

ABS ACTA BIOLOGICA SLOVENICA



VOL. 52 ŠT. 2 LJUBLJANA 2009

prej/formerly BIOLOŠKI VESTNIK

**ISSN 1408-3671
UDK 57(497.4)**

**izdajatelj/publisher
Društvo biologov Slovenije**

Acta Biologica Slovenica

Glasilo Društva biologov Slovenije – Journal of Biological Society of Slovenia

Izdaja – Published by

Društvo biologov Slovenije – Biological Society of Slovenia

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Acta Biologica Slovenica, Večna pot 111, SI-1001 Ljubljana, Slovenija

<http://bijh.zrc-sazu.si/abs/>

Oblikovanje – Design

Žare Vrezec

ISSN 1408-3671

UDK 57(497.4)

Natisnjeno – Printed on: 2009

Tisk – Print: Tiskarna Pleško d.o.o., Ljubljana

Naklada: 500 izvodov

Cena letnika (dve številki): 15 € za posameznike, 42 € za ustanove

Številka poslovnega računa pri Ljubljanski banki: 02083-142508/30

Publikacijo je sofinancirala Agencija za Raziskovalno dejavnost Republike Slovenije.

Acta Biologica Slovenica je indeksirana v – is indexed in: Biological Abstracts, Zoological records.

France Velkovrh, malakolog in speleobiolog

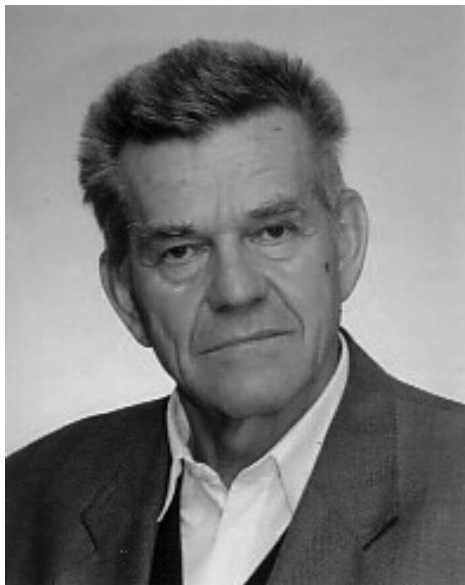
1934–2009

Vajeni smo bili, da je bil med najzagretejšimi udeleženci, kadar smo se odpravljali na raziskave po nekdanji 'širši domovini'. Tokrat je bil že več let v zasluženem pokoju, zadnje leto pa, žal ob strašno neprijetni bolezni, v bolnišnici. Na poti na 'naš' Ohrid me je dohitela vest, da se je poslovil.

France Velkovrh je bil mož, ki je vedno storil več, kot bi smeli pričakovati. Dobronamerni kolegi so to seveda spoštovali, marsikoga pa je tudi motilo, da se vmešava v zadeve, ki naj bi ga ne brigale. Ko pa je vendar bil le tehnični sodelavec. Francetovo prizadevnost so občutile – in iz nje povzele kak nauk – tudi generacije mladih jamarjev. Bil je dolga leta 'gospodar' ljubljanskega Jamarskega društva. Kaj pri jamarski dejavnosti pomeni skrb za brezhibno opremo, si lahko vsakdo predstavlja. In koliko prizadevnosti je potrebne, da najstnika pripraviš k vestnem čiščenju blatnih vrvi in žičnih lestvic, tudi. Do sebe je bil le v eni zadevi manj zahteven.

Francetova edina resna napaka je bila, da se ni mogel pripraviti, disciplinirati, za pravšnje dokončanje študija, čeprav navdušen za naravo in razgledan v naravoslovju. Ostal je pri 'višješolski' stopnji. Vseeno je bil biolog, bil je znanstvenik z znanstveno bibliografijo, ki je bila v sedemdesetih in osemdesetih letih kar spoštovanja vredna in je ni zmozel prenekateri raziskovalec ali učitelj. S svojim znanjem je sodeloval tudi pri pouku. Največ pri terenskih vajah. Pa tudi pri laboratorijskih vajah smo ga (res malce mimo pravil, a s pridom) zadolžili za vaje z mehkužci. Nek študent se je nekoč pritožil, da oni 'niso kaki stroji za določanje mehkužcev' – spet prevelika zagnanost? Merila so pač od osebe do osebe različna.

Bil je predvsem neprekosljiv tehnični pomočnik, v hiši, kot na terenu. Poseben izziv mu je bila priprava opreme in druge prtljage za terenske raziskave. Skupaj smo obšli kar lepe kose Jugoslavije. Zlasti lepo število kraških jam in seveda imenitno Ohridsko jezero. Po povratku je bila njegova osnovna zadolžitev obdelava grobo zajetih



vzorcev, iz katerih je pobiral živali za znanstveno obdelavo. Tudi temeljitost terenskih raziskav je bila v veliki meri prav njegova zasluga. In tako je v veliki meri njegova zasluga sorazmerno dobra raziskanost podzemeljske favne na dinarskem krasu in v intersticijskih vodah ob njem. Veliko tega gradiva je še neobdelanega v zbirkah in bo zaposlovalo taksonome še nekaj časa – če bodo na voljo 'sredstva' v ta namen. Seveda je tudi pri raziskavah presegel vlogo le tehničnega pomočnika, kar smo primerno upoštevali pri soavtorstvu znanstvenih prispevkov.

Francetu najljubše pa je bilo delo z mehkužci, zlasti s polži in spet zlasti s podzemeljskimi. Zaradi njih je ostajal v laboratoriju cele (neplačane) dneve. Prekuhal je kilograme jamske prsti, da je iz nje izbrskal po nekaj milimetrskih polžjih hišic. Tako je prispeval k poznavanju razširjenosti vrst, vendar pa je našel in znanstveno opisal tudi 13 novih, dotlej neznanjih vrst; opisal je tudi nove rodove, pod lepimi imeni *Phreatica*, *Istriana*, *Dalmatella*.

In rezultat te prizadevnosti je izjemno bogata malakološka zbirka, verjetno kar ena najbogatejših v Evropi, ki jo ima zdaj v oskrbi Prirodoslovni muzej Slovenije.

Za konec še nekaj pustih podatkov. France Velkovrh je bil rojen 24. februarja 1934 v Ljubljani. Njegov oče je padel leta 1943 kot partizan. Gimnazijo je zaključil z odliko. Študij biologije na UL je vpisal leta 1953, že naslednje leto pa se je zaposlil; najprej na Inštitutu za zdravstveno hidrotehniko FAGG UL, nato na Inštitutu za biologijo Univerze in od 1965 do upokojitve na Oddelku za biologijo Biotehniške fakultete UL. Študij biologije je zaključil s fakultetno izobrazbo I. stopnje (višjo strokovno izobrazbo).

Prejel je več priznanj:

1958: študentsko **Prešernovo nagrado Univerze v Ljubljani** za obdelavo teme 'Nove najdbe in pripombe k dosedanjim opisom podzemnih gastropodov iz porečja Ljubljaničice';

1970: **Srebrno značko** za dolgoletno jamarsko organizacijsko in raziskovalno delo (bil je član DZRJL od 1949);

1977: **Plaketo** v znak priznanja za prispevek k razvoju fakultete in njenih enot ter **Medaljo dela**;

1987: **Red dela s srebrnim vencem** za zasluge in uspehe pri delu, pomembne za napredek države.

Kot svojevrstno priznanje lahko štejemo tudi poimenovanje živalskih vrst, s katerim s(m)o ga želeli počastiti strokovni kolegi. Po njem smo poimenovali en imeniten rod in sedem vrst iz različnih živalskih skupin. Tako endemnega in edinega jamskega ožigalkarja, jamskega trdoživa ali velkovrhijo (*Velkovrhia enigmatica* Matjašič et Sket 1971). Takšni so tudi polžki jamski prilepek *Acroloxus velkovrhi* Bole 1965, pa *Truncatellina velkovrhi* Štamol 1995 in *Gyalina velkovrhi* Riedel 1985; deževnik *Dendrobaena velkovrhi* Mršič 1988 in podzemeljska rakca, ceponožec *Elaphoidella franci* Petkovski 1983 ter jamski ježek *Monolistra velkovrhi* Sket 1960.

France se je poslovil od nas

1. oktobra 2009.

O vsebini njegovega dela naj pričajo naslovi objavljenih znanstvenih in poljudnih člankov:

BOLE J. & F. VELKOVHR 1986: Mollusca from continental subterranean aquatic habitats; v: Botosaneanu L (editor): Stygofauna Mundi. A Faunistic, Distributional and Ecological Synthesis of the World Fauna inhabiting Subterranean Waters, Leiden. str. 177–208.

BOLE J. & F. VELKOVHR 1987: Nove vrste podzemeljskih polžev Jugoslavije. [Neue Arten der unterirdischen Schnecken Jugoslawiens]. Razprave (Dissertationes), classis IV, SAZU 28(3): 69–83.

BREZIGAR A. & F. VELKOVHR 1987: Malakofavna v: Paleontološke raziskave pliokvartarne skladovnice velenjske udorine. [Paleontological investigations of the Plio-Quaternary beds of the Velenje Depression]. Geologija 28/29 (1985/86): 98–99.

BREZIGAR A., G. KOSI, D. VRHOVŠEK & F. VELKOVHR 1983: Paleontološke raziskave pliokvartarne skladovnice velenjske udorine. Referat na simpoziju: Geologija Šaleške kotline, 1983. – Geološki zbornik 3: 31–33.

HERSHLER R. & F. VELKOVHR 1993: A new genus of hydrobiid snails (Prosobranchia: Rissoidea) from northern south America. Proceedings of the Biological Society of Washington, 106: 182–189.

KORNIUSHIN A. V. & F. VELKOVHR 2005: Two new species of the bivalve molluscs from the Balkan Lake Prespa. (verjetno neobjavljeno)

MORTON B., F. VELKOVHR & B. SKET 1998: Biology and anatomy of the »living fossil« *Congeria kuscerei* (Bivalvia: Dreissenidae) from subterranean rivers and caves in the Dinaric karst of the former Yugoslavia. Journal of Zoology London 245: 147–174.

NOVAK D., F. VELKOVHR, F. LEBAN & A. KRANJC 1988: Gradivo za Slovensko speleološko bibliografijo z bibliografijo. Naše jame 30: 1–192.

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- SKET B. & F. VELKOVRH 1979: The discovery of *Proteus*-eggs (*Proteus anguinus*) in seminatural conditions. *International Journal of Speleology* 10(2): 205–209.
- SKET B. & F. VELKOVRH 1981: Phreatische Fauna in Ljubljansko polje (Ljubljana–Ebene, Jugoslavi-en) – ihre ökologische Verteilungen und zoogeographische Beziehungen. *International Journal of Speleology* 11: 105–121.
- SKET B. & F. VELKOVRH 1981: Postojnsko–planinski jamski sistem kot model za preučevanje onesnaženja podzemeljskih voda. [The Postojna–Planina–CaveSystem as a model for the investigations of the polluted subterranean rivers]. *Naše jame* 22: 27–44.
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Boris Sket

**Razvoj zoofiziologije na Biološkem oddelku Biotehniške fakultete
in Inštitutu za biologijo do leta 1987 – osebni pogled**

**History of animal physiology at the Department of Biology and Institute
of Biology, Ljubljana – a personal view**

Matija GOGALA

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Izvleček: V tem pregledu podajam svoj osebni pogled na začetke in razvoj zoofiziološkega laboratorija na Univerzi v Ljubljani do mojega odhoda s fakultete toda s projekcijami v sedanji čas. Za popolnejšo zgodovino laboratorija bi bil potreben dolgotrajnejši študij dokumentacije in arhivov Univerze v Ljubljani, Biološkega oddelka BF, Biotehniške fakultete in Inštituta za biologijo.

Ključne besede: zoofiziologija, fiziologija živali, Biološki oddelek BF, Inštitut za biologijo, Univerza v Ljubljani

Abstract: This is a personal view to the development of the zoophysiology laboratory at the Biology department of Biotechnical faculty of the University of Ljubljana and the National Institute of Biology. This review mainly covers 27 years of my life in this institution with projections to the present state. For a comprehensive history one should thoroughly study documents in the archives of institutions, mentioned above.

Keywords: zoophysiology, animal physiology, Department of Biology BF, Institute of Biology, University of Ljubljana

Začetki

Fiziologija živali na ljubljanski univerzi ni imela srečnega začetka. Kmalu po ustanovitvi Univerze v Ljubljani so člani Filozofske fakultete leta 1921 izvolili za profesorja zoofiziologije priznanega slovenskega znanstvenika **Ivana Regna** (*1868, †1947) (sl. 1), ki je živel in delal na Dunaju in se je ukvarjal z bioakustiko žuželk, predvsem murnov in kobilic (GOGALA 2008). Vendar Regen kljub temu in kljub želji, da se vrne v domovino, tega mesta nikdar ni zasedel. Že pri volitvah ni šlo brez očitkov, da je Regen neki svoj članek po izidu popravljal, toda prof. Regen je dokazal, da ni naredil ničesar nečastnega. Iz njegovih zapiskov

pa je jasno, da je nekdo (Slovenec A. G.!) že po tej izvolitvi kandidata očrnil v Beogradu in to je bil verjetno glavni razlog, da Regen, izredno natančen in občutljiv človek, ni želel več priti v Ljubljano, kjer bi tako ali tako imel bistveno slabše razmere za raziskovalno delo kot na Dunaju.

Na Dunaju je pred 1. svetovno vojno deloval tudi **Franč Megušar** (*1876, †1916), biolog zoofiziolog, ki se je ukvarjal z razvojem žuželk, spreminjanjem barv pri žuželkah, rakih in ribah ter z ekologijo jamskih živali. Objavil je okoli 20 znanstvenih del. Od leta 1904 do 1913 je bil asistent na Dunajski univerzi, kjer je delal v vivariju, leta 1915 je dobil mesto na Kmetijski poskusni postaji v Gorici, njegovo kariero pa je



Slika 1: Prof. dr. Ivan Regen, nesojeni prvi profesor fiziologije živali na ljubljanski univerzi. Fotografija je verjetno nastala na Dunaju okoli leta 1910 (Knjižnica SAZU).

Fig. 1: Prof. Dr. Ivan Regen, first elected professor of animal physiology at Ljubljana University never took this chair. Photography has been probably done in Vienna around 1910 (SASA Library).

prekinila zgodnja smrt na fronti v 1. Svetovni vojni. Več o njem lahko beremo v Slovenskem biografskem leksikonu (SBL 1925–1991) oziroma na spletnem naslovu: <http://nl.ijs.si:8080/fedora/get/sbl:sbl/VIEW/>.

Zato je »občo« fiziologijo in primerjalno fiziologijo živali od leta 1927 do konca petdesetih let po drugi svetovni vojni predaval prof. dr. **Albin Seliškar** (*1896, †1973) (sl. 2), po raziskovalni usmeritvi nedvomno zoofiziolog, ki pa je imel svoje stalno mesto na Medicinski fakulteti. Ukvarjal se je z biologijo jamskih živali, eno od njegovih prvih del pa je na primer opis dišavnih (feromonskih) organov pri jamskih koblicah (SELIŠKAR 1923). Svoje morda najpomembnejše delo pa je objavil skupaj z A. O. Župančičem o sinaptičnem prenosu vzburjenja na živčno-mišičnem preparatu (SELIŠKAR & ŽUPANČIČ 1947). Več o njem lahko preberete v spominskih hčerke



Slika 2: Prof. dr. Albin Seliškar, fotografija iz leta 1965, ko je še predaval fiziologijo živali za študente biologije.

Fig. 2: Prof. dr. Albin Seliškar, photography from the year 1965, when he still lectured for the students of biology at the Ljubljana University.

Mojce Seliškar (1996) in v Zgodovinskem zborniku Medicinske fakultete (URLEP & al. 2003: 187–206 in 239–240). Okoli leta 1927 je s sodelavci iz Društva za raziskovanje jam uredil prvi jamski laboratorij v Podpeški jami. Po 2. svetovni vojni je organiziral jamski laboratorij v Postojnski jami in se posebej posvetil raziskavam človeške ribice. Na žalost pa o tem ni veliko objavil. Prof. Seliškar je bil zelo široko izobražen in je bil v času mojega študija biologije pri kolegih starejših letnikov na dobrem glasu kot organizator odličnih ekskurzij, kjer je študentom biologije odkrival raznolikost habitatov bivše skupne domovine in raznovrstnost živega sveta v njih. Na žalost pa se mu je na eni izmed teh ekskurzij nekaj zamerilo in ga naša generacija ni mogla več prepričati, da bi tudi nas in naslednje letnike študentov vodil na kakšno podobno pot.

Na Biološkem oddelku je proti koncu petdesetih let sicer poskušal vzpostaviti zoofiziološki laboratorij dr. **Dušan Lušicky** (*1918, †1997), asistent pri prof. dr. Jovanu Hadžiju v letih

1947–57. Imel je težave, tudi zdravstvene, in je Univerzo v letu 1957 zapustil. Iz tistega časa je ostalo nekaj raziskovalne opreme, predvsem preprost elektronski stimulator in kimograf za registracijo počasnih pojavov.

Lilijana Istenič (*12. 4. 1931) je doktorirala z ekofiziološko temo meritev porabe kisika pri vrbnicah (Plecoptera). Po njeni zaslugi je Oddelek nabavil Warburgov aparat za take meritve in smo ga kasneje uporabljali tudi v zoofiziološkem laboratoriju. V skladu s svojim delovnim mestom profesorice za primerjalno fiziologijo vretenčarjev se je v naslednjih letih usmerila v raziskave funkcionalne morfologije, še posebej v mehanizem plavanja hrustančnic in v študij močerila (*Proteus anguinus*), njegovih ampularnih organov, okušalnih brstičev in barvil.

Michielijevo obdobje

Naslednji biolog, ki je končno v letu 1961/62 tudi na Biološkem oddelku organiziral zoofiziološki laboratorij, je bil dr. **Štefan Sušec-Michieli** (1933–1968) (sl. 3). Bil je entomolog – lepidopterolog in široko izobražen biolog z veliko energije in z odličnimi organizacijskimi sposobnostmi. Promoviral je leta 1959, bil leta 1960 imenovan za docenta in leta 1965 za izrednega profesorja za primerjalno fiziologijo živali z osnovami fiziologije človeka na Biološkem oddelku BF. Dodatno se je izraževal v Nemčiji, v Tübingenu in Münchnu pri priznanih profesorjih Petru Möhresu in Hansjochemu Autrumu kot štipendist organizacije UNESCO. Doma je želel uvesti moderno zoofiziologijo z metodami tedaj novega področja elektrofiziologije, eksperimentalne etologije in kromatografije. Prednost prof. Michielija je bilo odlično poznavanje poskusnih živali, predvsem žuželk in sposobnost zastaviti poskuse tudi v izredno skromnih razmerah tako, da so rezultate s spoštovanjem sprejemali tudi tuji strokovnjaki. Tako za eksperimentalno delo na svoji disertaciji z naslovom Analiza skototaktičnih reakcij pri artropodih (MICHIELI 1959) poleg poskusnih živali ni potreboval mnogo več kot nekaj kartona, papirja, vodene barvice ter seveda poskusne živali. Uvedel je pojem perigramotaksis, razčistil pojem skototaksije, ugotavljal sposobnost barvnega gledanja pri različnih artropodih, ostrino vida



Slika 3: Prof. dr. Štefan Sušec-Michieli, ustanovitelj zoofiziološkega laboratorija Oddelka za biologijo Biotehniške fakultete in Inštituta za biologijo (sedaj NIB). Portret je delo pokojnega slikarja Florisa Obláka, ki je prof. Michielija poznal in je v tistih letih učil biološko risanje študente biologije.

Fig. 3: Prof. dr. Štefan Sušec-Michieli, founder of the zoophysiology lab at the Department of Biology, Biotechnical faculty and at the Institute of Biology (now National Institute of Biology). Portrait is a work of the late painter Floris Oblák (1969), who taught at that time drawing for biologists.

in še marsikaj drugega. Skupaj z dr. Miranom Vardjanom sta ustanovila katedro za zoo- in fitofiziologijo na Biološkem oddelku Naravoslovne in po vrsti reorganizacij Biotehniške fakultete. Prof. Michieli je v skladu s takratno dvotirno organizacijo ustanovil tudi zoofiziološki laboratorij Inštituta za biologijo Univerze v Ljubljani. Pravzaprav pa nam je bilo takrat vseeno, ali je kdo dobil mesto na inštitutu ali oddelku, delal je v istem zoofiziološkem laboratoriju. Do ločitve obeh inštitucij oziroma osamosvojitve Inštituta za

biologijo je prišlo šele proti koncu sedemdesetih let prejšnjega stoletja.

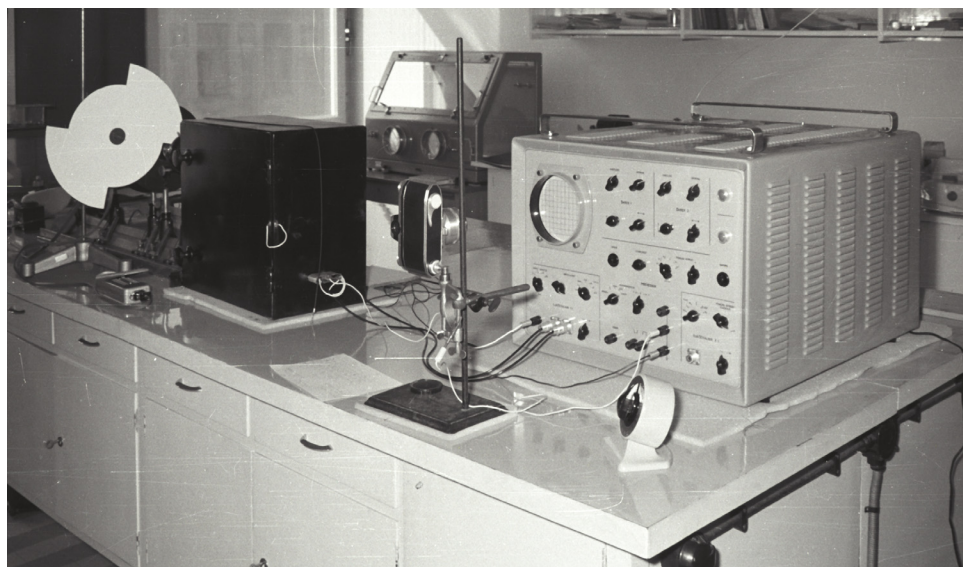
Področja raziskovanja so bila v začetku predvsem barve in menjavanje barv pri žuželkah, fotorepcija žuželk in njihova optična orientacija v prostoru. Za uvedbo elektrofizioloških metod je bilo takrat zelo težko nabaviti primerno opremo v tujini, zato se je prof. Michieli dogovoril s strokovnjaki na Fakulteti za elektrotehniko za razvoj prototipnega osciloskopa (sl. 4), katerega glavni konstruktor je bil dr. Lojze Vodovnik. Ta aparat nam je še dolga leta koristno služil, dopolnili pa smo ga še z dodatnimi predojačevalniki, delo dr. Lojzeta Kralja iz istega laboratorija in z drugo opremo. Sicer pa smo se takrat morali znati. Za vaje smo si kimografe najprej priredili s kombinacijo gramofona, mopedove transmisije in kimografske bobna. Sicer pa so bile bučike, slamice, papir, plastelin in podobne stvari takrat glavno gradivo v zoofiziološkem laboratoriju.

S Štefanom Michelijem sva se poznala že iz mojih dijaških in kasneje študentskih let zaradi obojestranskega zanimanja za žuželke. Hodil sem na entomološke sestanke, kjer sem pogosto srečeval tudi Štefana Michelija in nekajkrat sva

šla tudi skupaj na entomološke izlete. Po diplomi (1959) pred odhodom k vojakom sem bil pol leta pri njemu volonter v Biološkem inštitutu Slovenske akademije znanosti in umetnosti, kjer sem med drugim delal prva opazovanja zvočne komunikacije pri stenicah s preprostimi napravami, kot je stetoskop in z redkimi gostovanji na Radiu Ljubljana, kjer so mi naredili prve magnetofonske zapise teh signalov.

Po diplomi na ljubljanski univerzi leta 1960 mi je prof. Božo Škerlj, antropolog, ponudil možnost izpopolnjevanja v ZDA za genetiko, saj ta pomembna veja biologije takrat na Biološkem oddelku še ni bila zasedena. Ponudba je bila mamljiva, po temeljitem premisleku in presoji argumentov za in proti pa sem se odločil, da останem pri drugi možnosti, delu na fiziologiji živali, kar mi je ponujal takratni docent in odlični mentor dr. Štefan Michieli.

Leta 1961 sem postal prvi asistent pri Štefanu Micheliju. Po moji izvolitvi sva skupaj raziskovala barve in prebarvanje žuželk, predvsem stenic, ki sem jih dobro poznal in tudi favnistično preučeval, in ravnokrilcev. Pri mnogih vrstah zeleno obarvanih stenic in drugih žuželk pride jeseni do



Slika 4: Prvi osciloskop zoofiziološkega laboratorija in dodatne aparature za stimulacijo oči in registracijo elektroretinogramov. Slika je nastala v letu 1963 ali 1964.

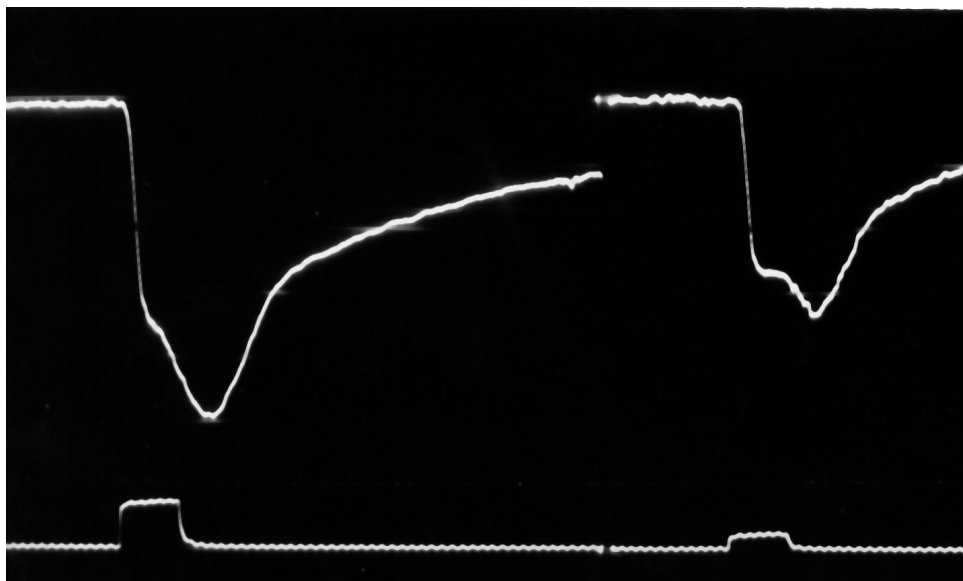
Fig. 4: First oscilloscope and additional equipment in the zoophysiology lab for electroretinography. Photography from the year 1963 or 1964.

sezonskega prebarvanja na rjavo ali rdečerjavo, kar je seveda primerna kriptična obarvanost. Zanimal nas je torej mehanizem tega prebarvanja in dejavniki, ki te procese sprožijo. Za ločevanje barvil smo uporabljali papirno kromatografijo (takrat »metodo revnih ljudi«), za študij okoljskih dejavnikov pa improvizirano klimatsko komoro. Ker boljše opreme takrat nismo premogli in tudi nismo uspeli pridobiti za sodelovanje kemika, pri teh raziskavah nismo bili posebej uspešni. Vsekakor pa smo uspeli pri vrsti *Nezara viridula* dokazati povezavo z zimsko diapavzo in svetlobne in temperaturne pogoje, ki sprožijo prebarvanje (GOGALA & MICHIELI 1966).

Ukvarjali smo se tudi s konstrukcijo svetlobnih pasti za žuželke (MICHIELI & GOGALA 1962), obenem pa smo pripravljali laboratorij za elektrofiziološke raziskave čutil. S skromno opremo, ki smo jo uspeli dobiti, ali jo je po naših nestrokovnih skicah izdelal mojster A. Debeljak na Oddelku za psihologijo Filozofske fakultete, sva v začetku šestdesetih let končno pričela z elektoretinografskimi meritvami občutljivosti

oči nevretenčarjev, predvsem žuželk. Iz tega je nastalo delo o monofazičnih in difazičnih retinogramih pri insektih (sl. 5) (GOGALA & MICHIELI 1964), nato publikacija o stopitveni frekenci žuželčjih oči (MICHIELI 1965) ter primerjalna študija spektralne občutljivosti žuželk (MICHIELI 1966). V okviru svoje doktorske disertacije (1964) pa sem histološko in elektrofiziološko proučeval oči jamskih kobilic, predvsem vrste *Troglophilus neglectus* (GOGALA 1966). Naj omenim, da sem pri tem kot prvi biolog v Sloveniji uporabil pri delu na disertaciji računalnik Zuse Z-23, seveda z izdatno pomočjo bratranca matematika dr. Zvonimirja Bohteta. Čeprav so bile računske operacije preproste, iskanje krivulje, ki bi se najlepše prilegala izmerjenim vrednostim amplitud ERG kot odgovor na različno intenziteto svetlobe, je bil s tem za naš laboratorij storjen prvi pomemben korak v digitalno dobo.

V letu 1964 sem tudi jaz odšel na izpopolnjevanje v inozemstvo kot štipendist ustanove Aleksandra von Humboldta. Eno leto sem bival na Zoološkem inštitutu Univerze v Münchnu pri



Slika 5: Dva zaporedna posnetka elektoretinogramov jamske kobilice (*Troglophilus neglectus*). Spodnja sled je zapis svetlobnega dražljaja, zgornja sled pa prikaz ERG dveh dražljajev različne jakosti. Ti zapisi so bili narejeni z osciloskopom, prikazanim na sliki 4.

Fig. 5: Two successive ERGs of the cave cricket (*Troglophilus neglectus*). The lower trace represents the light stimulus and the upper trace the ERGs to two light stimuli of different intensity. The recording has been reproduced from the screen of the oscilloscope, shown in Fig. 4.

svetovno znanem zoofiziologu prof. dr. Hans-jochemu Autrumu, ki se je s svojimi sodelavci vred ukvarjal s fiziologijo čutil. Tja sem prišel z lepo doto iz našega laboratorija, odkritjem specializiranih oči za ultravijolično svetlobo metuljčnice *Libelloides* (ali po starem *Ascalaphus*) *macaronius* (GOGALA & MICHIELI 1965 in GOGALA 1967). Tudi to je bil rezultat primerjalnih raziskovanj spektralne občutljivosti žuželk v našem zoofiziološkem laboratoriju. V Münchnu je oko metuljčnice vzbudilo veliko zanimanja, saj je spektralna občutljivostna krivulja dala slutiti, da gre za zelo drugačne mehanizme v očesu, kot na primer pri muhah *Calliphora erythrocephala* in drugih njihovih standardnih objektih. Zato sem lahko ponovil meritve na dveh merilnih napravah z mnogo večjo natančnostjo (GOGALA 1967). Ena od teh naprav je bila v laboratoriju dr. Kurta Hamdorfa (*4. 10. 1929 †21. 5. 2009), ki se je zelo zanimal za te poskuse in s katerim smo več let kasneje vzpostavili večletno tvorno sodelovanje z vrsto odmevnih skupnih publikacij tudi v reviji *Natura*.

Nekaj mesecev pa sem v Münchnu delal tudi v laboratoriju dr. Christiana Hoffmana, ki je takrat raziskoval delovanje čutilnih dlačic – trihobotrijev pri škorpionih. Izkušnje iz tega laboratorija so nam koristile kasneje v domačem laboratoriju, ko je Kazimir Drašlar, član zoofiziološke katedre Biološkega oddelka Biotehniške fakultete od leta 1969, pripravljal svoje magistrsko delo in kasneje doktorsko disertacijo na trihobotrijih stenic šuštarjev (*Pyrrhocoris apterus*) (DRAŠLAR 1977).

Na žalost je Štefan Michieli, ki je trpel za astmo, umrl zelo mlad 29. junija 1968.

Obdobje 1968–1987

Vodenje katedre, ki je pred smrtjo Štefana Michielija obsegala še asistenta Boruta Ženerja ter nepogrešljivi tehnični sodelavki Marjeto Grmič (sedaj M. Grmič Tkalec) in Malči (Amalija) Blažević, sem po smrti prof. Michielija prevzel že kot docent. **Borut Žener** (*13. 5. 1935 †6. 1. 1974) je zasedel mesto asistenta na katedri za zoo- in fito-fiziologijo leta 1964. Bil je odličen akvarist, ki je napisal prvo obsežnejšo slovensko knjigo o akvaristiki (ŽENER 1964), v našem laboratoriju se je posvetil meritvam porabe kisika med barvnimi

spremembami, ki spremljajo zimsko diapavzo pri stenici *Nezara viridula* (MICHIELI & ŽENER 1968). Kasneje je meril tudi spreminjanje stopitvene frekvence očesa med zimsko diapavzo teh žuželk (ŽENER 1971). Tudi on je bil Humboldtov štipendist v letih 1969/70 in sicer pri prof. Dietrichu Burkhardtju na univerzi v Frankfurtu in na Inštitutu za ribištvo v Wiesbadnu. Želel je raziskovati čutilne sposobnosti močerila (*Proteus anguinus*) vendar tega tedanje laboratorijske možnosti razen preliminarnih poskusov niso omogočale. Leta 1973 pa je zapustil laboratorij in se zaposlil kot profesor biologije na gimnaziji v Mostah v Ljubljani. Umrl je leta 1974, star niti 39 let (GOGALA 1973 in SBL 1925–91).

Omenil sem že **Kazimirja Drašlarja**, ki se nam je pridružil v zoofiziološkem laboratoriju najprej med študijem kot demonstrator, po diplomi leta 1965 kot štipendist Sklada Borisa Kidriča, po smrti prof. Michielija pa je postal asistent. Posvetil se je raziskavam funkcije trihobotrijev pri stenici *Pyrrhocoris apterus* z morfološkimi in elektrofiziološkimi metodami in iz te tematike sta njegovo magistrsko in doktorsko delo (DRAŠLAR 1972, 1977). Da se to delo nadaljuje do danes, dokazuje letošnja objava članka v uglednem časopisu (ŠKORJANC et al. 2009). Po njegovem prizadevanju je Biološki oddelek že leta 1975 nabavil opremo za vrstično elektronsko mikroskopijo (vrstični elektronski mikroskop Cambridge 600) in dr. Drašlar je postal tudi prvi strokovnjak Biološkega oddelka BF na tem področju (glej spletno stran: <http://web.bf.uni-lj.si/bi/mikroskopija/mikroskop-sem.php>). To je bilo seveda pomembno tudi za nadaljnji razvoj zoofiziološkega laboratorija (npr.: DRAŠLAR & GOGALA 1978). Kazimir Drašlar je sedaj izredni profesor za fiziologijo živali in v tem mandatu predstojnik Biološkega oddelka oziroma po novem prodekan Biotehniške fakultete.

Morda je prav, da tu omenim še obdobje, ko sem prevzemal vodstvo katedre in laboratorija za zoofiziologijo. Nenadoma sem imel toliko obveznosti, da sem zaprosil za pomoč kolege fiziologe na Medicinski fakulteti, s katerimi smo se pogosto srečevali na sestankih Društva za fiziologijo in raznih znanstvenih srečanjih. Ti so mi bili takoj pripravljeni pomagati in so v teh prvih letih prevzeli nekatere cikle predavanj za biologe. Posamezna predavanja so takrat in še v poznejših

letih prevzeli tudi kolegi iz Laboratorija za medicinsko elektroniko in biokibernetiko Fakultete za elektrotehniko pod vodstvom prof. Vodovnika in dr. Lojzeta Kralja. To je bilo še več vredno, ker so takrat nekateri biologi želeli preusmeriti raziskave laboratorija v povsem druge vode ...

Odkritje že omenjenega poskusnega objekta *Ascalaphus macaronius* je bilo povod za vrsto raziskav, večinoma v sodelovanju z laboratorijem prof. dr. Kurta Hamdorfa, ki se je s tem fenomenom seznanil že med mojim bivanjem v Münchnu, ko pa je zasedel profesorsko mesto na univerzi v Bochumu in je skupina študentov in profesorjev iz Ljubljane obiskala to univerzo, je to sodelovanje steklo. Eden najpomembnejših rezultatov skupnih raziskav je odkritje in izolacija očesnega pigmenta metuljčnice *Ascalaphus* z enakim retinalom, kot je v rodopsinskih molekulah vretenčarjev (GOGALA et al. 1970; HAMDORF et al. 1971; SCHWEMER et al. 1971). Zaradi odkritja termostabilnosti metarodopsina so se odprla tudi zanimiva vprašanja mehanizmov adaptacije v žuželčjih očeh (HAMDORF & GOGALA 1973). Na tem enkratnem poskusnem objektu še danes delajo sedanji sodelavci zoofiziološkega laboratorija, njihovi študenti in sodelavci iz drugih držav (BENTROP et al. 2001, PANGRŠIČ et al., 2005).

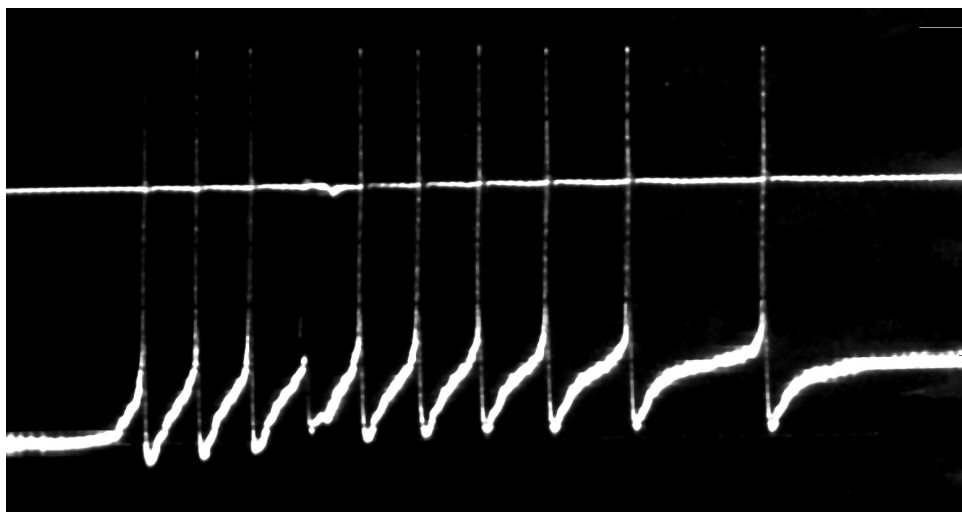
Peter Stušek je pričel z delom v zoofiziološkem laboratoriju leta 1970, do 1972 je bil zaposlen pri Inštitutu za biologijo, od leta 1973 pa na Biološkem oddelku, kjer je še danes. V začetku se je ukvarjal z elektrofiziološkimi meritvami oči in ocelov izbranih vrst žuželk (STUŠEK & GOGALA 1971), veliko energije je vložil v razvoj raznih poskusnih naprav, kasneje pa se je vključil v mednarodno sodelovanje z laboratorijem prof. Kurta Hamdorfa pri raziskavah adaptacijskih procesov v očeh nevretenčarjev (HAMDORF et al. 1978, STUŠEK & HAMDORF 1999). Je tudi avtor ali soavtor različnih srednješolskih učbenikov biologije oziroma fiziologije. Pomembno je njegovo delo pri razvoju eksperimentalnih metod, posebej mikrogazometričnih meritev očesnega metabolizma v različnih adaptacijskih stanjih (PANGRŠIČ et al., 2005). Mikrogazometrijo s Kartezičevim plavačem oziroma mikrogazometrično tehniko so v osnovi razvili na Patofiziološkem inštitutu Medicinske fakultete v Ljubljani, Stušek pa je to metodo dopolnil za meritve na očesnih preparatih ob različnih svetlobnih dražljajih in adaptacijskih

stanjih. Rezultati njegovih meritev porabe kisika na očeh žuželk so se precej razlikovali od meritev drugih avtorjev, predvsem tudi prof. Hamdorfa, ki je uporabljal drugačne merske metode. Po mnogo letih pa so drugi avtorji potrdili Stuškove meritve.

Skupaj smo člani laboratorija predavali in vodili vaje iz splošne fiziologije z osnovami fiziologije človeka ter primerjalne fiziologije. Kasneje so se naslovi predmetov in obseg snovi nekoliko spreminjali, osnovnim predmetom smo dodajali izbirne predmete, npr. nevrofiziologijo, fiziologijo čutil, orientacije in komunikacije ter fiziologijo človeka, posamezne teme ali predmete smo predavali tudi študentom iz Mariborske univerze in drugih fakultet in to se nadaljuje še danes. Od začetnih preprostih razmer ob izvedbi vaj smo počasi prišli do boljše opreme in smo na primer tudi pri vajah lahko pokazali intracelularne potencialne živčnih celic (sl. 6) in še marsikaj drugega.

Omeniti moramo še zanimivo tematiko, ki je povezana s spektralno občutljivostjo žuželčjih oči, namreč s skritimi ultravijoličnimi vzorci na telesu oziroma krilih nekaterih žuželk. S tem se je med drugim ukvarjal **Mitja Grosman**, član zoofiziološkega laboratorija v letih 1973/4 kot tehnični asistent na Biološkem oddelku in nato s presledki zaposlen na Inštitutu za biologijo v letih 1974 do 1990 (GROSMAN M. & P. STUŠEK 1982). Tema njegovega magisterija (1976) pa je bila raziskava posebnosti vidnega sistema pri gekonu *Hemidactylus turcicus*. V zvezi s tem je bil nekaj mesecev v Kotorju v Mednarodnem laboratoriju za raziskave možganov, ki ga je vodil dr. Robert Siminoff iz ZDA. Dr. Siminoff je tudi večkrat obiskal naš laboratorij in nam je posodil specializiran računalnik CAT (Computer of average transients), od nas pa si je tudi izposodil nekatere naprave. Pri nas je izšel slovenski prevod njegovega učbenika Bioelektrika, do tesnejšega sodelovanja pa ni prišlo. Mitja Grosman je bil v letih 1984 do 1988 direktor Inštituta za biologijo, kasneje je deloval v znanstveni redakciji RTV Ljubljana, sedaj pa vodi zasebno podjetje.

Druga tematika, s katero smo se začeli ukvarjati predvsem po moji vrnitvi iz Nemčije in po prvi donaciji opreme ustanove A. v. Humboldt v šestdesetih letih, je bioakustika žuželk. Končno smo s polprofesionalnim magnetofonom in primernimi mikrofoni lahko posegli tudi v svet akustične komunikacije pri živalih. To delo se je



Slika 6: Eden prvih zapisov intracelularnih akcijskih potencialov nevronov morskega polža iz rodu *Aplysia* narejenih pri vajah z našimi študenti v sedemdesetih letih prejšnjega stoletja.

Fig. 6: One of the first intracellular spike recordings from the slug *Aplysia* photographed during the laboratory training of our students in the seventies of the last century.

začelo z raziskovanjem zvočnih signalov stenic iz družine Cydnidae (GOGALA 1969, 1970), z ugotovitvijo, da gre za prenos teh signalov prek podlage (GOGALA et al. 1974) in nato s širjenjem raziskav tudi na druge družine in redove žuželk. Tako smo že v šestdesetih letih kot standardno poskusno žival začeli bioakustično raziskovati tudi zelene smrdljivke (*Nezara viridula*) (ČOKL et al. 1972). Tudi sedaj še potekajo raziskave na tem objektu v enoti NIB Entomologija pod vodstvom prof. dr. **Andreja Čokla** (ČOKL et al. 1999, 2000, 2005), ki prav tako izvira iz nekdanje zoofiziološke enote Inštituta za biologijo. Andrej Čokl (*16.6.1947) je od leta 1971 delal v zoofiziološkem laboratoriju, od leta 1974 pa je redno zaposlen na Inštitutu za biologijo, sedaj Nacionalnem inštitutu za biologijo. Raziskovalno je delal v zoofiziološkem laboratoriju na vibracijski in zvočni komunikaciji žuželk, največ na stenici *Nezara viridula*, pa tudi na drugih vrstah stenic in na kobilicah. Ukvarjal se je tudi s termorecepcijo pri žuželkah (ČOKL 1972). Bil je štipendist ustanove A. v. Humboldt v letih 1979/80 in se je takrat izpopolnjeval na Univerzi Philipps v Marburgu pri prof. dr. Klausu Kalmringu. Tudi on je po zaključku štipendije dobil kot darilo ustanove A. v. Humboldt dragoceno opremo. Sedaj vodi Oddelek za entomologijo Nacionalnega inštituta za

biologijo – NIB, predava pa kot habilitirani redni profesor na Biološkem oddelku BF in drugod. Prof. dr. Andrej Čokl je bil od leta 1988 do 1996 tudi direktor Inštituta za biologijo.

Če pišem o bioakustiki, moram omeniti tudi povezavo s svetovno znanim profesorjem z Univerze v Odenseju (Danska) Axlom Michelsenom. V našem laboratoriju je bil prvi tuji štipendist (jugoslovanske vlade), ki je leta 1963 dva meseca – takrat še študent – pri nas opazoval in snemal razmnoževalno vedenje hroščev roginov (Cerambycidae). Kasneje je še večkrat prišel v Slovenijo raziskovat akustično komunikacijo žuželk, pa tudi mi smo gostovali pri njemu in delali na skupnih projektih (MICHELSEN et al. 1982). Skupaj s prof. dr. Franzom Huberjem iz Nemčije pa smo bili tudi glavni organizatorji serije mednarodnih simpozijev o bioakustiki žuželk (Insect sound and vibration).

