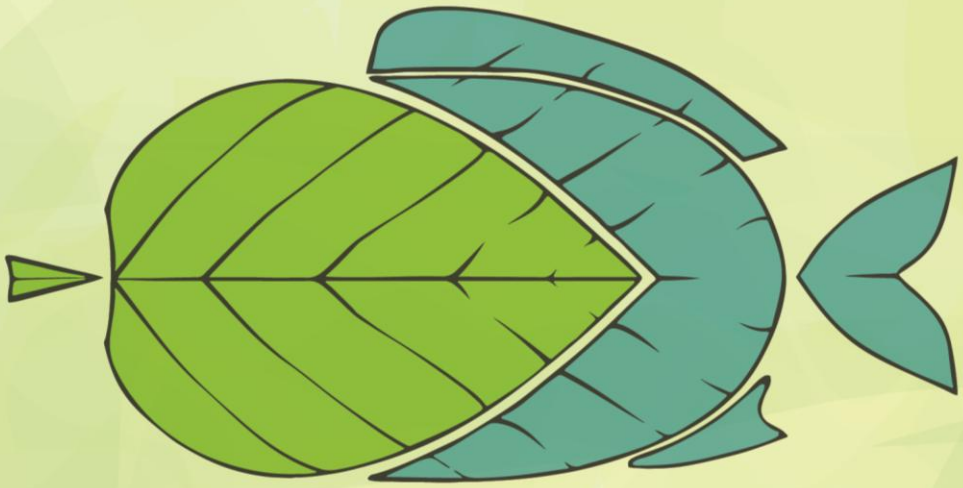

BIOSFERA

Konferenca študentov bioloških znanosti

ZBORNIK KONFERENCE

Ljubljana, 2016

BIOSFERA

Konferenca študentov bioloških znanosti

ZBORNIK KONFERENCE

Ljubljana, 2016

BIOSFERA – Konferenca študentov bioloških znanosti
ZBORNIK KONFERENCE

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UVODNIK

Kaj zanima študente biologije in sorodnih ved? S čim se ukvarjajo in kaj raziskujejo?

Ideja o organizaciji dogodka, ki bi ponudil odgovora na zgornji vprašanji in bil namenjen študentom, je nastala že lani spomladi, svojo podobo pa je začela dobivati z začetkom novega študijskega leta.

Organizatorji konference smo se tovrstnega projekta lotili prvič. Predvsem v jeseni se nam je ob premlevanju idej in mnenj porajalo nešteto vprašanj, na vsa pa smo bolj ali manj hitro (in seveda s pomočjo) našli odgovor. Želeli smo organizirati srečanje študentov različnih bioloških znanosti, ki bi vsem omogočilo predstavitev svojega raziskovalnega dela v sklopu diplomske, magistrske, doktorske ali drugačne projektne naloge oziroma dejavnosti.

Vseskozi smo si prizadevali, da bi bila udeležba dosegljiva vsem in, da bi konferenca pritegnila čim več ljudi, še posebej študentov. Pomembno se nam je zdelo, da študenti ob poslušanju predstavitev svojih vrstnikov, kolegov in povabljenih predavateljev tudi na tak način spoznajo, s čim vse se lahko v Sloveniji raziskovalno ukvarjajo kot študenti bioloških ved. Želeli smo, da bi s konferenco spodbudili tudi zanimanje začetnih letnikov fakultet za študentsko raziskovalno delo. Navsezadnje se jih večina prej ali slej znajde pred odločitvijo, na kakšno področje se usmeriti, kaj izbrati za temo svoje diplomske ali magistrske naloge in podobno.

K sodelovanju na konferenci smo povabili tudi že uveljavljene znanstvenike z različnih raziskovalnih ustanov, ki so svoje delo predstavili v obliki uvodnih predavanj sklopov. Našemu povabilu so se odzvali doc. dr. Marjetka Podobnik, doc. dr. Jernej Jorgačevski, prof. dr. Marko Kreft in dr. Matjaž Gregorič.

Ker pojem 'biološke znanosti' opredeljuje zelo širok in raznolik spekter raziskovalnih področij, smo želeli, da bi bil nabor vsebin uvodnih predavanj čim bolj pester. Tudi vsebine študentskih prispevkov so zelo raznolike, kar je po našem mnenju dobro in pripomore k temu, da je konferenca pritegnila toliko študentov. Vsak je tako lahko našel predstavitev s področja, ki mu je blizu. Konference so se udeležili študenti vseh treh slovenskih univerz in mnogih študijskih programov, pritegnila pa je tudi profesorje in raziskovalce s fakultet.

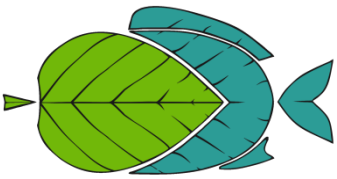
Na tem mestu bi se radi iskreno zahvalili vsem, ki so nam svetovali, odgovarjali na vprašanja tekom leta, pregledovali študentske prispevke in nam pomagali pri oblikovanju programa. V veliko, veliko pomoč so nam bili doc. dr. Gregor Belušič, doc. dr. Matej Butala, doc. dr. Jasna Dolenc Koce, prof. dr. Rok Kostanjšek, asist. dr. Aleš Škorjanc in prof. dr. Davorin Tome. Za finančno podporo se najlepše zahvaljujemo tudi vsem našim donatorjem in sofinancerjem, ki so s svojimi prispevki omogočili brezplačno udeležbo vsem sodelujočim. Hvala tudi vodstvu Oddelka za biologijo, ki nam je omogočilo celodnevno uporabo predavalnice, avle in panojev.

Veseli nas, da smo vedno naleteli na pozitiven odziv in odobravanje vseh, ki so za dogodek izvedeli. Naša želja je, da bi se tovrstni dogodki organizirali tudi v prihodnosti.

Organizacijski odbor

BIOSFERA – PROGRAM

23. september 2016
BF – Oddelek za biologijo



9.00 Uvodni nagovor (predavalnica B1)

9.15 ***doc. dr. Marjetka Podobnik***
B1 Kemijski inštitut
Toksini, ki tvorijo pore: njihova struktura, funkcija in uporaba

9.45 **Jerneja Kladnik**
Vpliv prostega polietilenimina na transfekcijo celic CHO z magnetnimi nanodelci

10.00 **dr. Maša Čater**
Optimizacija proizvodnje bioplina iz lignoceluloznega substrata z granulirano anaerobno biomaso iz UASB bioreaktorja z bioaugmentacijo

10.15 **Angelika Vižintin**
Priprava samomorilskega genetskega stikala za obvladovanje cianobakterije *Synechocystis* sp. PCC 6803

Odmor za kavo

11.15 ***doc. dr. Jernej Jorgačevski***
B1 Medicinska fakulteta, Univerza v Ljubljani
Meritve premerov mešičkov v astrocitih z mikroskopijo STED

11.45 **Urban Bogataj**
Ultrastruktura in diferenciacija bazalne površine črevesnega epitela med razvojem modelnega organizma *Porcellio scaber*

12.00 **Tajda Gredar**
Razvoj nedestruktivnih metod za citogenetske analize močerila (*Proteus anguinus*)

12.15 – 13.00 Odmor za kosilo

13.00 – 14.30 Predstavitve plakatov (avla Oddelka za biologijo)

14.30 ***prof. dr. Marko Kreft***
B1 Biotehniška fakulteta, Univerza v Ljubljani
Presnova glukoze v možganih

15.00 **Katja Hrovat**
Utišanje helikaze RECQ1 vpliva na proliferacijo glioblastomskih celic v možganih zarodkov cebric (*Danio rerio*)

15.15 **Rok Šturm**
Vibracijska slika naravnega okolja in vpliv biotskega šuma na vedenje samcev iz rodu *Aphrodes*

15.30 **Zarja Muršič**
Vedenjski odziv na svetlobo pri površinskih in podzemeljskih populacijah vodnega oslička *Asellus aquaticus*

Odmor za kavo

16.30 ***dr. Matjaž Gregorič***
B1 Biološki inštitut Jovana Hadžija, ZRC SAZU
Mreže in predivo pajkov: integracija vedenja, ekologije in biomehanike

17.00 **Eva Horvat**
Prispevek k poznavanju drsti sardona (*Engraulis encrasicolus* L. 1758) na območju Slovenskega morja

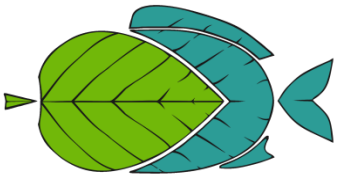
17.15 **Tjaša Zagoršek**
Gnezditvena gostota Velikega skovika (*Otus scops*) ob vzhodni jadranski obali Slovenije in Hrvaške

17.30 **David Škufca**
Podzemlje in površje: biomonitoring vodnih osličkov

17.45 **Katarina Šoln**
Alelopatija – biokemijsko orožje rastlin: zaviralni vpliv listnih in koreninskih izvlečkov invazivnih dresnikov (*Fallopia* sp.)

Zaključna pogostitev

BIOSFERA
PREDSTAVITVE PLAKATOV



Blaž Oblak	Interakcija med magnetnimi nanodelci oplaščenimi s polietileniminom in označenimi z rodaminom B ter celicami CHO
Jure Zabret	Homologi kaspaz cianobakterije *Microcystis aeruginosa*
Tinkara Rozina	Učinek elektrokemijske oksidacije na *Microcystis aeruginosa*
Maša Čater	Robotsko plovilo za nadzor cianobakterij
Sara Gorše, Metka Petrič	Vpliv starosti na pigmentacijo jeter pri neotenični jamski repati dvoživki *Proteus anguinus*
Maruša Rošer	Skeletokronološka analiza in interpretacija linij zavrte rasti v dolgih kosteh močerila
Saša Pušavec, Tadej Pliberšek	Določanje nivojev infliksimaba in protiteles proti infliksimabu med zdravljenjem KVČB
Špela Granda	Vpliv nanodelcev titanovega dioksida na preživetje celic THP-1
Patrik Prša, Rok Janža	Funkcionalne lastnosti fotoreceptorjev v akutnih conah oči samic in samcev obada (*Tabanus bromius* Linnaeus, 1758)
Nina Lozej	Razlike v izbiri podlage in iskanju zatočišč med površinskimi in podzemeljskimi vodnimi oslički
Žan Kuralt	Vpliv klimatskih sprememb na premike arealov mediteranskih vrst: Primer mediteranske črne vdove
Manca Velkavrh	Gnezditveni uspeh prib v povezavi s kmetijskimi dejavnostmi na izbrani popisni ploskvi na Ljubljanskem Barju
Nina Ražen	Skrivnosti iz življenja volkov – teritorialnost, raba prostora in aktivnost volkov (*Canis lupus*) v severnih Dinaridih
Rok Janža, Patrik Prša, Lucijan Skubic	Biogeografske korelacije velikosti genoma pri neotropskih pupkih rodu *Bolitoglossa*
Amela Kujović	Ocena genetskega materiala za študijo o razmerjih med spoloma pri *Salix viminalis* L.
Timotej Čepin, Tilen Sever	Timijanovo eterično olje kot naravni fungicid pri kalitvi pšenice
Andraž Dolar	Žlezni trihomi navadne in tatarske ajde pogojujejo vsebnosti njunih flavonoidov
Maja Križnik	Vloga malih RNA pri obrambnem odgovoru krompirja na okužbo z virusom krompirja Y
Tjaša Košir, Valentina Ivanovska Zelić, Patrik Prša	Vpliv onesnaženosti in rastlinske združbe na endofite pisane vilovine (*Sesleria caerulea*)

KAZALO

VABLJENO PREDAVANJE / INVITED LECTURE

Toksini, ki tvorijo pore: njihova struktura, funkcija in uporaba
Pore forming toxins: their structure, function and application

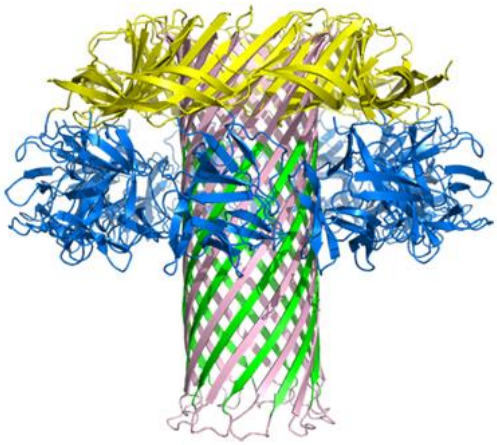
Marjetka Podobnik[1]

[1]Odsek za molekularno biologijo in nanobiotehnologijo, Kemijski inštitut, Hajdrihova 19, Ljubljana/Department for molecular biology and nanobiotechnology, National Institute of Chemistry, Hajdrihova 19, Ljubljana

Biološke membrane so zgrajene iz lipidnih dvoslojev, ki vsebujejo tudi različne oblike proteinov in sladkorjev. Naloga membran je, da ščitijo celice pred okoljem, obenem pa omogočijo potek mnogih bioloških procesov. Za celice je intaktnost membran ključna za njihovo preživetje, torej so tu tudi najbolj ranljive. Mnogi organizmi izkoriščajo ranljivost celičnih membran tako, da z aktivnimi komponentami napadejo druge organizme, ali pa se branijo pred njimi. Ena najbolj usepšnih metod razbijanja membran je preko tvorbe por v lipidne dvosloje. Molekule, ki tvorijo te pore so v večini toksični peptidi in proteini, ki jih izražajo organizmi v obliki topnih monomerov, ki se vežejo na gostiteljeve membrane preko specifičnih receptorjev. Najbolj preučevani proteini te vrste so mnogi bakterijski toksini, ki jih s kratico imenujemo PFTji (angl. pore-forming toxins). Bakterijski PFTji igrajo pomembno vlogo kot virulenčni dejavniki, ki bakterijam omogočajo širjenje preko gostiteljivih celic in tkiv. PFTji pa niso omejeni samo na bakterije, najdemo jih v vseh življenjskih kraljestvih.

PFTje delimo v dve veliki skupini glede na strukturo transmembranskega sodčka njihove pore. Le ta je lahko zgrajena iz α-vijačnic ali pa β-zank, zato se imenujejo α-PFTji in β-PFTji. Predstavljen bo pregled čez družine PFTjev, ki sestavljajo ti dve veliki skupini. Predstavljena pa bo tudi kristalna struktura in mehanizem delovanja β-PFTja iz deževnika *Eisenia fetida*, ki se imenuje *lizenin*, ki smo jo nedavno določili v naši skupini.[1]

Atomska struktura lizeninske pore in mehanizem njene sestave predstavljata velik doprinos k razumevanju delovanja sorodnih bakterijskih proteinov in bo služila kot pomembna osnova za razvoj novih pristopov preprečevanja bakterijskih obolenj, kjer jih bakterije uporabljajo za infekcijo ljudi in živali. Poleg tega pa je znanje o PFTjih zelo uporabno v nanobiotehnologiji, na primer kot del dostavnih sistemv zdravil ali pa kot nanosenzorji za različne substance.



Slika 1: Kristalna struktura pore lizenina.[1]
Figure 1: Crystal structure of the lysenin pore.[1]

Biological membranes are built of sheets of lipid bilayers including various forms of proteins and sugars. They protect cells from their environment as well as enable many important biological processes. Intact membranes are vital to cells, thus the disintegration of the membrane is one of the simplest ways to harm cells. Such destructive processes are used by many organisms, which by using membrane active compounds attack other organisms or defend themselves against them. One of the most effective ways to damage membranes is to form pores into the lipid bilayer. Pore forming compounds are almost exclusively toxic peptides or proteins, which are expressed by the producing organism as water-soluble monomers, attracted by the membranes via specific receptors. The most studied pore-forming proteins are various families of bacterial toxins, termed pore-forming toxins (PFTs). Bacterial PFTs serve as important virulence factors promoting bacterial spread through host cells and tissues. PFTs are not limited to bacteria, they can be found in all kingdoms of life.

PFTs can be divided in two major groups, based on a structure of the transmembrane barrel of their pore. This can be either built of α-helices or β-hairpins, and are named α-PFTs and β-PFTs, respectively. An overview of various families of PFTs will be presented. A crystal structure of β-PFT from the earthworm *Eisenia fetida*, called *lysenin*, that was determined recently in our department[1] will be discussed in detail, along with its mechanism of action.

The atomic structure of the lysenin pore as well as the mechanism of its assembly now crucially help to understanding of the action of many related bacterial toxins and will serve as an important basis for development of new approaches towards bacterial infections, where such toxins are being used to infect people and animals. Moreover, knowledge about PFTs is also extremely applicable in nanobiotechnology, for example as a part of drug delivery systems or nanosensors for various substances.

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Vpliv prostega polietilenimina na transfekcijo celic CHO z magnetnimi nanodelci

Effect of free polyethyleneimine on the transfection of CHO cells with magnetic nanoparticles

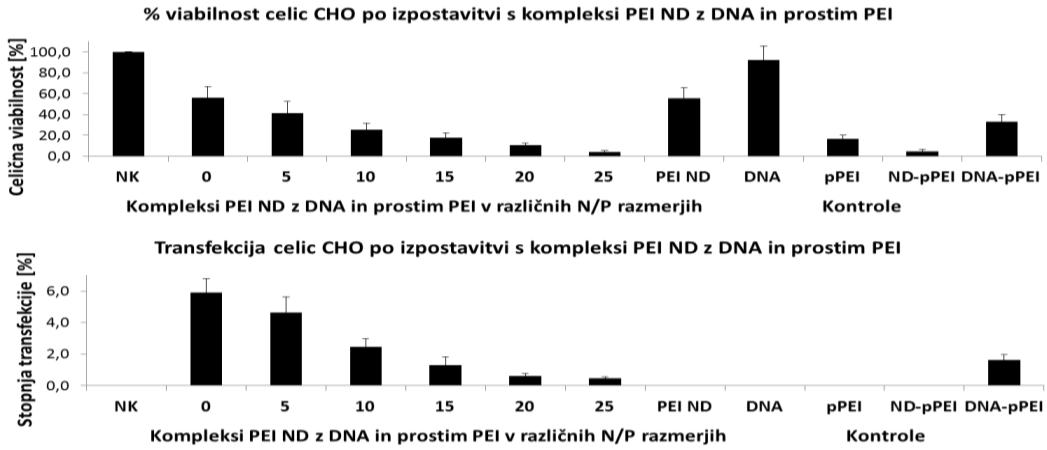
Jerneja Kladnik[1], Klemen Strojan[1], Mojca Pavlin[1]

[1]Skupina za nano in biotehnološke aplikacije, Fakulteta za elektrotehniko, Univerza v Ljubljani, Slovenija/Group for nano- and biotechnological applications, Faculty of Electrical Engineering, University of Ljubljana

Genska terapija izkazuje velik potencial za zdravljenje bolezni, ki imajo svoj izvor v okvarjenih genih (npr. rak).[1] Magnetni nanodelci (mND) so posebna vrsta nanodelcev z magnetnim, elektronsko gostim jedrom, ki se jih uporablja kot nevirusne vektorje za vnos genov. Transfekcija s pomočjo mND se mnogokrat imenuje magnetofekcija, saj so nekatere študije uporabile tudi zunanje magnetno polje za stimulacijo vnosa, mND pa prehajajo v celice tudi brez prisotnosti magnetnega polja z endocitozo. mND so zanimivi zaradi možnosti vizualizacije z elektronsko mikroskopijo in NMR vzporedno z vnosom ciljnih molekul in relativno enostavne modifikacije površine.[1, 2]

Polietilen imin (PEI) se pogosto uporablja kot zunanja plast mND za gensko transfekcijo z mND.[3] Kationski polimer PEI tvori komplekse z anionsko plazmidno DNA, hkrati pa se lahko veže na negativno nabito celično membrano. Tako PEI pomaga pri vstopu kompleksov v celico in sprostitvi DNA v citoplazmo.[1] V nekaterih študijah je bilo dokazano, da dodatek PEI v prosti obliki h kompleksom PEI mND/DNA zviša transfekcijo pri različnih celičnih linijah.[1,3] Vpliv prostega PEI smo želeli preveriti tudi pri PEI mND, razvitih v naši skupini.

Sintetizirali smo magnetne kobalt feritne nanodelce in jih najprej oplaščili s poliakrilno kislino in nato še s PEI ter dobili PEI mND. Nato smo preko elektrostatskih interakcij tvorili komplekse PEI mND/DNA in jim dodali prosti PEI. Naši rezultati so pokazali, da dodatek prostega PEI ne izboljša transfekcije na celicah CHO. Nasprotno, dodatek prostega PEI zniža tako celično viabilnost kot stopnjo transfekcije. Prost PEI se je izkazal za zelo toksičnega za celice CHO in najuspešnejšo transfekcijo smo dosegli pri optimalnem razmerju PEI mND/DNA brez dodanega prostega PEI.



Slika 1: Celična viabilnost in stopnja transfekcije po izpostavitvi celic CHO s kompleksi PEI mND/DNA in prostega PEI pri naraščajočem razmerju N/P (molarno razmerje dušika prostega PEI in fosforja v DNA).
Figure 1: Cell viability and transfection efficiency after exposure of CHO cells to PEI mNP/DNA complexes and free PEI at increasing N/P ratio (molar ratio of free PEI nitrogen to plasmide DNA phosphate).

Gene therapy has shown great potential in treating human diseases that originates from defective genes, e.g. cancer.[1] Magnetic nanoparticles (mND) are special type of nanoparticles with magnetic, electron dense core, which can be used as non-viral gene delivery vector. The transfection process mediated by mND is often called magnetofection, as in some studies external magnetic field was used to stimulate the uptake of mND, although mND can also enter the cells without magnetic field via endocytosis. mND are attractive because of possible tracking of the entry of target molecules in real time by electronic microscope or NMR and relatively simple surface modification.[1,2]

In gene transfection with mND polyethyleneimine (PEI) is often used as an external layer of mND.[3] Cationic PEI can form complexes with the anionic plasmide DNA and binds on negatively charged cell membrane and brings complexes in cells, where DNA is released into the cytoplasm.[1] In some studies, it has been shown, that the addition of free PEI to PEI mND/DNA complexes enhances transfection on different cell lines.[1, 3] Therefore, we wanted to investigate, how does the addition of free PEI influence the transfection in combination with PEI mND, which have been developed in our group.

In our study, magnetic cobalt ferrite nanoparticles were synthesized and coated, firstly with polyacrylic acid and after that with PEI, referred as PEI mND. Further we prepared magnetofectines via electrostatic interactions by mixing PEI mND with plasmide DNA followed by adding free PEI to the PEI mND/DNA complexes. Our results showed that extra free PEI did not improve transfection on CHO cells. Conversely, when more free PEI was added, cell viability and transfection efficiency were reduced. Free PEI proved to be very toxic for CHO cells and the best transfection was still achieved by PEI mND/DNA complexes at optimal ratio without free PEI.

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2. Lojk, J.; Bregar, V. B.; Rajh, M.; Miš, K.; Kreft, M. E.; Pirkmajer, S.; Veranič, P.; Pavlin, M. Cell type-specific response to high intracellular loading of polyacrylic acid-coated magnetic nanoparticles. *Int J Nanomedicine*. **2015,** *10*, 1449-1462.
3. Ma, Y.; Zhang, Z.; Wang, X.; Xia, W.; Gu, H. Insights into the mechanism of magnetofection using MNPs-PEI/pDNA/free PEI magnetofectins. *Int J Pharm*. **2011,** *419*, 247-254.

Interakcija med magnetnimi nanodelci oplaščenimi s polietileniminom in označenimi z rodaminom B ter celicami CHO

Interaction between polyethyleneimine coated rhodamine B labelled magnetic nanoparticles and CHO cells

Blaž Oblak[1,2], Klemen Strojan[1], Jurij Svete[2], Mojca Pavlin[1]

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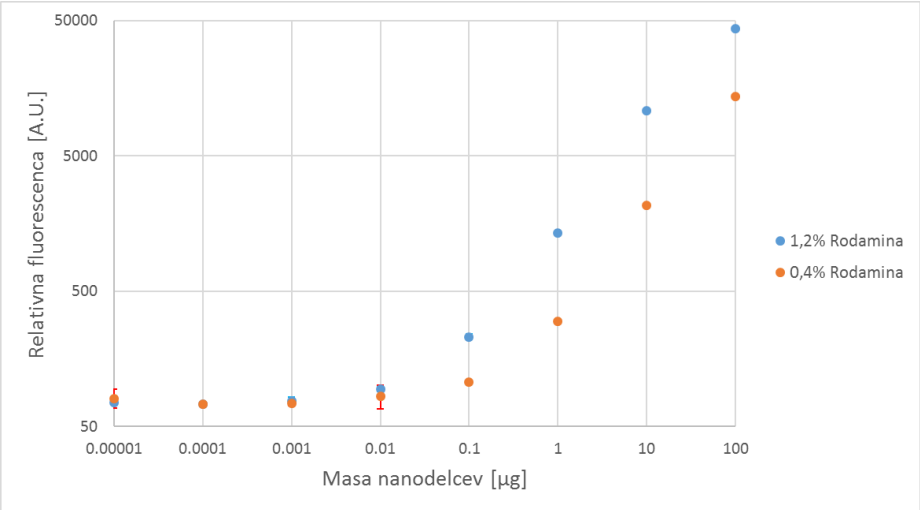
[2]Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani/Faculty of Chemistry and Chemical Technology, University of Ljubljana

Magnetni nanodelci (MND) so pridobili veliko pozornosti v biomedicinskih in industrijskih aplikacijah zaradi njihove biokompatibilnosti, relativno lahke površinske modifikacije in njihovih magnetnih lastnosti. MND se lahko uporabljajo podobno kot nemagnetni ND za različne aplikacije, od dostave učinkovin do vizualizacije, magnetne lastnosti pa jim dodajo še dodatne možnosti uporabe, saj manipulacija z magnetnim poljem omogoča še dodatne načine uporabe, kot je npr. hipertermija.[1]

Polietilenimin (PEI) je kationski polimer, uporaben za transfekcijo celic.[2] Uporablja se samostojno ali kot polimerni plašč različnih nanodelcev. Njegova uporaba je problematična zaradi visoke toksičnosti, mehanizmi toksičnosti niso povsem razjasnjeni. Fluorescentno označen PEI pa lahko pripomore pri analizi še nepojasnjenih mehanizmov.

