform in liver of all three frog species, out of which the highest activity was observed in species *Pelophylax* kl.*esculentus.* In the muscle, CAT isoform was detected only in species *Bombina variegata.* Comparative analysis of liver and muscle SOD profiles showed gender and species correlated differences. Obtained results are discussed in terms of differences in the environments which these species inhabit in correlation with the antioxidative metabolism.

Key words: antioxidative metabolism, *Rana graeca, Bombina variegata, Pelophylax* kl. *esculentus*

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ZMIJE U HERPETOLOŠKOJ ZBIRCI PRIRODOSLOVNOG MUZEJA U SPLITU, HRVATSKA

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Analizom herpetološke zbirke Prirodoslovnog muzeja u Splitu utvrđeno je da su zmije u herpetološkoj zbirci zastupljene kroz 183 inventarna broja sa 224 primjeraka. U navedenoj zbirci se nalaze primjerci 4 porodice, 5 potporodica, 11 rodova i 15 vrsta zmija. Najzastupljeniji su primjerci iz porodice Natricinae i Viperidae odnosno rodovi *Natrix* i *Vipera*. Najveći broj primjeraka prikupili su U. Girometta i A. Cvitanić sakupljanjem na području Dalmacije. Veliki dio zbirke ne sadrži podatke o sakupljaču kao ni datum sakupljanja. Zbirka je nastala u periodu od 20-tih godina XX. stoljeća do kraja 2014. godine.

Ključne riječi: zmije, herpetološka zbirka, prirodoslovni muzej u Splitu, Hrvatska

SNAKES IN HERPETOLOGICAL COLLECTION OF THE NATURAL HISTORY MUSEUM IN SPLIT, CROATIA

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Analysis of the herpetological collection of Natural History Museum in Split found that snakes were represented in the herpetological collection by 183 inventory numbers with 224 samples. In the herpetological collection there are samples of 4 families, 5 subfamilies, 11 genus and 15 snakes species. The most represented samples are from Natracinae and Viperidae family, infact genders *Natrix* and *Vipera*. The largest number of samples were collected by Girometta U. and Cvitanić A. collecting at the area od Dalmatia. A large part of the collection does not contain information of collector or collection date. The collection was created in period of 20-ies of XX. century to the end of 2014.

Key words: snakes, herpetological collection, natural history museum in Split, Croatia

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DISCOVERY OF AN AMPHIKINETIC AMPHIBIAN SKULL AND ITS LINKAGE TO NEOTENIC LIFESTYLE

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Among urodeles there is a significant variation in the degree of skull kinetics, with some species possessing a highly kinetic skull during their larval stages, which then ossifies in the course of the ontogenetic development. We used high-speed cinematography (420 fps) and micro-computed tomography (μCT) to study the feeding behaviour in the recently described Buresch's newt *Triturus ivanbureschi* Arntzen & Wielstra, 2013. In addition to the typical amphibian articulation between skull roof and palatoquadrate associated structures (pleurokinesis) there were also flexible connections between parietals and frontals (mesokinesis) and between parietals and the exocipitals (metakinesis). This morphological pattern is similar to that observed in modern lizards but the combination of pleuro-, meta-, and mesokinetic skull articulations found in the Buresch's newt represents a new kind of amphikinetics not described for any vertebrate to date. The functional analysis of the cranial kinesis in *T. ivanbureschi* indicates that the advantage of having less rigid skull morphology is to optimize for jaw closing mechanism. We propose that the cranial kinesis in *T. ivanbureschi* increases the efficiency of fast jaw closure and may represent a neotenic feature retained from the newt larvae. The presence of intracranial mobility may be influenced by specific (yet undetermined) environmental conditions.

Key words: Urodela, kinetics, metamorphosis, adaptation, biomechanics.

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"ZAMKE SMRTI" - NEPRIKLADNI GRAĐEVINSKI ZAHVATI U ZAŠTIĆENIM PODRUČJIMA MOGU UZROKOVATI VISOKU SMRTNOST VODOZEMACA I GMAZOVA

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Vodozemci i gmazovi spadaju među globalno najugroženije skupine životinja. Njihov je opstanak danas direktno vezan uz mogućnost prilagodbe na izmijenjena antropogena staništa. No, od presudne važnosti je i naša mogućnost prilagodbe zahvata da budu čim više ekološki prihvatljivi. Ovdje iznosimo problem građevinskih objekata koji zbog svoje neprilagođenosti prirodi uzrokuju vrlo visoku smrtnost jedinki različitih vrsta – "zamke smrti". Naše istraživanje obuhvatilo je primjer odvodnog sustava postavljenog na parkiralištu ispred zgrade JUPP Kopački rit u Kopačevu, Hrvatska. Građevinski objekt sastoji se od oko 80 m odvodnih kanala i velikog spremnika za sabirnu vodu (zapremine oko 15 m^3). Kanali djeluju kao prepreka životinjama koje tuda prolaze i njima se usmjeravaju u podzemni spremnik. Ovaj efekt je prvi put zabilježen u svibnju 2010. godine kada je slučajnim pregledom u spremniku pronađeno oko 200-tinjak jedinki vodozemaca od čega je 50% već bilo mrtvo. Tijekom 2015. godine proveli smo praćenje ovog sustava kako bi pokušali kvantificirati negativan utjecaj i mortalitet. Ukupno je iz spremnika izvađeno 245 jedinki, i to šest vrsta vodozemaca i jedna vrsta gmaza, od čega je oko 5% već bilo mrtvo, a preostale su spašene i puštene natrag u prirodu. Da jedinke nisu izvađene iz spremnika, sigurno bi sve stradale. Tijekom travnja i svibnja zbilježeno je da u

prosjeku dnevno u spremnik upadne 4,98 jedinki.Trenutno se razmatraju moguće mjere za smanjenje negativnog utjecaja, te generalne smjernice za buduće građevinske zahvate.

Ključne riječi: vodozemci, gmazovi, smrtnost, zamka smrti, građevinarstvo, parkiralište

"DEATH TRAPS" – INAPPROPRIATE CONSTRUCTIONS IN PROTECTED AREAS CAN CAUSE HIGH MORTALITY IN AMPHIBIANS AND REPTILES

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Amphibians and reptiles are among the most threatened groups of animals on our planet. Their future survival is directly connected to their ability to adapt to ever spreading anthropogenic habitats. But it is also important that we make our impact to be as ecologically friendly as possible. Here we describe the problem of urban constructions which cause very high animal mortality when used in natural habitats – called "death traps". Our research was focused on parking drainage system built in front of Kopačkirit Nature Park visitor centre in Kopačevo, Croatia. This construction is made out of around 80 meters of collection channels and a large underground tank (volume of around 15 m^3). Collection channels act as pitfall traps collecting animals from around parking surface and focusing them to underground tank. This effect was first observed in May 2010 when more than 200 animals were found within the underground tank (around 50% of animals were already dead). In 2015 we started a continuous monitoring on this location in order to quantify the negative effect and mortality. In total we collected 245 individuals, belonging to six amphibian and one reptile species, from which around 5% was already found dead. All animals were removed from the tank during each research occasion and live individuals were released back into nature. If not removed all animals would die eventually. During April and May sampling we calculated that in average 4.98 individuals fall into the tank each day. We give a set of conservation measures that can reduce the mortality and suggest general guidelines for development of more eco-friendly constructions.

Key words: Amphibian, reptile, mortality, construction, death trap, car parking

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SEXUAL DIMORPHISM, DIET AND REPRODUCTION OF THE GRASS SNAKE (*Natrix natrix*) IN THE REGION OF THE MARSHY-POND ECOSYSTEM BARDAČA (REPUBLIC OF SRPSKA, BOSNIA AND HERZEGOVINA)

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In this publication we present the first data concerning ecological characteristics of the grass snake, *Natrix natrix*, based on the data collected during the capture-mark-recapture study in the area of marshy-pond ecosystem Bardača between 2011 and 2014. We captured, processed

and marked 172 adult individuals (96 ♀ : 76 ♂), in which we analysed body weight, seven morphometric characteristics and two meristic traits. Females had larger average values of body weight and of all analysed morphometric traits; however, considered as a ratio of total body length, males had longer tails than females (22.3% compared to 19.3%). Additionally, males had significantly larger numbers of ventral and subcaudal scales. The analysis of diet of the snakes we processed was conducted on the contents spontaneously regurgitated by the animals, while by palpating abdomens of gravid females, we assessed the numbers of growing follicles/eggs they had. We found statistically significant differences in the type of consumed prey among different years, which indicates the grass snake's dietary plasticity. We also found differences in the diets of the two genders, where females more often consumed adult green frogs (*Pelophylax sp*.), while males usually consumed fish (especially *Pseudorasbora parva*). The smallest female with growing follicles had standard body length (SVL) of 62.6 cm, while the smallest male which was observed in mating had a SVL of 48.5 cm. Clutch size varied between 8 and 28.

Key words: grass snake, body size, diet, clutch size

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DETECTION OF CONTINUED ANNUAL MORTALITY IN THE VULNERABLE ALPINE SALAMANDER, *Salamandra atra prenjensis* (MIKŠIĆ, 1969) NOT ASSOCIATED WITH THE PRESENCE OF KNOWN AMPHIBIAN PATHOGENS

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The emerging disease, chytridiomycosis plays a key role in amphibian declines and species extinctions. Additional agents contributing to this issue are Rana virus (Rv) and a recently discovered fungus: *Batrachochytridium salamandrivorans* (Bs). Chytridiomycosis's etiological agent, the fungus *Batrachochytrium dendrobatidis* (Bd), has been reported on all continents inhabited by amphibians. In Europe it is detected in 17 EU Member states. Occurrence of the disease in Balkan area is almost unknown. In this study we report first results for chytridiomycosis analyzes in Bosnia and Herzegovina (B&H) for caudate endemic *Salamandra atra prenjensis*. During summer 2013, fieldwork was performed on two B&H Mountains. In total, 145 individuals of *S. a. prenjensis* were swabbed and screened for Bd infection. Analyzes were performed using quantitative real-time PCR (qPCR). Results were negative for all individuals. On Mt. Prenj, sick and dead individuals expressing specific symptoms (abnormal posture, cachexia) were found and collected. Besides for Bd, dead animals were additionally screened for Bs and Rv, which showed absence of these infections. Additional histopathology of skin also proved absence of chytridiomycosis. The phenomenon has been regularly registered during several years of fieldwork (2% incidence). Possible explanations of its occurrence are presented in this paper.

Key words: amphibian disease, Bosnia and Herzegovina (B&H), mountain ecosystem

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PRELIMINARY ASSESSMENT OF THE LACERTID SKULL MODULARITY – A CASE STUDY OF *Podarcis muralis*

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The concepts of modularity and morphological integration have gained prominent attention in the evolutionary biology in recent years. Here we present a study of dorsal and ventral cranium modularity within the single species – European wall lizard (*Podarcis muralis* Laurenti, 1768), on a sample of 25 neonates, 26 females and 26 males. For the dorsal cranium, we evaluated three hypotheses which were already suggested for lizard dorsal cranium: mammalian hypothesis, 'Anolis' hypothesis and tripartite hypothesis. For the ventral cranium, we tested two hypotheses: the developmental hypothesis (braincase *vs*. dermatocranium) and the functional hypothesis (jaw and palate *vs*. posterior cranium). Since the allometry was significant in the whole sample, we did our tests of modularity hypotheses on size corrected dataset. For the dorsal cranium only 'Anolis' hypothesis was supported for adult females and males, but not for the neonates. For the ventral cranium, only the functional hypothesis was supported for adult females and males, but not for neonates. Our conclusions would be that there is an onthogenetic shift in the pattern of skull integration, from neonates which have highly integrated skull, to adults in which functional constraints lead to distinct functional modularity.

Key words: cranium modularity, developmental hypothesis, European wall lizard, functional hypothesis

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RIĐOVKA, *Vipera berus* (SQUAMATA, VIPERIDAE) U HRVATSKOJ: POPULACIJSKA EKOLOGIJA, ODABIR MIKROSTANIŠTA I TERMOREGULACIJA

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Riđovka, *Vipera berus* (Linnaeus, 1785) je najšire, a ujedno i najsjevernije rasprostranjena terestrička vrsta zmije u svijetu. U Hrvatskoj nalazimo dvije podvrste u tri odvojene populacije. Terenska istraživanja provedena su na dva različita lokaliteta u Hrvatskoj: Okuje (Turopolje, dolina rijeke Save; *V. b. bosniensis*) i Begovo Razdolje (Gorski kotar; *V. b. berus*). Monitoring vrste proveden je Capture – Mark – Recapture metodom tokom četiri godine u Okuju (2009.-2012.), odnosno tri godine u Begovom Razdolju (2009.-2011.). Cilj istraživanja bio je utvrditi postoje li interpopulacijske i intrapopulacijske sličnosti i razlike u odabiru mikrostaništa, termoregulaciji, dnevnoj i godišnjoj aktivnosti, spolnoj strukturi te analiziranim morfometrijskim i merističkim karakteristikama. Analizirani uzorak obuhvaćao je ukupno 106 jedinki (50 iz Begovog Razdolja, 56 iz Okuja). Utvrđeno je da postoje statistički značajne razlike u duljini i visini glave te tjelesnoj masi jedinki. Jedinke riđovke u Okuju preferiraju zatvoreni tip, a jedinke u Begovom Razdolju otvoreni tip mikrostaništa. U obje populacije postoji razlika u

spolnoj strukturi te termoregulaciji prema spolu i tipu obojenja. U Okuju riđovke počinju svoju aktivnost u mjesecu ožujku, a u Begovom Razdolju u mjesecu lipnju.

Ključne riječi: Capture – Mark – Recapture metoda, *Vipera berus*, ekološke preferencije, spolna struktura, merističke karakteristike

COMMON ADDER, *Vipera berus* (SQUAMATA, VIPERIDAE) IN CROATIA: POPULATION ECOLOGY, MICROHABITAT PREFERENCES AND THERMOREGULATION

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The Adder, *Vipera berus* (Linnaeus, 1785) is the most widespread and also the northernmost distributed terrestrial snake species in the world. In Croatia, there are two subspecies of the adder in three separate populations. Field work was conducted on two different localities in Croatia: Okuje (Turopolje, river Sava Valley; *Vipera berus bosniensis*) and Begovo Razdolje (Gorski kotar; *Vipera berus berus*). The monitoring was conducted by Capture – Mark – Recapture method during a four year period in Okuje (2009 – 2012), and a three year period in Begovo Razdolje (2009 – 2011). The main goals of this study were to determine if there are intrapopulation or interpopulation similarities or differences in microhabitat preferences, thermoregulation, daily or yearly activity period, gender structure of population and meristic characteristics. We collected 106 adders (50 from Begovo Razdolje and 56 from Okuje). The analyses have shown that there are statistically significant differences between the two populations in head length, head height and weight. The individuals from Okuje prefer a closed type of microhabitat, unlike individuals from Begovo Razdolje that prefer an opened type of microhabitat. In both populations, there are differences in the gender structure, and thermoregulation which is sex and colour morph dependent. In Okuje, Adders begin their activity period in March as opposed to individuals from Begovo Razdolje whose activity period begins in June.

Key words: Capture – Mark – Recapture, activity period, gender structure, meristic characteristics.

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GMAZOVI I VODOZEMCI VUGROVEČKE OKOLICE

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Faunistička istraživanja gmazova i vodozemaca u Hrvatskoj nisu česta, pogotovo u kontinentalnom dijelu. U ovom radu su dani nalazi 11-godišnjeg inventariziranja gmazova i vodozemaca na području sela Vugrovca i okolice, nedaleko od Zagreba, provedenog od 2004. do 2014. godine. Dio istraživanog područja spada pod novo Natura 2000 područje "Vejalnica i Krč", a dio u Značajni krajobraz "Goranec". Objema upravlja Javna ustanova "Maksimir". Gmazovi su lovljeni rukom, ponekad uz uporabu zaštitnih rukavica i kuka za zmije. Vodozemci su lovljeni rukama i ručnim mrežama. Ulovljene jedinke su fotografirane i neozlijeđene puštene na mjesto ulova u najkraćem mogućem roku. Zabilježena je prisutnost 11 vrsta gmazova i sedam vrsta vodozemaca. Među gmazovima je prisutna jedna unešena vrsta – čančara, *Testudo hermanni* Gmelin, 1789, koja ne obitava prirodno u ovom dijelu Hrvatske. Nalaz žutog mukača, *Bombina variegata* (Linnaeus, 1758), je od posebnog značaja jer je na Dodacima II i IV Direktive

o staništima Europske komisije. Rezultati ovog istraživanja daju doprinos poznavanju prisutnosti i raširenosti gmazova i vodozemaca u zagrebačkoj okolici te na Natura 2000 području "Vejalnica i Krč" i u Značajnom krajobrazu "Goranec". Prikupljeni podaci mogu pomoći djelatnicima Javne ustanove "Maksimir" i prilikom upravljanja tim područjem.

Ključne riječi: herpetofauna, Natura 2000, "Vejalnica i Krč"

REPTILES AND AMPHIBIANS OF THE VUGROVEC AREA

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Faunistic research of reptiles and amphibians in Croatia are not so commonplace, especially in the continental part. Here I present the results of an 11-year inventarisation study of reptiles and amphibians in the village of Vugrovec and its environs, near Zagreb, conducted from 2004 till 2014. A part of the research area falls under the new Natura 2000 site "Vejalnica and Krč" and another one under the Significant landscape "Goranec". Both are being administered by the Public institution "Maksimir". Reptiles were caught by hand, sometimes with the use of special gloves and snake hooks. Amphibians were caught by hand and handheld nets. The caught individuals were photographed and released afterwards at the same spot in the shortest time possible. A total of 11 reptile and seven amphibian species were recorded. One of the reptile species is introduced – the Hermann's tortoise, *Testudo hermanni* (Gmelin, 1789). It does not occur naturally in this part of Croatia. Of special importance is the finding of *Bombina variegata* (Linnaeus, 1758), which is on Annexes II and IV of the European Comission's Habitats Directive. The results of this research contribute to the knowledge about the occurence and distribution of reptiles and amphibians in the Zagreb area, and within the Natura 2000 site "Vejalnica and Krč" and the Significant landscape "Goranec". They can also be of use to the employees of the Public institution "Maksimir" for the creation of management plans for the two sites.

Key words: herpetofauna, Natura 2000, "Vejalnica and Krč"

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GMAZOVI I VODOZEMCI KAMENOLOMA BIZEK

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Na ovom posteru predstavljeni su rezultati faunističkog istraživanja gmazova i vodozemaca u neaktivnom kamenolomu Bizek, od 2008. do 2014. godine. Na istraživanom području su raznolika staništa: mješovita šuma bukve, hrasta i graba okružuje kamenolom sa sjeverne, istočne i zapadne strane. Termofilni elementi dominiraju na zapadnoj strani, dok na južnoj šuma prelazi u dvorišta i stambene objekte. Dva stalna vodotoka i jedna lokva su također prisutni, uz nekoliko povremenih vodotokova i lokvica. Jedinke obiju skupina su lovljene rukom, a u nekim slučajevima koristilo se štap s omčicom za guštere, kuke i rukavice za zmije te mreža za vodozemce. Pronađeno je sedam vrsta gmazova i sedam vrsta vodozemaca. Sve pronađene vrste su već poznate iz zagrebačke okolice. Zbog složenosti i nemogućnosti određivanja zelenih žaba (*Pelophylax* spp.) do vrste na temelju vanjskog izgleda, sve nađene jedinke ostavljene su na razini

svojte. Od posebnog značaja je nalaz žutog mukača, *Bombina variegata* (Linnaeus, 1758), koji je na Dodacima II i IV Direktive o staništima Europske komisije. Rezultati ovog istraživanja daju doprinos poznavanju prisutnosti i rasprostranjenosti vrsta gmazova i vodozemaca na samome rubu grada Zagreba i unutar Parka prirode Medvednica.

Ključne riječi: herpetofauna, Natura 2000, Park prirode Medvednica

REPTILES AND AMPHIBIANS OF THE BIZEK QUARRY

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This was a study of reptiles and amphibians which lasted from 2008 till 2014 in the inactive Bizek quarry, where we collected information on the presence of species belonging to those two groups. A variety of habitats occur in the quarry: a mixed deciduous forest of *Quercus* sp., *Fagus sylvatica* and *Carpinus betulus* surrounds the quarry to the north, east and west. Thermophilic elements are predominant on the western side, while on the southern side the forest gives way to yards and housing. Two permanent streams and a pond are also present, along with some intermittent streams and puddles. We caught animals by hand and, in some cases, we also used a stick with a noose for lizards, gloves and hooks for snakes, and a net for the amphibians. We found seven reptile and seven amphibian species in the quarry, all of which are already known from the surroundings of Zagreb. Frogs belonging to the genus *Pelophylax* Fitzinger, 1843 were identified down to the genus level only, because of the complexity of the determination and the inability to reliably identify them on the basis of external morphology. The finding of *Bombina variegata* (Linnaeus, 1758), which is on Annexes II and IV of the European Comission's Habitats Directive, is of special significance. The results of this study contribute to the knowledge of the occurrence and distribution of suburban reptiles and amphibians within the Nature Park Medvednica.

Key words: herpetofauna, Natura 2000, Nature Park Medvednica

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NEW REVISION OF THE CHECKLIST OF AMPHIBIAN FAUNA IN BOSNIA AND HERZEGOVINA

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Research of batrahofauna of Bosnia and Herzegovina (B&H) has a long tradition. It can be traced back to the O. Möellendorf PhD dissertation in 1873. The batrachological investigations in B&H could be divide on four periods: (1) The period of Ottoman Empire/Austro-Hungarian Monarchy (until 1918), (2) The period of the Kingdom of Yugoslavia (1918-1943), (3) The period of the Socialist Federal Republic of Yugoslavia (1943-1992) and (4) The period of independent B&H. The main aim of this paper is to give an update of biosystematic information and to

present the new precise distribution maps for all amphibian species in B&H. With new information (literature and field data) we found significant differences comparative to the previous reviews. Two new amphibian species were found, so there is evidence that 21 amphibian species (including one klepton) occurs in B&H. Authors have confirmed existence of all 21 species. The most common species are *Pelophylax ridibundus* and *Bombina variegata* and the rarest ones are *Triturus carnifex*, *Triturus dobrogicus*, *Pelobates fuscus*, *Salamandra atra* and *Triturus macedonicus*, with just several findings. With 65% species of batrahofauna of Balkan penninsula and two subendemic taxa (*Proteus anguinus* and *Salamandra atra* prenjensis) B&H can be considered as important area of amphibian biodiversity in Southern Europe. New expected (sub)species and some biosystematic problems are also discussed in this paper.

Key words: distribution, biosystematics, batrachology, Bosnia and Herzegovina

BIOLOGIJA MORA
MARINE BIOLOGY

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UVIDI O EKOLOGIJI PREHRANE SREDOZEMNE MEDVJEDICE

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Sredozemna medvjedica je vrsta kritično ugroženih morskih sisavaca čije stanište i ponašanje su u velikom dijelu i dalje nepoznati u Mediteranu i Jadranu. U ovoj studiji smo predstavili neke uvide o prehrani ovog grabežljivca. Uzorci izmeta iz sjevernog Jadrana (Istra-Hrvatska, n=24, 2010.-2014.) prikupljeni su i analizirani za istraživanje o prehrani. U svim uzorcima pronađene su samo riblje kosti i ljuske. Većina plijena (36%) sastoji se od riba Ljuskavki, od čega su najčešći identificirani plijen bili ušata i trlja, tj. barbun. Ostale ostatke je moguće samo klasificirati kao Grgečke zbog stupnja probave kostiju. Iako je studija provedena s ograničenom veličinom uzorka, možemo istaknuti važnost ove informacije za ugroženog sisavca kao što je sredozemna medvjedica, te korištenje izmeta po prvi puta za ovu vrstu.

Ključne riječi: sredozemna medvjedica, uzorci izmeta, prehrana

INSIGHTS ON THE FORAGING ECOLOGY OF THE MEDITERRANEAN MONK SEAL

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The Mediterranean monk seal is a critically endangered marine mammal species whose habitat and behavior are in great part still unknown in the Mediterranean and Adriatic. In this study we present some insights about the foraging of this predator. Scat samples from north Adriatic

(Croatia, n=24, 2010-2014) were collected and analyzed for dietary investigations. In all samples, only fish bones and scales were recovered. Most of the preys items (36%) consist on Sparidae fish were saddle sea bream and surmullet were the most common identified prey. Other remains were only possible to classify as Perciforms due to the digestion degree of the bones. Although the study was carried out with a limited sample size, we underline the importance of this information for an endanger mammal such as the Mediterranean Monk Seal, and the use of scats for the first time in this species.

Key words: The Mediterranean monk seal, Scat samples, The foraging

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Brooksia lacromae sp. nov. (Tunicata, Thaliacea), NOVA VRSTA DVOOTVORKE U JADRANSKOM MORU

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Brooksia lacromae sp. nov. Garić & Batistić je opisana iz zooplanktonskog materijala sakupljenog na stalnoj postaji u južnom Jadranu u jesen 2014. Vrsta se morforloški značajno razlikuje od jedine dvije poznate vrste istog roda, *B. rostrata* i *B. berneri*. Genetička analiza bazirana na genu za 18S rRNA potvrdila je različitost vrste *B. lacromae* od vrste *B. rostrata* (p-vrijednost između sekvenci je bila 1,5%). U istim uzorcima pronađena je i atlantska vrsta repnjaka *Fritillaria helenae* pa možemo pretpostaviti da su obje vrste atlantskog/zapadnomediteranskog podrijetla te su mogući indikatori atlantsko/zapadnomediteranske struje.

Ključne riječi: Thaliacea, nova vrsta, *Brooksia lacromae*, Jadransko more

Brooksia lacromae sp. nov. (Tunicata, Thaliacea), A NEW SPECIES OF SALP FROM THE ADRIATIC SEA

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Brooksia lacromae sp. nov. Garić & Batistić was described from zooplankton material collected at a marine monitoring station in the South Adriatic in autumn 2014. This new species is morphologically markedly different from *B. rostrata* and *B. berneri*, so far the only two species in the genus *Brooksia*. Genetic analysis based on 18S rRNA gene confirmed distinctness (p-value between sequences 1.5%) of *B. lacromae* from *B. rostrata*. Occurrence of *B. lacromae* with an Atlantic appendicularian, *Fritillaria helenae*, in the same samples suggests its Atlantic or Western Mediterranean origin and its potential for being an indicator species of Atlantic/Western Mediterranean waters.

Key words: Thaliacea, new species, *Brooksia lacromae*, Adriatic Sea

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ZBIRKA RAKOVA PRIRODOSLOVNOG MUZEJA U SPLITU

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Zbirku rakova u Prirodoslovnom muzeju u Splitu započeo je skupljati profesor Umberto Girometta te njezin početak nastajanja možemo smjestiti u 20-te godine dvadesetog stoljeća. Zbirka se temelji na uzorcima osam sakupljača. Zbirka rakova Prirodoslovnog muzeja u Splitu broji 409 primjeraka raspoređenih u 124 inventarna broja. Analizom je utvrđeno da zbirka sadrži 70 morskih vrsta rakova unutar 53 roda, 40 porodica i 6 redova. Važno je istaknuti 89 primjeraka koji su sakupljeni u Jadranu na znanstvenim ekspedicijama Instituta za oceanografiju i ribarstvo: AdriaMed i MEDITS. Ostali primjerci su sakupljeni u osobnim terenskim istraživanjima sakupljača, a dva primjerka su darovana.

Ključne riječi: Zbirka rakova, rakovi, Prirodoslovni muzej, Umberto Girometta

NATURAL HISTORY MUSEUM CRUSTACEAN COLLECTION IN SPLIT

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Crustacean collection of the Natural History Museum in Split began to collect Professor Umberto Girometta and its origin can be dated in the early 1920-ies. The collection is based on samples from eight collectors. The collection of the Natural Museum in Split has 409 copies distributed to 124 inventory numbers. The analysis shows that the collection contains 70 marine species of crustaceans within 53 genus, 40 families and 6 orders. It is important to highlight 89 specimens that were collected in the Adriatic Sea on the scientific expeditions of the Institute of Oceanography and Fisheries: AdriaMed and MEDITS. Other specimens were collected in the personal field researches of collectors, and two copies were donated.

Key words: Crustacean collection, Crustaceans, Natural History Museum, Umberto Girometta

P-65
MORFOLOGIJA I FILOGENIJA RODA *Bacteriastrum* (BACILLARIOPHYTA) U JADRANSKOM MORU

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Četiri kolonijalne vrste planktonskih dijatomeja roda *Bacteriastrum* – *B. furcatum*, *B. hyalinum*, *B. jadranum* i *B. mediterraneum* analizirane su u uzorcima iz Jadranskog mora te su iz njih uzgojene kulture u laboratoriju. Opisana je morfologija i ultrastruktura frustula koristeći svjetlosni i elektronski mikroskop, te su dobivene sekvence velike podjedinice (LSU) rDNA. Opis vrste *B. jadranum* je dopunjen i ispravljen na osnovi otkrića novog i jedinstvenog načina formiranja kolonija kod te vrste koji uključuje povezivanje stanica u lance pomoću organske tvari. Dopunjeni su opisi dvije od tri preostale vrste; vegetativne stanice vrste *B. hyalinum* posjeduju kratke spinule

u obliku slova Y na spojenim dijelovima seta i izdanke na valve u obliku slova T, dok mirujuće spore posjeduju nepravilnu površinu vanjskog dijela omotača primarne valve. Za vrstu *B. furcatum* na đeno je da posjeduje specifičan raspored pora na površini valve. Filogenetski rezultati grupirali su LSU sekvence četiri vrste u dvije sestrinske grane; na prvoj se nalaze *B. furcatum* (odjel Sagittata) i *B. hyalinum* (odjel Isomorpha) dok su na drugoj smješteni *B. jadranum* (odjel Isomorpha) i *B. mediterraneum* (odjel Sagittata). Možemo zaključiti da je rod *Bacteriastrum* monofiletski rod, međutim odjeli ne dijele monofiletsko porijeklo te njihove pretpostavljene određujuće taksonomske značajke: izomorfne terminalne sete kod vrsta koje pripadaju odjelu Isomorpha te dimorfne kod vrsta odjela Sagitatta nisu sinapomorfije.

Ključne riječi: Planktonske dijatomeje, *Bacteriastrum*, Jadransko more

MORPHOLOGY AND PHYLOGENY OF THE GENUS *Bacteriastrum* (BACILLARIOPHYTA) IN THE ADRIATIC SEA

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Four colonial species of the multipolar centric diatom genus *Bacteriastrum: B. furcatum, B. hyalinum*, *B. jadranum* and *B. mediterraneum* were investigated using field samples from the Adriatic Sea and cultured strains obtained from these samples. Morphology and frustule ultrastructure were examined using light and electron microscopy, and nuclear-encoded large-subunit (LSU) rDNA sequences were obtained from the strains. The description of *B. jadranum* has been emended based on a novel mechanism of colony formation in which cells are joined through an organic cell jacket which holds together cells in chains. Descriptions for two of the three other species were supplemented; vegetative cells of *B. hyalinum* have short Y spinules on the fused part of the setae and T-shaped outgrowths, and the spores of this species possess a granular surface on the exterior side of t he primary valve mantle. Specific for *B. furcatum* is the location of pores scattered around the setae bases. Phylogenetic results grouped the sequences of the four species in a clade in which *B. furcatum* (section Sagittata) and *B. hyalinum* (section Isomorpha) formed a clade as sister to a clade with *B. jadranum* (section Isomorpha) and *B. mediterraneum* (section Sagittata). Apparently, neither section was monophyletic, and the supposed defining features of isomorphic terminal setae in Isomorpha and dimorphic ones in Sagittata were not synapomorphies. Nonetheless, the genus *Bacteriastrum* itself remained monophyletic.

Key words: planktonic diatoms, *Bacteriastrum*, Adriatic Sea

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SASTAV MAKROZOOBENTOSKIH BESKRALJEŠNJAKA U OBRAŠTAJNIM ZAJEDNICAMA U LUCI SPLIT (HRVATSKA, JADRANSKO MORE)

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Istraživanje obraštajnih zajednica u luci Split, važnoj hrvatskoj luci za međunarodni pomorski promet, obavljeno je na dva teretna terminala (Sjeverna luka i Sveti Kajo) u proljeće i jesen

2014. godine. Korištena je metodologija uzorkovanja koja se bazira na CRIMP protokolu, a na pojedinom terminalu uzorci su sakupljani na tri transekta, međusobno udaljena 15 m, s betonskih vertikalnih zidova, s dubina od 0,5 m, 3 m i 7 m, te iz sedimenta. Ukupno su zabilježene 132 vrste makrozoobentoskih beskralješnjaka, 84 na terminalu Sjeverna luka i 108 na terminalu Sveti Kajo. Zabilježene su dvije alohtone vrste: morski puž *Siphonaria pectinata* (Linnaeus, 1758), prvi put zabilježen u akvatoriju grada Splita 2003. godine; te kolonijalni kameni koralj *Oculina patagonica* de Angelis, 1908, prvi put zabilježen u luci Split 2011. godine.

