

ABS ACTA **BIOLOGICA** **SLOVENICA**



VOL. 58 ŠT. 1 LJUBLJANA 2015

prej/formerly BIOLOŠKI VESTNIK

ISSN 1408-3671
UDK 57(497.4)

izdajatelj/publisher
Društvo biologov Slovenije

Acta Biologica Slovenica

Glasilo Društva biologov Slovenije – Journal of Biological Society of Slovenia

Izdaja – Published by

Društvo biologov Slovenije – Biological Society of Slovenia

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

Zasnova oblikovanja – Design

Žare Vrezec

ISSN 1408-3671

UDK 57(497.4)

Natisnjeno – Printed on: 2015

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

Naklada: 400 izvodov

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

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

Publikacijo je sofinancirala Javna agencija za raziskovalno dejavnost Republike Slovenije

Acta Biologica Slovenica je indeksirana v – is indexed in: CAB Abstracts, Web of Knowledge – Thomson Reuters

β -lactam antibiotics, *recA* mutation and SOS response

β -laktamski antibiotiki, mutacija *recA* in odziv SOS

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Abstract: The claim that β -lactam antibiotics induce the SOS response, allowing *E. coli* survival in the presence of low antibiotic concentrations, was evaluated. No association between the *recA* gene and antibiotic survival rate was found. Disagreements with published observations are attributed to discrepancy in minimum inhibitory concentrations and growth characteristics of various strains carrying the *recA* mutation. Moreover, β -lactam antibiotics do not induce expression of the SOS regulated gene *cka*, encoding colicin K in wild-type strains.

Keywords: SOS response, *E. coli*, β -lactam antibiotics, antibiotic resistance

Povzetek: Preverili smo trditev, da β -laktamski antibiotiki sprožijo odziv SOS, kar naj bi omogočalo bakteriji *E. coli* preživetje v prisotnosti nizkih koncentracij antibiotikov. Opravljeni poizkusi niso pokazali nobene povezave med delovanjem produkta gena *recA* in stopnjo preživelosti ob prisotnosti antibiotika. Neskladje z objavljenimi opazovanji lahko pripišemo razlikam v minimalni inhibitorni koncentraciji in dramatično različnim lastnostim rasti pri doslej uporabljenih sevih z mutacijo v genu *recA*. Z uporabo genetsko neokrnjenega seva smo ugotovili, da β -laktamski antibiotiki ne sprožijo izražanje gena za kolicin K (*cka*), gena, ki je nadzorovan s sistemom SOS.

Ključne besede: odziv SOS, *E. coli*, β -laktamski antibiotiki, odpornost proti antibiotikom

Introduction

In most bacteria, DNA damage is addressed by a set of genes constituting the SOS response. The response can be triggered by diverse exogenous treatments that elicit DNA damage and physical stress such as high pressure. In addition, stalled replication forks, unrepaired defects following recombination or chromosome segregation, as

well as DNA damage caused by metabolic intermediates can also induce the SOS response. The LexA and RecA (recombinase A) proteins are its key regulators (Erill et al. 2007). RecA protein responds to a single-stranded DNA formed at sites of the DNA damage and triggers degradation of LexA transcriptional repressor. As a consequence, a number of LexA repressed genes are induced, covering among others synthesis and secretion of

colicins in *E. coli*. In 2004, Miller et al. reported that sublethal concentrations of β -lactam antibiotics trigger the SOS response through the DpiBA two-component signal transduction system. Although this effect is generally accepted, several facts question the nature of this induction.. First, the DpiA and DpiB proteins have been shown to be involved in anaerobic citrate metabolism (Kaspar and Bott 2002). DpiB only binds specifically to the promoter regions of *cit* and *mdh* operons involved in citrate metabolism. In addition, it was found that DpiBA may also control the hexuronate dissimilation pathway via the *exuTR* operon, which may possibly be linked to citrate fermentation (Yamamoto et al. 2008). Second, a transcriptome profiling demonstrated that *dpiA* was not induced as one of the SOS genes after exposure to UV, in fact, it was downregulated (Courcelle et al. 2001). Also, contrary to the observation by Miller *et al.*, *dpiA* and *dpiB* were found to be downregulated during ampicillin treatment (Sangurdekar et al. 2006).

The aim of this study was to elucidate the mechanism by which arrest of cell-wall synthesis by β -lactam antibiotics triggers the expression of the seemingly unrelated *dpi* operon. However, neither differences in survival rates of *recA* mutants nor β -lactam induction of the SOS response were

observed when reproducing certain experiments published by Miller et al. (2004).

Material and Methods

Bacterial strains and plasmids

Parental bacterial strains are listed in Table 1. Strains AB1157 *recA::Kn*, MG1655 *recA::Kn* and RW118 *recA::Kn* were constructed by transducing the respective wild-type strains with a P1 lysate, prepared from strain JW2669 carrying a *recA* deletion mutation, and by selecting for kanamycin resistance. Transductants were **verified** by **PCR** with primers flanking the *recA::Kn* ORF and for mitomycin-C sensitivity. Plasmid pKCT1-Tc, carrying the colicin K gene was constructed as follows. Firstly, a 1.8 kb blunt-ended fragment containing the Tc^r gene was obtained after digestion of vector pBR322 with restriction enzymes *Bsr*B I and *Bse*J I. Secondly, plasmid pKCT1 (Mulec *et al.*) was digested with *Psp*1406 I to remove the β -lactamase gene, and the final fragment was treated to generate blunt ends (CloneJet kit; Fermentas). Both fragments were subsequently ligated and used to transform *E. coli* DH5 α . Transformants were selected for tetracycline resistance.

Table 1: Bacterial strains and plasmid used in this study.

Tabela 1: Uporabljeni bakterijski sevi in plazmidi.

Strain	Genotype	Source/Reference
BW25113	<i>lacIq rrnBT14 DlacZ JW16 hsdR514 DaraBADAH33 DrhaBADLD78</i>	(Datsenko and Wanner 2000)
JW1889	BW25113 <i>araF::Kn</i>	Keio Collection
JW2669	BW25113 <i>recA::Kn</i>	Keio Collection
AB1157	<i>thr-1 leuB6 thi-1 supE44 lacY1 kdgK51 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 str-31 tsx-33</i>	(Bachmann 1987)
MG1655	<i>ilvG rfb-50 rph-1</i>	<i>E. coli</i> Genetic Stock Center (CGSC 6300)
RW118	<i>hr-1 araD139 D (gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1 sulA211</i>	(Ho <i>et al.</i> 1993)
RW464	RW118 <i>recA1</i>	(Ho <i>et al.</i> 1993)
Plasmid		
pKCT1-Tc	pKCT1 with Ap ^r gene replaced by Tc ^r gene	(Mulec <i>et al.</i> 2003); this study

Determination of minimum inhibitory concentration (MIC)

LB medium was inoculated with a 1 percent inoculum of an overnight culture of *E. coli* and incubated at 37 °C until the culture reached a density of 0.5 McFarland's turbidity standard (optical density at 600 nm (OD_{600}) of approximately 0.1). A portion of the culture was diluted ten-fold with fresh LB medium, incubated for an additional 30 minutes at 37 °C and subsequently used at a 1:1000 dilution to inoculate test tubes with gradually increasing concentrations of antibiotics. Thus, the final cell number was approximately 5×10^4 per milliliter. After 18-24 hours of growth at 37 °C with aeration, the lowest concentration of antibiotic that has inhibited the visible growth of particular strain was determined.

Survival rate determination

An overnight culture was used to inoculate 40 ml of LB medium and incubated at 37 °C to an OD_{600} of 0.5. The culture was then divided into four 10 ml aliquots in shake flasks, one was used as a control, while appropriate concentrations of antibiotics were added to the others. Growth was continued with standard aeration at 37 °C. Samples were withdrawn at desired intervals and used for viable cell number determination. It was found that more accurate results of viable cell number were obtained when the antibiotic was removed from the sample prior to analysis by brief pelleting in a bench-top centrifuge. The sample was then resuspended in the original volume of fresh LB medium, followed by incubation of test tubes at 37 °C for 15 minutes in a water bath and then used for serial dilutions. This additional step greatly eliminated the presence of long filamentous, undivided cells as observed by microscopy (data not shown). All experiments were repeated at least three times. In addition, stock solutions of antibiotics were kept at -80 °C and used immediately after thawing and then discarded.

Colicin K induction

All $RecA^+$ strains were transformed with the colicin K producing plasmid pKCT1-Tc. Typically, an overnight culture grown at 37 °C was

used to inoculate 40 ml of LB medium at a 1% inoculation level, which was incubated until an OD_{600} of 0.5 was reached. The culture was then divided into four 10 ml aliquots, one served as control, the second one was exposed to 0.5 μ g/ml of mitomycin C (Sigma), the third to ampicillin at $\frac{1}{4}$ x MIC and the fourth to piperacillin at $\frac{1}{4}$ x MIC. Minimal inhibitory concentrations for particular strain are shown in Table 2. After growth for another 4 hrs, a 1 ml sample was removed. The cells were lysed by sonication (3×20 s bursts) using a Vibra Cell (Sonics) sonicator and a lysate was prepared by subsequent centrifugation at $20,000 \times g$ for 10 min. Colicin K activity of the lysate was determined by agar diffusion on LB plates, seeded with *E. coli* DH5a as an indicator strain. A two-fold serial dilutions were used. It was found that the uninduced strain MG 1655 exhibited the lowest colicin K activity and thus, the highest dilution of its lysate still showing inhibition was defined as 1 arbitrary activity unit.

Results and Discussion

β -lactam survival rate of $RecA^-/RecA^+$ strains

Prior to comparing the survival of different *E. coli* mutants against β -lactam antibiotics, the minimum inhibitory concentrations (MIC) of the two antibiotics, ampicillin and piperacillin, was determined. Contrary to the results published by Miller et al. (2004), MICs for both antibiotics were substantially different, either lower or higher, in some commonly used isogenic $RecA^-/RecA^+$ strains (Tab. 2). To exclude the influence of possible secondary mutations, unintentionally introduced during preparation of *recA* defective strains, two fresh *recA* mutant strains, MG1644 *recA::Kn* and AB1157 *recA::Kn* were constructed. Introduction of a *recA* deletion mutation into strain MG1655, generally considered the least genetically manipulated *E. coli* strain, revealed that the observed MIC changes were indeed related to the *recA* mutation. As shown in Table 2, the MIC of ampicillin dropped from 7 μ g/ml to 5 μ g/ml and that of piperacillin from 2.5 μ g/ml to 1.5 μ g/ml. The observed strong sensitivity of all *recA* strains to the DNA damaging agent mitomycin C was as expected. However, MICs for unrelated targets (e.g.

Table 2: Growth characteristics of isogenic RecA⁻/RecA⁺ strains in presence of antibiotics. Final number of cells is expressed as mean $\pm$ standard deviation.

Tabela 2: Značilnosti rasti izogenih sevov RecA⁻/RecA⁺ v prisotnosti antibiotikov. Končno število celic je izraženo kot povprečna vrednost $\pm$ standardna deviacija.

Parent strain	Mutant strains	MIC for ampicillin (μ g/ml)	MIC for piperacillin (μ g/ml)	MIC for chloramphenicol (μ g/ml)	MIC for mitomycin (μ g/ml)	Doubling time (min)	Final number of cells ($\times 10^6$)
BW25113	w.t.	8.0	2.5	6.0	5.0	23	2.9 $\pm$ 0.6
	recA::Kn	5.0	1.5	3.5	0.2	40	0.3 $\pm$ 0.06
MG1655	w.t.	7.0	2.5	5.5	6.0	22	3.5 $\pm$ 0.9
	recA::Kn	5.0	1.5	4.0	0.4	29	2.1 $\pm$ 0.3
AB1157	w.t.	10.0	4.0	5.5	3.0	28	2.5 $\pm$ 0.2
	recA1	7.0	2.0	5.5	0.45	33	1.1 $\pm$ 0.2
	recA::Kn	7.0	2.5	5.5	0.4	37	1.2 $\pm$ 0.4
RW118	w.t.	9.0	2.5	3.5	6.0	25	2.6 $\pm$ 0.3
	recA1	7.0	2.0	3.0	0.25	40	0.75 $\pm$ 0.15

chloramphenicol) were also significantly reduced. In addition, some growth characteristics were also affected. All *recA* defective strains exhibited slower growth rates in comparison with the corresponding wild-type strains e.g., as much as twofold in the JW2669 (RecA⁻) when compared with the isogenic strain JW1889 (RecA⁺). Furthermore, the maximum cell concentration in a culture was also affected by mutation of *recA*, again most pronounced in strain JW2669 – the number of cells was reduced by as much as one order of magnitude. The striking difference between this particular strain and other *recA* mutants is not understood; none of the known mutations in JW1889, its parental strain, seem to be associated with any *recA* dependent process. Reduced viability of *recA* mutants was already observed by Miranda and Kuzminov (2003), although a direct comparison is not possible, as they cultivated their cultures under suboptimal conditions (at 28 °C).

Next, the survival rate of isogenic RecA⁻/RecA⁺ strains following addition of β -lactam antibiotics in the exponential growth phase was determined. The concentrations used were chosen in accordance with the obtained MIC data and at similar multiples (1/2, 1 and 2) of MICs to those used by Miller et al. (2004). The obtained results are presented in Table 3 for ampicillin and in Ta-

ble 4 for piperacillin, respectively. As shown for both antibiotics, no significant difference in the survival rate of the isogenic RecA⁻/RecA⁺ strains was found. Clearly, our data did not confirm the previously reported assertion that inactivation of *recA* increases bacterial susceptibility to ampicillin and piperacillin.

SOS induction

Colicins, bacteriocins produced by *E. coli* strains, are typically regulated by the SOS response. To test the ability of either ampicillin or piperacillin to induce the SOS response, we studied the effect of both antibiotics on colicin K expression. Although the *cka* gene is transcribed-late during the SOS response, induction resulted in an increase of *cka* expression spanning three orders of magnitude (Tab. 5). Thus, even a slight change in *cka* gene activity could be easily identified. Nevertheless, our results (Tab. 5) showed no *cka* induction by ampicillin or piperacillin at $\frac{1}{4} \times$ MIC. As expected, colicin K expression was fully induced by mitomycin C.

In conclusion, inactivation of *recA* results in important alterations in cell metabolism: (a) reduction of growth rate; (b) reduction of final cell density to only 10 % to 60 % of the wild-

Table 3: Survival of various isogenic RecA⁻/RecA⁺ strains following treatment with ampicillin. Percentage of survivors resembles the ratio of cell count before and after the addition of antibiotic and is expressed as mean $\pm$ standard deviation.

Tabela 3: Preživetje različnih izogenih sevov RecA⁻/RecA⁺ po izpostavljenju ampicilinu. Odstotek preživetih je razmerje med številom celic pred in po dodatki antibiotika ter je izražen kot povprečna vrednost $\pm$ standardna deviacija.