Pripravili smo nanodelce oplaščene s PEI na katerega smo predhodno kovalentno vezali rodamin B. Preučevali smo vpliv različnih koncentracij ND in rodamina na intenziteto fluorescence ter interakcijo MND z ovarijskimi celicami kitajskega hrčka (CHO). Na PEI je bilo v prvem primeru vezanega 0,4% v drugem pa 1,2% (n/n) rodamina B. Vplive na celice smo preučevali 24 ur po dodatku medija, ki je vseboval MND. Fluorescenčne lastnosti MND smo preučevali s fluorescenčno spektroskopijo, interakcije s celicami pa s svetlobno in fluorescenčno mikroskopijo. Kot pričakovano, so fluorescenčne lastnosti odvisne od koncentracije rodamina B in ND. Sama koncentracija rodamina vezanega na PEI nima vpliva na viabilnost celic, medtem ko koncentracija samih ND ima zelo jasen in logičen vpliv.



Slika 1: Relativna fluorescenca nanodelcev v odvisnosti od njihove mase.
Figure 1: Relative fluorescence of nanoparticles in relation to their mass.

Magnetic nanoparticles (MNP) have gained a lot of attention in biomedical and industrial applications due to their biocompatibility, ease of surface modification and magnetic properties. MNP can be utilized in many ways, very similar to those of nanoparticles in general. However, the magnetic properties set them apart and add a new dimension where they can be manipulated upon application of an external magnetic field; e.g. hyperthermia.[1]

Polyethyleneimine (PEI) is a cationic polymer, used for transfection.[2] It is used in a solution or coated on different types of nanoparticles. Its use is problematic due to high toxicity and the mechanisms responsible for the toxicity are not completely clear. Fluorescent doping of PEI could help studying the mechanisms involved in its toxicity.

We have prepared PEI MNP covalently doped with Rhodamine B and studied the effects of rhodamine and MNP concentration on their fluorescence properties and interactions with Chinese hamster ovary (CHO) cells. Functional loading of rhodamine B on PEI was 0.4 % and 1.2 % (n/n). The effects of MNP on cells were studied 24 hours after the addition of media containing PEI MNP. The fluorescence properties were determined by fluorescence spectroscopy and interactions with cells were studied with light and fluorescence microscopy. As expected the fluorescence properties were correlated with rhodamine B and MNP concentration. While the effects of MNP on cells were not dependant on rhodamine B concentration, they were on NP concentration.

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Optimizacija proizvodnje bioplina iz lignoceluloznega substrata z granulirano anaerobno biomaso iz UASB bioreaktorja z bioaugmentacijo

Optimisation of biogas production from lignocellulosic substrate with granulated anaerobic biomass from UASB bioreactor by bioaugmentation

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Bioplinarne se za proizvodnjo bioplina iz lignocelulloznih substratov zaradi majhnih izplenov metana ne odločajo pogosto, čeprav so široko dostopni in poceni. Vzrok je v zapleteni zgradbi lignoceluloze in ovirani hidrolizi.[1,2] Z obdelavo lahko močno izboljšamo njegovo razgradnjo in povečamo proizvodnjo bioplina. Najbolj raziskane so fizikalno-kemijske obdelave, medtem ko biološke obdelave predstavljajo poseben potencial, saj se izogibajo agresivnim kemikalijam, so okolju prijaznejše in poceni.[3] V raziskavi smo se osredotočili na biološko obdelavo in sicer na bioaugmentacijo s hidrolitičnimi bakterijami. Kot modelni lignocelulozni substrat smo uporabili pivovarske tropine,[4] anaerobno mikrobno biomaso iz bioreaktorja UASB Čistilne naprave Laško pa kot vir aktivnih mikroorganizmov za proizvodnjo bioplina. Za bioaugmentacijo smo izbrali dve vampni bakteriji (*P. xylanivorans* Mz5T in *F. succinogenes* S85) in eno iz metanogenega bioreaktorja, ki je tretiral topolov les (*C. cellulovorans*). S testom biokemijskega metanskega potenciala (30 dni, 37 °C, 120 rpm, v temi) smo testirali, kako dodatek posamezne bakterije ali kokulture (5 %) vpliva na povečanje proizvodnje metana. Izbranim bakterijam smo predhodno inducirali njihovo ksilanolitično in celulolitično aktivnost za hitrejše delovanje v bioplinskem procesu. Redno smo spremljali proizveden bioplin, delež metana, pH, kemijsko potrebo po kisiku in koncentracijo kratkoverižnih maščobnih kislin (KMK).

Proizvodnjo metana je najbolj povečal dodatek *P. xylanivorans* Mz5T (10-28 %) sledita dodatek *C. cellulovorans* in dodatek kokulture *C. cellulovorans* in *F. succinogenes* S85 (oba 11 %). Proizvodnja metana je bila povečana najverjetneje zaradi izboljšane hidrolize zaradi bioaugmentacije, kar dokazujejo tudi rezultati spremljanja koncentracije KMK v bioreaktorjih. Z analizo T-RFLP smo dokazali, da je dodatek *P. xylanivorans* Mz5T v bakterijski združbi povzročil večje spremembe (30-50 %) kot ostale bioaugmentacije. DGGE in qPCR analizi mikrobne združbe pa sta pokazali, da je *P. xylanivorans* Mz5T v bioreaktorju preživel najdlje, skoraj en teden, zato je bil najverjetneje najuspešnejši pri povečevanju proizvodnje metana, saj je najdlje izvajal hidrolizo substrata. *C. cellulovorans* in *F. succinogenes* S85 se v bioreaktorjih nista obdržala več kot en dan.

Rezultati kažejo, da so lahko biološke obdelave lignocelulloznega substrata po učinku primerljive s fizikalno-kemijskimi in da velja bioaugmentacija s *P. xylanivorans* Mz5T za izredno perspektivno. Za določitev optimalnega razmerja med stroškom obdelave substrata in povečanjem izplena metana za aplikacijo v bioplinarnah pa so potrebna nadaljnja testiranja na pilotnem in industrijskem nivoju.

Biogas production from lignocellulosic substrates usually results in very low methane yields, therefore it is not a favorable choice of biogas plants. Their complex structure disables a successful hydrolysis, yet they are worldwide available and inexpensive.[1,2] Lately lots of research is done on biological substrate pretreatments, which are very perspective because they do not use agressive chemicals, are environmentally friendly and relatively cheap in contrary to physico-chemical ones.[3] In our research we have focused on the development of a successful biological treatment involving bioaugmentation with anaerobic hydrolytic bacteria. Brewery spent grain was used as a model lignocellulosic substrate[4] and anaerobic microbial biomass from UASB bioreaktor from Wastewater treatment plant Laško as a source of active microorganisms. Two rumen bacteria (*P. xylanivorans* Mz5T and *F. succinogenes* S85) and one from a methanogenic bioreactor treating hybrid poplar wood (*C. cellulovorans*) were chosen for the study. The impact of bioaugmenting individual bacteria or cocultures of bacteria (5%) on methane production from brewery spent grain was measured by a biochemical methane potential assay. Their xylanases and cellulases were first for an accelerated start of hydrolysis in biogas bioreactor. The assays were run for 30 days in dark at 37 °C and 120 rpm. During the assay we have regularly measured the newly produced biogas, content of methane, pH, chemical oxygen demand and concentration of short-chain fatty acids (SCFA).

The addition of *P. xylanivorans* Mz5T resulted in best accelerating of methane production (10-28 %), while the additions of *C. cellulovorans* and coculture of *C. cellulovorans* with *F. succinogenes* S85 enhanced methane production for about 11 %. Methane production was improved most probably because of the accelerated hydrolysis of the brewery spent grain due to the bioaugmentation. The highest, but not inhibitory, SCFAs concentration was detected with added *P. xylanivorans* Mz5T during the assay. T-RFLP results showed that the addition of *P. xylanivorans* Mz5T, which showed to be the most successful in biogas production enhancement, makes bigger changes in bacterial community in the biogas bioreactor (30-50 %) than other tested bioaugmentations. DGGE and qPCR were used to determine the time of survival of the added bacteria in bioreactors. *P. xylanivorans* Mz5T survived in the bioreactors the longest, almost a week, which is obviously a reason for its successfulness in methane production acceleration. *C. cellulovorans* and *F. succinogenes* S85 did not survive in bioreactors more than one day after the bioaugmentation, which correlates to their lower impact on methane production.

These results show that biological substrate pretreatment methods can be as successful as physico-chemical ones. Bioaugmentation with *P. xylanivorans* Mz5T showed to be a very perspective technique for enhancing methane production from brewery spent grain. Further research at pilot and industrial level is needed for a determination of an optimal ratio between acceleration of methane production and the cost of the substrate pretreatment and its application in biogas plants.

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Homologi kaspaz cianobakterije Microcystis aeruginosa

Caspase homologues of the cianobacterium Microcystis aeruginosa

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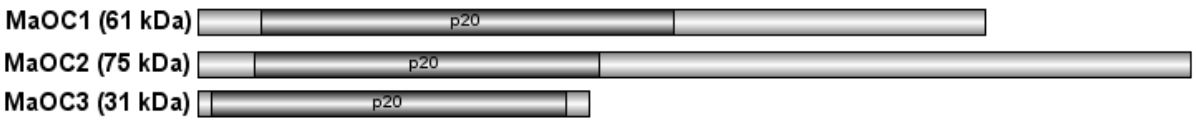
Programirana celična smrt (PCS) je ena izmed značilnosti mnogoceličnih organizmov, ki so jo raziskovalci šele pred kratkim opazili tudi pri ostalih oblikah življenja. Proteini, ki so pri živalih odgovorni za izvajanje tega procesa, so encimi, ki se imenujejo kaspaze. Zanimivo, te skupine proteaz ne najdemo pri rastlinah in glivah, saj pri teh organizmih enako funkcijo namreč opravljajo kaspazam podobni proteini, metakaspaze.[1] Le-te so vključene v PCS, ko je celica izpostavljena oksidativnemu stresu, virusnim toksinom in antibiotikom.

Raziskovalci so podobne encime odkrili tudi pri prokariontih.[2] Klemenčič in sod. so pred kratkim objavili raziskavo, ki predstavlja prvo biokemijsko karakterizacijo prokariontskih kaspaznih homologov. Kot modelni organizem so izbrali cianobakterijo *Microcystis aeruginosa* PCC 7806.[3] Omenjen rod cianobakterij je namreč eden izmed glavnih svetovnih povzročiteljev toksičnega cvetenja eutrofičnih sladkih vod.[4] Za toksičnost je odgovoren ciklični heptapeptid, imenovan mikrocistin, ki povzroča nekatera človeška obolenja, najpogosteje pa zastrupi domače in divje živali.[4]

Na podlagi stukturnih in funkcijskih lastnosti so to skupino kaspaznih homologov poimenovali ortokaspaze.[3] Te se zaradi prisotnosti katalitične domene p20 uvrščajo v družino cisteinskih proteaz C14. Glavni predstavniki te družine, kaspaze, cepijo substrate z visoko specifičnostjo z aspartatom na položaju P1.[1] Ortokaspaze pa tako kot metakaspaze z visoko učinkovitostjo cepijo substrate z argininom na položaju P1.[3] Poleg omenjene specifičnosti, je za proteine družine C14 značilna sinteza v obliki neaktivnega prekurzorja,[1,2] ki se kasneje aktivira preko proteolitične cepitve - kar je obenem značilno tudi za ortokaspazo MaOC1 *M. aeruginosa* PCC 7806.[3] Genom druega seva *Microcystis aeruginosa*, NIES843,[4] vsebuje tri odprte bralne okvirje, z ohranjeno katalitično domeno p20. Proteine smo poimenovali MaOC1, MaOC2 in MaOC3, katerih domenska razporeditev je prikazana na sliki 1. Od teh imata edino MaOC1 in MaOC3 ohranjeno aktivno mesto, kar je prvi pokazatelj njune katalitične sposobnosti, medtem ko MaOC2 ne vsebuje katalitične diade in po vsej verjetnosti v celici opravlja drugo regulativno vlogo. Prekomerno izražanje teh encimov v bakteriji *Escherichia coli* je pokazalo, da MaOC3 za svojo aktivacijo ne potrebuje proteolitičnega procesiranja, ampak se prevede kar v aktivni encim. Njegova preferenca za arginin na položaju P1 substrata pa nakazuje na skupne katalitične in morebitne funkcijske značilnosti ortokaspaz.



Slika 1: Domenska ureditev ortokaspaz cianobakterije *Microcystis aeruginosa* NIES843.
Figure 1: Domain organization of orthocaspases from the cyanobacterium *Microcystis aeruginosa* NIES843.

Programmed cell death (PCD) is an essential trait of multicellular organisms that has just recently been observed in other forms of life as well. In the animal kingdom this process is carried out by specialised enzymes called caspases. These are a group of proteases which are not found in other eukaryotes. Instead, their role is carried out by their distant homologues, called metacaspases.[1] Further research on theses enzymes has revealed that they play a role in PCD, which occurs as a consequence of oxidative stress, virus toxins and presence of antibiotics.[1]

Similar enzymes have been identified in prokaryotes as well.[2] Recently Klemenčič et al. published a paper in which they, for the first time, biochemically characterised prokaryotic caspase homologs. As the model organism they have chosen the cyanobacterium *Microcystis aeruginosa*, strain PCC 7806,[3] since this genus of cyanobacteria is one of the main world-wide causes for toxic freshwater blooms.[4] The reason for their toxicity is the synthesis of a cyclic heptapeptide, called microcystine.[4] It is responsible for some human diseases, but is mainly toxic to livestock and wild animals.[4]

Due to their structural and functional properties, the functional prokaryotic caspase homologues were termed orthocaspases.[3] Because of the presence of the p20 catalytic domain they are classified in the C14 family of cysteine proteases. The representatives of this family, caspases, are characterised by a high affinity for aspartate at the P1 position of the substrate.[1] Interestingly, metacaspases and orthocaspases do not cleave after negatively charged amino acid residues, but exhibit a greater affinity for arginine on the P1 position.[3] Beside the aforementioned affinity, activation trough proteolytic cleavage is also a characteristic of this protease family[1,2] in which orthocapases from *M. aeruginosa* are no exeption.[3] The genome of a different strain of *M. aeruginosa,* NIES843,[4] contains three open reading frames that contain a sequence coding for the catalytic p20 domain. We named these gene products MaOC1, MaOC2 and MaOC3, as shown on figure 1. Only MaOC1 and MaOC3 have a conserved active site, which is the first indicator of their catalytic capabilities. MaOC2 lacks the catalytic dyad and is therefore most likely involved in some other metabolic or signalling pathways. Overexpression of these enzymes in *Escherichia coli* and their isolation using different chromatographic techniques showed that MaOC3 does not require proteolytic activation, but is synthesized as a fully active enzyme with a preference for arginine at the P1 position. This indicates that various orthocaspases share catalytic and even functional similarities.

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Učinek elektrokemijske oksidacije na Microcystis aeruginosa

The effect of electrochemical oxidation on Microcystis aeruginosa

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Množičen pojav cianobakterij je globalen pojav, značilen za s hranili bogata vodna telesa. Med cvetenjem se toksini nekaterih toksičnih rodov cianobakterijski kopičijo, kar predstavlja izredno tveganje za okolje in zdravje ljudi. Nadzor nad številčnostjo cianobakterij je zato ključnega pomena. Poznamo številne metode za zmanjšanje škodljivih posledic cvetenja.[1] Največkrat uporabljena filtracija, koagulacija in sedimentacija, so učinkovite pri odstranjevanju cianobakterij, ne pa tudi njihovih toksinov. Novi pristopi kontrole cianobakterij v naravnem okolju morajo zato doseči tudi razgradnjo toksinov oziroma omejiti njihovo sproščanje.

Testirali smo vpliv elektrokemijske oksidacije na laboratorijsko kulturo *Microcystis aeruginosa* PCC 7806. Z uporabo naprednih oksidacijskih postopkov so že dokazali možnost razgradnje cianotoksinov.[2,3] V našem eksperimentu smo uporabil elektrolitsko celico opremljeno z borom dopiranima diamantnima elektrodama (BDD). BDD elektrodi sta bili v funkciji anode kot tudi katode. Za tovrstne elektrode je značilen visok nadpotencial, ko omogoča tvorbo hidroksilnih radikalov. V poskusu smo 500 ml vzorca, z začetno koncentracijo cianobakterij $5x10^6$ celic/ml, spustili skozi elektrolitsko celico. Učinek na koncentracijo celic ter koncentracijo zunajceličnih in celokupnih toksinov smo spremljali 72 ur. Za oceno primernosti izvajanje metode z vidika vpliva na preostali fitoplankton v naravnem okolju, smo tretirane cianobakterije izpostavili zeleni algi *Pseudokirchneriella subcapitata* po OECD smernicah (OECD Test No. 201).

Tretiranje toksičnih cianobakterij iz rodu *Microcystis* z elektrolitsko celico se odraža v popolni inhibicij proliferacije. Po 72 urah je število celic v tretiranem vzorcu padlo na 24% začetne koncentracije. Koncentracija zunajceličnih toksinov tretiranega vzorca ni presegla koncentracije zunajceličnih toksinov kontrolnega vzorca. Primernost metode za nadzor cianobakterijske populacije v naravnem okolju podpirajo tudi preliminarni rezultati testov toksičnosti, ki kažejo, da produkti tretiranja ne vplivajo na rast *P. subcapitata*. Laboratorijski rezultati kažejo velik potencial uporabe elektrokemijske oksidacije za zaviranje cvetenja cianobakterij v naravnem okolju, saj omogoča postopen upad koncentracije cianobakterij in nadzorovano sproščanje ter razgradnjo cianotoksinov.

The massive occurrence of cyanobacteria is a global phenomenon, typical for nutrient-rich water bodies. Most cyanobacterial genera produce toxins, which have negative impacts on human health and the environment. Reducing the massive proliferation of cyanobacteria is therefore crucial. There are a number of methods to reduce the harmful effects of cyanobacteria.[1] Most frequently used filtration, coagulation and sedimentation, are efficient in the removal of cyanobacteria, but not their toxins. The proposed new approaches in stoichiometric control of cyanobacteria in the natural environment should also be effective in degradation of toxins and/or in reducing their release into the environment.

In the experiment, the effects of electrochemical oxidation of a laboratory-grown toxic cyanobacterial culture of *Microcystis aeruginosa* PCC 7806 were tested. The electrochemical oxidation was already proven to be useful in the degradation of cyanotoxins.[2,3] In our experiment the electrolytic cell equipped with boron-doped diamond electrodes was used. This type of electrode is known to have the highest potential of forming hydroxyl radicals. 500ml of the sample, with an initial cyanobacterial concentration of $5 \cdot 10^6$ cells/ml was driven through the electrolytic cell. The effect on the concentration of cells and the concentrations of extracellular and of total toxins were monitored for 72 hours. To assess the suitability of the method in terms of impact on the rest of the phytoplankton in the natural environment, green alga *Pseudokirchneriella subcapitata* was exposed to the products of electro-oxidation. The test was performed according OECD Guidelines (OECD Test No 201).

The treatment of *M. aeruginosa* toxic strain with the electrolytic cell resulted in a complete inhibition of proliferation. After 72 hours, the cell concentration in the treated sample decrreased to 24% of the initial concentration. The concentration of extracellular toxins of the treated sample did not exceed the concentration of extracellular toxins of the control sample. The suitability of the methods to control cyanobacterial populations in the natural environment is also supported by the results of toxicity tests, which show that the products of the treatment do not affect the growth of *P. subcapitata*. Laboratory results show the great potential for the use of electrochemical oxidation in the inactivation of cyanobacteria and their products in the natural environment. It provokes a gradual decline in the concentrations of cyanobacteria and the degradation of cyanotoxins.

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Priprava samomorilskega genetskega stikala za obvladovanje cianobakterije Synechocystis sp. PCC 6803

Construction of a suicide genetic switch for containment of the cyanobacterium Synechocystis sp. PCC 6803

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Cianobakterije so skupina bakterij, ki so tako kot rastline zmožne pretvarjati ogljikov dioksid in vodo v sladkorje v procesu fotosinteze, ob čemer sproščajo tudi kisik. S fotosintezo pridobljeno energijo lahko uporabijo za rast in razmnoževanje ali pa za proizvodnjo raznih molekul, kot so na primer vodik, vitamini, barvila, biogoriva, idr.[1]

Zadnja leta narašča zanimanje za uporabo cianobakterij v biotehnologiji za trajnostno proizvodnjo dragocenih metabolitov. S pomočjo spoznanj sistemske biologije mikroalg lahko cianobakterije gensko spremenimo tako, da proizvajajo večje količine želenega produkta ali celo tako, da začnejo proizvajati nek nov produkt, ki ga divji tip ni zmožen proizvajati.[2]

Ob uporabi gensko spremenjenih cianobakterij pa se poraja vprašanje biološke varnosti - kaj bi se zgodilo, če bi te ušle iz zaprtega sistema, kot je bioreaktor, v naravo? Čeprav ima vprašanje biološke varnosti velik vpliv na splošno sprejemljivost uporabe gensko spremenjenih cianobakterij, ostaja to področje v veliki meri še neraziskano. Zato želimo imeti na voljo orodja za nadzor gensko spremenjenih cianobakterij. Radi bi, da bi se pobegle gensko spremenjene cianobakterije samouničile, če bi ušle v naravo. S tem namenom smo pripravili samomorilsko genetsko stikalo na osnovi nespecifične nukleaze NucA in njenega inhibitorja Nui iz cianobakterije iz rodu *Anabaena* in ga vstavili v modelno cianobakterijo *Synechocystis* sp. PCC 6803. To samomorilsko stikalo je prvi objavljeni biološki zadrževalni ukrep za obvladovanje gensko spremenjenih cianobakterij.[3]

Kadar nukleaza ni inhibirana, razgradi dedni material cianobakterije, s čimer ubije celico in hkrati tudi prepreči, da bi se transgeni lahko horizontalno prenesli v druge bakterije v naravi. Ker želimo, da nukleaza pobije celice le pri določenih pogojih, smo gen *nucA* vstavili pod različico inducibilnega promotorja P*copM*. Prepisovanje genov pod promotorjem P*copM* se močno poveča ob prisotnosti določenih kovinskih ionov. Hkrati smo v celice vstavili tudi gen za inhibitor nukleaze (Nui), ki je pod kontrolo šibkega konstitutivnega promotorja P*rnpB*, da možno puščanje nukleaze v odsotnosti induktorja ne bi motilo rasti cianobakterij. Po dodatku cinkovih ionov smo opazili uspešno samoubijanje celic *Synechocystis* s samomorilskim genetskim stikalom, medtem ko je divji tip *Synechocystis* še naprej rasel. Z RT-PCR smo dokazali, da se je ob dodatku cinkovih ionov prepisovanje gena za nukleazo NucA povečalo, medtem ko je prepisovanje gena za inhibitor Nui ostalo enako.

Cyanobacteria are a group of bacteria which, alike to plants, are able to convert carbon dioxide and water into sugars in the process of photosynthesis, while releasing molecular oxygen. The energy generated in the process of photosynthesis may be used for growth and reproduction or for production of various molecules, such as hydrogen, vitamins, pigments, biofuels, etc.[1]

In recent years, cyanobacteria have attracted interest for biotechnological applications for sustainable production of valuable metabolites. With knowledge of microalgal systems biology we are able to genetically engineer cyanobacteria to produce larger quantities of the desired product or even to start synthesizing a new product which the wild-type cyanobacteria are unable to produce.[2]

The use of genetically modified cyanobacteria raises the question of biosafety: what would happen if they escape from a closed system, such as a bioreactor, into the nature? Although biosafety issues can have a great impact on public acceptance of genetically modified cyanobacteria, they have remained largely unexplored. Therefore, we would like to develop tools for controlling genetically modified cyanobacteria. We wanted that unintentionally released genetically modified cyanobacteria start a suicide programme if they escape into the wild. For this purpose, we have prepared a plasmid-encoded suicide genetic switch on the basis of a non-specific nuclease NucA and its inhibitor Nui from the cyanobacterium *Anabaena* and incorporated the plasmid into the model cyanobacterium *Synechocystis* sp. PCC 6803. We were the first to report any such biocontainment implementation in cyanobacteria.[3]

When the nuclease NucA is not inhibited, it degrades cyanobacterial genetic material, thus killing the cell and simultaneously preventing horizontal transfer of the transgenes to other bacteria in the nature. Because we want that the nuclease kills cells only under specific conditions, the *nucA* gene was fused to a variant of the inducible promoter P*copM*. The transcription of genes under P*copM* promoter is greatly induced in the presence of metal ions. The gene for nuclease inhibitor Nui was fused to the weak constitutive promoter P*rnpB* to protect cells from possible leaky nuclease production in absence of the inducer. Successful autokilling of *Synechocystis* cells with the suicide switch was observed after addition of zinc ions, while the wild-type *Synechocystis* continued to grow. By RT-PCR, we showed that the addition of zinc ions increases transcription levels of the NucA nuclease gene while transcription of the gene for its inhibitor Nui remained unchanged.

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Robotsko plovilo za nadzor cianobakterij

Robotic floating platform for control of cyanobacteria

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Večina cianobakterijskih vrst proizvaja nevarne cianotoksine, ki imajo negativne socialno-ekonomske učinke ter vplivajo na zdravje ljudi in živali. Na ogroženih sladkovodnih telesih je zato potreben reden monitoring za zaznavo že nizkih koncentracij cianobakterij, zgodnje opozarjanje in pravočasno izvajanje kontrole številčnosti cianobakterijske populacije. Poznanih je več metod za kontrolo cianobakterijskih in algnih cvetenj ter razgradnjo njihovih toksinov,[1] nekatere od teh so tudi precej invazivne, saj uporabljajo agresivne kemikalije.[2] Robotsko plovilo (RP), ki ga predstavljamo, je namenjeno izvajanju monitoringa vodnih teles kot tudi izvajanju kontrole nad cianobakterijsko populacijo in predstavlja rešitev za številna jezera in zajetja pitne vode, ki imajo potencial za prekomerno rast cianobakterij.