Ključne riječi: obraštajna zajednica, *Siphonaria pectinata*, *Oculina patagonica*, luka Split, Jadransko more

COMPOSITION OF MACROZOOBENTHIC INVERTEBRATES IN FOULING COMMUNITIES IN THE HARBOUR OF SPLIT (CROATIA, ADRIATIC SEA)

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Investigation of fouling communities in harbour of Split, important Croatian harbour for international maritime transport, was performed on two cargo terminals (Sjeverna luka and Sveti Kajo) in spring and autumn in 2014. Sampling methodology was based on CRIMP protocol, and samples were collected on each terminal from three transects, 15 m apart from each other, from concrete vertical walls, at depths of 0.5 m, 3 m and 7 m, and from sediment. Total of 132 species of macrozoobenthic invertebrates was recorded, 84 in Sjeverna luka terminal and 108 in Sveti Kajo terminal. Two non-indigenous species were recorded: marine gastropod *Siphonaria pectinata* (Linnaeus, 1758), for the first time recorded in Split city aquatorium in 2003; and colonial stony coral *Oculina patagonica* de Angelis, 1908, for the first time recorded in harbour of Split in 2011.

Key words: fouling community, *Siphonaria pectinata*, *Oculina patagonica*, harbour Split, Adriatic Sea

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TAKSONOMSKI SASTAV MORSKIH BENTOSKIH DIJATOMEJA I NJEGOV ODNOS S GLAVNIM EKOLOŠKIM ČIMBENICIMA U BOSNI I HERCEGOVINI

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Na istočnojadranskoj obali postoji svega nekoliko studija o morskim bentoskim dijatomejama, koje su uglavnom bile usmjerene na istraživanje taksonomije i ekologije perifitona u hrvatskim estuarijima i albanskim slanim močvarama. Morske planktonske i bentoske dijatomeje do sada nisu nikada istraživane u Bosni i Hercegovini. Ova studija bila je dio većeg istraživačkog programa s ciljem utvrđivanja bioraznolikosti te funkcioniranja ekosustava u pograničnim obalnim vodama između Hrvatske i Bosne i Hercegovine. Program je uključivao algološka, zoološka i hidrografska istraživanja. U radu se prikazuju taksonomski sastav i sezonalnost morskih bentoskih dijatomeja u odnosu na osnovne ekološke parametre (temperatura,

salinitet, gustoća) u Neumskom zaljevu u Bosni i Hercegovini.

Ključne riječi: Neumski zaljev, bentos, dijatomeje, istočni Jadran, SI Sredozemlje

TAXONOMIC COMPOSITION OF MARINE BENTHIC DIATOMS IN THE RELATION TO SOME KEY ENVIRONMENTAL VARIABLES IN BOSNIA AND HERZEGOVINA

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In the eastern Adriatic, there are only a few studies on benthic diatoms mostly focused on ecology and taxonomy of periphytic diatoms in the Croatian estuaries or Albanian coastal wetlands. Until now, both planktonic and benthic marine diatoms have never been studied in Bosnia and Herzegovina. This study forms part of a research program investigating the biodiversity and ecosystem functioning in the border marine coastal waters between the two countries – Croatia and Bosnia and Herzegovina. The program includes phycological, zoological and hydrographical research. This paper considers the taxonomic composition and seasonality of marine benthic diatoms in the relation to some key environmental variables (temperature, salinity and density) in the Bay of Neum in Bosnia and Herzegovina on the south-eastern Adriatic coast.

Key words: Neum Bay, benthos, diatoms, eastern Adriatic, NE Mediterranean

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DUBINSKA RASPROSTRANJENOST KORALJA U KORALIGENSKOJ BIOCENOZI ISTOČNOG JADRANA

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Jedan od glavnih problema vezanih uz spoznaje o koraligenskoj zajednici u Jadranskom moru je nedostatak podataka o dubinskoj rasprostranjenosti pojedinih vrsta. Koraligen se smatra jednim od glavnih "vrućih točaka" biološke raznolikosti u Sredozemnom moru. Karakterizira ga i velik broj ugroženih dugoživućih vrsta (poput koralja), koje su vrlo osjetljive na antropogene promjene, pogotovo zbog sporog rasta i neizvjesnog oporavka. Početna dubina koraligenske zajednice ovisi o količini svjetlosti koja dopire do morskog dna. Uz smanjenu količinu svjetlosti (dovoljne za veći razvoj crvenih alga (red Corallinales)), ponegdje početak koraligena može biti vrlo plitko. U Jadranskom moru, na podmorskim strmcima, najplića početna dubina za koraligensku biocenozu je oko 20 metara. Raznolikost vrsta koralja, vezano uz dubinsku rasprostranjenost, ovisna je o nekoliko ekoloških čimbenika (morske struje, količina svjetlosti, temperatura i sedimentacija). Najčešće vrste koralja u ovoj biocenozi su *Parazoanthus*

axinellae, *Epizoanthus arenaceus*, *Alcyonium coralloides*, *Alcyonium acaule*, *Paramuricea clavata*, *Eunicella singularis*, *E. cavolini*, *Leptopsammia pruvoti*, *Hoplangia durotrix*, *Caryophyllia inornata*, *C. smithii* i *Madracis pharensis*. Crveni koralj (*Corallium rubrum*) komercijalno je iskorištavan u gotovo svim dijelovima istočnog Jadrana, te su njegove populacije znatno smanjene, pogotovo u plitkom koraligenu.

Ključne riječi: Koralji, koraligen, Jadransko more

DEPTH DISTRIBUTION OF CORALS IN THE CORALLIGENOUS OF THE EASTERN ADRIATIC SEA

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One of the major gaps concerning the current state of knowledge of coralligenous community in the Adriatic Sea is the scarcity of information on species bathymetrical distribution. The coralligenous is considered as one of the most important "hot spots" of species diversity in the Mediterranean. It is also characterized by harbouring a great number of endangered species, which are long-lived organisms, whose slow growth and recruitment rates make them very sensitive to disturbances. The minimal depth for the formation of coralligenous concretions depends on the amount of irradiance reaching the sea bottom. Coralligenous concretions can appear in very shallow waters if light conditions are dim enough to allow a great development of coralline algae. In the Adriatic Sea, on the vertical slopes, the minimal depth reaches about 20 meters. Differences among species of corals in tolerances to several physical factors (currents, light, temperature and sedimentation) are thought to determine the upper and lower limits of species depth distributions. The most common coral species are *Parazoanthus axinellae*, *Epizoanthus arenaceus*, *Alcyonium coralloides*, *Alcyonium acaule*, *Paramuricea clavata*, *Eunicella singularis*, *E. cavolini*, *Leptopsammia pruvoti*, *Hoplangia durotrix*, *Caryophyllia inornata*, *C. smithii* and *Madracis pharensis*. Populations of red coral (*Corallium rubrum*) declined in most areas in the Adriatic Sea, particularly in shallow coralligenous.

Key words: Corals, Coralligenous, Adriatic Sea

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TROFIČKE INTERAKCIJE U ZAŠTIĆENIM MORSKIM PODRUČJIMA: ULOGA STRANIH INVAZIVNIH VRSTA

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Cilj ovog istraživanja je procjena utjecaja invazivnih stranih vrsta (zelene alge *Caulerpa cylindracea*, crvene alge *Asparagopsis* sp., desetonožnog raka *Percnon gibbesi* i puža stražnjoškržnjaka *Aplysia dactylomela*) u zaštićenom morskom području Pelagijski otoci. Analizom stabilnih izotopa bentičkih organizama kvantificirane su trofičke interakcije između alohtonih i autohtonih morskih vrsta. Usporedbom širina izotopskih niša i modela miješanja izotopa temeljenih na Bayesovoj statistici, istražili smo preklapa li se izotopska niša herbivornih vrsta *P.*

gibbesi i *A. dactylomela* s nišama autohtonih herbivora i izračunali udio različitih vrsta alga u prehrani stranih i autohtonih vrsta. Rezultati su pokazali da je udio *C. cylindracea* između 9% i 22%, a najveći je u prehrani *P. gibbesi* i *A. dactylomela* (16% i 22%). Ovo istraživanje pokazalo je da invazivne strane alge povećavaju raznolikost raspoloživog plijena i vjerojatno olakšavaju širenje drugih stranih vrsta. Iako strane vrste nisu u izravnoj kompeticiji s autohtonim ježincima *Paracentrotus lividus* i *Arbacia lixula*, moguća posljedica njihove velike brojnosti je smanjenje energije dostupne organizmima na višem trofičkom nivou u hranidbenoj mreži.

Ključne riječi: strane invazivne vrste, trofičke interakcije, zaštićeno morsko područje, Sredozemno more

TROPHIC INTERACTIONS IN MARINE PROTECED AREAS: THE ROLE OF INVASIVE ALIEN SPECIES

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The objective of this study was to evaluate the effects of invasive alien species (green algae *Caulerpa cylindracea*, red algae *Asparagopsis* sp., herbivorous crab *Percnon gibbesi* and sea hare *Aplysia dactylomela*) in MPA Pelagie Islands. Here, we present the results of the stable isotope analysis of benthic organisms in order to characterise trophic interactions between native and alien species. Using comparisons of species' isotopic niche widths and Bayesian mixing models, we investigated whether the diet of alien herbivores overlapped with those of native species and quantified the food source contribution to diets of native and alien consumers. Mixing models showed that contribution of *C. cylindracea* is between 9% and 22%. Highest contributions are those in diets of *P. gibbesi* and *A. dactylomela* (16% and 22%, respectively). Results indicated that invasion of macroalgae is increasing the diversity of available prey and facilitates spread of another alien species. Although *P. gibbesi* is not in direct competition with native sea urchins *Paracentrotus lividus* and *Arbacia lixula*, high abundances of this invasive crab are likely to be reducing the energy available to higher consumers in the food web.

Key words: invasive alien species, herbivores, trophic interactions, marine protected area, Mediterranean

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DNEVNI PRIRASTI OTOLITA KAO METODA VALIDACIJE TOČNOG ODREĐIVANJA STAROSTI TRLJE OD KAMENA, *Mullus surmuletus*

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Postoji pojačana potreba za relevantnom, vjerodostojnom metodom određivanja starosti kod trlje od kamena, *Mullus surmuletus* kao ciljane vrste mediteranskog priobalnog ribarstva. Ukupno je analizirano 387 jedinki ukupnih dužina od 6,8 do 32,9 cm. Godišnja periodičnost nastanka prstenova potvrđena je analizom rubnog prirasta. Godišnji rast otolita je verificiran analizom tipa ruba. Udio otolita s tamnim rubom je bio najveći zimi (>80%). Mjesečni srednji rubni prirast je bio najmanji u lipnju. Analiza mjerenih udaljenosti je pokazala da se prvi pravi

prsten pojavljuje na udaljenosti od 0,78 mm (± 0,104 SD) od centra i pri starosti ribe od 0,83 godina. Analiza starosti je utvrdila 6 starosnih razreda i točnost je potvrđena brojanjem dnevnih prirasta. Svi su mjereni parametri otolita bili linearni sa starosti. Najpreciznija je procjena za analiziranu populaciju dobivena iz širine otolita, a slijedila je dužina i masa. Rast je procijenjen Von Bertalanffy-om jednadžbom rasta. Rast je bio najbrži do druge godine života, a onda usporava. Uzimajući u obzir utvrđene biološke odrednice, trenutno gospodarenje je potrebno nadopuniti cjelovitijim, ekološkim pristupom propisima, kako bi se osigurale učinkovite mjere zaštite za ovu ekonomski i ekološki važnu vrstu riba.

Ključne riječi: dnevni prirast, starost, otolit, *Mullus surmuletus*

DAILY OTOLITH INCREMENTS AS VALIDATION METHOD FOR AGE DETERMINATION OF THE STRIPED RED MULLET, *Mullus surmuletus*

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There is a high need to establish a method for reliable age determination of the striped red mullet, *Mullus surmuletus* as target fish species of Mediterranean artisanal fisheries. In total, 387 specimens ranging in total length from 6.8 to 32.9 cm were analyzed. The annual periodicity of annulus deposition was supported by the marginal increment analysis. The annual growth of otoliths was verified using edge-type analysis. The proportion of otoliths with opaque margins was the highest (>80%) in the winter. The monthly mean marginal increment showed a single minimum in June. A distance measurements analysis revealed that first regular ring appears at distance of 0.78 mm (± 0.104 SD) from otolith nucleus and fish age of about 0.83 years. The age analysis revealed 6 age classes. Age determination was validated by daily otoliths increments readings. All measured otolith morphometric parameters were linear with fish age. The most precise age estimations of analyzed population were obtained from the otolith width, followed by the otolith length and mass. The growth was estimated by the fit of the Von Bertalanffy growth function. The growth of *M. surmuletus* was rapid up to 2 years of age and then slowed. Considering the identified biological implications, existing management should be complemented by a more holistic, ecosystem approach to regulations, in order to assure effective conservation measures for this economically and ecologically important fish species.

Key words: daily increments, age, otolith, *Mullus surmuletus*

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PROCJENA BROJNOSTI ZAJEDNICE DOBRIH DUPINA (*Tursiops truncatus*) VIŠKOG AKVATORIJA

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U razdoblju od 2007. do 2014. istraživana je biologija i ekologija dobrih dupina (*Tursiops truncatus*) u akvatoriju otoka Visa (~5.500 km^2). Terensko prikupljanje podataka provedeno je

od proljeća do jeseni plovilom duljine 6 m. Opaženo je 327 skupina dobrih dupina, a metodom fotoidentifikacije je utvrđena prisutnost 723 jedinke. Brojnost populacije procijenjena je metodom ulova i ponovnog ulova za zatvorene populacije pri čemu je korišten Chao Mth procjenitelj. U analizu su uvrštene samo jedinke s izraženim prirodnim oznakama kako bi se umanjila vjerojatnost pogrešne identifikacije. Rezultati ukazuju na to da je brojnost populacije u ovom razdoblju bila stabilna budući da usporedbom utvrđenih vrijednosti nije ustanovljena statistički značajna razlika između pojedinih godina (Z-test; $z<1,829$, $P>0,0674$ za sve usporedbe). Na temelju podataka iz 2014. godine, procijenjena brojnost populacije iznosi 278 (95% CI=208-413) jedinki dobrih dupina. Budući da je dio istraživanog područja uvršten u Natura 2000 mrežu s ciljem očuvanja dobrih dupina, potrebno je osigurati nastavak praćenja brojnosti populacije u ovom području.

Ključne riječi: dobri dupin, procjena brojnosti, zaštićeno područje

ABUNDANCE ESTIMATES OF THE BOTTLENOSE DOLPHIN (*Tursiops truncatus*) COMMUNITY IN VIS ARCHIPELAGO

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We studied the biology and ecology of the resident population of bottlenose dolphins (*Tursiops truncatus*) in the area around Vis Island (~5,500 km^2) between 2007 and 2014. Data was collected in the spring and summer using a 6 meter rigid inflatable boat. We obtained 327 sightings and photo-identified 723 individuals. Abundance of the bottlenose dolphin population was estimated using the mark-recapture model for closed populations and Mth estimator of Chao. To avoid misidentification errors, only well-marked individuals were included in the analysis. No significant difference in the year-to-year abundance was detected (Z-test; $z<1.829$, $P>0.0674$ for all comparisons), so the population appeared stable during the study period. Based on data collected in 2014, the population abundance estimate is 278 (95% CI=208-413) bottlenose dolphins. As a part of the research area was included in the Natura 2000 network with the aim of conserving the local bottlenose dolphin population, it is necessary to continue with monitoring of the population abundance in this area.

Key words: bottlenose dolphin, abundance estimate, protected area

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STANJE POPULACIJE INĆUNA (*Engraulis encrasicolus* L. 1758) U ISTOČNOM DIJELU JADRANSKOG MORA

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Inćun (brgljun) *Engraulis encrasicolus* L. 1758., je jedna od najbrojnijih vrsta u ribarskim lovinama RH. Međutim, zadnjih godina se uočava pad ulova ove izrazito značajne vrste za gospodarstvo svih zemalja uz Jadransko more, kao i za ekosustav Jadranskoga mora. Ukupan

ulov inćuna u RH je 2011. godine bio 14163 t, dok je 2013. godine iznosio 8904 tone. Svrha je ovog rada utvrditi stanje populacije inćuna u istočnom dijelu Jadrana.Analizirano je ukupno 612 jedinki uzorkovanih od lipnja do prosinca 2014. godine. Svi uzorci su potjecali iz lovina plivarica, ostvarenih u ribolovnim zonama B, C, E i F istočnog, hrvatskog dijela Jadranskoga mora.Totalne dužine (LT) svih jedinki su se kretale u rasponu od 10,0 do 16,6 cm. Srednja vrijednost je iznosila 13,5 ± 0,99 cm, a modalni dužinski razred - 13,0 cm. Odnos spolova je bio u korist mužjaka; iznosio je m/ž=1,63. Analizom dužinsko-masenog odnosa utvrđen je pozitivan alometrijski rast (b=3,469; r^2=0,899). Kubični koeficijent kondicije (K) se kretao u rasponu od 0,52 do 0,69, te je pokazao, ukupno gledajući, povećanje vrijednosti s porastom dužine ribe. Početkom ovog istraživanja, u lipnju, inćun se mrijestio, a faza spolnog mirovanja je započela nakon rujna.

Ključne riječi: inćun, Jadransko more, dužinsko-maseni donos, kondicija

ANCHOVY (*Engraulis encrasicolus* L. 1758) POPULATION STATUS IN THE EASTERN ADRIATIC SEA

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Anchovy *Engraulis encrasicolus* L. 1758, is one of the most abundant species in Croatian fisheries. However, in the last few years, landings of this ecologically and commercially very important species have decreased from 14163 tons in 2011 to 8904 tons in 2013. The aim of this study was to analyse current state of anchovy population in the Eastern Adriatic Sea. In total, 612 anchovy specimens were sampled from June to December 2014. All fishes were sampled from purse seine landings, i.e. from fisheries zones B, C, E and F of the Eastern Adriatic Sea. Total length (LT) varied from 10.0 to 16.6 cm, the average length was 13.5 ± 0.99 cm, and modal length class - 13.0 cm. Number of male specimens exceeded the number of female specimens (m/f=1.63). Analyses of length-weight relation indicated positive allometric growth (b=3.469; r^2=0.899). Condition coefficient (K) ranged from 0.52 to 0.69. In general, condition values were higher in longer fishes. In the beginning of this study, in June, anchovy was spawning, and inactive phase of maturity cycle started after September.

Key words: Anchovy, Adriatic Sea, Length-weight relation, Condition coefficient

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PRAĆENJE RADIOEKOLOŠKOG STANJA SJEVERNOG JADRANA KORIŠTENJEM DAGNJE *Mytilus galloprovincialis* KAO INDIKATORA

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Izvor radionuklida u Jadranskom moru je atmosfersko zagađivanje, prvenstveno od Černobilske nesreće 1986. godine, a posredni izvori su donosi rijekama. Dagnja *Mytilus galloprovincialis* se koristi kao indikatorski organizam u monitoringu onečišćenja mora. Program sustavnog ispitivanja radioaktivnosti u morskim indikatorskim organizmima temelji se na određivanju koncentracije aktivnosti umjetnog radionuklida ^{137}Cs, te prirodnih ^{40}K, ^{232}Th (^{228}Ra), ^{226}Ra i ^{238}U i kozmogenog ^{7}Be u tkivu dagnje. Dagnje su uzorkovane u travnju i listopadu od 2008. do 2014. godine na

lokacijama pod izrazitim utjecajem dotoka slatkih voda: Limski kanal, Raški i Bakarski zaljev. U morskoj vodi Limskog kanala koncentracije aktivnosti ^{137}Cs su bile niske (1,83 Bq m^{-3}). Na svim lokacijama koncentracije aktivnosti ^{137}Cs u dagnji kretale su se ispod granica detekcije (<0,3 Bq kg^{-1}). Koncentracije aktivnosti ^{7}Be u dagnji su u proljeće više od aktivnosti mjerenih u jesen. Koncentracije aktivnosti ^{40}K u dagnji su nešto više u jesen sa prosječnom aktivnosti 313 Bq kg^{-1}. Koncentracije aktivnosti ^{232}Th (^{228}Ra), ^{238}U i ^{226}Ra u dagnji izrazito su niske i bitno se sezonski ne razlikuju. Povišene koncentracije aktivnosti pojedinih radionuklida u tkivu dagnji dokazuju njenu iznimnu sposobnost biokoncentracije/biomagnifikacije zagađivala i potencijalno korištenje kao indikatora. Radioekološko stanje sjevernog Jadrana je zadovoljavajuće i nema značajnijeg unosa radionuklida atmosferskim zagađivanjem i rijekama.

Ključne riječi: sjeverni Jadran, *Mytilus galloprovincialis*, radionuklidi

RADIOECOLOGY MONITORING OF THE NORTHEN ADRIATIC SEA USING MUSSELS *Mytilus galloprovincialis* AS AN INIDCATOR

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The source of radionuclides in the Adriatic Sea is global fallout primarily from the Chernobyl accident in 1986 and indirectly from the rivers discharges. Mussel *Mytilus galloprovincialis* is used as an indicator organism in monitoring marine pollution programs. Systematic radioactivity monitoring in marine indicator organisms was conducted by determination activity concentrations of artificial ^{137}Cs, natural ^{40}K, ^{232}Th (^{228}Ra), ^{226}Ra, ^{238}U and cosmogenic ^{7}Be in the tissues of mussels. The mussels were collected in spring and autumn periods from 2008 to 2014 at locations under significant fresh water discharges: Lim, Raša and Bakar bay. In Lim Bay ^{137}Cs activity concentrations in surface seawater were low (1.83 Bq m^{-3}). In mussels ^{137}Cs activity concentrations (< 0.3 Bq kg^{-1}) were below the detection limit in all locations. In the spring activity concentrations of ^{7}Be in mussels were greater than in autumn. In the autumn activity concentrations of ^{40}K in mussels were higher than in the spring, the average ^{40}K activity was 313 Bq kg^{-1}. Activity concentrations of ^{232}Th (^{228}Ra), ^{238}U and ^{226}Ra in mussels were mostly below the detection limit with no seasonal changes. Increased activity concentrations of individual radionuclides in the mussel tissues proved their exceptional ability of pollutant concentration and potential indicator usage. Radioecological condition of the northern Adriatic Sea is satisfactory without significant radionuclide discharges from fallout and rivers.

Key words: The northern Adriatic Sea, *Mytilus galloprovincialis*, radionuclid

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POJAVNOST I GUSTOĆA DOBRIH DUPINA (*Tursiops truncatus*) U MURTERSKOM MORU

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U vremenu od 2002. do 2007. (P1) i od 2013. do 2014. (P2) istražili smo pojavnost, stope opažanja i relativnu gustoću dobrih dupina (*Tursiops truncatus*) u Murterskom moru. Vizualne pretrage terena provedene su s jedrilice prosječne brzine kretanja od 3,8 čv u periodu P1, te gumenjakom prosječne brzine kretanja od 12,5 čv u periodu P2. Navigacijski podatci bilježeni su GPS uređajem,

a opažanja dupina kamerom s filmom od 2002 do 2004, te digitalnom kamerom od 2005. nadalje. Prijeđeni put tijekom pretraživanja iznosio je 1931 km u P1, te 2186 km u P2. U vremenu 2002.-2007. zabilježeno je 37 opažanja dobrih dupina, te 50 opažanja tijekom 2013.-2104. Prosječna veličina skupine iznosila je 6,2 jedinke u P1 i 5,5 jedinki u P2. Primjenom metode foto-identifikacije, na osnovi 84 jedinke opažene u P1 i 127 jedinki utvrđenih u P2 izrađen je katalog od 194 dobra dupina. Ukupno je 17 jedinki opažano u oba perioda (8,8%). Krivulja otkrivanja temeljena na svim opažanjima ne pokazuje asimptotski rast. Prosječna stopa opažanja, izražena kao broj opažanja po 1 km prijeđenog puta tijekom pretrage, iznosila je 0,0397 u P1 i 0,023 u P2. Relativna gustoća, izražena kao broj identificiranih jedinki po 1 km prijeđenog puta tijekom pretrage, iznosila je 0,0947 u P1 i 0,0797 u P2. Rezultati pokazuju da je Murtersko more značajano područje za dobre dupine. S obzirom na intenzitet nautičkog turizma u ovom području, ovo istraživanje pruža smjernice za daljnji monitoring i zaštitu.

Ključne riječi: dobri dupin, *Tursiops truncatus*, Jadransko more, foto-identifikacija, zaštita

OCCURRENCE AND DENSITY OF BOTTLENOSE DOLPHINS (*Tursiops truncatus*) IN MURTER SEA

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We studied bottlenose dolphins (*Tursiops truncatus*) in the waters surrounding the Island of Murter (Murter Sea, Croatia) in two periods: 2002-2007 (P1), and 2013-2014 (P2), and obtained baseline data on occurrence, encounter rates and relative densities. Visual area surveys in search for dolphins were conducted using a sailing boat with the average search speed of 3.8 kts in the P1, and 5.2 m rigid-hull inflatable boat with the average search speed of 12.5 kts in the P2. Navigation data were collected using GPS, while observations of dolphins were documented using film camera from 2002 to 2004 and digital camera from 2005 onwards. Research effort resulted in 1931 km in search and 37 sightings in P1, and 2186 km in search and 50 sightings in P2. Average group size was 6.2 individuals in P1, and 5.5 in P2. Photo-identification analysis resulted in a catalogue of 194 individuals. In P1, 84 individuals were identified, 127 individuals in P1, while 17 animals were seen in both periods (8.8%). Discovery curve based on all sightings shows no asymptotic growth. Relative encounter rate, expressed as number of sightings per km on effort, was 0.0397 in P1 and 0.023 in P2. Relative linear density, expressed as number of identified dolphins per km on effort was 0.0947 in P1 and 0.0797 in P2. The results show that the Murter Sea is important for local bottlenose dolphins. Given the intense levels of nautical tourism in this area, these results can serve as guidelines for subsequent monitoring and development of conservation plans.

Key words: Bottlenose dolphin, *Tursiops truncatus*, Adriatic Sea, photo-identification, conservation

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RASPROSTRANJENOST, ABUNDANCIJA I RAZMNOŽAVANJE VRSTE *Siphonaria pectinata* (GASTROPODA, PULMONATA) U AKVATORIJU GRADA SPLITA

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Morski puž *Siphonaria pectinata* (Linnaeus, 1758) (Gastropoda, Pulmonata) naseljava pojas plime i oseke na stjenovitim obalama u toplijim područjima s obje strane Atlantika, te Alboransko more. U srednjem Jadranu, u akvatoriju grada Splita, ova alohtona vrsta je prvi put zabilježena u proljeće 2003. godine gdje je najvjerojatnije unesena brodovima. Budući joj je populacija bila brojna, pretpostavljeno je da je to područje nastanila ranije. U 2009. godini zabilježena je na području od Rogoznice do Omiša te na području otoka Brača. Istraživanje rasprostranjenosti, abundancije, biometrijskih značajki i razmnožavanja ove alohtone vrste provedeno je u razdoblju od lipnja 2010. do svibnja 2011. godine, na tri lokaliteta na području grada Splita (poluotok Marjan). Na istraživanom području vrsta naseljava stjenovito dno mediolitorala, a brojnija je u gornjem mediolitoralu. Srednja vrijednost abundancije vrste je bila 6 ± 4 jedinki/400 cm^2 (n=720). Srednja vrijednost dužine jedinki iznosila je 1,1 ± 0,4 cm (n=4883). Vrsta se razmnožavala u razdoblju od svibnja do listopada, kada je polagala kokone.

Ključne riječi: *Siphonaria pectinata*, rasprostranjenost, abundancija, razmnožavanje, Jadransko more

DISTRIBUTION, ABUNDANCE AND REPRODUCTION OF THE SPECIES *Siphonaria pectinata* (GASTROPODA, PULMONATA) IN THE SPLIT CITY AQUATORIUM

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Marine snail *Siphonaria pectinata* (Linnaeus, 1758) (Gastropoda, Pulmonata) inhabits rocky intertidal habitats in warmer areas on both sides of the Atlantic, and the Alboran Sea. In the central Adriatic, in the waters of the Split area, this invasive species was recorded for the first time in the spring of 2003, probably introduced by ships. Since its population was numerous, it was assumed that it was settled in this area much earlier. In 2009 was recorded in the area from Rogoznica to Omiš and the island of Brač. A research of distribution, abundance, biometric features and reproduction period of this species had been conducted at the three stations in the city of Split (peninsula Marjan). In the investigated area this species inhabits rocky bottom of mediolittoral and it is more numerous in the upper mediolittoral. The mean abundance of the species was 6 ± 4 organisms/400 cm^2 (n=720). The mean length of individual organisms was 1.1 ± 0.4 cm (n=4883). The recorded reproduction period of *S. pectinata* in the study area was from May to October, when it laid cocoons.

Key words: *Siphonaria pectinata*, distribution, abundance, reproduction, Adriatic Sea

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UTJECAJ STRATEGIJE HRANJENJA NA DRUŠTVENU STRUKTURU DOBRIH DUPINA (*Tursiops truncatus*) U ISTOČNOM JADRANU, HRVATSKA

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Ranije studije opisuju utjecaj koćarenja ili marikulture na veličinu i sastav skupina dobrog dupina (*Tursiops truncatus*), no do sada nije promatran kombinirani utjecaj. Tijekom 2013. i 2014.

provedeno je istraživanje u tri područja Jadrana: cresko-lošinjski arhipelag, sjeverna Dalmacija (Zadarska i Šibensko-kninska županija) te viški arhipelag. Cilj istraživanja bio je utvrditi kombinirani utjecaj koćarenja i marikulture na veličinu, društveni i dobni sastav skupina u tri područja koja se razlikuju u intenzitetu koćarenja i marikulture. Ukupno je zabilježeno 176 opažanja grupa koje se hrane na otvorenom moru (OM), 59 grupa pri lovu iza koćarice (K) te 8 grupa pri lovu na ribljim farmama (RF). Prosječna veličina skupina bila je 5±0,56, najviša u cresko-lošinjskom (5,81±0,43), najniža u viškom arhipelagu (3,6 ±0.73), no nije se znatno razlikovala među OM, RF i K skupinama (K=0,138, df=2, p>0,05). Ukupan stupanj asocijacije jedinki bio je nizak (0,1±0,1), no viši za RF (0,07 ±0,03) i K (0,07±0,03), nego OM skupine (0,02±0,01). Hijerarhijska cluster analiza pokazala je visoko strukturiranu ukupnu populaciju (CCC=0,91), a prisutnost koćarica i ribljih farmi ima veći utjecaj na sastav skupina u sjevernoj Dalmaciji i viškom arhipelagu nego cresko-lošinjskom arhipelagu. Analiza dobnog sastava skupina pokazuje veću zastupljenost odraslih jedinki u T i RF, nego u OM skupinama (χ^2=9,21, p<0,001). Ovi rezultati pružaju temelj za daljnja istraživanja i osmišljavanje mjera zaštite.

Ključne riječi: dobri dupin, *Tursiops truncatus*, socijalna ekologija, ribarstvo, zaštita

INFLUENCE OF FORAGING STRATEGIES ON SOCIAL STRUCTURE OF BOTTLENOSE DOLPHINS (*Tursiops truncatus*) IN EASTERN ADRIATIC SEA, CROATIA

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Previous studies have shown that either bottom trawlers or fish farms influence bottlenose dolphin (*Tursiops truncatus*) group size and structure, however no study described combined effect. In 2013 and 2014 a photo-ID based study was conducted in three regions of the Adriatic sea: Cres-Lošinj, North Dalmatia and Vis. The aim was to examine combined effect of bottom trawlers and fish farms upon group size, social and age-class structure of bottlenose dolphin communities in the three regions that differ in intensity of bottom trawler and aquaculture fisheries. Research effort resulted in sightings of 176 groups engaged in "open water foraging" (OWF), 59 in "bottom-trawler foraging" (BTF) and 8 in "foraging at fish farm" (FFF). Overall group size was 5±0.56, being highest in Cres-Lošinj (5.81±0.43) and lowest in Vis region (3.6±0.73), but it did not vary significantly between the foraging strategies (K=0.138, df=2, p>0.05). Simple-ratio association index was overall low (0.1±0.1), but higher for FFF (0.07 ±0.03) and BTF (0.04 ±0.02) than OWF groups (0.02±0.01). Hierarchial cluster analyses showed overall highly structured population (CCC=0.91), structure being more affected by presence of bottom trawlers and fish farms in North Dalmatia and Vis than in Cres-Lošinj region. Age composition of groups varied, with more adults in BTF and FFF than in OWF groups (χ^2=9.21, p<0.001). These results provide baseline information for further research and conservation plans.