Parent strain	Mutant strain	% of survivors					
		1/2 x MIC		1 x MIC		2 x MIC	
		1 h	4 h	1 h	4 h	1 h	4 h
BW25113	w.t.	32.8 $\pm$ 18.0	3.5 $\pm$ 1.0	10.2 $\pm$ 3.7	1.0 $\pm$ 0.7	4.1 $\pm$ 1.4	0.6 $\pm$ 0.3
	recA::Kn	80.1 $\pm$ 32.7	35.5 $\pm$ 1.5	8.1 $\pm$ 1.4	5.2 $\pm$ 2.3	1.7 $\pm$ 1.3	0.4 $\pm$ 0.3
MG1655	w.t.	61.6 $\pm$ 25.7	44.9 $\pm$ 27.8	22.8 $\pm$ 13.5	0.8 $\pm$ 0.6	1.8 $\pm$ 0.8	0.42 $\pm$ 0.39
	recA::Kn	75.9 $\pm$ 14.6	40.4 $\pm$ 22.4	32.7 $\pm$ 7.6	0.55 $\pm$ 0.28	2.1 $\pm$ 0.9	0.14 $\pm$ 0.03
AB1157	w.t.	105.7 $\pm$ 19.7	32.7 $\pm$ 15.0	51.1 $\pm$ 14.4	4.4 $\pm$ 1.9	20.6 $\pm$ 7.7	0.1 $\pm$ 0.06
	recA1	105.1 $\pm$ 27.5	17.0 $\pm$ 2.2	41.2 $\pm$ 8.1	1.9 $\pm$ 0.8	35.3 $\pm$ 4.2	0.6 $\pm$ 0.4
	recA::Kn	90.8 $\pm$ 11.6	65.1 $\pm$ 22.0	38.5 $\pm$ 19.0	7.9 $\pm$ 4.8	33.9 $\pm$ 18.6	3.3 $\pm$ 1.1
RW118	w.t.	48.8 $\pm$ 25.3	5.2 $\pm$ 2.9	17.1 $\pm$ 8.5	0.7 $\pm$ 0.56	1.5 $\pm$ 0.8	0.32 $\pm$ 0.2
	recA1	40.2 $\pm$ 10.3	12.6 $\pm$ 11.0	13.0 $\pm$ 3.7	0.9 $\pm$ 1.3	0.9 $\pm$ 0.6	0.08 $\pm$ 0.05

Table 4: Survival of various isogenic RecA⁻/RecA⁺ strains following treatment with piperacillin. Percentage of survivors resembles the ratio of cell count before and after the addition of antibiotic and is expressed as mean $\pm$ standard deviation.

Tabela 4: Preživetje različnih izogenih sevov RecA⁻/RecA⁺ po izpostavljenju piperacilinu. Odstotek preživetih je razmerje med številom celic pred in po dodatki antibiotika ter je izražen kot povprečna vrednost $\pm$ standardna deviacija.

Parent strain	Mutant strain	% of survivors					
		1 x MIC		2 x MIC		4 x MIC	
		1 h	4 h	1 h	4 h	1 h	4 h
BW25113	w.t.	33.1 $\pm$ 12.1	12.4 $\pm$ 7.4	21.8 $\pm$ 12.9	6.5 $\pm$ 4.8	16.5 $\pm$ 8.2	1.4 $\pm$ 1.0
	recA::Kn	39.8 $\pm$ 12.3	33.9 $\pm$ 7.0	27.5 $\pm$ 15.7	19.9 $\pm$ 10.8	16.3 $\pm$ 3.3	10.7 $\pm$ 5.2
MG1655	w.t.	29.9 $\pm$ 11.9	8.4 $\pm$ 5.5	13.6 $\pm$ 4.9	6.9 $\pm$ 3.8	15.8 $\pm$ 6.1	4.9 $\pm$ 3.9
	recA::Kn	56.5 $\pm$ 10.9	3.4 $\pm$ 2.2	32.5 $\pm$ 7.9	3.3 $\pm$ 2.5	35.6 $\pm$ 8.0	2.8 $\pm$ 2.3
AB1157	w.t.	82.4 $\pm$ 16.5	7.5 $\pm$ 3.1	64.0 $\pm$ 19.5	9.0 $\pm$ 5.5	43.0 $\pm$ 21.6	4.2 $\pm$ 0.5
	recA1	68.0 $\pm$ 34.0	14.5 $\pm$ 4.5	52.2 $\pm$ 21.7	2.8 $\pm$ 1.3	23.5 $\pm$ 3.1	2.2 $\pm$ 1.6
	recA::Kn	59.1 $\pm$ 15.5	7.1 $\pm$ 2.2	37.2 $\pm$ 12.0	4.4 $\pm$ 1.3	27.2 $\pm$ 13.8	3.8 $\pm$ 1.4
RW118	w.t.	42.2 $\pm$ 7.1	7.5 $\pm$ 3.8	29.9 $\pm$ 11.9	2.1 $\pm$ 1.0	30.9 $\pm$ 8.4	2.5 $\pm$ 1.7
	recA1	34 $\pm$ 10.5	12.3 $\pm$ 5.3	24.9 $\pm$ 11.3	6.3 $\pm$ 3.8	19.3 $\pm$ 12.0	3.9 $\pm$ 1.6

Table 5: The activity of colicin K synthesized in *E. coli* RecA⁺ strains of different genetic backgrounds following addition of ampicillin (1/4 of MIC), piperacillin (1/4 of MIC) or mitomycin C (0.5 µg/ml), respectively.
 Tabela 5: Aktivnost kolicina K sintetiziranega v sevih *E. coli* RecA⁺ različnega genetskega ozadja po dodatku bodisi ampicilina (1/4 MIC), piperacilina (1/4 MIC) ali mitomicina C (0.5 µg/ml).

Strain	Colicin K activity (arbitrary units)			
	no inducer/ control	ampicillin	piperacillin	mitomycin C
JW1889	2	2	1	1280-2560
MG1655	1	1	1-2	1280
AB1157	8	8	8	1280
RW118	8	8	4	2560-5120

type strain, depending on the genetic background (Tab. 2); and (c) increased sensitivity to different antibiotics with unrelated mode of action. In this respect, the interpretation of an isolated phenomenon, such as the response to β -lactams, could be misleading if based solely on the comparison between RecA⁻/RecA⁺ strains.

Summary

Due to the claim that β -lactam antibiotics induce the SOS response in *E. coli*, the characteristics of various *recA* mutants were analyzed. It was shown that inactivation of the *recA* gene severely altered cell metabolism, reflected in its growth rate, final population density and its sensitivity to unrelated antibiotics. By using genetically "clean" wild-type strains, it was possible to show that there is no link between the presence of β -lactam antibiotics and the SOS response.

The observations presented here urge the reinterpretation of the published results based on the genetically over-manipulated strains.

Povzetek

Zaradi trditve, da β -laktamski antibiotiki sprožijo odziv SOS pri *E. coli*, smo analizirali značilnosti nekaterih mutantov *recA*. Pokazalo se je, da inaktivacija tega gena izzove dramatične spremembe v celičnem metabolizmu, ki se odražajo v stopnji rasti, končni populaciji celic in občutljivosti za različne, nesorodne antibiotike. Uporaba genetsko "čistega" seva je pokazala, da ni nobene povezave med β -laktamskimi antibiotiki in odzivom SOS.

Predstavljena opazovanja kličejo po nujni re-interpretaciji marsikaterega objavljenega rezultata, izvedenega na genetsko (pre)obremenjenih sevih.

Acknowledgement

This work was supported by the Slovenian Research Agency (ARRS).

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Taxonomy, phytogeography and phytosociology of *Laserpitium krapfii* Crantz. in Slovenia

Taksonomska, fitogeografska in fitocenološka oznaka vrste *Laserpitium krapfii* Crantz. v Sloveniji

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Abstract: The article discusses the occurrence, distribution and phytosociological affinity of *Laserpitium krapfii* in Slovenia. According to some literature sources (Tutin 1968, Fischer et al. 2008) and the distribution patterns, two subspecies of *L. krapfii* are expected in Slovenia: *L. krapfii* subsp. *krapfii* and *L. krapfii* subsp. *gaudinii*. The revision of the Slovene herbarium material in LJU and LJS herbaria confirmed only the occurrence of its type subspecies. It has a Dinaric pattern of distribution (NW-SE) in the Alpine, Prealpine, Dinaric and Predinaric phytogeographical regions, with most of its known localities in the hills south of Ljubljana, in the Snežnik mountains, in the Kočevje region with the Kolpa Valley and in the Gorjanci mountains. Since the species mostly thrives in the mountain beech forests, it can be considered as a diagnostic (differential) species of the Illyrian alliance *Aremonio-Fagion*.

Key words: *Laserpitium krapfii* subsp. *krapfii*, taxonomy, phytogeography, *Aremonio-Fagion*, Slovenia

Izvleček: V članku obravnavamo pojavljanje, razširjenost in fitocenološko navezanost vrste *Laserpitium krapfii* v Sloveniji. Glede na literaturne navedbe (Tutin 1968, Fischer et al. 2008) in vzorca razširjenosti, bi pri nas lahko pričakovali uspevanje dveh podvrst: *L. krapfii* subsp. *krapfii* in *L. krapfii* subsp. *gaudinii*. Z revizijo herbarijskega gradiva v LJU in LJS smo za Slovenijo potrdili le pojavljanje podvrste *L. krapfii* subsp. *krapfii*. Razširjena je v dinarski smeri (severozahod-jugovzhod), v alpskem, predalpskem, dinarskem in preddinarskem fitogeografskem območju, z največjo gostoto nahajališč v hribovju južno od Ljubljane, v Snežniškem pogorju, na Kočevskem s Kolpsko dolino in na Gorjancih. Največ nahajališč je v montanskih bukovih gozdovih, zato jo lahko štejemo za diagnostično (razlikovalno) vrsto ilirske zveze *Aremonio-Fagion*.

Ključne besede: *Laserpitium krapfii* subsp. *krapfii*, taksonomija, fitogeografija, *Aremonio-Fagion*, Slovenija

Introduction

Laserpitium krapfii is a member of the Umbelliferae family (*Apiaceae*). The identification of the members of this family is relatively difficult, especially since fully ripe fruits and flowers are very important for their identification. Among other members of the *Laserpitium* genus, *L. krapfii* is well characterized by its ovoid to oblong, dentate leaf-segments, 0-5 bracts, which are glabrous, and 5-15 rays of the umbel, which are distinctly of different lengths; flowers are usually greenish (Martinčič 2007).

The distribution of *L. krapfii* in Slovenia was discussed in Fleischmann (1844), Plemel (1862), Paulin (1904), Neugebauer (1932), Mayer (1952), T. Wraber (1966), Thellung (1975) etc.; contemporary knowledge is summarized by Martinčič (2007). According to this literature source, *L. krapfii* thrives in the Slovenian Alpine (Tolminsko), Dinaric and Predinaric (Gorjanci mountain range) phytogeographical region. In the work *Gradivo za Atlas flore Slovenije* (Jogan & al. 2001), the distribution map of *L. krapfii* has accidentally been omitted.

According to Flora Europaea (Tutin 1968) there are two subspecies of *L. krapfii*: *L. krapfii* subsp. *krapfii* (syn. *L. marginatum* Waldst. & Kit.; incl. *L. alpinum* Waldst. & Kit.) and *L. krapfii* L. subsp. *gaudinii* (Moretti) Thell. (*L. gaudinii* Moretti). The type subspecies has a Dinaric-Carpathian pattern of distribution: it thrives in the northern part of Balkan Peninsula, extending to NE Italy and in Carpathians (Tutin 1968). The other subspecies, *L. krapfii* subsp. *gaudinii* has an Alpine distribution. According to Flora Europaea (Tutin 1968), it grows in W Austria, E Switzerland, N Italy, and in the northwestern part of the former Yugoslavia, which implicitly include Slovenia. Similarly, Fischer et al. (2008) also indicate that *L. krapfii* L. subsp. *gaudinii* thrives in Slovenia (with its main distribution area in E Switzerland, N Italy and N Tyrol).

However, according to Martinčič (2007) there is only one subspecies present in Slovenia, that is the type species *L. krapfii* subsp. *krapfii*. There are no older Slovenian data referring to *L. krapfii* L. subsp. *gaudinii*. Only *L. krapfii* subsp. *krapfii* (sin. *L. marginatum*) is considered by the authors

(f. e. Paulin 1904, Neugebauer 1932, Mayer 1952).

It should be added, that in Austria, *L. krapfii* subsp. *gaudinii* is very rare. It is included in the red list of threatened species (Niklfeld and Schratt-Ehrendorfer; 1999), but not mentioned in *Verbreitungsatlas der Farn- und Blütenpflanzen Kärntens* (Hartl et al. 1992). In adjacent parts of Italy (Friuli Venezia Giulia) the species doesn't occur (Poldini 2002).

On the other hand, according to the distribution map in *Flora Alpina* (Aeschmann and al. 2004), the type subspecies has an eastern-Alpine-Illyrian pattern of distribution; it grows in Alpine areas of N Italy, Switzerland, W Austria, and (surprisingly) in Dinaric mountains, but not in Slovenia.

Morphologically, the two subspecies are well characterized (see Table 1) and they also differ in ecological preferences. *L. krapfii* subsp. *gaudinii* thrives in low-nutrient meadows, glacial gravel and bushes, in mountain and subalpine belt (Fischer et al. 2008), while the type subspecies prefers forests (Thellung 1975, Martinčič 2007).

Since Slovenia lies between the Alps and the Dinarides, we believe that the occurrence of both subspecies is expected. That was the reason why we revised all the available herbarium material to verify the taxonomic status of Slovene populations. The distribution map of *L. krapfii* has not yet been published, so we systematically gathered floristic data to produce an up-to-date distribution map. We also investigated the occurrence of the species in different forest communities and investigated its phytosociological preferences.

Material and methods

We reviewed all the available herbarium material of this species in Herbarium of the Ljubljana University (LJU) (22 herbarium sheets) and Herbarium of the Institute of Biology ZRC SAZU (LJS) (13 herbarium sheets). Data on the revised herbarium specimens (*Specimina visa*) are in Appendix. We checked the discriminative characters, provided by Pignatti 1982, Tutin 1968 and Thellung 1975 (Table 1).

Table 1: Discriminative characters for *Laserpitium krapfii* subsp. *krapfii* and *L. krapfii* subsp. *gaudinii* (Pignatti 1982, Tutin 1968, Thellung 1975).

Preglednica 1: Razlikovalni znaki med podvrstama *Laserpitium krapfii* subsp. *krapfii* in *L. krapfii* subsp. *gaudinii* (Pignatti 1982, Tutin 1968, Thellung 1975).

Character	<i>L. krapfii</i> subsp. <i>krapfii</i>	<i>L. krapfii</i> subsp. <i>gaudinii</i>
pruinosity of the stem	slightly pruinose	usually strongly pruinose
similarity of the upper cauline leaves with lower leaves	similar	markedly different
the shape of leaf-segments of upper cauline leaves	ovate, usually dentate	oblong, usually entire
roughness of the umbel rays	rays rough or shortly hispid on inner side	rays glabrous and smooth on inner side
presence of setae on primary ridges of fruit	ridges with short setae	ridges glabrous

To get further insight in the discrimination of the two subspecies, we also compared Slovene material with the available material of *L. krapfii* subsp. *gaudinii* from abroad.