RP omogoča avtomatiziran monitoring za kvantifikacijo cianobakterij, ob povišanih koncentracijah pa vrši tudi nadzor nad številčnostjo cianobakterij. V primerjavi s tradicionalnim pristopom monitoringa, ki je časovno potraten in stroškovno neugoden, je prednost uporabe RP-ja v hitrem izvajanju monitoringa kvalitete vode v realnem času. Druga prednost je ciljana oziroma lokalna kontrola rasti cianobakterij, ki je na področjih z večjo koncentracijo cianobakterij dosežena s tretiranjem vode z elektrokemično oksidacijo v elektrolitski celici z borom dopiranimi elektrodami. S povečanjem števila elektrod je možno učinkovitost elektro-oksidacijskega tretiranja še izboljšati.[3] V primerjavi z nekaterimi drugimi pristopi pri tretiranju vode z elektrolizo v vodo ne vnašamo nevarnih kemikalij in snovi, ki tvorijo sedimente. Predstavljen RP je okolju prijazen in opremljen s sončnimi celicami, GPS modulom, žiroskopom, pospeškometrom, kompaems, ultrazvočnimi senzorji razdalje in video kamero. Vsi nameščeni senzorji skupaj z mehkim regulatorjem omogočajo natančno navigacijo plovila, izogibanje oviram in avtomatsko pristajanje. Tehnična oprema v kombinaciji s senzorskim sistemom za zaznavanje alg in cianobakterij omogoča uvid v prostorsko razporeditev cianobakterij v vodnem telesu. Parametri vode se merijo v pretočni merilni komori, ki je s cevmi povezana z elektrolitsko celico in avtomatskim vzorčevalnikom vode, ki shrani vzorce vode za nadaljnjo laboratorijsko analizo. Poleg natančne prostorske informacije o stanju vodnega telesa, lahko z uporabo nameščenega vitla in cevnega sistema pridobimo tudi globinske meritve vodnega stolpca. Sistem avtonomnega odločanja se odloča ali naj izvede meritev parametrov vode, vzame vzorec vode ali naj tretira vodo. Sistem glede na prej zaznane lokacije z visoko koncentracijo cianobakterij določi tudi prednostne plovne poti. Vse aktivnosti RP-ja in merilni rezultati so posredovani TCP/IP strežniku, kjer so podatki prikazani v realnem času in shranjeni v SQL bazo. Na zemljevidu strežniške aplikacije sta prikazana prepotovana pot in trenuten položaj čolna na jezeru. Predstavljen sistem omogoča zgodnje opozarjanje na prisotnost cianobakterij in oddaljeno pošiljanje ukazov plovilu, kot na primer *tretiraj vodo*. Merjeni podatki se prikazujejo v realnem času tudi na spletni strani.

Most cyanobacteria species produce dangerous cyanotoxins, which have negative socio - economic impact, affecting human and animal health. Therefore, endangered freshwater bodies need regular monitoring and early warning system for reducing or even removal of cyanobacteria. Many methods for cyanobacterial and algal bloom control and toxin degradation have been developed,[1] some of them being invasive, as often chemicals are used.[2] We developed robotic floating platform (RFP) to monitor the water bodies and to control cyanobacteria population. The RFP represents a solution for numerous freshwater lakes and drinking water reservoirs that are subjected to excessive growth of cyanobacteria.

The proposed RFP combines an automated online monitoring of cyanobacteria population and control of cyanobacteria by electrolysis in a single platform. The first advantage of the RFP is real-time water quality monitoring and therefore early warning system compared to traditional approaches, which are very time consuming and costly. The second advantage is targeted control of cyanobacteria growth, which is achieved by treating the water with electrochemical-oxidation using an electrolytic cell. With the RFP, which is equipped with the electrolytic cell containing boron-doped diamond electrodes, the water can be treated locally in the areas of higher concentration of cyanobacteria. Electro-oxidation treatment efficiency can be improved by adding more electrodes.[3] By treating the water with electrolytic cell no dangerous chemicals and no sediment forming substances are added into water in comparison with some other approaches. The introduced RFP is environmentally friendly and solar powered. It is equipped with a GPS module, a gyroscope, an accelerometer, a compass, ultra sound distance sensors and a video camera. All these sensors together with a fuzzy logic control algorithm ensure precise navigation of the RFP, avoidance of obstacles and automatic docking. The water parameters are measured in a flow-through measuring chamber connected to electrolytic cell and automatic water sampler. Water sampler stores the samples for further laboratory analysis. In addition to detailed spatial information on water status, a depth profile can be obtained by a winch with a hose system. Autonomous decision-making system of the RFP decides whether to do a measurement, take a sample or treat the water. It also decides on preferential floating routes with previously detected locations of high incidence of cyanobacteria. All activities of the RFP and measurement results are transferred to TCP/IP server for real-time presentation and storage of the data in SQL database. On the server's map of the lake the travelled route and the current position of the boat are shown. This framework offers an early warning system for cyanobacteria and remote transmission of feedback commands to the RFP, like *treat the water*. The monitored data are displayed in real-time also on the web page.

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VABLJENO PREDAVANJE / INVITED LECTURE

Meritve premerov mešičkov v astrocitih z mikroskopijo STED

The diameters of vesicles in astrocytes studied by STED microscopy

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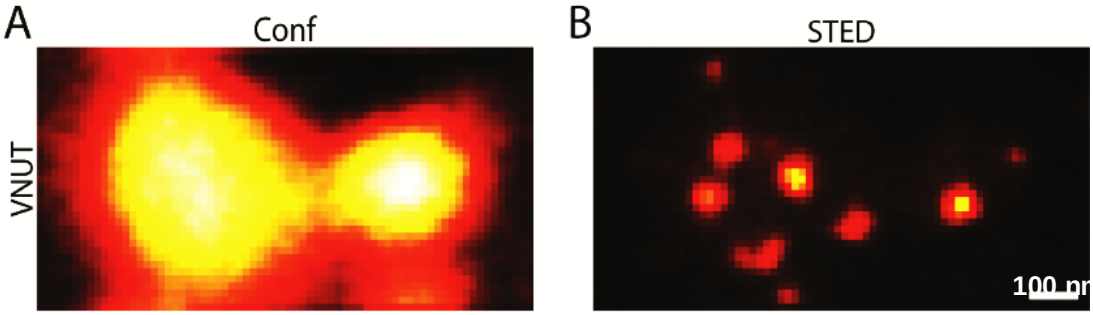
Fluorescenčna mikroskopija je tehnika, ki jo uporabljamo za neinvazivne raziskave celičnih struktur in fizioloških procesov v celicah. Pomembno omejitev fluorescenčne mikroskopije predstavlja uklonska limita, ki omeji ločljivost v ravnini fokusa na približno polovico valovne dolžine (~200 nm za nižje valovne dolžine vidnega dela spektra). Mikroskopija z vzbujenim praznjenjem emisije, bolj znana pod angleškim akronimom STED (STimulated Emission Depletion) je prva fluorescenčna mikroskopija, katere ločljivost je presegla uklonsko limito.[1] Mikroskopija STED nima teoretične omejitve ločljivosti, v praksi pa dosegamo ločljivosti do približno 20 nm. Zaradi tega uvrščamo mikroskopijo STED med t.i. superločljivostne mikroskopije, t.j. fluorescenčne mikroskopije z ločljivostjo vsaj 100 nm. Za prispevek k razvoju superločljivostnih mikroskopij, je izumitelj mikroskopije STED, Stefan W. Hell, skupaj z Ericom Betzigem in Williamom E. Moernerjem leta 2014 prejel Nobelovo nagrado za kemijo.

V predavanju bom razložil osnovni princip delovanja miroskopije STED in predstavil dvokanalni mikroskop STED, ki smo ga zgradili v sodelovanju z nemškimi kolegi. V nadaljevanju bom predstavil primerjavo meritev premerov mešičkov v astrocitih, ki smo jih izvedli s konfokalno mikroskopijo, s superločljivostno mikroskopijo s strukturirano osvetlitvijo vzorca (mikroskopija SIM; z ločljivostjo ~100 nm)[2] in z mikroskopijo STED.[3] Premere mešičkov izmerejene z različnimi fluorescenčnimi mikroskopijami bom primerjal z meritvami, ki so bile izvedene z elektronsko mikroskopijo.



Slika 1: Primerjava konfokalne mikroskopije in mikroskopije STED. Mešičke v astrocitih smo označili s fluorescenčnimi protitelesi proti vezikularnemu transporterju za nukleotide (VNUT) in posneli s konfokalno mikroskopijo (A, Conf) ter z mikroskopijo STED (B, STED). Sliki A in B predstavljata isti izsek astrocita.
Figure 1: Comparison of confocal microscopy and STED microscopy. Confocal (A, Conf) and STED (B, STED) images of fluorescently labelled vesicular nucleotide transporter (VNUT). Note that images A and B represent the same section of astrocyte.

Fluorescent microscopy has become an essential tool in the modern research of cell structures and physiological processes. In fluorescent microscopy, the resolution that can be obtained is limited by the diffraction of light to ~200 nm. Stimulated emission depletion microscopy (STED) was the first physical concept to break the diffraction barrier in the far-field fluorescence microscopy.[1] In STED microscopy, the diffraction-limited focal spot can be made infinitely small. Nonetheless, in biological samples lateral resolution of 20–40 nm is typically achieved. For his contribution to the invention of super-resolved fluorescence microscopy, Stefan W. Hell received the Nobel Prize in Chemistry in 2014, together with Eric Betzig and William Moerner.

In this presentation I will first explain the basic concept of STED microscopy, with special focus on the specific technical implementation of a two-colour STED microscope, which we developed in the collaboration with dr. Stefan W. Hell, dr. Alexander Egner and dr. Claudia Geisler. Then, I will present our study of the anatomy of single vesicles in astrocytes using confocal microscopy, structured illumination microscopy (SIM; lateral resolution of ~100 nm)[2] and STED microscopy.[3] These findings will contribute to the debate on gliotransmission.

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Ultrastruktura in diferenciacija bazalne površine črevesnega epitela med razvojem modelnega organizma Porcellio scaber

Ultrastructure and differentiation of the hindgut epithelium basal surface during development of the model organism Porcellio scaber

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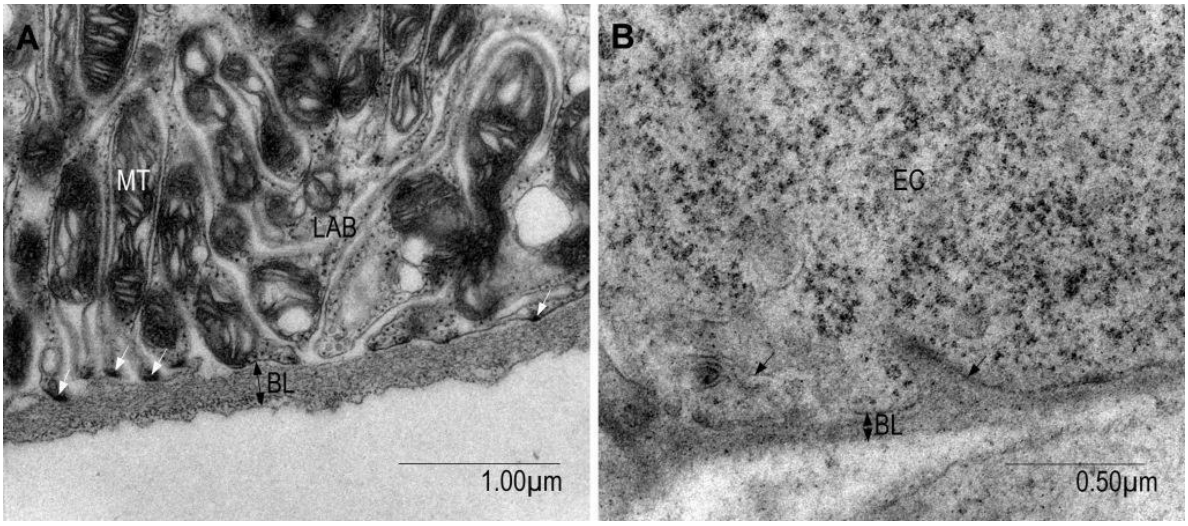
Med diferenciacijo epitelnih celic se v procesu polarizacije izoblikujeta tudi apikalna in bazalna celična površina, ki se razlikujeta po ultrastrukturi in molekularni sestavi. Pri tem ima pomembno vlogo tudi bazalna lamina. Interakcije molekulskih komponent bazalne lamine z njihovimi receptorji na plazmalemi predstavljajo signal za vzpostavitev celične polarnosti.[1,2,3] Med morfogenezo bazalna lamina tudi mehansko vpliva na oblikovanost epitelnih tkiv.[2,4]

Namen našega dela je ultrastrukturna analiza bazalne površine epitela zadnjega črevesa med diferenciacijo pri raku vrste *Porcellio scaber,* s poudarkom na analizi oblike celic in ultrastrukturi bazalnega labirinta ter bazalne lamine.

Za črevesni epitel papilatne regije odraslih živali je značilno, da so bazalni deli celic močno izbočeni v hemocel[5] in obdani z bazalno lamino fibrilarne strukture. Bazalna membrana epitelnih celic je nagubana v izrazit labirint. Ob citoplazemski strani bazalne membrane so vidne zgostitve, ki so verjetno mesta povezav celice z bazalno lamino (Slika 1A). Pri marzupijskih ličinkah črevesne celice v papilatni regiji še niso izbočene v hemocel, bazalna membrana pa tvori plitve invaginacije (Slika 1B). Površina za izmenjavo snovi med epitelom in hemolimfo je tako manjša kot pri odraslih osebkih. V črevesnem epitelu marzupijskih ličink je že razvidna homogena bazalna lamina.



Slika 1: (A) Bazalni del črevesne epitelne celice pri odraslem osebku. (B) Bazalni del črevesne epitelne celice pri marzupijski ličinki. BL – bazalna lamina, LAB – bazalni labirint, MT – mitohondriji, bele puščice – povezave celice z bazalno lamino, EC – citoplazma epitelne celice, črni puščici – invaginacije bazalne membrane.
Figure 1: (A) Basal part of the hindgut epithelial cell in adult specimen. (B) Basal part of the hindgut epithelial cell in marsupial larva. BL – basal lamina, LAB – basal labyrinth, MT – mitochondria, white arrows – cell-basal lamina junctions, EC – epithelial cell cytoplasm, black arrows – invaginations of basal membrane.

Polarization of epithelial cells during differentiation comprises also the establishment of the apical and basal cell surfaces that differ in ultrastructure and molecular composition. In this process basal lamina plays a crucial role. Interactions of the basal lamina molecular components with their receptors at the plasmalemma provide a signal for the establishment of cell polarity.[1,2,3] During morphogenesis basal lamina also mechanically shapes epithelial tissues.[2,4]

The aim of our study is ultrastructural analysis of the hindgut epithelium basal surface during its differentiation in crustacean species *Porcellio scaber*. Our focus is the analysis of the cell shape and ultrastructure of the basal labyrinth and basal lamina.

Hindgut epithelium in the papillate region of adults consists of dome shaped epithelial cells which bulge into the hemocoel[5] and are underlined by a basal lamina of fibrillary structure. The basal membrane of hindgut cells is folded into a pronounced basal labyrinth. At the cytoplasmic face of basal membrane electron dense patches are visible, which are likely the sites of junctions between the cell and basal lamina (Figure 1A). In the papillate region of marsupial larvae the basal parts of epithelial cells are not yet bulged into the hemocoel and basal membrane forms shallow invaginations (Figure 1B). Thus, the surface for the exchange of materials between the epithelium and the hemolymph is not as extensive as in adults. At the basal surface of the hindgut epithelium a basal lamina of homogenous appearance is already discerned in marsupial larvae.

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Razvoj nedestruktivnih metod za citogenetske analize močerila (Proteus anguinus)

Developing non-destructive methods for cytogenetic analysis in the European blind cave salamander (Proteus anguinus)

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Določitev spola močerila je po zunanjih morfoloških znakih zelo negotova, saj ni izraženega spolnega dimorfizma. Podatek o spolu pa je ključen za določitev strukture in dinamike populacije ter uspešen program razmnoževanja v ujetništvu. Eden od možnih načinov določitve spola je identifikacija heteromorfnih spolnih kromosomov.

Večina citogenetskih metod pri repatih dvoživkah zahteva žrtvovanje živali, kar pa ni zaželeno, če gre za ogroženo in zaščiteno vrsto. Zaradi tega smo razvili nedestruktivno metodo, povzeto po humani citogenetiki,[1] ki zagotavlja preživetje osebka, uporabljenega za raziskavo. Gre za *in vitro* metodo gojenja primarnih celičnih kultur krvnih celic in je že bila preizkušena na repatih dvoživkah.[2] Mitotske delitve v kulturi krvnih celic so spodbujene s fitohemaglutininom (PHA-M), to je mitogen, ki spodbudi celične delitve limfocitov. Po nekaj dneh v kulturi moramo celične delitve ustaviti v metafazi, da lahko analiziramo morfologijo kromosomov (velikost in obliko) in njihovo število. Za zaustavitev celičnih delitev v metafazi uporabimo kolhicin ali njegovo biosintezno predstopnjo kolcemid. Nato celice izpostavimo hipotonični raztopini, da ostanejo samo jedra ter kromosomi, in jih fiksiramo. Pripravljene preparate barvamo, kromosome lahko tudi progamo. Tako pridobljen material je primeren za nadaljnje citogenetske analize.

Ta metoda se v humani citogenetiki že dolgo uporablja, vendar zahteva veliko modifikacij, če jo želimo uporabiti na repatih dvoživkah. Močerilove celice vsebujejo veliko količino DNA, zato je njihov celični cikel mnogo daljši kakor pri ljudeh, kažejo tudi drugačno občutljivost na mitogen, koncentracijo soli (ozmolarnost), kolhicin, temperaturo in čase izpostavljenosti. Bili smo uspešni z optimizacijo omenjenih parametrov za kulturo krvnih celic močerila (Sl. 1). Razen kulture krvnih celic smo vzgojili tudi kulturo celic iz majhnega koščka repnega tkiva. Razvoj omenjenih metod je pomemben, saj odpira vrata novim pristopom raziskav na močerilu, ki do sedaj niso bile možne. Celične kulture niso le nepogrešljiv material za citogenetske analize, ampak potencialno tudi za genetske, biokemijske in toksikološke študije. Gre za nedestruktivno metodo, kar je še posebej pomembno za zaščiteno vrsto, kakor je močeril.

Sex identification in the European blind cave salamander (*Proteus anguinus)* using external morphological characters is very unreliable because of the lack of sexual dimorphism. Accurate identification of sex is crucial for the determination of population structure and dynamics, and for establishing a breeding program in captivity. One possible way for sex identification is to use cytogenetics to identify heteromorphic sex chromosomes.

Most published cytogenetic methods for salamanders require sacrificing the animals, which is not desirable especially if the species is vulnerable or protected. Therefore, we developed a non-destructive method derived from human cytogenetics, which ensures the survival of the specimens used for research. The method was based on one that was already used for salamander blood cells,[1] and it involves the *in vitro* cultivation of blood cells in culture. Mitotic cell division in the blood cell culture must be stimulated with phytohemagglutinin (PHA-M), a mitogen that induces cell division in lymphocytes. After a few days in culture, the cell divisions have to be arrested in metaphase for proper analysis of chromosome morphology and number. To stop cell divisions in mitotic metaphase it is necessary to use colchicine or its biosynthetic pre-step colcemid. Cells are then exposed to hypotonic solution so that only nuclei and chromosomes remain and then they are fixed. After the chromosomes are "splashed" and dried onto glass slides, the chromosomes are stained with or without banding, and the sample is then ready for further cytogenetic analyses.

This method has long been used for human cytogenetics, but required extensive modification for use with salamanders. Because the cells of *Proteus* contain so much DNA, their cell cycles are much longer than those of humans and they show different sensitivity to mitogen, salt concentration (osmolality) and colchicine, as well as cultivation temperature and incubation time. We were successful with the optimization of these parameters for *Proteus* blood cell culture (Fig. 1). In addition to the blood cell cultures, we also cultivated cells from tail explants. The importance of this development is that it opens the door to a whole new approach to research in *Proteus* that was simply not possible before. Cell cultures are not only indispensable material for cytogenetic analyses but potentially also for genetic, biochemical, and toxicological studies using a non-destructive method, which is especially important for protected species like *Proteus*.

Slika 1: Metafazni kromosomi močerila (*Proteus anguinus*), pridobljeni iz kulture krvnih celic. Merilo = 10 µm.
Figure 1: Mataphase chromosomes of *Proteus* (*Proteus anguinus*), obtained from blood cell culture. Bar = 10 µm.

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Vpliv starosti na pigmentacijo jeter pri neotenični jamski repati dvoživki Proteus anguinus

Effect of age on liver pigmentation in the neotenic blind cave salamander Proteus anguinus

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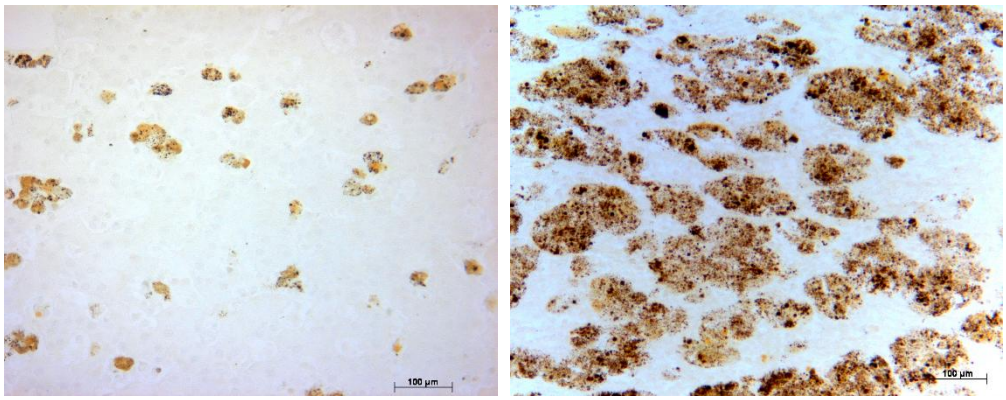
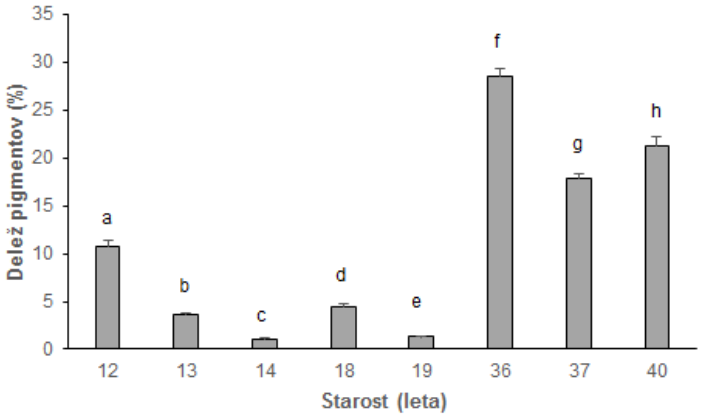
Človeška ribica (*Proteus anguinus*) je edini vretenčar v Evropi, ki stalno živi v jamah. Zanj je značilna dolga življenjska doba, saj lahko dočaka tudi več kot 70 let. Zaradi dolgoživosti je *P. anguinus* postal zanimiv za preučevanje procesov staranja.[1, 2] Na pospešeno staranje lahko vplivajo tudi reaktivne kisikove zvrsti (ROS), ki nastajajo tekom normalnega celičnega metabolizma. Še posebej so izpostavljeni oksidativnemu stresu notranji organi, ki so zelo metabolno aktivni, med njimi so tudi jetra.[3, 4] V jetrih človeške ribice so pigmentne celice, ki vključujejo melanin in železove pigmente, in so pomembna zaščita pred prostimi radikali.[5]

Z našo raziskavo smo preverili korelacijo med pigmentnim deležem v jetrih človeških ribic in starostjo osebkov. S pomočjo zajema slik in računalniškega programa *ImageJ* (Rasband) smo analizirali posnetke histoloških rezin jeter arhiviranih osebkov *P. anguinus* poznane starosti. Osebki so bili izleženi in vzgojeni v jamskem laboratoriju CNRS Station d'Ecologie Expérimentale v Moulisu, Francija. Ugotovili smo, da se delež pigmentnih celc v jetrih starejših živalih poveča (Sl. 1) in sicer za več kot 4x v primerjavi z mladimi osebki (Sl. 2). Pigmenti v jetrih človeške ribice so najverjetneje vključeni v pomembne mehanizme, ki prispevajo k njeni dolgoživosti.[6]



Slika 1: Pigmentne celice jeter pri mlademu (13 let, levo) in starejšemu (36 let) osebku (desno) *P. anguinus.*
Figure 1: Liver pigment cells in young (13 years, left) and old (36 let) specimen (right) of *P. anguinus.*



Slika 2: Delež pigmentnih celic v jetrih različno starih osebkov *P. anguinus*, prikazana so povprečja ± SN (N=2-3). Različne črke nad stolpci označujejo statistično značilne razlike pri p<0,001 s primerjavo glede na 12-letni osebek.

Figure 2: Liver pigmentation ratio of *P. anguinus* for each specimen. Average ± SN (N=2-3). Different letters represent statistically significant difference p<0,001 when compared with 12 years old animal.