Key words: bottlenose dolphin, *Tursiops truncatus*, social ecology, fisheries, conservation

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POVEZANOST PODVODNE BUKE U CRESKO – LOŠINJSKOM AKVATORIJU SA PROMJENAMA U GLASANJU DOBRIH DUPINA (*Tursiops truncatus*)

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U cresko – lošinjskom akvatoriju (sjeverni Jadran), tehnike pasivne akustike primijenjene su za praćenje buke u moru i utvrđivanje povezanosti podvodne buke s akustičnim ponašanjem (glasanjem) rezidentnih jedinki dobrih dupina. U razdoblju 2007.–2009., sustavno snimanje podvodne buke provodilo se na 10 lokacija predviđenima za akustični monitoring korištenjem hidrofona RESON TC 4032 povezanog sa kalibriranim Pioneer DC-88 DAT snimačem zvuka. Za vrijeme akustičnog monitoringa bilježeni su podaci o prisutnosti plovila, njihovom broju, tipu i udaljenosti plovila od lokacije snimanja buke. Analizirane su akustične snimke koje sadrže zvižduke dupina te su definirani sljedeći parametri: trajanje zvižduka, najniža i najviša frekvencija, frekvencijski raspon zvižduka, početna i završna frekvencija, broj točki fleksija kontura zvižduka i alikvotnih tonova. Parametri zvižduka uspoređeni su sa razinom i karakteristikama podvodne buke utvrđenim za akustične snimke koje ih sadrže. Rezultati ukazuju da dupini proizvode zvižduke na višim frekvencijama kada su razine podvodne buke na nižim frekvencijama više. Promjene u parametrima zvižduka povezane su i sa prisutnošću plovila na moru. U prisustvu plovila zvižduci dupina imali su značajno više frekvencije i duže trajanje. Ove promjene povezane sa i ponašanjem skupine te su učestale za vrijeme aktivnosti hranjenja i druženja dupina.

Ključne riječi: Dobri dupini, podvodna buka, zvižduk, frekvencija, plovila

SHIFTS IN BOTTLENOSE DOLPHIN (*Tursiops truncatus*) VOCALIZATION IN RELATION TO SEA AMBIENT NOISE IN THE CRES AND LOŠINJ WATERS, CROATIA

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In the Cres–Lošinj archipelago (northeast Adriatic Sea, Croatia) passive acoustic monitoring was applied to characterize local sea ambient noise (SAN) and assess the relationship between the underwater noise and the acoustic behaviour (whistles) of resident bottlenose dolphin groups. Between 2007 and 2009, systematic monthly sampling of SAN was made at ten locations using RESON TC 4032 hydrophone connected to a previously calibrated Pioneer DC-88 DAT recorder. Boat presence, type and distance from the monitoring station were noted along whilst acoustic monitoring was ongoing. Acoustic recordings containing dolphin whistles were analyzed. The following whistle parameters were considered: duration, maximum and minimum frequencies, delta frequencies, start and end frequencies, number of inflection points and the number of harmonics. Whistle parameters were compared to the characteristics of sea ambient noise of the acoustic samples containing them. Results indicated that dolphin produce whistles at higher frequencies when ambient noise levels at low frequencies were higher. Variations of the whistle structure resulted to be related to the overall presence of boats. When boats were present, dolphins produced whistles with longer duration at significantly higher freq uencies. These changes in whistle structure were found to be dependent on the group behavioural state, in particular during their foraging and socializing activities.

Key words: Bottlenose dolphins, sea ambient noise, whistle, frequency, boats

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MALAKOLOŠKA ZBIRKA PETRA NOVAKA

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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. Petar Novak (1879.–1968.), hrvatski agronom i stručnjak za zaštitu bilja, bio je ravnatelj Prirodoslovnog muzeja u Splitu (1946.-1952.). Cijenjen entomolog, specijalist za kornjaše (Coleoptera), otkrio je veći broj endemskih vrsta kukaca, a oko 30 vrsta dobilo je po njemu ime. Više od 40 stručnih i znanstvenih radova objavio je u Trstu, Beču, Rimu, Zagrebu, Sarajevu i Beogradu. Međutim, malo je poznato da je prikupio i malakološku zbirku. Zbirka je nastala neposredno nakon Drugog svjetskog rata (1946.-1949.) i čine je predstavnici puževa i školjkaša Jadranskog mora i kopnenih puževa. Primjerci su sakupljeni na području Splita i srednjodalmatinskih otoka (Sušac, Svetac, Palagruža, Šolta) i to poglavito u plitkom litoralnom području. Zbirka se sastoji od 364 primjerka raspoređenih u 45 inventarnih brojeva, svrstanih u 23 porodice i 32 roda. Najzastupljenije porodice su Veneridae, Donacidae, Tellinidae, Helicellidae, Nassariidae i Hygromiidae. Svi su primjerci determinirani, inventarizirani i uneseni u bazu podataka.

Ključne riječi: Petar Novak, malakološka zbirka, Prirodoslovni muzej

MALACOLOGICAL COLLECTION OF PETAR NOVAK

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The Natural History Museum and ZOO in Split holds 7 malacological collections of which one is permanently exhibited and the other ones are deposited: collections of P. Novak, A. Cvitanić, M. Kolander, J. Savo, V. Golubić and B. Cvitanović. Petar Novak (1879-1968), Croatian agronomist and an expert for plant protection, was the principle of The Natural History Museum in Split (1946-1952). This admired entomologist (coleopterologist) discovered many endemic species and around 30 species were named after him. He published more than 40 scientific papers in Trst, Vienna, Rome, Zagreb, Sarajevo and Belgrade. However, it's not known that he also had a malacological collection. The collection was created immediately after World War II (1946-1949) and contains molluscs and gastropods of The Adriatic Sea and land snails. The specimens were collected in the Split area, on middle dalmatian islands (Sušac, Svetac, Palagruža, Šolta) mostly in the shallow littoral area. Collection consists of 364 specimens organised in 45 inventory number, presenting 23 family and 32 genus. The most represented family were Veneridae, Donacidae, Tellinidae, Helicellidae, Nassariidae and Hygromiidae. All the specimens are determined, inventoried and stored in a database.

Key words: Petar Novak, malacological collection, Natural History Museum

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FOTOSINTETSKA UČINKOVITOST, UDIO FOTOSINTETSKIH PIGMENATA I FENOLNIH SPOJEVA TE ANTIOKSIDACIJSKA AKTIVNOST U ROSIKA (*Droseraceae*) PRI RAZLIČITIM UVJETIMA UZGOJA

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Kako bi nadoknadile manjak nutrijenata na staništu, rosike (*Droseraceae*) mame i love kukce ljepljivim kapljicama na tentakulima listova. U radu je istražen učinak različitih uvjeta uzgoja na fotosintetsku učinkovitost, količinu fotosintetskih pigmenata i fenolnih spojeva, te antioksidacijsku aktivnost plojki i peteljki listova u vrstama *Drosera spatulata* i *D. aliciae*. Dio biljaka bio je uzgajan u sobnim uvjetima pri niskim intenzitetima osvjetljenja uz smanjenu mogućnost hvatanja kukaca dok je drugi dio biljaka uzgajan na otvorenom prostoru pri visokim intenzitetima osvjetljenja uz veću dostupnost kukaca. Plojke listova imale su višu fotosintetsku učinkovitost te viši sadržaj fotosintetskih pigmenata i fenolnih spojeva od peteljki analiziranih vrsta. Iako je sadržaj klorofila i karotenoida bio viši u biljaka uzgajanih u sobnim uvjetima, fotosintetska učinkovitost bila je veća u biljaka koje su rasle na otvorenom, vjerojatno zbog izloženosti višim intenzitetima svjetlosti i/ili veće dostupnosti kukaca. Udio flavonoida i antocijana bio je veći u plojkama biljaka uzgajanih na otvorenom, što je rezultiralo povećanjem sadržaja ukupnih fenola u vrste *D. spatulata* dok je u vrste *D. aliciae* viši sadržaj ukupnih fenola nađen u biljaka uzgajanih u sobnim uvjetima. Antioksidacijska aktivnost u ekstraktima plojki i peteljki bila je visoka u biljkama koje su rasle u sobnim uvjetima kao i kod biljaka na otvorenom, osim u ekstraktima peteljki vrste *D. spatulata* uzgajane u sobnim uvjetima.

Ključne riječi: Droseraceae, fotosinteza, klorofili, fenolni spojevi

PHOTOSYNTHETIC EFFICIENCY, CONTENT OF PHOTOSYNTHETIC PIGMENTS AND PHENOLIC COMPOUNDS, AND ANTIOXIDATIVE ACTIVITY IN *Droseraceae* AT DIFFERENT GROWTH CONDITIONS

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Droseraceae (sundews) lure and capture insect prey using sticky mucilage of leaf tentacles, in order to obtain extra nutrients since growing in nutrient-poor peat bogs. In this work, the effects of different growth conditions on photosynthetic efficiency, content of photosynthetic pigments and phenolic compounds, and antioxidative activity of leaf blades and petioles of *Drosera spatulata* and *D. aliciae* were investigated. One set of sundews was grown indoors at low light intensities with scarce prey availability. Another set of plants was grown outdoors at high light intensities with greater insect availability. In general, leaf blades performed higher photosynthetic efficiency and contained more photosynthetic pigments and phenolic compounds than petioles of analyzed species. Despite of increased content of chlorophylls and carotenoids in indoor-growing plants, the p hotosynthetic efficiency was higher in outdoor-growing plants, probably due to high light intensities exposure and/or greater prey availability.

The contents of flavonoids and anthocyanins were higher in leaf blades of outdoor-growing plants, resulting in increased content of total phenolic compounds in *D. spatulata.* On the contrary, *D. aliciae* plants contained more phenolic compounds in indoor-growing conditions. Antioxidative activity was high in blade and petiole extracts of investigated species grown in both indoor and outdoor conditions, with exception of petiole extract of *D. spatulata* grown indoors.

Key words: Droseraceae, photosynthesis, chlorophylls, phenolic compounds

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ZAJEDNIČKE RAZVOJNE ZNAČAJKE SOMATSKE I ZIGOTNE EMBRIOGENEZE

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U mnogim vaskularnim biljkama, zigotna reprodukcija se izmjenjuje s različitim vrstama aseksualnih načina reprodukcije. Tako se biljni embriji mogu razviti ne samo iz oplođene jajne stanice, nego i iz induciranih somatskih stanica procesom somatske embriogeneze. Iako somatska i zigotna embriogeneza nisu izravno povezane, neka njihova zajednička obilježja ukazuju da inicijacija i razvoj somatskih embrija i morfološki i fiziološki podsjeća na razvoj zigotnog embrija. Početak embriogeneze u oba slučaja zahtijeva aktivaciju bilo jajne stanice oplodnjom ili somatske stanice specifičnim okolišnim čimbenicima. Tijekom indukcije dolazi do promjena razine metilacije DNA, aktivacije hormonskih regulatornih mehanizama i odgovora na stres, te promjena svojstava stanične stijenke. Povezanost ovih čimbenika s procesima somatske i zigotne embriogeneze, te njihova međusobna usklađenost biti će prikazana i raspravljena, s posebnim osvrtom na inicijaciju i razvoj ranog embrija.

Ključne riječi: somatska embriogeneza, zigotna embriogeneza, metilacija DNA

SOMATIC AND ZYGOTIC EMBRYOS SHARE COMMON DEVELOPMENTAL FEATURES

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In many vascular plants, zygotic reproduction regularly alternates with different types of asexual reproduction, so embryos can develop not only from fertilized egg cells, but also from induced somatic cells through the process of somatic embryogenesis. Although somatic and zygotic embryogenesis are not directly correlated, their common features will be presented, demonstrating that the origin and development of the somatic embryo morphologically and physiologically resembles zygotic embryogenesis at certain points. In order to initiate embryogenesis, both competent egg and somatic cells require activation either by fertilization or by specific environmental signals respectively. During induction of somatic and zygotic embryogenesis modulation of DNA methylation, activation of particular hormonal and stress-related mechanisms including changes in cell wall propertie s are triggered. We will give an overview and discuss the recent knowledge in the field of plant somatic and zygotic

embryogenesis, with special attention given to the onset of embryogenesis and early embryo development as well as to embryogenesis-related interconnections between plant hormones, stress responses, DNA methylation and regulatory gene expression.

Key words: somatic embryogenesis, zygotic embryogenesis, DNA methylation

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RASPROSTRANJENOST DRVENASTIH INVAZIVNIH VRSTA *Robinia pseudoacacia* L., *Reynoutria japonica* Houtt. I *Acer negundo* L. NA PODRUČJU SAMOBORA

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U radu su prikazani rezultati istraživanja rasprostranjenosti drvenastih invazivnih vrsta *Robinia pseudoacacia* L., *Reynoutria japonica* Houtt. i *Acer negundo* L. u urbanim dijelovima grada Samobora tijekom vegetacijskih sezona 2010. g. i 2011. g. Za svaku zabilježenu GPS poziciju utvrđen je i tip staništa prema Klasifikaciji staništa Republike Hrvatske. Zabilježili smo ukupno 31 stanište alohtonih drvenastih biljaka. Najviše je staništa vrste *Acer negundo* L., čak 16, a najmanje staništa *Reynoutria japonica* Houtt., samo tri. Najvećim udjelom staništa u ukupnom broju svih evidentiranih staništa alohtonih drvenastih biljaka ističe se *Acer negundo* L. sa 51%. Slijedi ga *Robinia pseudoacacia* L. koju nalazimo na 39% i *Reynoutria japonica* Houtt. koju nalazimo na samo 10% evidentiranih staništa.

Ključne riječi: invazivna flora, Samobor, Hrvatska

DISTRIBUTION OF NON-HERBACEOUS INVASIVE SPECIES *Robinia pseudoacacia* L., *Reynoutria japonica* Houtt. AND *Acer negundo* L. IN THE SAMOBOR AREA

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This work brings results of the research into distribution of non-herbaceous invasive species *Robinia pseudoacacia* L., *Reynoutria japonica* Houtt. and *Acer negundo* L. in urban parts of the town of Samobor during vegetation seasons of 2010 and 2011. For each marked GPS position we determined the habitat type according to the National Habitat Classification of Croatia. We recorded altogether 31 habitats of allochthonous non-herbaceous plants. The species *Acer negundo* L. had the greatest number of habitats – 16, and *Reynoutria japonica* Houtt. had only 3. *Acer negundo* L. also has the biggest portion of habitats in the total number of all recorded allochthonous non-herbaceous plant habitats – 51%. It is followed by *Robinia pseudoacacia* L. found in 39% and *Reynoutria japonica* Houtt. found in only 10% of recorded habitats.

Key words: invasive flora, Samobor, Croatia

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ULOGA TRANSPORTERA HMA U AKUMULACIJI Cd U VRSTI *Nicotiana glauca*

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Hiperakumulacija toksičnih metala, kao što je kadmij (Cd), rijedak je fenomen u biljaka. *Nicotiana glauca* vrsta je koja akumulira Cd, dok je obični duhan *N. tabacum* umjereno tolerantna ne-akumulatorska biljka. Transporteri metala HMA su identificirani kao ključne komponente akumulacije i tolerancije Cd u drugim hiperakumulatorskim biljkama, kao što su *Arabidopsis halleri* i *Nocacea caerulescens*. Nedavno su u vrsti *N. tabacum* sekvencirani geni za transportere HMA, ali njihova lokalizacija i funkcije nisu poznate. Istražili smo uzorke ekspresije gena HMA-A i HMA-B u obje vrste duhana kako bismo razjasnili ulogu koju ovi transporteri imaju u akumulaciji i toleranciji Cd. Razlika između dvije vrste duhana je važna zbog toga što one koriste dva bitno različita mehanizma tolerancije: hiperakumulaciju Cd (*N. glauca*) i isključivanje Cd (*N. tabacum*). HMA-A je jače eksprimiran u listovima vrste *N. glauca*, a HMA-B u listovima i korijenu vrste *N. tabacum*. Tretman s Cd inducirao je ekspresiju oba gena u listovima i korijenu vrste *N. glauca*, dok je u vrsti *N. tabacum* indukcija bila tkivno specifična: HMA-A u listovima i HMA-B u korijenu. S obzirom na ekspresijski profil i fenotip akumulacije Cd u vrsti *N. glauca*, predlažemo da je HMA-A uključen u translokaciju metala iz korijena u stabljiku, slično svom homologu iz *N. caerulescens* (NcHMA4). S druge strane, HMA-B bi mogao biti uključen u detoksikaciju Cd, tako što bi mogao unositi Cd u vakuole, slično drugom homologu u *A. halleri* (AhHMA3).

Ključne riječi: hiperakumulacija Cd, *Nicotiana glauca*, transporteri HMA

ROLE OF HMA TRANSPORTERS IN Cd ACCUMULATION IN *Nicotiana glauca*

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Hyperaccumulation of toxic metals, such as cadmium (Cd), is a rare phenomenon in plants. *Nicotiana glauca* is Cd-accumulating plant, while common tobacco *N. tabacum* is moderately tolerant non-accumulating plant. HMA metal transporters have been identified as key components of Cd accumulation and tolerance in other hyperaccumulator plants, such as *Arabidopsis halleri* and *Nocacea caerulescens*. Recently, HMA transporter genes have been sequenced in *N. tabacum*, but their localization and function are still not known. We have examined expression patterns of HMA-A and HMA-B genes in both species to clarify roles of these two transporters in Cd tolerance and accumulation. The difference among two tobacco species is important since they utilize vastly different tolerance mechanisms: Cd-hyperaccumulation (*N. glauca*) and Cd-exclusion strategy (*N. tabacum*). HMA-A was constitutively highly expressed in the leaves of *N. glauca*, and HMA-B was highly expressed in leaves and roots of *N. tabacum*. After Cd-treatment expression of both genes was induced in leaves and roots of *N. glauca*, while in *N. tabacum* induction was tissue specific: HMA-A in leaves and HMA-B in roots. Considering gene expression and Cd-accumulation phenotype in *N. glauca*, we propose that HMA-A is involved in metal root-to-shoot translocation, similar to its homologue in *N. caerulescens* (NcHMA4). On the other hand, HMA-B could be involved in Cd

detoxication while transporting Cd into vacuoles, similar to another homologue in *A. halleri* (AhHMA3)

Key words: Cd hyperaccumulation, *Nicotiana glauca*, HMA transporters

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HRANIDBENA VRIJEDNOST KORE, PLODOVA I SJEMENKI OSKORUŠE (*Sorbus domestica* L.): MINERALI I FENOLNI SPOJEVI

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Cilj ovog rada bio je i) istražiti sadržaj anorganskih (makro- i mikroelemenata) i organskih tvari (ukupni fenoli, flavonoidi i tanini) te ii) antioksidativnu aktivnost ukupnih fenola i tanina iz kore, plodova (odvojeno egzokarp i mezokarp) i sjemenki vrste *S. domestica* L. koristeći fitokemijske analize. Rezultati su pokazali da su svi testirani uzorci bogati kalijem. Kora se pokazala kao najbolji izvor kalcija i cinka, dok su sjemenke najbolji izvor magnezija. Ekstrakti nezrelog egzokarpa i kore sadrže najveću količinu ukupnih fenola. Zrenjem se količina fenolnih spojeva u plodovima (i egzokarpu i mezokarpu) značajno smanjila. Egzokarp je znatno bogatiji ukupnim flavonoidima nego mezokarp, kora i sjemenke. Najbolju antioksidativnu aktivnost pokazali su ukupni fenoli nezrelog egzokarpa i kore. Antioksidativna aktivnost ukupnih fenola i tanina egzokarpa, ali ne i mezokarpa, značajno se smanjila nakon zrenja. Na temelju dobivenih podataka zaključujemo da su kora i sjemenke oskoruše značajno bolji izvori makro- i mikroelemenata nego plodovi, dok su egzokarp i kora bolji izvori ukupnih fenola i flavonoida. Predlažemo nedovoljno korištenu vrstu *Sorbus domestica* L. kao odličan izvor minerala i antikosidativnih tvari važnih u ljudskoj prehrani.

Ključne riječi: makro- i mikroelementi, flavonoidi, tanini, antioksidativna aktivnost

NUTRITIONAL VALUE OF *Sorbus domestica* L. BARK, FRUITS AND SEEDS: MINERALS AND PHENOLICS

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The aim of this study was to i) investigate the content of inorganic (macro- and microelements) and organic compounds (total phenolics, total flavonoids and total tannins) and ii) evaluate the antioxidant activities of total phenolics and total tannins in *S. domestica* L. bark, fruits (separately exocarp and mesocarp) and seeds using phytochemical analyses. Results revealed all the tested service tree samples were rich in potassium. Bark, among the tested samples, was the best source of calcium and zinc, while seeds were the best source of magnesium. Extracts of immature exocarp and bark contained the highest amounts of total phenolics. Ripening significantly decreased the amount of phenolics in fruits (both exocarp and

mesocarp). Exocarp was significantly richer in total flavonoids than mesocarp, bark or seeds. The highest antioxidant activity showed phenolics from i mmature exocarp and then from bark. The antioxidant activity of phenolics and tannins from exocarp, but not from mesocarp, was significantly decreased after ripening. Based on the data we conclude that service tree bark and seeds are significantly better sources of macro- and microelements than fruits, while exocarp and bark are better sources of total phenols and flavonoids. We suggest that under-utilized species *Sorbus domestica* L. could be used as a great source of mineral elements and antioxidants important for human diet.

Key words: macro- and microelements, flavonoids, tannins, antioxidant activity

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FOTOSINTETSKA UČINKOVITOST, UDIO FOTOSINTETSKIH PIGMENATA I FENOLNIH SPOJEVA TE ANTIOKSIDACIJSKA AKTIVNOST U RAZLIČITIM DIJELOVIMA VRČEVA U TRI HIBRIDA RODA *Sarracenia*

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Sarracenia je rod mesojednih biljaka poznatih po listovima preobraženim u vrčeve kojima mame, love i probavljaju kukce. U radu je istražena fotosintetska učinkovitost, udio klorofila, karotenoida i fenolnih spojeva, te antioksidacijska aktivnost različitih dijelova vrča (krilo, poklopac, gornji i donji dio vrča) u tri hibrida roda *Sarrracenia*. Hibrid A ima mali otvor vrča, poklopac kojemu se lijevi i desni rub spajaju te izražene crvene žile na poklopcu i gornjem dijelu vrča. Hibrid B je veći u odnosu na druga dva i karakterizira ga poklopac s izraženim crvenim žilama. Hibrid C ima poklopac i gornji dio vrča koji su gotovo crveni, a veličinom je najmanji u odnosu na druga dva hibrida. Rezultati ukazuju na povezanost obojenosti dijela biljke s količinom fenolnih spojeva i fotosintetskih pigmenata. U poklopcu i gornjem dijelu vrča koji su crveni povećan je udio fenolnih spojeva, dok je u krilu i donjem dijelu vrča koji su zeleni povećan udio klorofila i karotenoida. Analiza pokazatelja fotosintetske aktivnosti ukazuje na nižu stopu fotosinteze u poklopcu i gornjem dijelu vrča hibrida B. Hibrid B također se ističe većim udjelom klorofila i karotenoida u krilu i donjem dijelu vrča. Može se zaključiti da je uočeni pad fotosintetske učinkovitosti uvjetovan nižim udjelom klorofila u gornjem dijelu vrča i poklopcu. Iako je najveći udio fenolnih spojeva i flavonoida utvrđen u poklopcima svih hibrida, osobito C, ekstrakti svih dijelova vrča imaju vrlo visoku antioksidacijsku aktivnost.

Ključne riječi: *Sarracenia*, klorofili, karotenoidi, fenolni spojevi, fotosinteza

PHOTOSYNTHETIC EFFICIENCY, PHOTOSYNTHETIC PIGMENTS, AND PHENOLIC COMPOUNDS, AND ANTIOXIDANT ACTIVITY IN DIFFERENT PITCHER PARTS OF THREE *Sarracenia* HYBRIDS

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Sarracenia is a genus of carnivorous plants known by leaves modified into pitchers which lore, hunt and digest insects. In this study, photosynthetic efficiency, content of chlorophylls, carotenoids and phenolic compounds, and antioxidant activity in different parts of the

pitcher (wing, operculum, pitcher upper and lower) in three *Sarracenia* hybrids were analyzed. Hybrid A has small pitcher opening, operculum in which opposite edges merge, and red veins on operculum and pitcher upper part. Hybrid B is the largest of three hybrids and has operculum with red veins. Hybrid C is the smallest, with red-colored operculum and pitcher upper part. Results indicate that the coloration of pitcher parts is related to amount of phenolic compounds and photosynthetic pigments; the red-colored operculum and the upper part contain higher amount of phenolic compounds, while green-col ored wing and the lower part contain higher amount of chlorophylls and carotenoids. Analysis of photosynthetic parameters shows lower photosynthesis rate in operculum and pitcher upper part of hybrid B. Hybrid B also has higher amount of chlorophyll and carotenoids in wing and pitcher lower part. In conclusion, decline in photosynthetic efficiency in pitcher upper part and operculum is caused by lower amount of chlorophyll. Although the operculum contains the highest amount of phenolic compounds and flavonoids, especially in hybrid C, the extracts of all pitcher parts show high antioxidant activity.

Key words: *Sarracenia*, chlorophylls, carotenoids, phenolic compounds, photosynthesis

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ANTIOKSIDACIJSKA, ANTIBAKTERIJSKA I CITOTOKSIČNA AKTIVNOST VODENOG EKSTRAKTA DUBROVAČKE ZEČINE (*Centaurea ragusina* L.)

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Dubrovačka zečina (*Centaurea ragusina* L.) je endemična hrvatska biljna vrsta bogata bioaktivnim spojevima. Cilj ovog istraživanja je odrediti sadržaj polifenola u vodenim ekstraktima biljne vrste *C. ragusina* sakupljene na prirodnom lokalitetu (Katalinić brig i Sustipan) i uzgojene u kulturi tkiva kao i njihovu antioksidacijsku, antibakterijsku i citotoksičnu aktivnost. U svrhu razjašnjenja biološke aktivnosti proučavane su interakcije vodenih ekstrakata s polinukleotidima. Najviši sadržaj ukupnih fenola, flavonoida, flavonola, hidroksicimetnih kiselina i proantocijanidina izmjeren je u vodenim ekstraktima kalusa uzgojenim u uvjetima *in vitro*. Gotovo svi ispitani ekstrakti pokazali su neznatnu antibakterijsku aktivnost na *Acinetobacter baumannii* i *Staphylococcus aureus*, umjerenu antioksidacijsku aktivnost kao i značajnu citototoksičnu aktivnost na stani čnim linijama SCCVII, FsaR, B16-F10 i V79. Interakcijama vodenih ekstrakata s polinukleotidima (ctDNA, poli A - poli U) mjerenim UV-VIS spektroskopijom, promjenom temperature mekšanja polinukleotida (DTm) te spektroskopijom cirkularnog dikroizma (CD spektroskopijom) utvrđen je značajan utjecaj vodenih ekstrakata na stabilnost ispitivanih polinukleotida kao i na njihovu konformaciju.

Ključne riječi: *Centaurea ragusina* L., fitokemikalije, biološka aktivnost, interakcije s polinukleotidima

ANTIOXIDANT, ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES OF WATER EXTRACT OF PLANT SPECIES (*Centaurea ragusina* L.)

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Centaurea ragusina L. is an endemic Croatian plant species rich in bioactive substances. The goal of this work is to determine polyphenol content in water extracts of *C. ragusina* collected at natural habitats (Katalinić brig i Sustipan) and cultivated in vitro as well as to estimate their antioxidant, antibacterial and cytotoxic activity. The interactions of extracts with double stranded polynucleotides were studied to elucidate the biological activity of *C. ragusina*. The highest level of total phenolics, flavonoids, flavonols, hydroxycinnamic acids and proanthocyanidins were measured in calli water extracts cultivated *in vitro*. Almost all tested extracts showed weak antibacterial activity against *Acinetobacter baumannii* and *Staphylococcus aureus*, moderate antioxidant activity as well as significant cytotoxic activity on cell lines SCCVII, FsaR, B16-F10 and V79. Interactions of water extracts with polynucleotides (ctDNA, poly A - poly U) measured by UV-VIS spectroscopy, melting experiments (DTm) and circular dichroism (CD) spectroscopy showed significant impact of water extracts on the stability of studied polynucleotides and on their conformation.

Key words: *Centaurea ragusina* L., phytochemicals, biological activity, interactions with polynucleotides

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DO SUGAR CONTENTS IN SELECTED MOSS SPECIES IMPLY TO THEIR SALT RESISTANCE?

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Mosses are the first plants that face the terrestrial way of living and the harsh environment conditions. Some species can be also recorded in brackish water or salty soils, i.e. facultative halophytes. For study purpose of the resistance, tolerance and adaptation to salt, we chose three moss species with similar life span and life cycle, namely: *Physcomitrella patens* (non halophytic control moss), *Entosthodon hungaricus* and *Henediella heimii* (two facultative halophytic mosses). The *in vitro* cultures were established and growth conditions optimized prior to experimentation. In such a conditions moss plants were treated with different concentration of NaCl (0-500 mM) for three weeks prior to analyzing sugar content by HPLC. It was shown that all species have high level of sucrose (up to 350 µl/mL), glucose (up to 280 µl/mL) and fructose (up to 400 µl/mL) in total sugar profiles. However, sugar contents vary differently in two halophyte mosses. *H. heimii* seems to have predefined sucrose (ca. 200 µl/mL) content which tend to be maintained during increase salt stress, while *E. hungaricus* react to salt stress in quick synthesis of fructose and glucose. The sugar level in *E. hungaricus* is predefined only for low level of salt stress, which postpone vigorous reaction to medium and high salt exposure. However, *P. patens* reacts immediately upon salt stress (5 mM) by increase of initially low level of sucrose, fructose

and glucose. *H. heimii* express more tolerance and resistance to salt stress comparing to other halophyte moss *E. hungaricus* and especially non halophyte moss *P. patens.*

Key words: bryophytes, halophytes, salt stress, sucrose, fructose, glucose

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UČINAK NANOČESTICA SREBRA NA POJAVU OKSIDACIJSKOG STRESA U KLIJANCIMA DUHANA (*Nicotiana tabacum*)

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Nanomaterijali imaju veliku primjenu u svakodnevnome životu, a u skupinu često primjenjivanih spadaju i nanočestice srebra (AgNP). One omogućavaju veću iskoristivost protubakterijskih i protugljivičnih učinaka srebra. Istraživanje njihovog štetnog djelovanja na ekosisteme, a time i na biljke, stoga je u fokusu zanimanja. U ovom radu istraženi su učinci AgNP na klijance duhana, modelne biljke u istraživanjima abiotičkoga stresa. Klijanci su istovremeno bili izloženi i istim koncentracijama ionskog srebra ($AgNO_3$). Za procjenu oksidacijskog stresa izmjeren je sadržaj malondialdehida (MDA) i proteinskih karbonila te su određene aktivnosti antioksidacijskih enzima pirogalol (PPX) i askorbat peroksidaze (APX) te katalaze (CAT), spektrofotometrijski i u gelu. Svi tretmani s $AgNO_3$ doveli su do smanjenja sadržaja MDA u odnosu na kontrolu, dok je isti trend zamijećen nakon izlaganja većim koncentracijama AgNP. Tretmani česticama AgNP pokazali su povećanje sadržaja karbonila, dok izlaganje ionskom srebru nije dovelo do promjena u usporedbi s kontrolom. PPX i CAT pokazale su uglavnom smanjenu aktivnost nakon tretmana i s AgNP i s $AgNO_3$, iako je smanjenje aktivnosti PPX bilo jače izraženo nakon tretmana ionskim srebrom. Povećana aktivnost APX izmjerena je u svim tretmanima. Ovim je istraživanjem utvrđeno da oksidacijski stres sudjeluje u mehanizmu toksičnosti AgNP, a rezultati ukazuju na praktičnost i potrebu uključivanja biljaka u razvoj sveobuhvatnog istraživanja toksičnosti nanočestica.

Ključne riječi: duhan, nanosrebro, oksidacijski stres

THE EFFECT OF SILVER NANOPARTICLES ON OXIDATIVE STRESS IN TOBACCO SEEDLINGS (*Nicotiana tabacum*)

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Nanomaterials are widely used in everyday life, and silver nanoparticles (AgNPs) are often applied. They provide better utilization of antibacterial and antifungal effects of silver. Numerous studies conducted to determine their toxic effect on ecosystems, and therefore on plants, are in the focus of interest. Tobacco is a model plant often used in abiotic stress studies. In this research

the effects of AgNPs on tobacco seedlings were analyzed. Seedlings were also exposed to the same concentrations of ionic silver ($AgNO_3$). For the evaluation of oxidative stress the content of malondialdehyde (MDA) and protein carbonyls as well as the activity of antioxidative enzymes pyrogallol (PPX), ascorbate peroxidase (APX) and catalase (CAT) were analyzed spectrophotometrically and in-gel. Compared to control, all $AgNO_3$ treatments led to a decrease of MDA, and the same trend was obser ved after exposure to the higher AgNP concentrations. The increase of carbonyls was recorded in seedlings treated with AgNPs, while exposure to $AgNO_3$ showed no change. Generally, PPX and CAT activity was reduced after the AgNP and $AgNO_3$ treatments, although the decrease of PPX activity was more expressed in $AgNO_3$ treatments. Increased activity of APX was determined in all treatments. The results revealed that oxidative stress participates in the toxicity mechanism of AgNPs and suggests that involvement of plants in the comprehensive research of nanoparticles toxicity is convenient and necessary.