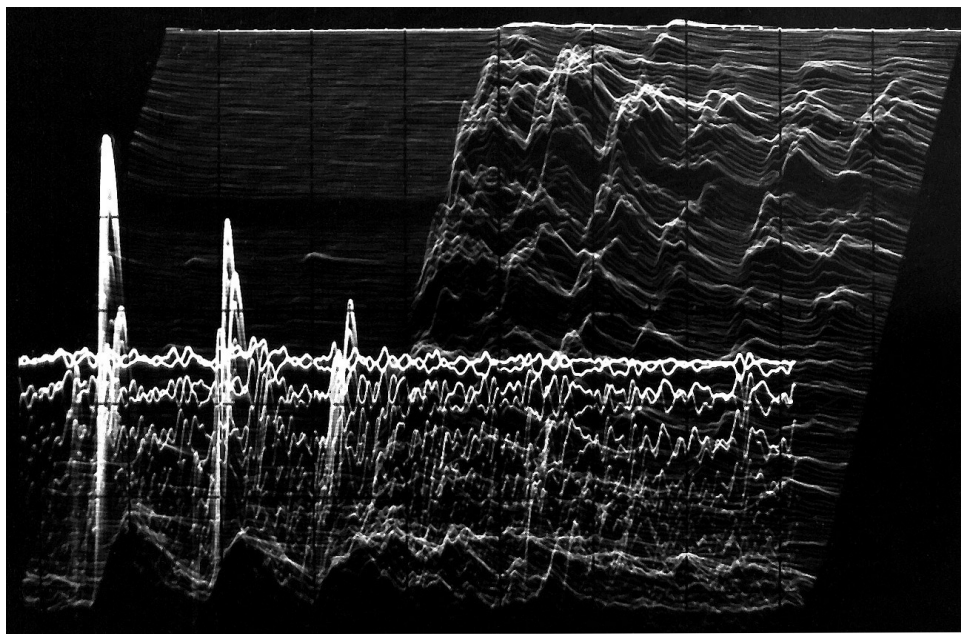
V šestdesetih in sedemdesetih letih prejšnjega stoletja je bil pri naših bioakustičnih raziskavah velik problem sonografija zvočnih signalov, torej trodimenzionalni prikaz frekvenčnega spektra v časovnem območju. Posebne naprave za to so bile izredno drage in na naši univerzi je edino aparaturo (Kay Sonagraph 6061 B) imel slovenist in jezikoslovec prof. Jože Toporišič. Občasno nam

je omogočil delo na tej napravi, potrebovali pa bi jo kar vsak teden ali dan. Včasih smo sonografske analize izvedli tudi v tujini, predvsem pri prof. Michelsenu na Danskem. Ko smo že imeli nekaj več elektronske opreme, sem enkrat prebil noč v kletnem laboratoriju (bivši potresomerni postaji) stare univerze, saj so me nepredvidneža zaklenili v hišo. Ko sem tuhtal, kaj naj počnem, sem ugotovil, da lahko s povezavo naših naprav, magnetofona z zanko, elektronskega filtra, stimulatorja in osciloscopa naredim neke vrste sonagraf. To sva z mlajšim kolegom Rajkom Razpotnikom tudi objavila (GOGALA & RAZPOTNIK 1974) (sl. 7). Dandanes pa lahko take spektrografske oz. sonografske analize z lahkoto izvedemo na vsakem osebнем računalniku.

Omenil sem že začetek uporabe računalništva na biologiji v Ljubljani v šestdesetih letih. Tudi kasneje smo tu orali ledino. Že leta 1972 smo z dr. Larsonom in prof. Alijem iz Univerze v Montrealu uporabila računalnik PDP-8 za vodenje mikrospektrofotometra Shimadzu za študij očesnih barvil (LARSON et al. 1972). Kasneje, v

osemdesetih letih smo si prizadevali za uvedbo prvih namiznih računalnikov (Hewlett-Packard 9820 z risalnikom, Commodore 64) tudi na Biološkem oddelku Biotehniške fakultete, v tistih časih smo veliko tudi sami pisali programe za razmeroma preproste izračune, za risanje grafov na risalniku in tudi za bolj zapletene procese, npr. za Fourierjevo transformacijo za analizo zvočnih signalov, saj primerni programi takrat niso bili splošno dosegljivi. Pri tem pa smo zaradi nerazumevanja nekaterih kolegov naleteli tudi na huda nasprotovanja. Dandanes si seveda delovnega mesta na univerzah brez dobrega računalnika ni mogoče predstavljati.

S prof. M. A. Alijem, ki sem ga omenil v prejšnjem odstavku, smo sodelovali še ob drugih priložnostih, med drugim sva se s Petrom Stuškom na njegovo vabilo udeležila mednarodnega simpozija Sensory Ecology v kraju Lennoxville, ki ga je finančno podprla organizacija NATO, kar je bilo takrat kar sumljivo. Vsekakor je iz tega gradiva nastala zanimiva knjiga, tudi z našim deležem (Gogala 1978).



Slika 7: Tridimenzionalni zapis zvočnih signalov stenice *Sehirus luctuosus*, narejen z našo metodo oscilografske sonografije, omenjene v besedilu.

Fig. 7: Three-dimensional graph of the acoustic signals of the bugs *Sehirus luctuosus*, produced with our method of oscillographic sonagraphy, described in the paper GOGALA & RAZPOTNIK, 1974.

V svetu bioakustike je bilo zanimivo odkritje oponašalskega vedenja stenice *Phymata crassipes*, ki odgovarja na zvočne in vibracijske signale iz okolja z lastnimi signali, katerih dolžino prilagaja trajanju dražljaja (GOGALA & ČOKL 1983, GOGALA et al. 1984). Pri teh raziskavah je v okviru diplomskega dela sodelovala tudi **Meta Virant Doberlet**, ki se je kasneje izpopolnjevala na Inštitutu Max Planck v Seewiesnu (Nemčija) pri prof. dr. Franzu Huberju in tam pripravila svoje magistrsko in doktorsko delo. Sedaj dela kot znanstvena svetnica v Oddelku za entomologijo Nacionalnega inštituta za biologijo.

Še eno zanimivo bioakustično temo smo raziskovali v zoofiziološkem laboratoriju in to v povezavi z dr. Annemarie Surlykke iz Univerze v Odense, Danska. To je oglašanje nočnega metulja, vrečenoske (*Rileyana* (= *Thecophora fovea*), katere predparitvene zvočne signale samcev na meji ultrazvoka smo registrirali, raziskali način proizvajanja teh signalov in lastnosti njihovih slušnih organov (SURLYKKE & GOGALA, 1986). Kasneje smo študirali tudi vedenje in slušne organe pri nekaterih dnevnih metuljih rodu *Erebia*.

Tine Valentinčič se je v času dodiplomskega izobraževanja ukvarjal z morsko biologijo in v okviru magistrskega študija raziskoval tunikate in iglokožce Severnega Jadrana. Kasneje se je pridružil zoofiziološkemu laboratoriju in začel raziskovati kemična čutila vodnih živalih, zanimala so ga kemična čutila in sposobnost učenja morskih zvezd (VALENTINČIČ 1982) in kasneje kačjerepov (VALENTINČIČ, 1991 a in b). Pri tem je uporabljal pretežno etološke metode. Začel je predavati etologijo in nevrotologijo ter je leta 1992 ustanovil Katedro za nevrotologijo na Biološkem oddelku BF. Leta 1989 je Valentinčiča povabil v ZDA znani fiziolog, raziskovalec ribjega voja in okusa dr. John Caprio, kjer je na Louisiana State University delal 6 let. Prvi je odkril in opisal okušalne reflekse, ki jih sprožijo nekatere aminokisline (CAPRIO et al. 1993; VALENTINČIČ & CAPRIO 1994 a, b). Iz te tematike so ti raziskovalci prijavi 4 patente. Odkril je, da se ribe naučijo prepoznavati posamezne aminokisline (VALENTINČIČ et al. 1994) in njihove zmesi. V zadnjem času sta doktorski študent Jurij Dolenšek in Tine Valentinčič elektrofiziološko potrdila, da je vsaka vohalna celica s svojo receptorsko beljakovino občutljiva za eno samo aminokislino: ([http://www.springerlink.com/](http://www.springerlink.com/content/1780576856m88048/fulltext.pdf)

[content/1780576856m88048/fulltext.pdf](http://www.springerlink.com/content/1780576856m88048/fulltext.pdf)). V isti enoti Biološkega oddelka BF dela Janko Božič, docent, ki se ukvarja s proučevanjem vedenja in gojenja čebel. Iz etološkega laboratorija sta izšla tudi profesor fiziologije na Medicinski fakulteti Univerze v Mariboru Dr. Marjan Rupnik in njegov asistent dr. Jurij Dolenšek.

V zoofiziološkem laboratoriju so opravljali vsaj diplomsko ali magistrsko delo še mnogi drugi biologi, ki pa so kasneje nadaljevali kariere v drugih okoljih in institucijah. Naj jih vsaj nekaj navedem: Gregor Serša je diplomu in delo za univerzitetno Prešernovo nagrado pripravil v zoofiziološkem laboratoriju in nadaljeval svojo kariero na Inštitutu za onkologijo MF. Tomaž Amon je magistriral leta 1981 pod mentorstvom dr. Čokla, doktorat pa je pripravil na Inštitutu Max Planck v Seewiesnu v Nemčiji in je leta 1988 na ljubljanski univerzi doktoriral. Danes vodi zasebni Center za znanstveno vizualizacijo AMNIM d.o.o.

Iz istega laboratorija na Biološkem oddelku Biotehniške fakultete je izšel **Dušan Devetak**, ki je diplomiral (1979) in magistriral (1985) pod mojim mentorstvom ter doktoriral pod vodstvom doc. K. Drašlarja (DEVETAK 1979, 1985, 1992). Sedaj v Mariboru vodi Katedro za fiziologijo živali in etologijo in predava zoologijo in fiziologijo živali (DEVETAK & SENČIČ 2008). V laboratoriju se ukvarjajo predvsem s problemi vibracijske orientacije in komunikacije pri žuželkah.

Tudi **Robert Zorec**, sedaj akademik in redni prof. na Medicinski fakulteti, je delal svojo diplomsko nalogo v zoofiziološkem laboratoriju iz področja fiziologije trihobotrijev pri stenicah leta 1981. Podiplomski študij pa je končal na Medicinski fakulteti.

Sam sem v jeseni leta 1987 zapustil univerzo, zato tu svoj pregled v glavnem končujem, kjer je bilo mogoče, pa sem nakazal povezave s sedanjim stanjem. Nastopil sem mesto znanstvenega svetnika v Prirodoslovnem muzeju Slovenije, kjer sem delal na področjih bioakustike, računalništva in entomologije. Od septembra 1992 do oktobra 2001 in upokojitve sem bil direktor tega muzeja. Član Slovenske akademije znanosti in umetnosti sem od leta 1991, glavni tajnik od leta 2002 do 2008 in podpredsednik od leta 2008. Znanstveno se zadnja leta ukvarjam predvsem z bioakustiko škrladov (Cicadidae) in njihovo taksonomijo. Po odhodu s fakultete sem še delno sodeloval pri pedagoškem

delu, predvsem na podiplomskem področju, sedaj, ko se že moji bivši učenci in asistenti pripravljajo na odhod v pokoj, pa je verjetno primeren čas za pisanje take male zgodovine laboratorija.

Summary

Shortly after a foundation of the Slovenian University in Ljubljana (1919) **Ivan (Johann) Regen** (*1868, †1947), one of the pioneers in the bioacoustics of insects, working in Vienna, has been elected as Professor of animal physiology at the Ljubljana University (1921). However, he never came to Ljubljana to teach and work here due to some unfortunate circumstances. Another possible candidate, working also in the field of animal physiology, was **Franč Megušar** (*1876, †1916), but he died very young in the first world war.

So lecturing of a general and comparative physiology has been taken over by **Albin Seliškar**, a biologist and physiology professor at the Medical Faculty of the same University of Ljubljana (from 1927 till late fifties). The zoophysiology lab at the Biology Department has been finally established in 1961 by **Štefan Sušec-Michieli** (*1933, †1968). He was an excellent entomologist – lepidopterologist, who spent some time in leading laboratories of animal physiology in Germany. After this he organized the research in sensory physiology, mainly in vision of insects, in colour change and related topics. Around 1962/63 he established an electrophysiology lab. During this time I was the first teaching assistant, preparing a dissertation on the vision of cave crickets *Troglophilus*. We were also working on comparative investigations of spectral sensitivity and other properties of insect eyes. During this research a discovery of specialized UV sensitive eyes of the owl-fly lead to further work on this interesting object – continuing during my Alexander von Humboldt fellowship (1964/65) and later by younger coworkers till now. Unfortunately, Prof. Michieli died very young, in 1968.

At that time, another teaching assistant was **Borut Žener** (*13. 5. 1935 †6. 1. 1974), another fellow of the A. v. Humboldt Stiftung who investigated by ERG the vision of bugs *Nezara viridula* during diapause and colour change. He

started also investigations of sensory properties of the cave salamander *Proteus anguinus*. The next coworker in the lab, already under my leadership, was **Kazimir Drašlar**, now vice-dean for Biology at the Biotechnical faculty. He has done important research on hair sensillas in fire-bug *Pyrhocoris apterus*. He was also the first to start using scanning electron microscopy at the Biology Department. The members of Zoophysiology group were in Slovenia also pioneers in using computers to solve biological problems.

Andrej Čokl, now professor and the head of the Entomology lab of the National Institute of Biology (NIB), worked for many years in the Zoophysiology lab. He mainly investigated acoustic communication and neurophysiological properties of the neurons in the central nervous system of selected insects (Hemiptera, Orthoptera). He and his students introduced many new neurophysiological techniques. He has now a team of younger coworkers in his lab at the NIB.

Another person, important for a development of zoophysiological laboratory, was **Peter Stušek**, Assistant Professor at the chair of Animal Physiology. His work is devoted to the vision of insects and especially to adaptation processes. He developed a method of a diver balance for measuring the oxygen consumption of eye preparations during illumination and various adaptation states.

Mitja Grosman who worked in our lab and at the National institute of Biology from 1973 till 1990 investigated hidden uv-patterns, visual system of gecko (*Hemidactylus turcicus*).

In the same lab began his work in chemosensory processes of aquatic animals (echinoderms and fishes) **Tine Valentinčič**. He uses now mainly ethological and neuroethological methods in his research and is now professor of ethology. Since 1989 he studies mainly chemoreception in fishes. In zoophysiology lab started also many other biologists their careers, now working in other labs or universities, like **Dušan Devetak**, now professor of zoology at the University of Maribor or **Robert Zorec**, now professor at the Medical faculty in Ljubljana.

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**Odnos študentov razrednega pouka do gensko spremenjenih organizmov
(GSO)**

**Opinion about Genetically Modified Organisms (GMOs) among Students
of Elementary Education**

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Izvleček: Prispevek obravnava odnos 359 študentov razrednega pouka treh slovenskih Pedagoških fakultet do genskega inženiringa in gensko spremenjenih organizmov (GSO). Izbor odgovorov oziroma soglašanje ali nesoglašanje s trditvami povezanimi z GSO izraža negotovost, nezaupanje in odklanjanje. S faktorsko analizo njihovih odgovorov smo prepoznali in ovrednotili nekatere skrbi in strahove, ki se pojavljajo v povezavi z razvojem novih tehnologij, njihov odnos do izobraževanja o GSO ter zaupanje do znanosti.

Ključne besede: gensko spremenjeni organizmi, GSO, bodoči učitelji, študenti razrednega pouka

Kratika: GSO – gensko spremenjeni organizem

Abstract: The objective of the article is to present the results of a survey of attitudes toward genetic modified organisms, conducted by questionnaire given to 359 prospective primary school teachers from three Slovene pedagogical faculties. Analysis of their answers, their agreement or disagreement with statements connected with GMOs, reveals uncertainty, distrust and rejection. By factorial analysis of their answers we identified and evaluated some of the concerns and fears connected with emerging new technologies, attitudes toward education about GMOs and levels of trust in scientific research.

Key words: genetically modified organisms, GMO, prospective primary school teachers, students of elementary education

Abbreviation: GMO – genetically modified organism

Uvod

Poučevanje naravoslovnih predmetov v današnjem času mora biti celovito. Količina znanj narašča (ZUPANČIČ 2005), sočasno pa si nekatera od naravoslovnih spoznanj utirajo pot na področja, ki klasično veljajo za družboslovna ali humanistična. Poleg sedaj že »tradicionalnih« tem kot je to npr. okoljska vzgoja so v zadnjih letih v ospredju tudi nove prakse in uporaba moderne biotehnologije s temami kot so genski inženiring, gensko spremenjeni organizmi (GSO), hrana iz GSO, terapevtsko in reproduktivno kloniranje, nadomestno materinstvo, potencialno kloniranje človeka, vpliv GSO na zdravje ljudi, živali, druge organizme in okolje, ipd..

Vsestranska in raznolika uporaba biotehno-loških znanstvenih in tehnoloških spoznanj na področjih kot so kmetijstvo, industrija ali medicina v teh primerih ni omejena le s tehnološkimi omejitvami ali nezadostnostjo znanstvenega instrumentarija, temveč še z etiko, moralo, vero, ekonomijo, okoljsko odgovornostjo, tveganji, političnimi odločitvami, ipd. (LAZAROWITZ & BLOCH 2005, PARDO S SOD. 2002, CHRISTOPH S SOD. 2008, FLORES & TOBIN 2002, STEWARD & McLEAN 2005, YUNTA S SOD. 2005). Zato se pogosto zgodi, da so ključni argumenti v razpravah za ali proti genski tehnologiji, izven območja znanstvene dokazljivosti in bližje predsodkom, misticizmu, praznoverju in paranormalnosti.

Oblikovanje stališč in vrednot se začne že v najzgodnejši otroški dobi, nadaljuje vse življenje in vsaj deloma jih lahko sooblikujejo učitelji in šolska praksa. Ker šole ne moremo obravnavati kot od družbenih dogajanj izoliranega sistema, se učitelji v sklopu pouka in drugih dejavnosti šole ne bodo mogli izogniti obravnavi nekaterih najbolj kočljivih tem s področja biotehnologije (DUNHAM S SOD. 2002), ki včasih premikajo tradicionalne civilizacijske temelje. Vloga šole v obravnavi novih praks in uporabe moderne biotehnologije ni povsem nevtralna, saj poleg znanj transparentno ali v obliki skritega kurikuluma oblikuje tudi vrednote učencev. Pri tem pa se pojavi dvojnost meril. Medtem, ko se za znanja pričakuje, da so strokovno neoporečna in utemeljena na znanstveni paradigmi pa je področje stališč in vrednot, še predvsem na mejnih področjih, praviloma povsem prepuščeno

učitelju in njegovi osebni presoji, velikokrat brez ustreznih strokovnih temeljev.

Ker lahko začnejo z oblikovanjem stališč in vrednot o temah, ki tradicionalno veljajo za naravoslovne, že učitelji razrednega pouka, nas je zanimalo, kakšna sta znanje in odnos študentov razrednega pouka treh slovenskih Pedagoških fakultet: Pedagoške fakultete Univerze v Mariboru (PeFMB), Pedagoške fakultete Univerze v Ljubljani (PeFLj) in Pedagoške fakultete Univerze na Primorskem (PeFKp), do genskega inženiringa in gensko spremenjenih organizmov, v nadaljnjem tekstu GSO.

Metode

Vzorec:

Raziskava smo izvedli na vzorcu 359 študentov drugega, tretjega in četrtega letnika razrednega pouka (RP), Pedagoških fakultet v Mariboru (N = 196; 54,4 %), Kopru (62; 17,2 %) in Ljubljani (102; 28,3 %). Ker so nas zanimala stališča bodočih učiteljev, ki bi lahko vplivala na poučevanje o gensko spremenjenih organizmih, drugih demografskih podatkov (npr. spol) nismo zbirali, saj so bili za namene raziskave nepomembni. Izhajali smo namreč iz povsem empirične ugotovitve, da učenec ne more izbirati učitelja na osnovi demografskih razlik.

Vprašalnik:

Da bi izvedeli kakšna so stališča študentov razrednega pouka do genskega inženiringa in gensko spremenjenih organizmov, smo pripravili vprašalnik, sestavljen iz 28 trditvev. Trditve so predstavljale več sklopov, in sicer smo preverjali, stališča študentov do prehranjevanja z GSO, uporabe GSO v medicinske namene, gojenja GSO, izobraževanja o GSO, uporabe GSO v svojih domovih, raziskav o GSO, vplivov GSO na okolje ter o njihovih strahovih in skrbih povezanih z GSO.

Stališča do GSO smo preverjali s pomočjo zaprtega vprašalnika, s petstopenjsko Likertovo ocenjevalno lestvico (1 – se zelo ne strinjam; 2 – se ne strinjam; 3 – nevtravno; mi je vseeno; 4 – se strinjam; 5 – se zelo strinjam), pri kateri so študentje pokazali svoje soglasje oziroma neso-glasje z 28 trditvami. Že ob sestavljanju vprašalnika

so bile trditve razvrščene v dvojne skupine: v prvi so bile trditve s katerimi so anketiranci izražali jezo, strahove, in skrb povezano z GSO, v drugem pa pripravljenost za akcijo. Da bi preprečili avtomatizem odgovarjanja smo uporabili mešan način, tako da je včasih nestrinjanje s trditvijo predstavljalo pozitivno stališče do trditve. Nasprotno postavljene trditve smo označili z zvezdico (*) v tabeli 1. Notranja zanesljivost vprašalnika je podana s Cronbachovim koeficientom alfa, ki je znašal 0,812, kar je sprejemljivo (KIND S SOD. 2007, CRONBACH 2004).

Obdelava podatkov:

Zbrane podatke smo računalniško obdelali s statističnim programom SPSS® 17.0. Iz nabora deskriptivne statistike smo izračunali aritmetično sredino ter standardni odklon odgovorov. Za preverjanje razlik med odgovori študentov različnih fakultete smo uporabili enosmerno analizo variance ter Kruskal Wallisov test. Statistične razlike med obema testoma so bile le minimalne, zato v zapisu predstavljamo le vrednosti analize variance. Latentne faktorje smo pridobili z enofaktorsko analizo podatkov z varimaks rotacijo. Primerčnost matrike za faktorsko analizo smo preverili s testoma KMO (Kaiser-Meyer-Olkin) (vrednost = 0,877) ter Barlettovim testom ($p < 001$). V faktorski analizi smo upoštevali celotno populacijo študentov.

Rezultati

Odgovori študentov bodočih učiteljev treh slovenskih pedagoških fakultet izražajo negotovost, nezaupanje ter odklanjanje GSO (Tabela 1). Negotovost in nezaupanje se najbolj izražata v trditvah, ki izražajo čustva kot so strah, skrb, sprejemanje, jeza in veselje. Študenti se v povprečju najbolj strinjajo (se strinjam, se zelo strinjam) s trditvami ki izražajo negativno stališče do GSO: »Ujezilo bi me, če na policah trgovin, živila iz GSO ne bi bila označena.« ($N = 358$; 84,6 %), »Skrbi me, da se bodo učinki uživanja GSO pokazali šele čez daljši čas« ($N = 352$; 78,4 %), ter z visoko stopnjo strinjanja s trditvami, ki izražajo negativno stališče do ravnanja z GSO: »Razvijalci GSO nam skrivajo podatke o njihovi škodljivosti.« ($N = 357$; 57,7 %) ter »Raziskovanje GSO bi morali

zamrzniti« ($N = 356$; 47,1 %). Odklonilen odnos študentov se kaže v nizkem sprejemanju trditev, ki izražajo pripravljenost na uporabo GSO: »Na lastnem vrtu bi zasadil(a) tudi gensko spremenjene rastline.« ($N = 358$; 16,5 %) ter »Dobro bi bilo, da bi kmetje sadili gensko spremenjene organizme, saj bi s tem uporabljali manj škropiv.« ($N = 356$; 22,5 %). Zelo visoko stopnjo strinjanja izkazujejo študentje do trditev povezanih z izobraževanjem, kar potrjuje visoka stopnja strinjanja (se strinjam, se zelo strinjam) s trditvami: »Poučevanje o GSO bi moralo poleg poznavanja dejstev vsebovati še vrednostno, moralno in etično komponento.« ($N = 359$; 68,2 %), »Učenci si niso sposobni ustvariti lastnega vrednostnega sistema o GSO, zato jih morajo pri tem usmerjati učitelji.« ($N = 359$; 75,8 %). Študentje se zavedajo pomena šole in izobraževanja učiteljev pri obravnavi aktualnih tem, ki se lahko vključujejo tudi v pouk naravoslovja v prvem in drugem triletnju ter se strinjajo s trditvijo: »Izobraževanje o GSO bi moralo biti organizirano za vse učitelje na šoli, ne glede na predmet, ki ga poučujejo« ($N = 358$; 68,4 %).

Študentje so se opredeljevali do trditev tako, da so pogostejše izbirali stališča, ki so izražala strinjanje ali nestrinjanje in redkeje drugače. Študentje so pri 20 od 28 trditev, torej večini trditev, izrazili svoje stališče, strinjanje ali nestrinjanje. Samo pri osmih od 28 trditev je najvišje frekvence dosegalo nevtralno stališče oziroma stališče »mi je vseeno«. Študenti bodoči učitelji treh slovenskih pedagoških fakultet ne izražajo skrajnih stališč do primerov novih biotehnologij. Za izražanje svojih stališč so redkeje odločajo med »se zelo ne strinjam« in »se zelo strinjam«. Prvi odgovor je izbralo v povprečju samo 6, 7 % študentov, drugega pa v povprečju samo 14,6 % študentov.

Stališčih študentov treh slovenskih fakultet do GSO so sicer zelo enotna, v posameznih stališčih (Tabela 1) pa se odgovori študentov treh Pedagoških fakultet med seboj statistično značilno razlikujejo (Tabela 2). Takih je sedem od 28 stališč. GS kakavovcu (čokoladi) so najbolj naklonjeni v Ljubljani in najmanj v Kopru ($p > 0,01$), s trditvijo, da bi zaradi GSO uporabljali manj škropiv se najbolj strinjajo v Kopru in najmanj Mariboru ($p > 0,02$), GS jabolka so najbolj sprejemljiva v Ljubljani pred ostalima fakultetama ($p > 0,001$), da bi morali imeti od GSO koristi vsi in ne samo proizvajalci ($p > 0,003$) se najbolj strinjajo v

Tabela 1: Aritmetična sredina (M) in standardni odklon (SD) odgovorov vrednotenih po pet-stopenjski lestvici (1 – se zelo ne strinjam; 2 – se ne strinjam; 3 – nevtraln; mi je vseeno; 4 – se strinjam; 5 – se zelo strinjam) študentov Pedagoških fakultet, Univerz v Mariboru, Ljubljani in Primorske (N = 359) pridobljene z vprašalnikom o stališčih do gensko spremenjenih organizmov. Trditve označene z indeksom*, so bile v nadaljnjih statističnih analizah prekodirane v nasprotni smeri, ker nestrinjanje s temi trditvami pomeni pozitivno stališče do take trditve.

Table 1: Means (M) and standard deviations (SD) of attitudes toward GMOs evaluated through a closed questionnaire, using a five-point Likert scale (5 Strongly agree, 4 Agree, 3 Neutral, 2 Disagree, 1 Strongly disagree) of students of Pedagogical faculties of Universities of Maribor, Ljubljana and Primorska (N = 359). For further analysis were statements marked with an asterix coded in the opposite direction, because disagreement with such statements means a positive attitude towards such statement.

	Stališče	N	M	SD	Me	Frekvence / N / %				
						1	2	3	4	5
1*	Bojim se, da se bo zaradi uporabe GSO povečalo število alergij.	356	3,73 *(2,23)	0,944	4	5 1,5	37 10,9	69 20,4	165 48,7	63 18,9
2*	Če bi ugotovil(a), da podarjena čokolada vsebuje maščobe iz gensko spremenjene soje, bi jo vrgel/vrgla stran.	351	2,83 *(3,17)	1,096	3	29 8,7	119 35,6	96 28,7	64 19,2	26 7,8
3	Če bi zbolel(a) zaradi bolezni povezane z gensko spremembo, bi izbral(a) zdravljenje z gensko terapijo.	354	3,02	0,971	3	26 7,7	58 17,1	157 46,3	78 23,0	20 5,9
4	Dobro bi bilo, da bi kmetje sadili gensko spremenjene organizme, saj bi s tem uporabljali manj škropiv.	356	2,57	1,092	2	63 18,5	110 32,3	88 25,8	71 20,8	9 2,6
5	Gensko spreminjanje rastlinskih celic je bolj sprejemljivo kot spreminjanje živalskih celic.	359	3,02	1,222	3	50 14,6	69 20,2	81 23,7	108 31,6	34 9,9
6	Izobraževanje o GSO bi moralo biti organizirano za vse učitelje na šoli, ne glede na predmet, ki ga poučujejo.	358	3,90	0,984	4	4 1,2	29 8,5	64 18,7	139 40,6	106 31,0
7*	Jabolka, ki so genetsko spremenjena z vnosom genov iz drugih sort jablan, zame niso sprejemljiva za prehrano.	359	2,82 *(3,18)	1,027	3	26 7,8	120 35,1	113 33,0	63 18,4	20 6
8*	Meso goveda, ki se je hranilo s krmo pridelano s pesticidi, je zame bolj sprejemljivo kot meso goveda, ki se je prehranjevalo z gensko spremenjeno krmo.	359	2,77 *(3,23)	0,922	3	26 7,6	105 30,7	141 41,2	61 117,8	9 2,6
9	Na lastnem vrtu bi zasadil(a) tudi gensko spremenjene rastline.	358	2,39	1,036	2	77 2,5	112 32,7	94 27,5	55 16,1	4 1,2
10	Od GSO bi morali imeti korist vsi, ne le njihovi proizvajalci.	356	3,46	1,016	4	20 5,9	22 6,5	126 37,1	125 36,8	47 13,8
11*	Pod nobenim pogojem ne bi kupoval(a) živil, ki vsebujejo GSO.	350	2,97 *(3,03)	1,011	3	16 4,8	102 30,4	127 37,9	61 18,2	29 8,7
12	Poučevanje o GSO bi moralo poleg poznavanja dejstev vsebovati še vrednostno, moralno in etično komponento.	359	3,93	0,863	4	4 1,2	12 3,5	81 23,7	152 44,4	93 27,2
13	Raje bi pojedel(la) živila iz gensko spremenjenih organizmov, če bi ta bila bolj zdrava od živil pridobljenih na standardni način.	358	3,47	1,084	4	21 6,1	46 13,5	79 23,1	145 42,4	51 14,9

	Stališče	N	M	SD	Me	Frekvence / N / %				
						1	2	3	4	5
14*	Raje bi umrl(a), kakor da bi vame presadili organ iz gensko spremenjene živali.	357	2,71 *(3,29)	1,282	3	63 18,5	104 30,5	84 24,6	44 12,9	46 13,5
15	Raziskovanje GSO bi morali še dodatno spodbujati.	357	3,31	1,040	3	19 5,6	42 12,4	132 38,8	101 29,7	46 13,5
16*	Raziskovanje GSO bi morali zamrzniti, dokler ne bi bilo nedvoumno dokazano, da so povsem neškodljivi.	356	3,40 *(2,60)	1,154	3	18 5,3	66 19,4	88 25,9	102 30,0	66 19,4
17*	Razvijalci GSO nam skrivajo podatke o njihovi škodljivosti.	357	3,66 *(2,34)	0,911	4	7 2,1	23 6,8	103 30,3	151 44,4	56 16,5
18*	Skrbelo bi me za zdravje otrok, če bi v šolski kuhinji pripravljali hrano iz GSO.	358	3,73 *(2,27)	0,935	4	3 0,9	40 11,7	69 20,2	165 48,4	64 18,8
19*	Skrbi me, da bi se GSO v okolju križali s sorodnimi vrstami	356	3,54 *(2,46)	0,836	4	2 0,6	27 7,9	136 39,9	135 39,6	41 12,0
20*	Skrbi me, da se bodo učinki uživanja GSO pokazali šele čez daljši čas.	352	4,15 *(1,85)	0,856	4	3 0,9	12 3,6	44 13,3	150 44,8	126 37,6
21*	Strah bi me bilo posledic za naravo, če bi zvedel(a) da na kmetijah gojijo GSO.	358	3,61 *(2,39)	0,924	4	4 1,2	35 10,2	107 31,3	141 41,2	55 16,1
22*	Strah me je, da bi se zaradi GSO povečala odpornost bakterij proti antibiotikom.	358	3,95 *(2,05)	0,852	4	1 0,3	19 5,6	68 19,9	164 48,0	90 26,3
23	Učenci si niso sposobni ustvariti lastnega vrednostnega sistema o GSO, zato jih morajo pri tem usmerjati učitelji.	359	4,01	0,947	4	4 1,2	28 8,2	38 11,1	157 45,9	115 33,6
24*	Ujezilo bi me, če na policah trgovin, živila iz GSO ne bi bila označena.	358	4,31 *(1,69)	0,731	4	1 0,3	7 2,1	30 8,8	157 46,0	146 42,8
25*	Ustvarjanje GSO je v nasprotju z zakoni narave, zato bi ga bilo treba prepovedati.	357	3,13 *(2,87)	0,961	3	10 2,9	70 20,5	167 48,8	55 16,1	40 11,7
26	Veseli me, če bi z genskim inženiringom uspeli vzgojiti živali, dajalce organov.	356	2,96	1,091	3	40 11,8	68 20,0	116 34,1	99 29,1	17 5,0
27*	Z GSO naj se ukvarjajo pri biologiji in gospodinjstvu, v drugih predmetih pa jim ni mesta.	358	2,41 *(3,59)	1,067	2	73 21,3	119 34,8	99 28,9	37 10,8	14 4,1
28	Že zaradi radovednosti bi si kupil(a) gensko spremenjeno lončnico.	358	2,95	1,212	3	53 15,5	71 20,8	85 24,9	103 30,1	30 8,8

Ljubljani pred ostalima fakultetama, živila, ki vsebujejo GSO bi najraje kupovali v Ljubljani, najmanj pa v Kopru ($p > 0,03$), da bi raziskave o GSO morali še spodbujati se najbolj strinjajo v Ljubljani in najmanj v Kopru ($p > 0,05$), da naj se z GSO ukvarjajo ne samo pri biologiji ampak tudi pri drugih predmetih se najbolj strinjajo v Ljubljani in najmanj Kopru ($p > 0,001$).

Dodaten vpogled v stališča učiteljev smo pridobili s faktorsko analizo. Prepoznali smo osem faktorjev (Tabela 3), s katerimi lahko pojasnimo 52,2 % variance.

Prvi faktor smo poimenovali »zaskrbljenost« in izraža skrb in strah pred neznanim. Drugi

faktor smo poimenovali »odnos do hrane in do prehranjevanja« in izraža odklanjanje take hrane. Tretji faktor smo poimenovali »skrb za zdravje« in povezuje GSO s skrbjo za zdravje. Enako pa lahko sklepamo iz strinjanja (se strinjam, se zelo strinjam) s trditvijo: »Raje bi pojedel(la) živila iz gensko spremenjenih organizmov, če bi ta bila bolj zdrava od živil pridobljenih na standardni način.« ($N = 358$; 54,7 %) ter nestrinjanjem (se ne strinjam, se zelo ne strinjam) s trditvijo »Raje bi umrl(a), kakor da bi vame presadili organ iz gensko spremenjene živali« ($N = 357$; 46,8 %). Četrty faktor smo poimenovali »vrednostni sistem« in peti faktor »organizacija izobraževanja«. Obravnavamo ju

Tabela 2: Statistično pomembne razlike v odgovorih na vprašalnik o odnosu do GSO med študenti pedagoških fakultet Univerz v Mariboru (PeFMb), Primorski (PeFKp) in Ljubljani (PeFLj). Razlike, ki niso značilne niso prikazane. Polna besedila trditev so podana v Tabeli 1.

Table 2: Statistically significant differences in reported attitudes toward GMOs among students of Pedagogical faculties of Universities of Maribor (PeFMb), Primorska (PeFKp) and Ljubljana (PeFLj). No-significant Differences among results are not presented. Full text of the answers is in the Table 1.

Trditev	Fakulteta	N	M	SD	F	p
2*	PeFMb	191	3,10	1,059	4,551	0,011
	PeFKp	60	2,97	1,301		
	PeFLj	100	3,44	0,988		
	Skupaj	351	3,17	1,096		
4	PeFMb	196	2,44	1,115	3,942	0,020
	PeFKp	59	2,88	1,084		
	PeFLj	101	2,63	1,017		
	Skupaj	356	2,57	1,092		
7*	PeFMb	196	3,06	1,004	6,917	0,001
	PeFKp	61	3,03	1,064		
	PeFLj	102	3,49	0,992		
	Skupaj	359	3,18	1,027		
10	PeFMb	196	3,39	1,015	3,453	0,033
	PeFKp	59	3,32	1,008		
	PeFLj	101	3,68	0,999		
	Skupaj	356	3,46	1,016		
11*	PeFMb	192	2,99	1,026	3,492	0,032
	PeFKp	58	2,81	1,034		
	PeFLj	100	3,23	0,941		
	Skupaj	350	3,03	1,011		
15	PeFMb	194	3,28	1,109	3,062	0,048
	PeFKp	61	3,10	0,926		
	PeFLj	102	3,50	0,941		
	Skupaj	357	3,31	1,040		
27*	PeFMb	196	3,74	1,055	8,929	0,000
	PeFKp	61	3,10	1,261		
	PeFLj	101	3,60	0,861		
	Skupaj	358	3,59	1,067		

skupaj, saj sta oba povezana z izobraževanjem. Bodoči učitelji menijo, da je izobraževanje o GSO velik problem, pri tem pa naj bi poseglo tudi na področje odnosa in stališč do GSO. Tako je bila prav trditev »Z GSO naj se ukvarjajo pri biologiji in gospodinjstvu, v drugih predmetih pa jim ni mesta.« deležna velikega nestrinjanja (N = 358; 53,6 %). Večina se tudi strinja s trditvijo, da bi morala po šolah potekati izobraževanja za učitelje ne glede na predmet, ki ga poučujejo (N = 358; 68,4 %). Šesti faktor smo poimenovali »odnos

do raziskovanja« je odnos do znanosti oziroma zaupanje v znanost, kjer naši študentje izražajo naklonjenost raziskavam. Sedmi faktor smo poimenovali »preživetje posameznika v družbeni skupnosti«. Izraža zaskrbljenost posameznika za lastno preživetje v družbeni skupnosti. Nanj se navezuje osmi faktor, ki smo ga poimenovali »možnost izbire«. Vsak študent si želi svobodno izbirati in se svobodno odločati o tem ali bo sprejel GSO hrano, zdravila, surovine ali ne.

Tabela 3: Model faktorjev zasnovan na trditvah študentov o odnosu do gensko spremenjenih organizmov (GSO).

Table 3: Model of factors based on the answers of the students toward genetic modified organisms (GMOs).

Faktor 1: ZASKRBLJENOST	Obtežitev
Strah me je, da bi se zaradi GSO povečala odpornost bakterij proti antibiotikom.	0,755
Skrbi me, da se bodo učinki uživanja GSO pokazali šele čez daljši čas.	0,711
Skrbi me, da bi se GSO v okolju križali s sorodnimi vrstami.	0,666
Strah bi me bilo posledic za naravo, če bi zvedel(a) da na kmetijah gojijo GSO.	0,621
Skrbelo bi me za zdravje otrok, če bi v šolski kuhinji pripravljali hrano iz GSO.	0,585
Razvijalci GSO nam skrivajo podatke o njihovi škodljivosti.	0,564
Bojim se, da se bo zaradi uporabe GSO povečalo število alergij.	0,533
Dobro bi bilo, da bi kmetje sadili gensko spremenjene organizme, saj bi s tem uporabljali manj škropiv.	0,406
Faktor 2: ODNOS DO HRANA IN PREHRANJEVANJA	
Če bi ugotovil(a), da podarjena čokolada vsebuje maščobe iz gensko spremenjene soje, bi jo vrgel/vrgla stran.	0,759
Pod nobenim pogojem ne bi kupoval(a) živil, ki vsebujejo GSO.	0,726
Jabolka, ki so genetsko spremenjena z vnosom genov iz drugih sort jablan, zame niso sprejemljiva za prehrano.	0,630
Ustvarjanje GSO je v nasprotju z zakoni narave, zato bi ga bilo treba prepovedati.	0,495
Faktor 3: SKRB ZA ZDRAVJE	
Veselilo bi me, če bi z genskim inženiringom uspeli vzgojiti živali, dajalce organov.	0,717
Raje bi pojedel(la) živila iz gensko spremenjenih organizmov, če bi ta bila bolj zdrava od živil pridobljenih na standardni način.	0,642
Že zaradi radovednosti bi si kupil(a) gensko spremenjeno lončnico.	0,571
Faktor 4: VREDNOSTNI SISTEM	
Učenci si niso sposobni ustvariti lastnega vrednostnega sistema o GSO, zato jih morajo pri tem usmerjati učitelji.	0,724
Poučevanje o GSO bi moralo poleg poznavanja dejstev vsebovati še vrednostno, moralno in etično komponento.	0,631
Ujezilo bi me, če na policah trgovin, živila iz GSO ne bi bila označena.	- 0,427
Faktor 5: ORGANIZACIJA IZOBRAŽEVANJA	
Z GSO naj se ukvarjajo pri biologiji in gospodinjstvu, v drugih predmetih pa jim ni mesta.	0,782
Izobraževanje o GSO bi moralo biti organizirano za vse učitelje na šoli, ne glede na predmet, ki ga poučujejo.	0,587
Faktor 6: ODNOS DO RAZISKOVANJA	
Raziskovanje GSO bi morali še dodatno spodbujati.	0,676
Raziskovanje GSO bi morali zamrzniti, dokler ne bi bilo nedvoumno dokazano, da so povsem neškodljivi.	0,580
Faktor 7: PPREŽIVETJE POSAMEZNIKA V DRUŽBENI SKUPNOSTI	
Če bi zbolel(a) zaradi bolezni povezane z gensko spremembo, bi izbral(a) zdravljenje z gensko terapijo.	0,792
Od GSO bi morali imeti korist vsi, ne le njihovi proizvajalci.	0,544
Na lastnem vrtu bi zasadil(a) tudi gensko spremenjene rastline.	0,401
Faktor 8: MOŽNOST IZBIRE	
Meso goveda, ki se je hranilo s krmo pridelano s pesticidi, je zame bolj sprejemljivo kot meso goveda, ki se je prehranjevalo z gensko spremenjeno krmo.	0,849

Razprava

Nezaupanje, negotovost in odklanjanje GSO, ki ga izražajo odgovori študentov bodočih učiteljev treh slovenskih pedagoških fakultet, ni nekaj novega in presenetljivega, srečamo jih kot vodilno nit različnih drugih študij narejenih ne samo na študentih ampak tudi na predstavnikih drugih družbenih skupin (CHRISTOPH S SOD. 2008, CAVANAGH S SOD. 2005, YUNTA S SOD. 2005, PARDO S SOD. 2002). Negotovost se kaže tudi v tem, da redkeje izbirajo skrajne odgovore »se zelo strinjam« kot »se strinjam« ali »se zelo ne strinjam« kot »se ne strinjam«. Zanimivo pri tem je to, da so s se strinjanjem ali nestrinjanjem opredelili do nekaj manj kot treh četrtin trditev in da so bili nevtralni ali jim je bilo vseeno do nekaj več kot četrtine trditev, kar kaže na željo po vključevanju v odločanje o aktualnih temah. Istočasno se študenti zavedajo pomena in vloge izobraževanja o temah, ki bi lahko vplivale na njihova življenja, družbo in okolje.

Študentje treh slovenskih fakultet so v svojih stališčih do GSO enotni, čeprav ne povsem. Značilne razlike v odgovorih so se pokazale v sedmih od 28 stališč. Pri tem so se študentje Pedagoške fakultete v Ljubljani izkazali za nekoliko bolj odprte do GSO ter bolj naklonjene raziskavam in vključevanju GSO v celotno izobraževanje. Večje odprtosti in naklonjenosti študentov PeFLj do GSO ne znamo zadovoljivo pojasniti, verjetno je povezana z odprtostjo mesta, ki je center tovrstnih raziskav in zato bližje učiteljem, študentom in profesorjem, vsem subjektom vključenim v izobraževanje na PeFLj in drugih fakultetah tega območja. GSO so najmanj sprejemljivi za študente PeFKp, ki je od vseh treh najmlajša in z najmanjšo tradicijo raziskovanja.