The blind cave salamander *(Proteus anguinus)* is the only permanently cave-dwelling vertebrate in Europe. It has an extended lifetime (70 years and more). High life expectancy makes *P. anguinus* an ideal model animal for studying aging processes.[1, 2] One reason for faster aging is the presence of reactive oxygen species (ROS), which are synthetized during normal cell metabolism. Metabolically highly active inner organs, such as livers, are exposed to oxidative stress as well.[3, 4] Pigment cells in the *P. anguinus* liver contain melanin and iron pigments, which represent important defense mechanism against free radicals.[5]

In order to determine whether a correlation exists between aging and pigmentation rate of *P. anguinus* liver, we analyzed images of histological slides of archived specimens with *ImageJ* software (Rasband). All the studied animals were hatched and raised in the cave laboratory of the CNRS Station d'Ecologie Expérimentale in Moulis, France, so specimens were of known age. It was shown that liver pigmentation in old animals increased (Fig.1) by 4-times when compared to younger animals (Fig. 2). Pigments in the liver of *P. anguinus* are likely to be included in the important mechanisms, which contribute to its longevity.[6]

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Skeletokronološka analiza in interpretacija linij zavrte rasti v dolgih kosteh močerila Proteus anguinus

Skeletochronological analysis and interpretation of lines of arrestet growth in long bones of blind cave salamander Proteus anguinus

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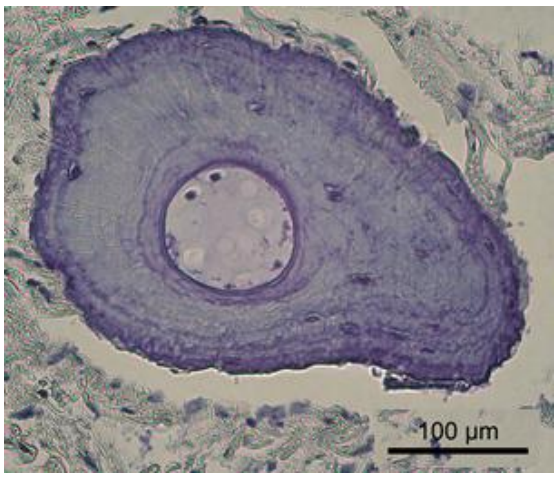
Skeletokronologija je metoda ugotavljanja starosti pri vretenčarjih na podlagi rastnih linij na dolgih kosteh[1]. Natančneje se za ugotavljanje starosti uporabljajo linije zavrte rasti oz. LAG (lines of arrested growth), ki so posledica začasne zaustavitve rasti osebka ob neugodnih okoljskih pogojih.[2] Ker je rast kostnine periodičen pojav, lahko s štetjem LAG-ov ugotovimo starost posameznega osebka.[2] Nastanek LAG je genetsko pogojen in močno ojačan z neugodnimi pogoji v okolju, zaradi česar je prisotnost LAG možno zaslediti tudi pri osebkih, ki ne živijo v okolju s sezonskimi cikli.[3]

V naši raziskavi smo želeli oceniti primernost skeletokronologije, kot metode za ocenjevanje starosti pri močerilu – *Proteus anguinus.* Analiza linij zavrte rasti je bila narejena na dolgih kosteh okončin 40-ih arhiviranih osebkov s poznano starostjo (8 – 40 let), ki so bili izleženi in vzgojeni v jamskem laboratoriju CNRS Station d'Ecologie Expérimentale v Moulisu, Francija. Pri vseh osebkih v vzorcu so bile zastopane linije zavrte rasti (LAG), vendar je bila večina neizrazitih, prav tako se njihovo število pri večini ni ujemalo z dejansko starostjo živali (sl. 1). Neizrazitost LAG pripisujemo specifični strukturi kostnine, kot tudi konstantnim abiotskim in biotskim pogojem jamskega laboratorija. Čeprav je močeril jamska dvoživka je zastopanost LAG posledica endogenih ritmov, ki so genetski ostanek prednikov živečih na površju.[3]



Slika 1: Prečni prerez tibije močerila (starost 30 let). Erhlich hematoksilin.
Figure 1: Cross-section of tibia from olm (age 30 years). Ehrlich hematoxylin.

Skeletochronology is a method for evaulating the age of vertebrates based on growth marks in long bones.[1] More specifically, it utilizes lines of arrested growth (LAG), which are the result of unfavourable environmental conditions.[2] Because the bone growth is a cyclical phenomenon, we can use the number of LAG for evaulating the age of individual specimens.[2] LAG formation is a genetically based, circannual rhythm synchronised with seasonal cycles. Therefore, LAGs are also formed in tropical and other habitats with very little seasonality.[3]

The purpose of this study was to evaluate the applicability of skeletochronology for evaluating the age of the blind cave salamander *Proteus anguinus*. Analysis of LAG was done on stained histological sections of long bones of the limbs of archived specimens of known age (8-40 years) that were hatched and raised in the cave laboratory of the CNRS Station d'Ecologie Expérimentale in Moulis, France. LAG were visible in all specimens, but in most cases there was no relationship between the number of LAG and the actual age of the animals (Fig.1). LAG were not clearly visible, which is probably connected with the constant biotic and abiotic conditions of the cave environment, as well as the specific structure and growth dynamics of the bone. We think that the visible LAG in the long bones of *Proteus* represent an intrinsic mode or cycle of bone growth retained from surface ancestors that lived in changing environmental conditions.[3]

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VABLJENO PREDAVANJE / INVITED LECTURE

Presnova glukoze v možganih

Glucose metabolism in brain

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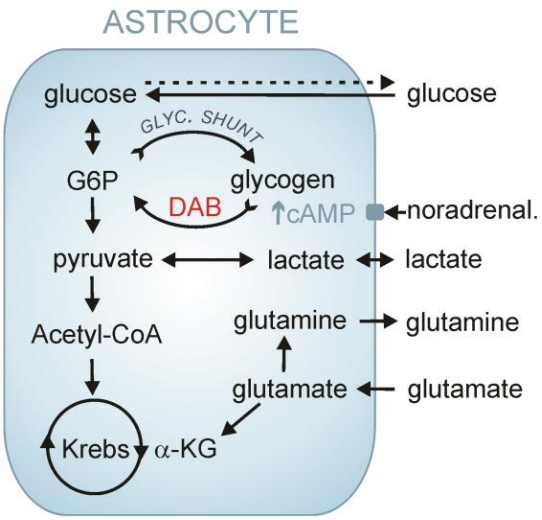
Možgani predstavljajo le dva odstotka telesne mase, porabijo pa kar 20 % vse glukoze in 25 % kisika. Če koncentracija glukoze v krvni plazmi pade pod 4 mM, to vpliva na kognitivne procese. Največji porabnik energije v možganih je Na^+/K^+-ATP-aza, ki vzdržuje gradiente ionov, ki so nujni za membranski potencial. Glukoza se prenese skozi krvno-možgansko pregrado, ki jo sestavljajo kapilarne endotelijske celice, bazalna membrana, ki ovija kapilare in izrastki astrocitov. Astrociti so najštevilčnejše celice glije, ki jih je v možganih enako kot nevronov. Astrociti imajo prvenstveno mesto pri privzemu glukoze iz plazme ob povečani možganski aktivnosti, saj so umeščeni med krvne žile in nevrone. S svojimi izrastki povezujejo obe strukturi ter zagotavljajo glukozo in druge molekule za energijski metabolizem v nevronih. Nevroni namreč nimajo neposrednega dostopa do glukoze, saj jih od notranjosti krvnih žil ločujejo štiri membrane – dve membrani endotelija žil in dve membrani astrocita. Glukoza se v astrocitih lahko uskladišči v obliki glikogena. Skoraj polovico vse glukoze, ki vstopi v astrocite, se presnovi posredno prek vgrajevanja v glikogen. Nevroni lahko ob pomanjkanju glukoze kot vir energije uporabljajo tudi laktat. Nekatere raziskave pa so pokazale, da je laktat za nevrone morda izključni vir energije. V taki glukozno-laktatni poti astrociti prevzemajo glukozo iz krvi, jo presnovijo v laktat in ga posredujejo nevronom. Razvoj senzorskih fluorescenčnih proteinov je omogočil dinamično spremljanje koncentracije glukoze in laktata v posamezni celici. Zaznamo lahko spremembe v koncentraciji po stimulaciji celic z nevrotransmiterji, agonisti kanabinoidnih receptorjev ali inzulina.

Brain represent only two percent of the whole body weight, and yet consume as much as 20% of glucose and 25% oxygen. If the concentration of glucose in the blood plasma falls below 4 mM, it affects the cognition. The most energy demanding process in the brain is maintaining the ion gradients by the Na^+/K^+-ATP-ase, crucial for membrane potential. Glucose is passed through the blood-brain barrier, consisting of capillary endothelial cells, basement membrane, and astrocyte endfeet. Astrocytes are the most abundant glial cells, equal in number as neurons. In increased brain activity, astrocytes are the primary site for glucose uptake. They provide glucose and other energy molecules to neurons. Neurons are separated from blood plasma by four membranes: two membranes of endothelial cells and two membranes of perivascular astrocytes. Glucose is stored in astrocytes as glycogen molecule. Almost half of all the glucose that enters the astrocytes is metabolized temporarily to glycogen. In glucose deficiency, neurons may use lactate as an energy source. Some studies have shown that in neurons the lactate may be the exclusive source of energy. In such a glucose-lactate pathway or shuttle, astrocytes receive glucose from the blood, which is then converted into lactate and transmitted to the neurons. The development of sensor fluorescent proteins has enabled the dynamic monitoring of blood glucose and lactate in single living cell. We recorded changes in concentration of glucose after stimulation of cells by neurotransmitters, agonists of cannabinoid receptor, or insulin.



Slika 1: Energijski metabolizem astrocitov. Zaloga glikogena v astrocitih lahko zagotavlja energijo med hipoglikemijo ali tudi med normalnim metabolizmom možganov. Inkubacija celic z DAB, ki je inhibitor glikogen fosforilaze a, zavre sproščanje te glikogenske zaloge. Glikogen je vir laktata, ki se lahko prenese k nevronom, ali pa se pretvori v piruvat, ki nadalje vstopi v Krebsov cikel. Glutamat je pomemben živčni prenašalec, hkrati pa tudi pomembna zaloga energije. Po stimulaciji z adrenalinom ali noradrenalinom se glikogen pospešeno razgrajuje, kot posledica zvišane koncentracije cAMP v celicah. To pa povzroči zvišano koncentracijo glukoze v citoplazmi.

Figure 1: Energy metabolism in astrocytes. The glycogen reservoir via glycogen shunt in astrocytes can provide fuel for energy production during hypoglycemia, as well as during normal brain metabolism. Incubation of astrocytes with DAB, a glycogen phosphorylase a (GPa) inhibitor inhibits the glycogen shunt. The glycogen serves as the source of lactate which may be transferred to neurons, or converted to pyruvate, which enters the Krebs cycle. In addition to neurotransmission, glutamate also serves as an important potential fuel reserve. Upon noradrenaline/adrenaline stimulation glycogen breakdown may be stimulated in response to increased cAMP level, leading to cytosolic glucose increase.

Utišanje helikaze RECQ1 vpliva na proliferacijo glioblastomskih celic v možganih zarodkov cebric (Danio rerio)
RECQ1 helicase depletion affects proliferation of glioblastoma cells in zebrafish (Danio rerio) embryonic brain

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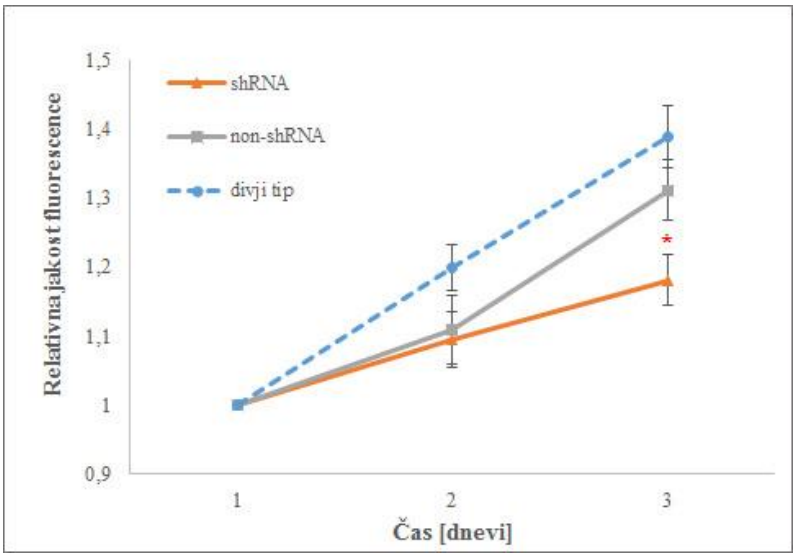
Cebrice (*Danio rerio*) so izredno pomemben vretenčarski modelni organizem za raziskave rakavih celic, saj imajo visoko genetsko homologijo z ljudmi, kar je poleg prosojnosti zarodkov njihova glavna prednost. Ksenotransplantacija fluorescenčno označenih tumorskih celic omogoča opazovanje le teh v živih zarodkih pri visoki ločljivosti v realnem času. V našem delu smo uporabili človeške glioblastomske celice U87-MG, ki izražajo gen za rdeč fluorescenčni protein DsRed, zanimalo pa nas je, ali utišanje helikaze RECQ1 vpliva na proliferacijo teh celic.

S fluorescenčno mikroskopijo smo spremljali jakost fluorescence celic tako *in vitro* kot tudi *in vivo* v zarodkih cebric. Implantacija celic v možgane zarodkov omogoča razvoj in preučevanje tumorja v okolju, ki je podobno tistemu v človeških možganih. V poskusih smo uporabili tri celične klone – celice s trajno utišano helikazo RECQ1 (shRNA), celice s praznim vektorjem za RNA-interferenco (non-shRNA) ter celice divjega tipa. S poskusom *in vitro* smo ugotovili, da odsotnost helikaze RECQ1 upočasni proliferacijo glioblastomskih celic, kar smo kasneje potrdili tudi z ksenotransplantacijo utišanih celic v možgane zarodkov cebric. RECQ1 namreč sodeluje pri replikaciji in popravljanju DNA ter na ta način ohranja stabilnost genoma in posredno vpliva tudi na proliferacijo celic. Posledici utišanja helikaze z RNA-interferenco sta upočasnjena rast in proliferacija, kar je ključnega pomena za zdravljenje bolnikov z glioblastomom, ki je najpogostejši možganski tumor z visoko stopnjo smrtnosti.

Rezultati naših poskusov kažejo na RECQ1 kot potencialno molekularno tarčo in poudarjajo pomembnost nadaljnjih raziskav za uporabo RNA-interference v zdravljenju rakavih bolnikov in uporabo cebric za raziskave glioblastomskih celic.



Slika 1: Spreminjanje relativne jakosti fluorescence v možganih zarodkov cebric. Vrednosti na grafu predstavljajo povprečje ± SE; * predstavlja $p < 0,05$ med shRNA in non-shRNA; n (shRNA) = 64; n (non-shRNA) = 55; n (divji tip) = 52.

Figure 1: Time courses of changes in relative fluorescence intensity in the zebrafish embryonic brain. Values represent average ± SE; * represents $p < 0,05$ in between shRNA and non-shRNA; n (shRNA) = 64; n (non-shRNA) = 55; n (wild type) = 52.

Zebrafish (*Danio rerio*) represents an important vertebrate model organism with several advantages, such as transparent embryos and high genetic homology to humans. Therefore, they are widely used for cancer research. Xenotransplantation of fluorescently labelled cancer cells into zebrafish embryos enables visualisation of cancer cells in live embryos at high resolution in real time. In our study, we used glioblastoma cells U87-MG DsRed, which express the red fluorescent protein DsRed. The aim of our work was to evaluate the effect of RECQ1 helicase depletion on proliferation of glioblastoma cells.

Fluorescence microscopy was used for evaluation of the fluorescence intensity of glioblastoma cells. We performed both *in vitro* and *in vivo* assays. Implantation of cells into zebrafish embryonic brain enables observation of these cells in an environment that is similar to that of humans. We used three different cell clones – cells with permanently depleted RECQ1 helicase (shRNA), cells, transfected with empty vector for RNA interference and wild type cells. Results show that RECQ1 depletion affects cellular proliferation both *in vitro* and *in vivo* in zebrafish brain. RECQ1 is involved in DNA replication and repair and thus maintains genome stability. Depletion of RECQ1 by RNA interference results in a reduction of cellular growth and proliferation. These two characteristics are vital for the treatment of glioblastoma, which is the most common brain tumour with the highest mortality rate.

Taken together, our results indicate that RECQ1 might represent potential molecular target. Application of RNA interference for cancer treatment should be focus of further investigations. Furthermore, we demonstrated that the zebrafish embryo could be used as a model organism in cancer research.

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Določanje nivojev infliksimaba in protiteles proti infliksimabu med zdravljenjem KVČB

Determination of infliximab trough levels and antibodies to infliximab during the treatment of IBD

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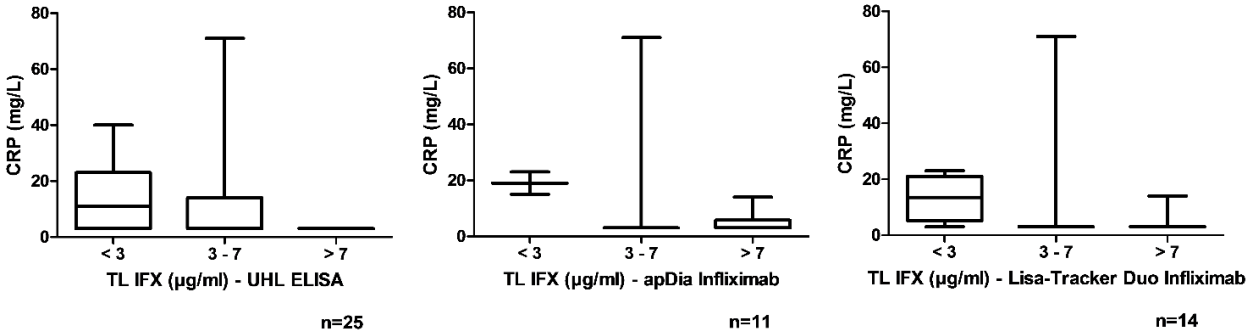
Kronična vnetna črevesna bolezen (KVČB) je bolezen prebavil, katere glavne oblike so Crohnova bolezen, ulcerozni kolitis in nedeterminirani kolitis. Ko standardno zdravljenje (5-aminosalicilna kislina, kortikosteroidi, imunosupresivi) ni več uspešno, sledi zdravljenje z biološkimi zdravili, med katera sodi tudi infliksimab (IFX).[1] Koncentracija IFX (TL; angl. trough level) in protiteles proti infliksimabu (ATI; angl. antibodies to IFX) v serumu sta pomembna podatka za odločitev o nadaljnjem zdravljenju bolnika.

Primerjali smo 2 komercialno dostopna ELISA testa ter nekomercialni ELISA test, ki so ga razvili na Univerzi v Leuvnu v Belgiji za določanje TL IFX. Za določanje ATI smo primerjali 2 testa ELISA - belgijskega ter komercialnega. TL IFX smo določali s testoma LISA-TRACKER Duo Infliximab in apDia Infliximab, dobljene rezultate pa smo primerjali z rezultati, pridobljenimi v Belgiji, z UHL ELISA. Koeficient determinacije (R^2) je pri primerjavi apDia Infliximab in UHL ELISE znašal 0,86, pri primerjavi LISA-TRACKER Duo Infliximab in UHL ELISE pa 0,76. S testom LISA-TRACKER Duo Infliximab smo določali tudi nivoje ATI, ki so bili predhodno že izmerjeni z UHL ELISO. Metodi za zaznavo protiteles se med seboj razlikujeta, zato dobljenih koncentracij ne moremo direktno primerjati med seboj. Test LISA-TRACKER Duo Infliximab je bil občutljivejši.

Z zbranimi nekaterimi kliničnimi podatki o pacientih, smo nato vse 3 teste za določanje TL IFX in testa za določanje ATI, primerjali še prek kliničnih podatkov.[2] Rezultati kažejo, da TL IFX pri vseh treh testih primerljivo korelirajo z vrednostmi C-reaktivnega proteina, vrednostmi fekalnega kalprotektina in endoskopsko remisijo. Visoki TL IFX so torej pri vseh treh testih povezani z boljšo kontrolo vnetja. Prav tako je odsotnost ATI pri obeh testih povezana z endoskopsko remisijo, prisotnost ATI pa z aktivno boleznijo, določeno z endoskopijo. Pri obeh testih so zaznavni TL IFX povezani z daljšim časom do ukinitve IFX, medtem ko rezultati obeh testov kažejo na povezanost visokih ATI z ukinitvijo IFX.



Slika 1: Povezava med serumskimi koncentracijami infliksimaba in CRP
Figure 1: Correlation of infliximab trough levels with CRP

Inflamatory bowel diseases are most commonly manifested as Crohn's disease, ulcerative colitis and indetermined colitis. When standard treatment (5-aminosalicylic acid, corticosteroids, immunosuppressants) is no longer efficient, patient is switched to therapy with biological medicines, most commonly infliximab (IFX).[1] Through levels of IFX (TL), and antibodies to infliximab (ATI) in blood are important factors for evaluation of treatment and development of disease.

In our research we compared 2 commercial and 1 in-house developed ELISA test for determination of TL IFX and 1 commercial and 1 in-house developed ELISA test for determination of ATI. TL IFX were measured with LISA-TRACKER Duo Infliximab, apDia and in-house developed UHL ELISA test (University hospital of Leuven, Belgium). Coefficient of determination (R^2) was 0,86 when comparing apDia Infliximab and UHL ELISA, and 0,76 when comparing LISA-TRACKER Duo Infliximab and UHL ELISA. ATI levels were detected with LISA-TRACKER Duo Infliximab and UHL ELISA. Because methods in detection differ we could not directly compare results. LISA-TRACKER Duo Infliximab however showed lower cut-off value.

After molecular determination we evaluated clinical data of patients treated for IBD.[2] Results imply that TL IFX correlate with concentrations of C-reactive protein and faecal calprotectin. Higher TL IFX measured with all three ELISA tests indicates better control of inflamation. Also the absence of ATI measured with both ELISA tests indicates endoscopic remission. Detectable TL IFX prolong efficient treatment of IBD patients and higher ATI level is a good indicator for termination of treatment with IFX.

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Vpliv nanodelcev titanovega dioksida na preživetje celic THP-1

Effect of titanium dioxide nanoparticles on survival of THP-1 cells

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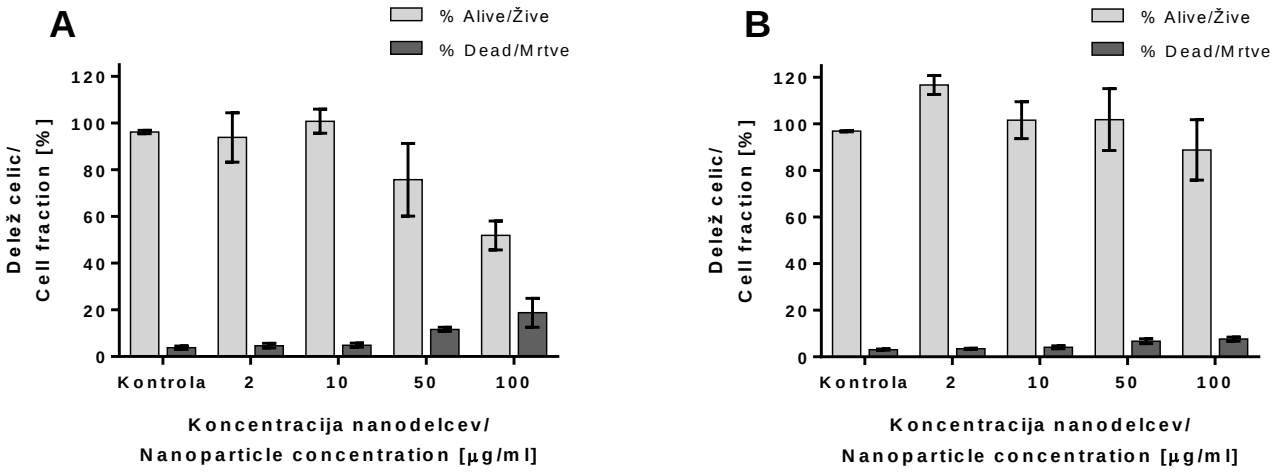
Nanodelci (ND) titanovega dioksida (TiO_2) so pomemben industrijski material, ki se uporablja tudi kot aditiv v kozmetiki, zdravilih in barvilih za živila, zaradi česar z njimi pogosto prihajamo v stik.[1] V telesu ND pogosto prve prevzamejo fagocitne celice imunskega sistema (npr. makrofagi), zato lahko pride do neželenih interakcij med ND in imunskim sistemom, ki lahko vplivajo na njegovo pravilno delovanje.[2] Izpostavljenost ND lahko med drugim vpliva na preživetje makrofagov, nastanek toksičnih reaktivnih kisikovih intermediatov ter na sposobnost fagocitiranja in predstavljanja antigenov limfocitom T, kar vpliva na nadaljnji potek imunskega odziva.[3,4]

V naši raziskavi smo na človeški monocitni celični liniji THP-1 ovrednotili citotoksičnost različnih koncentracij dveh tipov komercialno dostopnih TiO_2 ND (food-grade TiO_2 (FG) in TiO_2 P25). Upad viabilnosti makrofagov smo zaznali samo pri zelo visokih koncentracijah ND P25 (50 in 100 µg/ml), kjer smo opazili tudi porast v številu mrtvih celic. ND TiO_2 food-grade pri testiranih koncentracijah ne kažejo vpliva na viabilnost.

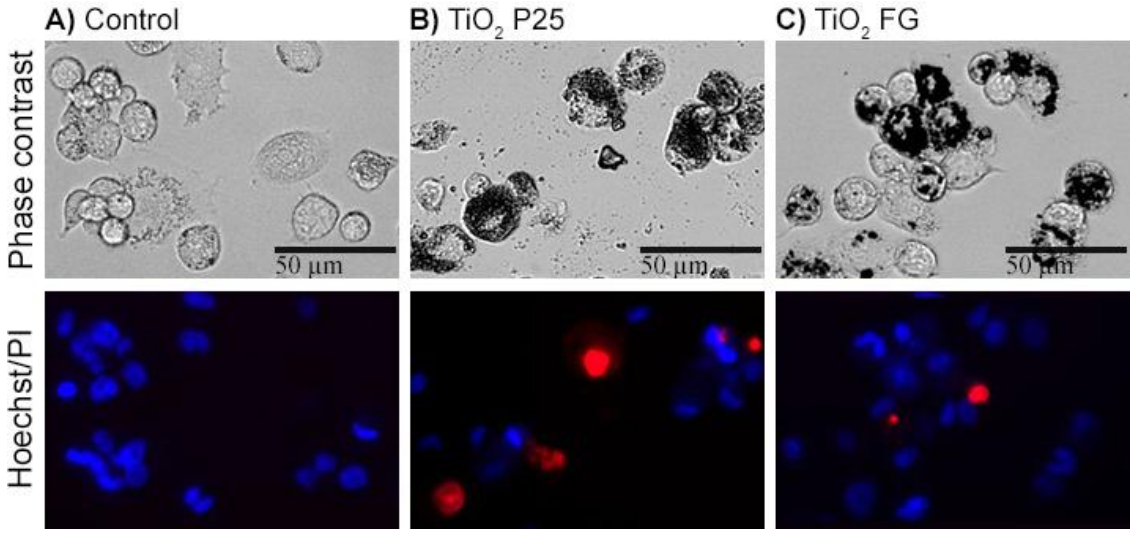


Slika 1: Vpliv naraščajoče koncentracije TiO_2 nanodelcev P25 (**A**) in FG (**B**) na diferencirane makrofage THP-1 po 24-urni izpostavitvi. Prikazan je delež živih celic (viabilnost) in mrtvih celic za posamezne koncentracije, določen z diferencialnim barvanjem Hoechst/PI. Prikazane vrednosti predstavljajo povprečje treh poskusov s standardno napako.