Key words: tobacco, nanosilver, oxidative stres

3. SIMPOZIJ HRVATSKOG ENTOMOLOŠKOG DRUŠTVA
3rd SYMPOSIUM OF THE CROATIAN ENTOMOLOGY SOCIETY

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USPOREDBA UČINKOVITOSTI METODA UZORKOVANJA VODENIH KUKACA (HETEROPTERA I COLEOPTERA) S OBZIROM NA ATRAKTANT, VELIČINU VRSTA I STANIŠTE

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Unatoč raznolikosti metoda uzorkovanja vodenih kukaca, usporedba njihove učinkovitosti na različitim stanišnim tipovima nije dostatna. Ovim istraživanjem usporedili smo učinkovitost tri metode uzorkovanja: velikom mrežom, metodom klopka-boca s konzerviranom tunjevinom i metodom klopka-boca s konzerviranom mačjom hranom. Glavni cilj ovog istraživanja jest utvrditi učinkovitost i selektivnost metoda uzorkovanja na različitim staništima te utvrditi učinkovitost novog atraktanta za vodene kukce - konzerviranu tunjevinu. Uzorkovanje je trajalo od 2010. do 2012. godine na dva različita staništa, melioracijskim kanalima i poplavnom području, u Parku Prirode Kopački rit. Rezultati su pokazali da stanište ima vrlo velik utjecaj na učinkovitost metode uzorkovanja. Naime, velika mreža je najučinkovitija metoda, osobito na melioracijskim kanalima. Konzervirana tunjevina se pokazala kao učinkovitiji atraktant, posebice za velike vrste porodice Dytiscidae, u odnosu na do sada korištenu konzerviranu mačju hranu u uzorkovanju vodenih kukaca. Naši rezultati potvrdili su potrebu za kombiniranjem nekoliko metoda uzorkovanja kako bi se u potpunosti moglo prikazati bogatstvo vrsta vodenih kukaca istraživanog područja.

Ključne riječi: vodeni kukci, učinkovitost i selektivnost metoda uzorkovanja, poplavno područje, melioracijski kanali

A COMPARISON OF METHODS FOR SAMPLING AQUATIC INSECTS (HETEROPTERA AND COLEOPTERA) CONSIDERING THE BAIT, BODY SIZE AND HABITAT TYPE

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Although various methods exist for sampling aquatic insects (Heteroptera and Coleoptera), comparisons of their performance across different habitat types are still scarce. In this study, we evaluated and compared the efficiency of three sampling methods: hand net (HN), bottle traps with canned tuna fish (BTF) and bottle traps with canned cat food (BCF). The aim of our investigation was to determine the efficiency and selectivity of sampling methods in different habitat types and also efficiency of newly introduced bait canned tuna fish in a study of aquatic insects. The methods were compared over the period 2010 – 2012 in two different habitat types, melioration canals and flooded areas, in the Nature Park Kopački rit, a floodplain area of the Danube River. The results show that the habitat type has a major influence on the effectiveness of the method. Overall, hand-netting was proven to be the most successful method, primarily at melioration canals. Canned tuna fish bait was more efficient than the commonly used cat food, especially for large and highly mobile Dytiscidae species. Our results reinforce the need for long-term sampling combined with multiple complementary methods to estimate the overall species richness of the area.

Key words: aquatic insects, sampling efficiency and selectivity, floodplain, canals, baits

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RASPROSTRANJENOST CRNE UDOVICE (*Latrodectus tredecimgutattus*, Rossi 1790.) NA PODRUČJU HRVATSKE I BIH

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Crnu udovicu, najotrovnijeg europskog pauka, na području Hrvatske nalazimo u Istri, Primorju i kvarnerskim otocima, te u različitim dijelovima Dalmacije. U BiH je rasprostranjena na području južne i zapadne Hercegovine. Kroz razdoblje od 2011. do 2014. godine pretražena su različita staništa na temelju literaturnih podataka, različitih podataka iz medija i drugih izvora te na temelju pretpostavke njihovog prisustva (krški travnjaci, klimatske značajke, kamenjarski travnjaci na visoravnima). Na temelju dobivenih podataka izrađena je karta rasprostranjenosti na području Hrvatske i BiH. Kako tijekom razdoblja istraživanja nije bilo masovnih gradacija na mnogim lokacijama jedinke su bile malobrojne a tako i teško uočljive. Zbog toga kartu ne možemo smatrati i konačnom.

Ključne riječi: crna udovica, karta rasprostranjenosti, staništa

DISTRIBUTION OF THE BLACK WIDOW SPIDER (*Latrodectus tredecimgutattus*, Rossi 1790.) IN THE AREA OF CROATIA AND BOSNIA AND HERZEGOVINA

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In Croatia, the black widow spider, which is known as the most poisonous European spider, can

be found in Istria, costal area and the Kvarnerian islands, as well as in different parts of Dalmatia. In Bosnia and Herzegovina the species can be found in the areas of south and west Herzegovina. Throughout the period from 2011 to 2014, different habitats have been researched based on the information from reference data and other sources including media and assumptions of the occurrence of the species (karst grasslands, climate). Based on the gathered data a map was created showing the distribution of the black widow spider in Croatia and Bosnia and Herzegovina. As there was no massive gradation on many of the locations trough the period of research the individuals were few and hard to spot. Therefore we cannot consider the map finite.

Key words: black widow spider, map of distribution, habitats

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FAUNA SKAKAVACA (INSECTA: ORTHOPTERA: CAELIFERA) HRVATSKE

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Fauna skakavaca (Orthoptera: Caelifera) Hrvatske nikad nije sistematično istraživana, a jedini popis vrsta koji postoji potječe iz vremena Jugoslavije i sadrži mnogo podataka koje je potrebno raspraviti. Ipak, podatci o skakavcima Hrvatske raspršeni su u mnogobrojnim publikacijama, a jedinke po mnogim muzejima. Cilj četverogodišnjeg istraživanja (2011.-2015.) bio je objediniti sve literaturne i dostupne muzejske podatke na jedno mjesto te na njih dati kritički osvrt, a u isto vrijeme znanje o ovoj zanemarenoj skupini kukaca nadopuniti novim terenskim i eksperimentalnim podatcima. Sakupljeno je preko 200 radova o fauni skakavaca Hrvatske, u suradnji sa stranim ekspertima i kustosima europskih muzeja objedinjeni su dostupni muzejski podatci, odrađeno je preko trideset terena diljem Hrvatske, a uzorci su preparirani i stvorena je nova mokra i suha zbirka skakavaca Hrvatske. Predstavljena je fauna skakavaca Hrvatske. Sveukupno, u Hrvatskoj je do sada zabilježeno 85 vrsta i 3 podvrste skakavaca. Predstavljen je broj vrsta po regijama (panonska, dinarska, mediteranska), raspravljeni su nalazi upitnih, rijetkih i zanimljivih vrsta. Predloženo je preko 15 sinonima i preko 10 taksonomskih promjena. Predstavljeni su također i neki zanimljivi rezultati bioakustičkih analiza te su dani prijedlozi za daljnja istraživanja taksonomije, nomenklature, ekologije, etologije, bioakustike i biogeografije ravnokrilaca.

Ključne riječi: faunistika, taksonomija, ravnokrilci, popis, revizija

CROATIAN SHORT-HORNED GRASSHOPPERS' (INSECTA: ORTHOPTERA: CAELIFERA) FAUNA

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Croatian Caeliferan fauna (Orthoptera) has never been systematically investigated, the only species' checklist originating from Yugoslavia and contains a lot of doubtful data. However, the data on Croatian short-horned grasshoppers are scattered within a lot of publications, while specimens are deposited within a lot of museums. The aim of the 4-years research (2011-2015) of this group was to gather all the literature and museums' data and to give critical review, and in the same time to fulfil the existing data with new – fieldwork and experimental information. More than 200 papers about Croatian Caeliferan fauna were gathered, via cooperation with foreign experts and museum curators' data from European museums were collected, as well,

more than 30 fieldworks all over Croatia have been conducted – this being the fundament for new wet and dry collection of Croatian Caelifera. Fauna of the Croatian Caelifera is presented. Hitherto, altogether 85 species and 3 additional subspecies have been recorded for Croatian fauna. Number of the species by region (Pannonian, Dinaric, and Adriatic) is presented; doubtful, new and interesting data are discussed. More than 15 synonyms and 10 taxonomical acts are proposed. Some interesting bioacoustical results are presented as well. Proposals and plans for future research on Orthopteran taxonomy, nomenclature, ecology, ethology, bioacoustics and biogeography are introduced.

Key words: faunistics, taxonomy, grasshoppers, checklist, review

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RAZNOLIKOST VRETENACA HRVATSKOGA ZAGORJA

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Fauna vretenaca Hrvatskoga zagorja nikada nije bila predmet sustavnih faunističkih istraživanja, sukladno tome su podatci o fauni vretenaca ove regije gotovo nepostojeći. U prilog tome govori činjenica da je do sada u fauni Hrvatske zabilježeno 75 vrsta vretenaca, dok je pregledom dostupne literature, za područje Hrvatskog zagorja utvrđeno samo njih 13. Budući da Hrvatsko zagorje obiluje mnoštvom vodenih tijela te zauzima veliki dio sjeverne Hrvatske, broj poznatih vrsta je daleko od stvarnog broja vrsta prisutnih na tome području. Tijekom 2012. i 2013. godine provedeno je prvo sustavno istraživanje faune vretenaca Hrvatskog zagorja. Utvrđene su 34 vrste od čega su 23 vrste po prvi puta zabilježene za područje Hrvatskog zagorja. Sedam pronađenih vrsta nalazi se u Crvenoj knjizi vretenaca Hrvatske, dok je jedna vrsta na Natura 2000 popisu. Nakon ovoga istraživanja Hrvatsko zagorje postaje jedno od najbolje istraženih područja u Hrvatskoj što se tiče faune vretenaca, s ukupno 37 zabilježenih vrsta.

Ključne riječi: vretenca, Hrvatsko zagorje, raznolikost, Natura 2000

DIVERSITY OF DRAGONFLY FAUNA OF HRVATSKO ZAGORJE

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Dragonfly fauna of Hrvatsko zagorje has never been systematically studied and accordingly literature data about dragonfly fauna of mentioned region are almost non-existent. This is corroborated by the fact that 75 dragonflies species are so far known from Croatia, while for the Hrvatsko zagorje 13 species have been recorded in the literature. Since Hrvatsko zagorje is rich in water bodies and takes up a large part of the northern Croatia, the number of known species is far from the expected number of species present in that area. During the years 2012 and 2013 we conducted the survey of dragonflies and damselflies fauna (Insecta, Odonata) of Hrvatsko zagorje. Thirty four species were found, of which 23 species were recorded for the first time for the region. Seven species are listed in the Red data book of dragonflies of Croatia, while one species is on Natura 2000 list. After this survey Hrvatsko zagorje becomes one of the best survey areas as regards dragonfly fauna, with a total of 37 recorded species.

Key words: Dragonfly, Hrvatsko zagorje, diversity, Natura 2000

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NOVE VRSTE TULARA (INSECTA: TRICHOPTERA) ZA FAUNU HRVATSKE IZ PARKA PRIRODE KOPAČKI RIT

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Odrasli tulari (Trichoptera) prikupljani su u razdoblju od 2010. – 2012. godine na području Parka prirode Kopački rit na ukupno 8 postaja. Ovo su prva istraživanja faune i ekologije tulara na području parka koja su obuhvatila različite tipove slatkovodnih staništa. Uzorkovanja su obavljena pomoću svjetlosne klopke (UV lampe BLB 15 W) jednom mjesečno, izuzev svibnja kada je uzorkovanje obavljeno dva puta. Dvije vrste zabilježene ovim istraživanjem nove su za faunu Hrvatske: *Oecetis furva* i *Ortotrichia tragetti*. Obje su vrste zabilježene na različitim tipovima staništa (riječni tok, jezero, kanali). Vrsta *Oecetis furva* zabilježena je na donjem toku rijeke Drave, zatim na eutrofnom jezeru (Sakadaškom jezeru), povremenom vodotoku s bazenčićima (Vemeljski dunavac) i kanalu s povremenim protokom (kanal Čarna). Vrsta *Ortotrichia tragetti* zabilježena je na eutrofnom jezeru (Sakadaškom jezeru) i kanalu s povremenim protokom (kanal Čarna). Ovi podatci predstavljaju značajan doprinos poznavanju faune tulara Hrvatske općenito, a posebice nedovoljno istraženih močvarnih i jezerskih staništa kontinentalnog dijela Hrvatske.

Ključne riječi: Trichoptera, *Oecetis furva*, *Ortotrichia tragetti*, močvarna staništa, jezera

NEW SPECIES OF CADDISFLIES (INSECTA: TRICHOPTERA) FOR CROATIAN FAUNA FROM THE KOPAČKI RIT NATURE PARK

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Adult caddisflies (Trichoptera) were collected from 2010 to 2012 in the Kopački Rit Nature Park. Collecting was conducted on a total of 8 collecting sites encompassing various freshwater habitats. The current study is the first study of faunistics and ecology of caddisflies in the park. Sampling was conducted using light traps (UV lamp BLB 15 W) that were operated monthly, with the exception of May when it was carried out on two occasions. Two species collected in this study are new for the Croatian fauna: *Oecetis furva*, and *Ortotrichia tragetti*. Both species were collected at various habitats (river course, lake, channels). *Oecetis furva* was collected at the lower reach of the Drava River, in the eutrophic lake (Sakadaško Lake), and Vemeljski dunavac and Čarna channels. *Ortotrichia tragetti* was collected at the eutrophic lake (Sakadaško Lake) and Čarna channel. These data represent a significant contribution to the general knowledge of caddisflies in Croatia, and particularly of insufficiently investigated wetlands and lakes in the continental region.

Key words: Trichoptera, *Oecetis furva*, *Ortotrichia tragetti*, wetlands, lakes

BIOLOGIJA KOPNENIH VODA I KOPNA
BIOLOGY OF FRESHWATER AND TERRESTRIAL ECOSYSTEMS

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STRUKTURA ZAJEDNICE I HRANIDBENE NAVIKE SLATKOVODNIH RIBA U PLITKOM JEZERU UMJERENOG PODRUČJA

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Ihtiocenoze predstavljaju jedan od ključnih elemenata u funkcioniranju lentičkih ekosustava, osiguravajući protok energije i proces recikliranja nutrijenata. Istraživanje sastava i strukture ihtiocenoze provedeno je tijekom srpnja 2014. godine u Kopačkom jezeru, Park prirode Kopački rit. Ispitan je sastav probavila riba kako bi se utvrdila važnost pojedinih kategorija hrane u njihovoj ishrani, s obzirom da ribe svojom ishranom mogu utjecati na bentos i obraštaj. Uzorkovanje je provedeno pomoću trostruke poponice, postavljene tijekom večeri da stoji 12 sati, koja je potom izvučena. Uzorkovani primjerci riba fiksirani su u 4%-tnom formaldehidu radi kasnije obrade u laboratoriju. Ribolovni napor (CPUE) iznosio je 2,71 kg/mreža-sati. Tijekom istraživanja su uzorkovane 143 jedinke, starosti između 1+ i 6+ godina. Utvrđeno je 9 različitih vrsta koje pripadaju porodicama: Esocidae, Cyprinidae i Percidae. Najbrojnija vrsta je bila *Blicca bjoerkna*, s uzorkovanih 50 jediniki. Također, zabilježen je veći broj mužjaka nego ženki. U probavilima riba utvrđene su različite kategorije hrane: vegetacija, detritus, beskralježnjaci, uz dominaciju ličinki kukaca, te ribe. Prema sastavu probavila, uzorkovane ribe možemo klasificirati kao omnivore, bentivore te piscivore. Također, pronađeni su i endoparaziti u jedinkama vrsta *B. bjoerkna*, *Alburnus alburnus* i *Gymnocephalus baloni*. Zabilježena ihtiofauna karakteristična je za područje i stanište istraživanja, a utvrđene hranidbene navike ukazuju na različite važne izvore hrane za pojedine vrste riba.

Ključne riječi: slatkovodna ihtiofauna, *Blicca bjoerkna*, trofička povezanost, ličinke kukaca

COMMUNITY STRUCTURE AND FEEDING HABITS OF FRESHWATER FISH IN A SHALLOW TEMPERATE LAKE

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Ichthyocenoses are one of the key elements in functioning of the lentic ecosystems, insuring the necessary energy flow and nutrient recycling process within a water body. Research of the composition and the structure of ichthyocenosis was conducted in July 2014, in Kopačko Lake, Kopački Rit Nature Park. To test the significance of certain food categories in the fish diet, their

stomach contents were examined, given that fish can significantly influence benthos and periphyton. Sampling was done using gill net placed in a stationary manner with a soaking time of 12h, after which it was retrieved. Sampled fish were fixed in 4% formaldehyde for later laboratory measurements. The catch per unit effort (CPUE) was 2.71 kg per net-hours. During the research, 143 fish specimens were sampled, aged from 1+ to 6+, belonging to 9 species of families Esocidae, Cyprinidae and Percidae. The most abundant species was *Blicca bjoerkna*, with 50 specimens recorded. In general, more males than females were found during the research. Different categories of food: vegetation, detritus, invertebrates, with the dominance of Insecta larvae, and fish were found in the alimentary canals. Results indicate that sampled fish can be classified as omnivores, benthivores and piscivores. We also recorded endoparasites in the representatives of *B. bjoerkna*, *Alburnus alburnus* and *Gymnocephalus baloni*. Recorded ichthyofauna is typical for this area and the type of habitat, whiles different feeding habits indicated different important food sources for the recorded species.

Key words: freshwater ichthyofauna, trophic relationships, Insecta larvae

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RELATIONSHIPS BETWEEN ALGAL PRODUCTION AND THE PHYSICAL AND CHEMICAL PROPERTIES OF A DRINKING WATER RESERVOIR

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Reservoirs in agricultural dominated watersheds are particularly vulnerable to algal blooms, but few results detail those drinking water reservoirs which has no agricultural impact however algal blooms often or regularly occur. During May 2007 to September 2008, we studied spatial and temporal patterns of algal abundance and composition with respect to a series of physical and chemical properties in Lázbérci-tározó, located in north-east Hungary. Our results suggest that nutrients (in particular TP, O-PO_4, TN, NO_3-N concentrations), turbidity, and hydrologic regime all played potentially important roles in regulating algal biomass. The low level of nitrogen coupled with the internal release of phosphorus from the lake sediment under brief periods of anoxia may have helped promote cyanobacterial algal bloom.

Key words: reservoirs, algal bloom

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TAKSONOMSKI SASTAV MORSKIH BENTOSKIH DIJATOMEJA I NJEGOV ODNOS S GLAVNIM EKOLOŠKIM ČIMBENICIMA U BOSNI I HERCEGOVINI

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Na istočnojadranskoj obali postoji svega nekoliko studija o morskim bentoskim

dijatomejama, koje su uglavnom bile usmjerene na istraživanje taksonomije i ekologije perifitona u hrvatskim estuarijima i albanskim slanim močvarama. Morske planktonske i bentoske dijatomeje do sada nisu nikada istraživane u Bosni i Hercegovini. Ova studija bila je dio većeg istraživačkog programa s ciljem utvrđivanja bioraznolikosti te funkcioniranja ekosustava u pograničnim obalnim vodama između Hrvatske i Bosne i Hercegovine. Program je uključivao algološka, zoološka i hidrografska istraživanja. U radu se prikazuju taksonomski sastav i sezonalnost morskih bentoskih dijatomeja u odnosu na osnovne ekološke parametre (temperatura, salinitet, gustoća) u Neumskom zaljevu u Bosni i Hercegovini.

Ključne riječi: Neumski zaljev, bentos, dijatomeje, istočni Jadran, SI Sredozemlje

TAXONOMIC COMPOSITION OF MARINE BENTHIC DIATOMS IN THE RELATION TO SOME KEY ENVIRONMENTAL VARIABLES IN BOSNIA AND HERZEGOVINA

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In the eastern Adriatic, there are only a few studies on benthic diatoms mostly focused on ecology and taxonomy of periphytic diatoms in the Croatian estuaries or Albanian coastal wetlands. Until now, both planktonic and benthic marine diatoms have never been studied in Bosnia and Herzegovina. This study forms part of a research program investigating the biodiversity and ecosystem functioning in the border marine coastal waters between the two countries – Croatia and Bosnia and Herzegovina. The program includes phycological, zoological and hydrographical research. This paper considers the taxonomic composition and seasonality of marine benthic diatoms in the relation to some key environmental variables (temperature, salinity and density) in the Bay of Neum in Bosnia and Herzegovina on the south-eastern Adriatic coast.

Key words: Neum Bay, benthic diatoms, Adriatic Sea, SE Mediterranean

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RASPROSTRANJENOST I PROCJENA VELIČINE POPULACIJE VIDRE (*Lutra lutra* L.) U ALPSKOJ BIOGEOGRAFSKOJ REGIJI HRVATSKE

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Postojeći podaci o rasprostranjenosti vidre u alpskoj biogeografskoj regiji Hrvatske bili su zastarjeli ili nepotpuni. Stoga je ovim istraživanjem analizirana prisutnost vidre u Gorskom Kotaru, Lici i dijelu Dalmacije. Korištena je Standardna metoda, preporučena od IUCN/SSC Otter Specialist Group. Prije terenskog dijela istraživanja, prethodno su procijenjena sva potencijalno pogodna staništa za vidru te su, pomoću topografskih karata i GIS aplikacija,

određene koordinate lokaliteta istraživanja. Terensko istraživanje je provedeno u listopadu i studenom 2013. godine. Kao znakovi prisutnosti vidre, dokumentirani su izmeti, želatinozne izlučevine, otisci stopala, ostaci hranjenja, staze, skloništa i brlozi. Ukupno je planirano istraživanje na 100 lokaliteta, no dio lokaliteta (23) nije bilo moguće istražiti zbog njihove nedostupnosti ili zbog opasnosti od mina. Utvrđena je prisutnost vidre na 25 lokaliteta, s ukupno 74 dokumentirana znaka njene prisutnosti. Ovim su istraživanjem potvrđeni stariji podaci o nestanku vidre iz sljevova Gacke i Cetine, te gornjeg toka rijeke Kupe. Prisutnost vidre je ponovno potvrđena u slijevu rijeke Like te u lovinačko-gračačkim tekućicama, Otuči i Ričici. Dobivena su nova saznanja o prisutnosti vrste u manjim krškim vodotocima u okolici Korenice, u potoku Sušik kod mjesta Drežnica, te u rijeci Butižnici. Veličina populacije vidre na istraženom području je procijenjena na 50 do 70 jedinki.

Ključne riječi: Gorski Kotar, Lika, Dalmacija, standardna metoda

DISTRIBUTION AND ESTIMATED POPULATION SIZE OF THE OTTER (*Lutra lutra* L.) IN THE ALPINE BIOGEOGRAPHICAL REGION OF CROATIA

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The data on distribution of the otter in the Alpine biogeographical region of Croatia were outdated or incomplete. Therefore, the aim of this study was to investigate the presence of otter in Gorski Kotar, Lika and a part of Dalmatia. The Standard method was used, as recommended by the IUCN/SSC Otter Specialist Group. Before the field survey, potentially suitable habitats for the otter were estimated, and the coordinates of the survey locations were determined using topographic maps and GIS applications. Field research was conducted in October and November 2013. The spraints, jellies, footprints, feeding remains, paths, hovers and holts were documented as signs of the otter presence. In total, 100 locations were planned to be surveyed, but a large part of them (23) were not investigated due to their inaccessibility or mine danger. The presence of otter was recorded at 25 localities, with a total of 74 signs of the otter presence. This research confirmed previous data on the species disappearance from the basins of Gacka and Cetina rivers, and from the upper Kupa River. The presence of otter was again confirmed in the Lika, Otuča and Ričica rivers. New data on the otter presence were recorded on small watercourses near Korenica, the Sušik Stream near Drežnica, and in the Butižnica River. The estimated otter population size is between 50 and 70 individuals.

Key words: Otter, Gorski Kotar, Lika, Dalmatia, Standard method

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BIOLOŠKO-EKOLOŠKA OBILJEŽJA ALUVIJALNIH POTOKA MEĐIMURJA

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Dosadašnja istraživanja aluvijalnih tekućica na području Međimurja su bila vrlo nedostatna. Ova istraživanja su prva sustavna istraživanja i to na po dvije postaje na potocima Boščak (BO) i Bistrec (BI), a uključivala su hidrološka, fizikalno-kemijska i faunistička obilježja. Potok Bistrec je veći, znatno bogatiji vodom, prosječan godišnji protok na postaji BI2 iznosi 2,3 m^3/sek, a na postaji BO2 tek 0,33 m^3/sek. Znatne razlike postoje i u fizikalno-kemijskim obilježjima. Osobito je značajna razlika u elektrovodljivosti koja u potoku Bistrecu iznosi 677-777 µS/cm, što je posljedica znatno većih vrijednosti Ca, Mg i sulfata te nešto viših vrijednosti Ni, Pb i nitrita. U potoku Boščaku vrijednosti elektrovodljivosti su znatno niže (288-382 µS/cm) uz nešto više vrijednosti pokazatelja organskog onečišćenja: KPK, Cr, nitrata, ukupnog N i ortofosfata, vjerojatno kao posljedica intenzivne poljoprivrede u slivu. Ukupno je utvrđena prisutnost 66 svojti makrozoobentosa. Značajnije razlike raznolikosti zajednica između istraživanih potoka nisu utvrđene. Na svim postajama izrazito je najbrojnija vrsta *Gammarus roeseli*. Jedino odstupanje je na postaji BI2 na kojoj je zabilježena najmanja brojnost makrozoobentosa (1092 jed./m^2) uz značajni udio predstavnika skupine Gastropoda, koji na ostalim postajama dolaze sporadično. Na ostalim postajama brojnost makrozoobentosa je od 4100 do 5765 jed./m^2. Prema Uredbi o standardu kakvoće voda temeljem makrozoobentosa utvrđena je dobra ekološka kakvoća.

Ključne riječi: aluvijalni potoci, biološko-ekološka obilježja, makrozoobentos, Međimurje

BIOLOGICAL-ECOLOGICAL FEATURES OF ALLUVIAL STREAMS OF THE REGION OF MEĐIMURJE

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Earlier studies of alluvial streams in the Region of Međimurje had been very insufficient so these current studies presented the first systematic research. The studies included hydrological, physical-chemical and faunistic characteristics of two streams. The Bistrec (BI) is larger and much richer in water than Boščak (BO). The average annual flow at the station BI2 was 2.3 m³/sec, and at the station BO2 it was only 0.33 m³/sec. Particularly significant is the difference in electrical conductivity, which in the stream of Bistrec was 677-777 µS/cm, and it is a result of much higher values of Ca, Mg and sulfate and a slightly higher values of Ni, Pb and nitrite. In the case of the creek of Boščak, the values of electrical conductivity were significantly lower (288-382 µS/cm) with slighltly higher values of the indicators of organic pollution: COD, Cr, nitrate, total nitrogen and orthophosphate, probably a consequence of intensive agriculture in the basin. The total presence of 66 species of macrozoobenthos have been found. At all stations the most numerous species was *Gammarus roeseli* (Amphipoda). The only exception was the station BI2 where the smallest number of macrozoobenthos have been registered (1092 Eq./m²), with a significant share of representatives of the Gastropoda groups, which at other stations come sporadically. At other stations the number of macrozoobenthos was in the range of 4100-5765 units/m². According to the Regulation on water quality standards, it is good ecological quality.

Key words: alluvial streams, biological-ecological features, macrozoobenthos, Međimurje

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VERTIKALNA RASPROSTRANJENOST, FAUNISTIČKE I BIOGEOGRAFSKE ZNAČAJKE DANJIH LEPTIRA (INSECTA, LEPIDOPTERA) PARKA PRIRODE UČKA

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Istraživanja danjih leptira (Insecta, Lepidoptera) Parka prirode Učka obuhvaćaju planinski masiv Učku i dio Čičarije a provedena su u razdoblju od 2004. do 2010. godine. Terensko istraživanje obuhvatilo je 23 lokalitet s različitim ekološkim, geografskim, pedološkim, vegetacijskim i hidrološkim značajkama. Leptiri su sakupljani od travnja do listopada, preparirani u laboratoriju te determinirani standardnim ključevima za danje leptire do nivoa vrste. Dio vrsta zabilježen je fotografiranjem na različitim lokalitetima ili determiniran je prilikom opažaja na terenu. Ovim istraživanjima utvrđeno je 90-tak vrsta danjih leptira iz svih 5 europskih porodica. Među najzanimljivijim vrstama utvrđenih tijekom ovih istraživanja ističu se: uskršnji leptir (*Zerynthia polyxena*), crni apolon (*Parnassius mnemosyne*), kupusov bijelac (*Pieris brassicae*), kleopatra (*Gonepteryx cleopatra*), močvarni plavac (*Maculinea alcon* – f. *rebeli*), žednjakov plavac (*Scolitantides orion*), trpučeva riđa (*Melitaea cinxia*), močvarna riđa (*Euphydryas aurina*), lažni sivac (*Arenthusana arethusa*), močvarni debeloglavac (*Heteropterus morpheus*). Biogeografskom analizom utvrđena je dominacija eurosibirskih vrsta te znatno manje mediteranskih vrsta. Fauna danjih leptira Učke uspoređena je s faunom danjih leptira: doline gornjeg toka rijeke Kupe, Biokova, Velebita, Dinare i Poštaka.

Ključne riječi: Učka, danji leptiri, vertikalna rasprostranjenost, fauna

VERTICAL DISTRIBUTION, FAUNAL AND BIOGEOGRAPHICAL FEATURES OF BUTTERFLIES (INSECTA, LEPIDOPTERA) FROM THE UČKA NATURE PARK

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Investigation of butterflies (Insecta, Lepidoptera) from the Učka Nature Park included habitats on the Učka and Čićarija mountains. Field work was conducted between 2004. and 2010., and it included twenty-three localities with different ecological, geographical, pedological and hydrological characteristics. Butterfly specimens were collected from April to October period, and determination was done in the laboratory according standard keys. Some species were

established with photo-data identification or determined directly in the field filed. In our investigation we established about 90 butterfly species. Especially interesting findings include: Southern Festoon (*Zerynthia polyxena*), Clouded Apollo (*Parnassius mnemosyne*), Large White (*Pieris brassicae*), Cleopatra (*Gonepteryx cleopatra*), Alcon Blue (*Maculinea alcon*), Chequered Blue (*Scolitantides orion*), Glanville Fritillary (*Melitaea cinxia*), Marsh Fritillary (*Euphydryas aurina*), False Grayling (*Arenthusana arethusa*), Large Chequered Skipper (*Heteropterus morpheus*). Biogeographical analyses of butterfly fauna in the Učka Nature Park showed domination of the Euro-Asian species, with smaller percentage of Mediterranean species. A comparison was also made between the fauna established in the Učka Nature Park, and upper part of the Kupa River, Biokovo, Dinara, Poštak and Velebit mountains areas.

Key words: Učka, butterflies, vertical distribution, fauna

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SEZONSKE RAZLIKE U UNOSU, SKLADIŠTENJU HRANE I TEŽINI DINARSKOG VOLUHARA (*Dinaromys bogdanovi*) U ZATOČENIŠTVU

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Zbog adekvatnog održavanja populacije u zatočeništvu, kao i budućih konzervacijskih mjera ili istraživanja biologije vrste, bilo je potrebno pribaviti podatke o prehrambenim potrebama dinarskog voluhara (*Dinaromys bogdanovi*). Stoga smo proveli jednogodišnje istraživanje unosa hrane te proizveli prvi popis prehrane ove vrste u zatočeništvu. Voluhari su prihvaćali širok raspon prehrane, uglavnom herbivorne, konzumirali su u prosjeku 41,27 g svježe mase po danu što iznosi 6,64 g suhe mase (0,54 g svježe mase i 0,088 g suhe mase po gramu tjelesne mase) te spremali u prosjeku 16,91 g svježe mase. Dnevna konzumacija svježe hrane po životinji značajno se razlikovala među sezonama. Životinje su konzumirale više hrane tijekom ljeta i jeseni u odnosu na zimu i proljeće. Također, postojale su i sezonske razlike u spremanju hrane. Tijekom proljeća spremano je značajno manje hrane u odnosu na zimu, tijekom ljeta značajno manje u odnosu na zimu. Životinje nisu imale konstantnu tjelesnu masu kroz godinu, bila je značajno manja tijekom zime i proljeća u odnosu na ljeto i jesen.

Ključne riječi: boljojed, prehrana, konzervacija rijetkih vrsta, biologija voluharica

SEASONAL DIFFERENCES IN FOOD INTAKE, CACHING AND BODY MASS OF THE ENDEMIC BALKAN SNOW VOLE (*Dinaromys bogdanovi*) IN CAPTIVITY

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For adequate maintenance of captive population, as well as for future conservation efforts or studies of species biology, it was necessary to obtain diet demands for Balkan snow vole

(*Dinaromys bogdanovi*). Therefore, we conducted one year monitoring of food intake of captive individuals of Balkan snow vole and provided the first list of food items eaten by this species in captivity. Voles accepted a wide array of food items, proven to be mainly herbivorous and consumed on average 41.27 g of food per day which corresponds to 6.64 g of dry mass (0.54 g of wet mass and 0.088 g of dry mass per gram of their body mass) and cached on average 16.91 g of wet mass of food. Daily consumption of wet mass per animal was significantly different between seasons. Animals consumed more food in summer and autumn than in winter and spring. There were also seasonal differences in food caching with amount of cached food significantly different in all seasons. In spring there was significantly lower amount of food cached then in winter; in summer, significantly lower than autumn and winter. Animals did not have constant body mass throughout the year; it was significantly lower in winter and spring than in summer and autumn.