Za predstavitev stališč študentov razrednega pouka smo izvedli faktorsko analizo in prepoznali osem faktorjev. Na osnovi analize njihove vsebine smo jih imenovali: zaskrbljenost, odnos do hrane in prehranjevanja, skrb za zdravje, odnos do raziskovanja in preživetje posameznika v družbeni skupnosti, vrednostni sistem in organizacija izobraževanja. Ker je prvi in zelo dominantni faktor pri izraženih stališčih do trditev zaskrbljenost, skrb in strah pred neznanim, bi lahko sklepali, da študentom ni vseeno in jih skrbi področje novih tehnologij in njeno ožje področje gensko spremi-

njanje organizmov. Ta zaskrbljenost je usmerjena na vplive GSO in te »moderne biotehnologije« na vsakdanje življenje. Skrbi jih odpornost na antibiotike, dolgoročni učinki, križanje s sorodnimi vrstami, zdravje potomcev in negativni družbeni pojavi kot je skrivanje podatkov o škodljivosti GSO. To znanje je razmeroma novo, gensko spremenjenih organizmov in njihovih produktov še niso preizkusili in ne poznajo nikogar ki bi to preizkusil. Strah in z njim povezanim odklanjanjem GSO bi lahko opisali kot strah pred tem česar ne vedo, pred neznanim in nepreizkušenim, podobno pa ugotavljajo tudi druge študije narejene na populaciji študentov in potrošnikov (CAVANAGH S SOD. 2005, CRISTOPH S SOD. 2008). Drugi faktor je povezan s hrano in prehranjevanjem, kjer lahko ugotovimo, da bi študenti takšno hrano in prehranjevanje odklonili. Študenti bi odklonili GS hrano in prehranjevanje z GSO, kar ugotavljajo tudi številne druge študije narejene na potrošnikih hrane (ROTENTALP S SOD. 2007) in na populaciji študentov (CAVANAGH S SOD. 2005). Odklonili pa ne bi le neposrednega vnosa GSO v telo, temveč tudi živila iz živali, ki bi se prehranjevala z GS krmo. V tem primeru bi bil lahko vzrok prastrah človeka pred zastrupitvijo z neznano hrano ali negotovost povezana z nasprotujočimi informacijami, ki jih posredujejo mediji, biotehnoška podjetja, vladne službe, znanstvena srenja in drugi bolj ali manj zanesljivi viri. Kljub polemikam in številnim raziskavam do danes nobena znanstveno veljavna študija ni pokazala, da bi bila hrana, ki vsebuje gensko spremenjene sestavine, manj varna kot hrana iz klasične pridelave (LEMAUX, 2008). Tretji faktor »skrb za zdravje« kaže na to, da se študentje se zavedajo pomena svojega zdravja in razmišljajo tudi o možnostih, ki jih ponujajo GSO za ohranjanje zdravja in zdravljenje. Da pa zdravje, kot tradicionalno močno izražena vrednota, ne more povsem pretehtati skrbi in strahu, lahko sklepamo po odgovorih na druge trditve povezane z zdravjem, ki so okoli nevtralnega stališča. Kot kaže je prav področje zdravja tisto, kjer bi GSO postali najhitreje sprejemljivi, kar se sklada s tujimi ugotovitvami (CAVANAGH S SOD. 2005). Četrti, peti in šesti faktor smo poimenovali vrednostni sistem, organizacija izobraževanja in odnos do raziskovanja izražajo zaupanje do izobraževanja in znanosti. Sedmi in osmi faktor preživetje posameznika v družbeni skupnosti in možnost

izbire lahko imenujemo tudi boj za preživetje v nestabilni družbi, kjer so lastne odločitve zelo pomembne za to ali se bo posamezniku uspelo vključiti v družbo tako da bo zadovoljen. Posamezni faktorji, prepoznani v našem vzorcu študentov, se skladajo z ugotovitvami drugih študij. Taki so npr. faktorji: zaskrbljenost in strah pred neznanim, odnos do hrane in prehranjevanja, skrb za zdravje in odnos do raziskovanja. Na novo prepoznani faktorji, ki jih dosedanje študije niso razkrile, so organizacija izobraževanja in vrednostni sistem, preživetje posameznika v družbeni skupnosti ter možnost izbire.

Sklep

Iz naše raziskave lahko izpeljemo nekaj praktičnih sklepov, ki bi lahko predstavljali temelj za nadaljnje izobraževanje učiteljev razrednega pouka o GSO. Odnos, ki ga imajo do GSO, je mešanica negotovosti in nezaupanja, ki se izraža v odklanjanju GSO. Negotovost in nezaupanje, ki ga izražajo v povezavi z GSO nikakor ne more biti dobra popotnica za objektivno presojanje, oblikovanje svojega sistema vrednot in uspešno izobraževanje otrok, ki jim bodo zaupani.

Povzetek

Oblikovanje stališč in vrednot se začne že v najzgodnejši otroški dobi, nadaljuje vse življenje in vsaj deloma jih lahko sooblikujejo učitelji in šolska praksa. Ker šole ne moremo obravnavati kot od družbenih dogajanj izoliranega sistema, se učitelji v sklopu pouka in drugih dejavnosti šole ne bodo mogli izogniti obravnavi nekaterih najbolj kočljivih tem, ki včasih premikajo tradicionalne civilizacijske temelje. Poleg sedaj že »tradicionalnih« tem kot je to npr. okoljska vzgoja je v zadnjih letih v ospredju tudi biotehnologija s temami kot so genski inženiring, gensko spremenjeni organizmi (GSO), hrana iz GSO, terapevtsko in reproduktivno kloniranje, nadomestno materinstvo, potencialno kloniranje človeka, vpliv GSO na zdravje ljudi, živali, druge organizme in okolje, ipd.. Ker lahko začnejo z oblikovanjem stališč in vrednot o temah, ki tradicionalno veljajo za naravoslovne, že učitelji razrednega pouka, nas je

zanimalo, kakšen je odnos študentov razrednega pouka treh slovenskih Pedagoških fakultet do genskega inženiringa in gensko spremenjenih organizmov (GSO). V raziskava izvedeni v letu 2008 je bilo vključenih 359 študentov drugega, tretjega in četrtega letnika razrednega pouka (RP), Pedagoških fakultet v Mariboru (54,4 %), Kopru (17,2 %) in Ljubljani (28,3 %). Stališča do GSO smo preverjali s pomočjo zaprtega vprašalnika, s petstopenjsko Likertovo ocenjevalno lestvico, pri kateri so študentje pokazali svoje soglasje oziroma nesoglasje z 28 trditvami.

Ugotovili smo, da bi lahko odnos študentov razrednega pouka do GSO označili kot negotovost, nezaupanje in odklanjanje. Negotovost in nezaupanje se kažeta v odgovorih, k izražajo čustva, odklanjanje pa v trditvah, ki izražajo stališče do ravnanja z GSO. Svoje strinjanje ali nestrinjanje so študenti izrazili s stališči, ki niso niti skrajna niti nevtralna. V posameznih odgovorih so se stališča lahko statistično značilno razlikovala. Tako so bili študenti Univerze v Ljubljani bolj naklonjeni GSO, kot ostali študentje. Dodaten vpogled v stališča smo dobili s faktorsko analizo in na osnovi analize njihove vsebine prepoznali in ovrednotili osem faktorjev, ki smo jih imenovali zaskrbljenost, odnos do hrane in prehranjevanja, skrb za zdravje, odnos do raziskovanja in preživetje posameznika v družbeni skupnosti, vrednostni sistem in organizacija izobraževanja. V prispevku smo ovrednotili nekatere skrbi in strahove, ki se pojavljajo zaradi razvoja novih tehnologij, njihov odnos do izobraževanja o GSO ter zaupanju do znanosti. Pridobljena vedenja bi bilo mogoče uporabiti za spremembe dodiplomskega kurikula izobraževanja učiteljev razrednega pouka ali uporabiti v sklopu izobraževanj za učitelje, ki že poučujejo.

Summary

Formation of attitudes and values is a lifelong process which starts in early childhood and is at least partially shaped by teachers and school practices. Because we cannot treat instruction and school activities as value-free systems, isolated from society, teachers cannot avoid classroom discussions of sensitive themes, which sometimes challenge the foundations of traditional civiliza-

tion. Besides already »traditional« themes such as environmental education, public interest has now shifted to biotechnology, with themes like genetic engineering, genetically modified organisms (GMOs), food from GMOs, therapeutic and reproductive cloning, surrogate maternity, cloning of humans, as well as the impact of GMOs on human health, the health of animals and of the environment, etc. Because the formation of attitudes and values in themes traditionally recognized as scientific is influenced by prospective primary school teachers, our interest lay in identifying attitudes of prospective primary school teachers from three Slovene pedagogical faculties towards genetically modified organisms. In a survey carried out in 2008, 359 students were included; these were prospective primary school teachers, in the second, third and fourth years of undergraduate university study from the pedagogical faculties of Maribor (54.4 %), Koper (17.2 %) and Ljubljana (28.3 %). Attitudes were checked using a five-point Likert scale, consisting of 28 items.

We found that the attitudes of the prospective primary school teachers can be described as uncertainty, distrust and rejection. Uncertainty and distrust can be recognized from trends in the answers concerning feelings, and rejection from answers concerning attitudes toward the handling and use of GMOs. The level of agreement

or disagreement among students is moderate or neutral in both directions. Statistically important differences among answers were recognized between the students from different faculties. In general, students from Ljubljana have more positive attitudes than students of other faculties. We gained additional insight with factorial analysis of answers. We were able to recognize eight factors: concerns, attitudes toward food and nutrition, health management, attitudes towards research and the survival of the individual in social communities, the research system and educational organization. In the paper, some of the concerns and fears were additionally evaluated, where the root cause is new technology, attitudes toward education about GMOs and trust in scientific research. The knowledge acquired can be used in future changes to the undergraduate curriculum for prospective primary school teachers and in the preparation of in-service training.

Zahvala

Zahvaljujemo se dr. Jelki Strgar, dr. Darji Skribe Dimec (Univerza v Ljubljani), mag. Claudiju Battelliju (Univerza na Primorskem), dr. Alenki Lipovec in mag. Martini Rajšp (Univerza v Mariboru) za njihovo pomoč pri anketiranju.

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Diatoms: Their strange evolution and remarkable properties

Kremenaste alge: Njihov nenavadni razvoj in izjemne lastnosti

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Abstract: We review some new literature on diatoms, with emphasis on genomics, evolution, ecology and biomimetic nanotechnical applications. Diatoms account for a substantial part of the photosynthetic production on this planet, and their genome is a mosaic of contributions from different sources. They occupy very diverse ecological niches, and may have been the first organisms to carry out C4 photosynthesis. Their frustules (silica enclosures) with their elaborate sculpturing make it possible to follow the occurrence of different forms back in time, and the frustules is also the main reason that they are interesting for biotechnology.

Keywords: C4 photosynthesis, chloroplasts, diatoms, dynamite, endosymbiosis, nanotechnology, omega-3 fatty acid, silica

Izveček: Prispevek je pregled novih virov o kremenastih algah s povdarkom na genomiki, evoluciji, ekologiji ter biomimetični nanotehnoški aplikaciji. Kremenaste alge prispevajo velik delež k fotosintezni produkciji našega planeta. Njihov genom je mozaik elementov različnega izvora. Zasedajo različne ekološke niše, in verjetno so bile prvi organizmi s C4 način fotosinteze. Njihove frustule (silikatni ovoji) z izdelanimi raznolikimi vzorci omogočajo sledenje različnih oblik v zgodovini in prav frustule so tiste, zaradi katerih so kremenaste alge zanimive za biotehnologije.

Ključne besede: C4 fotosinteza, kloroplasti, kremenaste alge, dinamit, endosimbioza, nanotehnologija, omega-3 maščobne kisline, silicij

Introduction

Diatoms are photosynthetic, unicellular organisms. In some species several cells remain attached in colonies, but without any differentiation or division of functions between cells (see HAYAKAWA & *al.* 1994). Diatoms belong to the so-called heterokonts which, together with oomycetes and others form the stramenopiles. Brown algae are among the most well-known close relatives of diatoms, and both groups have fucoxanthin as an accessory photosynthetic pigment.

Diatoms form one of the most successful groups of organisms on our planet. They are present in most niches of the biosphere where there is, at least from time to time, some water: in seas, lakes and stream water, hot springs (up to 50°C), salty brines up to saturated concentration, dry rock and stone walls, desert surface crusts, in the surface layer of other soils, and as symbionts inside dinoflagellates and foraminifers. Some diatoms harbour cyanobacteria as endosymbionts.

The diatoms of the sea are the most important ones in a global perspective. Marine dinoflagel-

lates produce about 40 percent of the biomass in the sea, and for sea and continents combined they produce about 20 percent of the biomass and the oxygen. Experts do not agree on the number of diatom species. Twenty-five thousand species have been described (ALVERSON 2008), but some of them have been shown to be different forms of the same species. Certainly there are more than 15 thousand species; MANN & DROOP 1996 say 200 thousand, and both DRUM & GORDON 2003 and STERRENBURG & *al.* 2007 give a range of one hundred thousand to one million. The upper limit of this interval appears unrealistic. A discussion is going on about how the species concept should be defined for diatoms, since it will be impossible to carry out mating experiments except in a few cases.

The first diatoms probably appeared on land about 280 million years ago, but the oldest un-

questionable fossils date from early Cretaceous, 120 million years ago. There is reason to believe in a radiation into different evolutionary lines between 160 and 150 million years ago. The first diatoms were »centric«, i.e. had radial symmetry (Figure 1), and the elongated and bisymmetric »pennate« forms (Figure 2) arose about 125 million years ago. It is thought that diatoms (as well as dinoflagellates) were favoured by the great extinction that marks the end of the Cretaceous, 65.5 million years ago, as this was a catastrophe not only for the dinosaurs, but also for coccolithophores and silicoflagellates, competitors of the marine diatoms. Diatoms and dinoflagellates could survive, probably thanks to their ability to form resistant resting cells. During their whole evolution the diatoms have also been favoured by the expanding terrestrial vegetation, which, because its roots and mycorrhiza have been expanding ever

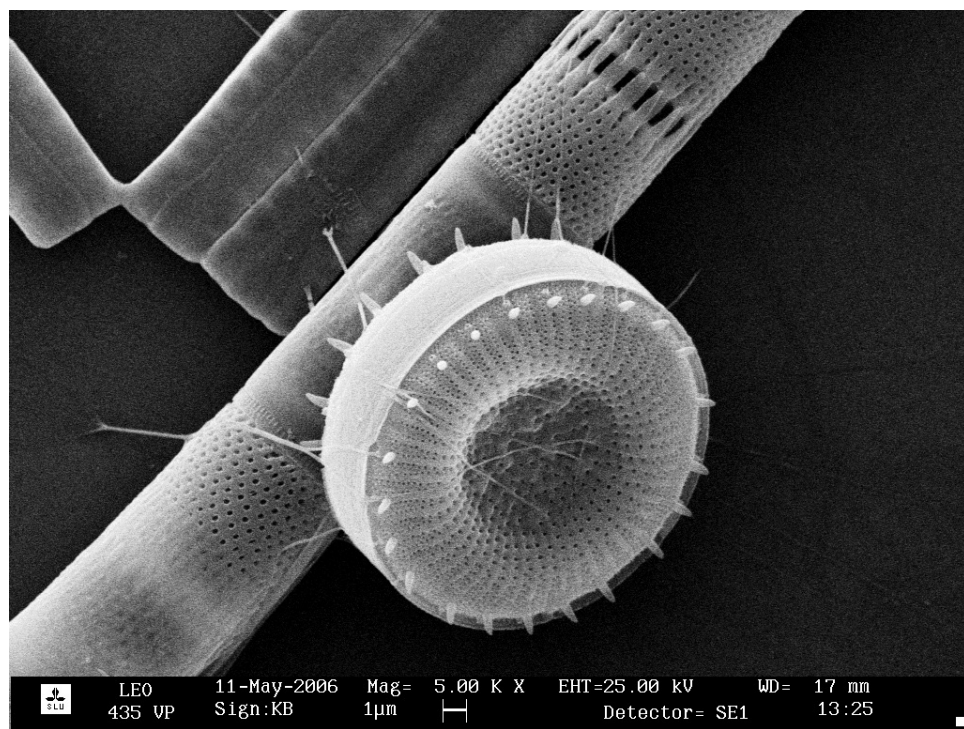


Fig. 1: Centric diatoms, *Cyclostephanos dubius* in the foreground and *Aulacoseira* sp. in the background (scanning electron microscope image by Gertrud Cronberg).

Slika 1: Kremenasti algi iz reda *Centrales*; vrsta *Cyclostephanos dubius* spredaj in predstavnica iz rodu *Aulacoseira* sp. v ozadju (foto: Gertrud Cronberg).

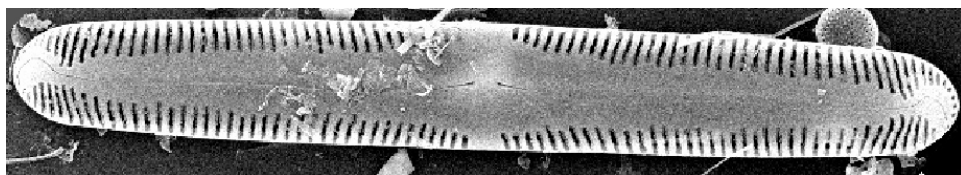


Fig. 2: Pennate diatom (*Pinnularia* sp.) (scanning electron microscope image by Gertrud Cronberg).
Slika 2: Kremenasta alga iz reda Pennales (*Pinnularia* sp.) (foto: Gertrud Cronberg).

wider and penetrating ever deeper, has contributed to increased weathering and transport of silicic acid to fresh waters and to the sea.

Peculiar genomes

Genomes have now been studied for both centric and pennate diatoms, and several strange circumstances have recently been brought to light. But let us begin what has been known for several years. Diatom chloroplasts, as most other chloroplasts with the exception of those of green algae and plants, derive from a red alga which has entered into an intimate symbiosis with an originally non-photosynthetic organism. This »secondary endosymbiotic event« is thought to have happened about a billion years ago, the chloroplasts of this and other red algae, in turn, being originally derived from a cyanobacterium (blue-green alga) which entered into »primary endosymbiosis« with a non-photosynthetic organism more than 1.2 billion years ago. But the recent analysis of diatom genes reveal that things are much more complicated than this.

Most of the genes originally present in the »engulfed« blue-green and red algae are not left in the chloroplast of the diatom, but have either disappeared or been transferred to the nucleus. This is because some of them were no longer needed, since similar genes were present in the nucleus of the original, non-photosynthetic organism. The genes which were still needed, for instance those necessary for photosynthesis, had better leave the chloroplast, because this is a dangerous place for DNA, as it has high concentrations of radicals and oxygen. And so they did, by the process of natural selection. Another »reason« to leave the chloroplast was that chloroplast genes cannot benefit from the advantages of sexual reproduction

(a tricky question discussed by many, and recently in Nick Lane's wonderful book »Life ascending«). A few genes were left in the chloroplast, because some functions related to photosynthesis require very rapid regulation of gene activity by signalling pathways originating in the photosynthetic apparatus.

The chloroplast origin of these genes that did move to the nucleus is recognized because these genes code for chloroplast proteins. If all of these genes in a diatom had arrived with the engulfed red alga, they would show greater similarity to genes in red algae (the »red line of evolution«) than with genes in green algae and plants (the »green line of evolution«). This is most often the case. But in quite a few cases the similarity is greater to the »green line of evolution«, for reasons that are not currently understood. One theory is that the diatoms (or diatom ancestors) have first harboured a »green« chloroplast, which at a later time has been exchanged for the present one. Another theory is that »horizontal gene transfer« has taken place by infection by viruses or bacteria. The sea is teeming with viruses, and several of them are known to harbour photosynthesis genes, although in the known cases these genes are from cyanobacteria (e.g., MANN & *al.* 2003; LINDELL & *al.* 2005; HELLWEGER 2009; SHARON & *al.* 2009). Horizontal gene transfer would have been a reasonable explanation if it had only been a few genes. But according to MOUSTAFA & *al.* 2009 more than 1,700 »green« genes have been transferred, which is 17% of the whole complement of a little more than 10,000 genes. This high transfer frequency is so surprising that not all workers in the field have wholeheartedly accepted it (DAGAN & MARTIN 2009).

But not only photosynthesis genes have been added by side-steps in the evolution of diatoms. In both centric and pennate diatoms BOWLER & *al.* 2008 have found hundreds of genes from

various prokaryotic organisms: cyanobacteria, various proteobacteria, and archaea. The more they are investigated, the more the diatoms appear as heaps of disconnected twigs from the great tree of evolution. Animals, fungi, and some microorganisms have, based on their molecular biology, been grouped together under the heading Opisthokonta, a rather thick branch on the tree of evolution. Scala et al. (2002) found as many genes in the diatom *Phaeodactylum tricornutum* being closely related to opisthokont genes as being related to genes in higher plants.

In contrast to chloroplasts of plants and green algae, which are surrounded by two membranes, there are four membranes around the chloroplasts of diatoms. This is understandable, as these chloroplasts have originated by two successive endosymbiotic events. Between the membranes one can in some cases find a »nucleomorph«, the remains of the nucleus of the engulfed red alga. The nucleomorph contains very few genes, but those that exist are typical chloroplast genes.

Motility, carbon assimilation, reproduction, and technical applications

One might think that diatoms, enclosed in glass jars as they are, would not be able to move actively. But for a long time people have studied how pennate diatoms can creep over a substratum, and now it is known that many centric diatoms possess this ability, too (SATO & MEDLIN 2006). Some diatoms leave a mucus track behind, as snails do, and it is thought that their movement is somehow connected to this slime exudation. The mucus exits through a slit called a raphe in the middle of one or both halves of the frustule (silica enclosure). The slime is set in motion by microfibrils which inside the cell are connected to filaments of actin and myosin, the same kinds of protein molecules that we have in our muscles (BERTRAND 2008). This view is, however, partly based on speculation, and slime trails are not to be seen after individuals of all species of motile diatoms. The movements are regulated by, among other things, various light-perceiving systems. Cells with a raphe can move with up to $25 \mu\text{m s}^{-1}$, but some species without a raphe can also move, albeit at a slower speed, about $1 \mu\text{m s}^{-1}$.

One might also imagine that it would be difficult for the armor-enclosed diatoms to take up carbon dioxide or bicarbonate ions for their photosynthesis, but they are, in fact, very efficient in doing this. The pretty perforations in the frustules contribute to this, and some species are able to carry out both C3-photosynthesis with incorporation of carbon dioxide into 3-phosphonoglycerate, and C4-photosynthesis with incorporation of bicarbonate into oxaloacetate (review by ROBERTS & al. 2007). Contributing to the efficient uptake of inorganic carbon is also an exudation of extracellular carbonic anhydrase, enabling a rapid interconversion between carbon dioxide and bicarbonate. Diatoms are unusual in that they have a carbonic anhydrase which can use cadmium instead of zinc without decrease in activity (STRASDEIT 2001; XU & al. 2008), which is very useful as zinc is depleted from the ocean surface. Cadmium can often replace zinc in enzymes, but usually with a large decrease or complete loss of activity.

The two halves of a diatom are of unequal size and form a box with lid (Figure 3). When cells divide, each daughter cell gets one half, and then forms a new half inside the existing one. Therefore one daughter cell will be of the same size as the original cell, while the other one will be smaller. With repeated divisions smaller and smaller cells will be created, and when a size of about one third of the original one has been reached meiosis takes place and sex cells (gametes) with half the number of chromosomes result. They can be of equal size, or half of them can be smaller and act as sperm cells, depending on the systematic position of the species. After fusion of gametes the resulting cell grows to the size we started the story with, and only then is a continuous silica enclosure produced again.

Diatoms have a long story of technical uses. The most well-known one from a Swedish perspective is as component in Alfred Nobel's dynamite, a mixture of diatom frustules and glyceryl trinitrate (»nitroglycerine«). Diatom frustules have also been used in toothpaste, but this use is declining since they are so hard that they damage the enamel of the teeth. By their ability to form patterns in silica (silicon dioxide) the diatoms have attracted interest in the field of nanotechnology (BOZARTH & al. 2009). One is more interested in understanding

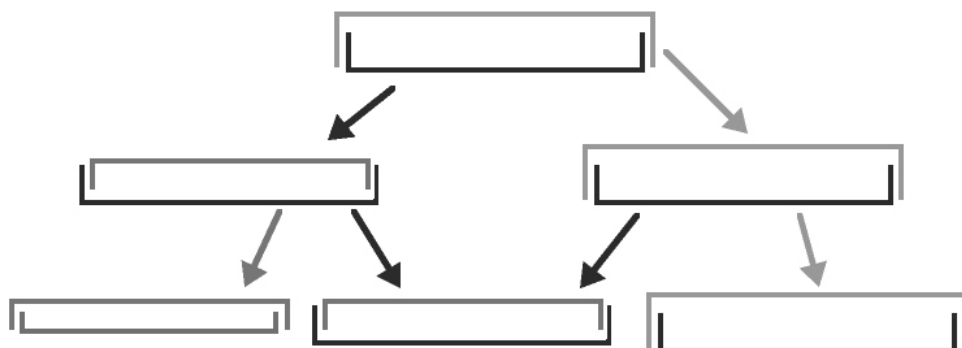


Fig. 3: The diagram shows repeated divisions of a diatom. The shades of the arrows indicate which of the two frustule halves is transmitted to the daughter cell. The cells on the right maintain the original size, while those to the left become smaller and smaller for each division. When they have reached about one third of the original size, sex cells are produced and the silica cover discarded.

Slika 3: Digram prikazuje ponavljajoče se delitve kremenastih alg. Barve puščic nakazujejo kateri dve polovicise preneseta na hčerinsko celico. Celice na desni ohranjajo originalno velikost, medtem ko tiste na levi postajajo po vsaki delitvi manjše.

how diatoms manage to do this, and copy their methods, than to use diatoms as such (DRUM & GORDON 2003; GORDON & *al.* 2009; NOYES & *al.* 2008). Nevertheless the frustule of a diatom has been used instead of a more conventional lens for focusing a 100 μm wide laser beam to a spot of 10 μm (DE STEFANO & *al.* 2007; Figure 4).

Diatom frustules can be regarded as photonic crystals, periodic structures with special properties with regard to light propagation (FUHRMANN & *al.* 2004).

The sculpturing of diatom frustules is such that they combine mechanical strength with low weight (the density of the silica in the frustules is ca. 2, i.e. about twice that of the surrounding water). The strength affords protection against predators, while the low weight is important in particular for planktonic species to avoid sedimentation out of the photic zone. The mechanical properties have been studied by HAMM & *al.* 2003.

For most diatoms silicon is an essential element, but an exception is afforded by *Phaeodactylum tricornutum* (BRZEZINSKI & *al.* 1990). The silicon is in most cases taken up as Si(OH)_4 (DEL AMO & BRZEZINSKI 1999), but *Phaeodactylum tricornutum* absorbs it as the anion SiO(OH)_3^- . Uptake takes place only during two phases of the cell cycle, namely at the end of G1 and throughout G2 (BRZEZINSKI 1992). The silicon concentration

($\approx 1 \text{ mM}$) in the Archaean sea was orders of magnitude greater than that of the contemporary (Konhauser & *al.* 2007), and due to volcanic eruptions during the Triassic the sea probably became saturated with silicic acid during the Jurassic. This is likely to have favoured diatoms, and their flourishing in the sea eventually again decreased the concentration to such an extent that many sponges with silica skeletons died out (MALDONADO & *al.* 1999). Silicon is now frequently limiting for diatom growth (e.g., SHIPE & *al.* 2007). A large drop in silica availability seems to have taken place in the late Eocene, 35 Ma ago, in connection with opening of the Southern Ocean, increased stratification, and increased abundance of diatoms. This is reflected in the decreased silification at this time of radiolarians (LAZARUS & *al.* 2009), which may have less efficient acquisition of silicate than diatoms do. Still, modern marine diatoms generally have less silica in their frustules than freshwater diatoms do, reflecting the generally higher silicic acid content in freshwater ($\approx 100 \mu\text{M}$) as compared to surface seawater ($\approx 10 \mu\text{M}$) (ALVERSON 2007). It is estimated that the silica input to the ocean is $6.1 \pm 2.0 \text{ Tmol/year}$ and the sedimentation $7.1 \pm 1.8 \text{ Tmol/year}$. Biogenic production is $240 \pm 40 \text{ Tmol/year}$, of which only a small fraction ends up as sediment. The residence time in surface waters is 400 years (TRÉGUER & *al.* 1995).

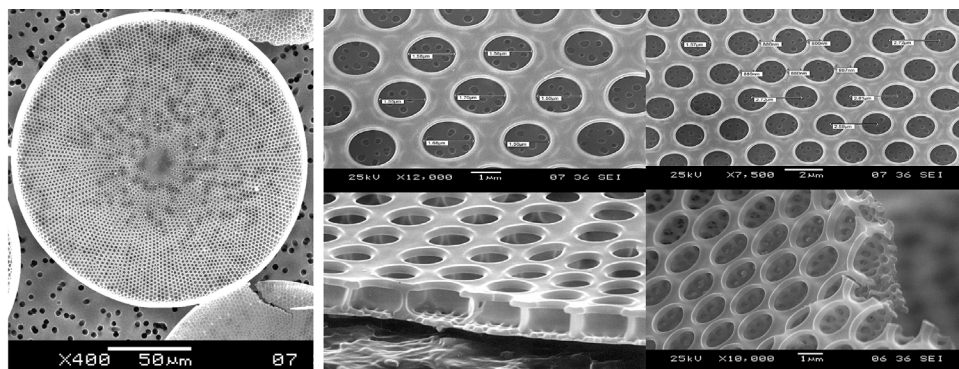


Fig. 4: Whole cell of the diatom *Coscinodiscus walesii*, used to focus laser light, and details of the regular perforations in the frustule that cause the diffractive bending of the light. The data on width of the perforations indicated in some places are printed in a font too small to be read here, but range 1.5 to 1.7 μm , and the distances between them 800–987 nm. From DE STEFANO L., I. REA, I. RENDINA, M. DE STEFANO & L. MORETTI 2007: Lensless light focusing with the centric marine diatom *Coscinodiscus walesii*. *Optics Express* **15**: 18082–18088.

Slika 4: Celotna celica kremenaste alge vrste *Coscinodiscus walesii*, uporabljena za fokusiranje laserskega žarka, in detajli luknjic v frustuli, ki povzročijo difrakcijo svetlobe. Podatki o širini luknjic, ki so na nekaterih mestih označeni so premajhni, da bi jih lahko prebrali. So v razponu od 1.5 do 1.7 μm , razdalje med njimi pa so od 800 do 987 nm. From DE STEFANO L., I. REA, I. RENDINA, M. DE STEFANO & L. MORETTI 2007: Lensless light focusing with the centric marine diatom *Coscinodiscus walesii*. *Optics Express* **15**: 18082–18088.

Diatoms are important also for their contents of omega-6 and omega-3 fatty acids, which reach us indirectly via fish in our diet. Both are essential for us, since they are needed for our brain and some other organs, and we cannot make them ourselves. The requirement of omega-6 acids is not very great, and too much inhibits the uptake in the brain cells of the omega-3 acids (Novak *et al.* 2008). Work is now in progress to circumvent the fish route and get the acids more directly into human diet, and at the same time to optimize

the omega-6/omega-3 ratio. The fats of diatoms have also been considered as biofuels for motor vehicles.

Acknowledgement

The authors stand in great debt to Professor Helen Ghiradella for her improvements of our original manuscript.

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Geological CO₂ affects microbial respiration rates in Stavešinci mofette soilsGeološki CO₂ vpliva na mikrobno dihanje v tleh na območju mofete StavešinciIrena MAČEK^{1*}, Urška VIDEMŠEK¹, Damijana KASTELEC¹, David STOPAR²,
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Abstract: Substrate-induced respiration (SIR) was used to estimate microbial respiration and microbial biomass in soils from Stavešinci natural CO₂ spring (mofette) exposed to different geological CO₂ concentrations. SIR measurements clearly demonstrated higher microbial respiration and microbial biomass in control sites compared to high soil CO₂ sites. Sampling in two different locations and in three different years also confirmed long-term stability of this pattern, which was found for both locations and in different sampling periods.

Keywords: substrate-induced respiration, SIR, microbial respiration, microbial biomass, soil respiration, natural CO₂ springs, mofette

Introduction

Soil CO₂ concentrations are about 50-times higher than ambient atmospheric CO₂ concentration and often fluctuate due to soil compaction, waterlogging and/or vegetation (BOUMA & BRYLA 2000, PFANZ & al. 2004). Natural CO₂ springs (mofettes) are extreme ecosystems with soil CO₂ concentrations that can reach values above 80 % (v/v) CO₂ in the upper 10-20 cm of soil at the most extreme sites (VODNIK & al. 2006). Most of the research at natural CO₂ springs in the past was focused on aboveground responses of vegetation (RASCHI & al. 1997, BADIANI & al. 1999, VODNIK & al. 2002, PFANZ & al. 2004, PFANZ & al. 2007). Much less work was done on the below ground responses of plants (MAČEK & al. 2005) or soil microorganisms (MAČEK 2004, MAČEK & al. 2008, VIDEMŠEK & al. 2009). Apart from the Stavešinci mofette, most of the reports on soil microbes come from the Haquanoa spring in New Zealand where arbuscular mycorrhizal (AM) fungi (RILLIG & al. 2000) and mineralization (ROSS & al. 2000,

ROSS & al. 2002, ROSS & al. 2003) were studied. In most of these studies, however, mofettes were used as long-term natural model systems for studying effects of elevated atmospheric CO₂ on ecosystems. Thus sampling was done according to the atmospheric CO₂ concentrations, which are much more dependent on weather conditions and do not always reflect soil CO₂ concentrations and their direct effects on soil microflora. Soil CO₂ concentrations were taken into consideration in the studies of soil microorganisms first at the Slovenian mofette Stavešinci (MAČEK 2004, MAČEK & al. 2008, VIDEMŠEK & al. 2009).

Soil microbial biomass can be estimated by adding an easily available substrate (e.g. glucose) to the soil (substrate-induced respiration – SIR) (JENKINSON & LADD 1981). ANDERSON & DOMSCH (1978) suggested that the initial maximal respiration rate induced by glucose was proportional to the size of the original soil microbial biomass. The method does not give an absolute value of the biomass, however, the results can be used for relative comparisons. The same authors also

report on highly significant correlation between fumigation-incubation technique and SIR for estimation of the microbial biomass. At 22 °C, 1 ml CO₂ h⁻¹ equals 40 mg microbial C (ANDERSON & DOMSCH 1978). In addition, ROSS & al. (2000) report on positive correlation between SIR and atmospheric CO₂ concentration up to 700 ppm at the New Zealand CO₂ springs, however, no attempt was made to calculate microbial biomass C from the resultant CO₂ values.

In this study substrate-induced respiration was used to estimate microbial biomass of soils exposed to different geological CO₂ concentrations in Stavešinci mofette ecosystem. Soil samples were taken in three different CO₂ regimes, defined as high, medium and low (control) geological CO₂ and in three different years 2003, 2004 and 2007.

Materials and methods

Site description and sampling

The study was conducted in Stavešinci mofette, NE Slovenia (see VODNIK & al. 2006, VODNIK & al. 2009, for detailed site description). Briefly, the site is a flat post-agricultural area where very pure, cold CO₂, without traces of sulphurous compounds, methane or carbon monoxide, is released into atmosphere through several vents. Atmospheric CO₂ concentrations largely depend on weather and wind conditions due to the topography of the site, and range from 0.036 % to 1 % (v/v) at 0.5 m aboveground (VODNIK & al. 2006). On the other side, soil CO₂ concentrations and CO₂ effluxes are more stable variables for measuring exposure to geological CO₂. Soil samples were taken from two separate locations (Location 1 and Location 2) *ca.* 40 m apart. Each sampling location covered an area of about 100 m² with

soil CO₂ concentrations ranging from high to low (ambient/control) CO₂ concentrations as measured by a portable gas analyzer (GA2000, Geotech, Germany) (VODNIK & al. 2006) and/or soil CO₂ flux measurements (LI-6400-09 Soil CO₂ flux chamber, LICOR, Lincoln, USA) (VODNIK & al. 2009). A good correlation between both methods has been confirmed before (VODNIK & al. 2009). Upper 10 cm of soil was sampled in Location 1 in March 2003 (*n* = 4-5 sampling points) and in April 2004 (*n* = 6-8 sampling points) in high CO₂ (73.6 % ± 2.7 v/v), medium (9.3 % ± 0.6 v/v) and low CO₂ (0.4 % ± 0.03 v/v) exposure. Location 2 soil was sampled in July 2007 (*n* = 4 sampling points) for high CO₂ (228.0 ± 50.4 μmol m⁻² s⁻¹), medium (42.4 ± 11.3 μmol m⁻² s⁻¹) and low CO₂ flux (21.1 ± 7.3 μmol m⁻² s⁻¹), see also VIDEMŠEK & al. 2009. Soil chemical properties for Location 1 are described by MAČEK 2004, MAČEK & al. 2005 and for Location 2 by VIDEMŠEK & al. 2009. In brief, the values for Location 1; pH 5.4 (control), 3.8 (high CO₂); organic matter 3.2 % (control), 3.8 % (high CO₂); total N 0.26 % (control), 0.32 % (high CO₂); available P₂O₅ 48 mg kg⁻¹ (control), 265 mg kg⁻¹ (high CO₂) and for Location 2; pH 5.7 (control), 4.9 (high CO₂); organic matter 3.3 % (control), 3.9 % (high CO₂); total N 0.32 % (control), 0.36 % (high CO₂); available P₂O₅ 22 mg kg⁻¹ (control), 44 mg kg⁻¹ (high CO₂). Fresh samples were transported and stored at 4 °C and all the measurements were performed within two days after sampling. Before measurements soil was thoroughly mixed and all visible plant particles were removed.

Soil water content

Soil water content was determined by drying soil samples over night at 110 °C and weighing.

Table 1: Sample water content. Avg ± SE are shown (*n* = 4-6).

Tabela 1: Vsebnost vode v vzorcih. Prikazano je povprečje ± SN (*n* = 4-6).

Sampling period	Soil water content (mass %)		
	High CO ₂	Medium CO ₂	Low CO ₂
March 2003	23.0 ± 3.1	22.3 ± 0.3	20.7 ± 0.7
April 2004	27.6 ± 0.3	no data	26.9 ± 0.6
June 2007	9.8 ± 1.2	9.8 ± 1.2	11.6 ± 2.0

Substrate-induced respiration (SIR)

Respiration rates were estimated by incubating 30 g of soil in 130-ml bottles sealed with rubber seals at room temperature (22 °C), for the 2003 and 2004 measurements, and at 28 °C in July 2007. All samples had equal dry weight. In order to avoid geological CO₂ background all samples were pre-aerated to equalize CO₂ concentrations to ambient concentrations. For SIR measurements the samples were amended with 25 mg glucose g⁻¹ dry soil and thoroughly mixed. Basal respiration was taken as the respiration rate of soils not amended with glucose and was subtracted from the SIR value. The concentrations of CO₂ in the headspace of the bottles were measured by gas chromatography, using a Becker Packard model 417 (Delft, Netherlands) gas chromatograph (GC), with thermal conductivity detector temperature 100 °C, 1.8-m column (2 mm inside diameter) packed with Prapak QS 180 cm column at 50 °C, injector temperature 100 °C, carrier gas (He) flow 20 ml min⁻¹ and Hewlett Packard 3392A integrator. Samples (2.5 ml) of headspace gas were taken with a gas-tight syringe and injected into the gas chromatograph. Since the pH of the aqueous phase was < 6.5, the effective gas headspace of the bottles was assumed to be the volume not occupied by soil or liquid (LIN & BROOKES 1999).

The amount of produced CO₂ in the measuring bottle was calculated as:

$$M_{CO_2} = (C_g * (V_g + V_v * \alpha)) / m$$

M_{CO_2} = total CO₂ (ml g⁻¹ soil), C_g = measured CO₂ concentration in the gas phase (%), V_g = volume of the gas phase (130 ml), V_v = volume of the liquid phase in the soil (ml), α = Bunsen coefficient for CO₂ = 0.758, m = dry weight of soil in the bottle.

For measurements performed at 22 °C microbial biomass was calculated according to ANDERSON & DOMSCH (1978) where 1 ml CO₂ h⁻¹ equals 40 mg microbial C.

Data analysis

Data of the microbial respiration at different CO₂ levels were analysed for each year/location separately. Because of the longitudinal nature of the data (each sample was measured consequently several times during a time interval and

the intervals between the measurements differ for different samples) the linear mixed models with restricted maximum likelihood method were used for the estimation of the parameters. Time and CO₂ exposure group (high, medium, low) and their interaction were included in the model as fixed effects and soil sample with its time dependence were included in the model as random effect. The compound symmetry structure of the within samples random effect covariance was used in the model (PINHERIRO & BATES, 2000). The calculations were done with the statistical package R (R DEVELOPMENT CORE TEAM, 2009).

Results and discussion

Microbial soil biomass is dependent on quantity and quality of soil organic matter (ZAK & al. 1993, CHENG 1999), which in turn depends on plant production. Both, plant roots and above ground vegetation are directly affected by high soil CO₂ concentrations (KALIGARIČ 2001, VODNIK & al. 2002, MAČEK & al. 2005, PFANZ & al. 2004, PFANZ & al. 2007). It has been shown that in the high CO₂ exposed mofette plants content of N is lower and C/N ratio in plant tissues is higher, compared to control (PFANZ & al. 2004). In addition, lower concentrations of several other elements (P, K, S, and Zn) have been reported for high geological CO₂ exposed plants (PFANZ & al. 2004). All this should have an effect on microbial biomass and respiration.

As given in Fig. 1 glucose addition stimulated CO₂ release from all soil samples, indicating that soil microorganisms were activated by the addition of the respiratory substrate. The respiration data show linear ($p < 0.0001$) increase of the CO₂ concentration. Different slopes of linear model lines indicate changes in microbial activities (Fig. 1). In 2003, SIR was significantly lower in high CO₂ soils, compared to control soils ($p = 0.0118$). A similar trend was found in 2004, however there was no significant difference between high and low CO₂ soils. Similar to findings from the previous two years also microbial respiration measured in 2007 in samples from the second mofette (Location 2) showed the lowest values in high CO₂ soils, followed by medium and low (control) soils. In this year, significant difference was found between

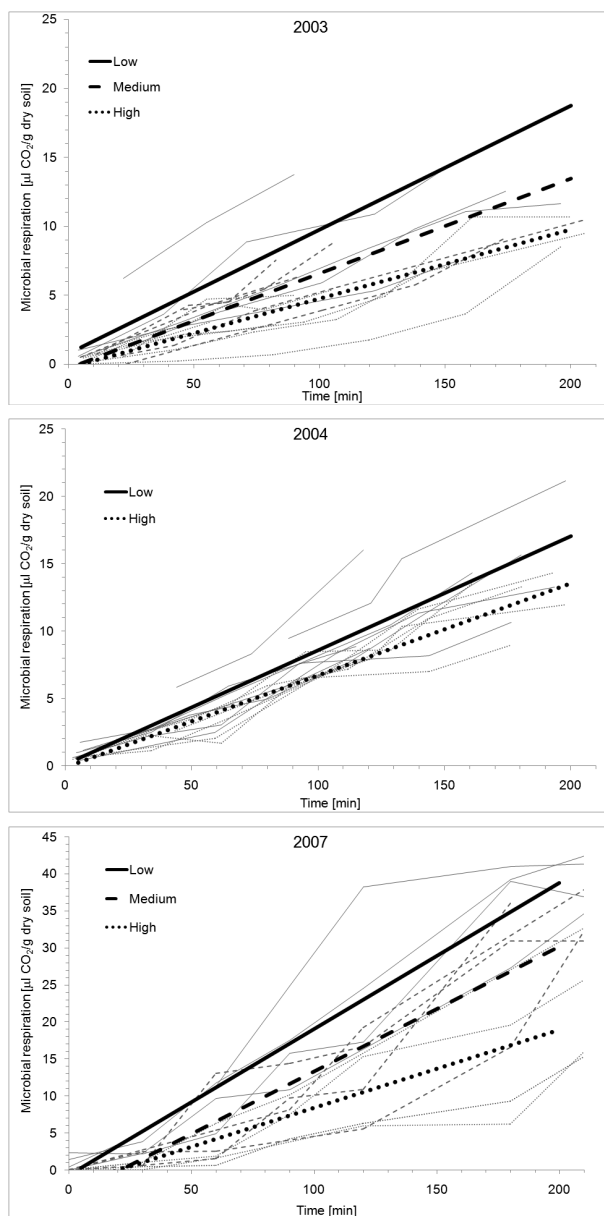


Fig. 1: Substrate induced microbial respiration (SIR), measurements of CO_2 production in soil samples from natural CO_2 springs in Stavešinci. Time course of microbial activity (respiration) after substrate addition measured on each sample (thin lines), linear model lines (thick lines); for low (full-lines), medium (dash-lines) and high (dot-lines) CO_2 concentrations.

Slika 1: S substratom inducirano mikrobno dihanje (SIR), meritve produkcije CO_2 v talnih vzorcih s področja naravnih izvirov CO_2 v Stavešincih. Časovna odvisnost mikrobne aktivnosti (dihanja) po dodatku substrata na posameznem vzorcu (tanke črte), premice linearnih modelov (debelejše črte); prikazano za majhne (polna linija), srednje (črtkana linija) in velike (pikčasta linija) koncentracije CO_2 .

Table 2: The estimated parameters of the linear mixed models with the 95 % confidence limits.

Tabela 2: Ocene parametrov linearnih mešanih modelov s 95 % intervali zaupanja.