Figure 1: Effect of increasing concentrations of TiO_2 nanoparticles P25 (**A**) and FG (**B**) on differentiated macrophages THP-1 after 24 hours of incubation. The results are presented as percentage of viable cells (viability) and dead cells for each concentration, determined by Hoechst/PI differential staining. Data shown represent the mean and standard error of three independent experiments.

Titanium dioxide (TiO_2) nanoparticles (NPs) are an important industrial material, also used as additives in cosmetics, pharmaceuticals, and as food colorants, therefore we are frequently exposed to them.[1] Once in the body, NPs are often first picked up by the phagocytic cells of the immune system (e.g. macrophages), which can lead to undesirable interactions between NPs and the immune system and affect its functionality.[2] Exposure to NPs can affect macrophage survival, formation of toxic reactive oxygen intermediates, engulfment of cellular debris by macrophages and antigen presentation to T lymphocytes, which also affects further immune reactions.[3,4]

In this study, human monocytic cell line THP-1 was used to evaluate the toxicity of different concentrations of two types of commercially available titanium dioxide NPs (food-grade (FG) TiO_2 and TiO_2 P25). Decrease in viability of macrophages was observed only at very high concentrations of P25 NPs (50 in 100 µg/ml), where we also observed an increase in the number of dead cells. Food-grade TiO_2 NPs do not exhibit any effect on viability.



Slika 2: Vpliv izpostavljenosti dvema tipoma TiO_2 ND na privzem ND in viabilnost diferenciranih celic THP-1. Prikazane so (**A**) kontrola (netretirane celice), in celice 24 ur inkubirane s (**B**) 50 µg/ml TiO_2 P25 in (**C**) 50 µg/ml TiO_2 food-grade (FG) ND. Na slikah faznega kontrasta je razviden precejšen privzem ND pri obeh tipih ND. Fluorescentne slike prikazujejo jedra obarvana s Hoechst 33342 (modro) in mrtve celice obarvane s propidijevim jodidom (PI) (rdeče) v istem vidnem polju.

Figure 2: The effect of exposure to two types TiO_2 NPs on NP uptake and viability of differentiated THP-1 cells. (**A**) Control (untreated cells), and cells incubated with (**B**) 50 µg/ml TiO_2 P25 and (**C**) 50 µg/ml TiO_2 food-grade NPs for 24 hours are shown. Phase contrast images show considerable NP uptake for both NP types. Fluorescent images show Hoechst 33342 stained nuclei (blue) and propidium iodide (PI) stained dead cells (red) of the same visual field.

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Vibracijska slika naravnega okolja in vpliv biotskega šuma na vedenje samcev iz rodu Aphrodes

Vibrational soundscape of natural enviroment and the effect of biotic noise on behaviour of male leafhoppers from genus Aphrodes

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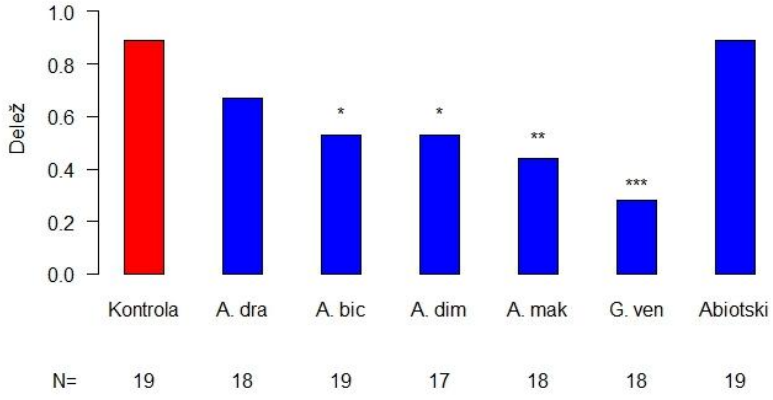
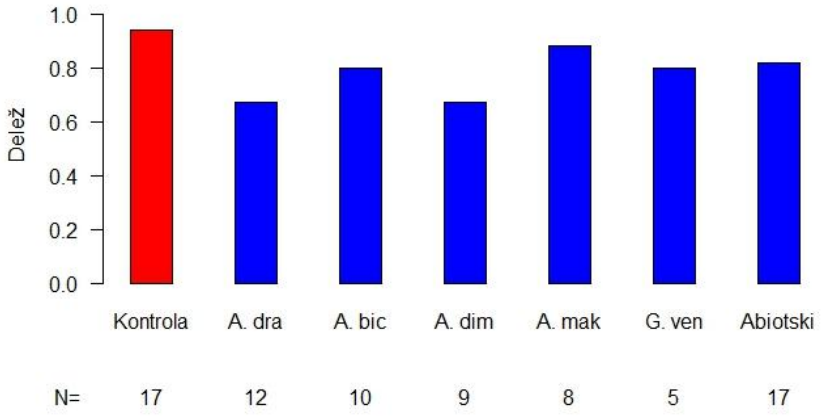
Pomemben element komunikacijskih omrežij so naključni sprejemniki (sprejemniki drugih vrst, ki živijo v istem okolju) za katere signal oddajnika ne predstavlja relevantne informacije temveč le biotski šum.[1] Vpliv biotskega šuma na sporazumevanje z vibracijskimi signali je slabo raziskan[1, 2, 3] in namen naloge je bil ugotoviti vpliv biotskega šuma na vedenje samcev iz rodu *Aphrodes* ter opisati naravno vibracijsko zvočno biotsko krajino teh škržatkov. V laboratorijskih poskusih smo na rastlino postavili samca in samico in z minaturnim vzbujevalnikom na rastlino v vlogi šuma predvajali signale škržatkov, ki živijo sintopično z našo modelno vrsto *Aphrodes 'Dragonja'*, duet lastne vrste ter abiotski šum. Rezultati kažejo, da ima biotski šum signifikanten negativen vpliv na proženje oddajanja pozivnega napeva in iskalnega vedenja pri samcih (slika 1), medtem ko vpliva na druge izmerjene parametre spolnega vedenja (čas iskanja, začetek petja samca, odstotek petja med iskanjem) nismo zasledili. Ob predvajanjem dueta lastne vrste *A. 'Dragonja'* samci niso izrazili rivalnega vedenja in signifikantno manj samcev je iskalo samico. Predvajanje abiotskega šuma ni imelo nobenega vpliva na vedenje samcev. Nivo okoljskega šuma pri snemanju v naravnem okolju (travniku) je bil visok, prevladovale so frekvence pod 50 Hz. Tekom dvodnevnega snemanja smo zabeležili 20 različnih signalov (tudi signale vrst *A. makarovi* in *A.* 'Dragonja'). Snemanje na terenu je pokazalo majhen delež prekrivanja signalov. V obeh dnevih snemanja smo opazili pozitivno korelacijo med prekrivanjem signalov in vibracijsko aktivnostjo v posameznem 30 minutnem intervalu (r = 0,80 in 0,85).



Slika 1: Delež samcev tipa *A. 'Dragonja'*, ki so pričeli s petjem. Zvezdice označujejo statistično značilne razlike med kontrolo (brez šuma) in tretmaji v prisotnosti šuma. Enostranski Fisherjev test, *P < 0,05, **P < 0,01, ***P < 0,001. N = število samcev vključenih v analizo.

Figure 1: Proportion of males *A.* 'Dragonja', which start with signaling. Asterisk label statistical difference in comparison to control (Kontrola). One way Fisher's test. N = numbers of individuals tested.

An important element of communication networks are incidental receivers (i.e. heterospecific receivers present in the same environment) that do not obtain any information from for the emitter and to which signals represent only biotic noise.[1] The impact of biotic noise on vibrational communication is largely unexplored[1, 2, 3]and the aim of the present study was to determine the effect of biotic noise on mating behaviour of the *Aphrodes* 'Dragonja' males and to describe the natural vibrational soundscape of these leafhoppers. In laboratory experiments we placed males and females of our model species on a plant and used vibration exciter to apply vibrations to the plant. As imitation of noise we used vibrational signals of species that live syntopically with *A.* 'Dragonja' (conspecific duet and 5 different heterospecific duets) and also abiotic noise (wind). Results showed significant negative impact of biotic noise on triggering of emission of advertisement calls and searching behaviour; however, we did not observe significant effect on other behavioural parameters (calling latency, searching time, proportion of calling during searching). When conspecific duet was applied, males did not express rivalry behaviour and significantly fewer males searched for the female. Playback of abiotic noise had no effect on male behaviour. The level of biotic noise in a natural environment (meadow) was high; and the main frequency band was below 50 Hz. We recorded vibrational soundscape on two consecutive days and we registered at least 20 different signals, including signals of *A. 'Dragonja'* and *A. makarovi*. In the field, we registered only few cases of overlapping signals and in both days signal overlap was positively correlated with the level of vibrational activity registered in the 30 minute time interval (r = 0,80 and 0,85).



Slika 2: Delež samcev tipa *A. 'Dragonja'*, ki so našli samico izmed tistih, ki so pričeli z iskanjem. Med tretmaji ni statistično značilnih razlik. Enostranski Fisherjev test. N = število samcev vključenih v analizo.
Figure 2: Proportion of males that locate female, from those which start searching. No significant difference to control. One way Fisher's test. N = numbers of individuals tested.

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Funkcionalne lastnosti fotoreceptorjev v akutnih conah oči samic in samcev obada (Tabanus bromius Linnaeus, 1758)

Functional properties of photoreceptors in the acute zones of female and male horseflies (Tabanus bromius Linnaeus, 1758)

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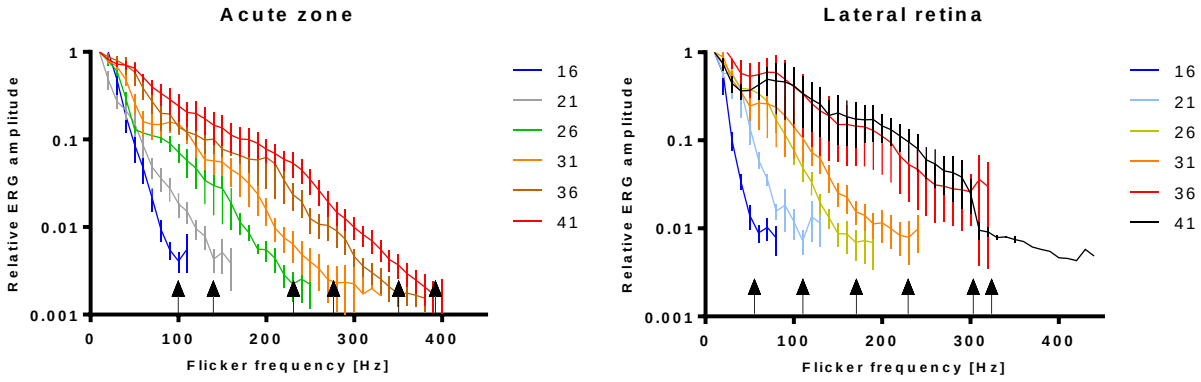
Obadi (Tabanidae) so družina dvokrilcev, ki parazitira na sesalcih (predvsem na pašnem govedu), saj samice potrebujejo kri za proizvodnjo jajčec. Svoje "žrtve" prepoznajo s pomočjo vidnih in vohalnih motivov. Samice z očmi zaznavajo vzorec polarizirane svetlobe na živalskih kožuhih,[1] samci pa zasledujejo obrise samic na nebu. Njihov uspeh je posledično v veliki meri odvisen od sposobnosti vida: hitrosti zaznav, kotne in kontrastne ločljivosti ter spektralne uglašenosti z okoljem. Vse te lastnosti so najbolj izražene v delu očesa, imenovanem akutna cona ali fovea. Odzive obadje mrežnice lahko raziskujemo s pomočjo elektroretinograma (ERG) in znotrajceličnih posnetkov iz fotoreceptorjev. Pri ERG-ju je hitrost zaznav izražena s fuzijsko frekvenco. Fuzijsko frekvenco smo skušali določiti na različnih predelih mrežnice samic in samcev in preveriti njeno odvisnost od temperature. Z znotrajceličnimi posnetki smo posneli aktivnost posameznih celic v različnih predelih mrežnice. Ugotovili smo, da receptorje v akutnih conah odlikujeta visoka kotna ločljivost in hitrost, ki pa pri je samcih nekoliko nižja, kot pri samicah. Domnevamo, da je nižja hitrost posledica velikih dimenzij fotoreceptorjev samcev. Zaradi velikosti so te celice zelo občutljive na svetlobo. Pokazali smo, da so sposobne zaznavati izjemno majhne svetlobne kontraste, pri čemer močno presegajo receptorje v akutni coni samic. Rezultati nam dajejo vpogled v specifične prilagoditve akutnih con samcev in samic, ki opravljata dve zelo različni vlogi: zaznavo živalskega plena na kompleksnem, zelenem ozadju, in zaznavo samic na UV in modrem nebu.



Slika 1: Relativna amplituda ERG v odvisnosti od frekvence bliskov v akutni zoni (levo) in lateralni retini (desno) pri različnih temperaturah pri samicah. Fuzijske frekvence so označene s puščicami.
Figure 1: Relative ERG amplitude in the acute zone and lateral retina at different temperatures in females. The flicker fusion frequencies are marked with arrows.

Horseflies (*Tabanidae)* are known cattle and horse parasites, because the females require blood for the production of eggs. Horseflies identify their victims using visual and olfactory motives. Females detect the polarized pattern on animal fur[1] and animal-related odors while males search for the silhouettes of females on the sky. Hence, their success critically depends on the performance of their visual system: the speed of reception, angular and contrast resolution and the spectral tuning to the environment. All these traits are optimally expressed in the part of the eye called the acute zone. Photoreceptor responses can be examined using electroretingram (ERG) and intracellular recordings. In ERG, the speed of vision is expressed in terms of the flicker fusion frequency (FFF). We determined the flicker fusion frequency of the horsefly eye in different parts of the retina of both females and males, and measured its temperature dependence. Using intracellular recordings, we examined the performance of single cells in different parts of the retina. Our results demonstrate a high angular resolution and speed in the acute zone receptors, the latter being lower in males than in females. We attribute the lower speed of male photoreceptors to their larger size, due to which these cells are very sensitive to light. We showed that they are capable of detecting minute light contrasts and are much better at it than females receptors in the acute zone. These results give us an insight into the specific adaptations of the acute zones in females are males which perform two very different tasks: in females it detects animal prey on a complex green background while in the males it detects females on an UV and blue sky.

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Vedenjski odziv na svetlobo pri površinskih in podzemeljskih populacijah vodnega oslička Asellus aquaticus

Behavioral response to light in surface and subterranean populations of the freshwater isopod Asellus aquaticus

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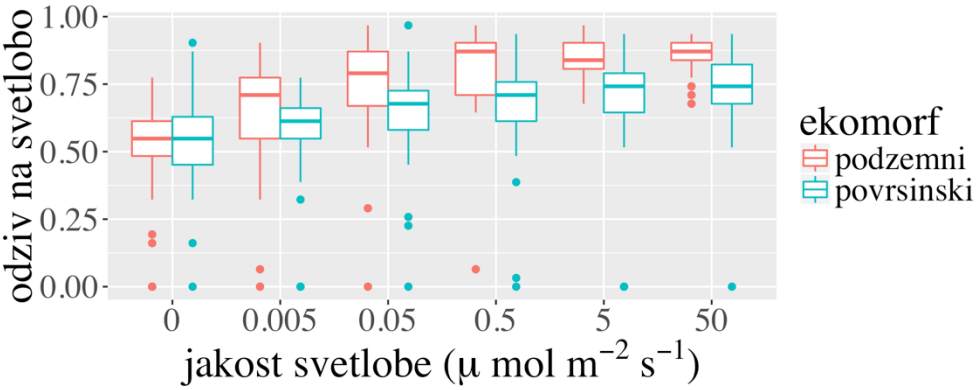
Izmed vseh okoljskih dejavnikov površinske in podzemeljske habitate najizraziteje loči svetloba. Podzemeljske živali imajo zaradi življenja v temi praviloma reducirane oči in pigment, a vendar jih večina še vedno lahko zazna svetlobo in se nanjo tudi vedenjsko odzove.[1] Vedenjski odziv na svetlobo so med podzemeljskimi živalmi največ raziskovali pri ribah, precej manj pri nevretenčarjih. Kljub temu iz dosedanjih ugotovitev ni jasno, kako se vedenjski odziv na svetlobo spremeni ob prilagajanju na podzemeljsko okolje. Podzemne vrste, ki še imajo površinske prednike in so podzemlje osvojile večkrat neodvisno, so za tovrstne raziskave najprimernejše. Tak je enakonožni rak vodni osliček (*Asellus aquaticus*).[2] Preverili smo, ali se vedenjski odziv na svetlobo podzemeljskih populacij vodnega oslička razlikuje od odziva predniških površinskih populacij.

Odziv na svetlobo smo izmerili površinskim vodnim osličkom iz Cerkniškega polja, Planinskega polja in Ljubljanskega barja ter podzemeljskim vodnim osličkom iz Zelških jam, jame Škratovke, Pivkinega rokava Planinske jame in Krške jame. Vedenje posameznih osebkov smo opazovali v petrijevki, ki smo jo razdelili na osvetljeno in zatemnjeno polovico, pri petih različnih jakostih svetlobe in v temi (kontrola).[3] Podatke smo statistično analizirali z uporabo posplošenih linearnih mešanih modelov (GLMM) v programu R.

Vse tri površinske populacije so se odzvale fotofobno in se med seboj niso zelo razlikovale. Po drugi strani pa je bil odziv med podzemeljskimi populacijami različen, saj so se vodni oslički iz Zelških jam in jame Škratovke odzvali izrazito fotofobno že pri nizkih jakostih svetlobe, tisti iz Pivkinega rokava Planinske jame in Krške jame pa šele pri višjih jakostih svetlobe. Odziv vseh podzemeljskih populacij se je razlikoval od predniških površinskih populacij, a vendar ne na enak način, kar priča o nekonvergentni spremembi tega vedenja ob prilagajanju na podzemeljsko okolje.

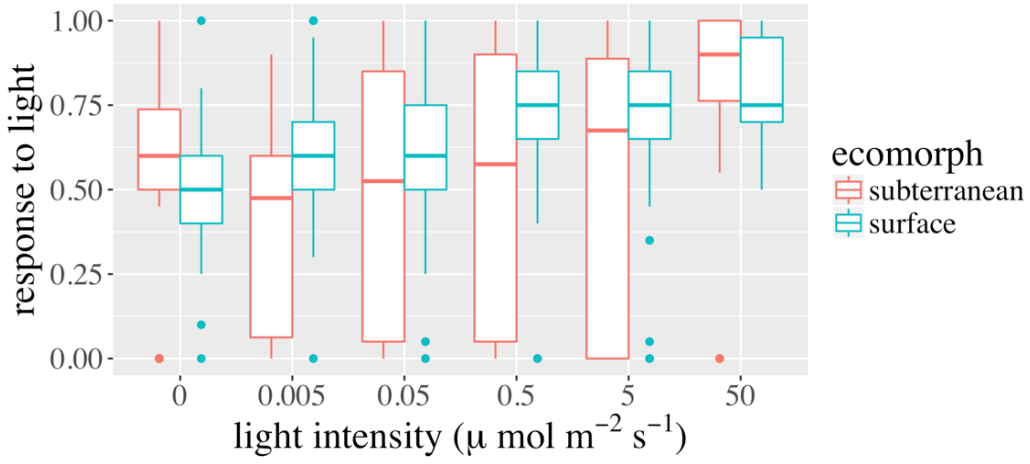


Slika 1: Vedenjski odziv na svetlobo površinskih (Cerkniško polje) in podzemeljskih (Zelške jame) osličkov.
Figure 1: Behavioral response to light of surface (Cerkniško polje) and subterranean (cave Zelške jame) *A. aquaticus*.

Light is the most noticeable out of all environmental factors that distinguish surface and subterranean habitats. Life in the dark caused subterranean animals to have reduced eyes and pigmentation. Despite this, most of them still have the ability to sense and behaviorally respond to light.[1] So far, the behavioral response to light was mostly studied in cave fish, much less in cave invertebrates. However, it is still not clear how this behavior changes during adaptation of surface species to the subterranean environment. The ideal subjects to answer that are species, such as the freshwater isopod *Asellus aquaticus*, with several independently evolved subterranean populations and still present surface ancestors.[2] We investigated whether behavioral response to light in subterranean populations of *A. aquaticus* differs from the response of their ancestral surface populations.

We measured the behavioral response to light of surface populations from Cerkniško polje, Planinsko polje and Ljubljansko barje, and subterranean populations from cave Zelške jame, cave Škratovka, Pivka river channel of Planina Cave and cave Krška jama. The behavior of each individual was observed in a Petri dish, divided into an illuminated and a dark half, at five different light intensities and in darkness (control).[3] Data were analyzed using generalized linear mixed models (GLMM) in R software.

All three surface populations displayed a similar photophobic response. On the other hand, the response of subterranean individuals differed between populations. Individuals from cave Zelške jame and cave Škratovka had a strong photophobic response already at low light intensities, while individuals from the Pivka river channel of Planina Cave and cave Krška jama responded photophobically only at high intensities of light. Although, the response of all subterranean populations differed from their ancestral surface populations, differences are not alike and suggest a nonconvergent evolution of this behavior during adaptation to the subterranean environment.



Slika 2: Vedenjski odziv na svetlobo površinskih (Ljubljansko barje) in podzemeljskih (Krška jama) osličkov.
Figure 2: Behavioral response to light of surface (Ljubljansko barje) and subterranean (cave Krška jama) *A. aquaticus*.

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Razlike v izbiri podlage in iskanju zatočišč med površinskimi in podzemeljskimi vodnimi oslički

Differences between surface and subterranean water lice in choosing the substrate and searching for shelter

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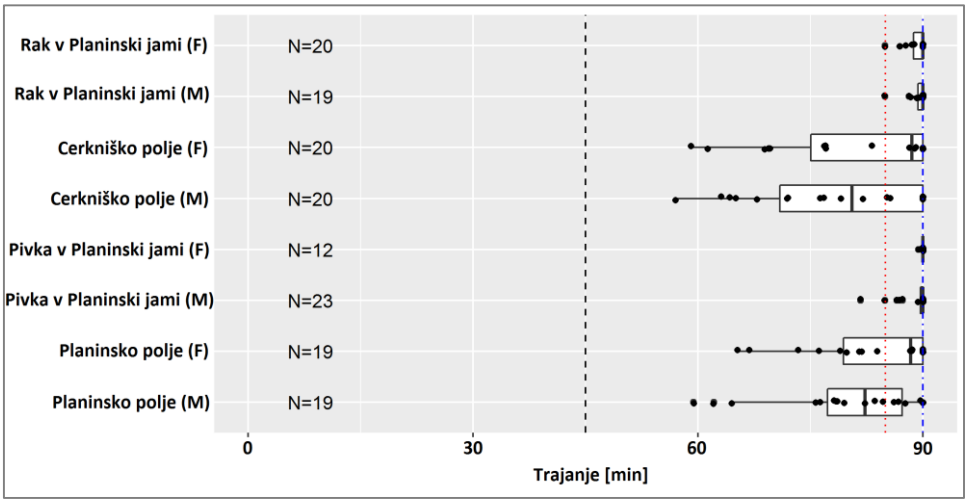
Uspešno naselitev jamskih živali v podzemlje in evolucijsko ločitev od površinskih prednikov lahko poleg morfoloških in fizioloških omogočajo tudi vedenjske prilagoditve na ta specifični habitat. Podzemne vrste, ki še imajo površinske prednike in so podzemlje osvojile večkrat, so za raziskave prilagoditev neprecenljive. Tak je enakonožni rak vodni osliček (*Asellus aquaticus* L.). Zaradi razlik v vedenjskih vzorcih bi lahko prišlo do reproduktivne izolacije med površinskimi in podzemnimi vodnimi oslički. Želeli smo preveriti ali so med omenjenima ekotipoma bistvene razlike v izboru podlage in zatočišča.

Analizirali smo po 20 vodnih osličkov (po 10 ♂♂ in ♀♀) iz dveh površinskih (Cerkniško in Planinsko polje) in dveh podzemnih populacij (Rakov in Pivkin rokav Planinske jame). Vedenjske poskuse smo izvedli v speleobiološkem laboratoriju (tema, 10 °C), v[1] petrijevkah z dnom iz hrapave in gladke polovice ter[2] petrijevkah z zatočiščem. Iz 2-urnih posnetkov vsakega osebka pri šibki rdeči svetlobi smo zabeležili čas nahajanja na hrapavi podlagi in v zatočišču. Za preverjanje razlik med ekotipoma in spoloma smo uporabili parametrične in neparametrične statistične teste v programu R.

Oba ekotipa vodnih osličkov sta izbrala hrapavo podlago, a so se podzemni tam pričakovano zadržali daljši čas. Med spoloma nismo ugotovili značilnih razlik. V nasprotju s predvidevanji se površinski oslički niso skrivali v zatočišču, oslički iz obeh ekotipov so se po petrijevkah gibali naključno ali pa so se mu celo izogibali. V obeh poskusih so bili površinski oslički gibalno aktivnejši od podzemnih. Potrebne bi bile nadaljnje raziskave tako individualnih kot skupinskih vedenjskih razlik.