Distributional data were obtained from the database of Centre for Cartography of Fauna and Flora and the FloVegSi database of the Institute of Biology of the SRC SASA. We used standard botanical and phytosociological methods (Ehrendorfer and Hamann 1965, Jalas and Suominen 1967, Braun-Blanquet 1964). Nomenclature source for the names of the taxa is Mala flora Slovenije (Martinčič et al. 2007) and for the names of the syntaxa (Šilc and Čarni 2012). The distribution map was made with the application FloVegSi (Seliškar et al. 2003).

Results and discussion

The herbarium revision in the determination of the subspecies

We found that all of the examined specimens, collected in the territory of Slovenia, belong to the type subspecies. Despite the fact that for now we do not have confirmation of the occurrence of *L. krapfii* subsp. *gaudinii* in Slovenia, we need to be aware of this possibility during the fieldwork in the northern part of the country, especially on scree, low-nutrient dry meadows and in tall herb and scrub communities of the mountain and subalpine belt.

Subspecies can reliably be identified, if the plants are flowering or fruiting, that is from mid-July onwards. The fruits are fully developed at the beginning of August, but for the determination of the subspecies they are not required. Especially useful characters are the roughness of the umbel rays (prickle trichomes on the inner side of the rays – Figure 1) and the presence of short setae on primary ridges of the fruit (Figure 2). Both are present only in the type subspecies. When observing these characters, we need to use a stronger magnifying glass (20-30 x magnification). In herbarium material, the characters pruinosity of the stem and the similarity of the upper cauline leaves with the lower ones proved hard to assess. The leaves seemed rather variable and there were not enough material of *L. krapfii* subsp. *gaudinii* to evaluate the differences in shapes of the leaf-segments of upper cauline leaves and their dentation in both of the subspecies.

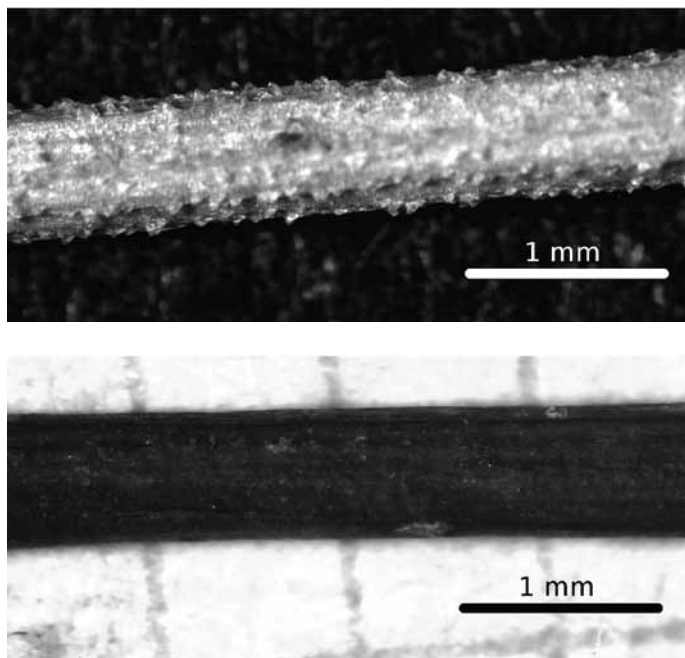


Figure 1: Prickle trichomes on the inner side of the rays present in *L. krapfii* subsp. *krapfii* (top) and absent in *L. krapfii* subsp. *gaudinii* (below).

Slika 1: Žarki kobula z bodičkami na notranji strani pri *L. krapfii* subsp. *krapfii* (zgoraj) in gladki kobulovi žarki pri *L. krapfii* subsp. *gaudinii* (spodaj).

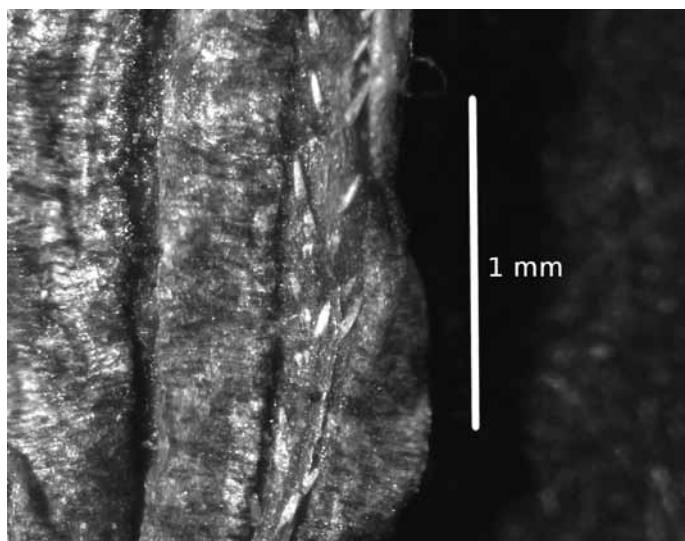


Figure 2: Short setae on primary ridges of the fruit in *L. krapfii* subsp. *krapfii*.

Slika 2: Ščetine na rebrih plodu pri *L. krapfii* subsp. *krapfii*.

The distribution of species in Slovenia

The distribution of L. krapfii subsp. krapfii in the Alpine and Prealpine phytogeographical region

According to the results of our study, in the Alpine phytogeographical region, the occurrence of *L. krapfii* is limited to the southern extensions of the Tolmin-Bohinj ridge of the Julian Alps. The localities, that are closest to this ridge, are on the slopes of the peak Žabijski Kuk above the Razor pasture. Most localities lie on the slopes of the side-lying, south-oriented ridge Žabijski Kuk–Vrh nad Sopotom–Krikov vrh–Tolminski Triglav–Kobilja glava–Jalovnik, above the valleys of streams Zadlaščica and Kneža (Knežica) with its right tributary Lipovšček (Dakskobler 1991, 1994, 2001, 2002, 2003, 2006, 2015). The reports for *L. krapfii* in extensions of the Savinja Alps (plateau Krašica above Šmartno at Dreta) were published by Diaci (1997). There are no other reports of *L. krapfii* in this mountain range and Diaci's records are not documented with herbarium material. We assess that these data need verification and that is the reason why we marked them as 'questionable' in our distribution map (Figure 3).

In the phytosociological tables published by Piskernik (1977), there are two additional records of *L. krapfii* for Alpine–Prealpine part of Slovenia. The first relates to Breginjski Stol. Although the flora of this area is relatively well-studied, *L. krapfii* was not observed there by other botanists so far (see Čušin 2006). The second record is from Paški Kozjak above Spodnji Dolič that is in the Prealpine phytogeographic region. These two localities are also marked as 'questionable', since the voucher herbarium material is not available. We here publish some new data from herbarium LJS (revision by Dakskobler and Vreš, February 2015) for Alpine and Prealpine region (Bača and Idrija Valleys: 9749/4, 9849/1, 9848/4 – for details see Appendix). There are also reliable records of *L. krapfii* in Prealpine phytogeographical region, on the Zaplana plateau between Vrhnika and Logatec (T. Wraber 1996, Rozman 2000).

The distribution of L. krapfii in Dinaric and Predinaric phytogeographical region

The localities of *L. krapfii* in the northwestern-most part of the Dinaric mountains – in Slovenian Dinaric phytogeographical region, were published

by Glowacki and Arnold (1870). This publication was summarized by Neugebauer (1932). According to these authors, the species thrived in Vojščica on the Vojskarska planota plateau in the Idrija mountains. We confirmed these records in the nearby hill Hudournik, on the edge of the plateau above the valleys of Kanomlja and Hotenja (Dakskobler 2001).

As far as we know, Paulin's (1904) report about locality under Čaven at Predmeja (Dol) has no recent confirmations. Paulin also reported that the species presumably grows near Idrija ("angeblich bei Idria"). Since surroundings of Idrija extend over several MTB quadrants, this record is not precise enough to put it in our distribution map.

The localities in SW part of the Trnovski gozd between Otlica and Col (under Kovk) (Dakskobler 1997, phytocoenological table 4 and herbarium LJU and LJS) and on the western edge of the plateau Nanos above the valley of Bela and at Podkraj (Dakskobler (1997), M. Wraber and Žigon, 16. 10. 1970 – personal notes of Wraber M. and J. Žigon) were recently confirmed.

The localities of *L. krapfii* on the hills south of Ljubljana (Krim, Mokrc, Rakitna plateau, Iška basin) have already been known to botanists for over 170 years (Fleischmann 1843, Paulin 1904). These populations are well documented with herbarium material LJU (authors Budnar, Mayer, Dolšak, Paulin, Zrimec – see Appendix). There are also other recent reports for this area (Robič 1960a, b and Accetto 2010, 2013) – the systematic mapping of flora was carried out particularly in Iška.

The records for the Snežnik mountains have been contributed by Paulin (1904), Justin (1923, LJU), Tregubov (1957), T. Wraber (1966, also herbarium material in LJU), Zupančič (1972, LJU), Piskernik (1977, 1991), Marinček (1996) and Surina and Rakaj (2007) – our observations during floristic fieldwork in this area showed the same (unpublished data BV).

Older data referring to the presence of *L. krapfii* in Kočevsko and Kolpa valley (Fleischmann 1844, Plemel 1862, LJU, Paulin 1904) are recently confirmed with numerous records: Martinčič (1958, LJU), Peterlin (1960, LJU), M. Wraber (1962, 1963, in litt.), Štimec (1982), Hočvar et al. (1985, 1995), Accetto (1995, 1999a, b, 2002a, 2003, 2007a, b, 2008), Accetto et al. (1996), Frajman (2001, LJU),

Trčak et al. (2002) and our own unpublished data (BV). According to these reports, the taxon is widespread in the Kočevsko: it thrives in Bela stena near Ribnica, Fridrihštajn, Podstene at Koprivnik in the Kočevski Rog, Goteniški Snežnik, Goteniška gora, the mountains of Borovška gora with Firstov rep, Krokav and Krempa and in their ravines above the Kolpa valley, Stružnica, Mirna gora near Semič, the slopes above the Čabranka valley (Strma reber, Belica) etc.

The presence of *L. krapfii* in the Gorjanci mountain range was noticed already by Paulin (1904). In the following decades, the plants were collected by Rataj (1954, LJU), Martinčič (1954, LJU), Strgar (1960, LJU), T. Wraber (1992, LJU) and recorded by Košir (1979), Hočevar et al. (1985) and Accetto (2002b). In Gorjanci, we recorded the species in forest reserve Kobile (Dakskobler et Grah, 2013, 2014, in litt.).

Distribution map

According to the distribution map (Figure 3), *L. krapfii* s. str. has a Dinaric pattern of the distribution, extending from NW to SE of Slovenia. It thrives in the Alpine, Prealpine, Dinaric and Predinaric phytogeographical region, with a concentration of its localities in the hills south from Ljubljana, the Snežnik Mountains, the Kočevje region with the Kolpa valley and in the Gorjanci mountain range.

Phytosociological characteristics of *L. krapfii*

According to Aeschmann et al. (2004), *L. krapfii* subsp. *gaudinii* is predominantly a scree taxon, a character species of the alliance *Petasi-tion paradoxi*. Zupančič (1999) characterizes *L. krapfii* as character species of the alliance *Vaccinio-Piceion* or the order *Vaccinio-Piceetalia*, while Accetto (2010, 2013) treats *L. krapfii* to be character species of the class *Erico-Pinetea*.

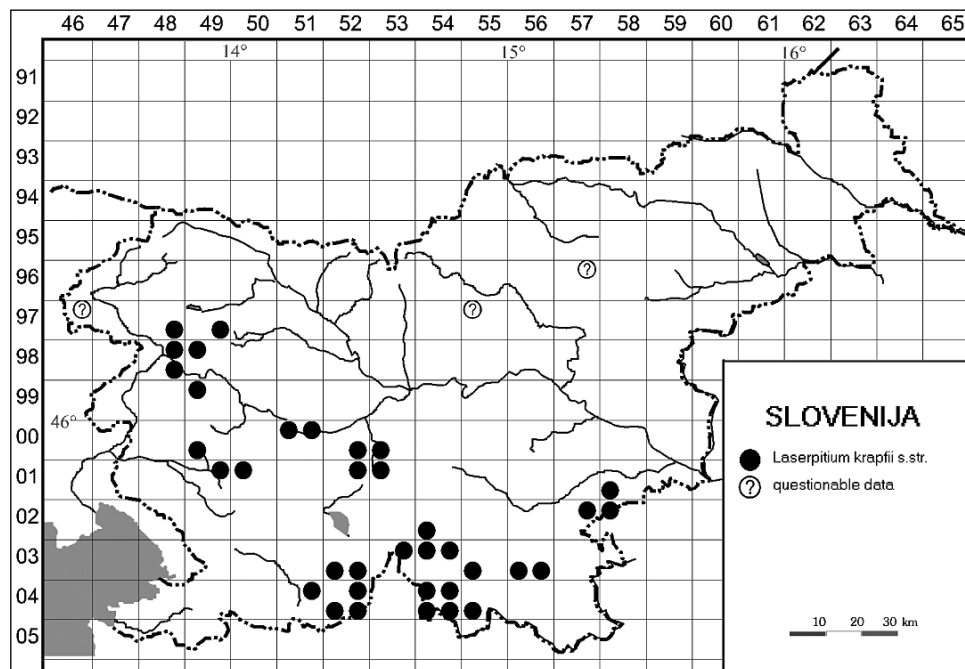


Figure 3: Distribution of *Laserpitium krapfii* subsp. *krapfii* in Slovenia.

Slika 3: Razširjenost vrste *Laserpitium krapfii* subsp. *krapfii* v Sloveniji.

Košir (1979) selected *L. krapfii* (= *L. marginatum*) as character species for the association *Arunco-Fagetum*. After this species, Vukelić et al. (2010) named the altimontane-subalpine spruce forest in northern part of the Velebit range – *Laserpitio krapfii-Piceetum*. In the Snežnik Mts., Tregubov (1957) considers *L. krapfii* as a differential species for the subassociation *Calamagrostio-Abietetum piceetosum*. Surina and Rakaj (2007) found the species in the same area in the stands of the subassociation *Polysticho lonchitis-Fagetum rhododendretosum hirsuti*. In the stands of the association *Polysticho lonchitis-Fagetum* under Snežnik, *L. krapfii* was recorded by T. Wraber (1966) and a few decades later also by Marinček (1996). In the Snežnik mountains, Marinček and Čarni (2010) recorded this species also in the stands of the syntaxon *Ranunculo platanifolii-Fagetum* var. geogr. *Calamintha grandiflora typicum* var. *Helleborus niger*.

In the southern Julian Alps, *L. krapfii* thrives in the stands of the following syntaxa: *Seslerio autumnalis-Fagetum*, *Ostryo-Fagetum* (Dakskobler 1991), *Arunco-Fagetum* (Dakskobler 1994, 2004), *Ranunculo platanifolii-Fagetum*, *Luzulo-Fagetum* (Dakskobler 2001), *Homogyno sylvestris-Fagetum* (Dakskobler 2002), *Rhododendro hirsuti-Fagetum* (Dakskobler 2003), *Rhodothamno-Laricetum ostryetosum* (Dakskobler 2006) and *Rhododendro hirsuti-Ostryetum* (Dakskobler 2015). In the summer 2004, we found the species on the north-west slopes of the peak Žabijski Kuk above the Razor pasture (ca. 1350 m – 1400 m a. s. l.), in the Alpine dwarf pine stands (*Rhodothamno-Pinetum mugo*).