Key words: herbivore, nutrition, rare species conservation, vole biology

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FITOBENTOS I VODENI MAKROFITI KAO ELEMENTI BIOLOŠKE KVALITETE I KLASIFIKACIJE STANIŠTA DUŽ SUBPLANINSKE KRŠKE RIJEKE

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Sastav i brojnost vodene flore (fitoplankton, fitobentos, makrofita) jedan je od 3 biološka elementa za utvrđivanje ekološkog stanja rijeke prema Okvirnoj direktivi o vodama, a uz nju je važna i kategorizacija staništa prema Nacionalnoj klasifikaciji staništa (NKS). Cilj istraživanja bio je opisati fitobentos te mjesta uzorkovanja prema NKS longitudinalno duž toka krške rijeke Like, jezera Krušćica te manjih tokova u širem području kroz dva vremenska perioda (svibanj i kolovoz 2014.). Uz to, istraživan je sastav zajednica te je kreiran popis svojti fitobentosa i vodenih makrofita. Svakoj postaji, na terenu s obzirom na vegetaciju uz rijeku/jezero, dodijeljen je kôd prema NKS. Na istraživanom području ukupno je zabilježeno 20 svojti vodenih makrofita koje su se koristile u karakterizaciji 9 različitih vodenih staništa na ukupno 11 obrađenih postaja. Potok kod mjesta Sitvuk pokazao se vegetacijski najzanimljivijom postajom sa dominantnom svojtom *Gallium* sp. te svojtama *Scirpus lacustris*, *Lysmiachia nummularia* i *Equisetum palustre*. U bentosu je ukupno zabilježeno 113 svojti. Najučestalije vrste bile su *Achnanthidium minutissimum* (17 uzoraka) i *Fragilaria capucina* (16 uzoraka) dok ih je 58 zabilježeno samo u pojedinim uzorcima. Trofički indeks dijatomeja varirao je između 1,7 do 2,5 što je ocijenjeno kao umjereno do dobro ekološko stanje vodenog tijela.

Ključne riječi: fitobentos, vodeni makrofiti, Okvirna direktiva o vodama, Nacionalna klasifikacija staništa

PHYTOBENTHOS AND AQUATIC VEGETATION AS BIOLOGICAL QUALITY AND HABITAT DESCRIPTION ELEMENTS ALONG SUBMOUNTAIN CARSTIC RIVER

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Composition and abundance of aquatic flora (phytoplankton, phytobenthos, macrophytes) is one of three biological elements for determining the ecological status of rivers according to the Water Framework Directive, and with it, National Habitat Classification (NHC) is important for categorization of habitats. The main objective of this study was to describe the phytobenthos and sampling sites according to the NHC along the river Lika, lake Kruščica and tributaries in the wider area at the two instances (May and August 2014). With the main objective, accompanying objectives were generated: comparison of the composition and creation of a taxonomical list of aquatic macrophytes and phytobenthos. In this investigation we used Croatian Trophic Diatom Index (CTDI) as potentially the best assemblage index for phytobenthos community. Each sampling site was characterized by the NHC. A total of 20 taxa of aquatic macrophytes were used in the characterization of nine different water habitats on a total of 11 stations processed. Tributary near the village Sitvuk was the most interesting with a dominant taxon *Gallium* sp. and species *Scirpus lacustris*, *Lysmiachia nummularia* and *Equisetum palustre*. A total of 113 taxa was recorded in benthic samples. The most common species were *Achnanthidium minutissimum* (17 samples) and *Fragilaria capucina* (16 samples) while 58 were recorded only for individual samples. CTDI was in the range from 1.7 to 2.5 indicating good to moderate conditions.

Key words: phytobenthos, aquatic flora, Water Framework Directive, National Habitat Classification

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ZNAČAKE IHTIOFAUNE RIJEKE SUTLE

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Rijeka Sutla istraživana je standardnom metodom elektroribolova na pet postaja nizvodno od ulaska rijeke Sutle u Hrvatsku do ušća u Savu. Ukupno je ulovljena 41 vrsta ribe. Porodica Cyprinidae najbrojnija je vrstama i ukupnim brojem ulovljenih jedinki. Najbrojnija vrsta na čitavom području istraživanja je dvoprugasta uklija, a brojnošću je slijede bezribica, plotica i klen. Od 33 zabilježene vrste 29 je autohtono, a 4 vrste su alohtone. Shannon-Wienerov indeks na svim istraženim postajama zajedno iznosi 3,96. Struktura ukupne zajednice opisana je i modelom logaritamske serije s parametrima α=5,3609; SE=0,8456 i x=0,9982. Od 33 zabilježene vrste tijekom istraživanja pet ih je prisutno na svih pet postaja, tj. duž gotovo čitavog toka rijeke, šest je vrsta prisutno na četiri postaje, sedam na tri postaje, šest na dvije postaje, a čak devet na samo jednoj postaji. Dominantna temperaturna grupa prema broju vrsta u zajednici riba rijeke Sutle je toplovodna grupa koja čini 54% svih vrsta. Posebno su zanimljive vrste koje smatramo rijetkima na području čitave Hrvatske poput Keslerove krkuše (*Romanogobio kessleri*), tankorepe krkuše (*Romanogobio uranoscopus*), malog vretenca (*Zingel streber*) i dunavske paklare (*Eudontomyzon vladykovi*). Zbog njih, kao i zbog gavčice (*Rhodeus amarus*), potočne mrene (*Barbus balcanicus*) i peša (*Cottus gobio*), rijeka je Sutla predložena kao jedno od značajnih Natura 2000 područja. Rijeka Sutla prema bioprodukciji spada u srednje produktivne ribolovne vode.

Ključne riječi: raznolikost riba, Natura 2000

FISH DIVERSITY OF THE RIVER SUTLA

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The river Sutla was investigated by the standard method of electrofishing on five stations, from the entrance of the river in Croatia to the mouth of the river. A total of 33 species of fish were recorded. The Cyprinidae family was the richest in species as well as total number of individuals caught. The most abundant species in the entire area of investigation was the Schneider and is followed by the Ston moroko, Danube roach and Chub. Of the 33 species recorded, 29 are indigenous and 4 species are allochthonous. The Shannon-Wiener index of all investigated stations combined was 3.96. The structure of the total community is described by a logarythmic series model with α=5.3609; SE=0.8456 and x=0.9982. Out of the 33 species, five were present on all five stations, six were present on four stations, seven on three stations, six on two stations, and nine on only one station. Ecologically, the warm-water group is dominant (54% of species). The Sutla river has species that we consider rare throughout the entire area of Croatia, as the Kessler's gudgeon (*Romanogobio kessleri*), Danube gudgeon (*Romanogobio uranoscopus*), Streber (*Zingel streber*) and the Danube lamprey (*Eudontomyzon vladykovi*). Due to these species, as well as the European bitterling (*Rhodeus amarus*) (*Barbus balcanicus*) and Bullhead (*Cottus gobio*), the river Sutla has been selected as a Natura 2000 area. According to its bioproduction, the river Sutla belongs to the category of medium productive fishing waters.

Key words: Fish community, Fish ecology, Natura 2000

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POČETNA ISTRAŽIVANJA VASKULARNE FLORE I STANIŠTA UZ RIJEKU MURU U HRVATSKOJ

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Tijekom razdoblja od travnja do rujna 2014., provedeno je botaničko istraživanje duž donjeg dijela rijeke Mure u Hrvatskoj. Istraživanje je započelo od sjeveroistočne okolice naselja Dekanovec, a završilo prije ušća rijeke Mure u Dravu blizu Legrada. Sva obrađena područja u ovom istraživanju nalazila su se između rijeke Mure i nasipa koji služi obrani od poplavnih voda. Ukupno je odabrano 19 interesnih područja kojima se nastojalo dobiti uvid u biljnu i stanišnu raznolikost. Obuhvaćene su mrtvice i močvare, zatim rukavci, jezera i dodirne šumske površine, a posjećivani su u nekoliko navrata tijekom vegetacijske sezone. Istraživanjem su zabilježene ukupno 94 biljne svojte te niz vrijednih stanišnih tipova koji prate nacionalnu klasifikaciju staništa (NKS). Analiza rezultata pokazuje da je 23 biljnih vrsta zaštićeno zakonom (15 zaštićenih, 8 strogo zaštićenih), a 10 vrsta posjeduje stanoviti status Crvene liste (1 ugrožena, 4 osjetljive, 3 gotovo ugrožene, 2 nedovoljno poznate). Valja naglasiti kako su određena područja posebno bogata biljnim vrstama, primjerice mrtvice Fučička i Gombakut. Vrste kao što su *Hottonia palustris*, *Stratiotes aloides* ili *Eleocharis quinqueflora* iznimno su rijetke. Istraživanje preostalog gornjeg dijela uz rijeku Muru trenutno je u provedbi.

Ključne riječi: Hrvatska, močvarno područje, rijeka Mura, staništa, vaskularna flora

INITIAL RESEARCH OF VASCULAR FLORA AND HABITATS ALONG THE MURA RIVER IN CROATIA

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During the period April-September of 2014, botanical research was conducted along the lower part of the Mura River in Croatia. The survey started in the northeast vicinity of the village Dekanovec and concluded just before the Mura-Drava confluence near Legrad. All surveyed areas in this research were located between the Mura River and the embankment that prevents flooding of the adjoining countryside. In order to gain insight into plant and habitat diversity, 19 areas of interest were selected. These consisted of oxbow lakes and marshes, followed by river forks, ponds and neighbouring woodland, which were visited on multiple occasions during the season. In this research, a total of 94 plant taxa were recorded along with a variety of valuable habitat types according to the national habitat classification (NKS). The analysis of the results shows that 23 plant species are protected by the law (15 protected, 8 strictly protected) and 10 species have a certain Red List status (1 endangered, 4 vulnerable, 3 near threatened, 2 data deficient). It is worth noting that certain areas, such as the oxbow lakes Fučička and Gombakut, were quite rich in plant species. Species such as *Hottonia palustris*, *Stratiotes aloides* or *Eleocharis quinqueflora* are particularly rare. The research of the remaining upper part along the Mura River is currently in progress.

Key words: Croatia, Mura River, habitats, vascular flora, wetlands

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STANIŠTA SLATKOVODNIH MEDUZA (*Craspedacusta sowerbyi* LANKESTER 1880.) NA PODRUČJU DONJEG MEĐIMURJA

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Istraživana je prisutnost slatkovodnih meduza (*Craspedacusta sowerbyi* Lankester 1880.) u vodenim ekosustavima donjeg Međimurja. Određivan je tip sedimenta dna, sastav ihtiofaune i fizikalna svojstva vode (pH, električna vodljivost i temperatura) na Perutnici (mrtvica rijeke Drave kraj naselja Otok) i grabi šljunčari kraj naselja Kotoriba. Na navedenim staništima prikupljene su jedinke meduznog oblika u površinskom sloju i na dubini 1,2 m. Terensko prikupljanje podataka je obavljeno od 12. srpnja do 17. listopada 2014. Na svakoj je istraživačkoj lokaciji prikupljan živi materijal osam puta (svaka dva tjedna). Prikupljeni uzorci su testirani na predatore, određivana je varijabilnost promjera meduznog oblika te broj jedinki slatkovodnih meduza u određenom volumenu vode svaka dva tjedna. Na obje lokacije je prikupljeno ukupno 1014 jedinki meduznog oblika. Na mrtvici Perutnici je prikupljeno 932 jedinke, a na šljunčari kraj Kotoribe svega 98 jedinki. Veći broj jedinki na obje lokacije u vremenu istraživanja je na dubini od 1,2 m, a kako se intenzitet svjetlosti smanjuje, jedinke se kreću sve više prema površini. Varijabilnost promjera tijela meduznog oblika povećava se tijekom istraživanja zbog rasta. Zabilježeni promjer je u rasponu od 0,8 cm do 2,5 cm. Istraživanjem je ustanovljeno da je jedini mogući testirani predator slatkovodne meduze riječni rak.

Ključne riječi: slatkovodna meduza, mrtvice, šljunčare, predatori

FRESHWATER JELLYFISH HABITATS IN LOWER MEĐIMURJE (*Craspedacusta sowerbyi* LANKESTER 1880)

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The topic of the research was the presence of freshwater jellyfish (*Craspedacusta sowerbyi* Lankester 1880) in the waters of lower Međimurje. The type of bottom sediment, ichtyofauna and water's physical properties (pH, electric conductivity and temperature) were determined in Perutnica (Drava's dead end near Otok) and gravel pit near Kotoriba. Units of jellyfish-like animals have been gathered at the mentioned habitats at the surface layer and at the depth od 1.2 meters. Field data gathering was done from 12th July to 17th October 2014. Alive material was gathered eight times at every research location (every 2 weeks). Collected samples were tested for predators, the variability of the diameter of jellyfish-like animals was determined, as well as the number of units of freshwater jellyfish in a certain water volume every two weeks. 1014 jellyfish-like animals were gathered at both locations, 923 in Perutnica and only 98 near Kotoriba. A larger number of units at both locations at the time of research was at the depth of 1.2 meters, and as the light intesity decreases, animals move up towards the surface. Variability of the body's diameter is increased during research due to growth and it varies from 0.8 to 2.5 centimeters. The research showed that the only possible tested predator of freshwater jellyfish is freshwater crayfish.

Key words: freshwater jellyfish, gravel, backwaters, predator

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QUALITATIVE ANALYSIS OF REPRESENTATIVES FROM ANNELIDA (OLIGOCHAETA AND HIRUDINEA) FROM KUMANOVO BATH

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From our research in the area of Kumanovo Bath, after conducting qualitative analysis of samples collected in 4 locations of Kumanovo Bath, we determinated 11 species of the type *Annelida* that belong to the classes *Oligochaeta* and *Hirudinea*. Based on our results, 5 species belong to the class of *Oligochaeta* and 6 to the class of *Hirudinea.* From the class of *Oligochaeta*, following species were determinated: *Stylaria lacustris, Tubifex tubifex, Limnodrilus hoffmeisteri, L. udekemianus* that belong to the family *Tubificidae* and *Haplotaxis gordioides* that belongs to the family *Haplotaxidae*. From the class of *Hirudinea* we determinated following species: *Piscicola geometra* that belongs to the family *Piscicolidae, Glossiphonia complanata* from the family *Glossiphonidae, Haemopis sangusuga* from the family *Haemopidae*, *Hirudo medicinalis* from the family *Hirudidae, Erpobdella octoculata* and *Dina sp*. from the family *Erpobdeliidae.* Dominant species from the class of *Oligochaeta* are *Limnodrilus hoffmeisteri* and *Haplotaxis gordioides,* and from the class of *Hirudinea* are *Hirudo medicinalis* and *Erpobdella octoculata*.

Key words: Kumanovo bath, class Oligochaeta, class Hirudinea, species

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VERTIKALNA DISTRIBUCIJA MORFOLOŠKIH OBLIKA VRSTE *Cylindrospermopsis raciborskii* U POPLAVNOM JEZERU

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Cijanobakterija *Cylindrospermopsis raciborski* (Woloszynska) Seenaya et Subba Raju je toksična, invazivna, tropska do suptropska vrsta koja se raširila u mnogim slatkovodnim ekološkim sustavima umjerenih područja. Cilj ovog rada bio je utvrditi vertikalnu distribuciju i morfološke promjene vrste *C. raciborskii* u ljetnom razdoblju 2011. godine u Sakadaškom jezeru (Park prirode Kopački rit). Uzorci su prikupljeni na površini te na svakih 1 m dubine od površine do dna jezera. Početak razvoja *C. raciborskii* utvrđen je u kolovozu (1,21 x 10^6 jed./L) kada je zabilježen samo jedan morfološki oblik, i to primarni filament. U rujnu se vrsta masovno razvila (27,81 x 10^6 jed./L), te je uz primarni utvrđeno i šest morfoloških oblika sekundarnog filamenta. Morfološki oblici međusobno su se razlikovali prema veličini (dužini i širini) filamenta i akineta. Trihomi s jednom heterocitom su bili najzastupljeniji sekundarni filamenti u cijelom vertikalnom stupcu vode, i činili su od 59,01% (u pridnenom sloju) do 84,85% (u površinskom sloju) ukupnog broja jedinki *C. raciborskii*. Kontinuirani monitoring ove invazivne vrste će pridonijeti kontroli širenja te definiranju mjera zaštite voda poplavnog područja i bioraznolikosti Kopačkog rita, a ujedno i drugih slatkovodnih ekoloških sustava srednje i južne Europe.

Ključne riječi: invazivne vrste, primarni i sekundarni filamenti, trihomi, akinete, heterocite

VERTICAL DISTRIBUTION OF MORPHOLOGICAL FORMS OF *Cylindrospermopsis raciborskii* IN A FLOODPLAIN LAKE

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The cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya et Subba Raju is a toxic, invasive, originally tropical to subtropical species, which has spread in many freshwater ecosystems of temperate regions. The aim of this study was to determine the vertical distribution and morphological changes of *C. raciborskii* in summer of 2011 in Lake Sakadaš (Kopački rit Nature Park). Samples were collected at the surface and at every meter depth to the lake bottom. At the beginning of the development (in August), the presence of only one morphological form (primary filament) of *C. raciborskii* was recorded (1.21 x 10^6 ind./L). In September, the massive development of this species was found (27.81 x 10^6 ind./L). Together with the primary filament, there were six other morphological forms of secondary filament. The morphological forms differentiated according to t he size (length and width) of filament as well as akinete size. Trichomes with one heterocyte were the most represented secondary filaments in the whole water column. Their presence was between 59.01% (in the bottom layer) to 84.85% (in the surface layer) of the total number of *C. raciborskii* individuals. Continuous monitoring of this invasive species will contribute to spread control and promote water protection measures and biodiversity of Kopački Rit floodplain, as well as other freshwater ecosystems of central and southern Europe.

Key words: invasive species, primary and secondary filaments, trichomes, akinetes, heterocytes

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ANTROPOGENI UTJECAJ NA RAZNOLIKOST BENTIČKIH BESKRALJEŽNJAKA SREDNJEG TOKA RIJEKE DRAVE I PRIPADAJUĆIH ŠLJUNČARA

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Makrozoobentos čine životinje koje žive na dnu slatkovodnih staništa koji se često koriste kao bioindikatori. Tako su primarni ciljevi istraživanja bili utvrditi sastav životinjskog svijeta dna u različitim tokovima rijeke Drave i pripadajućih šljunčara. Nadalje, utvrditi i utjecaj temperature vode i otopljenog kisika zajedno s antropogenim utjecajem na zajednicu makrozoobentosa. Istraživanje je provedeno na osam postaja na rijeci Dravi i pet pripadajućih šljunčara. Uzorkovanje je svaka dva mjeseca obavljano na rijeci Dravi od travnja do prosinca 2012., a na šljunčarama od srpnja 2013. do ožujka 2014. godine. Analizom odnosa zajednice makrozoobentosa i fizikalno-kemijskih pokazatelja vode utvrđena je značajna korelacija skupina Hirudinea, Copepoda i Coleoptera s temperaturom vode i količinom otopljenog kisika. Također je utvrđena veća brojnost indikatorskih skupina čistih voda Turbellaria, Hirudinea i Plecoptera na postajama gdje je bio najmanji antropogeni utjecaj. Na postajama gdje je kanalizirana rijeka Drava ili okružena poljoprivrednim zemljištima dominiraju skupine Copepoda i Diptera. Kod šljunčara koje su pod jakim antropogenim utjecajem prikupljene su skupine koje podnose veća organska onečišćenja poput trzalaca i vodenih grinja. S druge su strane u šljunčarama bez izraženog antropogenog utjecaja dominirali vodencvjetovi. Zaključno, makrozoobentos rijeke Drave, kao i okolnih šljunčara je pokazao visok stupanj bioraznolikosti, ali i osjetljivosti na različite antropogene utjecaje, što ukazuje na važnost očuvanja ovog područja.

Ključne riječi: rijeka Drava, šljunčare, makrozoobentos, bioraznolikost, osjetljivost

ANTHROPOGENIC IMPACT ON BENTHIC MACROINVERTEBRATE DIVERSITY IN THE MIDDLE REACH OF DRAVA RIVER AND THE ACCOMPANYING GRAVEL PITS

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As benthic invertebrates inhabit all types of freshwater habitats often are used as bioindicators, especially in areas of a special interest like Drava River. Therefore, the primary objective of this study was to determine the composition of macrozoobenthos in different sites of Drava River and the accompanying gravel pits. Secondly, to determine the impact of water temperature and dissolved oxygen together with anthropogenic pressure on studied communities. The research was conducted at eight stations on the Drava River and at five gravel pits. Sampling in the river was performed every two months from April to December 2012, and at the gravel from July 2013 to March 2014. Analysis of the relationship of macroinvertebrates and physico-chemical parameters of water resulted in significant correlation for Hirudinea, Copepoda and Coleoptera. Also, a higher number of clean water indicators, Turbellaria, Hirudinea or Plecoptera, were identified at stations where anthropogenic pressure was the least. At stations where Drava River

is canalised or agricultural lands occur Copepoda and Diptera dominated. In gravel that were under strong anthropogenic influence dominated Chironomidae and Hydrachnaellae, unlike in gravel with natural conditions where Ephemeroptera was a dominant group. In conclusion, macrozoobenthos of Drava River and surrounding gravel has shown a high level of biodiversity, but also the sensitivity on different anthropogenic impacts, which indicates the importance of preserving this area.

Key words: Drava river, gravel pits, macrozoobenthos, biodiversity, sensitivity

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DINAMIKA NASELJAVANJA I SUKCESIJE TREPETLJIKAŠA U PERIFITONU JEZERA POPLAVNOG PODRUČJA

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Istraživanje promjena u strukturi zajednice trepetljikaša u perifitonu razvijenom na umjetnim podlogama (predmetna stakalca) provedeno je u poplavnom području Dunava (Sakadaško jezero, Park prirode Kopački rit). Istraživanje je provedeno u razdoblju od travnja do prosinca 2009. godine. U svrhu praćenja procesa naseljavanja, uzorci su na početku istraživanja prikupljeni nakon 1., 3., 5., 7. i 14. dana ekspozicije, nakon čega je uzorkovanje provedeno svaka dva tjedna, kako bi se pratila sukcesija trepetljikaša u zajednici perifitona. Zabilježeno je ukupno 116 svojti trepetljikaša. Ustanovljene su tri faze u razvoju zajednice: inicijalna (1. – 5. dan), intermedijarna (7. – 14. dan) te zrela faza (28. – 224. dan). U inicijalnoj i intermedijarnoj fazi najznačajniji utjecaj na zajednicu imali su prozirnost i temperatura vode, pri čemu su karakteristični bili pokretni bakteriovorni i algivorni trepetljikaši. Organska tvar u perifitonu, fitoplankton i konduktivitet najviše su utjecali na zajednicu u zreloj fazi razvoja, kada su bile zastupljene različite trofičke skupine trepetljikaša, posebno sjedilački organizmi koji se hrane filtriranjem, te predatori. Na početku naseljavanja organizama na podlogu, abiotički parametri često imaju izraženiji utjecaj, dok su biotički parametri značajniji u oblikovanju već formirane zajednice perifitona.

Ključne riječi: trepetljikaši, razvoj perifitona, umjetne podloge, trofička struktura

COLONIZATION AND SUCCESSION DYNAMICS OF PERIPHYTIC CILIATES IN A FLOODPLAIN LAKE

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Changes in the community structure of ciliated Protozoa, settled in the periphyton developed on artificial substrata (glass slides) in a Danubian floodplain lake (Lake Sakadaš, Kopački Rit Nature Park), were investigated. The experiment was carried out from April to December 2009. To monitor the colonization process, the samples were collected on the 1st, 3rd, 5th, 7th and 14th day of exposure. After that, sampling was conducted biweekly, with the aim of studying succession patterns of the periphytic ciliate community. A total of 116 ciliate

taxa was recorded in periphyton during the investigation. Three phases in the development of periphytic ciliate community were observed: initial (days 1 to 5), intermediate (days 7 to 14) and mature (days 28 to 224). Water transparency and water temperature were the most important parameters influencing the initial and intermed iate phases of community development when mainly vagile bacterivorous and algivorous ciliates were present. The mature phase was most affected by periphyton organic matter, phytoplankton and conductivity, indicating food source as primary factor influencing periphytic ciliates. Ciliates with broader food spectrum characterized the community, especially sessile filter feeders and predators. It is often the case that abiotic parameters influence the initial colonization process, whiles biotic parameters can be more important for the structuring of the formed periphytic community.

Key words: ciliated Protozoa, periphyton development, artificial substrata, trophic structure

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MIKROBNI BIOFILM PERIODIČKOG SLATKOVODNOG KRŠKOG IZVORA

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Tijekom zime 2013. i proljeća 2014. godine sa izvora Krčić uzorkovani su uzorci mikrobne zajednice te fizičko-kemijski čimbenici kako bi se odredila promjena u sastavu zajednice izvora. Dijatomeje su brojane i taksonomski određene pomoću pretražnog elektronskog mikroskopa (SEM). Nakon izolacije DNA iz cijanobakterija, korištena je PCR (polimerazna lančana reakcija) metoda za amplifikaciju cijanobakterijskih 16S rRNA fragmenata kako bi se dobile čiste klonske sekvence za svaki pojedini uzorak. Usporedbom dobivenih sekvenci sa bazom podataka (GenBank) izrađena su filogenetska stabla sličnosti, pri čemu su sekvence pokazale visoki stupanj homologije (98-99%) sa 16S rRNA genima skupine Oscillatoriales (rodovi *Tychonema*, *Pseudoanabaena*, *Hydrocoleum*, *Wilmottia* te vrsta *Phormidium autumnale*). Rezultati međusobne usporedbe sekvenci pokazali su izrazito filogenetsko razdvajanje u 2 grupe: (i) "*Phormidium*", te (ii) "*Wilmottia*", sa 7-10% razlike u sastavu 16S rRNA. Uz to, grupa "*Wilmottia*", koju čini isključivo zimski uzorak, pokazala je 99% homolognost/srodnost s *Wilmottia* sp. CAWBG522 izolatom s Novog Zelanda, što predstavlja prvi nalaz tog cijanobakterijskog ribosomalnog tipa u Hrvatskoj. Također je, unatoč vrlo visokom stupnju homologije, došlo do dodatnog razdvajanja unutar samih grupa "*Phormidium*" i "*Wilmottia*" u pod-grupe s proljetnim i zimskim klonovima, što upućuje na mogućnost da cijanobakterije iz Krčića predstavljaju genotipove specifične za hrvatsku regiju.

Ključne riječi: mikrobne zajednice, krški izvor, *Phormidium*

MICROBIAL MATS IN INTERMITTENT FRESHWATER KARSTIC SPRING

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Live samples of microbial mat community, together with main ecological parameters, were taken during winter 2013 and spring 2014 in Krčić Spring. Diatoms were counted and identified using scanning electron microscopy (SEM). After DNA isolation of cyanobacterial species, PCR (polymerase chain reaction) was used for the amplification of cyanobacterial 16S rRNA fragments to obtain pure clonal sequences for each sample. The resulting sequences were compared to the database (GenBank) and based on the similarity phylogenetic trees were constructed. The sequences showed high homology (98-99%) with the 16S rRNA gene of the Oscillatoriales (genera *Tychonema*, *Pseudoanabaena*, *Hydrocoleum*, *Wilmottia* and *Phormidium autumnale*). Sequences grouped into 2 separate clusters: (i) "*Phormidium*", and (ii) "*Wilmottia*". Sequence comparison showed that there was 7-10% difference in 16S rRNA among the two clusters presented. The results indicate a distinct phylogenetic separation between the "*Phormidium*" and "*Wilmottia*" clusters. Furthermore, "*Wilmottia*" cluster originated exclusively from the sample collected in winter and showed 99% homology to *Wilmottia* sp. CAWBG522 isolate from New Zealand. Despite very high homology, sequences formed distinct sub-clusters inside "*Phormidium*" and "*Wilmottia*" cluster with spring clones being further separated from winter ones, which indicates that cyanobacteria from the karstic Krčić Spring represent genotypes specific for the Croatian region.

Key words: microbial mat, karstic spring, *Phormidium*

TOKSIKOLOGIJA I EKOTOKSIKOLOGIJA
TOXICOLOGY AND ECOTOXICOLOGY

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PROCJENA UTJECAJA OTPADNOG MULJA NA OKOLIŠ – MOGUĆI BIOMARKERI

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Povećana proizvodnja mulja u postrojenjima za pročišćavanje otpadnih voda (PPOV) zahtijeva razvitak alternativnih metoda u svrhu njihova zbrinjavanja. Budući da otpadni mulj sadrži hranjive tvari koje poboljšavaju svojstva tla, odlaganje na poljoprivrednim tlima jedna je od ekonomski najprihvatljivijih metoda zbrinjavanja. Međutim, otpadni mulj može sadržavati toksične spojeve (teške metale, lijekove i druge ksenobiotike) koji predstavljaju potencijalnu prijetnju fauni tla, a u konačnici i ljudima. Toksični potencijal ispitivan je na tekućem aktivnom mulju te mulju sa deponija (aktivni mulj odložen na za to određenim površinama tla) praćenjem histoloških promjena, promjena aktivnosti multiksenobiotičke otpornosti te mjerenjem razine lipidne peroksidacije nakon akutnog, semi-akutnog i sub-kroničnog izlaganja gujavice. Korišteni

biomarkeri ukazali su na značajne promjene na staničnoj razini i tkivnoj razini, potvrđujući kako otpadni mulj može predstavljati veliki rizik za organizme koji žive u tlu.

Ključne riječi: toksikologija tla, gujavica, mehanizam multiksenobiotičke otpornosti, histologija, lipidna peroksidacija

POTENTIAL BIOMARKERS FOR SEWAGE SLUDGE POLLUTION MONITORING

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The increased production of sewage sludge from municipal waste water treatment plants (WWTP) requires alternative methods for its final disposal. Since sewage sludge contains nutrients that enhance soil properties, land application is usually the most economic disposal alternative. However, sewage sludge may contain toxic substances (heavy metals, pharmaceuticals and other xenobiotics) that may pose a potential threat to terrestrial fauna, and consequently to humans. Toxic potential of liquid active sludge and semi-sold depot sludge (active sludge redeposited on a nearby land location) was evaluated by earthworm histopathological analysis, changes in the multixenobiotic resistance activity and lipid peroxidation analysis after acute, semi-acute and sub-chronic exposure period. Applied biomarkers demonstrated extensive alterations at the cellular and tissue levels, proving that sewage sludge can pose a serious risk for soil organisms.

Key words: soil toxicity, earthworm, multixenobiotic resistance mechanism, histopathology, lipid peroxidation

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PROCJENA TOKSIČNOSTI KOMUNALNIH OTPADNIH VODA IZ POSTROJENJA ZA PROČIŠĆAVANJE OTPADNIH VODA KORIŠTENJEM TESTA EMBRIOTOKSIČNOSTI NA ZEBRICAMA

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Embrij ribe zebrice (*Danio rerio*) sve je češće korišten model u ekotoksikološkim istraživanjima zahvaljujući kratkoći embriološkog razvoja i transparentnosti koriona. Embrij zebrice pokazao se kao koristan organizam za detekciju toksikanata, a postojeći rezultati upućuju na izvrsnu korelaciju s istraživanjima na sisavcima. Zbog navedenih razloga, toksični/teratogeni potencijal određen je izlaganjem embrija zebrica otpadnim vodama prije i nakon obrade u postrojenju za pročišćavanje otpadnih voda grada Virovitice. Postrojenje zaprima otpadne vode grada i gradske bolnice te otpadne vode šećerane. Dobiveni rezultati

potvrđuju da se embriji zebrica mogu koristiti za brzu i pouzdanu procjenu toksičnosti otpadnih voda.

Ključne riječi: embrij zebrice, otpadne vode, toksičnost, teratogenost

EVALUATION OF TOXIC POTENTIAL OF MUNICIPAL WASTE WATERS FROM WASTEWATER TREATMENT PLANT BY ZEBRAFISH EMBRYO TOXICITY TEST

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In recent years zebrafish (*Danio rerio*) embryo became frequently used model organism in ecotoxicology research. Due to their short embryonic development and transparent chorion zebrafish embryos have been shown to be a useful tool for the detection of toxic substances and obtained results correlate well with results obtained for mammals. For that reason, toxic potential of waste waters from wastewater treatment plant Virovitica that receives municipal, hospital and sugar plant wastewaters was evaluated on zebrafish embryos. By conducting fish embryo toxicity test (FET) we summarized embryotoxic/teratogenic effects and gained insight into the toxicity of pretreated and treated waste waters on aquatic organisms. Obtained results show that zebrafish embryo can be used as a valuable tool for potential toxic evaluation of waste waters.