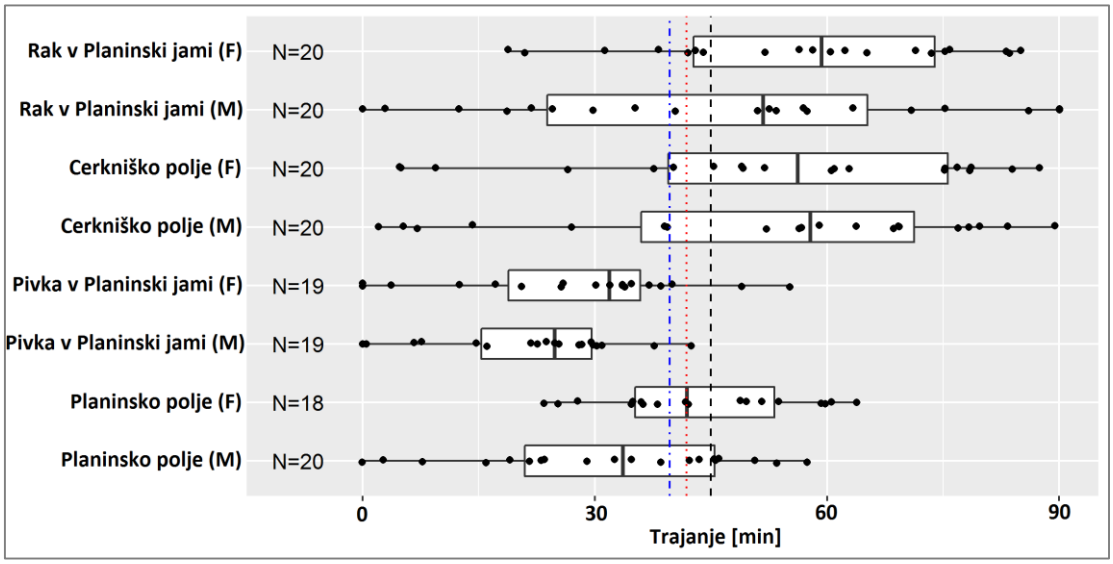
Slika 1: Časa zadrževanja površinskih in podzemnih vodnih osličkov (*Asellus aquaticus*) na hrapavi podlagi. N = število osebkov, F = samice, M = samci

Figure 1: Time spent on the rough surface in the surface and subterranean water lice (*A. aquaticus*). N = number of specimens, F = females, M = males

Successful colonization of subterranean habitats by cave animals and their separation from the surface dwelling ancestors depends on their morphological, physiological, but also behavioral adaptations to this specific habitat. Multiple cave invasions and extant surface ancestors make some cave species exceptional models for studying adaptations. Such species is the freshwater isopod water lice (*Asellus aquaticus* L.). Different habitat preferences may represent a barrier which caused the reproductive isolation between the cave and surface water lice. Our aim was to examine the behavioral differences in choosing of the rough surface and shelter between both ecotypes.

We used 20 specimens (10 males and 10 females) from each of the two surface (Cerkniško and Planinsko polje) and two underground populations (Rak and Pivka in the cave Planinska jama). Behavioral experiments were conducted in a speleolab (darkness, 10 °C) in the[1] petri dishes with bisected (rough vs. smooth) bottom and[2] petri dishes with sheltered halves. From 2-hour videos of each specimen recorded in dim red light, the time spent on the rough surface and under the shelter was recorded. To analyze differences both between ecotypes and genders, parametric and nonparametric tests were carried out in R.

The cave as well as the surface water lice preferred rough bottom over the smooth one, but as expected, the former stayed there significantly longer. There were no significant gender differences. Contrary to expectations, the surface water lice did not hide in the shelter; both ecotypes wandered around randomly or even avoided the shelter. In both experiments the surface water lice turned out as more active (moving faster and frequently). Further experiments are needed to analyze differences in individual as well as group behavior.



Slika 2: Čas zadrževanja površinskih in podzemnih vodnih osličkov (*Asellus aquaticus*) v zavetišču.
N = število osebkov, F = samice, M = samci
Figure 2: Time spent in the shelter in the surface and subterranean water lice (*A. aquaticus*).
N = number of specimens, F = females, M = males

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VABLJENO PREDAVANJE / INVITED LECTURE

Mreže in predivo pajkov: integracija vedenja, ekologije in biomehanike

Spider webs and silk: integrating behavior, ecology and biomechanics

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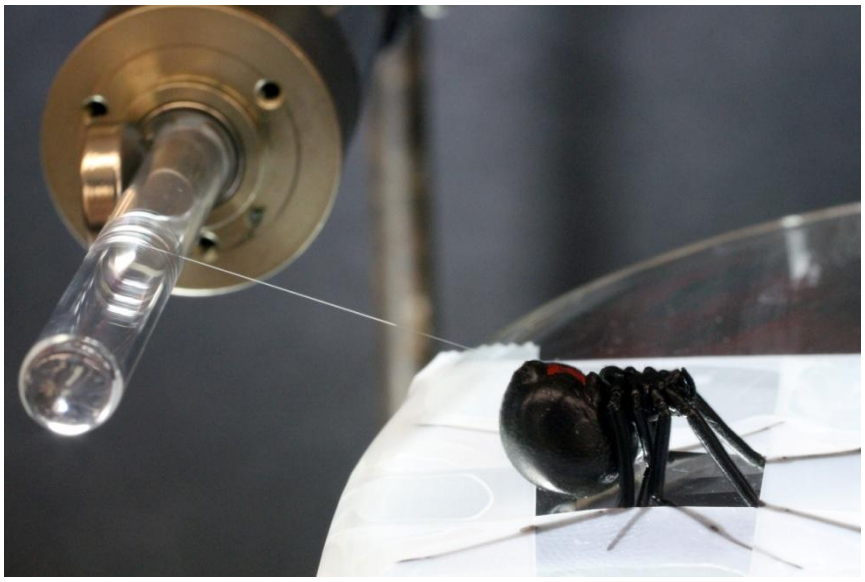
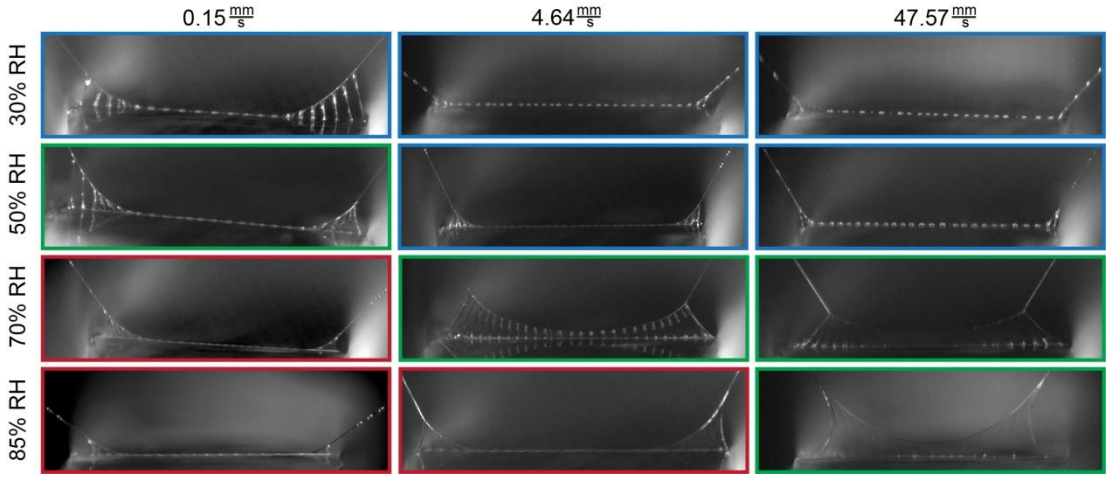
Pajki so izjemno raznovrstna skupina živali, ki poseljuje vse kopenske habitate na planetu. Ključna lastnost, ki jim omogoča izjemno raznovrstnost, je njihova sposobnost izdelovanja svilene niti. Najbolj karakteristična uporaba svilene niti je nedvomno gradnja lovilnih mrež. Kolesaste mreže so kompozitni materiali, sestavljeni iz več tipov prediva. Te se razlikujejo po svoji sestavi in mehanskih lastnostih, ki odsevajo njihovo funkcijo v mreži. V tem predavanju bom predstavil kako se biologija in vedenje pajkov v ekološkem kontekstu prepletata z zgradbo mrež in mehanskimi lastnostmi prediva. Natančneje, predstavil bom raznolikost in evolucijo mrež, od nivoja posameznega osebka, do širših koevolucijskih vzorcev mehanike prediva z vedenjskimi in ekološkimi lastnostmi pajkov. Predivo pajkov bom predstavil tudi v kontekstu zanimivih biomaterialov ter prikazal kako lahko raziskave mrež in prediva pajkov privedejo do novih aplikativnih spoznanj.

Slika 1: Pridobivanje pajčjega prediva na terenu.
Figure 1: Obtaining spider silk in the field.

Spiders are a megadiverse animal group that conquered all terrestrial habitats on our planet. The key feature that allowed for this spider diversity is their ability to produce silk threads, and the most characteristic use of silk is the building of prey-catching webs. Spider webs are physical manifestations of spider behavior and an extension of their senses. Also, they are composite structures made from multiple types of silk, each different in structure and material properties, reflecting their mechanical function in webs. In this talk, I will present how the natural history and behavior of spiders interact with web architecture and silk biomechanics in an ecological context. Specifically, I will present the diversity and evolution of spider webs, from the level of single individuals to broad coevolutionary patterns of biomaterial with behavioral and ecological traits. I will also present spider silks as interesting biomaterials and how web and silk research can identify novel applications.



Slika 2: Pridobivanje pajčjega prediva v laboratoriju.
Figure 2: Obtaining spider silk in the laboratory.



Slika 3: Predivo pajkov je material, ki se odziva na spremembe v okoljskih pogojih.
Figure 3: Spider silk is a material that responds to changes in environmental conditions.

Vpliv klimatskih sprememb na premike arealov mediteranskih vrst: Primer mediteranske črne vdove

Climate change induced range shift of Mediterranean species: A case study on the Mediterranean black widow

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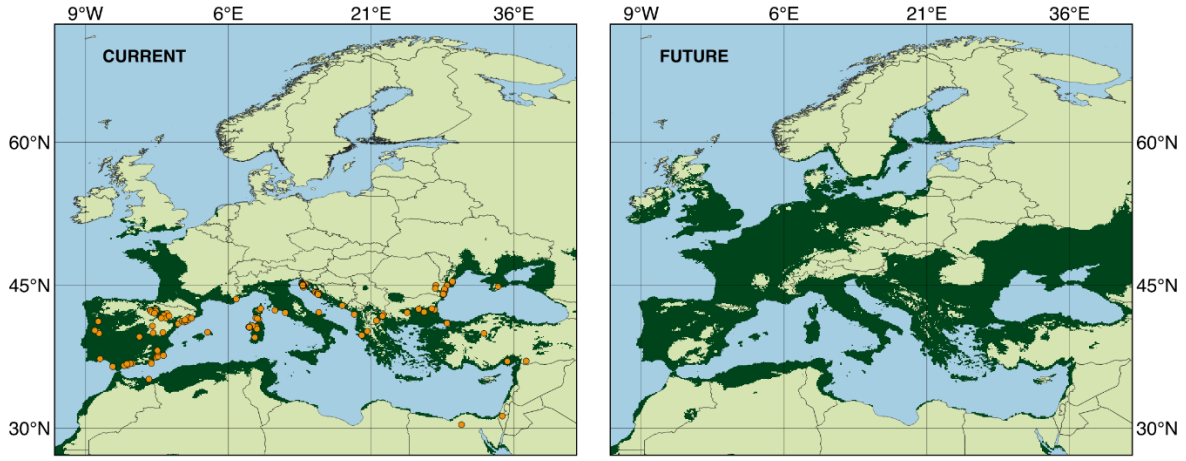
Rdečepikasti pajek *Latrodectus tredecimguttatus* (Rossi, 1790), bolje poznan kot mediteranska črna vdova, je pajek iz družine krogličarjev (Theridiidae).[10] Vse vrste rodu *Latrodectus* proizvajajo močan strup, ki je v nekaterih primerih lahko nevaren tudi človeku.[9] Vrsta je, kot nakazuje ime, razširjena v Sredozemlju, njen areal pa obsega tudi območje osrednje Azije.[4] Pajek v Sloveniji še ni bil najden,[3] čeprav se v zmernih gostotah pojavlja že v hrvaški Istri.

Areali mnogih vrst se zaradi klimatskih sprememb pomikajo proti severu.[5] Zaradi povečevanja količine toplogrednih plinov v atmosferi se spreminjajo ustaljeni vremenski vzorci, kar prinaša tudi spremembe pokrovnosti tal in biotskih odnosov med organizmi.[1] Tako se na območjih, kjer vrste prej niso bile prisotne zaradi abiotskih in biotskih omejitev, lahko vzpostavijo nove populacije.

Taki premiki območij razširjenosti, še posebej medicinsko pomembnih, vrst so vsekakor zanimivi in vredni obravnave.[8] V raziskavi smo želeli ugotoviti, katera območja bi lahko črna vdova poselila ob premiku areala. Kot vrsta z izrazito sredozemsko razširjenostjo je črna vdova dober model za tovrstno raziskavo.

S programoma Maxent[6] in QGIS[7] smo pripravili karte razširjenosti črne vdove v trenutnih razmerah ter razmerah, ki jih ob povečevanju količine toplogrednih plinov v ozračju napovedujejo klimatski modeli. Uporabili smo bioklimatske sloje dostopne na portalu WorldClim[2]. Napovedi kažejo opazen premik bioklimatske ovojnice proti severu.



Slika 1: Napovedi razširjenosti črne vdove v trenutnih ter prihodnjih razmerah (leto 2050 po scenariju RCP8.5). Na karti s trenutnimi razmerami so označena tudi najdišča vrste.
Figure 1: Species distribution maps in current and future conditions (predictions for year 2050 after RCP8.5 scenario). Known occurences are shown in the map with current conditions.

Mediterranean black widow *Latrodectus tredecimguttatus* (Rossi, 1790) is a spider from Theridiidae family.[10] All *Latrodectus* species are medically important species as they produce a strong toxin.[9] Species is present in the Mediterranean and its distribution area extends through Central Asia.[4] Even though the spider is quite common in Istria it has not yet been found in Slovenia.[3]

Climate change induced range shifts were observed in a number of species.[5] Rising concentrations of greenhouse gases in the atmosphere are one of the reasons for changing weather patterns that influence the composition of land cover and thus biotic interactions.[1] In that view, the species unable to survive in particular areas, either due to abiotic or biotic limitations, can extend their areals and form viable populations in new locations.

Such shifts of species range, especially ones of medical importance, deserve attention.[8] The aim of our research was to determine the potential areas of black widow expansion caused by climate changes. As a species with distinct Mediterranean distribution, the black widow provide a good model organism for such study.

We used Maxent[6] and QGIS[7] software to prepare species distribution maps for current and future conditions. Environmental layers used are available at WorldClim database.[2] Predictions show evident shift of bioclimatic envelope northwards.

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Potencialna uporaba vodnega oslička, Asellus aquaticus, v biomonitoringu kraškega vodnega sistema v Sloveniji

A potential use of waterlice, Asellus aquaticus, in biomonitoring of the Karst water system in Slovenia

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Sodobni pristopi v okoljskem monitoringu vključujejo tudi uporabo biomarkerjev, ki so običajno različne biokemijske, fiziološke ali tudi histološke, morfološke ali druge spremembe organizma. Biomarkerji ne odražajo le prisotnosti onesnažil v okolju ampak tudi biodostopnost in učinke onesnažil na organizme[1]. Pogosto so merljivi tudi po kratkotrajnem onesnaženju z razgradljivimi onesnažili, ki jih kemijski monitoring ne zazna, ali ob kronični izpostavitvi nizkim koncentracijam različnih onesnažil hkrati[1]. Za pravilno interpretacijo sprememb biomarkerjev zaradi onesnaženja je treba poznati kateri naravni okoljski dejavniki vplivajo na njihove vrednosti. Namen raziskave je bil analizirati naravno sezonsko dinamiko encimskih biomarkerjev in založnih snovi pri površinskem in jamskem vodnem osličku, *Asellus aquaticus* (L.). Vodni osliček je namreč zelo primerna vrsta za biomonitoring: je splošno razširjen, ne živi le v različnih tipih površinskih voda ampak tudi v podzemlju[2], je toleranten na nizko vsebnost kisika in zmerno organsko onesnaženje in ima pomembno vlogo v prehranskih spletih[3].

Vodne osličke smo vzorčili spomladi, poleti in jeseni, na treh lokacijah vzdolž ponikalnice Pivke: v Postojni pred vhodom v Postojnsko jamo, v Planinski jami in Jakovici na Planinskem polju, kjer teče kot Unica. Merili smo aktivnosti dveh encimov (acetilholinesteraze – AChE in glutation -S-transferaze – GST) ter količino založnih snovi (lipidov, ogljikovih hidratov in proteinov).

Encimska aktivnost AChE in GST je bila pri jamskih vodnih osličkih značilno nižja in manj sezonsko variabilna od tiste pri površinskih. Nizka aktivnost je verjetno posledica njihove nižje lokomotorne aktivnosti[4] in stopnje metabolizma[5] ter konstantno nizke temperature v jami. Encimska aktivnost GST površinskih živali je bila na splošno višja poleti, verjetno zaradi višje temperature, ki vpliva na metabolno aktivnost[6]. Količina založnih snovi se je med jamskimi in površinskimi živalmi le malo razlikovala. V živalih vzorčenih spomladi je bila količina ogljikovih hidratov na vseh lokacijah višja kakor v živalih vzorčenih poleti ali jeseni medtem, ko je bila količina proteinov spomladi najnižja. Pri jamskih živalih in živalih iz Postojne nismo ugotovili značilnih sezonskih razlik v količini lipidov medtem, ko so imele živali iz Jakovice največ lipidov poleti. Večja variabilnost v količini lipidov je verjetno posledica razmnoževanja živali.

Zaradi manjše variabilnosti encimskih aktivnosti jamskih osebkov bi lahko bile primernejše za morebitni monitoring vodotokov kot encimski biomarkerji površinskih, vendar je na tem področju potrebno še mnogo raziskav. Variabilnost založnih snovi je bila v obeh okoljih podobna. Poleg tega je treba upoštevati, da so jamske živali številčno manjše kot površinske, kar sproži pomisleke glede njihovega pretiranega izlova.

Modern approaches in environmental monitoring include also biomarkers that are usually various biochemical and physiological, but also histological, morphological or other changes in the organism. They reflect not only the presence of pollutants in the environment but also the bioavailability and effects of pollutants on organisms. They are often measurable even after short-term pollution with degradable pollutants or after chronic exposure to mixtures of various pollutants at low concentrations[1]. For the proper interpretation of biomarker response to pollutants we need to know which natural environmental factors affect biomarkers and to what extent. The aim of our research was to evaluate the natural seasonal dynamics of the enzyme biomarkers and energy reserves in waterlice *Asellus aquaticus* (L.) from surface and cave waters, with the potential to be used in biomonitoring of karst groundwaters in Slovenia. *Asellus aquaticus* is suitable indicator as it is: widespread, living in different types of surface as well as subterranean fresh waterswaters[2], tolerant to hypoxia and moderate organic pollution; an important segment of food webs[3].

Waterlice were sampled in spring, summer and autumn, at three locations along the sinking river Pivka: in Postojna near Postojna Cave entrance, in Planina Cave and near Jakovica on Planinsko polje. The activity of two enzymes (acetylcholinesterase - AChE and glutathione-S-transferase - GST) and the amount of energy reserves (lipids, carbohydrates and proteins) were measured.

The activity of AChE and GST enzymes was significantly lower and less variable between seasons in the cave animals than in the surface ones. Low activity is likely due to lower levels of locomotor activity[4] and metabolism[5] at constantly low temperature in the cave. The activity of GST enzymes in the surface animals was highest in the summer, probably due to higher temperatures, which affect metabolic activity[6]. The amount of energetic reserves in the cave animals did not differ much from energetic reserves in the surface ones. The amount of carbohydrates in animals sampled in spring was higher compared to animals sampled in summer or autumn, while the amount of proteins was lowest in spring. Higher variability of lipids is probably due to animal breeding.

The smaller variability in enzyme activity of cave waterlice could mean they are more suited to biomonitoring than enzyme biomarkers of its surface relative. However, much research is still needed in this field. The variability of energy reserves was similar in both environments. It should be noted, that cave animals are protected and their excessive sampling is inadmissible.

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Prispevek k poznavanju drsti sardona (Engraulis encrasicolus L. 1758) na območju Slovenskega morja

Contribution to the knowledge of anchovy spawning grounds in Slovenian sea

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Slovenija je obmorska država na severu Tržaškega zaliva, kjer se drstita gospodarsko pomembnejši vrsti sardela (*Sardina pilchardus* W. 1792) in sardon (*Engraulis encrasicolus* L. 1758).[4] Zaradi njunih razmnoževalnih strategij populaciji med leti močno nihata. Stanje dodatno poslabšujejo antropogeni vplivi, kar povratno negativno vpliva na gospodarstvo.[3,7,8] Poznavanje zgodnejših stadijev rib (iker, mladic) je nujno pri upravljanju in podajanju ocen o velikosti populacij v ribištvu in varstvenih študijah, saj se navadno drstitvena in vzrejna območja razlikujejo od kasnejših stadijev.[3]

Naš cilj je s podporo projekta AdriaMed prepoznati pomembnejša drstitvena območja sardona v Slovenskem morju in zabeležiti njegov drstitveni čas.

Vzorčenje je potekalo enkrat mesečno 11 mesecev (avgust 2014 – september 2014, november 2014 – julij 2015) na desetih postajah v Slovenskem morju, ki so med seboj oddaljene približno 3 milje.[5] Vzorčili smo po DEPM metodi z WP2 mrežo (velikost mrežnih okenc 200 µm in notranjim premerom 0,264 m^2).

V severnem Jadranu se sardon drsti od aprila do oktobra med 11,6 in 27,5 °C ter 9.1 – 38,5 ‰ slanosti.[6,7,8] V času naše raziskave nas je presenetil podatek o 13 ikrah v novembrskem vzorčenju, kar lahko najverjetneje pripišemo razmeroma visoki temperaturi morja v tistem letu, ki je še omogočala drst. Nasprotno, iker v aprilu nismo našli, čeprav smo jih pričakovali. Razlog gre iskati v razmeroma visoki slanosti (38,1 ‰) in prisotnosti sardele, s katero tekmujeta za drstitveni prostor.[2,8]

Višek drsti tekom vzorčenja je sardon dosegel v avgustu (2014). Med postajami je čez vzorčeno leto bila za drst najpomembnejša postaja 6, kjer smo ocenili kar 894 iker/m^2. Naši podatki kažejo na pričakovano višjo intenziteto drsti kot v južnem Jadranu (zaliv Kotor).

Slovenia is a coastal state in the northern part of the Gulf of Trieste, where commercially important fish species European pilchard (*Sardina pilchardus W. 1792*) and European anchovy spawn (*Engraulis encrasucolus L. 1758*).[4] Anchovy and sardine populations undergo large annual fluctuations in abundance because of their reproduction strategies. On top of that, increasing anthropogenic impacts in the last decades have intensified the natural fluctuations with negative effects on the economy.[3,7,8] Knowledge of early life history fish stages (eggs and larvae) is essential in recruitment, fisheries and conservation studies, because of different ecological requirements in comparison to the adult fish.[3]

With the support of AdriaMed project our goal is to recognize the most important spawning areas for anchovy in Slovenian sea and determine its spawning time.

Sampling was performed in the Slovenian sea once a month at 10 sampling stations, positioned app. 3 miles apart from Aug 2014 – July 2015. As suggested by the DEPM protocol, the sampling was conducted using WP2 net (mesh size of 200 µm and mouth diameter of 0,264 m^2).[5]

Data show that anchovy eggs were recorded in the Northern Adriatic from April to October, at temperatures ranging from 11,6 – 27,5 °C and 9.1 – 38,5 ‰ salinity.[6,7,8]

The 13 anchovy eggs found in the samples from November were not expected, but could probably be explained by the higher sea temperatures at the time. On the contrary, we didn't find any egg in April which could be explained by a relatively high salinity (38,1 ‰) and the presence of sardine, it's competitor for spawning areas.[2,8]

Anchovy's spawning peak was recorded in August (2014) on station 9. During our survey we found the highest number of eggs on station 6 (236 eggs or 894 eggs/m^2). Our data has confirmed expected higher spawning rate in relation to Southern Adriatic (Bay of Kotor).[1]

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Gnezditvena gostota velikega skovika (Otus scops) ob vzhodni jadranski obali Slovenije in Hrvaške

The breeding density of the Eurasian Scops Owl (Otus scops) along the eastern Adriatic coast of Slovenia and Croatia

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Veliki skovik *(Otus scops)* je vrsta sove, ki je široko razširjena v večjem delu južne in vzhodne Evrope. Naseljuje predvsem kulturno krajino - mozaike naselij, polj, travnikov, sadovnjakov in vinogradov. Pogost je tudi v parkih, drevoredih in vrtovih s starimi drevesi, saj ga bližina človeka ne moti.[9,3,8] Njegova številčnost v mnogih evropskih državah hitro upada,[1,6] sicer pa gre v Evropi za slabo poznano in malo proučevano vrsto.[6] Za območje vzhodne jadranske obale, predvsem za območje Hrvaške je bilo do sedaj narejenih le nekaj lokalnih popisov številčnosti velikega skovika,[9,4,2] prav tako tudi v Sloveniji.[3,7,8,10] Namen študije je predstaviti populacijsko gostoto in prostorsko razporejanje velikega skovika ob vzhodni jadranski obali v Sloveniji in na Hrvaškem. Metoda popisa je bila nočno beleženje oglašanja samcev velikega skovika z izzivanjem s predvajanim posnetkom samčevega teritorialnega petja.[5] Popisne točke so bile med seboj oddaljene od 500 do 1500 m, odvisno od odprtosti, razgibanosti in gozdnatosti terena.