Year	Parameter		95 % Confidence intervals		
			Estimates	Lower limit	Upper limit
2007	Intercept	H	-0.2115	-0.4532	0.0303
		M	-0.3576	-1.0006	0.2854
		L	-0.0690	-0.6954	0.5574
	Slope	H	0.0106	0.0068	0.0143
		M	0.0169	0.0078	0.0260
		L	0.0197	0.0108	0.0287
2004	Intercept	H	-0.0094	-0.0732	0.0545
		L	0.0107	-0.1475	0.1689
	Slope	H	0.0068	0.0054	0.0082
		L	0.0085	0.0051	0.0118
2003	Intercept	H	-0.0271	-0.0880	0.0338
		M	-0.0283	-0.2035	0.1470
		L	0.0791	-0.0965	0.2547
	Slope	H	0.0050	0.0032	0.0068
		M	0.0069	0.0021	0.0116
		L	0.0090	0.0041	0.0138

high soil CO₂ and control ($p = 0.0009$) and also between high and medium soil CO₂ ($p = 0.0210$), but there was no difference between medium soil CO₂ and control. The estimated parameters of the linear mixed models with the 95 % confidence limits are presented in Tab. 2. Calculated microbial biomass is given in Tab. 3 (only for years 2003 and 2004). There is a clear increase in microbial biomass in both years with decreased geological CO₂ concentrations in the soil.

The effect of elevated atmospheric CO₂ on soil microbial respiration was reported before for the mofette areas in New Zealand (Ross & al. 2000), however, to the best of our knowledge no study reports on the effect of the extreme soil geological CO₂ enrichment on microbial biomass. VIDEMŠEK & al. (2009) have shown a shift

in microbial community structure of CO₂-fixing bacteria in grassland soils from the Stavešinci mofette, depending on the soil CO₂ exposure. It has also been shown in the same mofette area that almost a complete turnover (β diversity) in community composition of symbiotic arbuscular mycorrhizal fungi occurs, depending on soil abiotic factors (soil CO₂ exposure and hypoxia) (MAČEK & al. 2008).

For the Stavešinci mofette, SIR measurements and microbial biomass C estimation, clearly demonstrate higher microbial respiration and microbial biomass in control sites with low soil CO₂ concentration compared to high CO₂ samples (Fig. 1, Tab. 3). Differences between the years could be partially explained with the soil water content (Tab. 1). It is possible that due to higher

Table 3: Calculated microbial biomass.

Tabela 3: Ocenjena mikrobna biomasa.

Year	* Microbial biomass ($\mu\text{g g}^{-1}$ dry soil)	
	2003	2004
High CO ₂	115	162
Medium CO ₂	159	no data
Low CO ₂	231	205

* Measured 2 h following glucose addition.

water content in 2004 the respiratory substrate glucose, introduced into the sample in a solid form, could not distribute evenly (formation of clumps during mixing of soil) and thus was not available to all the potential users. In the study on the evaluation of the SIR method by LIN & BROOKES (1999) glucose was added both in solid or liquid form, however, similar patterns of CO₂ evolution were found for both protocols. In addition, it was concluded in the same study, that no correction for CO₂ dissolved in the soil solution was needed for the soils below pH 6.5, which is also the case for Stavešinci soil. Higher absolute values of the microbial respiration measured in 2007 are probably due to higher incubation temperatures during the SIR experiment. Nevertheless, the same pattern in microbial respiration response to geological CO₂ as in the previous two years was observed. It is interesting to note that in 2007 samples originated from the second mofette (Location 2), which is about 40 m distant from the Location 1 (sampling in 2003 and 2004) with different soil properties and less extreme CO₂ regime (see the Methods section). The values for microbial biomass for the years 2003 and 2004 (Tab. 3) are in the range of those found for other grasslands (HABEKOST & al. 2008).

Conclusions

According to the results of this study we conclude that high concentrations of geological soil CO₂ decrease substrate induced microbial respiration and microbial biomass. This pattern

of microbial activity was stable and was not affected by different soil properties, different sampling periods, temperature of incubation, or soil water content.

Povzetek

Mikrobno dihanje in biomaso v talnih vzorcih lahko merimo z dodatkom lahko razgradljivega substrata npr. glukoze (s substratom inducirana respiracija – SIR). Respiratorni CO₂ merimo s plinsko kromatografijo. V naši raziskavi smo to metodo uporabili za oceno mikrobne dihanja in mikrobne biomase v vzorcih z območja naravnih izvirov CO₂ (mofet) v Stavešincih (SV Slovenija), izpostavljenih različnim koncentracijam geološkega CO₂. Meritve kažejo na manjše dihanje in mikrobno biomaso v vzorcih, izpostavljenih veliki koncentraciji CO₂, v primerjavi s kontrolo. Z vzorčenjem na dveh različnih lokacijah znotraj območja vrelcev v Stavešincih in obenem v treh različnih letih (2003, 2004 in 2007) pa smo pokazali tudi dolgoročno stabilnost opaženega vzorca mikrobnega odziva, ki se je pojavil na obeh lokacijah in v vseh treh letih vzorčenja.

Acknowledgements

The authors would like to thank to dr. Tjaša Danevčič and Simona Leskovec for technical assistance. The research was funded by the Slovenian Research Agency. The authors gratefully acknowledge all the given support.

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Changes in physico-chemical characteristics and the succession of phytoplankton in the lake Velenjsko jezero following its restoration

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Abstract: The species composition of phytoplankton in the artificial lake Velenjsko jezero has been monitored since 1994 while physico-chemical characteristics of the lake water since 1998. Before the year of lake remediation, 1994, the pH of lake water was around 12. In 1994, only filamentous cyanobacteria *Oscillatoria* ssp. were present in high abundance, with the rare appearance of *Synedra* sp. and *Ceratium* sp.. In 1995, the pH in the upper water layers decreased to 9, as a consequence of the construction of a fly ash system with a closed loop water cycle in October 1994. The number of algae taxons increased to 7 (*Coelosphaeria* sp., *Gomphosphaeria* sp., *Scenedesmus* sp., *Pediastrum* sp., *Asterionella* sp., *Synedra* sp. and *Ceratium* sp.). In 1996, when the pH fell to 8, it increased to 13. The lake provided good conditions for algal development since it was rich in nutrients. Since 1996 the level of nutrients in the upper layers of the water column has remained more or less the same, but in the deeper layers the reduced form of nitrogen (NH_4^+) has increased and the oxygen curve has become clinograd. Velenjsko jezero can be classified according to OECD, as hypereutrophic on the basis of the level of total phosphorus ($120 \mu\text{g L}^{-1}$) and total nitrogen ($1500 \mu\text{g L}^{-1}$), the average transparency of 5.38 m corresponds to mesoeutrophic status, and the average concentration of chlorophyll *a* at $1.03 \mu\text{g L}^{-1}$ to oligotrophic status. Despite the high availability of nutrients the primary production was not as high as in a similar natural lake ecosystem, which could be ascribed to the high concentration of ions Ca^{2+} , K^+ , Mg^{2+} , Na^+ , Cl^- and particularly, SO_4^{2-} . The predominant algae in the lake in 2007 were cyanobacteria *Pseudanabaena* cf. *catenata*, *Planktothrix rubescens*, from which the first bloom occurred in June and the second from November to January, and dynophyta *Ceratium hirundinella* and *Peridinium cinctum*.

Keywords: pH, lake water, nutrients, phytoplankton

Izvleček: Vrstna sestava in abundanca fitoplanktona se v Velenjskem jezeru določa že od leta 1994. Vrednost pH jezerske vode je bila 12 vse do leta 1994, ko so v Termoelektrarni Šoštanj uvedli zaprti krog transportne vode (oktober 1994). Ob visokem pH, so bile v jezeru v velikem številu prisotne le filamentozne cianobakterije *Oscillatoria* ssp., z redko prisotnostjo taksonov *Synedra* sp. in *Ceratium* sp.. V letu 1995, ko je pH zgornjih plasti jezera narasel do 9, je število taksonov zraslo na število 7 (*Coelosphaeria* sp., *Gomphosphaeria* sp., *Scenedesmus* sp., *Pediastrum* sp., *Asterionella* sp., *Synedra* sp. in *Ceratium* sp.) in v letu 1996, ko je pH padel na 8, smo lahko v jezeru določili že 13 taksonov. Jezero nudi zelo ugodne razmere za razvoj alg, saj je bogato s hranili. Od leta 1996 sicer ostajajo koncentracije hranil v epilimniju bolj ali manj enake, v spodnjih plasteh pa se z leti povečujejo koncentracije amonija (NH_4^+) in kisikova krivulja je postala klinograдна. Medtem ko je bilo jezero v letu 1996 še prezračeno do dna (45 m), je že v letu 2000 kisik skoraj popolnoma izginil pod globino 20 metrov. Velenjsko jezero lahko glede na OECD klasifikacijo uvrstimo glede na količino celotnega fosforja ($120 \mu\text{g L}^{-1}$) in celotnega dušika ($1500 \mu\text{g L}^{-1}$) med hiperevtrfna jezera, na osnovi povprečne prosojnosti (5.38 m) med mezoevtrfna jezera, in na osnovu povprečne koncentracije klorofila *a* ($1.03 \mu\text{g L}^{-1}$) med oligotrofna jezera. Kljub veliki koncentraciji hranilnih snovi v jezeru, pa primarna produkcija ni tako velika kot je v drugih podobnih jezerskih ekosistemi. Vzrok za to lahko iščemo

v drugačnem kemizmu, saj se zaradi bližine industrije jezero polni z ioni Ca^{2+} , K^+ , Mg^{2+} , Na^+ , Cl^- , še posebej pa z SO_4^{2-} . Prevladajoči algi v letu 2007 sta bili cyanobacteria *Pseudanabaena* cf. *catenata* in *Planktothrix rubescens*. Cvet prve vrste se je pojavil v juniju, druga vrsta pa je cvetela od novembra do januarja. Pogosti vrsti v letu 2007 sta bili tudi dynophyta *Ceratium hirundinella* in *Peridinium cinctum*.

Ključne besede: pH, jezero, hranila, fitoplankton

Introduction

The lake Velenjsko jezero can be used as a model of colonization of an empty habitat, since the high pH prevented the existence of most organisms before 1994. Colonization of macrophytes has been already described (MAZEJ & EPŠEK 2005, MAZEJ & GERM 2008). The massive development of submersed macrophytes indicated that Velenjsko jezero is very rich in nutrients.

Coexistence of a number of phytoplankton species is a conspicuous feature of fresh waters. Although a few species commonly dominate a phytoplankton assemblage, a number of rarer algae coexist with the dominant species. Many differences in algal physiological characteristics, requirements, and tolerances, together with seasonal and spatial variations in environmental parameters, permit an apparently multispecific equilibrium to exist for short periods. Algae have defined temperature optima and tolerance ranges that interact with other parameters to cause seasonal succession. For example, many diatoms can photosynthesize successfully at cooler water temperatures, whereas the temperature optima of many green algae and cyanobacteria are higher (WETZEL 2001).

Attributes considered to be symptoms of negative impacts of nutrient enrichment in many ecosystems include blooms of toxic algae, increased growth of epiphytic algae, the growth of macroalgae, the loss of submerged vegetation due to shading, the development of hypoxic (and anoxic) conditions due to the decomposition of accumulated biomass, and the changes in the community structure of benthic animals due to oxygen deficiency or the presence of toxic phytoplankton species (REVILLA & al. 2009). The phytoplankton, because of its relationship with the eutrophication processes, is one of the biological elements considered within the Water Framework Directive (WFD). Phytoplankton biomass, composition

and abundance, together with frequency and intensity of blooms, are the metrics to be assessed according to the WFD. Among the advantages of using phytoplankton to assess water quality are the rapid response of this group of organisms to the changes in the environment, their primary role in the food web and their influence on other organisms (WILLÉN 2001).

In this paper we evaluated the trophic status of Velenjsko jezero between 1996 and 2007, considering some physico-chemical and biological parameters and analysed the succession of algae species composition and abundance following completion of the restoration measures in 1995.

Materials and Methods

Study area

Velenjsko jezero is located in central Slovenia, in the Šalek Valley, at an altitude of 366 m. It has a surface area of 135,000 m² and a maximal depth of 54 m. It is an artificial lake resulting from mining activity. Whole settlements, meadows and fields were submerged and flooded as a result of subsidence. Until 1983, fly ash slurry from the Šoštanj Thermal Power Plant was transported by pipeline and emptied into Velenjsko jezero. This brought ash and calcium hydroxide to the lake, raising the pH of the water to 12. Since 1983 the ash has been used to build embankments, but effluent with a pH around 12 remained the predominant pollutant of the lake until 1994. After construction of a fly ash system with a closed loop water cycle in October 1994, biota appeared in the lake. It was colonized by phyto- and zooplankton, fish, macrophytes (MAZEJ & EPŠEK 2005) and other organisms. The pH of the lake is now around 8 and the lake is dimictic.

Physical and chemical parameters

The water samples from the different depths at deepest part of Velenjsko jezero were taken and analysed four times a year (spring, summer, autumn, winter) in the years 1996, 1998, 2000, 2002, 2004 and 2007. Transparency was measured by Secchi disk. Temperature, pH and oxygen profiles were obtained using a portable oxygen meter WTW multiline P4. Water samples for laboratory analysis were obtained from different depths using a depth (Van-Dorn) sampler. Before 2000, parameters were determined by following standard methods: total phosphorus (SIST ISO 6878:1996), ammonium nitrogen (SIST ISO 5664:1996), nitrate nitrogen and sulphate (SIST ISO 10304-2:1996), SEP (DIN 38404), magnesium and calcium (SIST EN ISO 7980). From 2000 onwards, parameters were determined by the following standard methods: total phosphorus (SIST ISO 6878:1996), ammonium nitrogen (SIST ISO 5664:1996), nitrate nitrogen, chloride and sulphate (SIST ISO 10304:1998), SEP (SIST EN 27888:1998), magnesium and calcium (SIST EN ISO 7980).

Biological parameters

Samples for Chl-a were obtained using a Van-Dorn sampler. After filtration through glass microfibre Watman GF/C filter they were analyzed by the standard method ISO 10260.

The plankton samples were taken and analysed four times a year (spring, summer, autumn, winter) in the years 1994, 1995, 1996, 2000, 2002, 2004 and 2007. Qualitative 20 µm mesh plankton net samples were taken as a vertical profile, preserved

in 3% formaldehyde and analysed for phytoplankton species community composition. The species were identified using a light microscope according to HINDAK (1978), Ettl & GARTNER (1988), Ettl & al. (1999), KRAMMER & LANGE-BERTALOT (1991, 1997, 2000a, 2000b, 2004), KOMAREK & al. (2005), STARMACH (1985), POPOVSKI & PFIESTER (1990), STREBLE & KRAUTER (2002), VRHOVŠEK & al. (2006). Their abundance was rated into three categories: present (1), subdominant (3) and dominant (5). Unicellular Cyanobacteria were counted like trichomes.

Results and Discussion

After construction of a fly ash system with a closed loop water cycle in October 1994, pH of water started to decrease (RAMŠAK & REJIC 1995, RAMŠAK 1996). Only filamentous cyanobacteria (*Oscillatoria* sp.) were present in higher abundance, and rare appearance of *Synedra* sp., and *Ceratium* sp. was observed in 1994, when the pH was still above 11. In 1995, when water quality improved, the pH in the upper water layers decreased to 9, the number of algae taxons increased to 7 (*Coelosphaeria* sp., *Gomphosphaeria* sp., *Scenedesmus* sp., *Pediastrum* sp., *Asterionella* sp., *Synedra* sp. and *Ceratium* sp.) and to 13 in 1996, when the pH fell to 8. The lake was rich in nutrients, providing good conditions for algae development (Table 1, Table 2). Velenjsko jezero is relatively deep lake, but accelerated eutrophication, due to non-point sources of nutrients from drainage areas, nevertheless occurred between

Table 1: OECD recommendations for classification of lakes into trophic categories (OECD 1982) based on total phosphorus, total nitrogen and chlorophyll contents and transparency of the water. The values for Velenjsko jezero in 2007 are shaded.

Tabela 1: Priporočila OECD (1992) za uvrstitev jezer v trofične kategorije na podlagi povprečnih letnih koncentracij celotnega dušika in fosforja, koncentracij klorofila a ter prosojnosti vode. Kategorije, v katere lahko uvrstimo Velenjsko jezero so osenčene.

OECD value	Total phosphorus (µg L ⁻¹)	Total nitrogen (µg L ⁻¹)	Transparency (m)	Chlorophyll (µg L ⁻¹)
Ultraoligotrophic	<4	<200	>12	<1
Oligotrophic	<10	200–400	>6	<2.5
Mesoeutrophic	10–35	300–650	6–3	2.5–8
Eutrophic	35–100	500–1500	3–1.5	8–25
Hyper eutrophic	>100	<1500	<1.5	>25

Table 2: Mean physico-chemical characteristics of water of 30 cm depth in Velenjsko jezero in the years of sampling; n=4, average value $\pm$ SDTabela 2: Povprečni rezultati fizikalnih meritev in kemijskih analiz vode iz globine 30 cm v Velenjskem jezeru v letih vzorčevanja; n=4, povprečna vrednost $\pm$ SD

	1996	1998	2000	2002	2004	2007
Transparency (m)	7.75 $\pm$ 1.77	4.83 $\pm$ 1.08	4.35 $\pm$ 0.92	4.50 $\pm$ 1.74	6.78 $\pm$ 2.81	5.38 $\pm$ 1.73
pH	8.75 $\pm$ 0.25	8.75 $\pm$ 0.26	8.40 $\pm$ 0.20	7.80 $\pm$ 0.10	8.20 $\pm$ 0.20	8.80 $\pm$ 0.40
SEP (μ S cm ⁻¹)	–	661 $\pm$ 126	905 $\pm$ 71.9	924 $\pm$ 68.2	1085 $\pm$ 81.0	1398 $\pm$ 29.9
NH ₄ ⁺ (mg L ⁻¹)	–	0.05 $\pm$ 0.005	0.33 $\pm$ 0.14	0.36 $\pm$ 0.07	0.30 $\pm$ 0.08	0.26 $\pm$ 0.26
NO ₃ ⁻ (mg L ⁻¹)	–	4.36 $\pm$ 0.51	2.90 $\pm$ 0.42	3.38 $\pm$ 0.52	4.70 $\pm$ 0.63	3.74 $\pm$ 0.75
P – total (mg L ⁻¹)	–	0.05 $\pm$ 0.011	0.05 $\pm$ 0.03	0.08 $\pm$ 0.05	0.09 $\pm$ 0.06	0.06 $\pm$ 0.03
Chlorophyll a (μ g L ⁻¹)	–	–	1.40 $\pm$ 0.51	2.10 $\pm$ 0.98	1.70 $\pm$ 0.10	1.03 $\pm$ 0.38
SO ₄ ²⁻ (mg L ⁻¹)	–	159 $\pm$ 65.5	369 $\pm$ 38.7	403 $\pm$ 43.1	595 $\pm$ 32.5	623 $\pm$ 31.2
Ca ²⁺ (mg L ⁻¹)	–	70.8 $\pm$ 8.10	81.3 $\pm$ 22.1	119 $\pm$ 14.2	175 $\pm$ 15.3	192 $\pm$ 18.6
K ⁺ (mg L ⁻¹)	–	42.4 $\pm$ 4.24	44.1 $\pm$ 9.5	40.0 $\pm$ 6.01	48.0 $\pm$ 3.52	43.1 $\pm$ 2.25
Mg ²⁺ (mg L ⁻¹)	–	11.8 $\pm$ 0.87	13.1 $\pm$ 1.27	14.6 $\pm$ 2.20	14.2 $\pm$ 1.06	16.2 $\pm$ 0.49
Na ⁺ (mg L ⁻¹)	–	41.6 $\pm$ 0.85	46.2 $\pm$ 5.40	48.2 $\pm$ 3.42	46.7 $\pm$ 2.70	63.7 $\pm$ 8.60
Cl ⁻ (mg L ⁻¹)	–	–	–	–	21.1 $\pm$ 0.22	34.2 $\pm$ 4.57

1996 and 2007. The transparency of the lake and the nutrient concentration, and the concentration of chlorophyll *a* in the epilimnium remained at the same levels from 1996 to 2007, while the concentrations of ions were increasing regularly, especially sulphate and calcium, and consequently the specific electrical conductivity (SEP). While in 1996 the lake water was fully aerated to the bottom (45 m), oxygen was almost completely exhausted below a depth of 20 m in August and in November 2000 and November 2007. As a consequence the concentration of NH₄⁺ started to arise, and the concentration of NO₃⁻ decreased below 10 metres.

Average values measured at different depths and the volume of each stratum was used to calculate average annual concentrations of parameters. On the basis of the levels of total phosphorus (120 μ g L⁻¹) and total nitrogen (1500 μ g L⁻¹) determined in 2007, Velenjsko jezero was classified as hyper eutrophic, while the average transparency of 5.38 m corresponded to meso eutrophic status and the average concentration of chlorophyll *a* 1.82 μ g L⁻¹ to oligotrophic status (OECD 1982). It was expected that primary production would be higher due to the relatively high concentration of nutrients, but it appeared that other factors limited development of phytoplankton. Concentration of chlorophyll *a* in Velenjsko jezero is smaller in

comparison with the lakes with the same trophic status (REMEC REKAR 2008). The concentration of chlorophyll *a* is directly connected with the presence of phytoplankton and cyanobacteria, which are holders of primary production in lake water. Chlorophyceae, Cryptophyceae and cyanobacteria have a high impact on the concentration of chlorophyll *a*, while Bacillariophyceae, Dinophyceae and Chrysophyceae are of lesser importance (KASPRZAK & al. 2008).

A small but general increase of all taxons was observed in the period from 1996 to 2007, but the increase of taxons of Bacillariophyceae, especially Chlorophyta was notable. Of the 66 species recognized, only a few contributed at least once during the year to the major percentage of total density (Cyanophyceae: *Pseudanabaena* cf. *catenata*, *Planktothrix rubescens* and *Phormidium* sp., Dynophyta: *Ceratium hirundinella* and *Peridinium cinctum* and Bacillariophyceae: *Cyclotella meneghiniana* and *Stephanodiscus* sp.) (Table 3). The phytoplankton assemblage, in which a number of rarer species were found among the dominant ones, shows the eutrophic status of the Lake. From 1996 onwards, a very significant part of the phytoplankton biomass consisted of dinoflagellates from genus *Ceratium* and *Peridinium*. They had been prevailing in the biomass till 2004, when the predominance of filamentous

Taxa	Spring					Summer					Autumn					Winter				
	May 1996	20. 04. 2000	06. 05. 2002	11. 05. 2004	17. 04. 2007	Aug. 1996	17. 08. 2000	18. 07. 2002	27. 07. 2004	08. 08. 2007	Sept. 1996	09. 11. 2000	23. 10. 2002	25. 10. 2004	08. 11. 2007	Dec. 1996	19. 12. 2000	16. 12. 2002	07. 12. 2004	20. 12. 2007
ph: Chlorophyta																				
<i>Ankistrodesmus acicularis</i> (<i>Monoraphidium griffithii</i>)		1	1								1									1
<i>Ankistrodesmus falcatus</i> (<i>Monoraphidium contortum</i>)															1					
<i>Chlorococcum infusionum</i>			1				1	1		1		1								
<i>Coelastrum microporum</i>										1		1	1							
<i>Coelastrum reticulatum</i>				1	1				1	1					1					1
<i>Coelastrum sphaericum</i>															1					
<i>Cosmarium bloculatum</i>							1													
<i>Monoraphidium convolutum</i>																				
<i>Microspora sp.</i>															1					
<i>Pandorina morum</i>							1	1											1	
<i>Pediastrum clathratum</i>					1			1					1					1		1
<i>Pediastrum boryanum</i>		1			1		1	1	1											
<i>Pediastrum gracillinum</i>							1													
<i>Pediastrum simplex</i>								1												
<i>Pediastrum sp.</i>	3					2					1									
<i>Planktosphaeria gelatinosa</i>																				1
<i>Scenedesmus obliquus</i>												1					1			
<i>Scenedesmus platydiscus</i>															1					
<i>Scenedesmus sp.</i>										1	1		1		1					1
<i>Sphaerocystis sp.</i>								1												
<i>Staurastrum gracile</i>											1		1							
<i>Staurastrum paradoxum</i>													1							
<i>Volvox sp.</i>																			1	
ph: Euglenophyta																				
Euglena sp.			1					1												
ph: Heterokontophyta																				
cl: Chrysophyceae																				
<i>Dinobryon divergens</i>															1					
<i>Dinobryon sp.</i>				1						1										
cl: Bacillariophyceae (Diatomeae)																				
Achnantes minutissima					1															
<i>Amphora veneta</i>			1					1												
<i>Amphora ovalis</i>												1								
<i>Amphora sp.</i>		1	1					1												
<i>Asterionella formosa</i>	1	1	1			1		1			1	1				1				1
<i>Caloneis amphisbaena</i>								1							1			1		
<i>Cocconeis sp.</i>										1										

Taxa	Spring					Summer					Autumn					Winter				
	May 1996	20. 04. 2000	06. 05. 2002	11. 05. 2004	17. 04. 2007	Aug. 1996	17. 08. 2000	18. 07. 2002	27. 07. 2004	08. 08. 2007	Sept. 1996	09. 11. 2000	23. 10. 2002	25. 10. 2004	08. 11. 2007	Dec. 1996	19. 12. 2000	16. 12. 2002	07. 12. 2004	20. 12. 2007
<i>Cyclotella meneghiniana</i> (<i>C. kuetzingiana</i> , <i>C. melosiroides</i>)		1				1				1					3					1
<i>Cyclotella</i> sp.				1	1			1	1											
<i>Cymatopleura solea</i> (<i>C. librilis</i>)					1															
<i>Cymbella lanceolata</i>										1										
<i>Diatoma elongatum</i> (<i>D. tenue</i>)					1															1
<i>Diatoma vulgare</i> (<i>D. vulgaris</i>)					1															
<i>Fragilaria crotonensis</i>		1						1												1
<i>Hantzschia amphioxys</i>																	1			
<i>Gyrosigma attenuatum</i> (<i>G. acuminatum</i>)															1					
<i>Melosira varians</i>																			1	
<i>Navicula cryptocephala</i>			1																	
<i>Navicula cuspidata</i>																				
<i>Navicula radiosa</i>																	1			
<i>Navicula</i> sp.			1							1	2				1	1				
<i>Nitzschia sigmoidea</i>															1					
<i>Nitzschia</i> sp.	2																			
<i>Stephanodiscus</i> sp. (<i>S. hantzschii</i>)												3					5			
<i>Surirella</i> sp.					1													1		
<i>Synedra acus</i>	3		1			3	1			1	3		1		1	2				1

Cyanobacteria was observed. Nutrient enrichment of lakes is usually accompanied by characteristic shifts within the phytoplankton community. During eutrophication, small flagellated taxa are replaced by increasing proportions of green algae, with cyanobacteria finally predominating (McQUEEN & al. 1986). The distribution of dinoflagellates as a function of major chemical and physical factors shows that most dinoflagellate species have restricted ranges with respect to calcium, pH, dissolved organic matter, and temperature (TAYLOR & POLLINGER 1987). Some are however highly tolerant and widespread, especially species of *Ceratium* and *Peridinium*, which were present in high abundance in Velenjsko jezero. Many other (micro) algae species, especially greens (22 species), occurred infrequently during warmer periods of the year.

Populations of filamentous cyanobacteria is increased in hypereutrophic lakes (WETZEL 2001). Although the number of cyanobacteria taxons was only 9 in 2007, their biomass was greater than that of other taxons almost all the year (REMEC REKAR 2008). Only in April 2007 the diatoms prevailed, in June the density of cyanobacteria *Pseudanabaena* sp. was very high especially in the metalimnium ($>24.42 \cdot 10^6$ cells/L). While in August Chlorophyta and Dinophyta constitutes 50% and Cyanobacteria 50% of the phytoplankton biovolume, in November a bloom of *Planktothrix rubescens* occurred prevailing over other taxons (REMEC REKAR 2008). It is generally recognized that cyanobacterial blooms are the direct consequence of eutrophication (REYNOLDS & PETERSEN 2000). In Velenjsko jezero massive, long-lasting blooms of *Planktothrix rubescens* were observed (from November 2007 to February 2008). *Planktothrix*

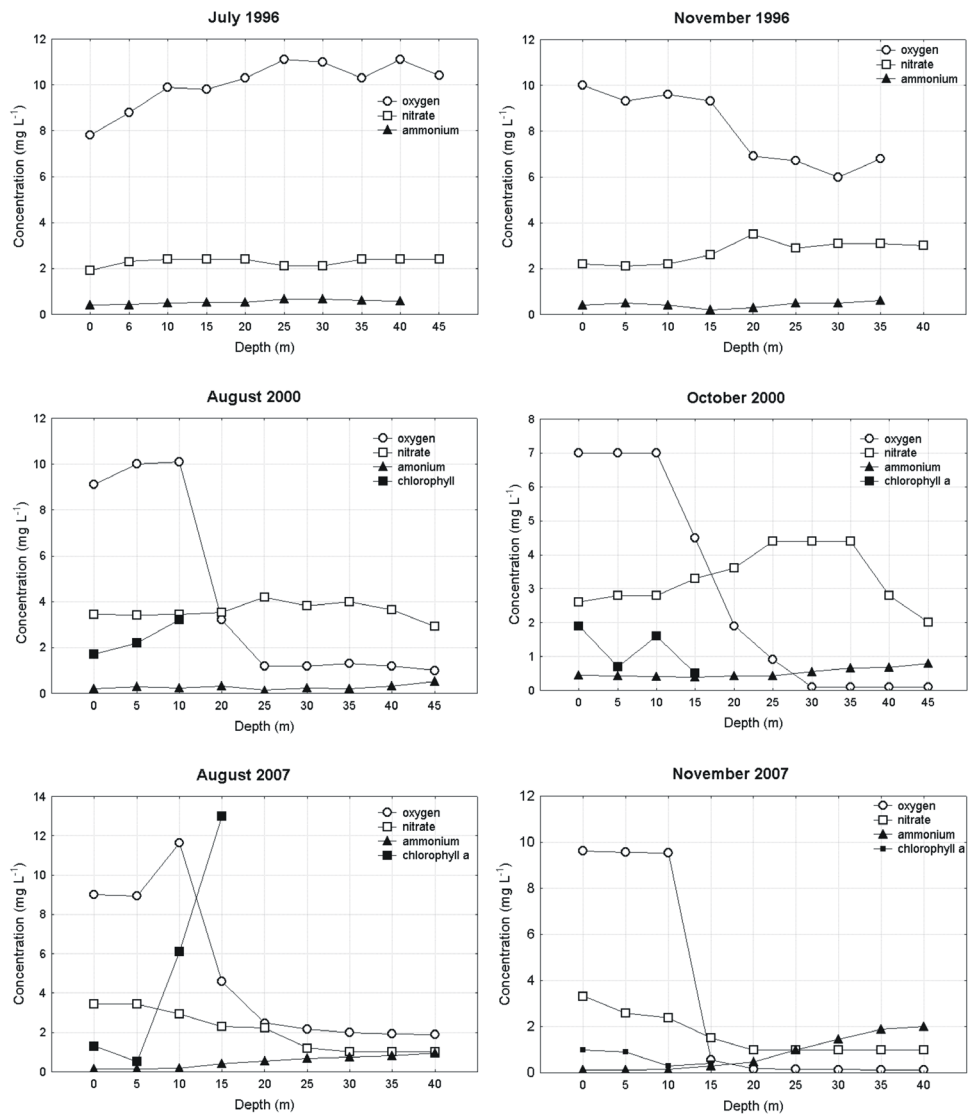


Fig. 1: Vertical distribution of oxygen, nitrate, ammonium and chlorophyll a concentrations in Velenjsko jezero in summer and autumn of 1996, 2000 and 2007.

Slika 1: Vertikalna razporeditev koncentracij kisika, nitrata, amonija in klorofila a poleti in jeseni v letih 1996, 2000 in 2007.

rubescens is a cold-water stenotherm species distributed mainly in middle European (REYNOLDS 1984) and Southern sub-alpine lakes. During the summer stratification it is usually located within the metalimnium (CHORUS & BARTRAM 1999, SEDMAK & KOSI 1997), where it is photosynthetically active (MICHELETTI et al. 1998). It usually grows at a depth where the penetrating PAR is around 1–5% of the surface values (CHORUS & BARTRAM 1999). In favourable meteorological and climatic conditions, it migrates to the surface forming a surface bloom frequently covering almost the entire lake surface. Such blooms can persist on the surface even in January and can grow under ice cover (SEDMAK & KOSI 2001). In such cases microcystin-YR can normally be detected in bloom samples (SEDMAK & KOSI 2001). Not all cyanobacteria blooms are toxic, and even blooms caused by known toxin producers may not actually produce toxins, or may only do so at undetectable levels. The triggers of toxin production are not known well. This type of toxin has been shown to persist in water for a week or more after the bloom has disappeared. No human deaths have been directly associated with these cyanotoxins, however they may cause skin irritations or nausea (CARMICHAEL 1997). The presence of microcystins can also influence the growth of other phytoplankton in the bloom. High densities of *Planktothrix* can inhibit the growth of other phytoplankton species and thus reduce the number of alternative food particles for zooplankton. The diversity in the blooms is thus low. It has been suggested that there this is due to the combined effect of light limitation and microcystin influence on susceptible phytoplankton species (SEDMAK & KOSI 2002).

Compared with other lakes (REMEC REKAR 2008), very high average annual concentrations of sulphate ($>590 \text{ mg L}^{-1}$), chloride ($>40.0 \text{ mg L}^{-1}$), sodium ($>60 \text{ mg L}^{-1}$) and potassium ($>50 \text{ mg L}^{-1}$) were detected in Velenjsko jezero (Table 2). Washing out of the ash disposal site is the most probable ion's source. The concentration of sulphate was almost four times higher than the maximum level in rivers provided for by Slovenian legislation (OGRS No. 11/2002). The usual concentration in lakes is in the range about of 5 to 30 mg L^{-1} , with an average value of about $11 \text{ mg SO}_4^{2-} \text{ L}^{-1}$. SO_4^{2-} has no influence on the trophic status of the water. Velenjsko jezero contains very high

concentrations of divalent cations, especially Ca^{2+} , providing good conditions for the development of green algae, which have high requirements for Ca^{2+} . Sodium can influence the development of large populations of cyanobacteria and maximal growth of several cyanobacteria species has been found at 40 mg L^{-1} (WETZEL 2001), but values in Velenjsko jezero were even higher (63.7 mg L^{-1}) in 2007. Diatoms were also the dominant species in the lake in early spring, since they dominate in very hard water lakes, like Velenjsko jezero, with ratios: monovalent cations:divalent cations much less than 1.5 (ROUND 1981). Distribution of most species of desmids of the Conjugales is limited to water with low concentrations of calcium and magnesium.

Conclusions:

1. The transparency of the lake and the nutrient concentration, and the concentration of chlorophyll *a* in the epilimnium remained at the same levels from 1996 to 2007, while the concentrations of ions were increasing regularly, especially sulphate and calcium, and consequently the specific electrical conductivity (SEP). Changes were detected in the phytoplankton community structure, blooms of toxic algae and the development of hypoxic (and anoxic) conditions in the hypolimnium occurred in the last years.
2. Concentration of chlorophyll *a* in Velenjsko jezero was smaller in comparison with the lakes with the same trophic status. Very high concentrations of sulphate, chloride, sodium and potassium can be one of the reasons for that phenomenon.
3. Filamentous cyanobacteria *Oscillatoria* sp., diatom *Synedra* sp., and dynophyta *Ceratium* sp. grew in lake even at pH 11. In recent years, following the first year after the normalisation of pH, Cyanobacteria have replaced Dinophyta as the predominant species. The predominant algae in the lake ten years later were cyanobacteria *Pseudanabaena* cf. *catenata* and *Planktothrix rubescens* as well as dynophyta *Ceratium hirundinella* and *Peridinium cinctum*. The first bloom of the former usually occurs in June and the second from November to January.

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Impact of simultaneous Cd and Zn substrate amendments on metal accumulation in two Cd/ Zn hyperaccumulating *Thlaspi* species

Vpliv interakcije Cd in Zn v substratu na njuno kopičenje pri dveh hiperakumulacijskih vrstah Cd in Zn iz rodu *Thlaspi*

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Abstract: The impact of simultaneous Cd and Zn amendments in the substrate on the accumulation of Cd and Zn were studied in a recently discovered Cd/ Zn hyperaccumulating *Thlaspi praecox* (Brassicaceae) and compared to a model hyperaccumulating plant species *T. caerulescens*. The plants were grown in pots with added Cd or Zn or both for three months in a greenhouse. The addition of Zn in the substrate increased Cd extractability in the substrate significantly without a significant pH change and this increase resulted in increased concentration and content of Cd in the shoots of both species indicating that species have similar abilities to extract Cd from the substrate. In the combined treatment (Cd and Zn) an increase in shoot biomass accompanied with a decrease in Zn concentration in roots and shoots of both species was observed, while no changes in total accumulated Zn in shoots were seen. These results suggest different uptake and translocation systems for Cd and Zn in *T. praecox*, positioning this plant species in the superior Cd hyperaccumulating league of *T. caerulescens* Ganges ecotype.

Keywords: *Thlaspi caerulescens*, *Thlaspi praecox*, cadmium uptake, hyperaccumulation, zinc uptake

Izvilleček: Preučevati smo vpliv sočasnega dodatka Cd in Zn v substrat na njuno akumulacijo pri nedavno odkriti hiperakumulacijski vrsti rani mošnjak (*Thlaspi praecox*, Brassicaceae) in jo primerjali z akumulacijo pri modelni hiperakumulacijski rastlini modrikasti mošnjak (*T. caerulescens*). Obe vrsti smo gojili v rastlinjaku tri mesece. Dodatek Zn v substrat je povečal dostopnost Cd v substratu, ne da bi se ob tem povečala pH vrednost substrata, posledično pa smo izmerili večje koncentracije in vsebnosti Cd v poganjkih pri obeh vrstah, kar pomeni, da imata vrsti podobno sposobnost odstranjevanja Cd iz substrata. V kombiniranem tretmaju (Cd in Zn) smo pri obeh vrstah izmerili največjo biomaso poganjkov in zmanjšano koncentracijo Zn v koreninah in poganjkih, vsebnost Zn pa se pri tem ni spremenila. Rezultati nakazujejo na ločen privzem in transport Cd in Zn pri vrsti *T. praecox*, kar jo postavlja ob bok ekotipu vrste *T. caerulescens* Ganges, za katero velja superiorna sposobnost hiperakumulacije Cd.

Ključne besede: *Thlaspi caerulescens*, *Thlaspi praecox*, privzem kadmija, hiperakumulacija, privzem cinka

Introduction

Accumulation of metals in plant shoots results from the mechanisms of both root uptake and root-to-shoot translocation. The interactions between metals in soil and in the plant itself are important factors in these processes. Soil interactions can be explained by simple ionic competition between metals for sorption sites (CHRISTENSEN 1987). An increase in bioavailable metal concentration of one after the addition of the other in the soil solution is frequently observed (UENO & al. 2004). In the plant, the competition for the metal binding sites in transport proteins normally leads to a decrease in the accumulation of one metal in the presence of other(s). Studies of these interactions are of immense importance in plants that are capable of taking up and storing high levels of metals without suffering from metal toxicity, the so-called hyperaccumulators (BAKER & BROOKS 1989) because of their potential application in phytoextraction technologies (REGVAR 2008).

Thlaspi caerulescens J. & C. Presl (Brassicaceae) is one of the most studied hyperaccumulators which occurs on metalliferous as well as on non-metalliferous soils (REEVES & BAKER 2000). Hyperaccumulation of non-essential metals Cd ($>0.01\%$ Cd in the shoot dry weight) and essential Ni ($>0.1\%$ Ni) has been reported in some populations of *T. caerulescens* and a superior ability to hyperaccumulate Cd was described in a population of *T. caerulescens* (Ganges) from Southern France (LOMBI & al. 2000, ZHAO & al. 2003). Recently, *T. praecox* Wulfen from a multi-metal polluted site in Žerjav (Slovenia) was reported to hyperaccumulate up to 0.6% Cd in shoots (VOGEL-MIKUŠ & al. 2005), up to 0.07% Cd in flowering and seeding stalks (PONGRAC & al. 2007), and up to 0.14% Cd in seeds (VOGEL-MIKUŠ & al. 2007) under field conditions. Besides the *T. praecox* population from Žerjav, populations from Mežica and Lokovec in Slovenia also exhibited Cd hyperaccumulating character (LIKAR & al. 2009).

Hyperaccumulation of Zn ($>1\%$ Zn in the shoot dry weight) was, unlike Cd hyperaccumulation, found to be a constitutive trait in *T. caerulescens* (ESCARRE & al. 2000, ASSUNÇÃO & al. 2003). In *T. praecox* plants collected in Žerjav, up to 1.5% Zn was found in shoots (VOGEL-MIKUŠ & al. 2005). The uptake and translocation of Cd and Zn were

studied in *T. praecox* and *T. caerulescens* Ganges ecotype in a hydroponic and in a pot experiment. The hydroponic experiment using radiolabels ^{109}Cd and ^{65}Zn showed that the short-term uptake rate of Cd and Zn was higher in *T. caerulescens* than in *T. praecox*, whereas the Cd but not Zn translocation efficiency was higher in *T. praecox* (XING & al. 2008). In the pot experiment the two species hyperaccumulated Cd in the shoots to a similar extent whereas Zn concentration in *T. praecox* shoots was lower than that in *T. caerulescens* (PONGRAC & al. 2009). However, the design of these experiments did not enable conclusions on the impact of interaction between Cd and Zn on the uptake and translocation of these two metals. Therefore a long-term pot experiment was set up in which *T. praecox* and *T. caerulescens* Ganges ecotype were treated with Zn, Cd or their combination (Cd + Zn) to study their interactions and are presented in this paper.

Material and Methods

Plant material and experimental design

Seeds of Zn/ Cd hyperaccumulating population of *Thlaspi praecox* Wulfen were collected from a heavy metal polluted site in Žerjav, Slovenia and seeds of *Thlaspi caerulescens* J. & C. Presl were collected from the Ganges area (south France). The seeds were germinated on a mixture of perlite and vermiculite (1:1 v/v) moistened with deionised water. Thirty days old seedlings were transplanted to plastic pots (three per pot) filled with 500 g of commercial peat-based substrate (Damjan Čamernik s.p., Biobrazda; pH 6.9–7.2, 7.45 g N kg^{-1} , $2.64 \text{ g P}_2\text{O}_5 \text{ kg}^{-1}$, $2.67 \text{ g K}_2\text{O kg}^{-1}$ and 251 g kg^{-1} organic matter). The substrate was amended 3 weeks before with Cd and/or Zn (both as a sulphate salt) to obtain the following treatments: the Zn treatment contained $100 \text{ mg Zn kg}^{-1}$, the Cd treatment contained 50 mg Cd kg^{-1} and the combined treatment contained $100 \text{ mg Zn kg}^{-1}$ and 50 mg Cd kg^{-1} . The control treatment did not receive the addition of Zn nor Cd. One batch of substrate was prepared per metal amendment treatment and used to fill four pots for each treatment and each plant species. Immediately before transplanting a sample of the substrate was taken

from each pot to determine metal availability using the extraction method with 1 M ammonium acetate (BAKER & al. 1994). The substrate pH in the water fraction was determined after diluting 1 g of dried soil in 20 ml of MiliQ water and shaking vigorously for 2 h (ÖHLINGER 1995). The plants were grown for three months in a growth chamber under controlled conditions with 16 h day period, light intensity of $160 \mu\text{mol m}^{-2}\text{s}^{-1}$, 18°C : 16°C day:night temperature and 50–60% relative humidity. Upon harvest, the plant material was carefully washed with deionised water; the shoots and roots were lyophilized and weighed (dry weight).

Cadmium and zinc determination

Subsamples (30 mg) of finely grinded plant tissue were digested with a mixture (7:1 v/v) of HNO_3 and HClO_4 . The concentrations of Cd and Zn in the digest were determined using atomic absorption spectrometry (AAS) (Perkin Elmer AAnalyst 100) (VOGEL-MIKUŠ & al. 2005).

Statistical analysis

The translocation factors (TF) were calculated as ratios of shoot and root concentration. The contents of Cd and Zn (μg) in the plant tissues were calculated by multiplying concentration and dry biomass. The effects of treatment on all the studied parameters were investigated using two-way analysis of variance (ANOVA) with species and treatment as independent factors (Tab. 1). When the within-species factor (effect of treatment) was significant, one-way ANOVA was undertaken with Tukey's honest significant difference (HSD) test to determine the significance of the differences between the treatments for both species ($p < 0.05$). When the between-species factor (effect of species) was significant, differences for each treatment between *T. praecox* and *T. caerulea* were determined separately using Student t-test at $p < 0.05$. All the tests were performed using Statistica Statsoft® (version 6.0) software.

Results

The addition of Zn significantly increased Cd extractability in the substrate without a significant change in pH, whereas the addition of Cd did not influence the extractability of Zn (t-test, $p < 0.05$; Tab. 2). The plant species did not differ significantly in the root nor shoot biomass; only the treatments influenced the plant biomass significantly (Tab. 1; Fig. 1A, B). The combination of Cd and Zn significantly increased the shoot biomass of both species in comparison to both control and Zn treated plants (Fig. 1B).

Overall the two species did not differ significantly in the Zn root and shoot concentration, nor in the Zn translocation factor (TF) (Tab. 1). Nevertheless, differences were observed between the species in particular treatments, e.g. *T. caerulea* accumulated higher shoot Zn concentrations in the Zn treatment compared to *T. praecox* (t-test; $p < 0.05$). In the combined treatment (Zn and Cd) decreased root and shoot Zn concentration when compared to the Zn treatment in both species was observed (Fig. 1C, D). Zinc TF was higher in the Zn and combined treatments in comparison to the control and Cd treatments (Fig. 2A). There was no significant change in the total accumulated Zn in the plant shoots between the Zn and the combined treatments (Fig. 3A).