Key words: zebrafish embryo, waste water, toxicity, teratogenic potential

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CAN HISTOPATHOLOGICAL INVESTIGATION BE A MARKER OF POLLUTION IMPACTS? A CASE STUDY OF SOME ECONOMICALLY IMPORTANT FISH SPECIES IN THE EUTHROPHIC LAKE MOGAN, CENTRAL ANATOLIA

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This study was carried out in Mogan Lake, during the breeding period of fish species between January and May 2013. Mogan Lake is an important recreational and fishing freshwater source, located in South of Ankara. The water quality parameters of: temperature, pH, dissolved oxygen, conductivity, total dissolved solids, salinity, alkalinity, bromine, copper, zinc, iron, floride, phosphate, calcium, chloride, chromium, magnesium, manganese, nitrite, nitrate, nickel, potassium, sulphate and total hardness were analyzed in Mogan Lake samples. Economic fish species of *Cyprinus carpio*, *Carassius carassius*, *Tinca tinca* and *Esox lucius* were

captured simultaneously. Gonads, gill, muscle, kidney, liver and peritonium tissues were examined histologically, and compared with a control group from an uncontaminated sampling area. Histological examination results: branchitis, hyperemia, epitelial lifting and fusion gill the secondary lamellae, hydropic degeneration and passive hyperemia in liver, hemorrhage in kidneys, oophoritis and seperation of the basal membrane on ovarium tissues, degeneration of testis tissue, peritonitis on peritonium of the intestine. The histopathology results showed them to be good biomarkers for studying and monitoring anthropogenic pollution/factors impacts on inland water systems.

Key words: Mogan Lake, ecotoxicology, histopathology, water quality

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PROCJENA TOKSIČNOSTI VODENOG EKSTRAKTA LISTA OBIČNE PLANIKE (*Arbutus unedo* L.) I ARBUTINA NA LJUDSKIM LIMFOCITIMA PERIFERNE KRVI U UVJETIMA *IN VITRO*

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Obična planika (*Arbutus unedo* L.) samonikla je vazdazelena biljka koja se tradicionalno koristi u pučkoj medicini. Mehanizmi djelovanja njenih sastavnica, osobito na staničnoj razini, još uvijek nisu u potpunosti razjašnjeni. Među brojnim fenolnim spojevima prisutnim u listu planike po ljekovitosti se izdvaja arbutin. Stoga je cilj naših istraživanja bio izmjeriti sadržaj tog fenolnog glikozida u ispitivanom ekstraktu lista planike te utvrditi koje biološke učinake ekstrakt lista planike i arbutin mogu izazvati u ljudskim stanicama. Biljni materijal prikupljen je na Malom Lošinju (5/2013.). Iz osušenih listova ekstrahirani su fenolni spojevi te je provedena kvantitativna analiza arbutina primjenom tekućinske kromatografije visoke djelotvornosti uz detekciju UV-detektorom s nizom dioda (HPLC-DAD). Ekstrakt je sadržavao 10,7 mg arbutina po gramu liofilizata. U *in vitro* pokusu na limfocitima periferne krvi istražili smo učinke arbutina i ekstrakta lista, primijenjenih u tri koncentracije: 11,4 mg/kg (odgovara najvećem dnevno prihvatljivom unosu ekstrakta u organizam), 200 mg/kg (koncentracija iznad koje su u ranijim istraživanjima uočeni toksični učinci) i 400 mg/kg. Nakon 24-satnog izlaganja limfocita procijenjen je citotoksični potencijal testiranih uzoraka (primjenom fluorescencijskog testa preživljenja); potencijal za izazivanje primarnih oštećenja DNA (primjenom komet-testa), odnosno citogenetičkih oštećenja (primjenom mikronukleus-testa). Ekstrakt lista planike i arbutin imaju niski citotoksični i genotoksični potencijal te ne izazivaju značajan porast učestalosti mikronukleusa u limfocitima. Pri istim koncentracijama, ekstrakt lista planike pokazuje veću citotoksičnost od arbutina. Međutim, arbutin, za razliku od ekstrakta, zamjetnije usporava stanični ciklus limfocita. Uočeni antiproliferacijski učinak vrijedan je daljnjih istraživanja na drugim vrstama stanica, a osobito tumorskim linijama.

Ključne riječi: citotoksičnost, HPLC, limfociti, ljekovito bilje, primarna oštećenja DNA

TOXICITY ASSESSMENT OF STRAWBERRY TREE (*Arbutus unedo* L.) WATER LEAF EXTRACT AND ARBUTIN IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES *IN VITRO*

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Strawberry tree (*Arbutus unedo* L.) is an evergreen tree commonly used in traditional medicine. The mechanisms that underlie the biological effects of its components, especially at the cellular level, have not yet been fully explained. Since arbutin constitutes the most important component of the plant's extract, our research focused mostly on this glycoside. This study aimed to determine arbutin content in strawberry tree water leaf extract, and evaluate the biological effects caused by the extract and arbutin in human cells. The plant material was collected on the island of Mali Lošinj, Croatia (5/2013). Phenolic compounds were extracted from dried leaves and extracts were subjected to quantitative analysis of arbutin using high performance liquid chromatography with UV diode array (HPLC-DAD) detection. The extract contained 10.7 mg arbutin per g of lyophilisate. Using human peripheral blood lymphocytes *in vitro*, we studied the effects of arbutin and strawberry tree water leaf extract applied at three concentrations: 11.4 mg/kg (corresponding to the maximum acceptable daily intake of the extract), 200 mg/kg (previous studies reported toxic effects above this concentration) and 400 mg/kg. After a 24-hour exposure, we evaluated the cytotoxic effects of the tested samples (using an acridine orange/ethidium bromide double staining viability assay); the potential for induction of primary DNA damage (using the alkaline comet assay), and cytogenetic damage (using the micronucleus assay) in treated and control lymphocytes. Both the strawberry tree leaf extract and arbutin had low cytotoxic and genotoxic potential. They also did not cause a significant increase in the frequency of micronuclei in the lymphocytes. At the same concentrations, strawberry tree leaf extract was slightly more cytotoxic than arbutin. However, arbutin, unlike the extract, caused delays in the lymphocyte cell cycle. The observed antiproliferative effect merits further research, which should focus on other cell types, in particular tumour cell lines.

Keywords: cytotoxicity, HPLC, lymphocytes, medicinal plants, primary DNA damage

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DETERMINATION OF ACUTE AND SUBLETHAL EFFECTS OF PERMETHRIN ON NARROW CLAWED CRAYFISH (*Astacus leptodactylus* Esch. 1823)

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In the present study, the acute and sublethal toxic effects of the synthetic pyhretroid permethrin, a toxic pollutant on aquatic systems from agricultural activities, on narrow clawed

crayfish (*Astacus leptodactylus*) were determined. The acute 96 h LC_{50} value was estimated as 0.091 (95% CI = 0.5042 -2.2734) µg/L. Static bioassay system was used in two replicates. 1/10 of 96 h LC_{50} value (0.0091 µg/L) was used as the sublethal exposure concentration and total hemocyte counts and tissue malondialdehyde (MDA) levels were determined after 48 and 96 hours. After 96 h sublethal exposure to permethrin, total hemocyte counts were significantly increased ($p < 0.05$). The MDA levels of muscle and gill tissues were significantly increased while hepatopancreas MDA levels decreased after 96 h sublethal permethrin concentrations ($p < 0.05$). In conclusion, permethrin was extremely toxic to narrow clawed crayfish, a non-target but indicator species, and affected biomarkers of health such as lipid peroxidation levels even at sublethal concentrations.

Key words: bioassay, permethrin

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CITOTOKSIČNI, GENOTOKSIČNI I CITOGENETIČKI UČINCI KLORPIRIFOSA NA LJUDSKE LIMFOCITE PERIFERNE KRVI U UVJETIMA *IN VITRO*

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Klorpirifos je jedan od najčešće korištenih organofosfornih insekticida. Zbog visoke lipofilnosti, molekula klorpirifosa lako ulazi u citoplazmu i oštećuje stanične makromolekule. U uvjetima *in vitro* istražili smo utjecaj klorpirifosa na apoptozu i nekrozu, oštećenja DNA te citogenetička oštećenja u ljudskim limfocitima periferne krvi, izlaganim 4 i 24 h koncentracijama koje odgovaraju: razini na kojoj je uočen štetan učinak, prihvatljivom dnevnom unosu te referentnoj razini izloženosti. Preživljenje stanica praćeno je primjenom testa s fluorescencijskim bojama. Primarna oštećenja DNA procijenjena su komet-testom, a citogenetička oštećenja mikronukleus-testom. Klorpirifos pri testiranim koncentracijama izaziva porast smrtnosti limfocita (najviše apoptozom), ovisno o koncentraciji i dužini izlaganja. Rezultati komet-testa upućuju na koncentracijski ovisan porast razine primarnih oštećenja DNA. Pomoću mikronukleus-testa utvrđena su značajna oštećenja kromosoma i diobenog vretena te poremećaji stanične kinetike i proliferacije limfocita. Citotoksični, genotoksični i citogenetički učinci klorpirifosa, uočeni čak i pri njegovim dopuštenim koncentracijama, pozivaju na povećani oprez, osobito jer neke namirnice mogu sadržavati visoke koncentracije ostataka ovog pesticida. Stoga je, radi smanjenja rizika od izloženosti klorpirifosu, potrebno nastaviti istraživanja s ciljem boljeg razjašnjenja mehanizama njegove toksičnosti.

Ključne riječi: toksičnost, oštećenja DNA, pesticid, ljudske krvne stanice

EVALUATION OF THE CYTOTOXIC, GENOTOXIC AND CYTOGENETIC EFFECTS OF CHLORPYRIFOS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES *IN VITRO*[*]

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Chlorpyrifos is one of the most widely used organophosphate insecticides. Due to its lipophilicity, chlorpyrifos reaches the cytoplasm easily and damages cellular macromolecules. This *in vitro*

study investigated the ability of chlorpyrifos to induce apoptosis/necrosis, DNA lesions and cytogenetic damage in human lymphocytes treated for 4 and 24 h with chlorpyrifos at doses equal to its observable effect level, acceptable daily intake, and reference exposure level. Cytotoxicity was tested using a viability assay with fluorescence dyes. Levels of primary DNA and cytogenetic damage were studied by comet assay and cytokinesis-blocked micronucleus (CBMN) assay, respectively. Following treatment, lymphocyte viability decreased in a time- and concentration-dependent manner. Apoptosis dominated over necrosis. The levels of primary DNA damage were also concentration-dependent, but longer exposure resulted in slightly lower values, both of comet tail lengths and of tail DNA. The findings of CBMN assay indicate significant DNA damage, aneugenic potential, and disturbances in cell-cycle kinetics. The fact that chlorpyrifos was, even at concentrations tolerable for human exposure, able to induce significant cytotoxic, genotoxic and cytogenetic effects calls for particular attention, since some food items often contain high residual levels of this pesticide. In this sense, further studies aimed at clarifying the mechanisms involved in chlorpyrifos toxicity to a greater extent are advised.

Key words: toxicity, DNA damage, pesticide, human blood cells

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DETERMINATION OF CELL DEATH MECHANISM ON HUMAN LUNG ADENOCARCINOMA CELLS EXPOSED TO CERANIB-2

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There is a fact that resistance to widely used chemotherapeutics occurs frequently. Thus novel agents for cancer treatment are required. In the present study we determined the cell death mechanism of ceranib-2, a ceramidase inhibitor, treated human lung adenocarcinoma cells (A549). The 50% inhibition concentration (IC_{50}) of ceranib-2 on A549 cells was determined in our previous study, and used for the treatments in the present study. The cell death analysis was realised on flow cytometry by annexin V-FITC and propidium iodide (PI) staining. The percentage of apoptotic cells was high compared to that of necrotic cells. Furthermore, the effect of ceranib-2 on the mitochondrial membrane potential of the treated A549 cells for 24 hours. Significant change on the mitochondrial membrane of A549 cells exposed to IC_{50} value of ceranib-2 for 24 hours was recorded. All experiments were realised in triplicates. In conclusion, ceranib-2 was highly cytotoxic to human lung adenocarcinoma cells in its IC_{50} concentration leading to apoptosis by changing the potential of the mitochondrial membrane.

Key words: Ceramide, cell death, ceranib-2, apoptosis, inhibitor

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UČINAK KADMIJA NA FOTOSINTEZU I SADRŽAJ SEKUNDARNIH METABOLITA U LIŠAJEVA *Parmelia sulcata*, *Evernia prunastri* I *Elavoparmelia caperata*

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Lišajevi se svrstavaju među najpoznatije bioindikatore kvalitete zraka. Utjecaj onečišćenja zraka očituje se fiziološkim promjenama, a osjetljivost lišaja razlikuje se između vrsta. S obzirom da se kadmij povezuje s onečišćenjem zraka iz prirodnih i umjetnih izvora, cilj istraživanja je bio otkriti njegov učinak na fotosintezu i sekundarne metabolite lišajeva. Količina sekundarnih metabolita često se mijenja ovisno o prisutnosti teških metala u talusu što ukazuje da ovi spojevi imaju značajnu ulogu u toleranciji na teške metale. U ovom smo radu tri vrste lišajeva (*Parmelia sulcata*, *Evernia prunastri* i *Flavoparmelia caperata*) izlagali otopini kadmija (50 µg/ml) u laboratorijskim uvjetima te smo nakon 1., 3. i 8. dana određivali učinkovitost fotosinteze i sadržaj sekundarnih metabolita. Vrijednosti koncentracija metabolita, usninske kiseline (*F. caperata*) te salazinske kiseline i atranorina (*P. sulcata*), su dan nakon izlaganja kadmiju značajno porasle, nakon tri dana su se snizile, te ponovno značajno porasle nakon osam dana izlaganja, što ukazuje na njihovu ulogu u imobilizaciji kadmija kod izlaganja u relativno kratkom trajanju. Rezultati pokazuju da su istraživane vrste relativno otporne na kadmij i da sekundarni metaboliti imaju značajnu ulogu u zaštiti primarnog metabolizma od negativnih učinaka ovog metala iako je nakon izlaganja kadmiju u trajanju od 8 dana utvrđena smanjena učinkovitost fotosinteze.

Ključne riječi: kadmij, onečišćenje, kvaliteta zraka, lišajevi, HPLC, fotosinteza

THE IMPACT OF CADMIUM ON PHOTOSYNTHESIS AND SECONDARY METABOLITES IN LICHENS *Parmelia sulcata*, *Evernia prunastri* AND *Elavoparmelia caperata*

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Lichens are one of the most common air quality bioindicators. Air pollution causes physiological changes in lichens, but sensitivity to air pollution is species specific. Since cadmium from natural and human activities is related to air pollution, the goal of our research was to reveal its impact on photosynthesis and secondary metabolite production. Secondary metabolites are sensitive to heavy metal accumulation indicating that they can have an important role in heavy metal tolerance. In this research three lichen species (*Parmelia sulcata*, *Evernia prunastri* and *Flavoparmelia caperata*) were exposed to cadmium (50 µg/ml) in laboratory conditions and after the first, third and eighth day photosynthesis efficiency and secondary metabolites content were determined. The content of the metabolites, usnic acid (*F. caperata*) and salazinic acid and atranorin (*P. sulcata*), increased a day after the cadmium exposure, then deceased after three days and increased again after eight days of exposure. This indicates the role of these metabolites in cadmium immobilization when exposure is relatively short. Results show that researched species are relatively resistant to cadmium and that secondary metabolites have significant role in

protection of primary metabolism from cadmium negative impacts although negative impact on lichen photosynthesis was recorded after eight days of the exposure.

Key words: cadmium, air pollution, air quality, lichens, HPLC, photosynthesis

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POLLUTION BIOMONITORING OF THE RIVER MORACA AND LAKE SKADAR USING CAGED MUSSELS *Unio* sp.

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The aim of this study was environmental pollution assessment of the lower flow of the river Moraca polluted by municipal and other waste waters. Assessment was done by applying biomarkers, bioassays and measuring the Biotic Index (BI). To test the genotoxic impact of pollutants on living organisms in this area, for the first time Comet assay and micronucleus test were applied on hemocytes of freshwater mussel *Unio* sp. cage exposed at 6 sites for a period of 3 weeks during summer. These methods can provide rather accurate information on level of DNA damage even if it is very low. The highest DNA damage detected by the Comet assay was at the site Collector (closest to the City of Podgorica), followed by location Grbavci, Vukovci and Vranjina. The Comet assay showed better resolution in detecting pollution at chosen locations then BI or physico-chemical water analysis. This confirms the assumption that BI and physico-chemical water analysis are insufficient for a complete assessment of pollution in aquatic ecosystems, mostly because the measurability of biological responses following the chemical contamination fall below analytical detection limits and / or after chemical exposure has finished. Moreover, the fact is that Comet assay and micronucleus test correctly pointed out the location polluted with industrial untreated wastewater justifies the introduction of these methods in the standard pollution monitoring system.

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BIOLOŠKA RAZGRADNJA ORGANSKIH TVARI U VODI-ODABIR METODE HRN EN ISO 9439:2000 ILI HRN EN ISO 10707:2000

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Biološka razgradnja je proces koji se neprestano odvija u prirodi i u kojem mikroorganizmi, prisutni u tlu ili vodi, razgrađuju organske tvari na jednostavnije molekule. Mikroorganizmima organska tvar koju razgrađuju služi kao izvor energije. Laboratorijski testovi biološke razgradnje imaju značajnu ulogu u procjeni utjecaja ispitivane tvari na okoliš. Supstance koje su biorazgradljive neće uzrokovati dugoročno štetne posljedice na okoliš, što podrazumijeva njihovu jednostavnu eliminaciju iz okoliša.Laboratorijski testovi opisuju kako možemo "izmjeriti" biorazgradivost organske tvari. Mjerenja mogu uključivati određivanje nastalog ugljik (IV)-oksida kao konačnog produkta razgradnje (HRN EN ISO 9439:2000); smanjenje koncentracije otopljenog kisika koji je potrošen u procesu razgradnje (HRN EN ISO 10707:2000), ili nekog drugog mjerljivog parametra. Konačni odabir metode biološke razgradnje (HRN EN ISO 9439:2000 ili HRN EN ISO 10707:2000) ovisi o fizikalno-kemijskim karakteristikama ispitivane tvari (topljivost u vodi,

hlapivost, svojstvo adsorpcije, toksičnost ispitivane tvari koja bi mogla inhibirati bakterijsku aktivnost i sl.), a koja je dostupna u tehničkoj dokumentaciji.

Ključne riječi: biološka razgradnja, metode, fizikalno-kemijske karakteristike

BIOLOGICAL DEGRADATION OF ORGANIC COMPOUNDS IN WATER-SELECTION OF THE METHOD HRN EN ISO 9439:2000 OR HRN EN ISO 10707:2000

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Biological degradation is process in the nature where microorganisms present in soil or water degrade organic substances to simpler molecules. Degraded organic substances serve as a source of energy for microorganisms. Biological degradation laboratory tests have significant role when estimating the influence of the substance under test to the environment. Biodegradable substances will not have long term and harmful impact on the environment, and the process of their removal from the environment is simple. Laboratory tests define methods to 'measure' the organic substance biodegradability. Measurements can include determination of carbon (IV) oxide, produced as a final degradation product; measurement of the dissolved oxygen reduction due to consumption in degradation process, or some other measurable parameter can be used. The final selection of biological degradation methods (HRN EN ISO 9439:2000 or HRN EN ISO 10707:2000) depends on chemical and physical properties of the substance (water solubility, volatility, adsorption, toxicity of test compound according to bacterial activity etc.) available in technical documentation.

Key words: biological degradation methods, physical and chemical properties

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INVESTIGATION OF CERANIB-2 INDUCED CYTOTOXICITY ON HUMAN LUNG ADENOCARCINOMA CELLS

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The incidence of cancer has been experiencing arise in recent years. Lung cancer is one of type common cases in the World. In this study we aimed to evaluate the cytotoxic and apoptotic effects of ceranib-2 on human lung adenocarcinoma cells (A549). For the cytotoxicity testing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay was used. Stock solution of ceranib-2 was prepared in dimethyl sulfoxide (DMSO) and further diluted in fresh culture medium. A549 cells were exposed to concentrations of ceranib-2 ranging from 5 to 110 µM for 24 hours. The percentages of viability and IC_{50} concentration of ceranib-2 for 24 hours were determined. The morphological alterations on A549 cells caused by IC_{50} concentration of ceranib-2 for 24 hours were intestigated on confocal microscope. For confocal microscopy, A549 cells were double stained with acridine orange and phalloidine. As a conclusion, ceranib-2 was found to be highly cytotoxic to A549 cells for 24 hours in dose dependent manner. Furthermore, ceranib-2 caused significant alterations on ceranib-2 treated

A549 cells in 24 hours. Consequently ceranib-2 inhibited the proliferation of A549 cells leading to morphological changes on the cells indicating apoptosis.

Key words: Ceranib-2, lung adenocarcinoma, cytotoxicity, sphingolipid, ceramidase inhibitors

MIKROBIOLOGIJA
MICROBIOLOGY

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BEZ NJIH SE NE MOŽE: EUKARIOTSKI ORGANIZMI - STANOVNICI AKTIVNOG MULJA

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Obrada otpadnih voda Grada Zagreba odvija se na Uređaju za pročišćavanje otpadnih voda Grada Zagreba u dva stupnja, gdje je drugi stupanj biološka obrada aktivnim muljem. Razgradnja organske tvari u biološki bazenima, koja je ključan faktor učinkovitosti pročišćavanja otpadne vode, odvija se uz pomoć kisika koji se dovodi u biološke bazene upuhivanjem atmosferskog zraka. Cilj istraživanja bio je utvrditi zastupljenost pojedinih rodova eukariotskih organizama aktivnog mulja kao i odnos prema određenim abiotičkim čimbenicima. Istraživanje je provedeno tijekom 2012. godine, određivanjem eukariotskih mikroorganizama u aktivnom mulju i njihove zastupljenosti tijekom pojedinih mjeseci. Ukupno je tijekom 2012. godine identificirano 35 rodova. Najviša biološka raznolikost zabilježena je u siječnju, kada je zabilježen 31 rod eukariotskih mikroorganizama u aktivnom mulju od kojih je najzastupljeniji bio rod *Epistylis sp.,* dok je najniža biološka raznolikost zabilježena u lipnju kada je zabilježen 21 rod od kojih je najzastupljeniji bio rod *Vorticella sp.*. Rodovi *Epistylis sp.*, *Opercularia sp.*, *Colurella sp.*, *Vorticella sp.*, *Rotaria sp.*, *Amphileptus sp.*, *Aspidisca sp.*, *Zooglea sp.* i *Euglypha sp.* bili su stalno zastupljeni tokom 2012. godine. Oscilacije u brojnosti mikroorganizama aktivnog mulja pojedinih mjeseci ovise o utjecaju abiotičkih faktora okoliša, od kojih su najznačajniji temperatura, kisik, protok te količina organske tvari u ulaznoj vodi.

Ključne riječi: aktivni mulj, eukariotski mikroorganizmi, Zagrebačke otpadne vode d.d.

WE CAN'T DO WITHOUT THEM: EUKARYOTIC ORGANISMS – INHABITANTS OF THE ACTIVATED SLUDGE

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Wastewater treatment of the City of Zagreb is performed at the Wastewater Treatment Plant of the City of Zagreb in two stages, where the second stage involves biological treatment by activated sludge. Decomposition of the organic matter in biological pools, which is the key factor of efficiency in wastewater treatment, is performed with the assistance of oxygen which is conveyed to the biological pools by insufflating atmospheric air. The purpose of the study was to establish representation of individual genera of eukaryotic organisms of activated sludge, as well as the relationship toward specific abiotic factors. The study was conducted during 2012, by determining eukaryotic microorganisms present in the activated sludge and their representation during particular months. In total, there were 35 genera identified during 2012. The highest biological diversity was recorded in January, when 31 genera of eukaryotic microorganisms were

recorded in the activated sludge, where the genus *Epistylis sp* had the highest representation, while the lowest biological diversity was recorded in June, when 21 genera was recorded, with the highest occurrence of *Vorticella sp.* genus. The genera *Epistylis sp.*, *Opercularia sp.*, *Colurella sp.*, *Vorticella sp.*, *Rotaria sp.*, *Amphileptus sp.*, *Aspidisca sp.*, *Zooglea sp.* and *Euglypha sp.* were constantly represented during 2012. Oscillation in abundance of eukaryotic microorganisms in activated sludge in certain months depends on the influence of abiotic environmental factors, the most important being temperature, oxygen, flow and amount of organic matter in the inflow water.

Key words: activated sludge, eukaryotic microorganisms, Zagreb Wastewater Treatment Plant Ltd.

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HETEROTROFNE BAKTERIJE U SEDIMENTU ŠIREG PODRUČJA SPLITA

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Iako ukupna debljina morskog sedimenta u pojedinim područjima može iznositi i do nekoliko kilometara, bakterije naseljavaju uglavnom površinske slojeve sedimenta. Postotak sedimenta koji naseljavaju bakterije je manji od 1%. Glavni razlog za to je opadanje količine organskog materijala s povećanjem dubine sedimenta. Istraživanje brojnosti bakterija u sedimentu provedeno je na širem području Splita, na postajama različitog trofičkog stupnja: Kaštelanski zaljev, Gradska luka Split, Splitski kanal. Uzorci sedimenta su uzimani do dubine od 15 cm i podijeljeni vertikalno na slojeve: 0-1 cm, 1-2 cm, 2-5 cm, 10-15 cm. Uzorci morske vode su uzimani na standardnim oceanografskim dubinama (0, 10, 20, 35 m). Cilj istraživanja bio je utvrditi brojnost i raspodjelu heterotrofnih bakterija po slojevima sedimenta, usporediti promjene među istraživanim postajama te odrediti odnos brojnosti bakterija vodeni stupac- sediment. Brojnost bakterija u sedimentu određena je metodom direktnog brojenja epifluorescentnim mikroskopom, dok je brojnost heterotrofnih bakterija u vodenom stupcu određena metodom direktnog brojenja protočnim citometrom. Najveće prosječne vrijednosti brojnosti bakterija u sedimentu utvrđene su na postaji u Kaštelanskom zaljevu, a najmanje na postaji u Splitskom kanalu. Analizom brojnosti bakterija na vertikalnoj skali u sedimentima ispitivanih postaja uočene su značajne razlike među postajama. Brojnost bakterija u Kaštelanskom zaljevu bila je gotovo ujednačena na cijeloj ispitivanoj dubini, dok je brojnost bakterija u sedimentu gradske luke pravilno opadala od površine do dubine od 15 cm. Ovakva raspodjela brojnosti bakterija u sedimentu može se objasniti utjecajem rijeka i bioturbacijom, odnosno eutrofikacijom. Usporedbom brojnosti bakterija vodenog stupca i sedimenta zabilježene su značajno veće prosječne vrijednosti brojnosti bakterija u sedimentu, i to za tri reda veličine.

Ključne riječi: brojnost bakterija, sediment, vodeni stupac

HETEROTROPHIC BACTERIA IN SEDIMENT OF A WIDER AREA OF SPLIT

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Although the total thickness of marine sediment in some areas can be up to several kilometers, bacteria inhabit mainly the surface layers of the sediment. The percentage of bacteria-settled sediment is less than 1%. The cause of this distribution is the decrease in organic material quantity with increasing sediment depth. The sediment bacteria abundance research was carried out in the wider area of Split, at stations of different trophic status: Kaštela bay, Split harbour, Split channel. The sediment samples were taken down to the depth of 15 cm and vertically divided into layers: 0-1 cm, 1-2 cm, 2-5 cm, 10-15 cm. The seawater samples were taken at standard oceanographic depths (0, 10, 20, 35 m). The purpose of the research was to determine the bacteria abundance and the distribution within the different sediment layers, to compare variations between the studied stations and to define ratio of bacterial abundance between the water column and the sediment. Epifluorescent microscopy direct counting method was used to determine sediment bacteria abundance, and the flow citometry direct counting method to determine water column heterotrophic bacteria abundance. The highest sediment bacteria abundance average values were found at the Kaštela bay station, the lowest at the Split channel station. Analysing the bacterial abundance on vertical scale, major differences in sampled stations sediments were noticed between the stations. The bacterial abundance in Kaštela bay was almost consistent at studied depths, while bacterial abundance in Split harbour sediment showed a consistent decline from the surface to the 15 cm depth. The river flow, bioturbation and eutrofication influence can explain such sediment bacteria distribution. Comparing the water column and sediment bacteria abundance, notably higher approximate vallues in sediment bacteria abundance, even in three orders of magnitude higher, were recorded.

Key words: bacterial abundance, sediment, water column

KOMPARATIVNA FIZIOLOGIJA, IMUNOBIOLOGIJA I BIOLOGIJA ČOVJEKA
COMPARATIVE PHYSIOLOGY, IMMUNOBIOLOGY AND HUMAN BIOLOGY

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ANIMALNI MODEL PSORIJAZE: ANTIUPALNA I ANTIOKSIDATIVNA UČINKOVITOST EPIGALOKATEHIN GALATA

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Istražili smo moguće korisno djelovanje pripravka epigalokatehin galata (EGCG) na animalnom modelu psorijaze, prouzročene iritantom di-n-propil disulfidom (PPD), tijekom 5 dana. Antiupalno i antioksidativno djelovanje EGCG-a pratili smo slijedećim metodama: broj stanica u trbušnoj šupljini, funkcionalna aktivnost makrofaga u peritonealnoj tekućini, patohistološka analiza promjena na koži pokusnih životinja, hematološki i biokemijski parametri krvi te metode za procjenu antioksidativne učinkovitosti (reducirani glutation, lipidna peroksidacija). Rezultati ukazuju da pripravak EGCG-a smanjuje ukupan broj upalnih stanica u peritonealnoj tekućini miševa. Hematološki i biokemijski pokazatelji kretali su se unutar normalnih vrijednosti dok je razina upalnih citokina u plazmi bila povećana u životinja obrađenih s PPD i/ili združenim s EGCG-om, posebice, čimbenik stimulacije kolonija granulocita (G-CSF) i interleukina-6 (IL-6). Patohistološka analiza uzoraka kože pokazala je da združena topička primjena iritanta s EGCG ima upalnu reakciju znatno slabijeg intenziteta od PPD-a. Genotoksični učinak istraživanih pripravaka procjenili smo pomoću komet-testa i mikronukleus-testa. Rezultati komet-testa i

mikronukleus-testa pokazuju da EGCG nije genotoksičan za stanice periferne krvi miševa. Temeljem rezultata zaključujemo da istraživani pripravak EGCG-a, pokazuje protuupalni učinak, te da bi njegova primjena mogla poslužiti u sprječavanju i liječenju upalnih promjena psorijaze, bez toksičnih posljedica.

Ključne riječi: mišji model psorijaze, kožne lezije, termografija, EGCG

ANIMAL MODELS OF PSORIASIS: ANTIINFLAMMATORY AND ANTIOXIDANT EPIGALLOCATEHIN GALLATE EFFICIENCY

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We examined positive effects of epigallocatehin gallate (EGCG) on an animal model of psoriasis, in which the psoriasis was caused by di-n-propyl disulphide (PPD) irritant during 5 days. Anti-inflammatory and antioxidative activity of EGCG preparations were followed by various methods: cell numbers in abdominal cavity, functional activity of macrophages in peritoneal fluid, pathohistological analyses of skin, haematological and biochemical parameters of blood and antioxidative efficiency by reduced glutathione and lipid peroxidation. The results indicate that EGCG preparations reduce number of inflammatory cells in peritoneal fluid of mice. All haematological and biochemical parameters indicate that it falls within the reference range while cytokines' values in plasma indicate that PPD alone, or combined with EGCG, caused a higher level of antiinflammatory cytokines, especially granulocyte-col ony stimulating factor (G-CSF) and interleukins-6 (IL-6). Pathohistological analysis of skin samples indicates that a topical use of the irritant with EGCG causes significantly lower inflammatory reaction than PPD itself. The results of comet and micronucleus test show no genotoxicity induced by EGCG preparations in peripheral blood cells of mice. We conclude that EGCG preparations show antiinflammatory effect and their use could be applicable in a suppression and treatments of inflammatory process in psoriasis, without any toxical effects.

Keywords: mouse models of psoriasis, skin lesions, thermography, EGCG

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SEPARACIJSKA ANKSIOZNOST U MLADUNACA ŠTAKORA S PERINATALNO PROMIJENJENIM METABOLIZMOM SEROTONINA

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Serotonin (5HT) je biogeni amin prisutan u mozgu (središnji odjeljak) i tjelesnim tkivima (periferni odjeljak). 5HT regulira razvoj živčanog sustava i kao neurotransmiter posreduje socijalno i anksiozno ponašanje. Kad su odvojeni od majke, mladunci štakora proizvode ultrazvučne dozive koji ukazuju na nelagodu zbog gubitka kontakta i mogu služiti kao mjera anksioznosti. Posljedice perinatalnog poremećaja metabolizma serotonina istražili smo tretirajući Wistar štakore tijekom razvoja neposrednim prekursorom serotonina, 5-hidroksitriptofanom (5-HTP, 25 mg/kg) koji povisuje samo periferne razine 5HT ili inhibitorom

monoamin oksidaze, tranilciprominom (TCP, 2 mg/kg), koji značajno povisuje razine 5HT u oba odjeljka, od 13. gestacijskog do 21. postnatalnog dana (PND). Dozivanje smo snimali 13. i 16. PND. Latenciju, broj i trajanje doziva koristili smo kao mjeru anksioznosti. Frekvenciju maksimalne energije koristili smo kao mjeru sazrijevanja. Povećana razina anksioznosti i odgođeno sazrijevanje bilo je blago i neznačajno nakon tretmana 5HTP-om, ali snažno i značajno nakon tretmana TCP-om. Ovi rezultati pokazuju da povišene razine serotonina tijekom razvoja mozga uzrokuju poremećaje ponašanja koji su izraženiji nakon promjene homeostaze 5HT u oba odjeljka i sugeriraju da sama hiperserotoninemija nije dovoljna da uzrokuje poremećaje vezane uz 5HT u neurorazvojnim sindromima.