Popis smo opravili na štirih območjih ob vzhodni jadranski obali (Slovenska Istra, Pula, Vransko jezero in Konavle) v gnezdilni sezoni od 2012 do 2016. Na območju Slovenske Istre smo popisali 308 točk, na območju Pule 44 točk, na območju Vranskega jezera 38 točk in na območju Konavel 88 popisnih točk. Gostota populacije v Slovenski Istri (0,71 samca/km^2) presega zabeleženo gostoto samcev iz Konavel (0,58 samca/km^2), ki je bila naša najbolj južna točka popisnega območja. Najnižja gostota samcev velikega skovika je bila na območju Vranskega jezera (0,19 samca/km^2), najvišjo gostoto populacije velikega skovika pa smo zabeležili na območju Pule (1,63 samca/km^2). Prostorska razporeditev velikega skovika ob vzhodni jadranski obali Slovenije in Hrvaške ni enakomerna, pač pa gručasta in vezana na posamezna območja, povečini naselja. Naselja so bila na vseh štirih območjih popisa tudi območja z zelo veliki lokalnimi gostotami pojočih samcev. Najverjetnejši razlog velikih gostot v naseljih je tudi ta, da veliki skovik v naseljih gnezdi in se v njih tudi prehranjuje predvsem z žuželkami, ki jih privabljajo ulične svetilke.[9] Kot verjeten razlog se navaja tudi odsotnost nekaterih plenilcev, kar se je pokazalo kot pomemben dejavnik pri izbiri habitata te majhne vrste sove.[6]

The Eurasian Scops Owl (*Otus scops*) is widespread breeder across much of southern and eastern Europe. It uses a wide type of semi-forest habitats, cultural landscape and urban areas,[3,8] where it can be particularly abundant.[9] Although its abundance is declining in many European countries, Eurasian Scops Owl remains one of least studied owl species in Europe.[1,6] From the area of eastern Adriatic coast there are only a few studies on the species available from Croatia[9,4,2] and Slovenia.[3,7,8,10] Therefore the aim of the study was to determine breeding density (abundance, spatial distribution and density) of Eurasian Scops Owl along the eastern Adriatic coast of Slovenia and Croatia. We searched for singing males at four study sites along the eastern Adriatic coast (Slovenska Istra, Pula, Vrana lake and Konavle) in the breeding seasons from 2012 to 2016. The playback method was used at all four study sites and count points were spaced 500 to 1500 m apart, depending on the openness and forest cover.[5]

We censused 308 points in Slovenian Istra, 44 points in Pula, 38 points at the coast of Vrana lake and 88 survey points in Konavle. The breeding density in Slovenian Istria (0.71 males/km^2) exceed the breeding density in Konavle (0.58 males/km^2) which was our most southern point. While breeding density was the lowest (0.19 males/km^2) at the coast of the Vrana lake, the highest breeding density of all study sites was in Pula (1.63 males/km^2). The spatial distribution of the Eurasian Scops Owl along the eastern Adriatic coast of Slovenia and Croatia is not uniform, but clustered and linked to local areas, mostly settlements. Settlements are a central part of the population and are in almost all survey sites located in residential areas. The most likely reason for large densities of Eurasian Scops Owls in settlements is assumed to be related to owl nesting in the settlements and feeding with insects that are attracted by the street lamps.[9] Most likely reason for such large densities in settlements can be attributed to predator release effect, which has been shown to be important factor in habitat selection pattern of this small owl species.[6]

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Gnezditveni uspeh prib v povezavi s kmetijskimi dejavnostmi na izbrani popisni ploskvi na Ljubljanskem Barju

Lapwing breeding success in relation to farming activities on chosen area in Ljubljana marshes

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Populacije pribe (*Vanellus vanellus*) v Evropi so bile stabilne med leti 1970 in 1990, nato pa je prišlo do upadanja številčnosti, kljub temu, da so nekatere manjše populacije ostale stabilne ali so imele celo pozitivne trende.[2] Populacija v Sloveniji je bila ocenjena na 2000 do 3000 parov, z manjšimi območji zgostitev, kot so Ljubljansko barje, Cerkniško in Sorško polje. Najmočnejša je bila populacija v severovzhodni Sloveniji, čeprav ta vrsta v preteklosti na tem območju ni bila tako pogosta. Ta sprememba je povezana z menjavo prebivališča. V dvajsetih letih prejšnjega stoletja je priba gnezdila na svojem primarnem gnezdišču – močvirnih travnikih, danes pa celotna štajerska populacija gnezdi v kmetijski kulturni krajini.[1] Z intenzifikacijo kmetijstva so se evropske in slovenske populacije srečale z novimi problemi. Kmetijska krajina izgublja na mozaičnosti, trava na intenzivnih travnikih raste hitreje in bolj zgoščeno, travnike izsušujejo, njive pa se obdeluje s težko mehanizacijo.[3] Vse to ima za posledico upadanje številčnosti prib.

Opazovanje prib je potekalo med 29. marcem in 18. majem 2015, na izbrani popisni ploskvi, ki je obsegala travnike in njive za železniško progo med Brezovico in Vnanjimi Goricami. Beležila sem število in kraj gnezdečih parov ter kmetijske dejavnosti na travnikih in njivah, ki bi lahko vplivale na gnezditveni uspeh prib. Rezultati so potrdili močno povezavo med kmetijskimi dejavnostmi in številčnostjo prib. V vrhuncu gnezditve je bilo na ploskvi 17 gnezdečih parov, proti koncu opazovanj pa je bilo zabeleženih le 6 mladičev, torej 0,35 mladiča na par.

Preglednica 1: Število parov/odraslih osebkov v povezavi s kmetijsko dejavnostjo ter število mladičev.
*Opomba: osebki označeni pod +(1) niso gnezdili.
Table 1: Number of pairs/individuals in correlation with farmland activities and number of waders.
*Comment: individuals marked as +(1), were not nesing.

Datum	Vsota parov/odraslih	Št. opaženih mladičev	Kmet. dejav.
29.3.	4+2		A, B: orani njivi
2.4.	13 +1		
6.4.	14+1		
7.4.	17 +1		
9.4.	17 +1		
12.4.	15+1		SVEŽE GNOJEN DEL NJIVE
15.4.	15		
21.4.	13		DOKAJ SVEŽE BRANANO
4.5.	14 odraslih	6	BRANANO V CELOTI (A, B, D, E)
7.5.	17 odraslih	4	
13.5.	13 odraslih	4	
18.5.	14 odraslih	1	

European populations of lapwing (*Vanellus vanellus*) had been stable between 1970 and 1990, but since then they keep declining, although some smaller populations are stable or have even positive trends.[2] Slovene population was estimated to 2000 to 3000 pairs, with smaller concentration zones like Ljubljana marshes, Cerknica Lake and Sorško polje. The strongest population was observed in north-eastern Slovenia, although the species had not been so common there before. This may be linked to habitat change – lapwing used to nest in its primary habitat, which was wet meadows, contrary to the present habitat, which is farmland.[1] With intensificat ion to agricultural changes: a review. UK Nature Conservation ion of the latter, lapwing ran into other problems. The mosaic of farmland is disappearing, grass is growing faster and thicker, meadows are being drained and arable land is being cultivated with heavy machinery.[3] All those are also the reasons for decline of lapwing population in Slovenia.

The monitoring was carried out from 29th of March to 18th of May on chosen area, which included meadows and arable land between Brezovica and Vnanje Gorice. The number of nesting pairs and farming activities were registered, with emphasis on those activities, which may influence breeding success. Results confirmed strong correlation between the number of lapwings and farming activities, since 17 nesting pairs were registered, but only 6 waders seen. Thus the breeding success was only 0,35 waders per pair.

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Skrivnosti iz življenja volkov- teritorialnost, raba prostora in aktivnost volkov (Canis lupus) v severnih Dinaridih

Secrets from wolves lives – habitat use, home range and activity of wolves (Canis lupus) in northern Dinaric Mountains

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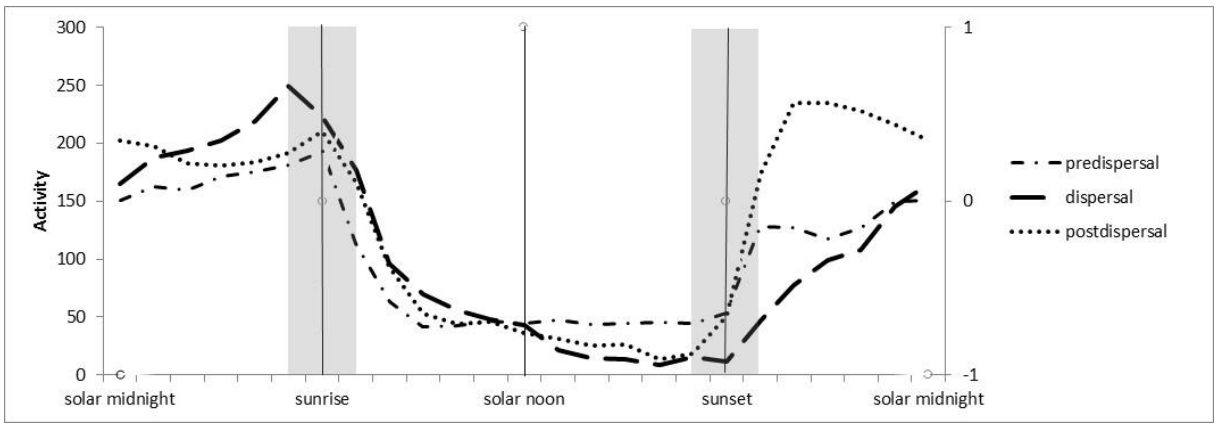
Osrednje območje razširjenosti volka (*Canis lupus)* v Sloveniji je zelo heterogeno, saj so biotske in abiotske razmere zelo raznolike. Preučili smo aktivnost in rabo življenjskega prostora volka v območju severnih Dinaridov v različnih življenjskih obdobjih volkov. V okviru projekta Slowolf (www.volkovi.si) je bilo z GPS-GSM ovratnicami opremljenih 8 volkov iz različnih volčjih tropov v starosti od 0.5 do 6 let. 4 samice in 4 samci so pripadali različnim socialnim statusom; spremljana je bila volčja samico v njenem prvem letu, nereproduktivna mlajša samica, nereproduktivni mlajši samec, nereproduktivni starejši samec, nedominanten član tropa, ki pozneje prevzame vlogo alfa samca v novem tropu, reproduktivni vodilni samec in reproduktivni vodilni samici. Popisan je primer krajše in daljinske disperzije volkulje in volka. Volkovi so bili spremljani od 79 - 407 dni. Njihovi domači okoliši so varirali od 259 - 560 km^2.

Kljub vedno bolj dodelanim tehnikam telemetričnega spremljanja živali, je ključna uporaba metode v kombinaciji s terenskim delom raziskovalcev. Slednje ne sme ostati zapostavljeno, saj je pomemben dejavnik pri sestavljanju mozaika o poznavanju ekologiji posameznih vrst živali. Pridobljena znanja je potrebno upoštevati pri varstvu in ohranjanju populacije prostoživečih vrst živali, tudi volka. Sobivanje s človekom je edina možnost za preživetje velikih zveri v Evropi, vključno s Slovenijo.[1]



Slika 1: Primerjava aktivnosti volka Slavca glede na relativni sončni čas v obdobju pred, med in po disperziji
Figure 1: Activity of wolf Slavc in comparison to relative sun time during predispersal, dispersal and postdispersal period.

Core area of wolves (*Canis lupus*) in Slovenia is very mixed, with large biotic and abitoic differences. In northern Dinaric Mountains, we studied wolf activity and habitat use in different period of wolves' life. During the SloWolf project (www.volkovi.si) 8 wolves, aged 0.5- 6 years, from different wolf packs were equipped with GPS-GSM radio collars. 4 female and 4 male wolves, were of different social status. We studied yearling female, younger subordinate female, younger subordinate male, older subordinate male, younger subordinate male that later dispersed and became alpha male in a new pack, breeding alpha male, two breeding alpha females. We witnessed short and long wolf dispersal. Wolves were studied from 79-407 days, their home ranges ranged from 259-560 km^2.

Although wildlife telemetry is getting more and more efficient, this method should be combined with field observations. Field work should not be forgotten when studying ecology of different species. Gained knowledge should be considered by conservation and regulation measure of free living species, also wolves. Coexistence with humans is only possibility for survival of large carnivores in Europe, including Slovenia.[1]

Preglednica 1: Osnovne informacije o spremljanih in proučenih volkovih
Table1: Information about collared and studied wolves

Volk *Wolf*	**Spol** *Sex*	**Starost ob odlovu** *Age (years)*	**Trop** *Pack*	**Mesto v tropu** *Position in the pack*	**Čas trajanja monitoringa (dni)** *Duration of Monitoring (days)*	**Velikost teritorija (km^2)** *Home Range (km^2)*
TINA	female	1 leto	Javorniki,po disperziji (after dispersion) Menišija	subordinate	207	370
BRIN	male	3 leta	Slavnik	subordinate	190	422
VOJKO	male	5 let	Vremščica-Nanos	subordinate	143	550
SLAVC	male	2 leti	Slavnik	subordinate, after dispersion breeding alpha	407	442
LUKA	male	1 leto	Gotenica	subordinate	262	560
TONKA	female	6 let	Vremščica-Nanos	breeding alpha	123	266
TIA	female	2 leti	Rog	subordinate	79	259
JASNA	female	4 leta	Gotenica	breeding alpha	330	320

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Biogeografske korelacije velikosti genoma pri neotropskih pupkih rodu Bolitoglossa

Biogeographical Correlates of Genome Size in Neotropical Bolitoglossine Salamanders

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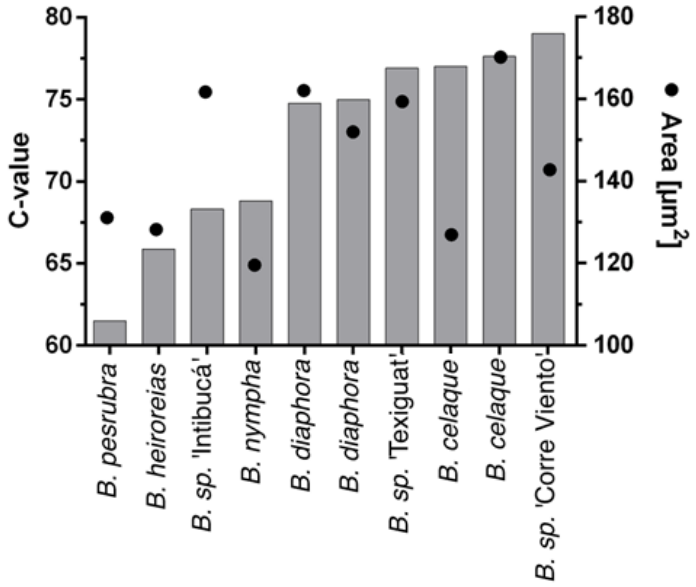
Raziskave na rastlinah so pokazale, da se v majhnih populacijah, parjenju v sorodstvu in populacijsko ozkih grlih hitreje povečuje velikost genoma, kot sicer.[1] Predpostavljamo, da so podobni mehanizmi udeleženi tudi pri večanju genoma geografsko izoliranih neotropskih brezplučarjih rodu *Bolitoglossa* (Plethodontidae). Družina brezplučarjev Plethodontidae vključuje predstavnike z različno velikostjo in obliko telesa, ki zasedajo različne niše in se razlikujejo tudi v velikosti genoma. Izmerili smo C-vrednosti (količina DNA v haploidnem genomu izražena v pikogramih) pri sedmih vrstah neotropskih brezplučarjev iz rodu *Bolitoglossa* (tri od njih so še neopisane), pri katerih velikost genoma še ni bil izmerjena. Naše meritve vključujejo doslej najvišje izmerjene vrednosti genoma pri omenjenem rodu in tudi družini Plethodontidae (Slika 1). Vse preučevane vrste so endemične za Honduras in Gvatamalo. Če naša hipoteza drži, bi se morali biogeografski dejavniki in populacijska dinamika odražati v velikosti genoma, predvsem pri izoliranih vrstah (npr. *B. celaque, B. heiroreias in B. diaphora*),[2] ki naj bi torej imele večji genom. Posebno pozornost smo posvetili vrsti *B. celaque*, ki je na podlagi filogenetskih dokazov najverjetneje kompleks dveh vrst, *B. celaque* in *B. sp.* 'Intibucá'. Populacija *B. celaque* je omejena le na del gorovja Lenca v JZ Hondurasu, medtem ko je *B. sp.* 'Intibucá' zastopana na več mestih vzdolž omenjenega gorovja (Itgen, osebna opažanja). Predvidevali smo, da bo genom pri *B. celaque* zaradi geografske izolacije večji kot pri sestrskih vrstah B. sp. 'Intibucá' in *B. heiroreias*, ki imata manj fragmentiran in večji habitat. Poleg navedenega naše meritve genoma potrjujejo tudi hipotezo, da sta *B. celaque* in *B. sp.* 'Intibucá' ločeni vrsti.

Evidence from plants shows that small population sizes, inbreeding and population bottlenecks are conditions that favor genome size expansion.[1] We speculate that a similar mechanism might be involved in the expansion of the genome size in geographically isolated Neotropical lungless salamanders (Plethodontidae, *Bolitoglossa*). The bolitoglossines display a diversity of body shapes and sizes, ecological niches, and genome sizes. We measured the C-values of seven Neotropical bolitoglossine salamander species, three of which are currently undescribed, whose genome sizes have never been measured before. Some of them include the largest measured genome sizes of any *Bolitoglossa* species reported so far (Fig. 1). All of them are endemic to Honduras and Guatemala. If our hypothesis is correct, then genome size should reflect biogeography and population dynamics, especially in biogeographically isolated species with small distributions (e.g. *Bolitoglossa celaque*, *B. heiroreias*, and *B. diaphora*)[2] which should have the largest genomes. We were particularly interested in *B. celaque* (celaque mushroomtongue salamander) because this species is hypothesized to be a species complex, based on two genetically distinct clades distributed across the Lenca highlands of southwestern Honduras, referred to as *B. celaque* and *B. sp.* 'Intibucá'. Interestingly, *B. celaque* is isolated to a single peak whereas *B. sp.* 'Intibucá' is distributed across various high elevation peaks (Itgen, pers. comm.). We predicted that highly restricted isolation of *B. celaque* would lead to inbreeding and genome size expansion such that its genome size would be larger than that of its sister species, *B. sp.* 'Intibucá' and *B. heiroreias*, whose breeding range is larger and less fragmented. We also find that genome size further supports the hypothesis that the *B. celaque* complex represents two distinct species.



Slika 1: C –vrednosti in površina jeder eritrocitov pri preučevanih vrstah rodu *Bolitoglossa*.
Figure 1: C-values and erythrocyte nuclear areas of species of *Bolitoglossa* salamanders measured in this study.

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Ocena genetskega materiala za študijo o razmerjih med spoloma pri Salix viminalis L.

Genetic assessment of plant material for studies of the genetic causes to female biased sex ratios in Salix viminalis L.

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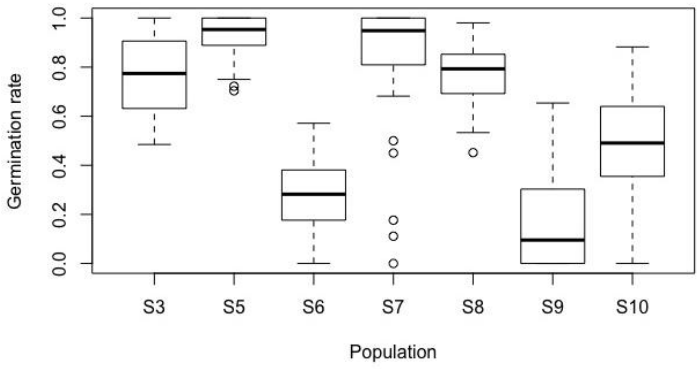
Vrsta *Salix viminalis* je dvodomno drevo ali olesenel grm s presežkom ženskih osebkov v populaciji. Pojav je verjetno posledica smrti zigot moškega spola zaradi genetske inkompatibilnosti. Vrsta ima ZW/ZZ sistem določanja spola, kjer W določa ženski spol. Vsi osebki ženskega spola imajo tako haplotip matere. V raziskavi smo producirali 7 populacij vrste *Salix viminalis*. Dve izmed teh sta bili že preučevani. Ena od teh ima razmerje med spoloma 1:1, pri drugi pa se pojavi povečano razmerje v prid ženskih osebkov. Cilj je bil preveriti, ali obstajajo razlike v uspehu germinacije semen med populacijami in ali na to vpliva inbreeding. Na podlagi testa germinacije smo preučevali korelacijo med pojavom inbreedinga in pojavnostjo belih semen, ki so manjša in ne vzkalijo. Možen vzrok za pojav teh je, da populacije z večjim inbreedingom producirajo več belih semen. Prešteli smo vse posamezne tipe semen (črna/bela, ki vzkalijo in črna/bela, ki ne vzkalijo) in naredili ANOVA test v programu JMP. Razvili smo tudi primerje za markerje na regiji za določanje spola na kromosomu 15. Markerji bodo uporabljeni v nadaljnjih raziskavah o razmerjih med spoloma pri tej vrsti.

Ugotovili smo, da so razlike med stopnjami germinacij med populacijami velike. Razlike so velike tudi med posameznimi cvetnimi mačicami, verjetno zaradi ekoloških razlogov (npr. količina oprašitve). Glede na rezultate domnevamo, da pojav belih semen ni v korelaciji s pojavnostjo inbreedinga pri obravnavanih populacijah. Možen vzrok za razlike v germinaciji je genetska inkompatibilnost med homozigotnimi alelnimi kombinacijami na »sex distorter« lokusu. Da bi natančno raziskali povezavo med belimi semeni in inbreedingom, je v nadaljnjih raziskavah možno sekvenciranje genomov različnih semen.



Slika 1: Stopnje kaljivosti produciranih populacij *Salix viminalis*
Figure 1: Germination rates of produced populations of *Salix viminalis*

Salix viminalis is a dioecious tree or a woody shrub with an overall female biased sex ratio. Most likely the bias is initiated in an early stage of development either by abortion of certain

male zygotes or by genetic incompatibility leading to failure of fusion between the sperm and the egg. *Salix viminalis* has a female heterogametic or ZW sexual system, where the W represents a female-specific chromosome. In this sexual system, all female offspring inherits the W haplotype from the mother.

In this work, we produced and studied seven bi-parental populations of *Salix viminalis*. Two of these had previousy been explored of which one had even sex ratio and one had female biased sex ratio. Our aim was to compare the germination rates between the populations and to estimate effects of inbreeding on the germination rates. Furthermore, based on the germination test the correlation between inbrreding and the frequency of white seeds was studied. One possibility is that more inbred populations produce relatively more white seeds than less inbred populations, since the white seeds are small and they do not germinate. All the types of seeds (black/white germinated and black/white non-germinated) were counted and the data was analysed in JMP program using ANOVA test. Moreover, we have designed primers for markers located in the sex determination region on chromosome 15. The markers will in the future be used to genetically determine the sex ratios in the studied populations.

The results show that there is significant difference between the germination rates between the crosses and between the catkins of each cross. The latter is most likely caused by ecological causes, such as sparse or abundant pollination. Based on assumptions, in this work it is suggested that the presence of white seeds is not correlated with levels of inbreeding. It is possible that genetic incompatibility between homozygous allelic combinations in the sex ratio distorter locus have led to the differences among the germination rates of the crosses. In future studies of the association between white seeds and inbreeding, one possibility would be to sequence the genomes of the different seeds.

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Alelopatija – biokemijsko orožje rastlin: zaviralni vpliv listnih in koreninskih izvlečkov invazivnih dresnikov (Fallopia sp.*)*

Allelopathy – plant's biochemical weapon: inhibitory effects of leaf and rhizome extracts of invasive knotweeds (Fallopia sp.*)*

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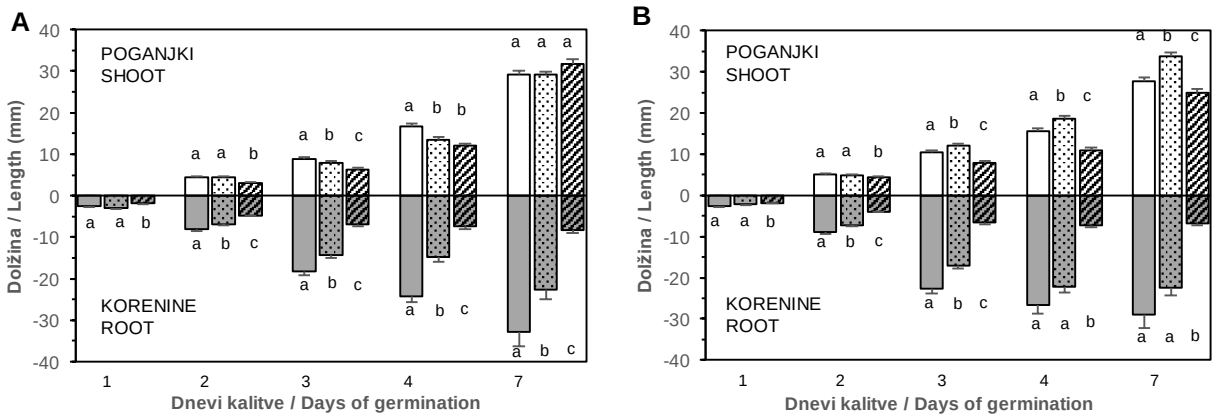
Alelopatija je posreden ali neposreden, praviloma negativen, vpliv rastline na rast in razvoj sosednjih rastlin preko sproščenih sekundarnih metabolitov (alelopatskih snovi). Te interakcije so lahko tudi pomemben mehanizem za uspešno rast tujerodnih invazivnih vrst v novem okolju. Ena takih vrst so dresniki (*Fallopia*),[1] zato smo z našo raziskavo poskušali ugotoviti, kakšen je alelopatski vpliv japonskega (*F. japonica*) in češkega dresnika (*F.* ×*bohemica*) na kalitev in rast testne vrste (redkvica, *Raphanus sativus*). Iz dresnikovih listov in korenik smo pripravili različne koncentracije (od 0,5 % do 10 % (w/v)) vodnih izvlečkov in testne rastline izpostavili njihovemu vplivu za 7 dni. Kontrolne rastline smo zalivali z destilirano vodo. Dnevno smo spremljali morfološke (dolžina poganjkov in korenin), biokemijske (antioksidanti, lipidna peroksidacija, fotosintezni pigmenti) in citološke (mitotski indeks) spremembe testnih rastlin.