Species, treatment and their interaction influenced the root Cd concentration and the Cd TF, but only the treatment influenced Cd concentration and content in the shoots (Tab. 1). In *T. caerulea* higher concentrations of Cd in the roots were measured in the Cd treatment. However, in the shoots the Cd concentration was not different between the two species (Fig. 1E, F). In the combined treatment increased Cd concentrations (Fig. 1F) and content (Fig. 3B) in the shoots of both species were observed when compared to the Cd treatment. The Cd TF was higher in *T. praecox* than in *T. caerulea* in the Cd and combined treatments (Fig. 2B).

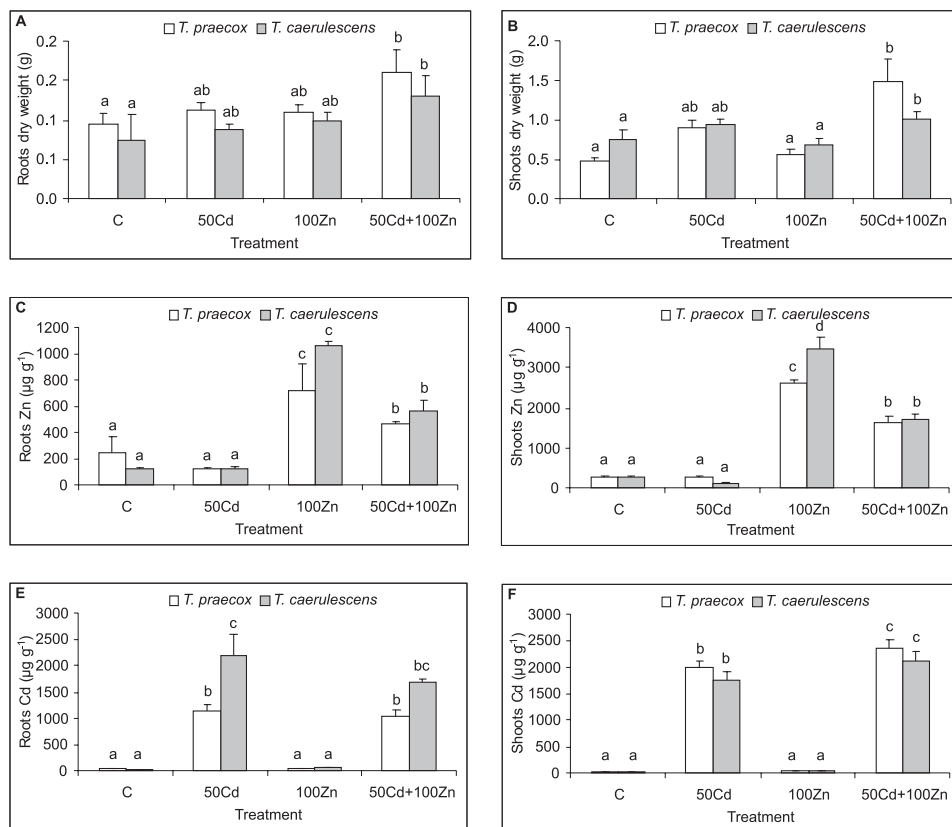


Fig. 1: Plant biomass (A and B), concentration of Zn (C and D) and Cd (E and F) in *Thlaspi praecox* and *Thlaspi caerulescens* grown in Cd and Zn amended substrates (means $\pm$ standard error, $n=4$), C – control treatment, 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters above the columns indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; $p < 0.05$).

Slika 1: Biomasa rastlin (A in B), koncentracija Zn (C in D) in Cd (E in F) pri vrstah *Thlaspi praecox* in *Thlaspi caerulescens*, ki smo ju gojili na substratu z dodanim Cd in Zn (povprečja $\pm$ standardna napaka; $n=4$), C – kontrola, 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke nad stolpci nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, $p < 0,05$).

Discussion

The accumulation capacity of Cd and Zn in response to the addition of Zn, Cd or their combination in the substrate was studied in Cd/Zn hyperaccumulating species *T. praecox* from Žerjav (Slovenia) and *T. caerulescens* Ganges ecotype. The addition of Zn in the combined treatment increased the Cd extractability significantly in the substrate as previously observed (UENO & al.

2004). In contrast, the extractability of Zn was not changed due to the presence of Cd in the combined treatment which may be contributed to a constantly high amount ($> 90\%$) of total Zn remaining insoluble in the substrate and thus unavailable for plant uptake (BROADLEY & al. 2007). The concentrations of ammonium-extractable concentrations of Zn and Cd were measured three weeks after amending the substrate to ensure homogenous distribution of these two metals and

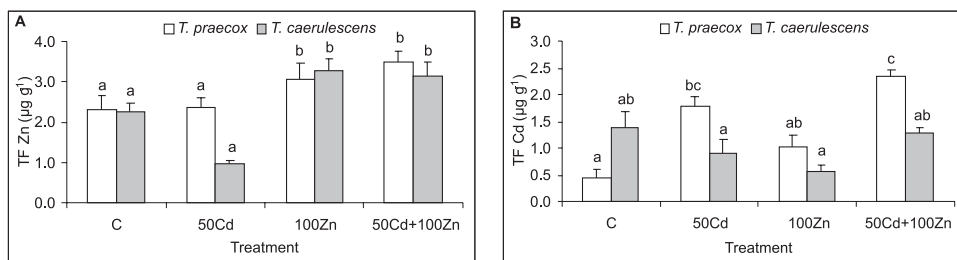


Fig. 2: Translocation factor (TF; the ratio between shoot and root concentration) for Zn (A) and Cd (B) in *Thlaspi praecox* and *Thlaspi caerulescens* grown in Cd and Zn amended substrates (means $\pm$ standard error, $n=4$); C – control treatment, 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters above the columns indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; $p<0.05$).

Slika 2: Translokacijski faktor (TF, razmerje koncentracij v poganjkih in koreninah) za Zn (A) in Cd (B) pri vrstah *Thlaspi praecox* in *Thlaspi caerulescens*, ki smo ju gojili na substratu z dodanim Cd in Zn (povprečja $\pm$ standardna napaka; $n=4$), C – kontrola, 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke nad stolpci nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, $p<0,05$).

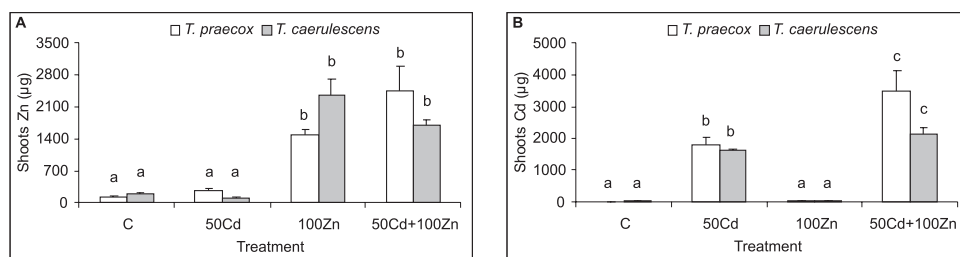


Fig. 3: Content (μg) of Zn (A) and Cd (B) in shoots of *Thlaspi praecox* and *Thlaspi caerulescens* grown in Cd and Zn amended substrates (means $\pm$ standard error, $n=4$); C – control treatment, 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters above the columns indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; $p<0.05$).

Slika 3: Vsebnost (μg) Zn (A) in Cd (B) v poganjkih vrst *Thlaspi praecox* in *Thlaspi caerulescens*, ki smo ju gojili na substratu z dodanim Cd in Zn (povprečja $\pm$ standardna napaka; $n=4$), C – kontrola, 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke nad stolpci nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, $p<0,05$).

before the substrate was used in the experiment thus the observed changes in the extractability of Cd were not a result of plant growth.

Increased shoot biomass of both species was observed in the combined treatment in comparison to only Zn treatment indicating that the combination of Cd and Zn is beneficiary to plant growth of these hyperaccumulating plant species. In our previous experiment increasing Cd in the

substrate resulted in the increase of the roots and shoots biomass of the same species (PONGRAC & al. 2009). However, in all the Cd treatments 100 $\mu\text{g Zn g}^{-1}$ was added as *T. caerulescens* is extremely sensitive to Zn deficiency in soils (OZTURK & al. 2003) and has higher requirement for Zn (SHEN & al. 1997). The growth enhancing effect of Cd in *T. caerulescens* was previously demonstrated (ESCARRE & al. 2000, ROOSENS & al. 2003, YANAI

Table 1: Two-way ANOVA table for analysed parameters with species and treatment as independent factors. Values $p < 0.05$ are given in bold.

Tabela 1: Preglednica rezultatov statistične analize podatkov z dvosmerno ANOVA pri čemer smo kot neodvisni spremenljivki uporabili vrsto in tretma. Statistično značilne vrednosti ($p < 0,05$) so odebeljene.

Source	df	F	p
Root dry weight			
Species	1	2.26	0.146
Treatment	3	3.39	0.035
Species $\times$ treatment	3	0.10	0.959
Error	24		
Shoot dry weight			
Species	1	0.02	0.885
Treatment	3	10.98	<0.000
Species $\times$ treatment	3	3.15	0.043
Error	24		
Root Zn concentration			
Species	1	1.58	0.220
Treatment	3	32.22	<0.000
Species $\times$ treatment	3	2.37	0.095
Error	24		
Shoot Zn concentration			
Species	1	4.05	0.056
Treatment	3	26.37	<0.000
Species $\times$ treatment	3	5.83	0.004
Error	24		
Zn translocation factor			
Species	1	3.75	0.066
Treatment	3	15.20	<0.000
Species $\times$ treatment	3	3.04	0.051
Error	22		
Shoot Zn content			
Species	1	0.0043	0.948
Treatment	3	39.19	<0.000
Species $\times$ treatment	3	3.96	0.020
Error	24		
Root Cd concentration			
Species	1	15.88	<0.000
Treatment	3	63.60	<0.000
Species $\times$ treatment	3	5.75	0.004
Error	24		
Shoot Cd concentration			
Species	1	2.14	0.157
Treatment	3	213.90	<0.000
Species $\times$ treatment	3	0.80	0.508
Error	23		
Cd translocation factor			
Species	1	7.09	0.014
Treatment	3	11.48	<0.000
Species $\times$ treatment	3	10.38	<0.000
Error	23		
Shoot Cd content			
Species	1	4.03	0.057
Treatment	3	50.78	<0.000
Species $\times$ treatment	3	2.95	0.054
Error	23		

Table 2: Ammonium acetate extractable concentrations ($\mu\text{g g}^{-1}$) of Zn and Cd and pH in the substrate before planting *Thlaspi praecox* and *Thlaspi caerulescens* in the pot experiment (means $\pm$ standard error; $n=4$). 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters beside the numbers indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; $p<0.05$).

Tabela 2: Koncentracija ($\mu\text{g g}^{-1}$) Zn in Cd po ekstrakciji z amonijevim acetatom in pH v substratu pred presaditvijo vrst *Thlaspi praecox* in *Thlaspi caerulescens* (povprečja $\pm$ standardna napaka; $n=4$). 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke ob številkah nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, $p<0,05$).

Treatment	<i>Thlaspi praecox</i>			<i>Thlaspi caerulescens</i>		
	Zn	Cd	pH	Zn	Cd	pH
Control	0.80 $\pm$ 0.12 a	-	7.09 $\pm$ 0.01	0.75 $\pm$ 0.12 a	-	7.05 $\pm$ 0.02
100Zn	5.83 $\pm$ 0.98 b	-	7.02 $\pm$ 0.01	5.01 $\pm$ 0.33 b	-	7.01 $\pm$ 0.02
50Cd	0.66 $\pm$ 0.05 a	6.37 $\pm$ 0.17 A	7.09 $\pm$ 0.04	0.60 $\pm$ 0.04 a	6.13 $\pm$ 0.18 A	7.06 $\pm$ 0.03
50Cd+100Zn	4.91 $\pm$ 0.33 b	7.56 $\pm$ 0.29 B	7.06 $\pm$ 0.02	4.82 $\pm$ 0.28 b	7.12 $\pm$ 0.19 B	7.04 $\pm$ 0.02

& al. 2006) and a physiological role of Cd in *T. caerulescens* was proposed (LIU & al. 2008). Our results indicate that also in *T. praecox* Cd may have a physiological role and that high Zn substrate concentrations are required for optimal growth.

The Zn hyperaccumulating concentration criterion set at 10,000 $\mu\text{g Zn g}^{-1}$ in dry weight of shoots (BAKER & BROOKS 1989) was not reached in either of the species in this experiment which may be a result of the length of the plant growth as well as low availability of Zn for the plants. Zinc concentrations from the field exceeding this criterion were reported repeatedly for *T. caerulescens* (BAKER & al. 1994, REEVES & al. 2001) and for *T. praecox* (VOGEL-MIKUŠ & al. 2005, LIKAR & al. 2009). The highest concentration of Zn in shoots was measured in *T. caerulescens* in the Zn treatment which was significantly higher than that in *T. praecox* as observed in our previous experiment (PONGRAC & al. 2009). However, the species did not differ in the Zn TF indicating equally efficient Zn transport in both species supporting the observation from the hydroponic experiment (XING & al. 2008). In the Cd treatment lower Zn concentrations in the shoots as well as Zn TF were found in *T. caerulescens* indicating a decreased Zn transport in this species in the case of high Cd to Zn ratio in the substrate that may lead to Zn deficiency in plants as previously suggested (CHANEY & al. 2006). A significant decrease in Zn concentration in the roots and shoots in both species was observed in the combined treatment when compared to the Zn treatment indicating a

competition of Cd and Zn in the substrate that may have led to a decreased Zn uptake into the roots. This however did not result in a changed translocation of Zn within plants. High external Cd concentration was already reported to inhibit Zn accumulation in *T. caerulescens* Ganges ecotype (LOMBI & al. 2001, ZHAO & al. 2002, ROOSENS & al. 2003), but not in Prayon ecotype (PAPOYAN & al. 2007). Thus different uptake systems for Cd and Zn in *T. caerulescens* Ganges ecotype were proposed (LOMBI & al. 2001) and based on our results may exist also in *T. praecox*. In another Cd/Zn hyperaccumulator *Sedum alfredii* H. the addition of Cd enhanced Zn translocation and partition to the shoots (YANG & al. 2004) indicating similar response to the one observed in *T. caerulescens* Prayon ecotype. On the other hand, no effect on Zn accumulation by Cd supply was observed in a Cd accumulating chamomile (*Matricaria recutita* L.) plants (CHIZZOLA & MITTEREGGER 2005). Increasing Cd application to Zn-deficient durum wheat plants (*Triticum durum* Desf. cv. Cakmak) tended to decrease Zn concentrations, whereas in plants with adequate Zn supply, the concentrations of Zn were either not affected or increased by Cd (KÖLELI & al. 2004). It seems that the concentration range may profoundly affect metal interactions and accumulation in both metal (hyper)accumulating and non-accumulating species.

The concentration of Cd of both studied species exceeded the hyperaccumulating Cd concentration set at 100 $\mu\text{g Cd g}^{-1}$ in shoot dry weight (BROOKS 1998) in the Cd and in the combined treatments, thus confirming the observations from our previ-

ous pot study in which *T. praecox* matched the superior Cd hyperaccumulation ability (PONGRAC & al. 2009) reported for *T. caerulescens* Ganges ecotype (LOMBI & al. 2000, ZHAO & al. 2003). However, higher concentrations of Cd in the roots of *T. caerulescens* were observed in the same two treatments and consequently lower Cd TFs were calculated. This supports the results from the hydroponic experiment where using radiolabels ^{109}Cd the short-term uptake rate of Cd was higher in *T. caerulescens* Ganges ecotype than in *T. praecox*, whereas the Cd translocation efficiency was higher in *T. praecox* (XING & al. 2008). Relatively high Cd concentrations were measured also in the control and Zn treatments which may be a consequence of Cd presence in the commercial substrate or presence of Cd in the seeds of these two hyperaccumulating plants, or both. High Cd concentrations have been reported in seeds of *T. praecox* that contained on average $1,000\ \mu\text{g Cd g}^{-1}$ when grown at the most polluted site in Žerjav (VOGEL-MIKUŠ & al. 2007) and seeds of *T. caerulescens* were reported to contain on average $3,200\ \mu\text{g Cd g}^{-1}$ (KACHENKO & al. 2009). The concentration of Cd in the substrate of these two treatments was however below the detection limit of the method used to determine ammonium-extractable Cd concentration in soil.

The interaction of Zn and Cd in the substrate in the combined treatment significantly increased the concentration and content of Cd in the shoots of both species which is probably a result of the increased Cd extractability in the substrate that was not a result of the pH change. In contrast, the Cd accumulation was not affected by the Zn addition in *T. caerulescens* Ganges ecotype (UENO & al. 2004) and an inhibition in the Cd accumulation was observed in *T. caerulescens* Prayon due to the Cd and Zn interaction in the substrate (LOMBI & al. 2001, ZHAO & al. 2002, ROSENS & al. 2003). Similarly, in a Cd accumulating *M. recutita* plants the addition of Zn to the soil led to a decreased Cd accumulation, whereas further increase in the Zn supply did not further decrease the Cd concentration in shoots (CHIZZOLA & MITTEREGGER 2005). Neither was additional Zn supply accompanied by a corresponding decrease in Cd shoot concentrations of Cd sensitive *T. durum* (KÖLELI & al. 2004). In the presence of Cd adding Mn to the solution significantly reduced the concentra-

tions of Cd in all organs of Mn hyperaccumulator *Phytolacca americana* L. (PENG & al. 2008). These observations indicate the importance of the metal in question and its concentrations used in the experiment as well as plant species and metal (hyper)accumulation properties of the plants when studying the elemental interactions in soil and their importance for plant uptake.

In conclusion, the interactions between the metals in the substrate may significantly affect their accumulation in the aboveground plant parts that are dependent on the metal(s) in question, its concentrations, plant species and/or even population and soil properties. The metal concentrations and their mode of accumulation may already indicate the underlying metal uptake and transport mechanisms in plants. Studies of combined pollution are therefore important as they are more likely to relate to the field conditions. The two studied hyperaccumulating plants showed similar responses to the interaction of Cd and Zn in the substrate with an observed decrease in Zn but an increase in the Cd concentration and content in the shoots. The results thus suggest that different uptake systems for Cd and Zn may also exist in *T. praecox* and that the two species have similar ability to extract Cd from substrate.

Povzetek

Na območjih obremenjenih s kovinami lahko življenjski cikel zaključijo le rastline, ki so na povečane koncentracije kovin v tleh prilagojene. Ena izmed prilagoditev na tovrstni stres je razvoj razstrupljevalnih mehanizmov, ki rastlinam omogočajo, da v nadzemnih delih kopičijo zelo velike koncentracije kovin. Ta redek pojav imenujemo hiperakumulacija.

V Sloveniji je bila v okolici Žerjava (Mežiška dolina), ki je zaradi delovanja talilnice in predelovalnice Pb obremenjena s Zn, Cd in Pb, nedavno odkrita hiperakumulacijska vrsta rani mošnjak (*Thlaspi praecox* Wulfen) (VOGEL-MIKUŠ & al. 2005). Za vrsto *T. praecox* smo že pokazali, da ima primerljivo dobre lastnosti kopičenja Cd kot modelna hiperakumulacijska vrsta za Cd modrikasti mošnjak (*T. caerulescens* J. & C. Presl) (PONGRAC & al. 2009). Po do sedaj znanih podatkih ima izmed različnih ekotipov vrste *T. caerulescens*

ekotip Ganges superiorno sposobnost hiperakumulacije Cd in pri njem obstajata različna sistema za privzem in transport Cd in Zn. V pričujočem delu smo pri vrstah *T. praecox* in *T. caerulescens* ekotip Ganges preučevali vpliv interakcije med Cd in Zn v substratu na privzem in translokacijo Cd in Zn, da bi ugotovili, ali sta ta dva sistema različna tudi pri vrsti *T. praecox*.

V rastlinjaku smo tri mesece gojili obe vrsti v substratu, ki smo mu dodali Zn ali Cd ali njuno kombinacijo. Pred poskusom smo v substratu izmerili pH in dostopnost Zn in Cd po ekstrakciji z amonijevim acetatom, po poskusu pa rastlinam izmerili biomaso in koncentracije Zn in Cd s pomočjo atomskega absorpcijskega spektrometra po mineralizaciji suhega rastlinskega materiala v mešanici kislin $\text{HClO}_4\text{:HNO}_3$ (7:1 v/v).

Primerjava dostopnih koncentracij Zn in Cd v substratu posameznih tretmajev in kombiniranega tretmaja je pokazala, da je dodatek Zn v substrat povečal dostopnost Cd, ki ni bil posledica sprememb pH vrednosti, dodatek Cd pa ni povečal dostopnosti Zn. Povečanje dostopnosti Cd v substratu je vplivalo na povečano kopičenje Cd v poganjkih preučevanih vrst. Pri tretmaju s Zn smo v poganjkih vrste *T. caerulescens* izmerili večje koncentracije Zn kot v poganjkih vrste *T. praecox*. V kombiniranem tretmaju (Cd in Zn) smo pri obeh vrstah izmerili največjo biomaso hkrati pa zmanjšano koncentracijo Zn v koreninah in poganjkih v primerjavi s Zn tretmajem. Ti rezultati nakazujejo na ločen privzem in transport Cd in Zn tudi pri vrsti

T. praecox, kar jo postavlja ob bok superiornem ekotipu vrste *T. caerulescens* (Ganges).

Poznavanje mehanizmov, ki omogočajo toleranco in kopičenje velikih koncentracij kovin je pomembno zaradi potencialne uporabe hiperakumulacijskih rastlin v fitoekstrakciji, eni izmed tehnik fitoremediacije, t.j. čiščenja okolja s pomočjo rastlin. Pri fitoekstrakciji bi z uporabo hiperakumulacijskih rastlin z veliko biomaso v relativno kratkem času odstranili presežne koncentracije kovin iz tal. S čiščenjem kmetijskih površin bi na ta sonaraven način preprečili prenos kovin naprej v prehranjevalno verigo. Izsledki naše raziskave potrjujejo, da sta vrsti *T. praecox* in *T. caerulescens* v enaki meri sposobni odstranjevanja Cd iz tal.

Acknowledgements

The authors are indebted to Prof. Dr. Damjana DROBNE for access to the AAS and to Dr. Fangjie ZHAO for the seeds of *Thlaspi caerulescens* Ganges ecotype. The work was supported by the following projects: MSZS P1-0212 »Biology of Plants« research programme, »Young researchers«, and EU COST 859. A scholarship from the World Federation of Scientists and a national fellowship awarded by l'oreal-UNESCO – The Slovenian Science Foundation to P. PONGRAC is gratefully acknowledged.

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Pathogenicity islands, plasmids and iron uptake systems in extraintestinal pathogenic *Escherichia coli* strains

Otoki patogenosti, plazmidi in sistemi za privzem železa v zunajčrevesnih patogenih sevih bakterije *Escherichia coli*

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Abstract: The aim of the presented study was to estimate the prevalence, distribution and associations of different pathogenicity islands (PAI I₅₃₆ to PAI IV₅₃₆, PAI I₉₆, PAI II₉₆, PAI I_{CFT073} and PAI II_{CFT073}), iron uptake systems (genes *iutA*, *iucD*, *iroN*, *iroCD*, *fyuA*, *irp2*, *iha*, *ireA*, and *hbp*) and plasmids among extraintestinal pathogenic *Escherichia coli* (ExPEC) strains isolated from Slovenian patients. Twenty-nine ExPEC isolates obtained from the Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana were investigated for the presence of different pathogenicity islands and iron uptake systems with PCR, the plasmid content of the investigated strains was determined by molecular biology techniques. The significance of the found associations of the studied PAIs and iron uptake systems was analyzed with the Fisher's exact test. PAI IV₅₃₆ was found in 19, PAI II_{CFT073} in 6, PAI I_{CFT073} in 4, and PAI II₉₆ in one of the studied isolates. PAI I₅₃₆, PAI II₅₃₆, PAI III₅₃₆ and PAI I₉₆ were not detected in any studied isolate. In 19 of the studied isolates plasmids were detected. The *irp2* was found in 20, *fyuA* in 19, *iucD* and *iutA* in 12, *iha* in 9, *iroN* in 8, *iroCD* in 7, *ireA* in 7 and *hbp* in 4 of studied isolates. PAI IV₅₃₆ was statistically significantly associated with the yersiniabactin siderophore system and PAI I_{CFT073} was statistically significantly associated with the aerobactin siderophore system as well as *iha*. To our knowledge this is the first report on PAIs and iron uptake systems among Slovenian ExPEC isolates, as well as a first report on PAIs, iron uptake systems and plasmids among isolates from skin and soft tissue infections.

Key words: extraintestinal pathogenic *Escherichia coli*, ExPEC, pathogenicity island, PAI, plasmid, iron uptake

Izvleček: Cilj raziskave je bil oceniti prevalenco, razporeditev in asociacije različnih otokov patogenosti (PAI I₅₃₆ do PAI IV₅₃₆, PAI I₉₆, PAI II₉₆, PAI I_{CFT073} in PAI II_{CFT073}), sistemov za privzem železa (geni *iutA*, *iucD*, *iroN*, *iroCD*, *fyuA*, *irp2*, *iha*, *ireA*, in *hbp*) in plazmidov v zunajčrevesnih patogenih sevih bakterije *Escherichia coli* (ExPEC) izoliranih iz slovenskih bolnikov. Devetindvajset izolatov ExPEC, ki so jih osamili na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani, smo s pomočjo PCR preiskali za prisotnost različnih otokov patogenosti in sistemov za privzem železa, z molekulkobiološkimi tehnikami smo preverjali prisotnost plazmidov v preučevanih sevih. Statistično značilnost povezave preučevanih PAI in sistemov za privzem železa smo ugotavljali s Fisherjevim eksaktnim testom. PAI IV₅₃₆ smo našli v 19, PAI II_{CFT073} v 6, PAI I_{CFT073} v 4, PAI II₉₆ v enem od preučevanih izolatov. PAI I₅₃₆, PAI II₅₃₆, PAI III₅₃₆ in PAI I₉₆ nismo odkrili v nobenem izmed preučevanih izolatov. V 19 sevih smo odkrili plazmide. Gen *irp2* smo našli v 20, *fyuA* v 19, *iucD* in *iutA* v 12, *iha* v 9, *iroN* v 8, *iroCD* v 7, *ireA* v 7 in *hbp* v 4 od preučevanih sevov. PAI IV₅₃₆ je bil statistično značilno povezan s sideroformnim sistemom jersiniabaktin in PAI I_{CFT073} je bil statistično značilno povezan s sideroformnim sistemom aerobaktin in *iha*. Kolikor nam je poznano, je to prva raziskava o PAI in sistemih za privzem železa na zbirki sevov ExPEC iz slovenskih bolnikov, in ki podaja podatke o PAI, sistemih za privzem železa in plazmidih izolatov ExPEC iz infekcij kože in podkožja.

Ključne besede: Zunajčrevesna patogena *Escherichia coli*, ExPEC, otoki patogenosti, PAI, plazmid, sistemi za privzem železa

Introduction

Escherichia coli (*E. coli*) is the most abundant facultative anaerobe of the human intestinal microflora. Despite the fact that it is a commensal bacterium, some *E. coli* strains have acquired specific virulence attributes that confer an increased ability to adapt to new niches and allow them to cause a broad spectrum of disease at either intestinal or extraintestinal sites (KAPER & al. 2004). Often the virulence attributes are genetically linked – located in a subgroup of genomic islands, in the so called pathogenicity islands (PAI) (SCHMIDT & HENSEL 2004). Typical characteristics of PAIs (Tab. 1), apart from encoding virulence genes, are: presence in pathogenic strains but absence or rareness in nonpathogenic strains of the same or related species; size ranging from 10 kb up to 200 kb; relative instability, different G+C content than the core genome, association with tRNA genes, presence of mobile genetic elements (insertion sequences, transposons, integrases, and bacteri-

ophage DNA), flanked by direct repeat sequences and due to different episodes of horizontal gene transfer a mosaic-like structure (HACKER & KAPER 2000, SCHMIDT & HENSEL 2004).

In addition to PAIs, plasmids, extrachromosomal DNA elements that range in size from approximately 300 bp to 2400 kbp, can carry genes encoding virulence factors (KADO 1998). Based on the overall genetic content, two types of plasmids are distinguished. One, designated as non-conjugative or non-transmissible, harbour genes for the initiation and regulation of its replication but do not possess genes required for conjugal transfer. The second type are conjugative or self-transmissible that also carry genes involved in conjugation (HELINSKI & al. 1996).

Iron is an essential cofactor for many basic metabolic pathways and bacteria have developed specialized iron uptake systems to capture iron. The most prominent are the siderophores, iron-binding molecules that are taken up by special siderophore receptors and ATP-consuming porin-

Table 1: Characteristics of studied PAIs
Tabela 1: Značilnosti preučevanih PAI

PAI name	Size (kbp)	Insertion position	Identified carried virulence (associated) factors	Ref.
PAI I ₅₃₆	76,8	<i>selC</i>	alpha-hemolysin, F17-like fimbriae, and CS12-like fimbriae	(SCHMIDT & HENSEL 2004)
PAI II ₅₃₆	102,2	<i>leuX</i>	P-related fimbriae, alpha-hemolysin, Hek adhesin, hemagglutinin-like adhesins	(SCHMIDT & HENSEL 2004)
PAI III ₅₃₆	68,1	<i>thrW</i>	S-fimbriae, <i>iro</i> siderophore system, a HmuR-like heme receptor, a Sap adhesin, a TSH-like hemoglobin protease	(SCHMIDT & HENSEL 2004)
PAI IV ₅₃₆ = HPI	30,2	<i>asnT</i>	yersiniabactin siderophore system	(SCHMIDT & HENSEL 2004)
PAI I ₁₉₆	> 170	<i>pheV</i>	alpha-hemolysin, P- fimbriae	(SCHMIDT & HENSEL 2004)
PAI II ₁₉₆	110	<i>pheU</i>	alpha-hemolysin, Prs- fimbriae, cytotoxic necrotizing factor I	(SCHMIDT & HENSEL 2004)
PAI I _{CFT073} = PAI-CFT073- <i>pheV</i>	123	<i>pheV</i>	alpha-hemolysin, P-fimbriae, Iha, autotransporter Sat, aerobactin siderophore system, antigen 43 precursor, capsule gene <i>kpsTM</i>	(LLOYD & al. 2007)
PAI II _{CFT073} = PAI-CFT073- <i>pheU</i>	52	<i>pheU</i>	P-fimbriae	(LLOYD & al. 2007)

like transporters in the bacterial outer membrane (SCHAIBLE & KAUFMANN 2004). Siderophores can be classified into three groups: (i) the catecholate type (enterobactin, salmochelin = enterochelin), (ii) hydroxamate type (aerobactin) and (iii) a mixed type – a combination of both (yersiniabactin) (GRASS 2006, SCHAIBLE & KAUFMANN 2004). In addition to siderophore synthesis strains can use siderophores produced and released into the extracellular medium by other bacteria and even fungi. In the host, bacteria may use iron sources such as heme, hemoglobin, hemopexin, and iron bound to transferrin and lactoferrin (BRAUN & BRAUN 2002). Apart from the siderophores and their receptors, autotransporters, virulence-associated proteins in gram-negative bacteria, can also play a role in obtaining iron for example, the hemoglobin protease Hbp (OTTO & al. 2002). All autotransporter proteins are energy-independent secreted via a type 5 secretion system and possess an overall unifying structure, comprising (i) an amino-terminal leader peptide (for secretion across the inner membrane), (ii) the secreted mature protein (or passenger domain), and (iii) a dedicated C-terminal domain, which forms a pore in the outer membrane through which the passenger domain passes to the cell surface (HENDERSON & NATARO 2001). Hbp, after it is autotransported out of the bacterial cell, interacts specifically with human hemoglobin, degrades it, and subsequently binds the released heme (OTTO & al. 1998).

E. coli isolates capable of causing disease outside the intestinal tract, e. g., uropathogenic *E. coli* (UPEC), sepsis-associated *E. coli*, and neonatal meningitis-associated *E. coli*, are classified as extraintestinal pathogenic *E. coli* (ExPEC) (RUSSO & al. 2000). Within the human intestinal tract, ExPEC may colonize without causing disease, but when they disseminate to other body sites, they elicit, due to encoded virulence factors, pathogenesis (WILES & al. 2008). In ExPEC isolates many pathogenicity islands were found, among the best known and studied are pathogenicity islands PAI I₅₃₆ to PAI IV₅₃₆ from the uropathogenic *E. coli* strain 536, PAI I₉₆ and PAI II₉₆ from the uropathogenic *E. coli* strain J96 and PAI I_{CFT073} and PAI II_{CFT073} from uropathogenic *E. coli* strain CFT073 (SCHMIDT & HENSEL 2004). Further, among ExPEC strains many iron uptake systems were found, characterized and associ-

ated with pathogenesis, among them aerobactin, salmochelin, yersiniabactin, Iha, IreA and Hbp. The aim of the present study was to characterize 29 ExPEC strains isolated from Slovenian patients suffering from extraintestinal *E. coli* infections for the presence of the best characterised PAIs and iron uptake systems. In addition, we aimed to estimate the prevalence of PAIs, iron uptake systems and plasmids, and to analyse the distribution and associations of PAIs and iron uptake systems among ExPEC strains isolated in Slovenia.

Material and methods

Bacterial strains and media

Twenty-nine randomly collected *E. coli* isolates from humans with extraintestinal infections isolated at the Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia were studied. Only one isolate from each patient was analyzed. Nineteen isolates were from patients with a urinary tract infection, 3 isolates were from decubiti, 2 isolates were from wound infections, 2 isolates were from surgical wound infections, 2 isolates were from foot ulci and one isolate was from a genital tract infection. All patients were older than 14 years. For cultivation of strains Luria Bertani medium or agar were used.

Detection of PAIs and iron uptake systems

The primers and PCR conditions used to amplify PAI markers; PAI I₅₃₆, PAI II₅₃₆, PAI III₅₃₆, PAI IV₅₃₆, PAI I₉₆, PAI II₉₆, PAI I_{CFT073} and PAI II_{CFT073}, and iron uptake genes; *iutA* – ferric aerobactin receptor gene, *iucD* – lysine: N⁶-hydroxylase gene (aerobactin biosynthesis), *iroN* – catecholate siderophore (ferric salmochelin, ferric 2,3-dihydroxybenzoic acid, ferric 2,3-dihydroxybenzoyl-D-ornithine) receptor gene, *iroCD* – salmochelin ATP-binding cassette ABC transporter gene (*iroC*), ferric salmochelin esterase gene (*iroD*), *fyuA* – ferric yersiniabactin receptor gene, *irp2* – yersiniabactin synthetase (yersiniabactin biosynthesis), *iha* – gene for a bifunctional protein: catecholate siderophore (ferric enterobactin, ferric 2,3-dihydroxybenzoylserine) receptor and adhesin, *ireA* – putative TonB-dependent siderophore receptor,

Table 2: Oligonucleotide primers and PCR conditions to detect PAIs and iron uptake systems

Tabela 2: Oligonukleotidni začetniki in pogoji PCR za ugotavljanje PAI in genov za sisteme za privzem železa

Target	Oligonucleotide name and sequence (5' to 3')	Size of product (kbp)	PCR conditions			Reference
PAI I ₅₃₆	I.9	1,8	94°C	5 min	1×	(SABATE & al. 2006)
	TAATGCCGGAGATTCATTGTC		94°C	1 min		
	I.10		56°C	1 min	30×	
	AGGATTTGTCTCAGGGCTTT		72°C	1 min		
			72°C	10 min	1×	
PAI II ₅₃₆	orfIup	1,0	94°C	5 min	1×	(SABATE & al. 2006)
	CATGTCCAAAGCTCGAGC		94°C	1 min		
	orfIldown		62°C	1 min	30×	
	CTACGTCAGGCTGGCTTT		72°C	1 min		
			72°C	10 min	1×	
PAI III ₅₃₆	sfaAI1	0,2	94°C	5 min	1×	(SABATE & al. 2006)
	CGGGCATGCATCAATTATCTTTG		94°C	1 min		
	sfaAI2		63°C	1 min	30×	
	TGTGTAGATGCAGTCACTCCG		72°C	1 min		
			72°C	10 min	1×	
PAI IV ₅₃₆	IRP2FP	0,3	94°C	5 min	1×	(SABATE & al. 2006)
	AAGGATTCGCTGTTACCGGAC		94°C	1 min		
	IRP2RP		61°C	1 min	30×	
	TCGTCGGGCAGCGTTTCTCT		72°C	1 min		
			72°C	10 min	1×	
PAI I _{J96}	papGI f	0,4	94°C	5 min	1×	(SABATE & al. 2006)
	TCGTGCTCAGGTCCGGAATT		94°C	0,5 min		
	papGI r		57°C	0,5 min	30×	
	TGGCATCCCACATTATCG		72°C	1 min		
			72°C	10 min	1×	
PAI II _{J96}	Hlyd	2,3	94°C	5 min	1×	(SABATE & al. 2006)
	GGATCCATGAAAACATGGTTAATG		94°C	1 min		
	cnf		61°C	1 min	30×	
	GATATTTTGTGGCCATTGGTTACC		72°C	2,5 min		
			72°C	10 min	1×	
PAI I _{CFT073}	RPAi	0,93	94°C	5 min	1×	(SABATE & al. 2006)
	GGACATCCTGTTACAGCGCGCA		94°C	1 min		
	RPAf		63°C	1 min	30×	
	TCGCCACCAATCACAGCGAAC		72°C	1 min		
			72°C	10 min	1×	
PAI II _{CFT073}	Cft073.2Ent1	0,4	94°C	5 min	1×	(SABATE & al. 2006)
	ATGGATGTTGTATCGCGC		94°C	0,5 min		
	Cft073.2Ent2		56°C	0,5 min	30×	
	ACGAGCATGTGGATCTGC		72°C	1 min		
			72°C	10 min	1×	
<i>iutA</i>	iutA f	0,3	94°C	4 min	1×	(JOHNSON & al. 1997)
	GGCTGGACATCATGGGAACCTGG		94°C	1 min		
	iutA r		68°C	1 min	35×	
	CGTCGGGAACGGGTAGAATCG		72°C	1 min		
			72°C	10 min	1×	

Target	Oligonucleotide name and sequence (5' to 3')	Size of product (kbp)	PCR conditions			Reference
<i>iucD</i>	Aer 1	0,6	94°C	4,5 min	1×	(YAMAMOTO & al. 1995)
	TACCGGATTGTCATATGCAGACCGT		94°C	0,5 min		
	Aer 2		62°C	0,5 min	35×	
	AATATCTTCTCCAGTCCGGAGAAG		72°C	50 sec		
			72°C	10 min	1×	
<i>iroN</i>	iroN f	0,7	94°C	2,5 min	1×	(JOHNSON & al. 2000)
	AAGTCAAAGCAGGGGTTGCCCCG		94°C	0,5 min		
	iroN r		68°C	0,5 min	25×	
	GACGCCGACATTAAGACGCAG		72°C	2 min		
			72°C	10 min	1×	
<i>iroCD</i>	P52-A	1,0	94°C	2,5 min	1×	This study
	GGCTGAGAAATATCAACATCCG		94°C	0,5 min		
	P52-B		63°C	1 min	30×	
	ATCGCACATCCGAAGAACGACT		72°C	1 min		
			72°C	10 min	1×	
<i>fyuA</i>	fyuA 1	0,8	94°C	2,5 min	1×	(JOHNSON & STELL 2000, SCHUBERT & al. 1998)
	TGATTAACCCCGCGACGGGAA		94°C	0,5 min		
	fyuA 2		63°C	0,5 min	25×	
	CGCAGTAGGCACGATGTTGTA		72°C	3 min		
			72°C	10 min	1×	
<i>irp2</i>	Irp2 f	0,3	94°C	5 min	1×	(SCHUBERT & al. 1998)
	AAGGATTCGCTGTTACCGGAC		94°C	1 min		
	Irp2 r		61°C	1 min	35×	
	TCGTCTGGGCAGCGTTTCTTCT		72°C	1 min		
			72°C	8 min	1×	
<i>iha</i>	iha f	0,8	94°C	4 min	1×	(JOHNSON & al. 2000)
	CTGGCGGAGGCTCTGAGATCA		94°C	0,5 min		
	iha r		58°C	0,5 min	30×	
	TCCTTAAGCTCCCGCGGCTGA		72°C	1 min		
			72°C	8 min	1×	
<i>ireA</i>	ireA f	0,4	94°C	2,5 min	1×	(RUSSO T. A. & al. 2001)
	TGGTCTTCAGCTATATGG		94°C	0,5 min		
	ireA r		55°C	1 min	25×	
	ATCTATGATTGTGTTGGT		72°C	0,5 min		
			72°C	7 min	1×	
<i>hbp</i>	Hbp f	0,9	94°C	4,5 min	1×	This study
	GGTGAAGGTACGCTGACGGT		94°C	0,5 min		
	Hbp r		65°C	1 min	35×	
	GCGTGACGCTGGAGTTATCT		72°C	1 min		
			72°C	10 min	1×	

and *hbp* – hemoglobin protease autotransporter gene with polymerase chain reaction (PCR) are listed in Tab. 2. DNA to be amplified was released from whole organisms by boiling according to LE BOUGUENEC & al. (1992). Amplification was performed in an automated thermal cycler (UN-OII, Biometra, Göttingen, Germany) in a 50 µl reaction mixture containing template DNA (10 µl of boiled lysate), 20 pmol of forward and reverse primer, 0,2 mM of dNTP mixture, 1,25 U *Taq* DNA polymerase and 2,5 mM MgCl₂ in 1× PCR buffer (Fermentas, Vilnius, Lithuania).

General molecular biology DNA techniques

Plasmid isolation, DNA digestion and agarose gel electrophoresis were performed by standard methods (SAMBROOK & RUSSELL 2001).

Statistical analysis

The significance of the results was established using the Fisher's exact test (2-tailed) available on-line on the web site <http://www.langsrud.com/fisher.htm> and the level of significance was set at a *P* value < 0.05.

Results

Prevalence of PAIs, plasmids and iron uptake systems

The PAI with the highest prevalence among the studied ExPEC isolates was PAI IV₅₃₆, also designated HPI (high-pathogenicity island), which was found in 19 studied strains. PAI II_{CFT073} was found in 6 strains, PAI I_{CFT073} in 4 strains and PAI II_{J96} in only one isolate. The PAIs: PAI I₅₃₆, PAI II₅₃₆, PAI III₅₃₆ and PAI I_{J96} were not detected in any studied isolate.

In 19 strains plasmids were detected, among them 9 harboured a plasmid larger than 30 kbp.

The iron uptake system with the highest prevalence was yersiniabactin, the *irp2* gene coding for the yersiniabactin synthetase was found in 20 strains and the *fyuA* gene coding for the ferric yersiniabactin receptor was found in 19 strains. The aerobactin iron uptake system genes *iucD*, coding for lysine: N⁶-hydroxylase needed

in aerobactin biosynthesis, and *iutA*, encoding the ferric aerobactin receptor, were detected in 12 strains. The *iha* gene was found in 9 strains. The salmochelin uptake system genes *iroN*, coding for the catecholate siderophore receptor, and *iroCD* coding for proteins needed in salmochelin transport, were found in 8 and 7 isolates, respectively. The *ireA* gene was harboured by 7 studied strains and the *hbp* gene by 4 isolates.

Distribution of PAIs and iron uptake systems among strains

As seen from Tab. 3 most of the strains harbouring PAI possessed one PAI (12 strains) and 7 strains harboured 2 or 3 PAIs. In 10 strains none of tested PAIs could be detected. The average PAI number per strain was 1.03.

The majority of tested strains possessed 1 to 3 iron uptake systems (22 strains). In 4 strains none of the tested iron uptake systems could be detected. Three strains possessed four or five different iron uptake systems. The average iron uptake system number per strain was 1.97.

Associations of PAIs and iron uptake systems

Since many PAIs are known to carry iron uptake systems (see Tab. 1), associations of PAIs and iron uptake systems were analyzed. As seen from Tab. 4, only 3 statistically significant associations of PAIs with iron uptake systems could be determined: the yersiniabactin siderophore system was associated with PAI IV₅₃₆, the aerobactin siderophore system and *Iha* were associated with PAI I_{CFT073}.

Discussion

Our findings showed that PAIs, plasmids and iron uptake systems are abundant, as the majority of the tested isolates harboured PAIs, plasmids and iron uptake systems.