On the northern edge of the Vojsko plateau and on the edges of the Trnovski gozd and Nanos plateaus, we recorded the species in the stands of the associations *Omphalodo-Fagetum*, *Ranunculo platanifolii-Fagetum*, *Arunco-Fagetum*, *Rhododendro hirsuti-Fagetum* and *Seslerio autumnalis-Fagetum*.

Near Zaplana, T. Wraber (1996) recorded the species in the stand of the association *Ostryo-Fagetum*. On Mokrc, Robič (1960) observed it in the stands of the associations *Arunco-Fagetum* and *Omphalodo-Fagetum*.

In Iška basin, Accetto (2013) found the species in the stands of the associations *Omphalodo-Fagetum* and *Ostryo carpinifolii-Piceetum*, but

it is also present in the stands of the association *Arunco-Fagetum*.

In the Gorjanci mountain range, Accetto (2002b) recorded the species in the stands of the association *Tanacetum clusii-Fagetum*. In the Kočevsko region, Zupančič and Accetto (1994) reported *L. krapfii* for the stands of the association *Ribeso alpini-Piceetum*, while Accetto (1995, 1999 a, b, 2002a, 2003, 2007b, 2008) observed the species in the stands of the syntaxa *Carici sempervirentis-Pinetum nigrae*, *Aconito lycoctoni-Fagetum*, *Omphalodo-Fagetum*, *Lamio orvalae-Fagetum*, *Allio victorialis-Fagetum*, *Rhododendro hirsuti-Fagetum*, *Arunco-Fagetum* var. geogr. *Acer obtusatum* and in the stands of two non-forest associations, *Seslerio kalnikensis-Arabidetum muralis* and *Neckero crispae-Campanuletum justinianae*.

Most of the localities are in the montane belt, between 400 m and 1200 m n. m. The highest localities of the species in the Julian Alps are in the alpine dwarf pine stands below the peak Žabijski Kuk, about 1400 m a. s. l., while in the Dinaric Mountains, the vertical distribution of *L. krapfii* reaches (current) upper timberline below Snežnik, at an altitude of about 1600 m.

The localities are mostly on calcareous bedrock (dolomite, dolomite limestone and limestone), occasionally with admixture of chert marlstone or claystone. The soils are shallow (mainly rendzina), sometimes due to the admixture of chert somewhat acidic.

Although this species occasionally thrives in some spruce, black pine and alpine dwarf pine stands, we ascertained that most localities are in the Illyrian beech forests and therefore we may reasonably consider it as diagnostic (differential) species of the Illyrian alliance *Aremonio-Fagion*.

Taking into account its habitat-type preferences in Slovenia, the species doesn't seem to have strong affinity to communities of the alliance *Vaccinio-Piceion*, except for the fact that it predominantly grows on shallow and often rocky ground with moder rendzina.

If (when) we rank it among the character species of spruce forests, in our opinion it should be associated with the suballiance *Abieti-Piceion*.

Conclusions

According to some literature sources (Tutin 1968, Fischer et al. 2008) and the distribution patterns, two subspecies of *L. krapfii* would be expected in Slovenia: *L. krapfii* subsp. *krapfii* and *L. krapfii* subsp. *gaudinii*. The revision of Slovene herbarium material in LJU and LJS confirmed only the presence of the type subspecies. However, we need to be aware of the possibility of finding the other subspecies, during the fieldwork in the northern part of the country, especially on screes, low-nutrient dry meadows, in tall herb and scrub communities of the mountain and subalpine belt.

In Slovenia, *L. krapfii* subsp. *krapfii* has a Dinaric pattern of distribution, extending from NW to SE of Slovenia. It has scattered distribution in the Alpine, Prealpine, Dinaric and Predinaric phytogeographical region, with a concentration of its localities in the mountains south of Ljubljana, the Snežnik Mountains, the Kočevje region with the Kolpa valley and in the Gorjanci mountain range. The north-westernmost localities in the whole distribution area of this taxon are in the southern Julian Alps (the southern extension of the Tolmin-Bohinj ridge), while the localities in the pre-Alpine region include those above the valleys of Bača and Idrija and on the Zaplana plateau between the towns of Vrhnika and Logatec.

Most of its localities are in beech forests extending from the submontane to the subalpine belt (300 m to 1600 m a.s.l.), on calcareous bedrock (dolomite, limestone, in places mixed with chert, marlstone or claystone) and on shallow soil (moder rendzina). The species was observed also in several spruce communities, in Dinaric black pine community, in Alpine dwarf pine and hop hornbeam communities, as well as in communities of moist rock crevices and screes.

Phytosociologists treat *L. krapfii* as a character species of either spruce or pine forests. With most of its localities in the montane beech forests it can legitimately be considered as a diagnostic (differential) species of the Illyrian alliance *Aremonio-Fagion*.

Povzetek

Vrsta *L. krapfii* je kobulnica, ki uspeva pri nas predvsem v dinarskih gozdovih. Od ostalih vrst tega rodu jo ločimo po jajčastih do podolgastih listnih segmentih, golem, malolistnem ogrinjalu in le 5-15 kobulovih žarkih, ki so izrazito različno dolgi. Zbirna evropska floristična dela (npr. Tutin 1968, Pignatti 1982, Thellung 1975, Fischer et al. 2008) navajajo dve podvrsti krapfovega jelenovca: *L. krapfii* subsp. *gaudinii* (*L. gaudinii*) in *L. krapfii* subsp. *krapfii* (*L. krapfii* s. str., sin. *L. marginatum*). Prvi takson ima predvsem alpsko razširjenost, drugi dinarsko-karpatško. Glede na lego Slovenije in nekatere navedbe (npr. Fischer 2008, Tutin 1968) naj bi pri nas uspevali obe. Da bi to preverili, smo pregledali ves dostopni herbarijski material te vrste v herbariju LJU in LJS. Opazovali smo razlikovalne znake, ki jih navajajo Pignatti (1982), Tutin (1968) in Thellung (1975). Ugotovili smo, da pregledani herbarijski material pripada tipski podvrsti, kar je v skladu z navedbami iz domače literature (npr. Mayer 1952, Martinčič 2007), po katerih naj bi pri nas uspevala le *L. krapfii* subsp. *krapfii*.

Iz podatkov, zbranih v podatkovnih bazah Centra za kartografijo favne in flore (CKFF) in FloVegSi Biološkega inštituta Jovana Hadžija ZRC SAZU, literaturnih podatkov in podatkov iz herbarijev LJU in LJS smo izdelali zemljevid znane razširjenosti Krapfovega jelenovca v Sloveniji. Ugotavljamo, da je v Sloveniji razširjen v alpskem, predalpskem, dinarskem in preddinarskem fitogeografskem območju. Najbolj severozahodna nahajališča v njegovem celotnem arealu so v južnih Julijskih Alpah. Glavnina nahajališč leži v dinarski smeri severozahod-jugovzhod, z največjo gostoto nahajališč v hribovju južno od Ljubljane, v Snežniškem pogorju, na Kočevskem s Kolpsko dolino in na Gorjancih. Največ nahajališč je v montanskih bukovih gozdovih, zato Krapfov jelenovec lahko štejemo za diagnostično vrsto ilirske zveze *Aremonio-Fagion*.

Vrsta največkrat raste v bukovih gozdovih od submontanskega do subalpskega pasu (300 m do 1600 m n. m.), na karbonatni podlagi (dolomit, apnenec, ponekod s silikatno primesjo) in plitvih tleh (prhlinasta rendzina). Popisali smo ga tudi v nekaterih smrekovih združbah, v dinarskem črnoborovju, v alpskem ruševju in črnogabrovju ter v združbah vlažnih skalnih razpok in melišč.

Acknowledgements

For friendly consultations and literature we sincerely thank doc. dr. Boštjan Surina. For sending us his doctoral dissertation with the reports of *L. krapfii* in extensions of the Savinja Alps we thank prof. dr. Jurij Diaci. We would also like to thank all the botanists who have contributed their herbarium sheets in the herbaria LJS and LJU.

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Appendix

Data on the revised herbarium specimens (*Specimina visa*) of *Laserpitium krapfii* subsp. *krapfii*

- 0051/2** Slovenija: In silvis lucidis declinalis septentrionali - orientalis montis Ulovka supre opp. Vrhnika, s. dolom., 610 m s. m.; leg. T. Wraber, 18. 7. 1994 (LJU10032497).
- 0052/4** Slovenija: Carniola. In silvaticis et dumetosis montis Krim (ditio Labacensis), s. calc.; 800 m, leg. Dolšak F., 6. 7. 1925 (LJU10032509).
- 0052/4** Slovenija: Carniola. In silvaticis et dumetosis montis Krim (ditio Labacensis), s. calc.; 800 m, leg. Dolšak F., 7. (LJU10032510).
- 0052/4** Slovenija: Krim. leg. Budnar, 8. 8. 1948 (LJU10032500).
- 0052/4** Slovenija: Carniola. In pratis et silvaticis lapidosi montis Krim prope Labacum; solo calcareo; 1000 m s. m. (Flora exsiccata Carniolica); leg. Paulin A., 7. (LJU10032496).
- 0149/2** Slovenija: Primorska, Otlica, Kovk: bukov gozd (*Seslerio autumnalis-Fagetum*), ekspozicija: NE, nagib: 30°; 740 m n. m.; leg. I. Dakskobler, 13. 7. 1989 (LJS03258).
- 0149/2** Slovenija: Primorska, Otlica, Kovk: bukov gozd (*Seslerio autumnalis-Fagetum*), ekspozicija: N, nagib: 30°. 830 m n. m.; leg. I. Dakskobler, 13. 7. 1989 (LJS03259).
- 0152/2** Slovenija: Ljubljanska okolica: Iški Vintgar - in fruticosis, solo calcareo, cca 330 m s. m., leg. E. Mayer, 19. 8. 1954 (LJU10032507).
- 0152/2** Slovenija: Rakiška planota JZ od Krima nad Ljubljano, ob umetnem jezeru J od vasi Rakitna; listnat gozd, 800 m n. m.; leg. A. Zrimec, 22. 7. 1991 (LJU10032498).
- 0153/1** Slovenija: Dolenjska, Mokrec pri Igu, tik pod vrhom: bukov gozd (*Omphalodo-Fagetum* (Treg. 1957) Marinček et al. 1993), ekspozicija: W, nagib: 25°, rendzina, apnenec. 972 m n. m.; leg. V. Babij, det. D. Robič, 17. 6. 1997 (LJS05987).
- 0158/3** Slovenija, Gorjanci: ob poti s Polma na Mirčev grič, leg. V. Strgar, 29. 6. 1960 (LJU10032506).
- 0257/2** Slovenija: Gorjanci: Gospodična - pr. Miklavž, gozd, 920 m, leg. J. Rataj, 23. 6. 1954 (LJU10032490).
- 0257/2** Slovenija: In silvis umbrosis humidis prope refugium alpinum Paderšičeva koča in monte Gorjanci, solo dolomitico, 850 m s. m., A. Martinčič, 24. 6. 1954 (LJU10032508).
- 0257/2** Slovenija, Gorjanci: In silvis prope locum Gospodična dictum supra vicum Gabrje. 830 m s. m.; leg. T. Wraber, 26. 6. 1992 (LJU10032501).
- 0257/2** Slovenija: Dolenjska, Gorjanci, Gabrje (Novo mesto), Gospodična: v bukovem gozdu (*Arunco-Fagetum*), karbonat, ca. 900 m n. m.; leg. I. Dakskobler, 23. 8. 1989 (LJS03257).
- 0352/4** Slovenija: Snežnik, Peklo: solo dolomitico; 1230 m s. m., leg. M. Zupančič, 31. 8. 1972 (LJU10032502).
- 0353/1** Slovenija: Kranjsko-notranjska flora: Biva med grmovjem ob cesti pod Mašunom pod Snežnikom, leg. R. Justin, 15. 7. 1923 (LJU10032511).
- 0356/3** Slovenija: Podstene v Kočevskem Rogu pri Koprivniku pri Kočevju, leg. V. Plemel, 2. 8. 1849 (LJU10032495).

- 0356/3** Slovenija: Podstene v Kočevskem Rogu pri Koprivniku pri Kočevju, leg. V. Plemel, 2. 8. 1849 (LJU10032499).
- 0356/4** Slovenija: Kočevski Rog, Semič, Planina - Mirna gora, 800-1000 m n. m. v.; suh, topel travnat pas pod el. daljnovodom (RTŠB Semič 01), leg. B. Frajman, 26. 7. 2001 (LJU10130873).
- 0452/2** Slovenija, Notranjski Snežnik: Veliki Snežnik, in fagetis */Fagetum subalpinum/* declivitatibus septentrionalis; 1550 n s. m.; leg. T. Wraber, 12. 8. 1965 (LJU10032503).
- 0454/1** Slovenija, Kočevsko: Goteniški Snežnik, gozdna jasa, 1100 m n. m.; leg. I. Štivec, 16. 7. 1981 (LJU10032491).
- 0454/1** Slovenija: Dolenjska, Bezgovica (Osilnica), Bezgarska planina: travnik, opuščen pašnik, 894 m n. m.; leg. B. Vreš B. & T. Čelik, 13. 7. 2013 (LJS11851).
- 0454/2** Slovenija: Kočevsko: pobočje Krempe, med grmovjem, 900 m n.m. (Flora osnovnega polja 0454 Cerklje), leg. I. Štivec, 4. 7. 1982 (LJU10032494).
- 0454/2** Slovenija: Kočevsko: Ravne, na gozdni poseki ob poti v Krokarski pragozd, 840 m n. m. (Flora osnovnega polja 0454 Cerklje), leg. I. Štivec, 17. 7. 1981 (LJU10032493).
- 0454/4** Slovenija: In silvis montis Krempa supra vallem fluvii Kolpa, 800 m s. m. leg. A. Martinčič, 9. 7. 1958 (LJU10032504).
- 0454/4** Slovenija: Potok nad Mitroviči v dolini Kolpe, peščenno apnenčasto pobočje; 270 m n. m.; leg. S. Peterlin, 5.-10. 8. 1960 (LJU10032505).
- 9748/4** Slovenija: Primorska, Julijske Alpe, Krikov vrh: *Arunco-Fagetum*, 1150 m n.m.; leg. I. Dakskobler, 29. 7. 1992 (LJS03164).
- 9748/4** Slovenija: Primorska, Julijske Alpe, Krikov vrh: *Arunco-Fagetum*, 1150 m n. m.; leg. I. Dakskobler, 30. 7. 1992 (LJS03171).
- 9748/4** Slovenija: Julijske Alpe, Krikov vrh, pobočja nad Mirno grapo: bukov gozd (*Arunco-Fagetum*), strmo gruščnato pobočje, dolomit z rožencem, rendzina, ekspozicija: NE, nagib: 40°, 940 m n.m.; leg. I. Dakskobler, 21. 7. 1989 (LJS03260).
- 9748/4** Slovenija: Primorska, dolina Zadlaščice, Pod Sopotom: listopadni gozd (*Rhododendro hirsutum-Ostryetum*), strmo kamnito pobočje, ki se prelomi v steno, ob lovski poti, ekspozicija: NNW, nagib: 40°, rendzina, apnenec z rožencem. 780 m n. m.; leg. I. Dakskobler, 21. 7. 1993 (LJS03261).
- 9749/4** Slovenija: Baška dolina, Podbrdo, Batava, pobočje Robarjevega griča, 760 m n. m., bukov gozd, *Arunco-Fagetum* s. lat., leg. I. Dakskobler, 24. 6. 1990 (LJS, study collection). New locality in Prealpine phytogeographical region.
- 9848/4** Slovenija: dolina Idrijce, Slap ob Idrijci, osovina pobočja Špehovega brda, Vresnica nad domačijo Bukovca, 350 m n. m., bukov gozd, *Arunco-Fagetum*; leg. I. Dakskobler, 14. 5. 2002, (LJS, study collection) New locality in Prealpine phytogeographical region, but actually in northwesternmost edge of Trnovski gozd plateau (Dinaric mountains).
- 9849/1** Slovenija: Baška dolina, vznožje Koriške gore nad cesto Humar-Hudajužna, 390 m in 470 m n. m., bukov gozd, *Ostrya-Fagetum*; leg. I. Dakskobler, 29.6. 1989 (LJS, study collection) (new locality in Alpine phytogeographical region).
- 9849/1** Slovenija: Baška dolina, vznožje Kobilice nad čuvajnico ob železniški progi Grahovo ob Bači-Hudajužna, okoli 400 m n. m., bukov gozd, *Ostrya-Fagetum*; leg. I. Dakskobler, 29.6. 1989 (LJS, study collection) New locality in Prealpine phytogeographical region.