Ključne riječi: serotonin, ultrazvučne vokalizacije, hiperserotoninemija, tranilcipromin, štakori, 5-hidroksitriptofan

SEPARATION ANXIETY IN RAT PUPS WITH PERINATALLY ALTERED SEROTONIN METABOLISM

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In mammals, serotonin (5HT) is present both in the brain (central compartment) and peripheral tissues (peripheral compartment). 5HT regulates neurodevelopment and as a neurotransmitter mediates essential behaviors including social and anxiety behaviors. Rat pups separated from their mother produce ultrasonic calls which indicate distress from loss of affiliate contact and may serve as a measure of anxiety. Effects of perinatal disturbances in 5HT metabolism were studied by exposing developing Wistar rats to treatments with the immediate 5HT precursor 5-hydroxytryptophan (5HTP, 25 mg/kg), which significantly raises only peripheral 5HT concentrations, or the monoamine oxidase inhibitor tranylcypromine (TCP, 2 mg/kg), which significantly elevates 5HT in both compartments, from gestational day 12 until postnatal day (PND) 21. Separation calls were recorded on PNDs 13 and 16. Latency, number and duration of calls were used as measures of anxiety. Frequency of maximal energy was used as a measure of maturation. Increase in separation anxiety and delay in maturation were slight and non-significant after 5HTP treatment but strong and significant after TCP treatment. The results indicate that exposure of developing brain to increased 5HT concentrations leads to behavioral abnormalities that were more pronounced after perinatal alteration in both 5HT compartments, indicating that hyperserotonemia alone is not sufficient to cause 5HT-related disturbances in neurodevelopmental syndromes.

Key words: serotonin, ultrasonic vocalizations, hyperserotonemia, tranylcypromine, rats, 5-hydroxytryptophan

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RAZLIKE U SASTAVU MASNIH KISELINA U MURINE, UGORA I JEGULJE IZ JADRANSKOG MORA BLIZU DUBROVNIKA

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Omjer ω-6 / ω-3 masnih kiselina u prehrani i njihovi učinci na zdravlje ljudi dobro su poznati. Prehrana ribom važan je izvor različitih masnih kiselina. Sastav masnih kiselina u ribama izravno je povezan s raznolikosti organizama koji prevladavaju u prehrani pojedinih vrsta riba. U prethodnim radovima pokazali smo razlike u sastavu prehrane između predatorskih vrsta murine i ugora, a u ovom radu analiziramo razlike u sastavu masnih kiselina između dvije vrste. Rezultati su uspoređeni i sa sastavom masti u obične jegulje jer je sastav masnih kiselina u te vrste dobro poznat. Sve su ribe prikupljene u Jadranskom moru i riblje ulje je ekstrahirano iz mišićnog tkiva (n = 6 / vrsti). Analiza masne kiseline napravljena je plinskom kromatografijom. Rezultati su pokazali najveću količinu proteina u murine, slično jegulji, a najmanju količinu u ugora. Najveće količine ekstrahiranih masti zabilježene su u jegulje i murine, dok je ukupna količina masti bila izrazito niža u ugora. U jegulja je zabilježen najveći omjer zasićenih/nezasićenih masti, najviši omjer ω-6 / ω-3 masnih kiselina i najviši postotak višestruko nezasićenih masnih kiselina (% PUFA) u odnosu na murinu ili ugora koji su imali slične vrijednosti u ova tri parametra. Međutim, postotak jednostruko nezasićenih masnih kiselina (% MUFA) bio je sličan u murine i ugora, i viši nego u jegulje. Ove razlike upućuju da različita ekofiziologija i različiti sastav prehrane istraženih srodnih vrsta riba utječu na različiti sastav masnoća u njihovim tkivima.

Ključne riječi: masne kiseline, fiziologija prehrane, ekofiziologija

DIFFERENCES IN FATTY ACID COMPOSITION BETWEEN MORAY, CONGER AND COMMON EEL FROM ADRIATIC SEA NEAR DUBROVNIK

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Ratio of ω-6/ω-3 fatty acid in the human nutrition and their beneficiary effects on health are well known. Fish consumption is an important source of fatty acids. The composition of fatty acids in fish is directly linked to the diversity of organisms devoured by particular fish species. Previously we showed the differences in pray composition between top-order predators the Moray and Conger eel and here present the differences in fatty acid composition between two species. Results are compared to Common eel since in this species a fatty acid composition is well known. All fish were collected in the Adriatic sea and the fish oil was extracted from muscular tissue (N=6/species). The fatty acid analysis was done by gas chromatography (GC). This work is the first existing report on Moray eel fatty acid composition. The results showed that Moray had highest amount of protein, similar to Common eel and followed by Conger eel. Total fat content was the highest in Common eel, followed by Moray and distinctively lower in Conger. Common eel had the highest saturated/unsaturated ratio, the highest ω-6/ω-3 fatty acids ratio and the highest percentage of polyunsaturated fatty acids (PUFA %) compared to Moray or Conger which had similar values in these three parameters. However, percentage of monounsaturated fatty acids (MUFA %) was higher and similar in Moray and Conger eel than in Common eel. These differences

implicate that different ecophysiology and pray composition influence the fat composition in kin Anguilliform species.

Key words: fatty acids, nutritional physiology, ecophysiology

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UČINKOVITOST KAFEINSKE KISELINE NA PERITONEALNU ANGIOGENEZU U EHRLICHOVOM ASCITESNOM TUMORU

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Napredovanje razvoja tumorskih stanica uključuje preživljavanje, širenje, invaziju, angiogenezu i metastaziranje. Kafeinska kiselina (CA) je biološki aktivna sastavnica propolisa koja pokazuje antioksidativna, protuupalna, antiproliferativna, citostatska, antiangiogena i antineoplastična svojstva. U ovom radu istraživali smo učinak kafeinske kiseline na rast tumora i tumorsku angiogenezu u miševa nositelja Ehrlichovog ascitesnog tumora (EAT). Stanice EAT ($2,5x10^6$) su injicirane intraperitonealno (*i.p.*) u Swiss albino miševe. Nakon inokulacije tumora, miševi su injicirani *i.p.* CA u dozi od 40 i 80 mg kg^{-1} tjelesne težine u fazi eksponencijalnog rasta od 5 dana nakon injekcije stanica tumora (na dan 5, 7, 9). Četrnaesti dan analizirali smo ukupni broj stanica ascitesa u peritonealnoj šupljini, volumen ascitesa, diferencijalnu analizu stanica prisutnih u peritonealnoj šupljini, funkcionalna aktivnost makrofaga, NO i prožiljenost peritonealne stijenke. CA je inhibirala rast stanica EAT i nastanak ascitesa u peritonealnoj šupljini miševa nositelja EAT. Nadalje, rezultati smanjenja peritonealne angiogeneze i mikrožilne gustoće pokazuju antiangiogeni potencijal CA *in vivo*. CA smanjuje razinu NO u stanicama EAT, dok razina NO je povećana u peritonealnim makrofagima. Sve to upućuje na zaključak da CA može aktivirati makrofage i povećati njihov citotoksični učinak kroz povećanu proizvodnju NO te spriječiti rast tumora i angiogenezu.

Ključne riječi: Ehrlichov ascitesni tumor, kafeinska kiselina, antiangiogeneza, NO, makrofagi: M1, M2 i TAMs

THE EFFICIENCY OF CAFFEIC ACID ON OXIDATIVE STRESS AND ANGIOGENESIS IN EHRLICH ASCITES TUMOR

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Progression of tumor cell development involves survival, proliferation, invasion, angiogenesis, and metastasis. Caffeic acid (CA) is an active component of propolis extract, which exhibits antioxidant, antiinflammatory, antiproliferative, cytostatic, antiangiogenic and most improtantly, antineoplastic properties. In the present study we investigated the effect of of caffeic acid on tumor growth and tumor angiogenesis in mice bearing Ehrlich ascites tumor (EAT). EAT cells ($2.5x10^6$) were implanted intraperitoneally (*i.p.*) in Swiss albino mice. After tumor inoculation, mice were injected *i.p.* with CA at dose of 40 and 80 mg kg^{-1} bw in exponential growth phase from the 5 days after tumor cell injection (on day 5, 7, 9). On day 14, ascites volume, the total number of cells, differential count of the cells present in the

peritoneal cavity, functional activity of macrophages, NO and antiangiogenic parameters were determined. The growth of EAT cells and formation of ascites in the peritoneum of EAT-bearing mice was inhibited by CA. Further, results on decrease in the peritoneal angiogenesis and microvessel density show the antiangiogenic potential of CA *in vivo*. CA decreased NO level in tumor cells whereas NO level was increased in peritoneal macrophages. Taken together, we conclude that CA may increase the cytotoxic actions of macrophages by increasing NO and inhibit tumor growth and angiogenesis.

Keywords: Ehrlich ascites tumor, caffeic acid, antiangiogenesis, NO, macrophages: M1, M2 and TAMs

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GALNA KISELINA SMANJUJE TUMORSKI RAST I ANGIOGENEZU U MIŠEVA S EHRLICHOVIM ASCITESNIM TUMOROM

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Galna kiselina (GA), također poznata kao 3,4,5-trihidroksibenzojeva kiselina, je fenolna kiselina prisutna u biljkama diljem svijeta, uključujući i ljekovito bilje Hrvatske. Ranija su istraživanja pokazala citotoksične učinke galne kiseline na određene vrste stanica raka bez oštećenja normalnih stanica. Cilj ovog istraživanja bio je utvrditi može li galna kiselina inhibirati rast Ehrlichovog ascitesnog tumora (EAT) u miša. Stanice EAT ($2,5x10^6$) su injicirane intraperitonealno (*i.p.*) u Swiss albino miševe, koji su sljedećeg dana počeli dobivati GA *i.p.* u dozi od 40 i 80 mg kg^{-1} tijekom 10 dana. Četrnaestog dana određeni su volumen ascitesa, ukupni broj stanica u peritonealnoj šupljini, gustoća krvožilja, funkcionalna aktivnost makrofaga i proizvodnja dušikovog oksida (NO). Obrada s GA normalizira razinu VEGF-a u ascitesu i smanjuje neoangiogenezu, što posljedično smanjuje rast tumora i poboljšava preživljavanje životinja. Životinje obrađene s GA u dozi od 40 mg kg^{-1} razvijaju manji volumen ascitesa u odnosu na kontrolu, što upućuje na smanjenu vaskularnu permeabilnost i smanjenje VEGF ekspresije u stanicama EAT.

Ključne riječi: galna kiselina, rast tumora, angiogeneza, VEGF

GALLIC ACID REDUCES TUMOR GROWTH AND ANGIOGENESIS IN MICE WITH EHRLICH ASCITES TUMOR

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Gallic acid, also known as 3,4,5 trihydroxybenzoic acid, is a type of phenolic acid, which is present in plants worldwide, including Croatian medicinal herbs. Gallic acid has been shown to have cytotoxic effects in certain cancer cells, without damaging normal cells. The objective of the present study was to determine whether gallic acid is able to inhibit growth of Ehrlich ascites tumor (EAT) in mice. EAT cells ($2.5x10^6$) were implanted intraperitoneally (*i.p.*) in Swiss albino mice. One day after tumor inoculation, mice were injected *i.p.* with GA at dose of 40 and 80 mg kg^{-1} bw during 10 days. On day 14, ascites volume, the total number of cells in the peritoneal cavity, microvessel density, functional activity of macrophages and nitric oxid production (NO) were determined. GA therapy normalizes VEGF in ascites and decreases

neoangiogenesis, thereby reducing tumor growth and im proving survival. GA-treated animals at dose of 40 mg kg^{-1} developed ascites in a smaller volume compared to the control, suggesting decreased vascular permeability by reducing VEGF expression in EAT cells.

Keywords: gallic acid, tumor growth, angiogenesis, VEGF

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POVEZANOST ŽIVOTNIH NAVIKA I PRIMARNIH OŠTEĆENJA DNA SPERMIJA S REPRODUKTIVNOM SPOSOBNOSTI MUŠKARACA

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Nastanak spermija, kao i kvaliteta spermija, ovise o genetskoj predispoziciji, fizičkim traumama te mnogim egzogenim i endogenim čimbenicima u testisima i okolnom tkivu te organima koji čine dio reproduktivnog sustava. Cilj ovog istraživanja je utvrditi kako su uobičajene metode analize ejakulata (pokretljivost, koncentracija i građa spermija) povezane s analizom fragmentacije DNA spermija, te povezanost životnih navika (konzumiranje duhanskih proizvoda, lakih droga i alkohola) ispitanika s kvalitetom spermija. Analiza sjemenih parametara: koncentracija spermija, pokretljivosti spermija i građa spermija vršena je prema smjernicama World Health Organization (WHO). Za utvrđivanje primarnih oštećenja DNA spermija korišten je komet-test. Analizirana je međusobna povezanost navedenih parametara, kao i povezanost životnih navika i navedenih parametara. Oštećenje DNA spermija nije statistički značajno povezano s tradicionalnim sjemenim parametrima (koncentracija, pokretljivost i građa spermija, dob ispitanika). Konzumiranje više od deset cigareta dnevno, kao i konzumiranje kanabisa više od tri puta tjedno, ne utječe značajno niti na jedan od sjemenih parametara. Konzumiranje više od dva alkoholna pića dnevno značajno smanjuje progresivnu pokretljivost i koncentraciju spermija. Istovremeno konzumiranje više od dva alkoholna pića dnevno i više od deset cigareta dnevno značajno smanjuje progresivnu pokretljivost spermija.

Ključne riječi: spermiji, oštećenja DNA, komet-test, životne navike

RELATIONSHIP BETWEEN LIFESTYLE HABITS AND PRIMARY DNA DAMAGE OF THE SPERM AND REPRODUCTIVE ABILITY OF MEN

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Spermatogenesis and sperm quality depend on genetic predisposition, physical trauma, and numerous exogenous and endogenous factors in the testicles and surrounding tissue and

organs that are part of the reproductive system. The aim of this study was to determine how common method of analysis of sperm (motility, concentration and sperm morphology) are associated with the analysis of DNA fragmentation of sperm, and the connection between lifestyle habits (consumption of tobacco products, soft drugs and alcohol) of respondents to the quality of sperm. The analysis of seminal parameters: sperm concentration, sperm motility and sperm morphology was performed according to the guidelines of the World Health Organization (WHO). To determine the primary DNA damage sperm comet assay was used. We analysed the interrelation of the sperm parameters, as well as the connection between lifestyle. DNA damage of sperm was not significantly associated with traditional seminal parameters (concentration, motility and sperm morphology and age of the respondents). Consuming more than ten cigarettes a day, as well as consuming cannabis more than three times a week, do not significantly affect any of the seminal parameters. Drinking more than two alcoholic drinks per day significantly reduces the progressive motility and sperm concentration. At the same time consuming more than two alcoholic drinks per day and more than ten cigarettes a day significantly reduces the progressive sperm motility.

Key words: sperm, DNA damage, comet assay, lifestyle habits

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UČINKOVITOST KVERCETINA NA BIOMARKERE ZA OSTEOPOROZU

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Oksidativni stres može dovesti do opsežnog gubitka koštane mase i izazvati lomnost kostiju, kao ključne značajke osteoporoze. Istražili smo zaštitni učinak kvercetina (QU) na kosti u osteoporozi izazvanoj retinoičnom kiselinom (13cRA) u štakora te učinak QU na kosti zdravih životinja. Analizirali smo mineralnu gustoću kostiju (BMD), serumske biljege koštane pregradnje (osteokalcin; OC i C-terminalnog fragmenta tipa I kolagena; CTX), ukupni sadržaj kalcija (Ca) i fosfora (P) te geometrijska i fizikalna obilježja bedrenih kostiju, histologiju, hematološke i biokemijske parametare, lipidnu peroksidaciju (MDA) i razinu glutationa (GSH) u stanicama jetre i bubrega. Retinoična kiselina primjenjena u dozi od 80 mg kg^{-1} uzrokovala je značajan porast biokemijskih parametara, MDA, OC i CTX vrijednosti i značajan pad vrijednosti BMD, Ca i P u bedrenim kostima te razine GSH u jetri i bubrezima u usporedbi s kontrolom. Intragastrična primjena QU (100 mg kg^{-1}) istodobno s 13cRA rezultirala je znatnim smanjenjem vrijednosti CTX i MDA u odnosu na RMO. QU je polučio pozitivne učinke na razinu Ca i P, BMD u distalnom dijelu femura, težinu i dužinu femura, biokemijske odrednice i razinu GSH. Istovremena obrada štakora s 13cRA i alendronatom imala je toksični učinak i rezultirala značajnim smanjenjem tjelesne mase od 5,67% nakon dva tjedna obrade životinja. Zaštitna djelotvornost QU protiv toksičnosti inducirane s 13cRA u štakora daje nadu da bi kvercetin mogao imati sličan zaštitni učinak na kosti u ljudi.

Ključne riječi: retinoična kiselina, osteoporoza, kvercetin, alendronat, štakor

THE EFFECTIVENESS OF QUERCETIN ON BIOMARKERS FOR OSTEOPOROSIS

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Oxidative stress may lead to extensive bone loss and skeletal fragility, two characteristics of osteoporosis. We studied the bone-protective effect of quercetin (QU) in the retinoic acid induced model of osteoporosis (RMO) and in healthy rats. Densitometry, osteocalcin (OC), C-terminal cross-linked telopeptide (CTX), calcium (Ca) and phosphorus (P) content of bone femur, geometric measurement on the femur, histology, hematological and biochemical parameters as well as lipid peroxidation (MDA) and glutathione levels (GSH) of liver and kidney cells were applied to all groups. Retinoic acid administration (80 mg kg^{-1}) revealed significant increase in biochemical parameters, MDA level, OC and CTX values and a significant decrease in values of BMD, Ca and P in the femur and GSH levels in liver and kidney compared to control. Administration of QU intragastrically (100 mg kg^{-1} bw) daily concomitant with retinoic acid resulted in significantly reduction of CTX values and MDA in comparison to RMO. QU also exerted positive effects on biochemical parameters, values of Ca and P, BMD values in distal part, femoral weight and length and the GSH level. Concomitant treatments of rats with retinoic acid and alendronate had toxic effect to animals and resulted in a significant decrease in body mass of 5.67% after two weeks. The protective role of the QU against the toxicity of retinoic acid-induced osteoporosis in rats gives a hope that QU may have similar protective action in humans.

Key words: retinoic acid, osteoporosis, quercetin, alendronate, rat

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INHIBICIJA POLARIZACIJE MAKROFAGA KAFEINSKOM KISELINOM: MEĐUODNOS ROS, ANTIOKSIDANSA I UPALNIH CITOKINA

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Makrofagi su važne stanice prirođenog i stečenog imunosnog odgovora koje se nakon aktivacije mogu diferencirati u M1 ili M2 fenotip. M1 makrofagi posjeduju pro-upalnu, mikrobicidnu i antikancerogenu djelotvornost; M2 makrofagi i njima srodni tumoru-pridruženi makrofagi (TAMs) sudjeluju u remodeliranju tkiva, angiogenezi i imunomodulaciji. Proizvodnja reaktivnih kisikovih spojeva (ROS), kritična za aktivaciju i funkciju M1 makrofaga, nužno je potrebna i za diferencijaciju M2 makrofaga i TAMs. Poznato je da obrada s antioksidansima blokira diferencijaciju TAMs i tumorogenezu u mišjem tumorskom modelu. Da bi proučili kako kafeinska kiselina (CA), prirodni antioksidans, utječe na funkciju i polarizaciju makrofaga te rast tumora, miševi su injicirani sa stanicama Ehrlichovog ascitesnog tumora (EAT) i obrađeni tijekom 10 dana s kafeinskom kiselinom u dozi od 40 i/ili 80 mg kg^{-1}. Polarizacija makrofaga dalje je potvrđena prema razini izlučenih pro- i anti-upalnih citokina. Kafeinska kiselina može povećati citotoksičnu aktivnost makrofaga i inhibirati rast tumora. Inhibicijska djelotvornost CA na TAMs mogla bi se zasnivati na njenoj antioksidacijskoj aktivnosti. Temeljem navedenog,

možemo zaključiti da kontinuirana primjena CA kao inhibitora ROS-a učinkovito blokira pojavnost TAMs i znatno potiskuje razvoj tumora u miša. Pojavnost TAMs može biti spriječena blokiranjem razine ROS-a, što bi mogla biti potencijalno učinkovita metoda za liječenje raka.

Ključne riječi: tumor, polarizacija makrofaga, kafeinska kiselina, oksidacijski stres, imunomodulacija

INHIBITION OF MACROPHAGE POLARIZATION BY CAFFEIC ACID: INTERPLAY BETWEEN ROS, ANTIOXIDANS, AND INFLAMMATORY CYTOKINES

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Macrophages are important both to innate and adaptive immune responses and are known to differentiate into M1 or M2 phenotypes following activation. While M1 macrophages are highly pro-inflammatory, microbicidal and anticancer; M2 macrophages and the related tumor associated macrophages (TAMs) regulate tissue remodeling and angiogenesis and can display immunomodulatory activity. It is demonstrated that production of reactive oxygen species (ROS), critical for the activation and functions of M1 macrophages, is also necessary for the differentiation of M2 macrophages and TAMs, and that antioxidant therapy blocks TAMs differentiation and tumorigenesis in mouse models of cancer. In order to study how caffeic acid, a natural antioxidant, affects macrophage function, polarization and tumor growth, we injected mice with Ehrlich ascites tumor (EAT) cells and treated them for 10 days with caffeic acid in a dose of 40 and/or 80 mg kg^{-1}. Macrophage polarization was further characterized by quantifying secreted pro- and anti-inflammatory cytokines. Caffeic acid may increase the cytotoxic actions of macrophages and inhibit tumor growth; inhibitory activity on TAMs may be mediated through its antioxidative activity. Taken together, we conclude that the continuous administration of the ROS inhibitor caffeic acid efficiently blocked the occurrence of TAMs and markedly suppressed tumorigenesis in mouse cancer models. Targeting TAMs by blocking ROS can be a potentially effective method for cancer treatment.

Key words: tumor, macrophage polarization, caffeic acid, oxidative stress, immunomodulation

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UČINAK FLAVONOIDA I HIPERTERMALNE INTRAPERITONEALNE KEMOTERAPIJE (HIPEC) NA RAST TUMORA I INDUKCIJU MIKRONUKLEUSA U MIŠJEM MODELU TUMORA

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Hipertermija pojačava klastogenost alkilirajućih spojeva. Istražili smo mogu li kvercetin (QU) ili naringenin (NAR) senzibilizirati Ehrlichov ascitesni tumor (EAT) na hipertermalnu intraperitonealnu kemoterapiju (HIPEC) s cisplatinom te mogu li flavonoidi u kombinaciji s cisplatinom smanjiti cisplatinom prouzročen broj mikronukleusa u retikulocitima periferne krvi miševa. QU i NAR su injicirani miševima 7 i 3 dana prije unosa stanica EAT, dok je cisplatina (5 ili 10 mg kg^{-1}) injicirana

miševima intraperitonealno pri normalnim ili hipertermalnim uvjetima 3 dana nakon unosa stanica EAT (2×10^6). Naše istraživanje podupire tvrdnju da QU i NAR u združenom liječenju s cisplatinom imaju potencijal za inhibiranje rasta tumora u normalnim i hipertermalnim uvjetima te smanjuju broj mikronukleusa u retikulocitima periferne krvi miševa u normalnim uvjetima, dok pri hipertermalnim uvjetima flavonoidi pojačavaju klastogenost cisplatine.

Ključne riječi: hipertermija, kemoterapija, flavonoidi, Ehrlichov ascitesni tumor, genotoksičnost

EFFECT OF FLAVONOIDS AND HYPERTHERMAL INTRAPERITONEAL CHEMOTHERAPY (HIPEC) ON TUMOUR GROWTH AND MICRONUCLEUS INDUCTION IN MOUSE TUMOUR MODEL

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Hyperthermia enhanced the clastogenicity of alkylating agents. We investigated whether quercetin (QU) or naringenin (NAR) can sensitize Ehrlich ascites tumour to cisplatin hyperthermal intraperitoneal chemotherapy (HIPEC) treatment and whether these flavonoids in combination with cisplatin can ameliorate cisplatin-induced micronuclei in peripheral blood reticulocytes of mice. QU or NAR were administered to mice 7 and 3 days before implantation of EAT cells, while cisplatin (5 or 10 mg kg^{-1}) was injected intraperitoneally to normothermic or hyperthermic-treated mice 3 days after implantation of EAT cells (2×10^6). Our study supports the claim that the QU or NAR in combined treatment with cisplatin has the potential to inhibit tumour growth in both normothermic and hyperthermic conditions and attenuate number of micronuclei in the peripheral blood reticulocytes of mice at normothermic condition but enhanced the clastogenicity of cisplatin agents in hyperthermal condition.

Keywords: hyperthermia, chemotherapy, flavonoids, Ehrlich ascites tumour, genotoxicity

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ZAŠTITNI I REPARACIJSKI UČINAK PENTADEKAPEPTIDA BPC 157 NA STANICE KRVI, JETRE I BUBREGA MIŠA

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Istražili smo zaštitni i reparacijski učinak pentadekapeptida BPC 157 na stanice krvi, jetre i bubrega miša nakon oštećenja prouzročenog cisplatinom (CP). Miševe smo podijelili u 4 skupine: kontrola, BPC 157, BPC 157 i CP te CP. Pratili smo oštećenje DNA stanica krvi, jetre i bubrega nakon 0, 1, 6 i 24 sata, te brzinu popravka DNA. Rezultati komet-testa pokazali su da je najveće oštećenje DNA prouzročila CP nakon 1 sata u svim analiziranim tkivima, a postotak oštećenja bio je vidljiv i nakon 24 sata što upućuje na velik broj dvostrukih lomova. Najveći broj apoptotičnih stanica nađen je u stanicama bubrega. BPC 157 prouzročio je oštećenja DNA nakon 1 i 6 sati što ukazuje na mogućnost stvaranja oksidativnog stresa ulaskom BPC 157 u stanicu. Nakon 24 sata došlo je do potpunog oporavka stanica. Zajednička primjena BPC 157 i CP pokazala je smanjenje

CP-prouzročenih oštećenja. Broj mikronukleusa u skupini obrađenoj CP povećava se proporcionalno vremenu izloženosti i najveći je nakon 24 sata. BPC 157 združen s CP značajno smanjuje broj mikronukleusa. Kao najosjetljiviji organ na toksični učinak CP pokazao se bubreg, a nakon njega jetra te limfociti. Dobiveni rezultati ukazuju da BPC 157 ima zaštitni učinak na zdrave stanice vjerojatno temeljen na smanjenom unosu toksičnih metabolita CP u stanicu ili sudjelovanjem u procesu popravka DNA u oštećenim stanicama, što zahtjeva daljnja istraživanja.

Ključne riječi: cisplatina, BPC 157, miš, mikronukleus, komet-test

PROTECTIVE AND REPARATIVE EFFECT OF PENTADECAPEPTIDE BPC 157 ON MICE BLOOD, LIVER AND KIDNEY CELLS

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We investigated the protective and reparative effect of BPC 157 on blood, liver and kidney cells of mouse, after cisplatin (CP) treatment. The mice were divided into 4 groups: control, BPC 157, BPC 157 and CP and CP. We monitored the levels of DNA damage in blood, liver and kidney cells after 0, 1, 6, and 24 h and the speed of DNA repair. The comet test shows that the DNA was mostly damaged by CP after 1 h in all analysed tissues and the % of damage was visible after 24 h, which indicated a large number of double-strand breaks. The highest number of apoptotic cells was found in kidney cells. BPC 157 caused the DNA damage after 1 and 6 h as a result of oxidative stress incurred by BPC 157 administration. The values returned to normal after 24 h. The combined treatment with BPC 157 and CP resulted in lower CP-caused DNA damage. A number of micronuclei, which is a double-strand break indicator, in the CP group increased proportionally to the period of exposure and had the highest value after 24 h. The combined treatment with BPC and CP decreased the micronuclei count. It was shown that the most sensitive organ to the toxic effects of CP was the kidney, followed by liver and lymphocytes. Results indicated that BPC 157 has a protective effect on healthy cells, which is possibly based on a reduced intake of CP into the cell or BPC 157 participation in the process of DNA-repair in damaged cells, which requires further research.

Key words: cisplatin, BPC 157, mouse, micronucleus test, comet test

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KVERCETIN SMANJUJE IRITACIJU KOŽE INDUCIRANU HEKSIL-SALICILATOM ILI DI-N-PROPIL DISULFIDOM: OKSIDATIVNI STRES I UPALA

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Ovo istraživanje temelji se na procjeni mogućeg pozitivnog učinka kvercetina na mišjem modelu psorijaze prouzročene heksil salicilatom (HXS) ili di-n-propil-disulfidom (PPD). Istražena je i mogućnost primjene termografije u regresiji psorijaznih lezija. Proces upale pratili smo primjenom slijedećih metoda: patohistološkom analizom kože, praćenjem temperature kože

termografskom kamerom, analizom ukupnog broja upalnih stanica i diferencijalnom analizom stanica u peritonealnoj šupljini, praćenjem indeksa makrofagnog širenja, hematoloških i biokemijskih parametara, učestalosti pojave mikronukleusa u retikulocitima, lipidne peroksidacije i glutationa u tkivu kože, jetre i bubrega. Lokalno primjenjeni kvercetin s HXS ili PPD može smanjiti psorijazne lezije kože potiskivanjem broja upalnih stanica u koži, inhibicijom funkcionalne aktivnosti makrofaga i nastanka reaktivnih kisikovih spojeva (ROS-a). Dodatno, ukupni broj upalnih stanica u peritonealnoj šupljini, indeks širenja makrofaga, lipidna peroksidacija u jetri i bubregu, te enzimska aktivnost ALP i AST su smanjeni u miševa sa psorijazom obrađenim kvercetinom. Temeljem svega, rezultati podupiru činjenicu da kvercetin, kao snažni antiupalni i antioksidativni spoj, može biti djelotvoran u liječenju upalnih bolesti kože, bez toksičnih posljedica. Termografija kao metoda može biti primijenjena u praćenju upalnog procesa kože u psorijazi kao i u praćenju učinkovitosti kvercetina u obradi psorijaznih lezija.

Ključne riječi: mišji model psorijaze, kožne lezije, kvercetin, termografija

QUERCETIN ATTENUATES N-HEXYL SALICYLATE OR DI-N-PROPYL DISULFIDE-INDUCED SKIN IRRITATION: OXIDATIVE STRESS AND INFLAMMATION

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This study examined the possible positive effects of quercetin on animal model of psoriasis, induced by the Hexyl salicylate or Di-n-Propyl Disulfide irritant (PPD). Furthermore, we evaluated the usefulness of thermography in psoriatic lesion regression. We monitored the inflammation process by histopathological assessment of skin, thermographic scanning, total number of inflammatory cells in peritoneal cavity, differential analysis of cells in peritoneal cavity, macrophage spreading index, hematological and biochemical parameters, frequencies of micronucleated reticulocytes, lipid peroxidation and glutathione assay in skin, liver and kidney. Topically applied quercetin with HXS or PPD may improve psoriatic-like skin lesions by suppressing number of inflammatory cells in skin, functional activity of macrophages, and ROS production. In addition, total number of inflammatory cells in peritoneal cavity, macrophage spreading index, lipid peroxidation in liver and kidney, and enzymatic activity of ALP and AST were reduced in psoriatic mice treated with quercetin. Taken together, these results support the quercetin as an antiinflammatory and antioxidative agent and open up new possibilities for its use in skin disorders without causing any toxic effect. Thermography as a method can be applicable in examining of the inflammatory process in psoriasis and in evaluating the effectiveness of quercetin and other tested substances.

Keywords: mouse models of psoriasis, skin lesions, quercetin, thermography

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NARINGENIN UBLAŽAVA PATOLOŠKE PROMJENE U JETRIMA I BUBREGU MIŠEVA S DIJABETESOM

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Istraživan je učinak naringenina na razinu lipidne peroksidacije i histopatološke promjene u jetrima i bubrezima miševa s aloksanom izazvanim dijabetesom. Osim toga, istraženi su i njegovi antioksidativni učinci *in vitro* (sposobnost vezanja slobodnih radikala, sposobnost reduciranja i sposobnost keliranja Fe^{2+} iona). Dva dana nakon davanja aloksana (75 mg kg^{-1}, *i.v.*), miševi su, tijekom 7 dana, intreperitonealno (50 mg kg^{-1} dnevno), primili alkoholnu (0,5% v/v) otopinu naringenina. Iako je pokazao ograničeno antioksidativno djelovanje *in vitro*, terapija alkoholnom otpopinom naringenina prouzročila je značajno sniženje razine lipidne peroksidacije u jetrima i bubrezima kao i smanjenje stupnja vakuolizacije te broja vakuoliziranih stanica jetara. Naznake popravka tkiva bubrega navode na zaključak da se blagotvoran učinak na bubrege može postići terapijom kroz dulje vrijeme. Zaštitni učinak naringenina u razvoju komplikacija dijabetesa u miševa daje nadu da može pokazati slične učinke kod ljudi.