Since we analysed a relatively small number of isolates it is difficult to compare our results with results obtained by other authors on larger collections of strains, such as the study of SABATE & al. (2006) on the prevalence of PAIs among 100 UPEC strains and 50 commensal strains and the

Table 3: PAIs, iron uptake systems and plasmids in studied strains
Tabela 3: PAI, sistemi za privzem železa in plazmidi v preučevanih sevih

Strain	Patient's diagnosis ^a	Patient's gender ^b	PAI					Iron uptake system							Plasmid (bp)					
			I ₅₃₆	II ₅₃₆	III ₅₃₆	IV ₅₃₆	I ₉₆	II ₉₆	I _{CF1073}	II _{CF1073}	iutA	iucD	iroN	iroCD		fyuA	irp2	ihf	ireA	hbp
DL2	UTI	F	-	-	-	+	-	-	-	-	+	-	-	+	+	+	-	+	-	>30,000
DL6	UTI	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DL7	UTI	F	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	<30,000
DL8	UTI	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
DL14	UTI	F	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	-	+	>30,000
DL17	UTI	F	-	-	-	+	-	-	-	-	-	+	-	+	+	+	-	-	-	<30,000
DL22	UTI	M	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	+	<30,000
DL37	UTI	F	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	<30,000
DL41	UTI	F	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
DL43	UTI	F	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-
DL46	UTI	F	-	-	-	+	-	-	-	-	-	+	-	+	+	+	-	-	-	<30,000
DL48	UTI	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DL56	UTI	F	-	-	-	+	-	-	-	-	-	+	-	-	+	+	+	-	-	>30,000
DL76	UTI	F	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	>30,000
DL81	UTI	F	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	<30,000
DL84	UTI	F	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	>30,000
DL108	UTI	F	-	-	-	+	-	-	-	-	+	-	-	+	+	+	-	-	-	<30,000
DL109	UTI	F	-	-	-	+	-	-	-	-	-	+	+	+	+	+	-	+	+	>30,000
DL110	UTI	F	-	-	-	+	-	+	-	-	-	+	+	+	+	+	+	-	-	>30,000
TA10	WI	F	-	-	-	+	-	-	-	-	-	-	+	+	+	+	-	-	+	-
TA49	WI	F	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
TA50	D	M	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	-	-	>30,000
TA71	SWI	F	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
TA74	GTI	M	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-
TA103	SWI	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TA160	SU	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>30,000
TA171	D	F	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	<30,000
TA174	UC	M	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
TA212	D	M	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	-	-	<30,000

^aUTI – urinary tract infection, W1 – wound infection, D – decubitus, SW1 – surgical wound infection; SU – skin ulcer; UC – ulcer cruris

^bF – female; M – male

Table 4: Associations of PAIs with iron uptake systems

Tabela 4: Povezave PAI in sistemov za privzem železa

Iron uptake system	PAI (no. of strains)							
	IV ₅₃₆ +(19)	IV ₅₃₆ – (10)	II _{J96} +(1)	II _{J96} –(28)	I _{CFT073} +(4)	I _{CFT073} –(25)	II _{CFT073} +(6)	II _{CFT073} –(23)
Aer	9	2	1	10	4	7*	4	7
Sal	4	3	1	6	0	7	2	5
Yer	19	0***	1	18	4	15	6	13
Iha	7	2	0	9	4	5*	4	5
IreA	6	1	1	6	0	7	2	5
Hbp	4	0	0	4	0	4	0	4

P values (Fisher's exact test) of statistically significant associations ($P < 0.05$) are indicated by asterisks. Symbols: *, $P < 0.05$; **, $P < 0.005$; *** $P < 0.0005$. Aer, aerobactin siderophore system; Sal, salmochelin siderophore system; Yer, yersiniabactin siderophore system.

Statistično značilna vrednost *P* (Fisherjev eksaktni test) $< 0,05$ je nakazana z zvezdicami: simboli *, $P < 0,05$; **, $P \leq 0,01$; ***, $P \leq 0,001$. Aer, aerobaktinski sideroforni sistem; Sal, salmohelinski sideroforni sistem; Yer, jersiniabaktinski sideroforni sistem.

study of JOHNSON & al. (2005) on the prevalence of several virulence factors, including *iha*, *fyuA* and *iutA*, among 83 cystitis and 170 pyelonephritis *E. coli*. A further difficulty for comparison of our results with others' is the fact that differences might also be due to geographical differences. Differences in virulence factor profiles between distinct populations have previously been reported among cat populations from distant locations on feline uropathogenic *E. coli* strains from the United Kingdom and feline uropathogenic *E. coli* strains from New Zealand (FREITAG & al 2005). However, it is interesting that among all our studied strains none carried PAI I₅₃₆, PAI II₅₃₆, or PAI III₅₃₆ and that among the UTI strains included in this study no strain carrying PAI I_{CFT073} could be detected, while SABATE & al. (2006) reported detection of all PAIs except PAI I_{J96}. The overall lower prevalence in our study could be due to the fact, that our study incorporated only 29 strains.

In our study 19 ExPEC strains harboured at least one plasmid. Since it is commonly known that plasmids are abundant in all bacterial species, the obtained prevalence is of no surprise. It is also not unexpected, that 9 strains in our study harboured large plasmids (>30 kbp), since virulence factors enabling a strain to cause pathogenicity as well as antibiotic resistances can be encoded on large plasmids.

The prevalence of iron uptake system in our study is comparable to data presented in studies on cystitis *E. coli* strains (JOHNSON & al. 2005, KANAMARU & al. 2003). However, uroseptic and pyelonephritic *E. coli* isolates have a higher prevalence of iron uptake systems (JOHNSON & STELL 2000), (JOHNSON & al. 2005), also this is not unexpected since uroseptic and pyelonephritic strains are in general more virulent and possess more virulence factors.

It is known that iron uptake systems can be encoded either chromosomally, sometimes as a part of a genomic island, or on plasmids. The yersiniabactin siderophore system is known to be carried on the HPI (=PAI IV₅₃₆) (SCHMIDT & HENSEL 2004) and in our study the association of PAI IV₅₃₆ with this siderophore system was highly statistically significant, all 19 strains encoding the yersiniabactin siderophore system also harboured PAI IV₅₃₆. The aerobactin siderophore system is known to be part of the PAI I_{CFT073} (SCHMIDT & HENSEL 2004), but it was also found to be carried on a plasmid (CARBONETTI & WILLIAMS 1984). In our study all 4 strains that possessed the PAI I_{CFT073} also harboured the aerobactin siderophore genes, and their association proved to be statistically significant. However, in 7 strains that encoded the aerobactin siderophore system, PAI I_{CFT073} could not be detected, but all 7 strains harboured plasmids large enough to carry the aerobactin siderophore

system therefore, we could assume that the aerobactin siderophore system is more often encoded on plasmids than chromosomally. The same was found for the salmochelin siderophore system. This siderophore system can also be carried by a PAI, the PAI III₅₃₆ (SCHMIDT & HENSEL 2004), but also on a plasmid (SORSA & al. 2003). In our study in 7 strains we detected the salmochelin siderophore system however, none of these strains harboured the PAI III₅₃₆ and 6 strains carried plasmids large enough to encode the salmochelin siderophore system. The *iha* is known to be part of the PAI I_{CFT073} (SCHMIDT & HENSEL 2004) and in our study 4 out of 9 strains possessing *iha* also possessed the PAI I_{CFT073} (the association was statistically significant). However, the other 5 *iha* encoding strains did not harbour the PAI I_{CFT073}, in 4 of them plasmids large enough to encode *iha* were found. To our knowledge it has not yet been reported that *iha* could be plasmid encoded. The *ireA* and *hbp* were never associated with PAIs, *ireA* was found to be chromosomally encoded (RUSSO & al. 2001) and *hbp* was found to be plasmid encoded (OTTO & al. 1998). In 6 from 7 *ireA*-encoding strains and in 3 from 4 *hbp*-encoding strains plasmids were detected, so we might assume that both *ireA* as well as *hbp* could be either chromosomally encoded or carried by plasmids however, further studies to confirm the location of *iha*, *ireA* and *hbp* genes are needed.

To our knowledge this is the first study of the prevalence of PAIs in a collection of ExPEC strains that included not only UPEC isolates, but also isolates from other extraintestinal infections. Thus, this is the first report of UPEC associated PAIs, PAI I_{CFT073} and PAI II_{CFT073}, that were originally found in UPEC strain CFT073 isolated from blood and urine of a woman with pyelonephritis (SCHMIDT & HENSEL 2004), in strains isolated from skin and soft tissue infections. Further, to our knowledge this is the first study on molecular epidemiology including more than 4 iron uptake systems in ExPEC strains and it is worth to be emphasized that this is the first report on the association of iron uptake systems with PAIs and plasmids. However, since in this study only 29 ExPEC isolates were investigated, further studies examining a large number of ExPEC strains should be performed.

Conclusions

To summarize and conclude:

1. 29 ExPEC strains were screened with PCR for the presence of well characterized PAIs and iron uptake systems as well as with molecular biology techniques for the presence of plasmids;
2. the prevalence, the distribution and the genetic associations of the tested PAIs and iron uptake systems were determined;
3. PAI IV₅₃₆ was found in 19, PAI II_{CFT073} in 6, PAI I_{CFT073} in 4, PAI II₉₆ in 1 of the studied isolates, while PAI I₅₃₆, PAI II₅₃₆, PAI III₅₃₆ and PAI I₉₆ were not detected in any studied isolate;
4. in 19 of the studied isolates plasmids were detected;
5. *irp2* was found in 20, *fyuA* in 19, *iucD* and *iutA* in 12, *iha* in 9, *iroN* in 8, *iroCD* in 7, *ireA* in 7 and *hbp* in 4 of the studied isolates;
6. PAI IV₅₃₆ was statistically significantly associated with the yersiniabactin siderophore system and PAI I_{CFT073} was statistically significantly associated with the aerobactin siderophore system and *Iha*;
7. to our knowledge this is the first report on PAIs and iron uptake systems in an ExPEC collection including isolates from skin and soft tissue infections.

Povzetek

Bakterija *Escherichia coli* (*E. coli*) je najpogostejši fakultativni anaerob med človeškimi črevesnimi mikrobioti. Kljub temu, da je *E. coli* komenzalna bakterija, lahko določeni sevi, ki so pridobili genske zapise za virulentne dejavnike, povzročajo zelo širok spekter okužb, tako črevesnih kot zunajčrevesnih. Genski zapisi za virulentne dejavnike so pogosto vezani – umeščeni v otoke patogenosti (PAI), ki predstavljajo podskupino genomskih otokov. Genski zapisi za virulentne dejavnike pa se pogosto nahajajo tudi v plazmidih, v izvenkromosomskih molekulah DNA. Železo je pomemben element, saj nastopa kot kofaktor v mnogih metabolnih poteh tako gostitelja kot mikroorganizma. Ker je v gostitelju malo prostega železa, imajo mikrobi različne

sisteme za privzem železa, med najbolj znanimi so t. i. sideroforji. Naša raziskava je vključevala 29 sevov zunajčrevesnih patogenih *E. coli* (ExPEC), osamljenih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani iz diagnostičnih vzorcev 19 bolnikov z urinarnimi infekcijami, 9 bolnikov z infekcijami kože in podkožja in 1 bolnika z infekcijo spolovil. S pomočjo PCR smo preučevane seve preiskali za prisotnost različnih otokov patogenosti, ki so jih prvotno našli in opisali v uropatogenih sevih *E. coli* (PAI I₅₃₆ do PAI IV₅₃₆, PAI I₉₆, PAI II₉₆, PAI I_{CFT073} in PAI II_{CFT073}) in sisteme privzema železa (gena *iutA* in *iucD* aerobaktinskega sideroformnega sistema, geni *iroN*, in *iroCD* salmohelinskega sideroformnega sistema, gena *fyuA* in *irp2* jersiniabaktinskega sideroformnega sistema, gen *iha* receptorja kateholatnega sideroforja Iha, gen *ireA* od TonB odvisnega sideroformnega receptorja IreA in gen *hbp* hemoglobinske proteaze Hbp). Nadalje, smo z molekulskobiološkimi tehnikami preverjali prisotnost plazmidov v preučevanih sevih. Ugotavljali smo prevalenco in povezave preučevanih PAI in sistemov za privzem železa. PAI IV₅₃₆ smo našli v 19, PAI II_{CFT073} v 6, PAI I_{CFT073} v 4, PAI II₉₆ v 1 od preučevanih izolatov.

PAI I₅₃₆, PAI II₅₃₆, PAI III₅₃₆ in PAI I₉₆ nismo odkrili v nobenem izmed preučevanih izolatov. V 19 sevih smo odkrili plazmide, v 9 sevih smo našli plazmide, ki so bili večji od 30 kb. Gen *irp2* smo našli v 20, *fyuA* v 19, *iucD* in *iutA* v 12, *iha* v 9, *iroN* v 8, *iroCD* v 7, *ireA* v 7 in *hbp* v 4 izmed preučevanih sevov. PAI IV₅₃₆ je bil statistično značilno povezan s sideroformnim sistemom jersiniabaktin in PAI I_{CFT073} je bil statistično značilno povezan s sideroformnim sistemom aerobaktin in Iha. Kolikor nam je poznano je to prva raziskava o PAI in sistemih za privzem železa na zbirki sevov ExPEC, ki vključuje tudi izolate iz infekcij kože in podkožja.

Acknowledgements

The authors thank Veronika Križan-Hergouth, M.D., M.Sc. and Marija Gubina, M.D., Ph.D. from the Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana for providing the investigated strains. This research was supported by Grant PO-0508-0487 of the Ministry of Education, Science and Technology, Slovenia.

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Toxicity of the organophosphorous insecticide chlormephos to the earthworm *Eisenia andrei* and the terrestrial isopod *Porcellio scaber*

Strupenost organofosfatnega pesticida klormefosa za deževnike *Eisenia andrei* in kopenske enakonožce *Porcellio scaber*

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Abstract: In the present study we determined the toxicity of chlormephos to two common soil organisms – earthworms (*Eisenia andrei*) and isopods (*Porcellio scaber*) using Lufa 2.2 soil. The LC₅₀ values for the effects on earthworm and isopod survival were 58 and 76 mg/kg dry soil, respectively. Mass change of earthworms and behaviour of isopods were more sensitive end points as survival. Based on earthworm body mass changes, NOEC and LOEC values were 1 and 3 mg/kg dry soil, respectively. The earthworms did not reproduce even at the lowest chlormephos concentration (LOEC < 1 mg/kg dry soil). Isopods significantly avoided burrowing in soil treated with ≥40 mg/kg dry soil. Compared with some other organophosphate insecticides, chlormephos was highly toxic to beneficial terrestrial invertebrates.

Keywords: ecotoxicity; organophosphates; chlormephos; soil exposure; soil invertebrates

Izvleček: Toksičnost klormefosa smo določili na deževnikih (*Eisenia andrei*) in raki enakonožcih (*Porcellio scaber*). Obe vrsti sta pogosto uporabljani v tovrstnih študijah. Živali smo izpostavili klormefosu preko standardizirane zemlje Lufa 2.2. Vrednost LC₅₀ za preživetje deževnikov je bila 58 mg/kg suhe zemlje in 76 mg/kg suhe zemlje za rake enakonožce. Ugotovili smo, da so sprememba telesne teže in razmnoževanja pri deževnikih ter vedenjski odziv rakov enakonožcev bolj občutljivi parametri kot preživetje. Na podlagi sprememb telesne teže pri deževnikih, so bile določene vrednosti NOEC in LOEC, in sicer 1 in 3 mg/kg suhe zemlje. Deževniki se niso razmnoževali niti pri najnižji koncentraciji klormefosa (LOEC < 1 mg/kg suhe zemlje). Raki enakonožci so se značilno manj zakopavali v zemljo, v kateri je bilo ≥40 mg/kg suhe zemlje (LOEC). V primerjavi z nekaterimi drugimi organofosfatnimi insekticidi, je bil klormefos izjemno toksičen za testirane kopenske nevretenčarje.

Ključne besede: ekotoksičnost; organofosfati; klormefos; izpostavitve preko zemlje; kopenski nevretenčarji

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Introduction

Organophosphates (OPs) are a large class of chemicals. Since World War II, several thousand OPs have been synthesized for various purposes. A lot of these OPs are still in use, mainly as pesticides in agriculture, but some are used as flame retardants and parasiticides in veterinary medicine (GUPTA 2007). OP insecticides are highly effective in pest control, and relatively non persistent in the environment but potentially toxic also to non-target species, including vertebrates (GUPTA 2007). OP insecticides cause acute toxicity by inhibiting acetylcholinesterase (AChE) through phosphorylation, in both invertebrate and vertebrate organisms (GUPTA 2007).

Symptoms of high level exposure to OPs include muscle twitching, hyperactivity, paralysis, loss of equilibrium and eventually death (FULTON & KEY 2001, SANDAHL & al. 2005), whereas low-level exposure has been implicated in various behavioural and physiological impairments (BAYLEY 1995, ENGENHEIRO & al. 2005).

Chlormephos (*S*-chloromethyl *O,O*-diethyl phosphorodithioate) is an OP insecticide that was introduced on the market in 1973 to control soil-dwelling pests like **cockchafer**, **click beetles**, **mole cricket**, wireworms, millipedes and other pests (FOOTPRINT 2006, LYNCH 1978). It has been sold mostly under the trade name Dotan (Aventis, France). The World Health Organization (2004) classified chlormephos as extremely hazardous due to its high toxicity to mammals. In rats, acute lethal dose (LD_{50}) of chlormephos after oral administration was 7 mg/kg body weight (BW). It has been withdrawn from the market in all member states of the European Union not later than 2006 (EU 2006). Despite its prohibition in Europe, possibility of its abuse exists as it still might be in use in some less developed countries.

Although chlormephos was in use in Europe for more than 30 years only scarce data are available on its ecotoxicity and fate in the environment. Chlormephos is highly toxic to some aquatic crustaceans (*Echinogammarus tibaldi*, $LC_{50} = 0.04$ mg/L) (PANTANI & al. 1997) and fish (*Rasbora heteromorpha*, acute $LD_{50} = 2.5$ mg/L) (FOOTPRINT 2006), but generally less toxic to birds (*Coturnix japonica*, acute $LD_{50} = 260$ mg/kg) (FOOTPRINT 2006). No data are available on the toxicity to non-target soil organisms.

The aim of this study was to determine the toxicity of chlormephos to some soil non-target invertebrates (earthworms and isopods) upon exposure to contaminated soil. Earthworms are standardised test organisms in toxicity testing and isopods are a model test species, commonly used to study the mode of action as well as for risk assessment. Both animals have an important ecological role as decomposers of organic material.

Besides, both species are convenient for analyzing the response to contaminants at different levels of biological organisation. In earthworms, we followed survival, mass gain/loss and reproduction in relation to the degree of soil contamination with chlormephos. In isopods, mortality and behavioural response to contaminated soil was observed. The effects of chlormephos were compared with those of some other OP insecticides.

Materials and Methods

Test species

In the experiments we used the earthworm species *Eisenia andrei* (Oligochaeta: Annelida, Lumbricidae) originating from a laboratory culture at the Veterinary Faculty, University of Ljubljana. Animals were kept in a climate chamber at 20 ± 1 °C with a 12/12 h photo period and 80% relative humidity (RH). Plastic containers were filled with a bedding of potting soil and peat, adjusted to pH 6. The cultures were regularly fed with ground dried horse faeces. Sexually mature animals with clearly visible clitellum and weighing between 200 and 300 mg were used in the experiment.

Porcellio scaber, Latr. (Isopoda, Crustacea), originated from an unpolluted environment in the vicinity of Ljubljana, Slovenia. Animals were kept in a climate chamber at 20 ± 1 °C with a 16/8 h photo period, caged in glass containers with moist sand and peat on the bottom.

They were fed with leaves from various trees (mainly hazel), with periodical addition of commercial food designed for experimental animals (Altromin 1324, Germany), fresh vegetable and apples. All tests were performed on animals of both sexes, having body masses of 18–30 mg.

Soil preparation

Analytical grade chlormephos in liquid form was obtained from Riedel de Haën (45386 3045X), Germany. Its purity was 99.2%.

The tests were performed using Lufa 2.2, a standardized natural soil having 3.7% organic matter, 6.8% clay and a pH (1 M KCl) of approx. 6.0. The test substance was introduced into the soils using acetone as a solvent.

A small portion of the soil (approx. 25c) was spiked with the acetone solution (25 ml acetone per 30 g soil), thoroughly mixed and incubated over night in a fume cupboard. After evaporation of the acetone, the remainder of the soil was added, carefully mixed and the moisture content was adjusted to 40–50% of the Water Holding Capacity (WHC). The control soil was treated with acetone in the same way.

Experimental design

Tests were performed with earthworms and isopods, lasting 28 days. Chlormephos concentrations in the Lufa 2.2 soil were 0-1-3-9-15-30-45-60-100-200 mg/kg dry soil for earthworms and 0-10-40-60-100-200 mg/kg dry soil for isopods.

The chlormephos concentrations in soil were checked randomly at the beginning of the test and did not differ for more than 15% from nominal concentrations.

The deviation in concentrations due to degradation or volatilisation during the experiments was not checked.

Earthworm tests

Glass jars (0.8 L) were filled with 500–600 g moist soil. Ten adult pre-weighed earthworms were randomly introduced into the test containers. There were 4 replicates per test concentration and a control. Incubation took place in a climate chamber at 21 °C, with 80% RH and a 12/12 h light/dark cycle. A small amount of finely ground dried horse manure was added for food once a week and by weighing water loss was compensated where necessary. After 28 days, test containers were emptied and surviving adults were counted and weighed.

Soil was returned into the test containers to allow for hatching of the cocoons for an additional 28 days (OECD 2004). To count the number of juveniles produced, the test containers were placed in a water bath at 60 °C; after approx. 15 minutes, the juvenile earthworms appeared on the soil surface and were gently transferred to a separate jar and counted manually.

Isopod tests

Glass jars (100 ml) were filled with approx. 30 g moist soil, and three adult isopods were introduced. There were six replicates for each treatment, including the control. Twice a week, we observed the presence of isopods on the surface of the soil and statistically evaluated the obtained results.

As test jars were filled with three organisms, their presence was evaluated in shares – if there was one organism present that meant 0.33, if two 0.66 and if three 1.00. Isopods received food pellets, consisting of maple leaves (50%), ground commercial rabbit food (40%) and potato powder (10%), on the soil surface (HORNUNG & al. 1998).

Test jars were covered with perforated aluminium foil and placed on trays in a climate chamber at 21 °C, with 75% RH and a 16/8 h light/dark cycle. Additional food was given when needed but at least once a week. Moisture content was checked weekly by weighing the containers and replenishing the water loss with deionised water. The animals were extracted from the tested substrates after 28 days of exposure period by emptying the test jars, hand sorting the test substrates and counting the surviving animals.

Data analysis

LC₅₀, the concentration causing a 50% reduction in survival, was estimated applying the trimmed Spearman-Kärber method (HAMILTON & al. 1978). EC₅₀ and EC₁₀, the concentrations causing 50 and 10% reduction, respectively in the number of juveniles produced, were estimated using a logistic model (HAANSTRA & al. 1985). Calculations were done using the software package Excel for Windows (Microsoft, USA, 2002).

No observed effect concentrations (NOECs) and lowest observed effect concentrations (LOEC) were estimated using T-test and ANOVA followed by the Dunnett's test (pair-wise comparison of means with the control) in the TOXSTAT® software package (GULLY & al. 1991). All LC₅₀, EC_x and NOEC values are based on nominal concentrations in the Lufa 2.2 test soil.

Results

Test with earthworms

Mortality and abnormalities

After 28 days of exposure, survival in the control group was 100%, whereas at 200 mg/kg there were no survivals. Significant increase in mortality was observed at chlormephos concentrations ≥ 45 mg/kg dry soil (Tab. 1). The LC₅₀ value was 58 mg/kg dry soil (confidence interval: 50–67).

At chlormephos concentrations of ≥ 45 mg/kg organisms were swelled and red coloured, but at higher concentrations, their bodies softened and became covered with a large amount of yellow liquid.

Body mass change

Earthworms lost mass during the 28 days of exposure in all groups, apart from the control and

the lowest chlormephos concentration (Fig. 1). The average mass gain in the control group was 10%, while at 1 mg/kg chlormephos mass gain was 4.7%.

The increase in mass loss was dose-related and was most prominent at 60 mg/kg dry soil (56.6%). It was noted during the experiment that food, offered in upper parts of jars, remained uneaten as earthworms mainly stayed in the bottom of the test jars. NOEC and LOEC for the effect of

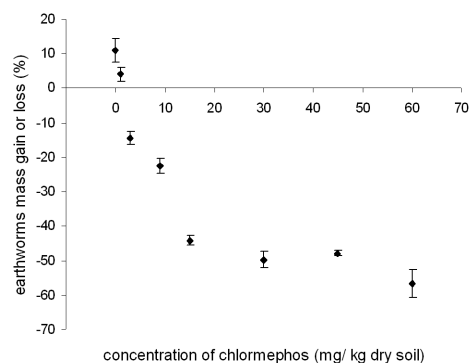


Fig. 1: Mass change (gain or loss) in earthworms (*Eisenia andrei*) exposed for 28 days to different concentrations of chlormephos in Lufa 2.2 soil (AVR $\pm$ SE).

Slika 1: Sprememba teže deževnikov, ki so bili 28 dni izpostavljeni različnim koncentracijam klormefosa v zemlji (POVP $\pm$ SN).

Table 1: Mortality rate of earthworms (*Eisenia andrei*) and isopods (*Porcellio scaber*) after 28 days of exposure to chlormephos in Lufa 2.2 soil, expressed in percentage of the number of individuals exposed.

Tabela 1: Stopnja smrtnosti deževnikov (*Eisenia andrei*) in enakonožcev (*Porcellio scaber*) po 28 dneh izpostavitve klormefosu, izraženo v odstotkih.

<i>Eisenia andrei</i>	
Chlormephos (mg/kg dry soil)	Mortality %
0	0
1	0
3	2.5
9	0
15	7.5
30	7.5
45	37.5
60	42.5
100	95
200	100

<i>Porcellio scaber</i>	
Chlormephos (mg/kg dry soil)	Mortality %
0	0
10	5
40	22
60	16.5
100	89
200	100

chlormephos on earthworm mass loss were 1 mg/kg and 3 mg/kg dry soil, respectively.

Reproduction

After 56 days, in the control groups the number of juveniles was 44 ± 11 (AVR $\pm$ SD), while already at the lowest test concentration (1 mg/kg) no juveniles were found. Therefore no EC₅₀ value could be established.

Test with isopods

Mortality

After 28 days of exposure, the survival in control groups was 100%, while at 200 mg/kg all isopods died (Tab. 1).

Significant increase in mortality was observed at chlormephos concentrations of ≥ 40 mg/kg dry soil. The LC₅₀ was 75 mg/kg dry soil (confidence interval: 67–84).

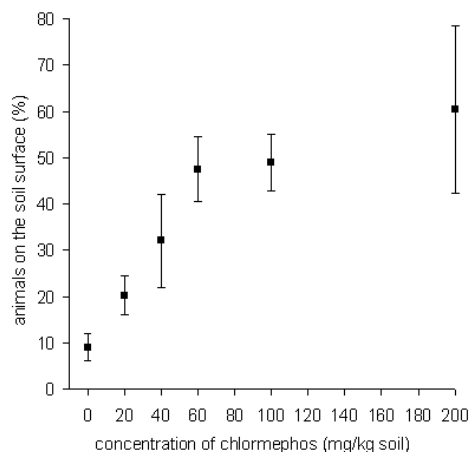


Fig. 2: Influence of chlormephos on the behavioural response of isopods (*Porcellio scaber*) during 28 days of exposure in soil. Shown are the average percentages (AVR $\pm$ SE) of animals detected on the soil surface as a function of exposure concentration in Lufa 2.2 soil.

Slika 2: Vpliv klormefosa na vedenje enakonožcev (*Porcellio scaber*) med 28-dnevno izpostavitvijo onesnaženi zemlji. Prikazani so povprečni deleži (POVP $\pm$ SN) živali, ki smo jih opazili na površini zemlje, v odvisnosti od koncentracije klormefosa v Lufa 2.2 zemlji.

Behavioural response

During observations, control animals were found mostly hidden in the soil (Fig. 2). Animals exposed to soil with chlormephos concentrations of ≥ 40 mg/kg dry soil were more frequently observed on the soil surface (t-test, $p < 0.05$). LOEC for behaviour is therefore 40 mg/kg dry soil.

Discussion

Organophosphates are still quite often used in agriculture as pesticides. Ecotoxicological studies related to OP's and using earthworms and isopods as model organisms exist (YASMIN & D'SOUZA 2007, DROBNE & al. 2008) but in comparison to the numerous studies on aquatic organisms (HANAZATO 1998, BARRY 1999, DUTTA & MAXWELL 2003, SHERRARDA & al. 2004) they are not that abundant.

This study revealed high sensitivity of both tested organisms to chlormephos. The NOEC for effects on earthworm body mass gain/loss was 1 mg/kg soil, while no reproduction occurred at this concentration.

The exposed earthworms probably lost weight due to their feeding and locomotor dysfunctions, as was noticed previously for ethyl-parathion in experiments with *Aporrectodea caliginosa* (OLVERA-VELONA & al. 2008).

The authors reported affected burrowing behaviour of *A. caliginosa* already at concentration 0.07 mg/kg dry soil. Behavioural response was recorded also in the presented experiment with *Porcellio scaber*. Isopods exposed to chlormephos were observed on the soil surface more frequently compared to control animals found mostly burrowed in the soil. The burrowing activity was probably affected by a locomotor dysfunction previously reported by ENGEHEIRO & al. (2005) but it can also be explained by avoidance behaviour response that is known for these animals (LOUREIRO & al. 2005, ZIDAR & al. 2005).

Terrestrial isopods are able to avoid soil (LOUREIRO & al. 2005) or food (ZIDAR & al. 2005) contaminated with pesticides or metals if they have an alternative. LOUREIRO & al. (2005) found behavioural response parameters to be equally or even more sensitive than other sublethal parameters like growth or reproduction.

Table 2: Literature data on the toxicity for earthworms and isopods of different organophosphorous insecticides.
 Tabela 2: Podatki iz literature o toksičnosti nekaterih organofosfatnih insekticidov za deževnike in enakoževce.

Test substance OP	Test organism(s)	Time (Days)	Test substrate	End-Point	Concentration (mg/kg dry soil)	Reference(s)
Chlorfenvinphos	<i>Eisenia fetida</i>	14	Sandy loam (pH 7.2)	LC ₅₀	204	WEYMAN (1997)
				NOEC (growth)	123	
				LOEC (growth)	234	
				LOEC (behaviour)	62	
Dichlorvos	<i>Eisenia fetida</i>	14	Sandy loam (pH 6.9)	LC ₅₀	80.9	VIAL (1991)
				NOEC (growth)	12.3	
Methamidophos	<i>Eisenia fetida</i>	18	Natural soil	LC ₅₀	29.5	QI-XING & al. (2006)
Diazinon	<i>Lumbricus terrestris</i>	21	Natural soil *	LC ₅₀ (different soil)	32, 233, 59	LANNO & al. (1997)
Ethyl-parathion	<i>Aporrectodea caliginosa</i>	14	Natural soil **	LC ₅₀ (different soil)	30, 24, 11, 32	OLVERA-VELONA & al. (2008)
				LOEC (growth)	7 (all soils)	
Malathion	<i>Drawida willsi</i> (juvenile, adult)	4	Sandy loam (pH 6.8)	LC ₅₀	15.1 (juv.); 18.8 (adult)	PANDA & SAHU (1999)
		15		LOEC (growth)	2.2	
Chlormephos	<i>Eisenia andrei</i>	28 56	Lufa 2.2	LC ₅₀	58	This study
				NOEC (growth)	1	
				LOEC (growth)	3	
				NOEC (reproduction)	<1	
Dimethoate	<i>Porcellio scaber</i>	18	Lufa 2.2	LC ₅₀	34 (juvenile)	FISCHER & al. (1997)
Diazinon	<i>Porcellionides pruinosus</i>	35	Sand	LC ₅₀	3.03	VINK & al. (1995)
Dimethoate	<i>Porcellionides pruinosus</i>	2	Lufa 2.2	NOEC (behaviour) [§]	10	LOUREIRO & al. (2005)
				LOEC (behaviour) [§]	40	
Dimethoate	<i>Porcellio dilatatus</i>	2	Silt loam	LOEC (behaviour) [#]	5	ENGENHEIRO & al. (2005)
		10		LOEC (behaviour) ^{##}	10	
Chlormephos	<i>Porcellio scaber</i>	28	Lufa 2.2	LC ₅₀	75	This study
				NOEC (behaviour)	20	
				LOEC (behaviour)	40	

* three different natural soils (Broxton Clay, Fox Sand, Guelph Loam); ** natural soil from three different locations in Mexico and one from France (Vertisol 1, Vertisol 2, Andosol, Calcisol); § avoidance; # pathlength, active time; ## stops per path

Earthworms (MOSLEH & al. 2003), isopods (ZIDAR & al. 2004) and probably also some other invertebrates can avoid accumulation of toxic substance in their body by reducing or even stopping food intake. This leads to diminished intake of necessary nutrients, resulting in starvation and upon long-term exposures to death of the organisms. This shows how important it is to also determine sublethal effects, with an emphasis on physiological parameters, besides the "classical" LC_{50} determination.

Reproduction of adult earthworms was completely inhibited already at 1 mg/kg dry soil. Besides direct OP poisoning, this might also be caused by lack of energy due to starvation in adult organisms or due to body abnormalities registered.

Namely, bodies of earthworms showed some typical changes for organophosphate exposure, observed also by other authors (QI-XING & al. 2006, VENKATESWARA & al. 2003).

In Table 2, data on the toxicity of different OP insecticides for earthworms and isopods are listed. Toxicity depends on the test species, soil type used and duration of exposure, which makes the comparison more difficult. For earthworm survival and growth, chlormephos was as toxic as malathion, parathion and methamidophos, but more toxic than dichlorvos and chlorphenvinpros. All these insecticides are already prohibited in the EU (EU 2008) but (like dichlorvos and parathion for example) may still be in use in some parts of Africa and Asia (FLO 2007). Chlormephos is less toxic to isopods compared to diazinon, which was withdrawn from the European market recently (EU 2008) and compared to dimethoate, which is still in use. Isopods seem less susceptible to chlormephos than earthworms, probably due to the avoidance response mentioned above.

Conclusions

- Chlormephos is highly toxic to earthworms, especially affecting their growth and reproduction, with an LC_{50} of 58 mg/kg dry soil, and NOEC growth of 1 mg/kg dry soil, and no reproduction at the NOEC growth.

- Isopods were less sensitive, with an LC_{50} of 76 mg/kg dry soil and burrowing behaviour significantly reduced at ≥ 40 mg/kg dry soil.

Povzetek

Uporaba toksičnih organofosfornih snovi se v zadnjem času zmanjšuje, vendar je njihova uporaba v kmetijstvu še vedno obsežna. Slednja lahko predstavlja resen ekološki problem, saj pogosta uporaba organofosfornih snovi negativno učinkuje na neciljne talne organizme, ki so sestavni člen prehranjevalnih verig. Glede na znane lastnosti organofosfornih snovi – visoka reaktivnost, hitro delovanje in toksičnost, smo za modelno substanco izbrali klormefos, ki v Evropi ni več v uporabi, vendar za katerega v literaturi ni veliko podatkov. V raziskovalnem delu smo spremljali učinke klormefosa na deževnike (*Eisenia andrei*) in rake enakonožce (*Porcellio scaber*), ki so bili 28 dni izpostavljeni kontaminirani standardizirani zemlji Lufa 2.2.

Določili smo koncentracijo klormefosa, ki povzroči smrt polovice izpostavljenih živali (LC_{50}). Ta je bila za deževnike 58 mg/kg suhe zemlje, pri rakah enakonožcih pa 76 mg/kg suhe zemlje. Spremljali smo tudi subletalne učinke, ki so občutljivejši pokazatelji toksičnosti snovi, zlasti ob izpostavitvi nižjim koncentracijam.

Na osnovi spremembe v masi deževnikov smo ugotovili, da je 1 mg/kg suhe zemlje koncentracija klormefosa brez opaznega učinka (NOEC), 3 mg/kg suhe zemlje pa koncentracija z opaznim učinkom na maso živali (LOEC). Pri izpostavljenih deževnikih smo opazili tudi telesne spremembe kot so nabrekanje, izžemanje, rumenkasti izločki ipd. (LOEC = 45 mg/kg suhe zemlje) ter prizadet proces reprodukcije (LOEC < 1 mg/kg suhe zemlje). Pri rakah enakonožcih smo poleg smrtnosti spremljali tudi vzorce obnašanja in ugotovili, da so se organizmi pri višjih koncentracijah zadrževali na površini in niso bili zakopani v zemlji kakor kontrolne živali.

Najnižja koncentracija z opaznim učinkom na vedenje živali je bila 40 mg/kg suhe zemlje.

Testiranje je pokazalo visoko toksičnost klormefosa za uporabljena testna organizma, pri čemer so bili enakonožci manj občutljivi na prisotnost klormefosa v zemlji od deževnikov, saj je pri enakonožcih bolj izražena sposobnost izogibanja

onesnaženi hrani. Toksičnost klormefosa je primerljiva z nekaterimi sorodnimi organofosfornimi insekticidi, pri čemer so nekateri že umaknjeni s trga, nekateri celo bolj strupeni za talne organizme

pa so še v uporabi (npr. dimetoat). Pridobljeni podatki lahko služijo tudi za izdelavo natančne ocene tveganja za okolje v primeru nenadzorovane rabe klormefosa.

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Mehanizmi eksocitoze/Mechanisms of Exocytosis

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Abstract: Vesicles are cellular organelles, in which signaling molecules (neurotransmitters or hormones) are stored and are essential for the function of neurons and endocrine cells in supporting the communication between tissues and organs. Upon stimulation the signaling molecules stored inside vesicles are released from cells by exocytosis. This fundamental biological process consists of membrane fusion between the vesicles and the plasma membrane, leading to the formation of an aqueous channel – the fusion pore – through which signaling molecules exit into the extracellular space or blood stream. The vesicle cargo discharge initially requires the delivery of vesicles to the plasma membrane, where vesicles dock and get primed for fusion with the plasma membrane. Classical view holds that stimulation initiates the fusion pore formation and vesicle cargo discharge in an all-or-none fashion. Once formed the fusion pore may close (transient, “kiss-and-run” exocytosis) or expand, leading to the full collapse of the vesicle membrane into the plasma membrane (full fusion exocytosis). However, recent studies indicate that exocytosis may not be as simple. Here we highlight the novel findings which indicate that transient fusion pore is subject to regulations, which affect the release competence of a single vesicle. Our recent studies have shown that in pituitary lactotrophs vesicle release of peptide signaling molecules involves modulation of fusion pore kinetics and fusion pore conductance.

Keywords: exocytosis, vesicle, fusion pore, transient/full fusion, pituitary lactotrophs, peptide hormones

Izvleček: Mešički so celični organeli, v katerih so shranjene signalne molekule (živčni prenašalci, hormoni), ki so nujno potrebne za delovanje živčnih in endokrinih celic, saj omogočajo komunikacijo med tkivi in organi. Po stimulaciji se signalne molekule izločijo iz mešičkov s pomočjo eksocitoze. Eksocitoza je temeljni biološki proces, pri katerem pride do zlitja membrane mešička in plazemske membrane, pri čemer nastane kanal – fuzijska pora, skozi katerega se izločijo signalne molekule v zunajcelični prostor ali krvni obtok. Pred eksocitozo mešički potujejo v neposredno bližino plazemske membrane, kjer se vsidrajo in pripravijo za zlitje s plazemsko membrano. V naslednjih fazah naj bi stimulacija sprožila nastanek fuzijske pore in popolno sprostitev vsebine mešička skozi fuzijsko poro. Fuzijska pora se lahko po odprtju zapre (*t.i.* prehodna, angl. »kiss-and-run« eksocitoza) ali pa razširi, kar vodi do popolnega zlitja membrane mešička s plazemsko membrano (*t.i.* popolna, angl. »full fusion« eksocitoza). Nedavne raziskave so pokazale, da proces eksocitoze ni tako preprost. V preglednem članku se bomo osredotočili na najnovejše raziskave, ki kažejo, da je prehodna eksocitoza lahko uravnavana, kar vpliva na zmožnost izločanja signalnih molekul iz posameznega mešička. Naše nedavne raziskave so pokazale, da v laktotrofih iz hipofize, sproščanje peptidnih signalnih molekul iz mešičkov vključuje tako modulacijo kinetike fuzijske pore kot tudi uravnavanje prevodnosti (premera) fuzijske pore.

Ključne besede: eksocitoza, mešiček, fuzijska pora, prehodna/popolna fuzija, hipofizni laktotrofi, peptidni hormoni

Introduction

Exocytosis is a fundamental cellular process used by eukaryotic cells to secrete different biological compounds. The basic machinery required for exocytosis has been well conserved throughout evolution from yeast to man. However, the precise molecular mechanisms underlying the process of exocytosis are still poorly understood and therefore extensively studied in different cell systems.

The secretory process consists of few different stages. First vesicles packed with the secretory compounds are transported to the plasma membrane. Then the vesicles are tethered or docked to the appropriate sites at the plasma membrane and prepared for fusion (priming). In the last step the fusion of vesicle with the plasma membrane (exocytosis) occurs and the vesicle content is released through the fusion pore (Fig. 1 and 2) and/or the vesicle membrane components are incorporated into the plasma membrane.

Exocytosis occurs in almost all cells in the form of constitutive exocytosis, which serves to release the components of the extracellular matrix, or just to deliver and incorporate newly synthesized membrane lipids and proteins into the plasma membrane (*i.e.* constitutive exocytosis). In many cells an alternative secretory pathway exists, where an extracellular stimulus is required to trigger exocytosis, therefore allowing a controlled release of peptide hormones and neurotransmitters. The stimulus typically triggers an increase in intracellular Ca^{2+} activity ($[\text{Ca}^{2+}]_i$), which activates vesicle fusion with the plasma membrane (*i.e.* regulated or Ca^{2+} -triggered exocytosis; Fig. 2).

In recent years new techniques have allowed direct measurements of elementary exocytotic events in real time in neurons and neuroendocrine cells. By measuring membrane capacitance (C_m) one can reveal changes in cell surface area, which are reflecting vesicle fusion and fission (NEHER & MARTY 1982, LINDAU & NEHER 1988). Detection of vesicle fusion with amperometry is based on the indirect electrochemical detection of released molecules with a suitable oxidation potential (WIGHTMAN & al. 1991, CHOW & al. 1992). Furthermore optical imaging with different fluorescence markers can provide a direct readout of vesicle fusion and recycling (BETZ &

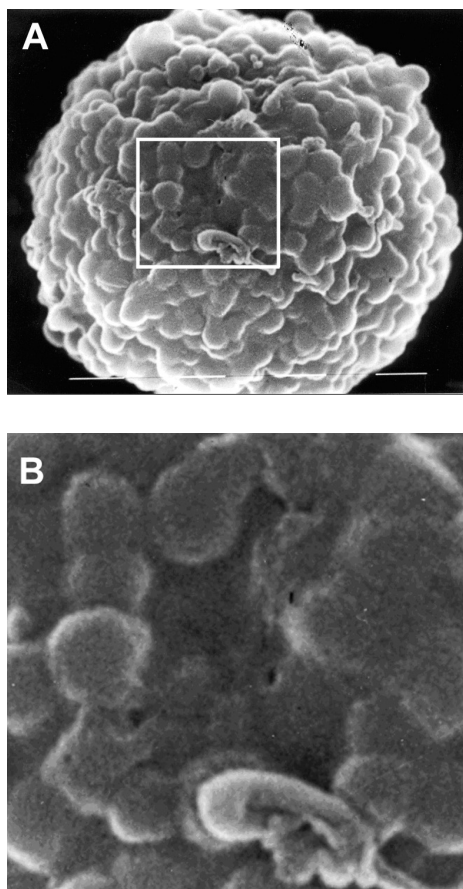


Figure 1: Scanning electron micrograph of the pituitary lactotroph surface exhibits fusion-pore-like formations. (A) Panel shows a view of the whole cell with a diameter of 10 μm . (B) Panel is a magnified view of the framed region in panel A. Several small openings in the cell surface (black) are seen, which may be related to the fusion pore-like structures.

BEWICK 1992, MIESENBOCK & al. 1998, SHANER & al. 2005). These multidisciplinary approaches led to the discovery that the rate (ALBILLOS & al. 1997, STENOVEC & al. 2004) and the amount of vesicle cargo release (ANGLESON & al. 1999) are controlled by the stimulus before the vesicle fusion, and more importantly, also after the fusion pore formation (*i.e.* post-fusion regulation of a release, RAHAMIMOFF & FERNANDEZ 1997).

In this article we focus on the mechanisms of regulated exocytosis. We first present an overview

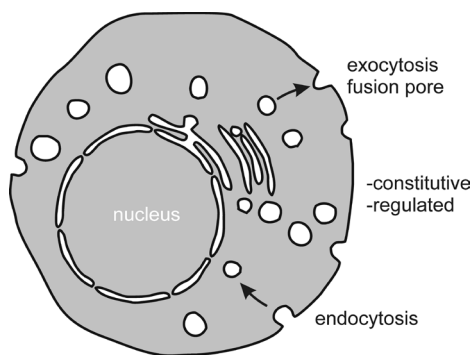


Figure 2: Exocytosis involves fusion of the vesicle membrane with the plasma membrane.

Membrane fusion leads to the formation of the fusion pore – an aqueous channel connecting the vesicle lumen with the extracellular space. Constitutive exocytosis does not require a stimulus to occur, whereas regulated exocytosis is triggered by a stimulus, such as an increase in the activity in cytosolic Ca^{2+} . Endocytosis is a process of plasma membrane retrieval, often balancing exocytosis to keep the surface area of a cell constant over a longer period of time.

of the present knowledge on single vesicle fusion events in lactotrophs, neuroendocrine cells of anterior pituitary, which secrete peptide hormone prolactin. We then discuss mechanisms of post-fusion regulation of vesicle cargo release from neurons and neuroendocrine cells.