Cytological analysis of *Fallopia japonica* and *Fallopia ×bohemica* shoots during growth season

Citološka analiza poganjkov japonskega in češkega dresnika med rastno sezono

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Abstract: *Fallopia japonica* and *Fallopia ×bohemica* are two very invasive plant species in Europe and North America. Their main mode of spread is vegetative reproduction. In spring new shoots emerge from the overwintering rhizome, grow rapidly and develop broad leaves which shade undergrowth plants. We studied cell size and starch accumulation in three stem regions at five sampling times during one growth season to determine possible differences in growth dynamics of both *Fallopia* species. On average *F. ×bohemica* had somewhat larger cells than *F. japonica* but the differences were not significant, except in the internodes of the middle stem region with differentiating cells. Also, cell growth dynamics of both species was similar and the only difference was detected at the 2nd sampling when cells of *F. ×bohemica* were more elongated. *F. ×bohemica* also accumulated starch earlier in the growth season and in younger tissues than *F. japonica*.

Keywords: *Fallopia japonica*, *Fallopia ×bohemica*, cell size, starch, growth

Izvleček: Japonski (*Fallopia japonica*) in češki dresnik (*F. ×bohemica*) sta v Evropi in Severni Ameriki zelo invazivni tujerodni rastlinski vrsti. Nespolno razmnoževanje je glavni način njunega razširjanja in vsako pomlad iz korenike požene brst, ki hitro zraste in razvije široke liste, ki zasenčijo podrast. V raziskavi smo preučevali velikost celic in tvorbo škrobnih zrn v treh območjih stebela pri petih vzorčenjih, ki smo jih opravili v eni rastni sezoni in tako ugotavljali morebitne razlike v dinamiki rasti pri obeh vrstah dresnika. V povprečju so bile celice češkega dresnika nekoliko večje kot celice japonskega dresnika, vendar razlike niso bile statistično značilne razen v členkih srednjega dela stebela z diferencirajočimi se celicami. Tudi dinamika celične rasti je bila podobna pri obeh vrstah dresnika razen pri 2. vzorčenju, ko so bile celice češkega dresnika bolj podaljšane. Češki dresnik je v primerjavi z japonskim kopičil škrob prej in v mlajših tkivih.

Ključne besede: japonski dresnik, češki dresnik, velikost celic, škrob, rast

Introduction

Fallopia japonica (Houtt.) Ronse Decr. (*Polygonaceae*) and its close relatives in the section

Reynoutria are well known and problematic invasive plant species worldwide. In Slovenia are present octoploid Japanese knotweed (*F. japonica* var. *japonica* (Houtt.) Ronse Decr.), tetraploid giant

knotweed (*F. sachalinensis* (F. Schmidt) Ronse Decr.) and their hybrid hexaploid Bohemian knotweed (*F. ×bohemica* (Chrtěk and Chrtěková) J. P. Bailey) (Strgulc Krajšek and Jogan 2011). Japanese knotweed is cytologically and genetically uniform in Europe and has successfully spread since its early introduction in Europe in the 19th century. Now it is considered as one of the most invasive plant species. On the other hand, giant knotweed has limited genetic variation and is less invasive. Their hybrid Bohemian knotweed exhibits the highest genetic variation (Bailey et al. 2009) and recent study shows that it also has higher invasive potential than Japanese knotweed (Parepa et al. 2014). All three knotweed species are approx. 2–4 m tall herbaceous perennials with high growth rate and broad leaves which shade undergrowth plants (Herpigny et al. 2012). The main competitive traits of knotweeds against the native flora were shown to be their vegetative reproduction with rhizomes and high regeneration success, but allelopathy and genome plasticity also account. All these characteristics enable knotweeds successful colonisation of new habitats, especially ruderal habitats and river banks (Parepa et al. 2014).

Japanese knotweed is up to 2 m high and has up to 15 cm long leaves, giant knotweed is higher, from 2 to 4 m, and has up to 30 cm long leaves while Bohemian knotweed is intermediate in size (Vreš 2007, Bailey et al. 2009). The size and shape of organs are related to cell division, cell elongation and cell differentiation which take place in the meristematic and submeristematic tissues. Environmental (water and nutrient supply) as well as genetic factors control organ growth (Mizukami 2001, John and Qi 2008, Krizek 2009). Cell size can be correlated also to the ploidy level (Kondorosi et al. 2000, Sugimoto-Shirasu and Roberts 2003) and

has been used in the taxonomic studies to estimate nuclear DNA content and ploidy level by the size of the stomatal guard cells (Šturm and Bačič 2013) even in the fossil plants (Masterson 1994). It was also shown that polyploidization can represent a mean to accelerate the growth of the plant species in niches that require and support fast development (Barow and Meister 2003) and can facilitate plant invasions (te Beest et al. 2012).

The aim of our study was to compare the growth of Japanese and Bohemian knotweed during one season. Different cytological traits (cell size, presence of starch grains) were determined and the following questions were asked: (i) Are the cells in the octoploid Japanese knotweed larger cells than in the hexaploid Bohemian knotweed? (ii) Is the cell size different in the meristematic, young and mature regions of the stem, which would lead to different growth dynamics in both *Fallopia* species? (iii) Is the starch accumulation species specific and correlated to cell size dynamics?

Material and methods

Knotweed sampling

Shoot samples of five plants of Japanese knotweed (*F. japonica* var. *japonica* (Houtt.) Ronse Decr.) and Bohemian knotweed (*F. ×bohemica* (Chrtěk and Chrtěková) J. P. Bailey) were collected in Ljubljana, Slovenia (46° 2' 33.98" N, 14° 27' 0.91" E and 46° 3' 0.3" N, 14° 28' 44" E, respectively) from April to October 2013. According to the developmental stage of the shoot, apical meristem (upper region, 1st internode), young (medium region, 5th internode) and mature internodes (lower region, 10th and 15th internode) were collected for further cytological analyses (Table 1).

Table 1: Sampling data.
Tabela 1: Potek vzorčenja.

Sampling	Date	Collected material	Developmental stage
1	15.4.2013	Shoot apex	Emerging shoot bud from the rhizome
2	24.4.2013	Shoot apex, internode 5	Young plants approx. 50 cm high
3	5./10.7.2013	Internodes 1, 5, 10	Fully grown plants approx. 2 m high
4	29.8.2013	Internodes 5, 10, 15	Flowering plants
5	25.10.2013	Internodes 5, 10, 15	Senescent plants at the end of the season

Fixation and preparation of microscopic slides

Approx. 1 cm long tissue samples were cut from the stem and fixed in FAA (3.7% formaldehyde, 50% ethanol, 5% glacial acetic acid) for at least 24 hours at 4°C. For longer storage samples were transferred to 70% ethanol at -20°C.

Thin transversal and longitudinal sections of fixed material were hand-cut using a razorblade. Sections were put in a drop of distilled water on the objective glass, covered and analysed with a light microscope (Axioskop 2 MOT, Carl Zeiss, Germany) combined with a colour digital camera (AxioCam MRc, Carl Zeiss, Germany) and AxioVision 4.8 software (Carl Zeiss, Germany). For better resolution of cell walls, autofluorescence (at UV excitation 365/12 nm band pass and emission 397 nm long pass) was also recorded when necessary.

Starch identification

The presence of starch grains was determined by staining the sections with the indicator iodine solution (3% KI/I₂ (w/v)).

Image analysis and statistics

In the longitudinal sections, width and length of cells (Fig. 1) were measured for at least 20 cells using ImageJ software (Rasband, 1997-2014). The obtained data were used to calculate cell volume according to the formula $V = \pi r^2 l$ (r – half of the cell width, l – cell length). Mean values and standard errors were calculated and the samples were compared by t-test and ANOVA (GraphPad Prism). The level of significance was set at p -value < 0.05.

Results

Cell size of *F. japonica* and *F. ×bohemica* gradually increased during the growth season from the smallest meristematic cells at the shoot apex (average width 40.73 µm, average volume $30.95 \times 10^3 \mu\text{m}^3$) to the largest mature cells of the 10th and 15th internode (average width 64.38 µm, average volume $593.05 \times 10^3 \mu\text{m}^3$). Cell width in the middle shoot region increased to 123% and to 158% in the lower mature region when compared

to the meristematic cells in the upper shoot region (Fig. 2A). Cell volume increased more intensely indicating higher level of cell elongation than expansion of cell width; the volume increased to 758% in the middle shoot region and to 1917% in the lower mature region when compared to the meristematic cells in the upper region (Fig. 2B).

The width and volume of the cells in both *Fallopia* species were not significantly different except for the volume of the cells in the middle shoot region ($p=0.020$). Dynamics of the cell growth during the season was estimated by a trend line and was similar in case of cell width and cell volume expansion for both *Fallopia* species (cell width trend line equation for *F. japonica* $y=14.10x$, $R^2=0.86$; for *F. ×bohemica* $y=15.18x$, $R^2=0.15$; cell volume trend line equation for *F. japonica* $y=99.76x$, $R^2=0.31$; for *F. ×bohemica* $y=110.13x$, $R^2=0.16$).

Upper shoot region

In the upper shoot region cells of the shoot apical meristem and the youngest internodes (1st) were measured. These cells were collected only during first three samplings. Later in the growth season at the 4th and 5th sampling, the apical region developed in reproductive tissues without the shoot apical meristem (Tab. 1).

The cell width was very similar for both *Fallopia* species ($p=0.974$) but the difference was more pronounced in cell volume ($p=0.351$) where cells of *F. ×bohemica* were on average larger ($39.66 \times 10^3 \mu\text{m}^3$) than of *F. japonica* ($25.31 \times 10^3 \mu\text{m}^3$) (Fig. 3, Suppl. Tab. 2).

In this region, starch granules were present in *F. ×bohemica* at all sampling times while in *F. japonica* starch occurred only at the 3rd sampling when plants were fully developed (Fig. 4).

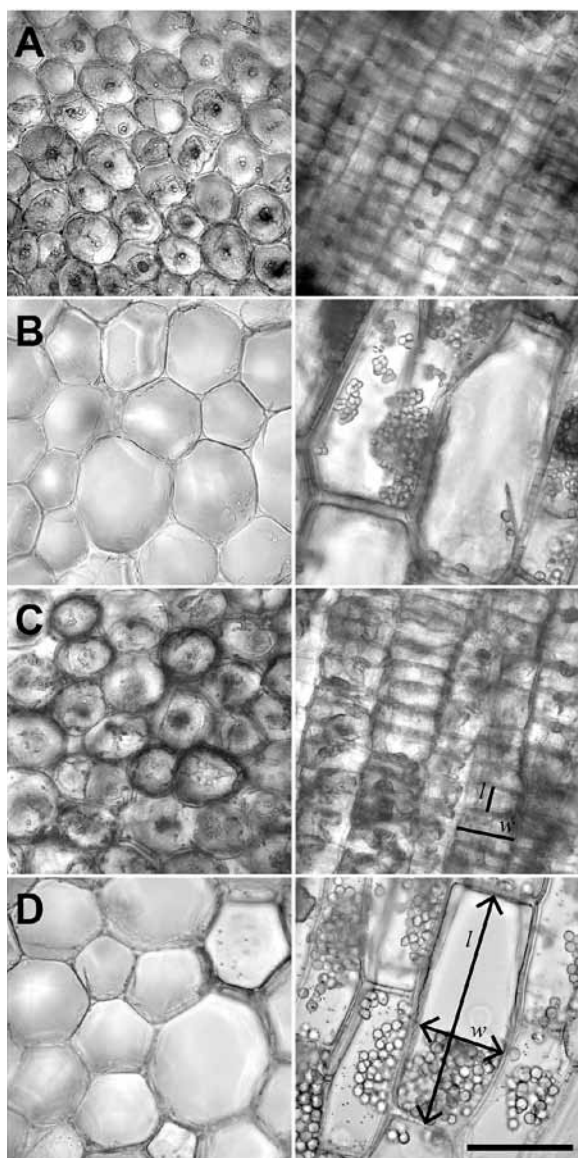


Figure 1: Transversal (left) and longitudinal (right) sections of *F. japonica* and *F. x bohemica* shoots. **A** – Sections of *F. japonica* meristematic region at the 1st sampling; **B** - Sections of *F. japonica* mature shoot region at the 3rd sampling. **C** - Sections of *F. x bohemica* meristematic region at the 1st sampling. **D** - Sections of *F. x bohemica* mature shoot region at the 3rd sampling. Measurement of width (w) and length (l) of cells is indicated by lines in panels C and D. Bar represents 100 μ m.

Slika 1: Prečni (levo) in vzdolžni (desno) prerez poganjka pri *F. japonica* in *F. x bohemica*. **A** – Prereza zgornjega meristemskega območja pri *F. japonica* pri 1. vzorčenju. **B** - Prereza spodnjega zrelega območja pri *F. japonica* pri 3. vzorčenju. **C** - Prereza zgornjega meristemskega območja pri *F. x bohemica* pri 1. vzorčenju. **D** - Prereza spodnjega zrelega območja pri *F. x bohemica* pri 3. vzorčenju. Meritev širine (w) in dolžine (l) celic je prikazana z oznakami na slikah C in D. Merilo predstavlja 100 μ m.