Ugotovili smo, da dresnikovi izvlečki zavirajo rast korenin do 75 % (Sl. 1). Rast poganjkov je bila manj prizadeta. Vpliv izvlečkov je bil koncentracijsko odvisen. Izvlečki korenik so imeli močnejši alelopatski vpliv kot izvlečki listov. Mitotski indeks v apikalnem meristemu korenin je bil po 7 dneh zmanjšan za 66 %, na kar lahko vplivajo alelopatske snovi.[2] Aktivnosti antioksidativnih encimov katalaze (CAT) in guaiakol peroksidaze (G-POD) so bile v kontrolnih vrednostih. Vsebnost neencimskih antioksidantov (TAC) je bila 6. in 7. dan do 3 % višja v koreninah izpostavljenih rastlin (Sl. 2). Med TAC spada tudi α-tokoferol, ki predstavlja pomembno zaščito membran pred lipidno peroksidacijo,[3] posledično je lipidna peroksidacija (izražena kot vsebnost MDA) v izpostavljenih rastlinah le rahlo narasla.

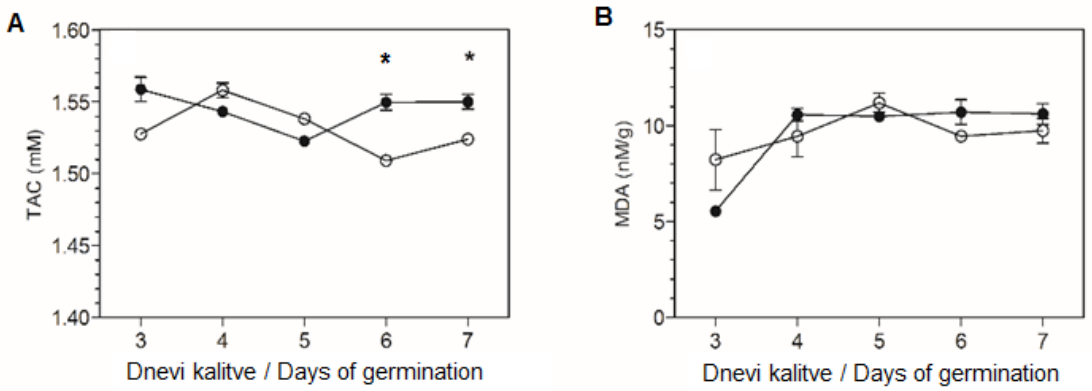


Slika 1: Rast kalic redkvice, ki so bile izpostavljene listnemu izvlečku (A) japonskega in (B) češkega dresnika. Koncentracije izvlečkov: 0 % (brez polnila); 0,5 % (pike); 5 % (črte). Povprečja ± SN (N = 50). Različne črke predstavljajo statistično značilno razliko ($p<0,05$).

Figure 1: Growth of radish seedlings exposed to leaf extract of (A) Japanese and (B) Bohemian knotweed. Extract concentrations: 0 % (no fill); 0.5 % (dotted fill); 5 % (striped fill). Mean value ± SE (N = 50). Different letters represent statistically significant differences ($p<0.05$).

Allelopathy is indirect or direct, mostly negative, plant´s influence to its neighbour plants´ growth and development via released secondary compounds (allelochemicals). These interactions can be important mechanism for successful growth of invasive alien species in their new environment. Knotweeds (*Fallopia*) are one of those species,[1] therefore we tried to evaluate allelopathic potential of Japanese (*Fallopia japonica*) and Bohemian knotweed (*F. ×bohemica*) on germination and growth of tested plants (radish, *Raphanus sativus*). Aqueous leaf and rhizome knotweed´s extracts of different concentrations (0.5 % to 10 % (w/v)) were prepared. Tested plants were exposed to extracts for 7 days. Control plants were watered with distilled water. Daily morphological (shoot and root length), biochemical (antioxidants, lipid peroxidation, photosynthetic pigments) and cytological changes (mitotic index) of tested plants were obtained.

It was shown that knotweed´s extracts inhibited root growth up to 75 % (Fig. 1). Shoots growth were less affected. Allelopathic power depended on extract concentration. Rhizome extracts had stronger allelopathic potential than leaf extracts. Mitotic index in the root apical meristem of exposed plants was inhibited up to 66 % after 7 days, what can be effect of allelochemicals.[2] Activity of two antioxidative enzymes catalase (CAT) and guaiakol peroxidase (G-POD) were at the control values. Total antioxidative capacity (TAC) increased for 3 % in the roots of exposed plants on the 6th and 7th day (Fig. 2). TAC also included α-tocopherol, which is important membrane protector,[3] consequently lipid peroxidation (measure as the content of MDA) only slightly increased in exposed plants.



Slika 2: Dnevno spreminjanje (A) vsebnosti neencimskih antioksidantov in (B) lipidne peroksidacije v koreninah redkvic, ki so bile izpostavljene 5 % listnemu izvlečku japonskega dresnika (●) in kontrola (○). Povprečja ± SN (N = 50). Zvezdica (*) predstavlja statistično značilno razliko (p<0,05).

Figure 2: Daily changes of (A) total antioxidative capacity and (B) lipid peroxidation in radish roots exposed to 5 % leaf extracts of Japanese knotweed (●) and control (○). Mean value ± SE (N = 50). Asterisk (*) indicated statistically significant differences (p<0.05).

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Timijanovo eterično olje kot naravni fungicid pri kalitvi pšenice

Thyme essential oil as natural fungicide for germination of wheat

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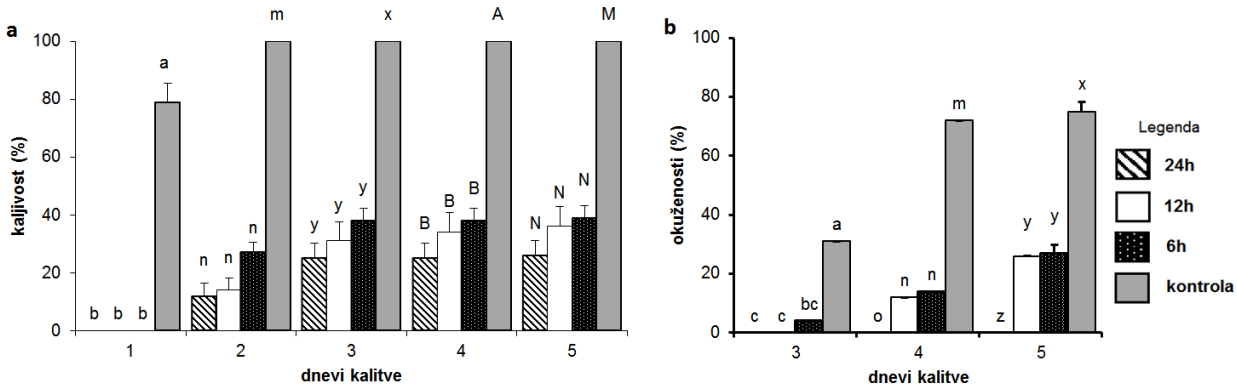
Pšenica (*Triticum aestivum*) je kulturna rastlina, ki je najpomembnejše krušno žito. Na polju ali v procesu nepravilnega shranjevanja lahko zrnje okužijo različne vrste gliv.[1] Rastline sintetizirajo različne sekundarne metabolite, s katerimi se branijo pred herbivori in patogeni.[2] Mednje sodijo tudi eterična olja (EO) in timijan (*Thymus vulgaris*) je ena izmed vrst z EO, ki zavira rast gliv in bakterij.[3] Namen našega dela je bilo zato določiti optimalen čas izpostavitve pšeničnih zrn timijanovemu EO, tako da bi zmanjšali glivne okužbe in hkrati ohranili čim višjo stopnjo kalivosti zrn.

Zrna pšenice smo namakali v EO timijana 6, 12 in 24 ur. Namočena zrna smo po koncu tretmaja osušili, jih prenesli na Petrijeve plošče z gojiščem PDA in 5 dni inkubirali v temi, pri sobni temperaturi. Dnevno smo spremljali kaljivost semen in glivne okužbe. Po koncu poskusa smo stehtali biomaso kalic. Potrdili smo fungicidno aktivnost timijanovega eteričnega olja,[3] saj je daljša izpostavitev semen eteričnemu olju močno zmanjšal število glivnih okužb, pri 24-urnem tretmaju celo do 100 % (Sl. 1b). Po drugi strani je daljša izpostavitev eteričnemu olju zavrla kalitev semen pšenice (Sl. 1a) in maso kalic zmanjšala za 86 % (Sl. 2). Najbolj optimalna je bila 6-urna izpostavitev pšeničnih zrn timijanovemu EO, saj je bilo pri tem tretmaju razmerje med številom kaljenih semen (39 % kaljivost) in glivnih okužb (27 % okuženih semen) najbolj ugodno.

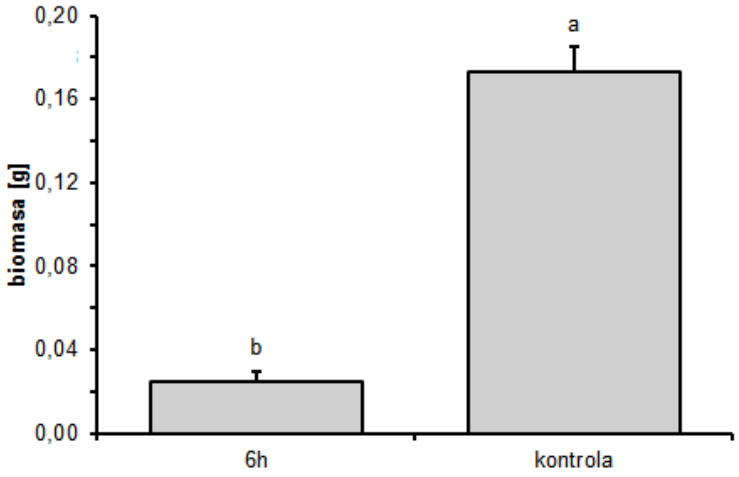


Slika 1: a) Kalitev zrn pšenice (SV ± SN, n = 10), ki so bila različno dolgo izpostavljena timijanovemu eteričnemu olju. b) Dinamika pojavljanja glivnih okužb (mean ± SN, n = 10). Različne črke nad stolpci označujejo statistično značilne razlike pri $p < 0,05$.

Figure 1: a) Germination of wheat grain (mean ± SE, n = 10) exposed to thyme essential oil for different time. b) Dynamics of fungal appearance (mean ± SE, n = 10). Different letters above columns present statistically significant differences at $p < 0.05$.

Wheat (*Triticum aestivum)* is a cultural plant, which is the most important baking cereal. Wheat grains can be infected with different fungi on the field or during the storage processes.[1] In order to protect themselves against herbivores and pathogens, plants produce secondary metabolites,[2] including essential oils (EO). Thyme (*Thymus vulgaris*) is one of plant species with EO that inhibit growth of fungi and bacteria.[3] The aim of our research was to determine optimal time of grain exposure to thyme EO in order to reduce fungal contamination and at the same time preserve seed germination rate as high as possible.

Wheat grain were incubated under shaking in thyme EO for 6, 12 and 24 hours. After the exposure, grain were dried, transfered to PDA plates and incubated for 5 days in dark, at room temperature. Fungal contamination and seed germination were observed daily. At the end of experiment, seedling biomass was measured. Fungicidal activity of thyme EO was confirmed,[3] therefore longer exposure of grain to EO reduced fungal contamination up to 100% by 24-hours treatment (Fig. 1b). On the other hand, longer exposure to EO inhibited germination of wheat grain (Fig. 1a) and the seedlings biomass up to 86% (Fig. 2). The most optimal treatment was 6 hours of exposure, where the ratio between seeds germination (39% germination) and fungal contamination (27% infection) was most favourable.



Slika 2: Biomasa kalic pšenice po 3 dneh rasti (SV ± SN, n = 100). Zrna so bila predhodno izpostavljena timijanovemu eteričnemu olju za 6 ur. Različni črki nad stolpci označujeta statistično značilno razliko pri $p < 0,05$.

Figure 2: Biomass of wheat seedlings after 3 days of growth (mean ± SE, n = 100). Grain were previously exposed to thyme essential oil for 6 hours. Different letters above columns present statistically significant differences at $p < 0.05$.

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Žlezni trihomi navadne in tatarske ajde pogojujejo vsebnosti njunih flavonoidov

Glandular trichomes of common and tartary buckwheat are the key to their flavonoid specificity

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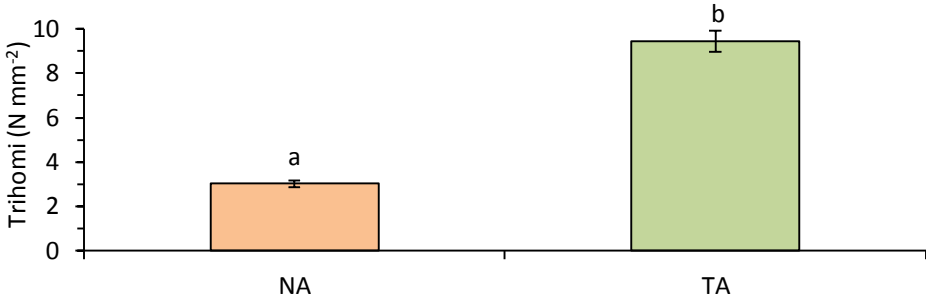
Navadno (*Fagopyrum esculentum* Moench) in tatarsko ajdo (*F. tataricum* Gaertn.) odlikujeta ugodna prehranska kakovost, zaradi vsebnosti fenolnih snovi pa tudi odpornost na škodljivce, bolezni in vplive UV sevanja, kar ju uvršča med funkcionalna živila.[1] Žlezni trihomi so prisotni pri vseh štirih vrstah ajde z razliko v vzorcu razporeditve na zgornji in spodnji povrhnjici.[2] Znano je, da tatarska ajda vsebuje 55-krat več flavonoidov kot navadna.[3] V raziskavi smo preučili prisotnost flavonoidov v žleznih trihomih in njihovo povezanost z vsebnostjo flavonoidov.

Številčno smo ocenili gostoto žleznih trihomov na spodnji in zgornji povrhnjici listov obeh vrst ajde. Njihovo vsebino smo dokazovali z barvili za dokazovanje lipidov, flavonoidov, fenolnih komponent, mrtvih celic in sekundarnih celičnih sten. Predpostavili smo odvisnost trihomov od ontogeneze in preverili njihovo prisotnost v treh razvojnih stopnjah listov (mladi, zreli, stari). Sveže preparate smo opazovali s svetlobnim mikroskopom Axioskop 2 MOT (Carl Zeiss).

Dokazali smo prisotnost žleznih trihomov na spodnji in zgornji povrhnjici listov obeh ajd. Trihomi se pritrjujejo preko dvoceličnega peclja, njihova sekretorna glava pa je večcelična. Podobno so pri rodu *Polygonum* opisali trihome, zgrajene iz kratkega peclja in 2–20 celične sekretorne glave.[4] Pri osvetlitvi trihomov z belo svetlobo so le-ti rjavo obarvani, medtem ko ob ekscitaciji z UV in modro svetlobo celice peclja, katerih stene so obogatene s fenolnimi komponentami, sevajo svetlo modro, celice sekretorne glave pa zaradi prisotnosti flavonoidov sevajo zeleno. Značilno večje število žleznih trihomov je na spodnji povrhnjici zrelih listov. Potrdili smo hipotezo, da je gostota trihomov 3-krat večja pri tatarski ajdi, kar je najverjetneje povezano z večjo vsebnostjo flavonoidov.



Slika 1: Gostota žleznih trihomov (N mm^{-2}) pri *F. esculentum (NA)* in *F. tataricum (TA)*. Prikazane so povprečne vrednosti ±SE, črke nad stolpci prikazujejo statistično značilno razliko (*p<0,05*).

Figure 1: Density of glandular trichomes (N mm^{-2}*)* of *F. esculentum (NA)* and *F. tataricum (TA)*. The average values ±SE are shown, letters above columns represent statistically significant difference (*p<0.05*).

Common (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* Gaertn.) are distinguished by favourable food quality, due to the presence of phenolic compounds also by

the resistance to pests, diseases and the effects of UV radiation, which classifies them among the functional food.[1] Glandular trichomes are observed in all four species of buckwheat with variation in the distribution pattern on upper and lower leaf epidermis.[2] It is known that Tartary buckwheat contains 55-times more flavonoids as common buckwheat.[3] In this study we have examined the presence of flavonoids in glandular trichomes and their connection with the content of flavonoids.

We have quantitatively assessed the density of glandular trichomes on the upper and lower epidermis of the buckwheat leaves. Their content was demonstrated with stains for the lipids, flavonoids, phenolic components, dead cells and secondary cell walls. We assumed that trichomes depends on the ontogenesis and their presence was verified in three developing stages of leaves (young, mature, old). Fresh samples were observed under a light microscope Axioshop 2 MOT (Carl Zeiss).

The presence of glandular trichomes on the upper and lower epidermis of the *F. esculentum* and *F. tataricum* leaves was confirmed. The trichomes are fixed over two-cell stalk, their secretory head is multicellular. Similar trichomes consisted from short stalk and 2–20 cell secretory heads have been described in the genus *Polygonum*.[4] When illuminated with the white light, glandular trichomes are brown, while the excitation of stalk cells, which walls are enriched with phenolic components, with UV and blue light, emit light blue and the cells of secretory heads, due to the presence of flavonoids, emit green. There are significant more glandular trichomes on the lower epidermis of the mature leaves. We have confirmed the hypothesis that the density of trichomes is 3-times higher in Tartary buckwheat, which is most likely associated with a higher content of flavonoids.

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Vloga malih RNA pri obrambnem odgovoru krompirja na okužbo z virusom krompirja Y

Role of small RNAs in defense response of potato to PVY infection

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Virus krompirja Y (PVY) je eden najpomembnejših povzročiteljev bolezni krompirja, ki pri občutljivih sortah povzroča obročkasto nekrozo gomoljev krompirja, bolezen, ki močno vpliva na zmanjšanje kakovosti in količine krompirja ter posledično vodi v hude ekonomske izgube. Znano je, da imajo pri odgovoru na okužbo z virusi pomembno vlogo hormoni, eden ključnih je salicilna kislina (SA), ki povzroči kopičenje inhibitorjev virusnega pomnoževanja in zavira razširjanje virusa po rastlini.[1] Ker so v zadnjem času številne raziskave dokazale pomembno vlogo malih nekodirajočih zaporedij RNA (miRNA, siRNA) pri modulaciji obrambnega odgovora rastline na mnoge povzročitelje bolezni, smo želeli ugotoviti kakšna je njihova vloga pri interakciji krompir - PVY.

V okviru raziskave smo uporabili tolerantno sorto krompirja Désirée in transgene rastline NahG-Désirée. Slednje zaradi vstavljenega gena *nahg,* ki kodira salicilat hidroksilazo, niso sposobne akumulirati SA. Rastline NahG-Désirée zaradi pomanjkanja SA razvijejo močna bolezenska znamenja in vsebujejo večje koncentracije virusa v primerjavi s tolerantnimi netransgenimi rastlinami Désirée.[1,2] Z uporabo teh rastlin smo želeli ugotoviti vpliv pomanjkanja SA na regulatorni nivo malih RNA in poiskati povezave v regulaciji, ki bi lahko pripevale k pojavu simptomov in/ali k povečani koncentraciji virusa.

Z uporabo sekvenciranja naslednje generacije in metodo reverzne transkripcije in verižne reakcije s polimerazo v realnem času (RT-qPCR) smo našli številne virusno regulirane male RNA, katerih količina se je spremenila po virusni okužbi pri obeh genotipih krompirja. Poleg tega smo našli mnoge male RNA s spremenjeno količino le v z virusom okuženih rastlinah Désirée. Dodatno smo z uporabo sekvenciranja naslednje generacije in bioinformatskih analiz določili več kot 100 novih zaporedij miRNA ter več kot 2000 zaporedij siRNA.

Da bi določili njihovo funkcionalno vlogo smo za vse male RNA poiskali tarčne mRNA z uporabo *in silico* pristopa in sekvenciranja degradoma. Regulacijo na nivoju malih RNA smo primerjali tudi s podatki o izražanju njihovih tarčnih genov[2] in odkrili kar nekaj negativnih korelacij. Pokazali smo, da male RNA, povezane s tolerantnim odgovorom krompirja, negativno regulirajo številne mRNA, ki kodirajo imunske receptorje, ključne biosintezne encime in transkripcijske faktorje iz številnih hormonskih signalizacijskih poti.

Potato virus Y (PVY) is one of the most devastating pathogens of potato, causing the potato tuber necrotic ringspot disease, which is responsible for huge decreases in the quality and quantity of potato production. It is known that salicylic acid (SA) is the crucial component for inhibition of the multiplication and spread of PVY.[1] However, recent findings reveal that also small RNAs, broadly divided into microRNAs (miRNAs) and small interferning RNAs (siRNA) are important regulators of gene expression and could play crucial roles in defence responses against various pathogens. The growing body of evidence suggests that sRNAs are important in plant defense immunity albeit none of the studies performed so far investigated small RNA regulatory level in potato. Therefore, the aim of our study was to investigate the role of different small RNAs in potato exposed to PVY infection.

In the study, tolerant plants of cv. Désirée and transgenic, SA deficient potato genotype NahG-Désirée were used. NahG-Désirée plants are unable to accumulate the SA and thus develop strong disease symptoms in reponse to PVY and allow faster onset of PVY multiplication.[1,2] Using this plants we aimed to evaluate the lack of SA on sRNA regulatory level and revealed the connections that may contribute to symptoms development and/or to increased level of PVY.

Employing next generation sequencing and reverse transcription-quantitative real-time PCR (RT-qPCR) many PVY-responsive small RNAs were found in both genotypes. Moreover, certain differentially expressed sRNAs have been found only in tolerant plants of Désirée. Using NGS we additionally identified more than 100 novel miRNAs and more than 2000 siRNAs.

In order to understand the functional role of sRNAs, their target transcript were predicted using *in silico* plant target prediction tool and degradome sequencing approach. Finally, regulation at the sRNA level was compared with the experimental transcriptomics data of measured in the same system.[2] Interestingly, many negative correlations have been found. sRNAs associated with tolerant response of potato were found to negatively regulate several mRNAs encoding immune receptors, key biosynthetic enzymes and transcription factors of various hormone signaling pathways.

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Vpliv onesnaženosti in rastlinske združbe na endofite pisane vilovine (Sesleria caerulea)

Influence of pollution and the plant community on the endophytes of blue moor-grass (Sesleria caerulea)

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V našem projektnem delu smo ugotavljali pojavnost endofitskih gliv. Zanimala nas je pojavnost glivnih endofitov trave (*Sesleria caerulea*) glede na bližino druge rastlinske vrste (*Pinus sylvestrus, Salix caprea, Betula pendula*) in vpliv onesnaženosti rastišča s težkimi kovinami (Cd, Pb in Zn). Razumevanje glivnih endofitov in njihovih interakcij z rastlinami je zelo pomembno, predvsem za pospešitev sukcesije degradiranih območji s pomočjo fitoremediacijskih tehnik. Pričakovali smo, da bo stopnja kolonizacije korenin z mikoriznimi glivami večja na manj onesnaženem območju v primerjavi z bolj onesnaženim. Glive bi lahko na bolj onesnaženem območju razvile več veziklov, kot na manj onesnaženem. Predvidevali smo tudi, da bomo v bližini vrbe (*Salix*) in breze (*Betula*), zaradi njihove sposobnosti bioakumulacije težkih kovin,[1] opazili večjo stopnjo kolonizacije korenin z mikoriznimi glivami. Ker je znano, da temno septirani endofiti (DSE) uspevajo v bolj onesnaženih okoljih,[2] smo pričakovali da bo njihova kolonizacija korenin večja na bolj onesnaženem območju v primerjavi z manj onesnaženim. Korenine smo barvali s tripan modrim,[3] ter z metodo hitrega skeniranja s svetlobno mikroskopijo ocenili stopnjo kolonizacije, prisotnost arbuskulov, veziklov, svitkov (arbuskularno mikorizne glive) in mikrosklerocijev (septiranih temnih endofitov)[4]. Ugotovili smo, da stopnja onesnaženosti zaviralno vpliva na mikorizo. Izkazalo se je tudi, da drevesne vrste zaviralno vplivajo na arbuskularno mikorizo pisane vilovine, vendar podatki niso statistično dovolj različni, da bi lahko bili podlaga za trdnejše sklepe. Prav tako, nismo potrdili hipoteze o večji kolonizaciji DSE na bolj onesnaženih območjih. Potrebne bi bile dodatne raziskave o vplivu drevesnih vrst s svojo ektomikorizo na okoliško arbuskularno in DSE mikorizo.

In this project we investigated the occurence of endophytic funi. We were interested in the occurence of mycorrhizal endophytes of blue moor-grass (*Sesleria caerulea*) relative to the distance of other (arboreal) plant species (*Pinus sylvestrus, Salix caprea, Betula pendula*) and habitat pollution with heavy metals (Cd, Pb in Zn). Knowledge of fungal endophytes and their interactions with plants is crucial for the acceleration of the succession process in degraded areas with the help of phytoremediation techniques.According to our hypothesis there should be a higher root colonisation rate and fewer vesicles in the less polluted soil. Bioaccumulation of heavy metals is present in willows (*Salix*) and birch (*Betula*),[1] therefore we expected a higher colonisation rate in their vicinity. Dark septate endophytes (DSE) grow in more polluted areas, consequently we expected a higher colonisation rate relative to the less polluted areas. We stained the roots with trypan blue[2] and evaluated the colonisation rate, the presence of arbuscules, vesicles, coils (of arbuscular mycorrhizal fungi) and microsclerocia (of dark septate endophytes) with the fast scanning technique using a light microscope.[3] Our results confirm the lesser extent of root colonisation of blue moor-grass in polluted areas. Arboreal species seemed to inhibit arbuscular mycorrhiza, but the difference was not statistically significant. We didn't confirm our hypothesis about the higher colonisation rate of DSE in more polluted soil. More research is needed on the influence of trees and their ectomycorrhiza on the surrounding arbuscular and DSE mycorrhiza.

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