Models of exocytosis

One of the first models of regulated vesicle exocytosis, introduced by del Castillo and Katz, predicts that in neurons the vesicles fuse transiently with the membrane and that the vesicle cargo is released in an all-or-none fashion (KATZ 1969). In the early 1970s, the first systematic studies on vesicle recycling were performed on frog neuromuscular junction by electron microscopy in the presence of the marker horseradish peroxidase. The studies were performed independently by two research groups, interestingly, their results yielded different interpretations (CECCARELLI & al. 1973, HEUSER & REESE 1973).

Heuser and Reese discovered that vesicle fusion is followed by full collapse of the vesicle membrane into the plasma membrane (*i.e.* full fusion exocytosis) emptying the entire vesicle content to the extracellular space (all-or-nothing release). The retrieval of vesicle membrane, which is necessary for maintaining the cell size and integrity, was proposed to occur at the location distal to the fusion site (HEUSER & REESE 1973). On the other hand, Ceccarelli and his co-workers proposed that vesicle fusion involves the opening of a small fusion pore, followed by its fast closure at the same site of fusion, without full pore dilation and vesicle membrane collapse into the plasma membrane (CECCARELLI & al. 1973). The model they proposed was similar to Katz's initial model, that vesicles fuse with the plasma membrane only

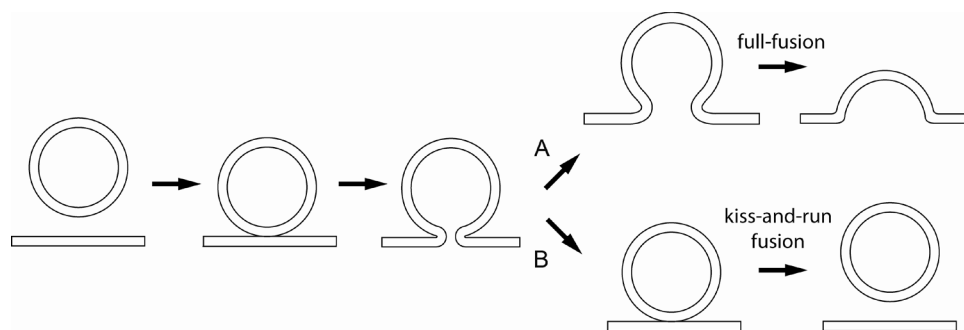


Figure 3: Models of exocytosis. Schematic drawing of exocytosis modes. (A) Full fusion of a vesicle after the initial formation of a narrow fusion pore. After the fusion pore widening the vesicle membrane is fully incorporated into the plasma membrane. (B) An example of transient fusion (a "kiss-and-run" event), where a vesicle forms a transient fusion pore with the plasma membrane and releases only part of its content. After the fusion pore closure, the vesicle lumen is reloaded and vesicle reused.

transiently (*i.e.* transient or “kiss-and-run” exocytosis as proposed by FESCE & al. (1994)). Further studies have revealed that the transient opening of the fusion pore may lead only to the partial release of vesicle cargo (STENOVEC & al. 2004, reviewed in HARATA & al. 2006) and that once opened, the fusion pore can change its dimensions dynamically and even close and reopen repetitively (fusion pore flickering, the pulsing fusion pore, FERNANDEZ & al. 1984, STENOVEC & al. 2004, VARDJAN & al. 2007). More than 30 years after, these two models (Fig. 3) have been still intensively debated (reviewed in HE & al. 2006, LOGIUDICE & MATTHEWS 2006, SMITH & al. 2008).

Stimulus increases the rate of cargo release from a single vesicle in lactotrophs

Figure 4 shows immunolabelled prolactin hormone accumulation at the surface of the plasma membrane following cell stimulation. Recently, it has been observed that in lactotrophs stimulated hormone discharge from a single vesicle is up to 20 times faster than spontaneous peptide hormone discharge (STENOVEC & al. 2004). Time-lapse con-

focal imaging was performed on cells expressing fluorescently tagged peptide ANP.emd (HAN & al. 1999) in their vesicle lumen and in the presence of the extracellularly added fluorescent styryl dye FM 4-64 (BETZ & BEWICK 1992). In lactotrophs, FM-membrane dye stains not only the plasma membrane when in the extracellular medium, but also the matrix of individual prolactin vesicles (ANGLESON & al. 1999, STENOVEC & al. 2005), upon exposure to the extracellular solution. The studies were carried out in resting conditions and following stimulation by exposing cells to a high potassium-containing solution. When exocytotic cargo release occurred, the fluorescence intensity of the ANP.emd probe decreased at the vesicle site and the vesicle was loaded through the same fusion pore with the FM 4-64. In resting lactotrophs, in 50% of spontaneously releasing vesicles, the peptide hormone release and the FM 4-64 loading were slow (~3 min). However, high potassium stimulation triggered hormone release and FM 4-64 loading within seconds, indicating that in lactotrophs stimulation increases the rate of vesicle cargo release, very likely at the stage when the fusion pore is already formed (post-fusion regulation of a release; RAHAMIMOFF & FERNANDEZ 1997, STENOVEC & al. 2004).

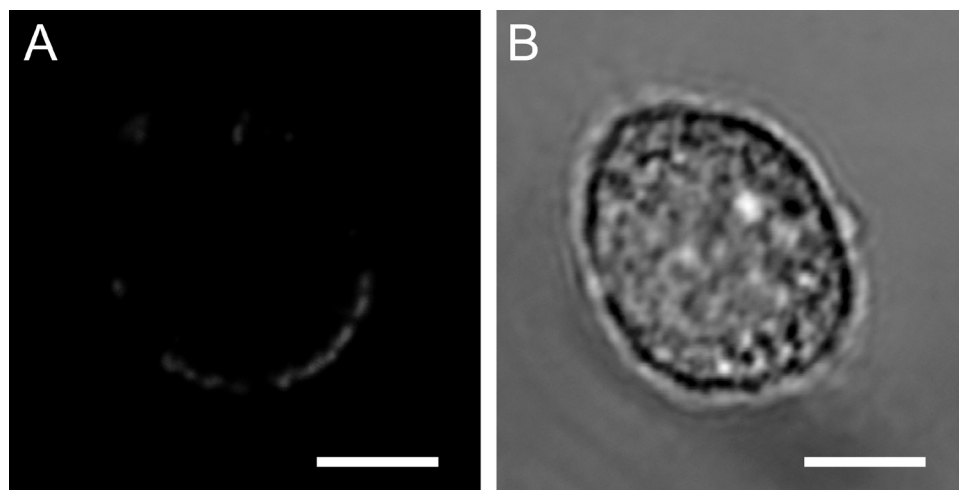


Figure 4: (A) Confocal image of secreted rat prolactin (rPRL) visualized on the surface of the plasma membrane by anti-rPRL antibody and fluorescent secondary antibody (red). The cell was exposed to a solution containing 100 mM K^+ , to depolarize the membrane and stimulate the cells prior to the immunocytochemical reaction. (B) Transmitted light image with DIC contrast of the cell displayed in A. Bar: 5 μ m.

Mechanisms of post-fusion regulation of vesicle cargo release in lactotrophs

Recent studies have shown that fusion pores are subject to regulations, which affect the release competence of a single vesicle (STENOVEC & al. 2004, reviewed in HARATA & al. 2006). In the next two chapters we will discuss two mechanisms that control the rate of vesicle cargo release in lactotrophs: (I) fusion pore kinetics and (II) fusion pore diameter (Fig. 5).

or “kiss-and-run” exocytosis) (NEHER & MARTY 1982, FERNANDEZ & al. 1984, SCEPEK & LINDAU 1993, LOLLIKE & al. 1995).

The majority of exocytotic events (>99%) observed by C_m measurements in resting lactotrophs were transient fusion events (*i.e.* transient fusion pore openings). The occurrence of non-reversing steps, representing full vesicle fusion (NEHER & MARTY 1982), was very low (~0.4% of all events). The fusion pore open-state duration of a single transient event was at rest ~50 ms (STENOVEC & al. 2004, VARDJAN & al. 2007), similar as in rest-

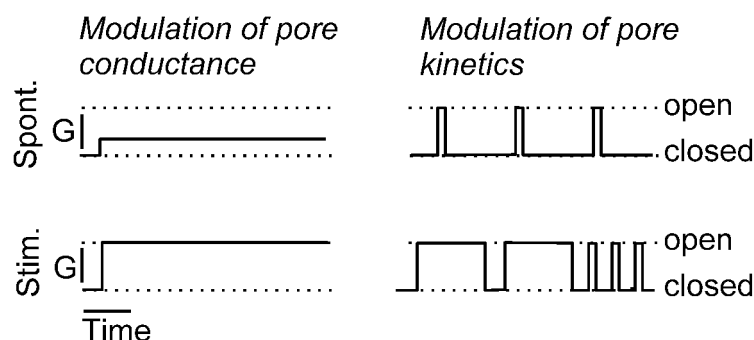


Figure 5: Mechanisms of vesicle cargo discharge modulation.

The permeation of molecules through the fusion pore depends on the fusion pore conductance (G ; diameter) and/or fusion pore kinetics. A wider fusion pore (higher G) and/or faster frequency of fusion pore openings with longer fusion pore dwell-times (faster kinetic of fusion pore openings) leads to increased release of peptides from a single vesicle in stimulated conditions.

I. Fusion pore kinetics

It has been proposed that transient, “kiss-and-run”, mode of vesicle fusion can limit or completely prevent peptide hormone release because of the relatively large size of peptide molecules and the consequent low diffusion mobility of these molecules (BARG & al. 2002, TSUBOI & RUTTER 2003, OBERMÜLLER & al. 2005). To determine, if transient mode of vesicle fusion is slowing the release of vesicle cargo in resting lactotrophs, C_m measurements of single vesicle fusion events were performed by cell-attached patch-clamp technique (NEHER & MARTY 1982). A stepwise increase in C_m (on-step) indicates full fusion exocytosis. If the on-step is followed by a downward, off-step in C_m , of a similar amplitude to the preceding on-step, the fusion of a vesicle with the plasma membrane is transient (transient

ing synapses of calyx of Held (SUN & al. 2002). These transient events appeared repetitively in bursts lasting for as long as 760 s (“the pulsing pore”; STENOVEC & al. 2004, VARDJAN & al. 2007), some of them were more complex with multiple amplitude on- and off-steps representing repetitive compound vesicle-to-vesicle-to-plasma membrane fusion events (VARDJAN & al. 2009). Regular repetitive fusion pore openings and slow, but synchronous, loading and unloading of fluorescent probes in lactotrophs, likely indicate that in resting lactotrophs the slow probe exchange through the fusion pore may be constrained kinetically by regular fusion pore openings (fusion pore flickering, also termed fusion pore gating; STENOVEC & al. 2004). Amperometric studies on neurons revealed that fusion pore flickering can limit the release of dopamine from synaptic terminals (STAAL & al. 2004). This indicates that flickering transient

fusion pores may also be involved in the regulation of small molecular weight transmitter release in synapses despite the relatively fast diffusion mobility of small chemical molecules.

Transient and full fusion exocytosis were reported to coexist in some systems and it has been suggested that switching between the transient to full fusion mode due to cell stimulation can result in the modulation of the amount of cargo release from a single vesicle (reviewed in HARATA & al. 2006). Interestingly, we have observed by C_m measurements that transient fusion pore openings with estimated mean burst duration of >100 s were the predominant mode of exocytosis not only in resting but also in high potassium stimulated lactotrophs (STENOVEC & al. 2004, VARDJAN & al. 2007). Full vesicle fusion occurred with practically the same relatively low incidence before and after stimulation (VARDJAN & al. 2007). However, transient events occurred fourfold more frequently in stimulated compared to resting lactotrophs. Moreover, the fusion pore dwell time was twofold longer after stimulation. Stimulus thus prolongs the effective open time of the transient pores, facilitating hormone secretion without full fusion (VARDJAN & al. 2007). More frequent transient events with prolonged fusion pore open state dwell-time were observed also in lactotrophs after hypotonic stimulation (JORGACEVSKI & al. 2008). These results are in contrast to previous studies in chromaffin cells where low levels of stimulation triggered “kiss-and-run” exocytosis, whereas with stronger stimulation the predominant mode of exocytosis was full fusion (ELHAMDANI & al. 2001, FULOP & al. 2005).

II. Fusion pore diameter

Recently, it has been shown that the fusion pore diameter can be the limiting factor preventing the permeation of molecules through the fusion pores (BARG & al. 2002, TAKAHASHI & al. 2002, TSUBOI & RUTTER 2003, FULOP & al. 2005). Therefore, the release of vesicle cargo from vesicles undergoing transient, “kiss-and-run”, exocytosis may depend also on the diameter of the fusion pore. We used electrophysiological and optical methods to measure the size of the effective fusion pore diameter in exocytic vesicles in lactotrophs before and after stimulation (VARDJAN & al. 2007).

The effective diameter of a fusion pore can be calculated from the fusion pore conductance (G_p ; SPRUCE & al. 1990, LOLLIKE & LINDAU 1999). Cell-attached patch-clamp technique can be used to monitor the time course of the G_p by modeling a vesicle fusing with the cell membrane as a conductance (the fusion pore) in series with a capacitor (the vesicle membrane) (ZIMMERBERG & al. 1987). We have measured the G_p as described in LOLLIKE & LINDAU (1999) and then we calculated the effective fusion pore diameters from the G_p values (described in SPRUCE & al. 1990). At rest, the G_p values in transient events ranged from 8 to 200 pS (the average 53 pS), corresponding to fusion pore diameters of 0.4 nm to 2.0 nm. After stimulation, the G_p values of transient events increased to an average measurable G_p of 81 pS. The maximal measurable G_p value after stimulation was ~ 530 pS (*i.e.* 3.2 nm), indicating that the diameter of fusion pore in the majority of stimulated events had effective pore diameters >3.0 nm. In $>98\%$ stimulated events the G_p values increased after stimulation to an unmeasurable final conductance, representing fusion pore expansion, and then decreased again to measurable values, indicating fusion pore narrowing/closing. This is consistent with previous results observed in different cell systems, where fusion pores enlarge their diameters several folds and close completely afterwards (FERNANDEZ & al. 1984, MONCK & al. 1990, SPRUCE & al. 1990, MELIKYAN & al. 1995, LARINA & al. 2007). Events with the measurable size of the fusion pore were more frequent in resting than in stimulated conditions (25% vs. 2%), indicating that the stimulus increases the size of the transient fusion pore (VARDJAN & al. 2007).

To confirm our observations, we performed optical fusion pore studies. Permeation of FM 4-64 (molecular diameter ~ 0.9 nm) and HEPES (molecular diameter ~ 0.5 nm) through spontaneously forming fusion pores was studied in lactotroph vesicles expressing synaptobHluorin (spH; VARDJAN & al. 2007). spH is a pH-dependent protein consisting of the vesicle membrane-targeted protein VAMP2 (synaptobrevin-2) with a pH-sensitive enhanced green fluorescent protein (superecliptic pHluorin) fused to its luminal side (MIESENBOCK & al. 1998). At the acidic pH of resting vesicles, spH fluorescence is quenched by protons because of the H^+ -ATPase activity. After

fusion with the plasma membrane, the vesicle interior becomes accessible from the relatively alkaline extracellular pH environment, allowing the protons to escape. The fluorescence intensity of spH increases rapidly and remains elevated until the pore closes and the vesicle is reacidified (MIESENBOCK & al. 1998).

Confocal imaging showed that half of the spontaneous exocytotic events exhibited fusion pore openings associated with an increase in spH fluorescence, indicating permeation of protons, however the pores were impermeable to FM 4-64 and HEPES molecules. Together with the results on G_p measurements these findings indicate an open fusion pore diameter in resting peptidergic vesicles of <0.5 nm. This is much narrower than the size of neuropeptides stored in these vesicles (prolactin molecular diameter ~ 5.2 nm; VARDJAN & al. 2007), indicating that a narrow open fusion pore (lower conductance - G_p) prevents or slows down the release process in resting lactotrophs. Probes used in the earlier fusion pore permeability studies in resting secretory cells (FM-dyes, horseradish peroxidase, antibodies) were of relatively large size >0.9 nm (MALGAROLI & al. 1995, RYAN & al. 1997, SARA & al. 2005), leading to the underestimation of fusion pore diameter and the extent of spontaneous fusion.

In stimulated lactotrophs, $>70\%$ of exocytotic events exhibited a larger, FM 4-64-permeable pore (>0.9 nm) consistent with previous fusion pore permeation studies (BARG & al. 2002, TAKAHASHI & al. 2002, TSUBOI & RUTTER 2003, FULOP & al. 2005) and our G_p measurements (VARDJAN & al. 2007, JORGACEVSKI & al. 2008). Stimulation-induced fusion pore widening is consistent with a facilitated vesicle cargo discharge in the majority of stimulated fusion events recorded (VARDJAN & al. 2007). Similarly, stimulation led to the fusion pore expansion in chromaffin cells, therefore increasing the efficiency of release of small classical transmitters (ELHAMDANI & al. 2001, FULOP & al. 2005).

Summary

Transient fusion was considered to be an event mediating complete discharge of the vesicle content (KATZ 1969), however recent findings indicate that

vesicle content may be emptied incompletely in transient fusion events. Partial vesicle discharge appears to occur at the post-fusion stage. Transient fusion pores are subject to physiological regulation which affects the amount of vesicle cargo discharge from a single vesicle. Current knowledge of regulatory mechanisms at the post-fusion stage, involving fusion pore diameter and/or fusion pore open-time modulation, is still limited (RAHAMIMOFF & FERNANDEZ 1997, VARDJAN & al. 2007). However, in lactotrophs the transient “kiss-and-run” mode of exocytosis is a robust physiological phenomenon and thus represents a model for further studies towards the unravelling the nature of the exocytotic fusion machinery. Based on the current results the release of vesicle cargo may be restrained kinetically and/or due to a narrow fusion pore. Under stimulation, the pre-formed fusion pore may retain the transient nature, but with a longer dwell-time, increased frequency of re-openings and a wider effective fusion pore diameter. All of these changes will facilitate the vesicle cargo release (Fig. 5; VARDJAN & al. 2007). Regulation of fusion pore dynamics is important, as any modulation of release processes could have an impact on specific physiological functions of cells.

Membrane fusion involves high energy barrier, therefore it is unlikely that transient fusion events represent cycles of fusion/fission of a single vesicle. Transient fusion events may represent fluctuations of an open fusion pore between states where the pore is extremely narrow. A narrow fusion pore is likely composed of molecules with highly negative curvatures (CHURCHWARD & al. 2008). It was reported recently that the structure of plasma membrane sites where prolactin vesicles undergo exocytosis are distinct from the plasma membrane areas devoid of docked prolactin vesicles (GONÇALVES & al. 2008). In the future the determination of the structure of the fusion pore and of the cellular mechanisms underlying the nature of the repetitive transient fusion events will be critical for a better understanding of exocytosis of peptidergic vesicles.

Povzetek

Dolgo je veljalo, da se pri procesu prehodne eksocitoze vsebina mešička popolnoma izloči skozi fuzijsko poro (KATZ 1969), vendar pa so novejša raziskava pokazale, da se lahko vsebina mešička pri prehodni eksocitozi sprosti le delno. Delno izločanje vsebine mešička je najverjetneje uravnvano po zlitju mešička s plazemsko membrano oz. na stopnji že oblikovane fuzijske pore. Fuzijska pora je torej prehodna struktura, ki je po samem nastanku lahko fiziološko uravnavana, kar vpliva na sproščanje količine vsebine mešička. Dosedanje znanje o postfuzijskem uravnavanju izločanja vsebine mešička na ravni modulacije premera fuzijske pore in časa odprtja fuzijske pore je pomanjkljivo (RAHAMIMOFF & FERNANDEZ 1997, VARDJAN & al. 2007). V laktotrofh je prehodna eksocitoza robusten fiziološki fenomen, zato lahko celice laktotrofov uporabljamo kot model za študij prehodne eksocitoze, kar bi v prihodnje pripomoglo k razjasnitvi osnovnih mehanizmov eksocitoze. Na podlagi dosedanjih rezultatov je izločanje vsebine mešička lahko ovirano kinetično in/ali zaradi ozke fuzijske pore. Po stimulaciji lahko fuzijska pora ohrani svojo prehodno naravo odpiranja, vendar pa je prehodna fuzijska pora po stimulaciji dlje časa odprta, se pogosteje odpira in zapira in ima večji premer. Vse te spremembe pospešijo izločanje vsebine mešička (Fig. 5; VARDJAN & al. 2007). Uravnavanje dinamike izločanja vsebine mešička skozi fuzijsko poro je fiziološko zelo pomembno,

saj ima lahko vsaka modulacija izločanja vpliv na specifične fiziološke funkcije celic.

Fuzija membran je energijsko neugoden proces, zato prehodna narava odpiranja fuzijske pore najverjetneje ni posledica ponavljajoče fuzije/fisije posameznega mešička. Prehodno odpiranje fuzijske pore je bolj verjetno posledica fluktuacije odprte fuzijske pore med stanji, ko je fuzijska pora skrajno ozka in stanji, ko se le-ta prehodno bolj odpre. Ozka fuzijska pora je zelo verjetno sestavljena iz molekul, ki imajo močno negativno ukrivljenost (CHURCHWARD & al. 2008). Nedavno je bilo pokazano, da se struktura plazemske membrane na področjih, kjer poteka eksocitoza prolaktinskih mešičkov razlikuje od plazemske membrane, kjer ni pripetih prolaktinskih mešičkov (GONÇALVES & al. 2008). V prihodnje bo določitev strukture fuzijske pore in pa razjasnitev celičnih mehanizmov, ki uravnava prehodno odpiranje fuzijske pore, kritično pripomoglo k boljšemu razumevanju mehanizmov eksocitoze peptidnih hormonov.

Acknowledgements

This work was supported by grants P3 310 381, Z3 7476 from the Ministry of Higher Education, Sciences and Technology of the Republic of Slovenia, NS36665 from the National Institutes of Health, EC grant #QLG3 2001-2004, EC support DECG, CLG3-CT-2001-02004, and EC GROWBETA QLG1-CT-2001-02233.

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Centipede catch in pitfall traps with leading boards

Ulov strig v talnih pasteh z vodili

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Abstract: In investigations of soil arthropods, different methods are used for collecting specimens. During centipede community investigation in unevenly aged beech stand in Dinaric forests in Slovenia pitfall traps with leading boards were used to catch centipedes that walked in a certain direction. In present paper these traps and catching centipedes using them is presented. In studied stand 30 pitfall traps (each having 2 collecting vessels) with leading boards were placed and emptied through the whole year of 2003. 2367 centipedes from 37 species (out of 44 species already found in this stand) were caught. The majority of catch presents well mobile, bodily bigger epigeic lithobiids that prefer litter layer. The portion of juveniles was very low.

Keywords: sampling methods, pitfall-trapping, migration, community dynamics, soil arthropods

Izvleček: Pri raziskavah združbe strig uporabljamo različne metode vzorčenja za zbiranje osebkov. Med raziskavo združbe strig raznomernega bukovega sestoja v Dinarskih gozdovih Slovenije so bile uporabljene talne pasti z vodili za lov strig, ki so hodile v določeni smeri. V prispevku so predstavljene te pasti in ulov strig z njimi. V raziskovanem sestoji je bilo postavljenih 30 talnih pasti z vodili (vsaka je imela po 2 lovilni posodici). Praznjenje posodic z ulovom je potekalo skozi vse leto 2003. Ujetih je bilo 2367 strig iz 37 vrst. Večina ulova predstavljajo dobro mobilni, telesno večji lithobiidi ki preferirajo sloj stelje. Delež mladostnih osebkov je zelo majhen. Osebkovi vrste *Eupolybothrus tridentinus* predstavljajo 45% celotnega ulova v pasteh.

Ključne besede: metode vzorčenja, lov s pastmi, migracija, dinamika združbe, talni členonožci

Introduction

In investigations of soil arthropod communities one of the first obstacle we face is usually the selection of adequate sampling method(s). Different methods differ by effort required, efficiency, time required for realisation, suitability for a certain group or its part, quality of gathered data, its quantity and applicability.

In centipede community studies different common sampling methods are used for collecting specimens. Pitfall trapping, soil sampling, litter sifting and hand collecting are mainly employed. Using different methods, differently large and differently active centipedes with different body structure and way of life can be caught. That is why mostly different sampling methods give dissimilar impressions of the structure of arthropod

communities at the certain location (KOS 1988, 1995a,b, MESIBOV & al. 1995, GRGIČ & KOS, in preparation). During centipede investigation using two different sampling methods GRGIČ (2005) distinguished two larger groups of centipedes: smaller lithobiomorphs and geophilomorphs that appear in deeper soil layers and larger epigeic lithobiomorphs that prefer litter layer. But TUF (in preparation) during comparative study of four different collection methods divided centipedes into five groups with different biology: larger abundant lithobiomorphs, larger less frequent lithobiomorphs, smaller soil lithobiomorphs, abundant geophilomorphs and not frequent geophilomorphs.

While some sampling methods are more effective than others, the ideal sampling method for a particular project is ultimately based on the goals of that project (SNYDER & al. 2006). During investigation of active centipede migrations between different forest development phases, which were found to have certain influence on their communities (GRGIČ & KOS 2003, 2005), we used pitfall traps with leading boards (GRGIČ 2005). In present paper these traps and catching centipedes using them is presented.

So called Barber traps (BARBER 1930) are commonly used for collecting soil invertebrates. These are plastic or glass vessels embedded in soil with margins in the surface level or a bit lower. They are filled with conservation solution. In this form traps are simple, time and effort saving, and can be exposed for a long time. TUF (in preparation) found pitfall trapping one of the most effective collection methods for centipedes. FRÜND and co-workers (1997) found that efficiency of catching is higher with leading boards in comparison with traps alone. Even more, with such traps we are able to establish the dynamics of surface centipedes, and their active migration in certain direction (GRGIČ 2005).

Material and methods

Soil trap with leading boards

For catching surface dwelling centipedes, pitfall traps with leading boards were used. These are somewhat modified and adapted traps as FRÜND and co-workers (1997) used for catching walking centipedes. Traps were made up of three

one meter long and 30 cm high plastic plates that were placed in Z-shape at right angle to each other (Fig. 1). About 20 cm of plates were under the ground and 10 above the ground. Opened parts of the traps were oriented towards particular phase. In each of 2 angles the 8x8 cm plastic vessel with Monoethylene Glycol that kills and partly conserves animals was placed. Margins of the vessels were about 5 cm under the surface level. Vessels were partly covered with leaves and bark, so that falling leaves and precipitations couldn't fill up the vessel and prevent the trapping.

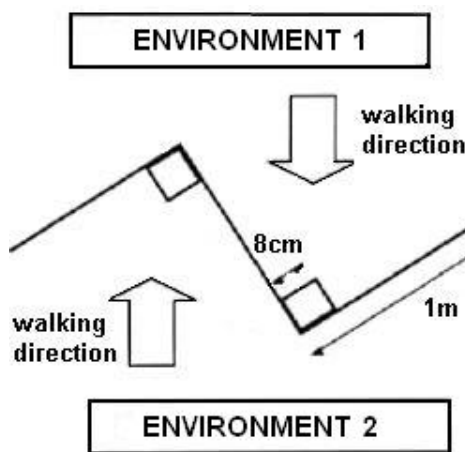


Fig. 1: Sketch of soil trap with leading boards.
Slika 1: Skica talne pasti z vodili.

In autumn 2002, 30 traps were placed in investigated unevenly-aged beech stand in Iška, a village 15 km south of Ljubljana, Slovenia. On each of the five borders between two different phases we placed 6 pitfall traps (each having 2 collecting vessels) with leading boards. On 25 November 2002 we started with trapping. We emptied traps after 17–40 days depending on the amount of precipitation till 5 January 2004.

Results

Leading boards directed specimens that walked in the certain direction into vessels, so we were able to separately catch animals that walked between two phases. We caught 2367 centipede from 37 species (out of 44 species already found

Table 1: Taxonomical review and specimens number (N) of Chilopoda species caught in pitfall traps with leading boards and currently known distribution (D) (en-endemic, il-Illyric, pa-Palearctic, se-South (-East) European, me-Mediterranean, eu-European, mi- Middle European).

Tabela 1: Taksonomski pregled in število osebkov (N) strig ujetih v talnih pasteh z vodili in trenutna znana razširjenost (D) (en-endemična, il-ilirska, pa-palearktična, se-jugo (-vzhodno) evropska, me-mediteranska, eu-evropska, mi- srednje evropska).

	SPECIES	N	D
Scolopend.	<i>Cryptops</i> cf. <i>anomalous</i> Newport, 1844	1	pa
	<i>Cryptops hortensis</i> Leach, 1815	33	pa
	<i>Cryptops parisi</i> Brolemann, 1920	47	eu
	<i>Cryptops</i> cf. <i>umbr icus</i> Verhoeff, 1931	2	en
Geophilomorpha	<i>Clinopodes flavidus</i> C. L. Koch, 1847	4	?
	<i>Dicelophylus carniolensis</i> C. L. Koch, 1847	72	il
	<i>Stenotaenia sorrentina</i> (Attems, 1903)	1	?
	<i>Geophilus electricus</i> (Linne, 1758)	2	eu
	<i>Geophilus</i> cf. <i>proximus</i> C. L. Koch, 1847	24	eu
	<i>Henia illyrica</i> (Meinert, 1870)	2	il
	<i>Schendyla carniolensis</i> (Verhoeff, 1902)	30	en
	<i>Schendyla montana</i> Attems, 1895	1	mi
	<i>Strigamia acuminata</i> (Leach, 1815)	121	eu
	<i>Strigamia crassipes</i> (C. L. Koch, 1835)	30	pa
	<i>Strigamia transsilvanica</i> (Verhoeff, 1928)	77	il
Lithobiomorpha	<i>Eupolybothrus tridentinus</i> (Fanzago, 1874)	1067	se
	<i>Harpolithobius</i> cf. <i>anodus</i> (Latzel, 1880)	109	eu
	<i>Lithobius agilis</i> C. L. Koch, 1847	27	pa
	<i>Lithobius borealis</i> Meinert, 1868	1	pa
	<i>Lithobius castaneus</i> Newport, 1844	260	me?
	<i>Lithobius</i> cf. <i>cyrtopus</i> Latzel, 1880	1	se
	<i>Lithobius dentatus</i> C. L. Koch, 1884	126	pa
	<i>Lithobius forficatus</i> (Linne, 1758)	22	pa
	<i>Lithobius lapidicola</i> Meinert, 1872	16	pa
	<i>Lithobius latro</i> Meinert, 1872	15	se
	<i>Lithobius</i> cf. <i>melanops</i> Newport, 1845	3	pa
	<i>Lithobius</i> cf. <i>muticus</i> C. L. Koch, 1847	19	pa
	<i>Lithobius</i> sp. (cf. <i>silvagus</i>)	3	?
	<i>Lithobius nodulipes</i> Latzel, 1880	97	mi
	<i>Lithobius</i> cf. <i>pelidnus</i> Haase, 1880	4	mi
	<i>Lithobius pygmaeus</i> Latzel, 1880	42	il?
	<i>Lithobius</i> cf. <i>subtilis</i> Latzel, 1880	4	?
	<i>Lithobius tenebrosus</i> Meinert, 1872	3	eu
	<i>Lithobius validus</i> Meinert, 1872	57	mi
	<i>Lithobius</i> (M.) <i>aeruginosus</i> L. Koch, 1862	4	eu
	<i>Lithobius</i> (S.) n. sp. (<i>anici</i>)	5	?
	<i>Lithobius</i> (S.) <i>burzenlandicus carinthiacus</i> Koren, 1992	9	?
undet.	<i>Lithobius</i> juven.	15	
	<i>Lithobius</i> sp.	10	
	<i>Schendyla</i> sp.	1	
TOTAL		2367	

in this stand; GRGIČ 2005): 22 from the group Lithobiomorpha, 11 from Geophilomorpha and 4 from the group Scolopendromorpha (Tab. 1). In 11 species, more than 40 specimens were caught and represent 87.6% of the whole catch in traps. The most frequent were: *Eupolybothrus tridentinus*, *Harpolithobius anodus*, *Lithobius castaneus*, *Lithobius dentatus*, *Lithobius nodulipes*, *Lithobius validus*, *Strigamia acuminata* and *Strigamia transylvanica*. These are mostly well mobile, bodily bigger species, which body in adults is larger than 15 mm. The portion of juveniles in traps was very low. The most frequent were specimens of *Eupolybothrus tridentinus* that present 45% of the whole catch in traps. Specimens of this species are the biggest lithobiids found in investigated stand. We also caught some specimens of two for science new, not yet described species: *Lithobius* (*Sigibius*) n. sp. ("*anici*") and *Lithobius* (*L.*) n. sp. (cf. *silvivagus*).

The catch was highest in summer, and lowest in winter months (Fig. 2), but in each month some centipedes were caught. Some individuals were caught even in the period when snow cover was present all the time.

Traps with leading boards proved to be convenient for assessment of centipede migrations

(GRGIČ 2005). Species differ among each other in frequency, season and directions of migrations. It has been found that migrations of centipedes depend on period of the year, as well as on forest phase and environmental conditions. Differences in catch among months and among directions were found (GRGIČ & KOS, in preparation).

Discussion

The catch was highest in summer (Fig. 2), when centipedes most intensively walked on the surface. Centipedes are exothermic animals and thus active mostly in the warmer period of the year. But some individuals were caught even in the period when snow cover was present all the time. We assume that centipedes walk under the snow cover.

The main reason for high catch of most frequent species *Eupolybothrus tridentinus* in our study probably lays in large body size, walking abilities and suitability of the sampling method. We caught mainly bodily bigger centipedes, as traps are not convenient for catching small ones. For larger centipedes it is hard to avoid the vessel when they enter the trap area. But smaller centipedes

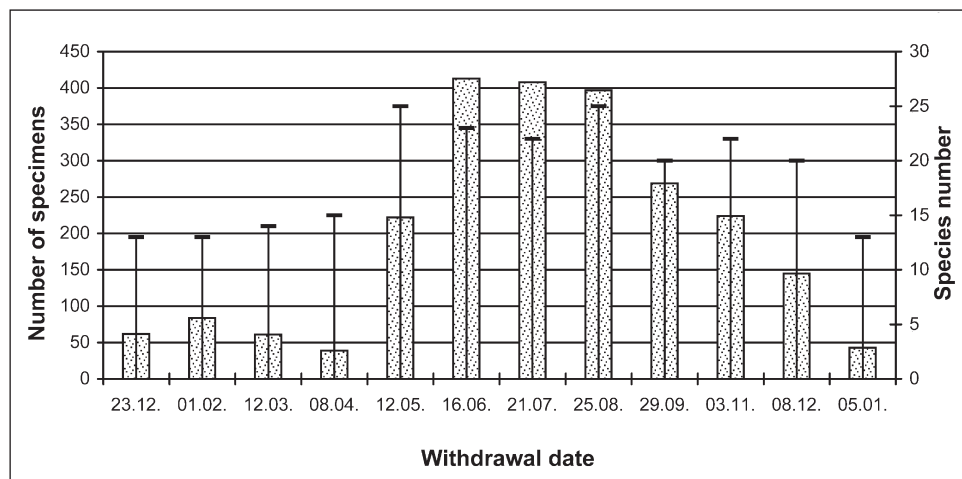


Fig. 2: The comparison of species number (black line) and specimens number (bars) of centipedes caught in pitfall traps with leading boards in a certain period.

Slika 2: Primerjava števila vrst (črne črte) in števila osebkov (stolpci) strig ujetih v talnih pasteh z vodili v določenem obdobju.

easily find a crack in the soil or between the soil and vessel and this way avoid the trap. In case that they come above the vessel on leaves or bark with which vessels were covered, small centipedes can walk back on the other side of leaves or bark and again avoid the vessel. Larger centipedes because of their body structure and weight in such case fall into the vessel. In our research vessels were under the surface level, in small cavities, so we caught also some representatives of species that live deeper in the soil. Plastic plates 20 cm deep in the soil also contributed to this. Low portion of juveniles shows that migrating individuals are mainly adults. Also FRÜND and co-workers (1997) already came to this conclusion.

According to BLOWER (1955) large lithobiids prefer litter layers whereas small lithobiids and geophilomorphs occur mostly in deeper layers. With traps mostly larger epigeic lithobiids that prefer litter layers can be caught. But for small centipedes that live deeper in the soil the method of soil samples is selective (KOS 1995a). FRÜND and co-workers (1997) also found great difference in catch between soil samples and pitfall traps. Species that are usually frequent in soil samples rarely appear in traps and vice versa (GRGIČ 2005).

Also LOCK and co-workers (2001) quote that with pitfalls as a rule only the epigeic species can be captured. TUF (in preparation) found that larger abundant lithobiomorphs with mainly epigeic life style can be recorded by several methods, but with higher probability by pitfall trapping, while larger less frequent lithobiomorphs can be recorded by pitfall trapping exclusively. Also smaller soil lithobiomorphs with low epigeic activity and abundant geophilomorphs can be often found in traps, but are mainly recorded by soil sampling. These statements correspond also to our species list from traps (Tab. 1).

Advantages and disadvantages of traps with leading boards

Pitfall traps with leading boards have their advantages, but also some weaknesses. Pitfall traps are one of the most effective methods for collecting walking centipedes and leading boards even increase the effect of catch in traps. At the same time these traps enable to determine the

walking direction of ground-dwelling arthropods (GRGIČ & KOS, in preparation). Traps can be installed throughout the year so that we can also determine yearly dynamics and compare activity among months or seasons.

Perhaps the main weakness is the trap's difficult installation. The appeal of pitfall trapping generally lies in the ease with which traps can be set and the replicability of trapping over space and time, but to place leading boards in very stony soil can be very hard and time consuming, as it is necessary to dig 3 meters of ditch for the boards. When the trap is already in place, big animals (e.g. bears, wild boars, badgers) can destroy or dig out both leading boards and vessels. This actually happened in few occasions during our investigation. Also if precipitation is high, the vessels can be full very quickly and for not losing the catch collecting must be done immediately.

Such traps can not give the information about species that live deeper in the soil or are too small to be caught in traps, as small specimens can easily avoid the trap. Unfortunately mainly surface-active species are caught by pitfall traps, so selecting animals of desired group can be long-lasting. The size of catch is affected by environmental factors (climate, microclimate, precipitation, temperature, type of soil, structure of soil, vegetation and others) and trap parameters. As the number of caught specimens doesn't depend only on density of species, therefore is not possible to estimate the density directly on the basis of catch in traps (PERNER & SCHUELER 2004). For this defined designs of sampling and mathematical models are needed.

Conclusion

Traps with leading boards proved to be suitable to establish active migrations of centipedes inside the stand (GRGIČ 2005). We found that pitfall traps with leading boards are one of the most effective collection methods for centipedes and they also enable to determine other centipede community characteristics. But with pitfall traps mostly epigeic species can be captured.

The centipede collection from a certain area in a great extent depends on sampling methods used. That is why in centipede community investigations

it is a must to exactly define the methods. The decision about which and how many sampling methods should be used in an investigation must be directly linked with the aim of the study, so it is necessary to know advantages and disadvantages of different methods and to know what kind of information can be expected from the catch with certain method.

Povzetek

Pri raziskavah združbe strig uporabljamo različne metode vzorčenja za zbiranje osebkov. Največkrat so uporabljene talne pasti, vzorčenje tal, sejanje tal in ročno pobiranje. Z različnimi metodami ujamemo različno velike in različno aktivne strige z različno telesno zgradbo in načinom življenja. Zato dobimo običajno z uporabo različnih metod vzorčenje različne predstave o zgradbi talnih združb na določenem mestu (Kos 1988, 1995a,b, MESIBOV in sod. 1995, GRGIČ & Kos, v pripravi).

Ker so različne metode različno učinkovite, idealna metoda za določeno raziskavo temelji direktno na ciljih raziskave (SNYDER in sod. 2006). V raziskavi aktivnih migracij strig med različnimi razvojnimi fazami gozda smo uporabili talne pasti z vodili, ki so opisane v tem prispevku. TUF (v pripravi) je ugotovil, da so talne pasti ena najučinkovitejših metod vzorčenja strig. FRÜND in sodelavci (1997) pa navajajo, da vodila še povečajo samo učinkovitost lova.

Talne pasti z vodili so nekoliko spremenjene in prilagojene pasti, ki so jih uporabljali FRÜND in sodelavci (1997) za lov strig. Pasti so sestavljene iz treh en meter dolgih in 30 cm širokih plastičnih plošč v obliki črke Z (Fig. 1). Približno 20 cm širine plošč je zakopanih v tleh, 10 cm pa je nad površino tal. Odprta kraka pasti sta bila obrnjena proti določenima fazama. V vsakem od dveh kotov je bila plastična lovilna posodica velikosti 8x8 cm, napolnjena z monoetilen glikolom, ki ubije in delno konzervira živali.

Na vsaki od petih meja med različnimi razvojnimi fazami je bilo postavljenih šest talnih pasti v vodili (vsaka past je imela 2 lovilni posodici). Jeseni leta 2002 je bilo postavljenih 30 pasti v raziskovanem raznomernem bukovem sestoju v vasi Iška, 15 km južno od Ljubljane. Novembra

leta 2002 smo začeli z lovom. Lovilne posodice smo praznili na 17–40 dni (odvisno od količine padavin) do januarja 2004.

Vodila so živali, ki so hodile v določeno smer, usmerjala v lovilne posodice, tako da smo lahko ločeno ujeli živali, ki so hodile v določeno smer med dvema fazama. Ujeli smo 2367 strig iz 37 vrst (od 44 vrst, ki so že bile najdene v raziskovanem sestoju, GRGIČ 2005): 22 iz skupine Lithobiomorpha, 11 iz skupine Geophilomorpha in 4 iz skupine Scolopendromorpha (Tab. 1). Najpogostejše sobile vrste: *Eupolybothrus tridentinus*, *Harpolithobius anodus*, *Lithobius castaneus*, *Lithobius dentatus*, *Lithobius nodulipes*, *Lithobius validus*, *Strigamia acuminata* in *Strigamia transsylvanica*. To so večinoma dobro mobilne strige, katerih telo pri odraslih je večje od 15 mm. Delež mladostnih osebkov v pasteh je bil majhen. Najpogostejša je bila vrsta *Eupolybothrus tridentinus*. Verjetno je glavni razlog za to velika telesna velikost, mobilnost in primernost metode vzorčenja. Ulov strig v pasteh je bil največji poleti in najnižji pozimi (Fig. 2), vendar noben mesec ni prišlo do izpada ulova strig. Strige so ektotermne živali in zato najbolj aktivne v toplem delu leta.

Lov strig s talnimi pastmi z vodili se je izkazal za primerno metodo vzorčenja za ocenjevanje aktivnih migracij strig (GRGIČ 2005). Vrste so se razlikovale v frekvenci, obdobju in smereh migracije. Ugotovljeno je bilo, da so migracije strig odvisne od sezone in strukture gozda. Najdene so bile razlike v migracijah strig med meseci in med smermi migracij (GRGIČ & Kos, v pripravi).

Metoda lova s talnimi pastmi z vodili ima določene prednosti, pa tudi nekatere slabosti. Je ena od najučinkovitejših metod lova talnih členonožcev, hkrati pa omogoča določitev smeri aktivnih migracij osebkov (GRGIČ & Kos, v pripravi). Pasti so lahko postavljene skozi vse leto, tako da lahko ugotavljamo letno dinamiko in primerjamo aktivnost v različnih delih leta. Verjetno glavna slabost teh pasti je težavnost postavitve na mesto vzorčenja, saj je treba izkopati tri metre dolg in 20 cm globok jarek za vodila, kar lahko pri zelo kamnitih tleh predstavlja veliko oviro. Ko so pasti postavljene, pa jih lahko velike živali (npr. medved) uničijo in izkopljejo tako vodila kot tudi lovilne posodice. Slabost teh pasti pa je tudi v tem, da nem ne morejo dati informacij o vrstah, ki živijo globlje v tleh ali so premajhne za lov s pastmi. Po

BLOWER-ju (1955) večji lithobiidi preferirajo sloj stelje, medtem ko se manjši lithobiidi in geophilidi pojavljajo večinoma v globljih plasteh. S pastmi tako večinoma ulovimo površinske lithobiide, za majhne strige v tleh pa je bolj primerna metoda lova talno vzorčenje (Kos 1995a). Na ulov v talnih pasteh z vodili vplivajo okoljski dejavniki (klima, mikroklima, padavine, temperatura, tip in zgradba tal, vegetacija in drugi).

Ker je vzorec strig z določenega vzorčnega mesta odvisen od uporabljene metode vzorčenja, je pri raziskavah združbe strig nujno treba natančno definirati uporabljeno metodo. Odločitev o metodi je povezana s cilji raziskave, zato je treba poznati prednosti in slabosti posamezne metode in vedeti, kakšne informacije lahko z njimi pridobimo.

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Bibliografija revije Acta Biologica Slovenica (1997–) prej Biološki vestnik (1952–1995)

The bibliography of the journal Acta Biologica Slovenica (1997–) formerly Biološki vestnik (1952–1995)

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Izvleček. Predstavljena je strokovna bibliografija revije Acta Biologica Slovenica prej Biološki vestnik. V bibliografski pregled je vključenih vseh 918 znanstvenih in strokovnih člankov, ki so bili objavljeni v obdobju od 1952 do 2008. Članke je napisalo 642 avtorjev iz 29 držav, pisali so v 6 jezikih. Citiranih je 15.584 enot virov uporabljene literature.