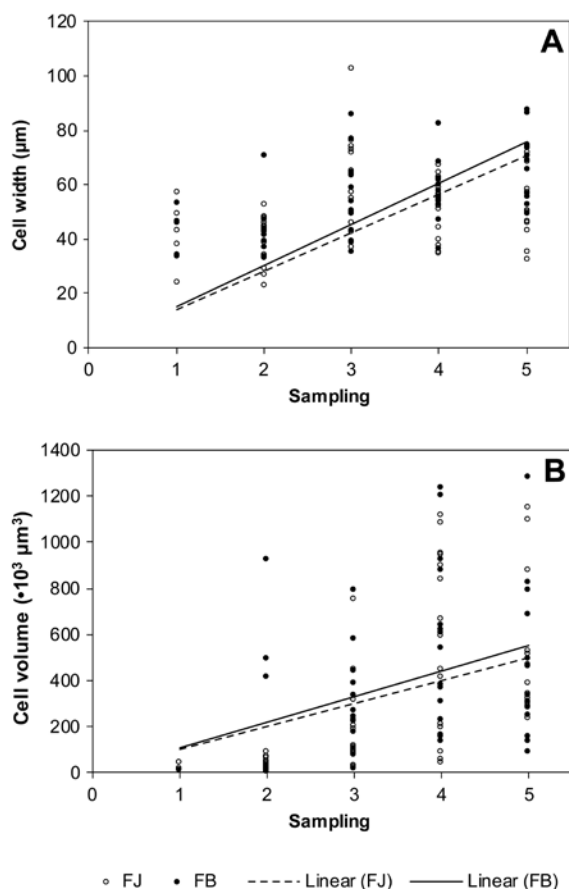


Figure 2: Dynamics of cell growth of *F. japonica* (FJ) and *F. x bohemica* (FB) during growth season. Data present A - cell width and B - cell volume (N=5-25). Trendline is a linear regression line (intercept set at 0.0).

Slika 2: Dinamika rasti celic pri *F. japonica* (FJ) in *F. x bohemica* (FB) med rastno sezono. Rezultati prikazujejo A - širino in B - prostornino izmerjenih celic (N=5-25). Trendna črta je linearna regresijska premica (izhodišče nastavljeno na 0,0).

Table 2: Cell size in the upper shoot region/meristem of *F. japonica* and *F. x bohemica*. Mean value $\pm$ standard error are presented (N=5-10).

Tabela 2: Velikost celic v zgornjem/meristemskem delu stebela pri *F. japonica* in *F. x bohemica*. Rezultati prikazujejo povprečno vrednost $\pm$ standardno napako (N=5-10).

Sampling	<i>F. japonica</i>		<i>F. x bohemica</i>	
	Volume ($\times 10^3 \mu\text{m}^3$)	Width (μm)	Volume ($\times 10^3 \mu\text{m}^3$)	Width (μm)
1	19.33 $\pm$ 6.16	2.46 $\pm$ 5.56	9.64 $\pm$ 0.49	42.66 $\pm$ 3.83
2	19.76 $\pm$ 6.06	33.35 $\pm$ 2.18	7.57 $\pm$ 3.77	35.82 $\pm$ 1.44
3	43.88 $\pm$ 17.26	50.62 $\pm$ 6.30	93.23 $\pm$ 33.39	42.91 $\pm$ 3.12

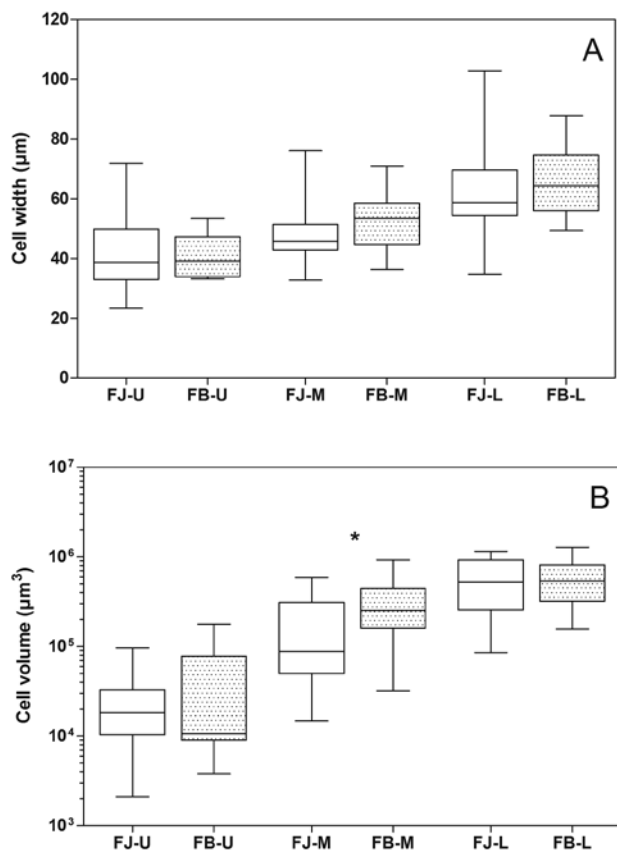


Figure 3: Cell size of *F. japonica* (FJ) and *F. x bohemica* (FB) in the upper (U), medium (M) and lower (L) shoot region; **A** - cell width, **B** - cell volume.

* statistically significant ($p < 0.05$) difference between *F. japonica* and *F. x bohemica*.

Slika 3: Velikost celic pri *F. japonica* (FJ) in *F. x bohemica* (FB) v zgornjem (U), srednjem (M) in spodnjem (L) delu poganjka; **A** - širina celic, **B** - prostornina celic.

* statistično značilna ($p < 0.05$) razlika med vrstama *F. japonica* in *F. x bohemica*.

Middle shoot region

In the middle shoot region, differentiating cells of the intermediate (5th) internode were measured. These cells were collected during four samplings and were missing only at the 1st sampling because plants were too small (Tab. 1).

The width of *F. x bohemica* cells was on average larger ($52.95 \mu\text{m}$) than of *F. japonica* cells ($47.51 \mu\text{m}$) and the difference was almost significant ($p = 0.067$). On the other hand the cell volume was significantly ($p = 0.020$) larger in *F.*

x bohemica ($321.48 \times 10^3 \mu\text{m}^3$) than in *F. japonica* ($178.75 \times 10^3 \mu\text{m}^3$) mostly because of the differences at the beginning of the growth season at the 2nd and the 3rd sampling. Later (4th and 5th sampling) the volume size was similar in both *Fallopia* species (Fig. 3, Suppl. Tab. 3).

In the middle region, there was no difference in the accumulation of the starch granules which were present in cells of both *Fallopia* species from the 3rd sampling on.

Table 3: Cell size in the middle shoot region of *F. japonica* and *F. ×bohemica*. Mean value ± standard error are presented (N=5-10).

* statistically significant ($p < 0.05$) difference between *F. japonica* and *F. ×bohemica*

Tabela 3: Velikost celic v srednjem delu stebila pri *F. japonica* in *F. ×bohemica*. Rezultati prikazujejo povprečno vrednost ± standardno napako (N=5-10).

* statistično značilna ($p < 0.05$) razlika med vrstama *F. japonica* in *F. ×bohemica*.

Sampling	<i>F. japonica</i>		<i>F. ×bohemica</i>	
	Volume ($\times 10^3 \mu\text{m}^3$)	Width (μm)	Volume ($\times 10^3 \mu\text{m}^3$)	Width (μm)
2	51.96 ±8.03*	45.97±0.94	465.79 ±182.65*	49.27±5.50
3	173.04±73.764	57.47±6.13	293.36±42.58	54.04±3.59
4	309.89±110.42	42.13±3.26	305.94±92.37	49.29±3.56
5	256.18±33.90	46.03±5.89	249.69±69.78	59.22±2.64

Lower shoot region

In the lower shoot region cells of fully differentiated and mature (10th and 15th) internodes were measured. These cells were collected in the fully developed plants from the 3rd sampling on (Tab. 1).

At the 4th and 5th sampling, the cells of *F. japonica* had significantly ($p=0.038$) larger volume than earlier in the growth season. The same pattern was observed also in *F. ×bohemica* but the difference was not significant ($p=0.292$). On the other hand, the cell width was similar during all mature period (Fig. 3, Suppl. Tab. 4).

In the lower region, starch granules were present in cells of both *Fallopia* species at all sampling times.

Discussion

Japanese and Bohemian knotweed are important invasive species in Slovenia and Europe. They form large and dense monospecific stands along rivers, railways, roads and other ruderal habitats, and severely decrease native biodiversity. In spring they restore the shoot from the underground rhizome and very quickly develop high stem and broad leaves. In our previous study (Strgulc Krajšek and Dolenc Koce 2015) it was shown that only octoploid Japanese knotweed (*F. japonica* var. *japonica*) and hexaploid Bohemian knotweed (*F. ×bohemica*) present highly invasive

populations in Slovenia which form monospecific as well as mixed population with both species. Giant knotweed (*F. sachalinensis*) is less common and at the moment does not present such severe invasive threat. The same observations were reported also for Belgium (Herpigny et al. 2012).

Since level of ploidy can affect the cell and organ size as well as plant fitness (Barow and Meister 2003) we aimed to compare the dynamics of cell growth during the season in *F. japonica* and *F. ×bohemica* species to determine if cytological traits are correlated to morphological differences of adult plants (*F. ×bohemica* is taller and has bigger leaves than *F. japonica*).

Cell size gradually increased from the smallest apical cells to the fully developed cells in the lower shoot regions as plants developed during the growth season (Fig. 1). Cell width increased less than cell volume which indicates that cells primarily elongated. At the end of the season both *Fallopia* species had cells of similar size. The dynamics of cell growth shows that the only significant difference was measured at the 2nd sampling when middle size cells of *F. ×bohemica* had 896% higher volume than of *F. japonica* (Fig. 2). Nevertheless, when cell data for all shoot regions and all sampling times at are pulled together and compared for both *Fallopia* species it is revealed that *F. ×bohemica* has bigger cells than *F. japonica* and differences are close to significant ($p=0.065$ for

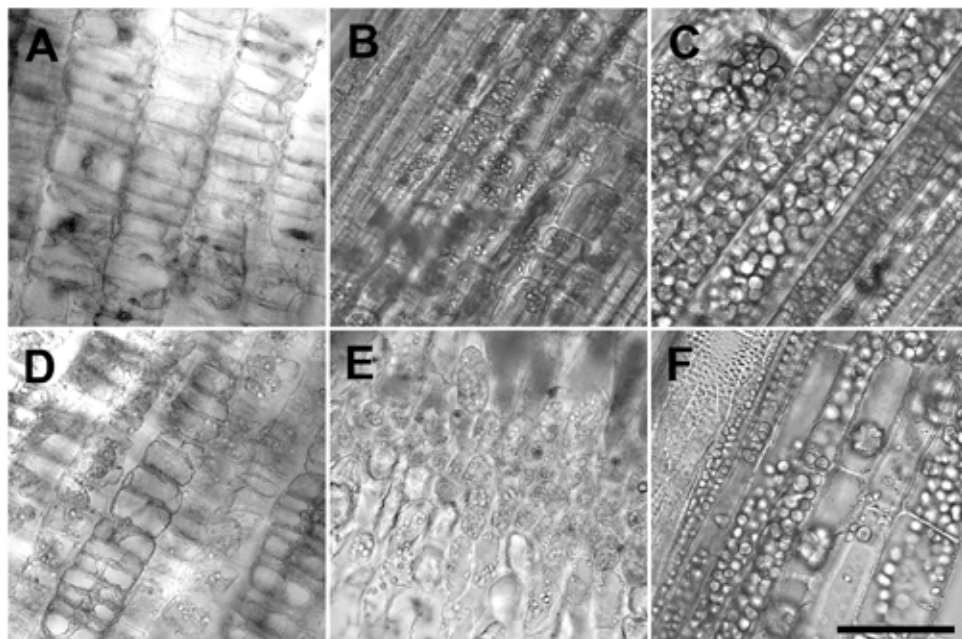


Figure 4: Stem cells with starch grains in *F. japonica* (A-C) and *F. ×bohemica* (D-E). A, D - meristematic cells at the 1st sampling; B, E - meristematic cells at the 3rd sampling; C, F - cells of the middle shoot region at the 4th sampling. Bar presents 100 μ m.

Slika 4: Celice stebela s škrobnimi zrni pri *F. japonica* (A-C) in *F. ×bohemica* (D-E). A, D - meristemske celice pri 1. vzorčenju; B, E - meristemske celice pri 3. vzorčenju; C, F - celice v srednjem delu stebela pri 4. vzorčenju. Merilo predstavlja 100 μ m.

cell width and $p=0.135$ for cell volume). Similar growth kinetics was described for tested *Fallopia* species in Belgium with *F. japonica* and *F. ×bohemica* having more comparable kinetics than *F. sachalinensis* with lower competitive ability for light and nitrogen (Herpigny et al. 2012). In that study it was also shown that *F. ×bohemica* had more variable growth and functional traits (shoot height, ramification, leaf size, foliar nitrogen concentration) which could be related to its hybrid origin. In our study, the variability of the cell width and cell volume was generally higher in *F. japonica* (CV range from 6.5 to 95.3%) than of *F. ×bohemica* (CV range from 8.0 to 78.4%) but only one cell trait was tested which is not enough to generalize the conclusion about cell growth.

We also tested correlation between cell size and ploidy level which was not significant therefore we conclude that polyploidy in investigated *F. japonica* and *F. ×bohemica* species had no effect on

cell size and consequently on the size of the shoot.

Additionally, the presence of starch grains in all plant material was observed from the initial developmental phases until the fully developed plants. Cells of *F. ×bohemica* accumulated starch already at the beginning of the growth season which could contribute to its higher growth rate when compared to *F. japonica* plants. In the fully developed and mature stems there were no differences in starch accumulation between *Fallopia* species.

Conclusions

Fallopia japonica and *F. ×bohemica* have similarly large stem cells and their size is not correlated to the ploidy level.

Cell size of both *Fallopia* species gradually increased during the growth season from the smallest meristematic cells at the shoot apex to the

Table 4: Cell size in the lower/mature shoot region of *F. japonica* and *F. ×bohemica*. Mean value ± standard error are presented (N=5-10).Tabela 4: Velikost celic v spodnjem/zrelem delu stebela pri *F. japonica* in *F. ×bohemica*. Rezultati prikazujejo povprečno vrednost ± standardno napako (N=5-10).

Sampling	<i>F. japonica</i>		<i>F. ×bohemica</i>	
	Volume ($\times 10^3 \mu\text{m}^3$)	Width (μm)	Volume ($\times 10^3 \mu\text{m}^3$)	Width (μm)
3	292.53±121.31	78.47±6.38	455.54±103.63	71.06±4.49
4	698.03±123.22	57.34±3.14	685.04±115.98	61.07±2.80
5	630.55±102.06	58.18±3.09	577.60±103.38	71.26±4.21

largest mature cells of the 10th and 15th internode

Cell volume increased more intensely indicating higher level of cell elongation than expansion of cell width

F. ×bohemica accumulated starch earlier in the growth season and in younger tissues than *F. japonica*.

Povzetek

Japonski (*Fallopia japonica*) in češki dresnik (*F. ×bohemica*) sta v Evropi in Severni Ameriki zelo invazivni tujerodni rastlinski vrsti. Spomladi iz prezimujoče korenike požene poganjek, ki hitro raste do končne višine (2-4 m) in ima široke liste, ki zasenčijo podrast. V raziskavi smo preučevali celično rast in tvorbo škrobnih zrn v treh območjih stebela, da bi ugotovili: (i) Ali ima oktoploidni japonski dresnik večje celice kot heksaploidni češki dresnik? (ii) Ali je velikost celic v meristemu, mladem in zrelem območju stebela različna in imata vrsti dresnika različno dinamiko celične rasti? (iii) Ali vrsti dresnika različno kopičita škrob?