Ključne riječi: dijabetes, naringenin, lipidna peroksidacija, histopatologija

NARINGENIN AMELIORATES PATHOLOGICAL CHANGES IN LIVER AND KIDNEY OF DIABETIC MICE

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The effect of naringenin on lipid peroxidation and histopathological changes in liver and kidney of alloxan-induced diabetic mice were investigated. In addition, its *in vitro* antioxidant effects (DPPH free radical scavenging activity, reducing power and the capability to chelate Fe^{2+} ions) were assessed. Two days after alloxan injection (75 mg kg^{-1}, *i.v.*), naringenin ethanolic solution (0.5% v/v) was given to mice intraperitoneally (50 mg kg^{-1} per day) for 7 days. Although it has shown limited antioxidant activity *in vitro*, naringenin administration resulted in a significant decrease of lipid peroxidation level in liver and kidney tissue as well as in decreased number of vacuolated liver cells and degree of vacuolization. Indications of tissue repair in kidney suggest that amelioration of diabetes-induced renal damage could be achieved through a longer period of time. The protective effect of naringenin in the development of diabetic complications in mice, gives a hope that it could show similar effects in treatment of diabetes in humans.

Key words: diabetes, naringenin, lipid peroxidation, histopathology

GENETIKA, STANIČNA I MOLEKULARNA BIOLOGIJA
GENETICS, CELLULAR AND MOLECULAR BIOLOGY

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PRIMJENA ANALIZE MIKROSATELITNIH BILJEGA NA POPULACIJAMA VRSTA *Astacus astacus* I *Astacus leptodactylus* U HRVATSKOJ – PRELIMINARNI REZULTATI

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Mikrosatelitni biljezi su uspješno primijenjeni u populacijsko genetičkim istraživanjima vrsta slatkovodnih desetoronožnih rakova. U ovom istraživanju upotrijebili smo mikrosatelitne lokuse za genotipizaciju vrsta *Astacus astacus* (plemeniti rak) i *Astacus leptodactylus* (uskoškari rak) kako bismo provjerili njihovu moguću upotrebu u otkrivanju njihovih hibrida, koji su već dobiveni u laboratorijskim uvjetima, a njihovo postojanje u prirodnim staništima je spominjano u literaturi. Prethodnim istraživanjima mogućih hibrida iz prirodnih staništa analizirane su morfometrijske značajke, no rezultati nisu bili jednoznačni i postojanje hibrida nije dokazano. U ovom istraživanju korištena su četiri mikrosatelitna biljega za genotipizaciju čistih populacija *A. leptodactylus* i *A. astacus*, miješanih populacija, te hibrida iz eksperimentalnog uzgoja. Dobiveni rezultati su kompleksni, te bi se za njihovo sveobuhvatno objašnjenje trebalo analizirati veći broj jedinki. Bez obzira na to, dokazano je da se mikrosateliti razvijeni na vrsti *A. astacus*, kao i novi mikrosatelitni biljeg, razvijen na vrsti *A. leptodactylus*, uspješno umnažaju na obje vrste. Analiza uzoraka iz rijeke Mrežnice (nizvodno populacija *A. leptodactylus*; uzvodno populacija *A. astacus*), daje uvid u populacijsku strukturu obje vrste. Za populaciju vrste *A. astacus* utvrđen je niži stupanj heterozigotnosti, što je vjerojatno posljedica uskog grla kojeg je ova populaciji prošla tijekom masivnog ugibanja uzrokovanog račjom kugom.

Ključne riječi: plemeniti rak, uskoškari rak, jezgrini biljezi, unakrsno umnažanje

APPLICATION OF MICROSATELLITE ANALYSIS ON *Astacus astacus* AND *Astacus leptodactylus* POPULATIONS FROM CROATIA – PRELIMINARY RESULTS

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Recently, microsatellite markers have been successfully applied to reveal genetic patterns in populations of crayfish species. In this research, we used four microsatellites primarily aiming to check their possible usefulness in detecting hybrids between noble and narrow-clawed crayfish. These hybrids have been previously obtained under laboratory conditions, and their occurrence in natural habitats has been mentioned in the literature. Existence of possible hybrids between noble and narrow-clawed crayfish in natural habitats was approached by morphometric analyses. Results on morphometry were ambiguous, and the existence of hybrids has not been approved with certainty. Application of microsatellites in this research was done on the samples from pure *A. leptodactylus* and *A. astacus* populations, from the mixed population, and on the samples of hybrids obtained from an

experimental trial. Obtained results are complex, and their comprehensive interpretation would require inclusion of more individuals. Nevertheless, it was proved that microsatellites developed for *A. astacus*, and novel microsatellite marker isolated from *A. leptodactylus*, gave successful amplification for both species. Analysis of samples from the Mrežnica River (downstream *A. leptodactylus* population; upstream *A. astacus* population), gave insight into the population structure for both species. *A. astacus* shows lower degree of heterozygosity, what could be a consequence of a crayfish plague outbreak.

Key words: noble crayfish, narrow-clawed crayfish, nuclear markers, cross-amplification

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ISTRAŽIVANJE ULOGE mikroRNA KOD HERPES SIMPLEKS VIRUSA 1

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Herpes simpleks virus 1 (HSV-1) je humani patogen koji tijekom produktivne i latentne infekcije eksprimira velik broj mikroRNA (miRNA), malih regulatornih RNA molekula. HSV-1 miR-H1 je miRNA obilato i isključivo eksprimirana tijekom produktivne infekcije. Zanimljivo je da je druga miRNA, miR-H6, kodirana na istom lokusu ali eksprimirana u suprotnom smjeru i u potpunosti komplementarna miR-H1, obilato eksprimirana tijekom obje faze infekcije. Dosadašnja istraživanja dovela su do osnovne karakterizacije kinetike ekspresije ovih miRNA, međutim njihova biogeneza i uloga u replikaciji virusa je nepoznata. Transkripti koji su ishodište miR-H1 i -H6 kao i regulacija njihove genske aktivnosti nisu poznati, a naši preliminarni rezultati pomoću Northern blota ukazuju na relativno kratke transkripte koji bi mogli biti ishodište ovih miRNA. Detaljna karakterizacija det ektiranih transkripata je u tijeku. S druge strane, kako bismo odredili ulogu miR-H1 i -H6, generirali smo mutantu sa delecijom u regiji koja kodira za ove miRNA i trenutno smo u procesu generiranja povratnih mutacija. Preliminarna ispitivanja pokazala su da delecijska mutanta nema značajni replikacijski nedostatak *in vitro*, u odnosu na divlji tip, međutim kod eksperimenata *in vivo* uočena je slabija replikacija mutiranog virusa. Ovi rezultati ukazuju da miR-H1 i -H6 imaju važnu ulogu u replikaciji HSV-1 te će disekcija njihovih uloga biti diskutirana u detalje.

Ključne riječi: Herpes simpleks virus 1, mikroRNA, primarni transkript

INVESTIGATING THE ROLES OF microRNAs IN HERPES SIMPLEX VIRUS 1 INFECTION

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Herpes simplex virus 1 (HSV 1) is an important human pathogen that during productive and latent infection expresses a large number of microRNA (miRNA), small regulatory RNA molecules. HSV 1

miR-H1 is abundantly expressed miRNA during productive infection. Interestingly, another miRNA, miR-H6, which is encoded at the same locus but transcribed in the opposite direction, is expressed during both stages of infection. Previous studies have determined the kinetics of expression of miR-H1 and -H6, but their biogenesis and their roles in HSV 1 replication are unknown. In our preliminary analysis using Northern blot we have detected relatively short transcripts which could represent the origin of these miRNAs. We are currently characterizing these transcripts. To determine the function of miR-H1 and -H6, we have generated the mutant virus with a deletion in the coding region for this two miRNAs, and we are currently in process of generating a revertant. Our preliminary results showed that mutant virus replicates similar to wild type virus in cultured cells. However in a mouse model we have observed slightly impaired replication of the mutant virus. This results indicate that miR-H1 and -H6 have important role in HSV 1 replication, and the dissection of their roles will be discussed in details.

Key words: Herpes simplex virus 1, microRNA, primary transcript

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KARAKTERIZACIJA GENA/PROTEINA *FAU* IZ MORSKE SPUŽVE *Suberites domuncula*

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Gen *FAU* je smanjeno eksprimiran kod karcinoma prostate, dojke i jajnika. Štoviše, njegova deregulacija je povezana s lošom prognozom kod raka dojke. Spužve (Porifera) su životinje bez pravog tkiva koje su se prve odvojile od zajedničkog pretka svih životinja. Velika većina gena povezana s nastankom raka kod ljudi ima homologe u spužvinom genomu. Naša studija pokazuje da gen *FAU* iz spužve *Suberites domuncula* odražava karakteristike gena pretka, koji se neznatno promijenio tijekom evolucije životinja. FAU protein iz spužve, kao i njegov ljudski homolog, povećava apoptozu u ljudskim stanicama HEK293T. To upućuje da biološka funkcija proteina FAU, obično povezana s "višim" životinjama, osobito u etiologiji raka, ima biokemijsku podlogu uspostavljenu tijekom rane evolucije Metazoa. Najvjerojatnije je predak svih životinja posjedovao protein FAU sa strukturom i funkcijama sličnim onima u recentnim verzijama proteina, čak i prije pojave pravih tkiva i nastanka tumora i metastaza. Naši rezultati upućuju na mogućnost korištenja bazalnih životinja kao jednostavnijeg modela za proučavanje složenih interakcija u ljudskoj kancerogenezi.

Ključne riječi: snoRNA, FAU, RPS30, SNORA62, Porifera

CHARACTERIZATION OF *FAU* GENE AND PROTEIN FROM MARINE SPONGE *Suberites domuncula*

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FAU gene is down-regulated in human prostate, breast and ovarian cancers. Moreover, its dysregulation is associated with poor prognosis in breast cancer. Sponges (Porifera) are animals without tissues which branched off first from the common ancestor of all metazoans. A large majority of genes implicated in human cancers have their homologues in the sponge genome. Our study suggests that *FAU* gene from the sponge *Suberites domuncula* reflects characteristics of the FAU gene from the metazoan ancestor, which have changed only slightly during the course of animal evolution. We found pro-apoptotic activity of sponge FAU protein. Same as its human homologue, sponge FAU increases apoptosis in human HEK293T cells. This indicates that biological functions of FAU, usually associated with "higher" metazoans, particularly in cancer etiology, possess biochemical background estab lished early in metazoan evolution. The ancestor of all animals possibly possessed FAU protein with the structure and the function similar to evolutionarily more recent versions of the protein, even before the appearance of true tissues and the origin of tumors and metastasis. It provides an opportunity to use pre-bilaterian animals as a simpler model for studying complex interactions in human cancerogenesis.

Key words: snoRNA, *FAU*, RPS30, SNORA62, Porifera

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DISTRIBUCIJA PROTEINA UKLJUČENIH U POLI(ADP-RIBOZIL)ACIJU U SVIM DOMENAMA ŽIVOTA

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Poli(ADP-ribozil)acija je proces reverzibilne post-translacijske modifikacije proteina koja ima ključnu ulogu u regulaciji važnih staničnih funkcija kao što su popravak DNA, transkripcija, održanje strukture kromatina i dužine telomera, starenje, razgradnja proteina, programirana stanična smrt. Ovu modifikaciju kataliziraju enzimi poli-ADP-riboza polimeraze (PARP) koristeći NAD^+ kao izvor ADP-riboze koja se veže na specifične aminokiselinske ostatke ciljnih proteina tvoreći lanac ponavljajućih jedinica ADP-riboze. Ova modifikacija može se ukloniti hidrolitičkim djelovanjem poli(ADP-riboza) glikohidrolaze (PARG) i ADP-ribozilhidrolaze 3 (ARH3). Hidrolitičko djelovanje proteina s makro domenom (MacroD1, MacroD2 i TARG1) uklanjanja terminalnu jedinicu ADP-riboze. Naši rezultati upućuju da su PARP proteini prisutni u predstavnicima svih šest glavnih eukariotskih supergrupa, a samo mali broj eukariotskih vrsta ne posjeduje PARP gene. Posljednji zajednički predak svih eukariota posjedovao je najmanje

pet različitih PARP proteina koji uključuju i mono- i poli-(ADP-ribozil)transferaze. Distribucija PARG proteina strogo slijedi raspodjelu PARP proteina. Najmanje jedan od proteina s makrodomenom koji hidrolizira terminalnu ADP-ribozu uvijek je prisutan. Možemo pretpostaviti da je posljednji zajednički predak svih eukariota posjedovao potpuno funkcionalan i reverzibilan PAR metabolizam i da je PAR signalizacija osiguravala uvjete neophodne za njegov opstanak u drevnom okolišu.

Ključne riječi: makro domena, odgovor na oštećenje DNA, PARG, PARP, Poli(ADP-riboza)

DISTRIBUTION OF PROTEIN POLY(ADP-RIBOSYL)ATION SYSTEMS ACROSS ALL DOMAINS OF LIFE

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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.

Key words: DNA damage response, Macrodomain, PARG, PARP, Poly(ADP-ribose)

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VALPROAT NARUŠAVA RAST ŠTAKORSKOG ZAMETKA I EKTOPLACENTALNOG STOŠCA *EX VIVO*

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Naš je cilj bio istražiti *ex vivo* utjecaj valproata, inhibitora histonske deacetilaze (HDI), na rast zametka štakora i ektoplacentalnog stošca. 9,5 dana stari zameci štakora Fisher (tri zametna listića) i ektoplacentalni stošci mikrokirurški su izolirani iz decidua. Zameci su kultivirani redom 14 dana, a stošci tri dana, u Eagleovom MEM-u i štakorskom serumu (1: 1) na granici tekućine i zraka. Valproat (2 mM ili 1 mM) i trihostatin A otopljen u DMSO (66 nM) i DMSO (1,2%) korišteni su u pokusima. Praćen je ukupni rast eksplantata tako da su veliki i mali promjeri mjereni svaki drugi dan, izračunate su aree (A= 1/2 veliki promjer x 1/2 manji promjer x π) i normalizirane na početne vrijednosti (A/A_0). U statističkoj obradi podataka korišten je Studentov t-test ili ANOVA. Ukupni rast zametaka i ektoplacentalnih stožaca kultiviranih s valproatom bio je značajno manji u odnosu na kontrole, posebno pri većoj koncentraciji valproata (2 mM). Trihostatin A također je smanjio rast zametaka u odnosu na kontrolu (bez statističke značajnosti), dok su u DMSO-u zameci bili veći nego u trihostatinu A, ali manji od kontrolne skupine. Dobiveni rezultati o utjecaju valproata na zametak i ektoplacentalni stožac, koji doprinosi razvoju posteljice, mogu biti važni za razumijevanje teratogenog učinka antieptileptika valproata.

Ključne riječi: valproat, štakorski zametak, ektoplacentalni stožac, rast, *ex vivo*

VALPROATE IMPAIRS GROWTH OF THE RAT EMBRYO AND THE ECTOPLACENTAL CONE *EX VIVO*

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Our aim was to investigate *ex vivo* impact of histone deacetylase inhibitor (HDI) valproate on growth of the rat embryo and the ectoplacental cone. 9.5-day-old Fisher rat embryos (three germ layers) and the ectoplacental cones were microsurgically isolated from deciduas. They were separately cultivated for 14 days or three days, respectively, in Eagle's MEM and rat serum (1:1) at the air-liquid interface. Valproate (2 mM or 1 mM) or trichostatin A dissolved in DMSO (66 nM) or DMSO (1.2%) were used in experiments. To follow overall growth, major and minor diameters were measured every other day, areas were calculated (An= 1/2 major diameter x 1/2 minor diameter x π) and normalized to the initial value (A/A_0). Data were statistically evaluated by Student's t-test or ANOVA. Overall growth of embryos and ectoplacental cones cultivated with valproate was significantly impaired in comparison to controls, being more impaired by higher valproate concentration (2 mM). Trichostatin A also impaired growth of embryos in comparison to controls (no statistical significance) while in DMSO they were constantly larger than with trichostatin A but smaller than controls. The obtained results about valproate impact upon the embryo and ectoplacental cone (contributing to placental development) could be important for understanding the teratogenic effect of the antiepileptic drug valproate.

Key words: valproate, rat embryo, ectoplacental cone, growth, *ex vivo*

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GENETSKA ANALIZA POPULACIJE ATLANTSKE PLAVOPERAJNE TUNE, *Thunnus thynnus* (Linnaeus, 1758) U KAVEZNOM UZGOJU PRIMJENOM MIKROSATELITNIH LOKUSA

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Atlantska plavoperajna tuna *Thunnus thynnus* (Linnaeus, 1758) je migratorna riba koja nastanjuje Atlantski ocean i Sredozemno more. Od 1996. godine prakticira se njen kavezni uzgoj u Hrvatskoj. Uzgoj tune donosi ekonomsku dobit, ali ulov, transport i adaptacija na kaveze ribama su stresni što može rezultirati padom imunosti i mortalitetima juvenilne ribe. Mikrosateliti su najčešći oblik ponavljajuće DNA u eukariotskom genomu, lako se amplificiraju PCR reakcijom i predstavljaju odlične genetičke biljege u istraživanju populacijske strukture, evolucije, identifikacije jedinki, određivanju srodstva među jedinkama te se često koriste u genetičkim i medicinskim istraživanjima. Koristeći mikrosatelitne motive identificirane na sekvencama normalizirane cDNA knjižnice plavoperajne tune kao inicijalnog kalupa, razvili smo multipleks PCR protokol s 15 lokusa smještenih u blizini kodirajućih regija gena koji se eksprimiraju prilikom stresa i imunosne reakcije. Na uzorcima DNA izoliranim iz fragmenta peraja 324 tune uzorkovanih prilikom ulaza populacije u uzgoj, te prilikom izlova iste populacije nakon 1,5 godišnjeg uzgoja utvrđena je razlika u učestalosti polimorfizama ovih mikrosatelitnih lokusa, što se može dovesti u vezu s fitnessom jedinki u uzgoju. Istraživani lokusi ubuduće mogu poslužiti kao biljezi za otkrivanje jedinki unutar populacije koje su uslijed polimorfizama unutar imunosnih gena sklonije promjenama imunosnog sustava i imaju izraženiji rizik od ugibanja kroz uzgojni ciklus.

Ključne riječi: Atlantska plavoperajna tuna, mikrosateliti, polimorfizam

GENETIC ANALYSIS OF CAGE-REARED ATLANTIC BLUEFIN TUNA, *Thunnus thynnus* (Linnaeus, 1758) POPULATION BY APPLICATION OF MICROSATELLITE LOCI

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Atlantic bluefin tuna *Thunnus thynnus* (Linnaeus, 1758) is a migratory fish that inhabits the Atlantic Ocean and the Mediterranean Sea. Since 1996s, Croatia practices cage breeding of this species. Tuna farming brings economic benefits, but the catch, transportation and adaptation to the cages are stressful and might result in decreased immunity and mortalities in fish. Microsatellites are the most common form of repetitive DNA in eukaryotic genomes, they can be easily amplified by the PCR reaction and they represent excellent genetic markers in the study of population structure, evolution, identification of individuals, determination of kinships between individuals and they are widely used in various fields of genetics and medical research. Using microsatellite motifs identified in the sequences of a normalized cDNA library of tuna used as the initial template, we developed a multiplex PCR with 15 loci located near the coding region of genes expressed during stress and immune reaction. DNA was isolated from fin clip of 324 tuna individuals at the moment of entrance in the cages and at harvest after 1.5 years of rearing. Differences in the frequency of polymorphisms of these microsatellite loci, which can be related to individual fitness of fish through the breeding cycle were detected. Those loci in the future may

be useful as markers for detecting individuals within the population which, due to polymorphism within target genes, have a higher risk of death through the breeding cycle.

Key words: Atlantic bluefin tuna, microsatellites, polymorphism

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EKSPRESIJA I IDENTIFIKACIJA NOVIH PROTEINA DJELOMIČNO NEUREĐENE TERCIJARNE STRUKTURE (IDP) IZ BILJKE UROČNJAK (*Arabidopsis thaliana*) NAKON IZLAGANJA ABIOTIČKOM STRESU

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Nativno neuređeni proteini (engl. *intrinsically disordered proteins*, IDP) pripadaju nedavno priznatoj skupini proteina koji su biološki aktivni usprkos nedostatku jasno definirane tercijarne strukture. Takvi proteini često su adapterske ili signalne molekule i šaperoni. Smatra se da imaju važnu ulogu u odgovoru biljaka na stres, no o tome nema mnogo podataka. Od velike je važnosti razjasniti molekularne mehanizme odgovora na stres jer on značajno ograničava rast i prinos industrijskih biljaka. Cilj ovog istraživanja bio je analizirati ekspresiju i identificirati IDP u biljci uročnjaku *Arabidopsis thaliana* nakon izlaganja hladnoći. Globularni proteini eliminirani su zagrijavanjem uzoraka na 99 °C. IDP i ostali globularni proteini razdvojeni su nativnom i denaturirajućom 2D PAGE s 8M urejom. U biljaka tretiranih hladnoćom, denzitometrijska analiza ukazala je na izostanak ekspresije 10 proteina, smanjenje ekspresije jednog i povećanje ekspresije četiri proteina u odnosu na kontrolu. Masenom spektrometrijom identificirano je 29 različitih proteina unutar 40 proteinskih mrlja. Bioinformatičkim alatom PrDOS za procjenu neuređenosti proteina na osnovi analiza primarne strukture otkriveno je 13 novih IDP. Analizom prema rječniku Gene Ontology pokazano je da većina proteina sudjeluje u abiotičkom stresu vezivanjem različitih bioloških molekula, kao što su DNA, RNA i proteini. Kao prvo istraživanje IDP u proteomu biljnog organizma ovaj rad je značajan za čitavo područje IDP.

Ključne riječi: adapterske molekule, šaperoni, procjenitelji neuređenosti, stres hladnoća

EXPRESSION AND IDENTIFICATION OF NOVEL INTRINSICALLY DISORDERED PROTEINS IN *Arabidopsis thaliana* DURING ABIOTIC STRESS

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Intrinsically disordered proteins and protein regions (IDP/IDR) are a recently recognized group of proteins which are biologically active despite their lack of a well-defined three-dimensional structure. IDPs are often chaperones, adapter or signaling molecules. They are thought to have important roles in abiotic stress response, but supporting data is still scarce. It is important to clarify the molecular mechanisms of abiotic stress response because stress can severely limit industrial plant growth and yield. The aim of this study was expression analysis and IDP identification in the model plant *Arabidopsis thaliana* during cold stress. Globular proteins were eliminated by heating extracts at 99 °C. IDPs and remaining globular proteins were separated using a native/8M urea 2D polyacrylamide gel electrophoresis. Densitometric

analysis indicated that, when compared to the control group, cold-treated plants lacked expression of ten proteins, while four were upregulated and one was downregulated. Mass spectrometry identified 29 different proteins from a total of 40 excised spots. 13 novel IDPs were discovered using PrDOS, a sequence-based bioinformatic disorder prediction tool. Gene Ontology analyses indicated that the majority of the proteins were involved in abiotic stress through binding of different ligands, such as DNA, RNA and proteins. As the first study of IDPs in a plant proteome, our research will have a significant impact on the whole IDP field.

Key words: adapter molecules, chaperones, disorder predictors, disorder prediction software, cold stress

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GENOMI ŠKOLJKAŠA UKAZUJU NA TRANSPOZICIJSKU MOBILNOST SATELITNIH PONAVLJANJA

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Satelitna DNA (satDNA) i transponirajući elementi (TE) čine dvije najznačajnije grupe ponovljene DNA u eukariotskim genomima. S obzirom na nedostatak informacija o DNA sekvencama koje okružuju nizove satDNA, naš je cilj bio istražiti sekvence vezane za satelitna ponavljanja koristeći se školjkašima kao modelnim organizmima. Asemblirani genom kamenice *Crassostrea gigas* (Zhang et al. 2012) pretražen je na sekvence koje okružuju njezinu satDNA Cg170 (Clabby et al. 1996). Također, u tri druge vrste školjkaša (*Ruditapes decussatus*, *R. philippinarum* i *Donax trunculus*) eksperimentalnim pristupom dobivene su sekvence nekoliko ranije opisanih satDNA i njihovih okruženja. Nekoliko fragmenata je nasumično odabrano i detaljno opisano. Dobiveni rezultati pokazuju da se TE-slične sekvence (naročito DNA transpozoni) nalaze u blizini satDNA te ukazuju da je intenzivno širenje satDNA sekvenci u genomu vjerojatno uzrokovano procesima koji su vezani za transpoziciju. Navedeno promiče satDNA u vrlo dinamične sekvence koje bi mogle predstavljati građevne jedinice genoma na sličan način te usporedivog značaja kao što su to TE. Stoga, iako se tradicionalno smatraju dvjema odvojenim grupama, satDNA i TE se vrlo vjerojatno intimno isprepliću u procesu širenja po genomu, mnogo više nego što se to dosad smatralo.

Ključne riječi: satelitna DNA, transponirajući elementi, okruženja uzastopnih ponavljanja, školjkaši

BIVALVE GENOMES SUGGEST TRANSPOSITION-DRIVEN MOBILITY OF SATELLITE DNA REPEATS

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Satellite DNAs (satDNAs) and transposable elements (TEs) are two most abundant classes of repetitive DNAs found in eukaryotic genomes. Since knowledge regarding DNA sequences that surround satDNA repeats in the genome is mostly missing, our aim was to explore satDNA-associated sequences using bivalve genomes as a model system. Here, we searched the genome assembly of the oyster *Crassostrea gigas* (Zhang et al. 2012) to explore the surroundings of its satDNA Cg170 (Clabby et al. 1996). In addition, surroundings of several satDNA sequences already characterized in three other bivalve species (*Ruditapes decussatus, R. philippinarum* and *Donax trunculus*) were gained experimentally. For a detailed study, some fragments were randomly selected and annotated. Obtained results revealed TE-like

sequences, mostly DNA transposons, in the closest vicinity of satDNAs and indicate that intensive shuffling of satDNA sequences in the genome is predominantly driven by a transposition-related process. These findings promote satDNAs as highly dynamic sequences that might represent genome builders in a similar way and with a comparable relevance as TEs. Thus, although traditionally considered as two distinct classes, satDNAs and TEs seem to be much more intimately interlinked in the process of spreading throughout the genome than previously thought.

Key words: satellite DNA, transposable elements, tandem repeat flankings, bivalves

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RAZLIKE U OTPORNOSTI NA ANTROPOGENI STRES IZMEĐU INVAZIVNE I NATIVNE VRSTE ŠKOLJKAŠA

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Otpornost na promjene u okolišu često se navodi kao jedan od ključnih čimbenika uspjeha invazivnih stranih vrsta. No, empirijski dokazi za pretpostavku kako invazivne vrste bolje od autohtonih podnose nepovoljne uvjete u okolišu su malobrojni. U ovom smo istraživanju usporedili otpornost na toplinski stres i onečišćenje cinkom ($ZnCl_2$) kod dvije filogenetski srodne vrste slatkovodnih školjkaša, autohtone vrste *Anodonta anatina* i invazivne strane vrste *Sinanodonta woodiana*. Procijenili smo njihov odgovor na stres koristeći nekoliko testova staničnog odgovora: stopu metabolizma (sustav prijenosa elektrona, eng. *electron transport system* - ETS), aktivnost mehanizma multiksenobiotičke otpornosti (eng. *multixenobiotic resistance* - MXR), i enzimatske biomarkere stresa kolinesterazu (ChE), glutation-S-transferazu (GST) i katalazu (CAT). Sveukupno, invazivna *S. woodiana* je mnogo bolje podnosila nepovoljne uvjete. Ovo je bilo vidljivo iz (i) značajno veće aktivnosti MXR mehanizma; (ii) značajno više ETS aktivnosti, i (iii) nižeg odgovora enzimatskih biomarkera stresa u uvjetima toplinskog stresa i onečišćenja cinkom. Veća otpornost na toplinski stres je vrlo važna fiziološka osobina za invazivni uspjeh vrste *S. woodiana*, posebice u kontekstu klimatskih promjena.

Ključne riječi: invanzivni uspjeh, toplinski stres, onečišćenje cinkom, slatkovodni školjkaši, stopa metabolizma, aktivnost MXR mehanizma

DIFFERENCES IN TOLERANCE TO ANTHROPOGENIC STRESS BETWEEN INVASIVE AND NATIVE BIVALVES

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Tolerance towards environmental stress has been frequently considered as one of the key determinants of invasion success. However, empirical evidence supporting the assumption that invasive species endure better unfavourable conditions compared to native species is limited and has even yielded opposing results. We examined the tolerance to thermal stress and heavy metal zinc pollution ($ZnCl_2$) in two phylogenetically related and functionally similar freshwater bivalve species, native *Anodonta anatina* and invasive *Sinanodonta woodiana*. We assessed their response to stress using several cellular response assays: metabolic rates (ETS - electron transport system), efficiency of the multixenobiotic resistance (MXR) mechanism activity, and enzymatic biomarkers (ChE - cholinesterase, GST - glutathione-S-transferase and CAT - catalase). Overall, invasive S. woodiana coped muc h better with unfavourable conditions. This was evident from (i) significantly more pronounced MXR mechanism activity; (ii) significantly higher ETS activity, and (iii) lower response of stress-related enzymes (ChE, GST and CAT) under thermal stress and $ZnCl_2$ pollution. The overall better tolerance to thermal extremes is an especially important physiological benefit for further invasion success of *S. woodiana* in European freshwaters, especially in the context of climate change.

Key words: invasion success, thermal stress, heavy metal zinc pollution, freshwater mussels, metabolic rate, MXR mechanism activity

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ISPITIVANJE ULOGE STANIČNIH miRNA U REPLIKACIJI HERPES SIMPLEKS VIRUSA 1

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Tijekom infekcije virusi značajno mijenjaju biologiju stanice te pritom određuju i usmjeravaju njezine funkcije u smjeru efikasne replikacije. Tako je nedavno pokazano da virusna infekcija dovodi i do značajnih promjena u ekspresiji miRNA domaćina, a za što se smatra da bi moglo dovoditi do stvaranja pogodnih uvjeta za replikaciju virusa. Promjene u ekspresiji staničnih miRNA kod infekcije herpes simpleks virusa 1 (HSV-1) uočene su u nekoliko studija, međutim istraživanja disregulacije miRNA domaćina te uloga takvih promjena nisu detaljno analizirane. Naša grupa je, koristeći se masivnim paralelnim sekvenciranje uzoraka produktivne i latentne infekcije HSV-1, uočila značajan porast ekspresije određenih staničnih miRNA u svim analiziranim uzorcima. Porast ekspresije miRNA dodatno smo potvrdili pomoću kvantitativne lančane reakcije polimerazom u realnom vremenu (qRT-PCR). Ovi rezultati mogli bi ukazivati na to da HSV-1 ima sposobnost indukcije ekspresije miRNA radi postizanja optimalnih uvjeta za replikaciju, ili alternativno, povećanje ekspresije miRNA domaćina moglo bi predstavljati obrambeni odgovor stanice na infekciju. Kako bi odgovorili na ova pitanja generirali smo vektore za transdukciju stanica kojima ćemo povećati ekspresiju miRNA od interesa, te smo trenutno u procesu generiranja stabilno transduciranih staničnih linija na kojima ćemo testirati virusnu replikaciju. Metodološki pristup i tehnički detalji studije biti će prikazani u ovom radu.

INVESTIGATING THE ROLES OF HOST microRNAs IN HERPES SIMPLEX VIRUS 1 INFECTION

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Viruses significantly perturb cell metabolism during infection enabling an efficient virus replication. Recently, it has been shown that virus infection leads also to significant changes in the expression of cellular miRNAs, which in turn might result in a favorable environment for the virus. Similar to other viruses studied thus far, herpes simplex virus 1 (HSV-1) induces differential expression of cellular miRNA, however the exact role of such up- or down-regulation has not been elucidated. Using massive parallel sequencing, our group has observed a significant increase in the expression of certain cellular miRNA in several different cell lines and cells latently infected with the virus. Moreover, we confirmed the increase in the expression of host miRNAs by quantitative real time polymerase chain reaction (qRT-PCR). These results indicate that HSV-1 has the ability to induce expression of the miRNAs to achieve optimal conditions for efficient replication; or alternatively, the increase in expression of host miRNAs may represent a defense response to the infection. To answer these questions, we generated a set of vectors for transduction of cells to elevate the expression of miRNAs of interest, and we are currently in the process of generating stably transduced cell lines to test viral replication. The methodological approach and the technical details of the study will be presented in this paper.

KAZALO AUTORA

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