Ključne besede: Acta Biologica Slovenica, Biološki vestnik, strokovna bibliografija

Abstract: The bibliography of the journal Acta Biologica Slovenica formerly Biološki vestnik is presented in the article. Bibliographical review from 1952 to 2008 comprises 918 scientific and professional articles. 642 authors from 29 countries, writing in 6 languages contributed their articles to the journal. They cited 15.584 references.

Keywords: Acta Biologica Slovenica, Biološki vestnik, bibliography

Uvod

Slovenski biologi v letu 2009 praznujemo 57. obletnico izhajanja stanovske revije Biološki vestnik (BV) oziroma Acta Biologica Slovenica (ABS). Ob različnih tematskih številkah, ki predstavljajo razvoj in raziskovalce v biologiji, je to delo posvečeno pregledu znanstvenih in strokovnih objav, ki so izšle v BV in ABS.

Namen članka je predstaviti bibliografski pregled revije ABS prej BA od 1952 do 2008. V bibliografijo so vključeni znanstveni in strokovni članki, ki so bili objavljeni v omenjenem obdobju izhajanja BA in ABS. V dopolnitev bibliografske predstavitve so še zbrani podatki o obsegu člankov (število strani), o številu uporabljenih referenc (število uporabljene literature), o številu avtorjev, seznam držav iz katerih so avtorji objavljali, pregled jezikov v katerih so članki napisani in

indeksiranje v bazah podatkov ter odmevnost (citiranost) v Science Citation Index (SCI).

Material in metode

Gradivo zbrano v strokovno bibliografijo revije BV in njenem nadaljevanju ABS obsega vse članke od 1. letnika iz leta 1952 do 51. letnika, ki je izšel leta 2008.

Po tipologiji dokumentov/del za vodenje bibliografij v sistemu COBISS (www.cobiss.si) so vključeni: izvirni znanstveni članki (1.01), pregledni znanstveni članki (1.02), kratki znanstveni članki (1.03) in strokovni članki (1.04).

Bibliografski zapisi so oblikovani tako, da so navedeni nujni identifikacijski elementi za prepoznavanje bibliografskih enot (člankov). Vsi zapisi zbrani v bibliografiji so izčrpani iz primarnega dokumenta, tj. iz izvirnih zvezkov revije.

V bibliografijo niso vključena dela in prispevki, ki so bili objavljeni v stalnih ali občasnih rubrikah: *Predgovor*, kasneje tudi *Urednikov uvodnik*; *Jubileji*; *Odmevi* rubrika se je v 27. letniku (1979) preimenovala v *Razmišljanja in odmevi-Contemplations and responses*; *Drobni prispevki-Miscellany*; *Nove metode- New methods*; *Favna in flora*; *Beleške*; *Kronika in društvene vesti*, kjer so bila objavljena poročila in zapisniki sestankov, vabila na kongrese, tečaje ipd.; *Srečanja*; *Biologija v šolah*; *Iz bioloških ustanov*, ki se je začela v 3. številki 38. letnika (1990) in je izhajala do 4. številke 39. letnika (1991), v slednji rubriki so bili prispevki o delovanju takrat novega Biološkega središča; *Iz domačega in tujega slovstva*, rubrika se je preimenovala v *Prikaze knjig*; *AIK listki* (to je kartonček standardizirane velikosti z bibliografskim zapisom članka) so bili prvič objavljeni v 20. letniku (1972) BV, ta način zbiranja in urejanja informacij se je v BA ohranil do 30. letnika (1982), ko se je začel razvoj računalniško podprtega knjižničarstva v Sloveniji; *In memoriam*; *Vsebina*; *Našim sodelavcem*, ki se je preimenovala v *Navodila avtorjem*.

Na tem mestu izpostavljam, kot dokumenta časa, dva prispevka iz 15. letnika (1967), to sta *Bibliografija poljudnoznanstvenih in strokovnih knjig s področja biologije* avtorja Izidorja Koblarja na straneh 143–146; ter *Abecedni seznam znanstvenih časopisov, ki jih prejema knjižnica Inštituta za biologijo v zameno za Biološki vestnik* avtorice Marije Majcen, na straneh 147–149. V naslednjem letniku je ista avtorica, Marija Majcen, objavila *Seznam kupljenih znanstvenih in strokovnih časopisov Knjižnice Inštituta za biologijo*. Iz teh treh člankov je v 20. letniku (1972) bila vpeljana rubrika *Centralna biološka knjižnica je leta ... prejela*, ki se je obdržala do 22. letnika (1974).

Opis vsebine in struktura bibliografskega zapisa za posamezni članek je prilagojena širšemu krogu uporabnikov, kar pomeni, da zapisi niso strogo v skladu s pravili in standardi za bibliografske zapise. Kljub temu so ohranjeni vsi elementi potrebni za identifikacijo posameznega članka, ti so:

- zaporedna številka bibliografskega zapisa;
- priimek in ime prvega avtorja (oziroma edinega) sledijo imena s priimki vseh nadaljnjih avtorjev, ki so med seboj ločeni z vejicami;

- naslov članka in vzporedni naslov v tujem jeziku in podnaslov (če so navedeni);
- letnica izida;
- skrajšan naslov revije: BV za Biološki vestnik in ABS za Acta Biologica Slovenica;
- letnik revije;
- številka v letniku; kjer je bila le ena številka na letnik je povzeta številka 1; če je izšla dvojna številka v enem zvezku je označeno z 2/3;
- strani od – do obsega članka; ponekod so dodane še oznake za priloge (zemljevidi, vegetacijski popisi, skice in tabele) in
- COBISS ID številka, ki omogoča povezavo z Vzajemno bibliografsko-kataložno bazo podatkov (COBIB.SI), v katero so vneseni vsi članki iz BV in ABS.

Klasificiranje in urejanje bibliografskih zapisov je urejeno s pomočjo računalniškega programa COBISS (Kooperativni Online Bibliografski Sistem in Servis, spletni naslov: www.cobis.si; Vzajemna katalogizacija/Izpisi). Program omogoča izpis bibliografskih enot, torej člankov, po abecednem redu priimkov prvih avtorjev. Zaradi bibliotekarskega pravila, da se objave z več kot tremi avtorji, razvršča med abecedni red avtorjev po začetnici iz naslova, zato izpis ni povsem dosleden. Zato so bibliografski zapisi s tremi ali več avtorji razvrščeni po abecednem redu naslovov. Bibliografija je opremljena s kazalom avtorjev, kjer so po strogem abecednem redu navedeni vsi avtorji (vključno s sinonimi in dvojnimi priimki, kjer so mi bili poznani) in opremljeni z zaporedno številko bibliografskega zapisa.

Predmetno kazalo in kazalo naslovov prav tako sodita v standardni prikaz bibliografije, vendar jih tukaj posebej ne predstavljam. Vsi obravnavani članki so vneseni v COBISS in so dosegljivi po svetovnem spletu.

Rezultati

Z izdajanjem strokovnega in znanstvenega glasila BV je v letu 1952 začela Biološka sekcija Prirodoslovnega društva Slovenije, izdala je 16 letnikov. Leta 1968 je izdajateljstvo prevzelo Društvo biologov Slovenije, ki ga izdaja še sedaj.

V 57 letih izhajanja BV in ABS je izšlo 51 letnikov (volumnov) v obsegu 97 rednih in ena posebna številka, torej skupno 98 zvezkov. Do

20. letnika, ki je izšel leta 1972 so izhajale po ena številka na letnik. V obdobju od 1973 do 1987 sta izhajali po 2 številki na letnik, v obdobju 1988 – 1990 so izhajale po 4 številke na letnik. Z letom 1991 so se začele težave s kontinuiteto v izhajanju. Začele so izhajati dvojne številke s 3 zvezki na letnik. V letih od 1991 do 1997 so izšli le trije letniki. Po letu 2002 spet izhajata po dve številki in dva zvezka na letnik.

Bibliografija BV in ABS obsega 918 znanstvenih in strokovnih člankov, tipologije 1.01 izvirni znanstveni članek, 1.02 pregledni znanstveni članek, 1.03 kratki znanstveni članek ter 1.04 strokovni članek (Priloga 1). Obravnavani članki so natisnjeni na skupno 9.317 straneh. Razpon strani posameznih člankov je od 2 do 239 strani, v povprečju obsega članek 10,1 strani, z mediano 9 strani na članek.

918 znanstvenih in strokovnih člankov je ustvarilo 642 posameznikov, vseh avtorstev s ponovitvami vred pa je 1.519 (Priloga 2). Posamezni članek je v povprečju napisalo 1,7 avtorja, mediana 1. Število avtorjev na članek je v razponu od 1 do 29 avtorjev.

Iz poštnega naslova avtorja oziroma avtorjev članka sem razporedila avtorje po državah, pri tem me ni zanimalo dejansko državljanstvo ali narodnost posameznega pisca temveč institucija oziroma domač naslov, ki ga je navedel avtor v članku. Vseh 1.519 avtorjev izvira iz 29 držav Evrope, Amerike, Avstralije in Azije. Največ avtorjev 1.230, kar je 80,7 %, izvira iz Slovenije. Po številu sledijo avtorji iz: Hrvaške, Srbije in Avstrije (Tabela 1).

Članki so pisani v 6 različnih jezikih. Prevladujoči jezik je pričakovano slovenščina v kateri je napisano 540 člankov, oz. 58,8 % vseh. Po številu objav v katerem jeziku je članek, si sledijo: angleščina s 304 članki, oz. 33,11 %; nemščina s 43 člankov, oz. 4,6 %; hrvaščina z 18 članki, oz. 1,9 %; francoščina s 7 članki, oz. 0,7 %; v srbsčini je napisan en članek, oz. 0,1 %; 5 člankov pa je dvojezičnih v slovenščini in angleščini oz. 0,54 %.

Avtorji so v 918 člankih BV in ABS citirali 15.584 enot virov literature. V povprečju je v posameznem članku 17,2 citiranih virov z mediano 14, razpon virov pa je od 0 do 221 enot.

Mednarodno odmevnost BV oz. ABS sem ugotavljala glede na indeksacijo in citiranost v

mednarodnih bazah podatkov. Revija je indeksirana samo v Biological Abstract in Zoological Records. Citiranost posameznih člankov BV in ABS sem preverjala v Web of Science, v bazi SCI je bilo v juniju 2009 skupno 51 citatov 30 različnih člankov. Najbolj citirana avtorji BV so Andrej Martinčič, Janez Matjašič ter Boris Sket. Žal pa novejših citatov z navedbo ABS še ni.

Diskusija

Biološki vestnik, iz predgovora v 1. letniku Biološkega vestnika:

»Biološki vestnik« je znanstveno in strokovno glasilo Biološke sekcije Prirodoslovnega društva v Ljubljani.

List bo objavljal izvirne razprave in članke s povzetkom v katerem od svetovnih jezikov, poročila iz domače in tuje strokovne literature, novosti z bioloških področij, poročila o kongresih, zborovanjih, društvene vesti in podobno.

Posebna pozornost bo posvečena našim biologom, ki so izven inštitutov; te bo vestnik povezal z znanstvenimi ustanovami v večjih centrih in jim tako omogočil spremljanje znanosti v svetu, strokovni dvig in kvalitetnejše delo na terenu.

Važna naloga Biološkega vestnika in njegovih sodelavcev je predvsem boj za znanstveno resnico proti vsem neznanstvenim idealističnim pojmovanjem v biologiji. Z boljšo strokovno vzgojo kadrov bo tudi širokim ljudskim množicam pripomogel h kulturnemu dvigu.

Prvi urednik BV je bil Viktor Petkovšek, pri urednikovanju mu je pomagal uredniški odbor v sestavi: Jovan Hadži, Hubert Pehani, Peter Us, Maks Wraber in Miroslav Zei. Prvi članek BV je *Nauk o celicah nekoč in danes* avtorja Jovana Hadžija in je skrajšano predavanje, ki ga je avtor imel 24. novembra 1951 v Biološki sekciji Prirodoslovnega društva. Uredništvo ga je sprejelo v objavo 26. maja 1952. Članek je razdeljen na »poglavja« označena z rimskimi številkami, opremljen je s povzetkom v nemščini in s seznamom uporabljene literature označene s *Slovstvo*.

V prvi številki je objavljenih 13 člankov. Sledi jim rubrika *Iz domačega in tujega slovstva*, ker so sezname objavljene domače zoološke literature po letu 1945 in še predstavitev treh knjig. Nadaljuje se z rubriko *Biologija v šolah*, ki se začinja s

seznamom poklicnih biologov v LR Sloveniji in s predstavitvami 3 učbenikov. Prva številka se nadaljuje z rubriko *Kronika in društvene vesti*, kjer so predstavljene naloge in delo Biološke sekcije Prirodoslovnega društva. Zadnji prispevek je obvestilo o Kongresu biologov Jugoslavije, na notranji strani platnice pa je natisnjeno navodilo avtorjem z naslovom *Našim sodelavcem*.

Kot vsak dokument časa je tudi prva številka BV izredna. S prebiranjem člankov je opazen drugačen jezikovni slog, kot ga imajo znanstveni in strokovni članki danes. S čemer se nakazuje pozitiven razvoj ne le znanstvene in strokovne discipline, ampak tudi razvoj znanstvenega in strokovnega slovenskega jezika.

Izpostavljam še 40., zadnji, letnik BA, ki je s številko 1 izšel leta 1992 in se zaključil z dvojno številko 3/4, ki je izšla leta 1995. Ta letnik sestavljajo 3 zvezki, ki so izšli s precejšnjim časovnim zamikom. S tem letnikom se tudi izteka ime BV.

V štirih desetletjih izhajanja revije z imenom BV je bilo objavljenih 749 člankov.

Acta biologica slovenica, iz *Uvodnika* k 1. številki 41. letnika, ki je izšel leta 1997:

»Društvo biologov Slovenije je več kot štiri-deset let izdajalo Biološki vestnik, ki je objavljaj znanstvene in strokovne članke pretežno domačih avtorjev. Tiskane prispevke so spremljali tudi po svetu, odmevna je bila tudi citiranost. V obdobju zadnjih nekaj let je izdajateljska aktivnost precej zamrla, vzroki so bili različni, nekateri tudi opravičeni. Potreben je bil nov zagon.

Leta 1995 smo izbrali nov uredniški odbor, ki ga je potrdilo tudi Društvo biologov Slovenije. Pod starim imenom smo izdali še štiri številke 40. zvezka, določili pa tudi že novo ime *Acta Biologica Slovenica* (ABS). K temu nas je med drugim vodila misel, da bo novo ime sprejemljivejše za tujino. K sodelovanju smo povabili tuje strokovnjake različnih področij biologije, nekateri so člani uredniškega odbora, drugi možni recenzenti. Glasilo bo s tem pridobilo na vrednosti, saj vodilni domači in tuji sodelavci zagotavljajo kvaliteto publiciranja...«.

Uvodnik je napisal prvi glavni in odgovorni urednik ABS Mihael Jožef Toman. Uredniški odbor so sestavljali: Branko Vreš (Tehnični urednik), Peter C. Dall, Matija Gogala, Nada Gogala, Peter Maček, Alenka Malej, Andrej Martinčič, Harald

Niklfeld, Livio Poldini, Boris Sket, Robert Zorec, Mitja Zupančič. V prvem zvezku ABS je bilo objavljenih 5 člankov, Urednikov uvodnik in v rubriki Jubileji prispevek: *Zoolog in speleobiolog Boris Sket, šestdesetletnik* v avtorstvu Kazimirja Tarmana na straneh 55–57 in z obsežno bibliografijo jubilaranta. V prvem letniku ABS je v četrti številki izšel jubilejni članek: *Šestdesetletnica prof. dr. Nada Gogala* v avtorstvu Maje Kovač na straneh 69–74 tudi z obsežno bibliografijo. V tej številki je bila še objavljena rubrika Nove knjige. 43. letnik številka 2/3 je bila izdana kot tematska številka – *Zbornik iz 3. srečanja slovenskega biokemijskega društva z mednarodno udeležbo*, Portorož, 25. – 29. september 1999. Uredila sta ga: Tamara Lah in Tom Turk. V nadaljnjih številkah so objavljeni še nekateri življenjski jubileji, obletnice institucij in žal tudi spomini na preminule.

V desetletju in pol izhajanja je ABS objavil 169 člankov.

Povzetek

V delu so predstavljene znanstvene in strokovne objave člankov (strokovna bibliografija) revije Biološki vestnik oziroma *Acta Biologica Slovenica*. V 57 letih izhajanja revije BV oz. ABS je izšlo 918 znanstvenih in strokovnih člankov, ki smo jih uredili v bibliografijo. Za izpis bibliografije smo uporabili programsko opremo COBISS (Kooperativni Online Bibliografski Sistem in Servis segment Vzajemna katalogizacija/Izpis). Revijo je začela izdajati Biološka sekcija Prirodoslovnega društva v letu 1952. Od 1968. leta naprej je izdajateljstvo prevzelo Društvo biologov Slovenije.

V 57 letih izhajanja revije je izšlo 51 letnikov v obsegu 97 rednih in ene posebne številke, torej 98 zvezkov. Članki so natisnjeni na 9.317 straneh. Razpon strani posameznih člankov je od 2 do 239 strani, v povprečju obsega članek 10,1 strani, z mediano 9 strani na članek.

Članke je napisalo 1.519 avtorjev, dejanskih piscev člankov brez ponovitev pa je 642. Posamezni članek je v povprečju napisalo 1,7 avtorja (mediana 1). Število avtorjev na članek je v razponu od 1 do 29 avtorjev. Avtorji člankov delujejo v 29 državah. Največ avtorjev 1.230 (80,7 %) izhaja iz Slovenije. Po številu avtorjev sledijo: Hrvaška, Srbija in Avstrija. Članki so napisani v 6 jezikih,

največ 540 (58,8 %) v slovenščini sledijo pa angleščina, nemščina, hrvaščina, francoščina ter srbščina.

V obravnavanih člankov so avtorji uporabili 15.584 enot virov literature. V povprečju je v posameznem članku 17,2 citiranih virov (mediana 14), razpon virov pa je od 0 do 221 enot.

Revija ABS prej BV je indeksirana v Biological Abstract in Zoological Records. V Web of Science, v bazi Science Citation Index je bilo v juniju 2009 skupno 51 citatov 30 različnih člankov BV in ABS.

Summary

The article summarises the scientific and professional articles (special bibliography) published in Biološki vestnik (BV) formerly Acta Biologica Slovenica (ABS). In 57 years, 918 scientific and professional articles were published. We used COBISS (Co-operative Online Bibliographic System & Services segment shared cataloguing/print) for extracting the bibliography.

In 1952 the Biology section of *Natural History Society of Slovenia* started publishing the journal. The Biological Society of Slovenia took over the publishing in 1968.

51 volumes of 97 regular and one special number (together 98 numbers) were published in 57 years of publishing. The articles are printed on 9,317 pages. The range of individual articles is from 2 to 239 pages, the average length of the article is 10.1 pages (median 9 pages) per article.

Altogether 1.519 authors wrote these articles. Taking into account different articles of the same author, this is 642 different authors. 1.7 authors wrote only one article (median 1). The number of authors per article ranges from 1 to 29 authors. The authors of these articles work in 29 countries. The majority of authors are from Slovenia (80.7%), followed by Croatia, Serbia and Austria. The articles are written in 6 languages, mostly (540 or 58.8%) in Slovene, followed by English, German, Croatian, French and Serbian language.

The authors of all discussed articles used 15,584 units of bibliography. In average there are 17.2 cited references in the individual article (median 14), the references range from 0 to 221 units.

The journal ABS formerly BV is indexed in Biological Abstract and Zoological Records. In Web of Science, in the base Science Citation Index there were 51 citations from 30 different articles of BV in ABS in June 2009.

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Tabela 1: Število avtorjev člankov iz različnih držav v reviji Acta biologica Slovenica prej Biološki vestnik.

Table 1: The number of authors of the articles from different countries in Acta Biologica Slovenica formerly Biološki vestnik.

Zap. št.	Država avtorja	Število avtorjev	Delež avtorjev
1.	Slovenija	1230	80,7
2.	Hrvaška	78	5,1
3.	Srbija	31	2,1
4.	Avstrija	30	2,0
5.	Italija	18	1,2
6.	Francija	14	0,9
7.	Madžarska	13	0,9
8.	Združene države Amerike	13	0,9
9.	Češka	11	0,7
10.	Bosna in Hercegovina	10	0,7
11.	Velika Britanija	10	0,7
12.	Nemčija	8	0,5
13.	Švica	8	0,5
14.	Nizozemska	7	0,5
15.	Slovaška	7	0,5
16.	Makedonija	6	0,4
17.	Avstralija	5	0,5
18.	Črna gora	5	0,5
19.	Belgija	3	0,2
20.	Kosovo	3	0,3
21.	Kanada	3	0,3
22.	Romunija	3	0,3
23.	Jugoslavija	2	0,1
24.	Španija	2	0,1
25.	Poljska	1	0,1
26.	Venezuela	1	0,1
27.	Kuba	1	0,1
28.	Japonska	1	0,1
29.	Liechtenstein	1	0,1

**Priloga 1: Bibliografija 918 znanstvenih in strokovnih člankov,
volumna od 1 do 51, revije Acta biologica Slovenica prej Biološki vestnik.**

Supplement 1: The bibliography of 918 scientific and professional articles from
1st to 51st volume of Acta Biologica Slovenica formerly Biološki vestnik.

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3. Accetto, Marko 1973: Prispevek k poznavanju razširjenosti vrste *Gagea spathacea* v Sloveniji. BV 21 (2): 111–115. COBISS.SI-ID 6187565
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NAVODILA AVTORJEM

1. Vrste prispevkov

ZNANSTVENI ČLANEK je celovit opis originalne raziskave in vključuje teoretični pregled tematike, podrobno predstavljene rezultate z diskusijo in sklepe ter literaturni pregled: shema IMRAD (Introduction, Methods, Results And Discussion). ABS v tej kategoriji objavlja tudi metodološke članke, v kolikor gre za izvirno metodo, ki še ni bila objavljena drugje ali pa gre za nov in izvirni način uporabe sicer že znane metode. Oceno o izvirnosti sprejme uredništvo, če je potrebno po posvetu z recenzenti. Priporočena dolžina članka je do 15 strani s dvojnimi razmikom vrstic vključno s tabelami in slikami. Recenzirata ga dva recenzenta.

PREGLEDNI ČLANEK objavi revija po posvetu uredniškega odbora z avtorjem. Število strani je lahko večje od 15.

KRATKA NOTICA je originalni prispevek z različnih bioloških področij (sistematike, biokemije, genetike, fiziologije, mikrobiologije, ekologije itd.), ki ne vsebuje podrobnega teoretičnega pregleda. Njen namen je seznaniti bralca s preliminarnimi ali delnimi rezultati raziskave. Dolžina naj ne presega 5 strani. Recenzira ga en recenzent.

KONGRESNA VEST seznaja bralce z vsebinami in sklepi pomembnih kongresov in posvetovanj doma in v tujini.

DRUŠTVENA VEST poroča o delovanju slovenskih bioloških društev.

2. Originalnost prispevka

Članek, objavljen v reviji Acta Biologica Slovenica, ne sme biti predhodno objavljen v drugih revijah ali kongresnih knjigah.

3. Jezik

Besedila naj bodo pisana v angleškem jeziku, izjemoma v slovenskem, če je tematika zelo lokalna. Kongresne in društvene vesti so praviloma v slovenskem jeziku.

4. Naslov prispevka

Naslov mora biti kratek, informativen in razumljiv. Napisan mora biti v angleškem in slovenskem jeziku. Za naslovom sledijo imena avtorjev in njihovi polni naslovi (če je le mogoče, tudi številka faxa in/ali e-pošta). Jasno mora biti označeno, kdo je korespondenčni avtor in k kateremu avtorju spada kateri naslov, če je naslovov več.

5. Izvleček – Abstract

Podati mora jedrnat informacijo o namenu, uporabljenih metodah, dobljenih rezultatih in zaključkih. Dolžina za znanstveni članek naj bo do 250 besed, za kratko notico pa 100 besed. Članek mora imeti izvleček napisan tako v angleščini kot v slovenščini.

6. Ključne besede – Keywords

Število naj ne presega 10 besed, predstavljati morajo področje raziskave, predstavljene v članku. Člankom v slovenskem jeziku morajo avtorji dodati ključne besede v angleškem jeziku.

7. Tekoči naslov – Running title

Krajša verzija naslova, ki naj nima več kot 60 znakov s presledki

8. Uvod

Nanašati se mora le na tematiko, ki je predstavljena v članku ali kratki notici.

9. Slike in tabele

Tabele in slike (grafi, dendrogrami, risbe, fotografije idr.) naj v članku ne presegajo števila 10, v članku naj bo njihovo mesto nedvoumno označeno. Ves slikovni material naj bo v elektronski obliki. Tabele naj bodo tipkane na posebnih straneh (v tabelah naj bodo le vodoravne črte). Naslovi tabel in slik ter legende so v slovenskem in angleškem jeziku. Pri citiranju tabel in slik v besedilu uporabljamo okrajšave (npr. Tab. 1 ali Tabs. 1–2, Fig. 1 ali Figs. 1–2; Tab. 1 in Sl. 1). V naslovu legende uporabimo polno ime (npr. **Figure 1**, **Table 2** itd.) pisano krepko, ki mu sledi kratek naslov slike ali tabele prav tako pisan krepko. Če ima slika več panelov, morajo biti le-ti nedvoumno označeni z velikimi tiskanimi črkami (A, B, ...). V legendi mora biti vsak del slike pojasnjen v abecednem vrstnem redu. Pojasnilo za vsak panel se začne s krepko veliko tiskano črko (A), pomišljajem in nato nadaljuje z besedilom.

10. Kvaliteta slikovnega materiala.

S prvo številko 53. letnika ABS prehaja na povsem elektronski način obdelave slikovnega materiala. Vse slike je tako potrebno poslati izključno v elektronski obliki. Vse slike v reviji so bodisi popolnoma črno-bele bodisi v sivinah (ang. halftone). Avtorje naprošamo, da že v osnovi slike pripravijo v pravilni obliki in se s tem izognejo nepotrebnim zamudam pri pripravi za tisk, predvsem zaradi morebitnih problemov s kontrasti in ločljivostjo. Jasnost in ločljivost na slikah in grafih predstavljenih informacij je odgovornost avtorja. Uredništvo si pridržuje pravico, da zavrne nejasne in slabo berljive slike in grafične prikaze. Ločljivost slik s sivinami mora biti najmanj 300 d.p.i., za popolnoma črno-bele pa 600 d.p.i. Najmanjše črke in številke na sliki ne smejo biti manjše od 8 pik (višina 2 mm). Debelina črt naj ne bo tanjša od 0,5 pike. Družine pisav, ki so dovoljene na slikah, so Times, Times New Roman, Helvetica in Arial, pri čemer naj bodo vse slike v posameznem članku opremljene z istim tipom pisave. Slike naj bodo pripravljene v TIFF, EPS ali PDF formatu, pri čemer je najprimernejši TIFF format (končnica *.tif). Pri shranjevanju slik v *.tif datoteke avtorjem predlagamo uporabo LZW ali ZIP kompresije za zmanjšanje velikosti datotek. Za fotografije je sprejemljiv tudi JPEG format (končnica *.jpg) z nizko stopnjo kompresije, pri čemer si uredništvo prav tako pridržuje pravico zavrnitve fotografij slabe kvalitete. Preden pošljete sliko v EPS formatu se prepričajte, da so vsi znaki v njej zapisani pravilno (npr. odprite in si oglejte datoteko s programom Ghostview oz. GSview – odvisno od operacijskega sistema) ali s programom Adobe Photoshop). Pri formatu PDF se prepričajte, da ste za pripravo *.pdf datoteke uporabili kompresijo, ki ne spreminja njene vsebine (primeri sta LZW ali ZIP, neprimerna pa JPEG, ki je sicer privzeta nastavitvev). Slik narejenih v programih Microsoft Word, Excel, PowerPoint ipd. brez pretvorbe v enega od zgoraj navedenih formatov ne bomo sprejeli v tisk, enako velja za slike iz drugih grafičnih programov (Corel Draw, Adobe Illustrator, ipd.). Slike naj bodo pripravljene v končni velikosti, ki bo objavljena v reviji. Širina slike je lahko največ 12,5 cm, višina pa 19 cm (širina in višina besedila na strani).

11. Zaključki

Članek končamo s povzetkom glavnih ugotovitev, ki jih lahko zapišemo tudi po točkah.

12. Povzetek – Summary

Članek, ki je pisan v slovenskem jeziku, mora vsebovati še obširnejši angleški povzetek. Velja tudi obratno.

13. Literatura

S prvo številko 53-ega letnika ABS prehaja na nov, poenostavljen način citiranja. Pomembne razlike so:

Ni več uporabe malih velikih črk (»small caps«).

V besedilu uporaba 'and' namesto '&' pri dveh avtorjih in 'et al.' namesto '& al.' pri več avtorjih.

Pri citiranju člankov v besedilu v slovenskem jeziku se 'in' uporablja namesto 'and', 'in sod.' pa namesto 'et al.'.

V seznamu literature si pri vseh avtorjih sledijo priimek in začetnica oz. začetnice imen.

Uporabljene literaturne vire citiramo med tekstom. Če citiramo enega avtorja, pišemo Allan (1995) ali (Allan 1995), če sta dva avtorja (Trinajstić and Franjić 1994) oziroma pri članku v slovenščini (Trinajstić in Franjić 1994), če je več avtorjev (Pullin et al. 1995) oziroma v slovenščini (Pullin in sod. 1995). Kadar navajamo citat iz večih del hkrati, pišemo (Honsig-Erlenburg et al. 1992, Ward 1994a, Allan 1995, Pullin et al. 1995). V primeru, če citiramo več del istega avtorja, objavljenih v enem letu, posamezno del označimo s črkami a, b, c itd. (Ward 1994a,b). Če navajamo dobesedni citat, označimo dodatno še strani: Toman (1992: 5) ali (Toman 1992: 5–6). Seznam literature uredimo po abecednem redu. Vsak zapis začnemo s priimkom prvega avtorja, vejico ter začetnico(ami) imen(a) in nadaljujemo na enak način s preostalimi avtorji ločenimi z vejicami. Sledi leto izdaje in naslov članka, mednarodna kratica za revijo (časopis), volumen, številka v oklepaju (neobvezno) in strani po naslednjem vzorcu:

Mielke, M.S., Almeida, A.A.F., Gomes, F.P., Aguilar, M.A.G., Mangabeira, P.A.O., 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. *Experimental Botany*, 50 (1), 221–231.

Knjige, poglavja iz knjig, poročila, kongresne povzetke citiramo sledeče:

Allan J. D. 1995: *Stream Ecology. Structure and Function of Running Waters*, 1st ed. Chapman & Hall, London, 388 pp.

Pullin A. S., McLean I. F. G., Webb M. R., 1995. *Ecology and Conservation of Lycaena dispar*: British and European Perspectives. In: Pullin A. S. (ed.): *Ecology and Conservation of Butterflies*, 1st ed. Chapman & Hall, London, pp. 150–164.

Toman M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1–7.

14. Format in oblika članka

Članke sprejemamo izključno v elektronski obliki. Format naj bo Microsoft Word (*.doc) ali obogateno besedilo (*.rtf) v pisavi Times New Roman 12. Med vrsticami naj bo dvojni razmak, besedilo naj bo poravnano levo, robovi besedila naj bodo 3 cm na vseh straneh, format papirja naj bo A4. Odstavki naj bodo med seboj ločeni s prazno vrstico. Naslov članka in naslovi poglavij naj bodo pisani krepko in v velikosti pisave 14 prav tako v pisavi Times New Roman. Morebitni naslovi podpoglavij naj bodo pisani v velikosti 12 ležeče. Vsa latinska imena morajo biti napisana ležeče. Uporabljene nomenklature vire navedemo v poglavju Metode. Glavnemu uredniku je potrebno oddati besedilo in ves slikovni material kot priponko elektronske pošte. Za namene recenzije so slike in tabele vključene v glavni *.doc ali *.rtf file (vsaka na svoji strani). Vseeno pa je ob oddaji rokopisa potrebno kot ločene priponke poslati tudi slike v obliki opisani pod točko 10. Vse strani (vključno s tabelami in slikami) morajo biti oštevilčene. Vse članke je potrebno pregledati glede strokovnih in jezikovnih napak pred pošiljanjem.

Kontrolni seznam elementov članka v angleškem jeziku (Za članek v slovenskem jeziku se smiselno uporablja enaka shema z zrcalnim zaporedjem slovenskih in angleških delov):

Angleški naslov – (Times New Roman 14, krepko)

Slovenski naslov – (Times New Roman 14, krepko)

Imena avtorjev, kjer morajo biti jasno označeni naslovi oz. pripadnost instituciji in ime korespondenčnega avtorja – (Times New Roman 12)

Naslov(i) avtor(jev) / naslovi institucij – (Times New Roman 12)

fax in/ali e-poštni naslov korespondenčnega avtorja – (Times New Roman 12)

Ključne besede v angleščini (keywords) – (Times New Roman 12)

Ključne besede v slovenščini – (Times New Roman 12)

Tekoč naslov (running title) – (Times New Roman 12)

Abstract v angleščini – (Times New Roman 12)

Izvleček v slovenščini – (Times New Roman 12)

Uvod – (Times New Roman 12, naslov – Times New Roman 14 krepko)

Material in metode – (Times New Roman 12, naslov – Times New Roman 14 krepko)

Rezultati – (Times New Roman 12, naslov – Times New Roman 14 krepko)

Diskusija – (Times New Roman 12, naslov – Times New Roman 14 krepko)

Povzetek v slovenščini – (Times New Roman 12, naslov – Times New Roman 14 krepko)

Legende slik; vsaka v angleščini in v slovenščini – (Times New Roman 12, naslov – Times New Roman 14 krepko,

Oznaka za posamezno sliko in naslov slike – Times New Roman 12 krepko)

Legende tabel; vsaka v angleščini in v slovenščini – (Times New Roman 12, naslov – Times New Roman 14

krepko, Oznaka za posamezno tabelo in naslov slike – Times New Roman 12 krepko)

Zahvale (Times New Roman 12, naslov – Times New Roman 14 krepko)

Literatura (Times New Roman 12, naslov – Times New Roman 14 krepko)

Slike, ena na stran; oznaka slik levo zgoraj (Times New Roman 12 krepko)

Tabele, ena na stran; oznaka tabele levo zgoraj – (Times New Roman 12 krepko)

Oštevilčenje vseh strani – desno spodaj – (Times New Roman 12)

15. Recenzije

Vsak znanstveni članek bosta recenzirala dva recenzenta (en domači in en tuji), kratko notico pa domači recenzent. Pri člankih z izrazito lokalno tematiko, ki so izjemoma pisani v slovenskem jeziku, sta oba recenzenta domača. Avtor mora v spremnem dopisu, ki mora obvezno spremljati rokopis, predlagati najmanj enega tujega in enega domačega recenzenta. Končna izbira recenzentov je kljub vsemu diskrecijska pravica uredništva. Recenzenti ostanejo za avtorje anonimni. Možno izidi recenzij so 1. Sprejeto brez pripomb, 2. V osnovi sprejeto a potrebuje manjše spremembe, 3. V osnovi sprejeto a potrebuje pomembnejše spremembe, 4. Sprejemljivo a potrebuje večjo predelavo, 5. Nesprejemljivo v predloženi obliki. V primeru ocen 3 in 4 pred sprejetjem v tisk ustreznost popravkov potrdijo še enkrat recenzenti, ki so jih zahtevali. V primeru zavrnitve korespondenčni avtor prejme pisno negativno odločitev glavnega urednika, originalne materiale se izbriše iz arhiva ABS na posebno zahtevo pa se jih tudi vrne avtorju. Po objavi korespondenčni avtor prejme članek v elektronski *.pdf obliki.

INSTRUCTIONS FOR AUTHORS

1. Types of Articles

SCIENTIFIC ARTICLES are comprehensive descriptions of original research and include a theoretical survey of the topic, a detailed presentation of results with discussion and conclusion, and a bibliography according to the IMRAD outline (Introduction, Methods, Results, and Discussion). In this category ABS also publishes methodological articles, in so far as they present an original method, which was not previously published elsewhere, or they present a new and original usage of an established method. The originality is judged by the editorial board if necessary after a consultation with the referees. The recommended length of an article including tables, graphs, and illustrations is up to fifteen (15) pages; lines must be double-spaced. Scientific articles shall be subject to peer review by two experts in the field.

REVIEW ARTICLES will be published in the journal after consultation between the editorial board and the author. Review articles may be longer than fifteen (15) pages.

BRIEF NOTES are original articles from various biological fields (systematics, biochemistry, genetics, physiology, microbiology, ecology, etc.) that do not include a detailed theoretical discussion. Their aim is to acquaint readers with preliminary or partial results of research. They should not be longer than five (5) pages. Brief note articles shall be subject to peer review by one expert in the field.

CONGRESS NEWS acquaints readers with the content and conclusions of important congresses and seminars at home and abroad.

ASSOCIATION NEWS reports on the work of Slovene biology associations.

2. Originality of Articles

Manuscripts submitted for publication in *Acta Biologica Slovenica* should not contain previously published material and should not be under consideration for publication elsewhere.

3. Language

Articles and notes should be submitted in English, or as an exception in Slovene if the topic is very local. As a rule, congress and association news will appear in Slovene.

4. Titles of Articles

Titles must be short, informative, and understandable. It must be written in English and in Slovene language. The title should be followed by the name and full address of the authors (and if possible, fax number and/or e-mail address). The affiliation and address of each author should be clearly marked as well as who is the corresponding author.

5. Abstract

The abstract must give concise information about the objective, the methods used, the results obtained, and the conclusions. The suitable length for scientific articles is up to 250 words, and for brief note articles, 100 words. Article must have an abstract in both English and Slovene.

6. Keywords

There should be no more than ten (10) keywords; they must reflect the field of research covered in the article. Authors must add keywords in English to articles written in Slovene.

7. Running title

This is a shorter version of the title that should contain no more than 60 characters with spaces.

8. Introduction

The introduction must refer only to topics presented in the article or brief note.

9. Illustrations and Tables

Articles should not contain more than ten (10) illustrations (graphs, dendrograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided in electronic form. Tables should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1–2, Fig. 1 or Figs. 1–2; Tab. 1 and Sl. 1). A full name is used in the legend title (e.g. **Figure 1**, **Table 2** etc.), written bold, followed by a short title of the figure or table, also in bold. Subpanels of a figure have to be unambiguously indicated with capital letters (A, B, ...). Explanations associated with subpanels are given alphabetically, each starting with bold capital letter (**A**), a hyphen and followed by the text.

10. The quality of graphic material

Starting with the first issue of the 53rd volume the ABS will be processing the graphic material only electronically. All the figures have to be submitted in the electronic form. The ABS publishes figures either in pure black and white or in halftones. Authors are kindly asked to prepare their figures in the correct form to avoid unnecessary delays in preparation for print, especially due to problems with insufficient contrast and resolution. Clarity and resolution of the information presented in graphical form is the responsibility of the author. Editors reserve the right to reject unclear and poorly readable pictures and graphical depictions. The resolution should be 300 d.p.i. minimum for halftones and 600 d.p.i. for pure black and white. The smallest numbers and lettering on the figure should not be smaller than 8 points (2 mm height). The thickness of lines should not be smaller than 0.5 points. The permitted font families are Times, Times New Roman, Helvetica and Arial, whereby all figures in the same article should have the same font type. The figures should be prepared in TIFF, EPS or PDF format, whereby TIFF (ending *.tif) is the preferred type. When saving figures in TIFF format we recommend the use of LZW or ZIP compression in order to reduce the file sizes. The photographs can be submitted in JPEG format (ending *.jpg) with low compression ratio. Editors reserve the right to reject the photos of poor quality. Before submitting a figure in EPS format make sure first, that all the characters are rendered correctly (e.g. by opening the file first in the programs Ghostview or GSview – depending on the operation system or in Adobe Photoshop). With PDF format make sure that lossless compression (LZW or ZIP) was used in the creation of the *.pdf file (JPEG, the default setting, is not suitable). Figures created in Microsoft Word, Excel, PowerPoint etc. will not be accepted without the conversion into one of the before mentioned formats. The same goes for graphics from other graphical programs (CorelDraw, Adobe Illustrator, etc.). The figures should be prepared in final size, published in the magazine. The dimensions are 12.5 cm maximum width and 19 cm maximum height (width and height of the text on a page).

11. Conclusions

Articles shall end with a summary of the main findings which may be written in point form.

12. Summary

Articles written in Slovene must contain a more extensive English summary. The reverse also applies.

13. Literature

Starting with the first issue of the 53rd volume the ABS is introducing a new simplified citation system. The important differences are:

No more usage of small caps.

In text 'and' is used instead of '&' when referring to two authors and 'et al.' instead of '& al.' when referring to many. In the literature section the initials of name(s) follow the surname with all authors.

References shall be cited in the text. If a reference work by one author is cited, we write Allan (1995) or (Allan 1995); if a work by two authors is cited, (Trinajstić et Franjić 1994); if a work by three or more authors is cited, (Pullin et al. 1995); and if the reference appears in several works, (Honsig-Erlenburg et al. 1992, Ward 1994a, Allan 1995, Pullin et al. 1995). If several works by the same author published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc.: (Ward 1994a,b). If direct quotations are used, the page numbers should be included: Toman (1992: 5) or (Toman 1992: 5–6). The bibliography shall be arranged in alphabetical order beginning with the surname of the first author, comma, the initials of the name(s) and continued in the same way with the rest of the authors, separated by commas. The names are followed by the year of publication, the title of the article, the international abbreviation for the journal (periodical), the volume, the number in parenthesis (optional), and the pages. Example:

Mielke, M.S., Almeida, A.A.F., Gomes, F.P., Aguilar, M.A.G., Mangabeira, P.A.O., 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. *Experimental Botany*, 50 (1), 221–231.

Books, chapters from books, reports, and congress anthologies use the following forms:

Allan J. D. 1995: *Stream Ecology. Structure and Function of Running Waters*, 1st ed. Chapman & Hall, London, 388 pp.

Pullin A. S., McLean I. F. G., Webb M. R., 1995. *Ecology and Conservation of Lycaena dispar: British and European Perspectives*. In: Pullin A. S. (ed.): *Ecology and Conservation of Butterflies*, 1st ed. Chapman & Hall, London, pp. 150–164.

Toman M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1–7.

14. Format and Form of Articles

The manuscripts should be sent exclusively in electronic form. The format should be Microsoft Word (*.doc) or Rich text format (*.rtf) using Times New Roman 12 font with double spacing, align left only and margins of 3 cm on all sides on A4 pages. Paragraphs should be separated by an empty line. The title and chapters should be written bold in font size 14, also Times New Roman. Possible sub-chapter titles should be written in italic. All scientific names must be properly italicized. Used nomenclature source should be cited in the Methods section. The text and graphic material should be sent to the editor-in-chief as an e-mail attachment. For the purpose of review the main *.doc or *.rtf file should contain figures and tables included (each on its own page). However, when submitting the manuscript the figures also have to be sent as separate attached files in the form described under paragraph 10. All the pages (including tables and figures) have to be numbered. All articles must be proofread for professional and language errors before submission.

A manuscript element checklist (For a manuscript in Slovene language the same checklist is appropriately applied with a mirroring sequence of Slovene and English parts):

English title – (Times New Roman 14, bold)

Slovene title – (Times New Roman 14, bold)

Names of authors with clearly indicated addresses, affiliations and the name of the corresponding author – (Times New Roman 12)

Author(s) address(es) / institutional addresses – (Times New Roman 12)

Fax and/or e-mail of the corresponding author – (Times New Roman 12)

Keywords in English – (Times New Roman 12)

Keywords in Slovene – (Times New Roman 12)

Running title – (Times New Roman 12)

Abstract in English – (Times New Roman 12)

Abstract in Slovene – (Times New Roman 12)

Introduction – (Times New Roman 12, title – Times New Roman 14 bold)

Material and methods – (Times New Roman 12, title – Times New Roman 14 bold)

Results – (Times New Roman 12, title – Times New Roman 14 bold)

Discussion – (Times New Roman 12, title – Times New Roman 14 bold)

Summary in Slovene (Times New Roman 12, title – Times New Roman 14 bold)

Figure legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, figure designation and figure title – Times New Roman 12 bold)

Table legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, table designation and table title – Times New Roman 12 bold)

Acknowledgements – (Times New Roman 12, title – Times New Roman 14 bold)

Literature (Times New Roman 12, title – Times New Roman 14 bold)

Figures, one per page; figure designation indicated top left – (Times New Roman 12 bold)

Tables, one per page; table designation indicated top left – (Times New Roman 12 bold)

Page numbering – bottom right – (Times New Roman 12)

15. Peer Review

All Scientific Articles shall be subject to peer review by two experts in the field (one Slovene and one foreign) and Brief Note articles by one Slovene expert in the field. With articles written in Slovene and dealing with a very local topic, both reviewers will be Slovene. In the compulsory accompanying letter to the editor the authors must nominate one foreign and one Slovene reviewer. However, the final choice of referees is at the discretion of the Editorial Board. The referees will remain anonymous to the author. The possible outcomes of the review are: 1. Fully acceptable in its present form, 2. Basically acceptable, but requires minor revision, 3. Basically acceptable, but requires important revision, 4. May be acceptable, but only after major revision, 5. Unacceptable in anything like its present form. In the case of marks 3 and 4 the reviewers that have requested revisions have to accept the suitability of the corrections made. In case of rejection the corresponding author will receive a written negative decision of the editor-in-chief. The original material will be erased from the ABS archives and can be returned to the submitting author on special request. After publication the corresponding author will receive the *.pdf version of the paper.