V eni rastni sezoni smo petkrat (dvakrat na začetku rastne sezone, ob vegetativni zrelosti rastlin, v času cvetenja in ob zaključku rastne sezone)

vzorčili celice stebela na vršičku, v srednjem delu stebela, kjer se celice še diferencirajo, in v nižje ležečih zrelih delih stebela. Rastlinski material smo fiksirali v fiksativu in ročno pripravili prečne in vzdolžne prereze za svetlobno mikroskopijo z vidno in UV-svetlobo. Na posnetih slikah smo z računalniškim programom za analizo slike (ImageJ) izmerili širino in dolžino celic, iz česar smo izračunali prostornino celic. Prisotnost škroba v tkivu smo dokazali z raztopino jodovice.

V povprečju so bile celice češkega dresnika nekoliko večje kot celice japonskega dresnika, vendar razlike niso bile statistično značilne razen v členkih srednjega dela stebela z diferencirajočimi se celicami. Tudi dinamika celične rasti je bila podobna pri obeh vrstah dresnika razen pri 2. vzorčenju, ko so bile celice češkega dresnika bolj podaljšane. Češki dresnik je v primerjavi z japonskim kopičil škrob prej in v mlajših tkivih.

Acknowledgements

This study was financially supported by the Slovenian Research Agency through research programme Plant Biology P1-0212.

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The effect of different compounds of selenium and iodine on selected biochemical and physiological characteristics in common buckwheat and pumpkin sprouts

Vpliv različnih oblik selena in joda na izbrane biokemijske in fiziološke lastnosti pri kalicah navadne ajde in buč

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Abstract: There is little data about possible interactions between selenium and iodine on plants. Se is essential for I metabolism in the thyroid in mammals. Thus, it is of great importance to carry out the research with simultaneous application of both elements in plant cultivation that are used for human consumption. Seeds of common buckwheat and pumpkins were soaked in solutions: 10 mgSe/L in the form of selenite or selenate, and 1000 mgI/L in the form of iodide or iodate and their combinations. The content of chlorophyll *a* and *b*, and carotenoids were measured. Further, the measurements of fluorescence of chlorophyll *a* were performed. Control buckwheat sprouts and sprouts from seeds soaked in Se(VI) and Se(VI)+I(-I), had the lowest and similar amount of chlorophyll *a* and carotenoids. There was little effect of different treatments on potential photochemical efficiency of photosystem II (PS II) in common buckwheat sprouts. In pumpkin sprouts neither of treatment affected the amount of photosynthetic pigments, as well as potential photochemical efficiency of (PS II) which was around 0.8.

Key words: sprouts, common buckwheat, pumpkins, selenium, iodine

Izvleček: Zelo malo podatkov obstaja o interakciji med selenom in jodom pri rastlinah. Selen je bistvenega pomena za delovanje ščitnice. Zato je pomembno, da preučujemo hkraten vpliv obeh elementov na rastline, ki jih uporabljamo za hrano ljudi. Seme navadne ajde in buč smo namakali v različnih raztopinah Se in I; selenata in selenita ter jodida oz. jodata ter vseh njunih kombinacij. Merili smo sledeče fiziološke in biokemijske lastnosti kontrolnih in obravnavanih rastlin: fotokemično učinkovitost fotosistema II (FS II) ter vsebnost fotosinteznih barvil (klorofil *a*, klorofil *b* in karotenoidi). Kontrolne kalice ajde in kalice, zrastle iz semen, namakanih s Se(VI) in se(VI)+I(-I), so imele najnižjo in podobno vsebnost klorofila *a* in karotenoidov. Obravnavanja so malo vplivala na potencialno fotokemično učinkovitost fotosistema II pri kalicah navadne ajde (kalice iz semen, namakanih v Se(IV)+I(-I), so imele najnižjo potencialno fotokemično učinkovitost fotosistema II). Nobeno obravnavanje ni vplivalo na vsebnost fotosinteznih barvil in potencialno fotokemično učinkovitost

fotosistema II pri kalicah buč. Potencialna fotokemična učinkovitost fotosistema II pri kalicah buč je bila okoli 0,8.

Ključne besede: kalice, navadna ajda, buče, selen, jod

Introduction

Several minerals and trace elements e.g., iodine, iron, selenium, and zinc, are essential for normal thyroid hormone metabolism (Zimmermann and Köhrle 2002). Iodine (I) and selenium (Se) are not essential nutrients for plants but both play important roles in human and animal organisms (Smoleń et al. 2014). Plant roots can take up Se as selenate, selenite or organoselenium compounds, such as selenocysteine (SeCys) and selenomethionine (SeMet) (White et al. 2004). Selenite is rapidly converted to organoselenium compounds in the root, whereas selenate is delivered to the xylem and transported to the shoot, where it is assimilated into organoselenium compounds and redistributed within the plant (Terry et al. 2000). Role of Se is beneficial in plants capable of accumulating large amounts of this element. It acted as an antioxidant, inhibiting lipid peroxidation in ryegrass and increased yield under ambient radiation conditions in pumpkins (Hartikainen et al. 2000, Germ et al. 2005). There is scarce information about the effect of iodine on plants neither possible interaction with selenium fertilization. Uptake of iodine from the soil to the plants depend from adsorption–desorption processes in the soil (Zia et al. 2014). Leaf vegetables have higher absorption capacity than fruit vegetable in ten chosen plants in the study from Weng et al. (2013). Plants take up iodine through the root system, preferably as iodide (Fuge 2005, Smoleń et al. 2011). Dai et al. (2006) evidenced that iodide (I(-I)) and iodate (IO₃⁻) added to the soil, do not significantly affect spinach biomass production.

Application of high doses of I(-I) to lettuce has a phytotoxic effect on plant physiology. In contrast, IO₃⁻ treatments increased the biomass of the plants which showed an elevated photosynthetic rate, stomatal conductance, and transpiration comparing to control plants. Blasco et al. (2010) reported about the response of lettuce to iodine biofortification. They found out that application of IO₃⁻, in contrast to I(-I), increased biomass

production, stimulated NO₃⁻ reduction and NH₄⁺ incorporation and optimised the photorespiratory process. Zhu et al. (2003), who studied the effect of iodine on spinach found out that iodine is not beneficial to the growth of spinach (*Spinacia oleracea*), but level of iodide above 10 µM was detrimental to yields, while iodate had little effect on the biomass production. Authors suggest that the detrimental effect of iodide on plant growth is probably due to its excessive accumulation in plant tissues. Voogt et al. (2010) reported that when they treated lettuce with I(-I) and IO₃⁻ no impact on plant biomass or quality was found, and accumulation of I(-I) was more effective than IO₃⁻. Iodine was mainly distributed to the outer leaves in lettuce. Landini et al. (2011) treated tomato (*Solanum lycopersicum*) with I(-I) and found out that tomato plants were not sensitive to high levels of iodine, stored both in vegetative tissues and fruits. They also reported that iodine was taken up better when supplied to the roots using hydroponically grown plants. However a considerable amount of iodine was also stored after leaf treatment that indicated that iodine is transport also through phloem.

Buckwheat is a good source of nutritionally important elements (Ikeda et al. 2006). It contains proteins that have a better balance of amino acids compared with cereals (Yoon et al. 2009). Buckwheat sprouts appeared some years ago as a new vegetable (Kim et al. 2004). Seeds of buckwheat and pumpkins can be used as additives to improve the quality of bread or other products (Stibilj et al. 2004). Pumpkin seeds have been used for centuries in traditional medicine, mainly in cases of problems of the kidney or the urinary tract (Kreft et al. 2002).

Despite its importance, studies focused on the effect of Se and I on plant physiological and biochemical characteristics are scarce (Zhu et al. 2004; Smoleń et al. 2014).

The aim of the study was to determine the possible simultaneous effect of iodine and selenium on the biochemical and physiological characteristics

on common buckwheat and pumpkin sprouts from seeds soaked in Se and I solution.

Materials and methods

Common buckwheat and pumpkin seeds were soaked in solution for 4 h in 200 mL distilled water (MilliQ) (control), or in solutions of sodium selenate (10 mg Se(VI) /L), sodium selenite (10 mg Se(IV) /L), potassium iodide (1000 mg I(-1) /L), potassium iodate (1000 mg I(V) /L) and combinations: 10 mg Se(VI) /L+ 1000 mg I(-1) /L; 10 mg Se(VI) /L+ 1000 mg I(V) /L; 10 mg Se(IV) /L+ 1000 mg I(-1) /L; 10 mg Se(IV) /L+ 1000 mg I(V) /L. After soaking seeds were distributed in plastic bowls, which were covered with filter paper. During germination, seeds were watered with tap water as needed. Sprouts were grown in controlled conditions in the growth chamber with constant temperature 22°C and 65–70% relative air humidity. Measurements were done after 12 and 10 days of growing common buckwheat and pumpkin sprouts, respectively.

Contents of chlorophyll *a* and *b* and carotenoids were determined using a UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). The total chlorophyll content was determined as described in Lichtenthaler and Buschmann (2001a, 2001b).

Fluorescence measurements were performed on the cotyledons of randomly selected sprouts using the fluorometer (PAM 2500 Portable Chlorophyll Fluorometer, WALZ). Prior to measurements samples were dark adapted for 20 min. The fluorescence parameters recorded included minimal (F_0) and maximal (F_m) chlorophyll fluorescence that were provided by dark-adaptation clips. The difference between F_m and F_0 is called the variable fluorescence F_v . $F_v/F_m = F_m - F_0/F_m$. F_v/F_m ratio is common parameter used in fluorescence which reflects the capacity to trap electrons by the photosystem (PS) II reaction centre (Schreiber et al. 1995).

Statistical analysis

The data were evaluated by ANOVA (Statgraphics Version 4) and the differences were tested using the Duncan test with a significance level of 0.05.

Results

Amount of chlorophyll *a* in control buckwheat sprouts was lower and statistical significantly different from amount of chlorophyll *a* in sprouts from seeds, soaked in Se(IV), I(V) and Se(IV)+I(V). The lowest amount of chlorophyll *a* was measured in the sprouts from seeds, soaked in Se(VI) and statistically different from sprouts from seeds, soaked in Se(IV), I(-1), I(V), Se(IV)+I(-1), Se(IV)+I(V) and Se(VI)+I(V) (Fig. 1). The pattern was similar regarding chlorophyll *b* although not statistically significant (data not shown).

Amount of carotenoids in control buckwheat sprouts were statistically different from the amount of carotenoids from seeds, soaked in Se(IV), I(-1), I(V), Se(IV)+I(-1) and Se(IV)+I(V). The lowest amount of carotenoids was measured in the sprouts from seeds, soaked in Se(VI) and was statistically lower from sprouts from seeds, soaked in Se(IV), I(-1), I(V), Se(IV)+I(-1), Se(IV)+I(V) and Se(VI)+I(V) (Fig. 2).

Neither of treatment affected the amount of chlorophyll *a* (Fig. 3) and carotenoids (Fig. 4) in pumpkins sprouts. Similar pattern appeared for chlorophyll *b* (data not shown).

Photochemical efficiency of PS II

There was little effect of different treatment on potential photochemical efficiency of photosystem II (PS II) in common buckwheat sprouts. The lowest potential photochemical efficiency of PS II was measured in common buckwheat sprouts from seeds, soaked in Se(IV)+I(-1). In pumpkin sprouts, neither treatment affected potential photochemical efficiency of PS II. Values were similar and around 0.8 (Table 1).

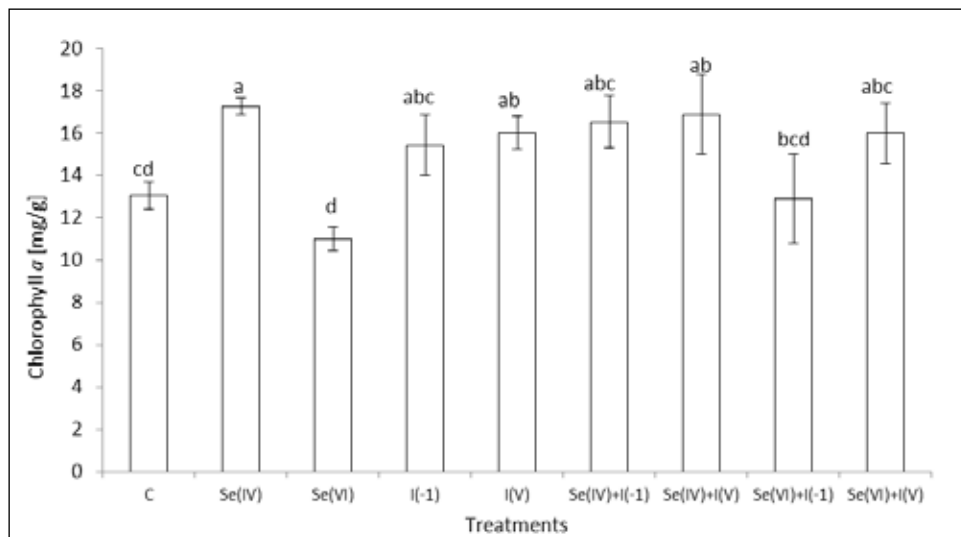


Figure 1: Concentration of chlorophyll *a* per DM in common buckwheat sprouts. Mean $\pm$ SE, $n = 6$, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 1: Koncentracija klorofila *a* na SM v kalih navadne ajde. Predstavljene so povprečne vrednosti $\pm$ SE ($n = 6$). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$.

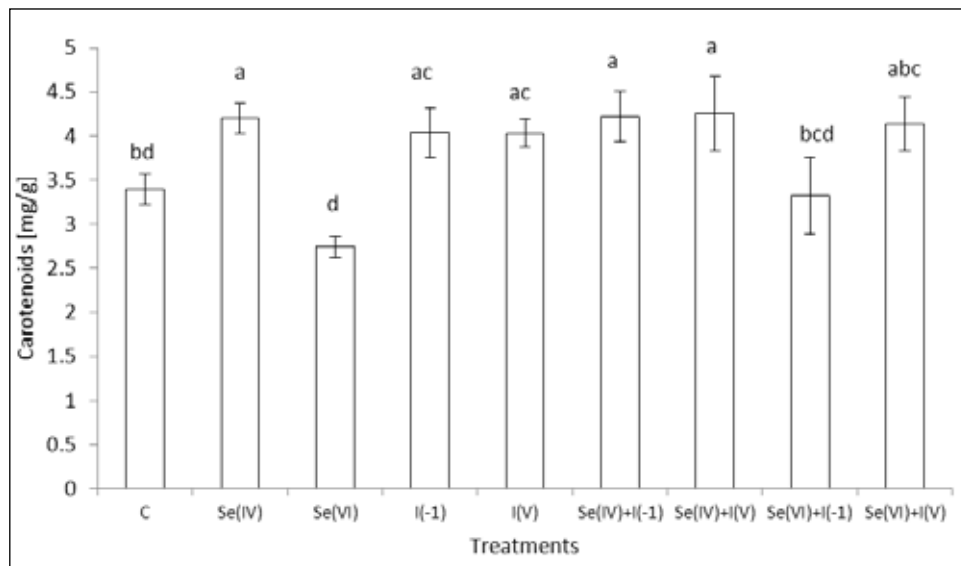


Figure 2: Concentration of carotenoids per DM in common buckwheat sprouts. Mean $\pm$ SE, $n = 6$, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 2: Koncentracija karotenoidov na SM v kalih navadne ajde. Predstavljene so povprečne vrednosti $\pm$ SE ($n = 6$). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$.

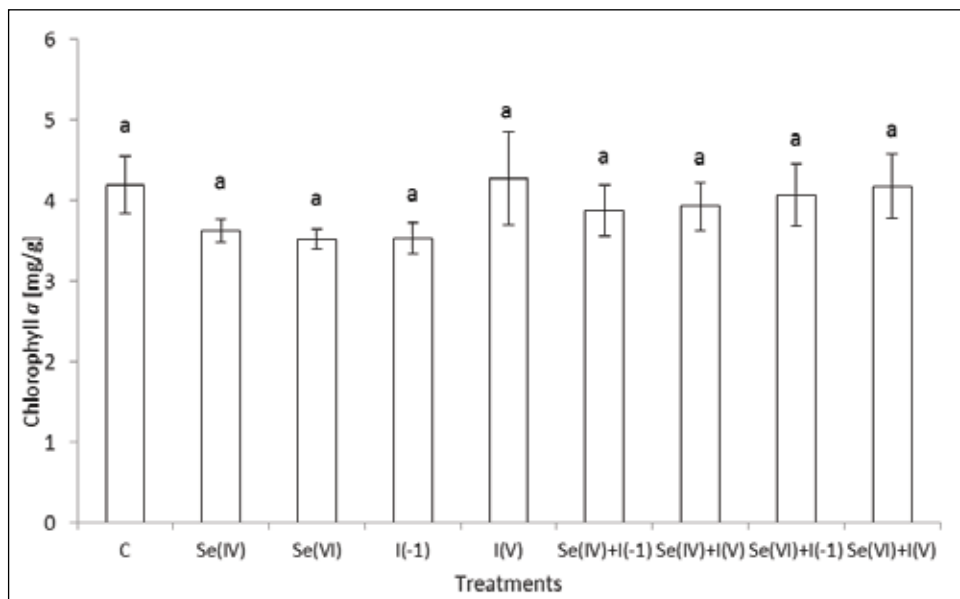


Figure 3: Concentration of chlorophyll *a* per DM in pumpkin sprouts. Mean $\pm$ SE, $n = 6$, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 3: Koncentracija klorofila *a* na SM v kalichah buč. Predstavljene so povprečne vrednosti $\pm$ SE ($n = 6$). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$.

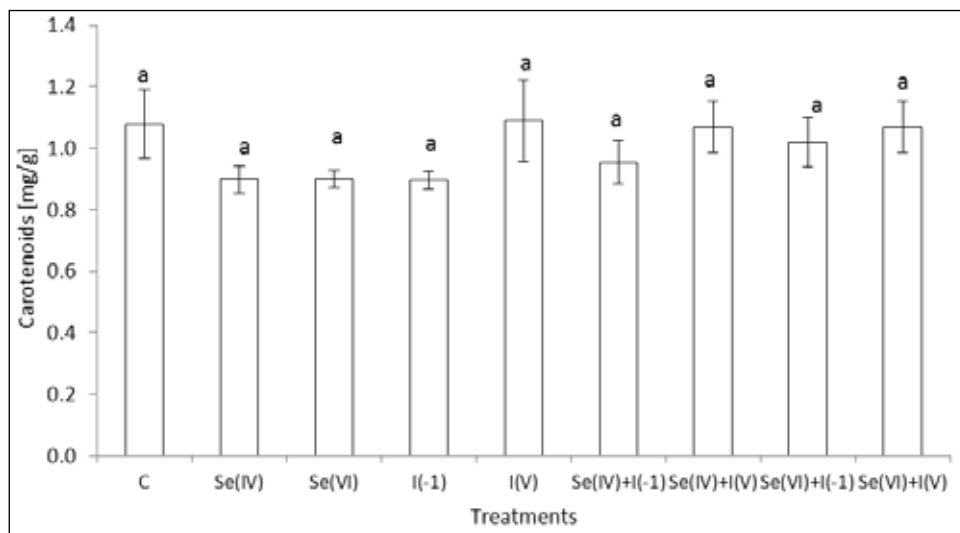


Figure 4: Concentration of carotenoids per DM in pumpkin sprouts. Mean $\pm$ SE, $n = 6$, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 4: Koncentracija karotenoidov na SM v kalichah buč. Predstavljene so povprečne vrednosti $\pm$ SE ($n = 6$). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$.

Table 1: Potential photochemical efficiency of PS II in common buckwheat and pumpkin sprouts. Mean values, marked with the same letter for each species, are not significantly different at $p \leq 0.05$, SE – standard error, $n = 8$.

Tabela 1: Potencialna fotokemična učinkovitost FS II v kalicah navadne ajde in buč. Srednje vrednosti, označene z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$, SE – standardna napaka, $n = 8$.

Treatment	Species			
	Pumpkins		Common buckwheat	
Treatment	mean	SE	mean	SE
Control	0.83a	0.006	0.72abc	0.02
SeIV	0.83a	0.003	0.75ab	0.02
SeVI	0.82a	0.002	0.70bc	0.02
I(-I)	0.83a	0.004	0.78ab	0.01
I(V)	0.83a	0.002	0.71abc	0.05
Se(IV)+I(-I)	0.83a	0.003	0.43d	0.06
Se(IV)+I(V)	0.83a	0.004	0.73ab	0.04
Se(VI)+I(-I)	0.83a	0.002	0.80a	0.01
Se(VI)+I(V)	0.83a	0.006	0.63c	0.03

Discussion

Biochemical response

Different forms of Se and I had different effect on the amount of chlorophyll *a*, *b*, and carotenoids. Control buckwheat sprouts and sprouts from seeds, soaked in Se(VI) and Se(VI)+I(-I) had the lowest and similar amount of chlorophyll *a* and carotenoids. There were no differences in the amount of photosynthetic pigments in pumpkin sprouts. Similar results were given by Mechora et al. (2011, 2014) who reported about similar amount of chlorophyll *a* and *b* in red cabbage and cabbage, treated with Se(VI). Cabbage was fertilized *via* the soil with an aqueous solution of Na selenate (33 mL per plant) containing $2 \mu\text{g Se L}^{-1}$, every second day for 2 months and second group was foliarly sprayed with 20 mg Se L^{-1} in the form of Na selenate ($\sim 0.30 \text{ mL per plant}$) twice in the growing season. Red cabbage was as cabbage fertilized at a rate of 33 mL per plant, in the form of an aqueous solution containing Na selenate at a concentration of $\text{Se } 2 \mu\text{g L}^{-1}$, every second day for two months. The second group of red cabbage was fertilized with $\text{Se } 0.5 \text{ mg L}^{-1}$ twice

in the growing season. In addition, on the study, performed on hydroponically cultivated lettuce, the addition of Se(IV) (2–30 μM) and Se(VI) (2–60 μM) did not affected the amount of photosynthetic pigments (Hawrylak-Nowak 2013). In sweet basil foliarly treated with (1–50 $\text{mg Se} \cdot \text{dm}^{-3}$) selenite, no significant effect on the content of chloroplast pigments was detected (Hawrylak-Nowak 2008a). Similarly, the concentration of Se(VI) < 10 mg/kg did not affect the amount of chlorophylls in *Lolium perenne* cultivated in a soil (Hartikainen et al. 2000). Foliarly spraying with higher concentration of I(-I) and I(V) in the solution as in this study and soil fertilization (15 mg I dm^{-3}) to the radish plants with I(-I) and I(V) did also not affect the amount of photosynthetic pigments (Strzetelski et al. 2010). On the other hand Xue et al. (2001) found out that the addition of Se(VI) in lower and higher concentration (0.1 mg/kg and 1.0 mg/kg respectively) induced synthesis of chlorophyll in young lettuce leaves, while in older leaves of lettuce, only higher concentration of Se(VI) induced the amount of chlorophyll. In younger leaves of *Lolium perenne* the addition of Se(VI) in concentration $\geq 10 \text{ mg/kg}$ in the soil lowered the amount of chlorophylls while on the

other hand enhanced the amount of chlorophyll in older leaves (Hartikainen et al. 2000).

In hydroponic experiment barley seedlings were subjected to 2, 4, 8, 16 ppm Se in the form of Se(VI). Chlorophyll content of the seedlings was affected significantly in a dose dependent manner (Akbulut and Çakır 2010). Significant decrease was observed in the chlorophyll content at ≥ 4 ppm Se applications, similar concentrations as used in the present study. Decrease of the total chlorophyll concentration depending on the Se form (selenate or selenomethionine) and dosage (25, 50, and 100 μM Se) were detected in hydroponically grown maize (Hawrylak-Nowak 2008b). Similarly in Tartary buckwheat, the foliar addition of Se(VI) (1 g Se/m³) at 10 times lower concentration in comparison to our used concentration of Se(VI), also lowered the amount of chlorophylls (Breznik et al. 2005). Under hydroponic cultivation in lettuce plants a marked decrease in photosynthetic pigments concentration was found after passing the toxicity threshold, which has been designated at a level of 15 μM for selenite and 20 μM for selenate (Hawrylak-Nowak 2013). In the study from Strzetelski et al. (2010), soil fertilization with iodine was carried out before radish sowing to the level of 15 mg I·dm⁻³ soil. Foliar application of this element was performed twice using iodine solution in a concentration per pure element of 0.2%, in dose of 0.4 dm³ · m⁻². Iodine foliar and soil application in radish, regardless of iodine forms (I(-1), IO₃⁻), dose and application method, had no significant effect on the content of dry matter, as well as on the level of photosynthetic pigments in leaves. The objective of study from Blasco et al. (2011) was to determine the effect of the application of different doses (20, 40 and 80 μM) and forms of iodine (iodate [IO₃⁻] and iodide [I(-1)]) on photosynthesis and carbohydrate metabolism in lettuce plants. The Chl *a* content did not differ between I(-1)-treated lettuce plants and controls but was significantly reduced in plants treated with 80 μM IO₃⁻.

Physiological response

Potential photochemical efficiency in common buckwheat (except sprouts from seeds, soaked in Se(IV)+I(-1)) and pumpkin sprouts was mainly around 0.7 and 0.8 respectively. Values, close to theoretical maximum 0.83 (Schreiber et al.

1995) meant that different forms of Se and I and their combination did not damage photosynthetic apparatus. Similar results were given regarding common buckwheat (Breznik et al. 2005, Tadina et al. 2007) and Tartary buckwheat (Breznik et al. 2005), foliarly treated with Se(VI) (1 g Se/m³). In addition, in the experiment, where Se(VI) was added to red cabbage (Mechora et al. 2011) and chicory (Germ et al. 2007), potential as well as effective photochemical efficiency were similar in treated and control plants. In pumpkins foliar spraying with Na-selenate solution (1.5 mg Se L⁻¹) did not influence the potential photochemical efficiency of PS II (Germ et al. 2005). A positive effect of Se on potential photochemical efficiency was reported for the strawberries, cultivated in soil enriched with Se (0.1 mg Se kg⁻¹ soil and 1 mg Se kg⁻¹ soil in the form of H₂SeO₄), but the same treatment had no positive effect on barley (Valkama et al. 2003).

Conclusions

There is scarce information about the effect of different forms of I on plants and particularly with the combination with Se. Concentrations, used in the present study, mainly caused no negative effect on the biochemical and physiological characteristics of sprouts.

Povzetek

Pri nas in po svetu se je močno povečalo zanimanje za ajdo, tudi zaradi izredno skladne sestave hranilnih snovi v njenih zrnih. Vsebuje zelo kakovostne beljakovine. V tradicionalni medicini se uporabljajo bučna semena že stoletja predvsem v primerih težav z ledvicami ali sečili. Kalice vsebujejo veliko beljakovin, vitaminov, ogljikovih hidratov, olj, rudninskih snovi in s tem pripomorejo k biološki polnovredni prehrani. Selen in jod sta elementa, ki sta ključnega pomena za pravilno delovanje ščitnice in sta nujno potrebna pri izgradnji tiroidnega hormona, njegovi aktivaciji in metabolizmu. Ker je Se neobhodno potreben za metabolizem I v ščitnici raziskovalci menijo, da je smiselno gojenim rastlinam sočasno dodajati oba elementa.

Kalice navadne ajde in buč smo gojili v rastni komori s stalno temperaturo 22°C in 65–70% r.z.v. Seme ajde in buč smo namakali v različnih raztopinah selen in joda. Preučevali smo vpliv naslednjih obravnavanj: selenat (10 mg Se(VI) /L)), selenit (10 mg Se(IV) /L)), jodid (1000 mg I(-1) /L)), jodat (1000 mg I(V) /L)) in kombinacije 10 mg Se(VI) /L+ 1000 mg I(-1) /L; 10 mg Se(VI) /L+ 1000 mg I(V) /L; 10 mg Se(IV) /L+ 1000 mg I(-1)/L; 10 mg Se(IV) /L+ 1000 mg I(V) /L in K (kontrola – destilirana voda brez dodanega selen in/ali joda).

Merili smo sledeče fiziološke in biokemijske lastnosti kontrolnih kalic in kalic, zrastlih iz obravnavanih semen: fotokemično učinkovitost fotosistema II (FS II) ter vsebnost fotosinteznih barvil (klorofil *a*, klorofil *b* in karotenoidov). Kontrolne kalice in kalice, zrastle iz semen, namakanih v Se(VI) ter Se(VI)+I(-1), so imele najnižjo in podobno vsebnost klorofila *a* in karotenoidov. Obravnavanja so imela majhen vpliv na potencialno fotokemično učinkovitost fotosistema

II pri kalicah navadne ajde. Namakanje semen v različnih raztopinah Se in I ni vplivalo na vsebnost fotosinteznih barvil in potencialno fotokemično učinkovitost fotosistema II pri kalicah buč.

Z raziskavo smo želeli ugotoviti, ali selen in jod, ki ju dodajamo hkrati, vplivata na kalice navadne ajde in buč. Raziskava je zanimiva zato, ker je malo podatkov o hkratnem delovanju selen in joda na biokemijske in fiziološke parametre rastline in tudi zato, ker sta selen in jod elementa, ki sta zelo pomembna za delovanje ščitnice.

Acknowledgments

This research was financed by the Ministry of Education, Science and Sport, Republic of Slovenia, through the programmes “Biology of plants” (P1-0212), “Young researchers” (34326) and projects J4-4224 and J4-5524.

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Biodiversity, the present ecological state of the Aral Sea and its impact on future development

Vrstna pestrost, sedanje ekološko stanje Aralskega jezera in njegov vpliv na prihodnji razvoj

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Abstract : The Aral sea used to be the fourth largest lake in the world. Its catchment area is huge, two main rivers (Amu Darya and Syr Darya) feed the lake. The balance of hydrological regime changed drastically after 1960 due to regulation of both main rivers and diversion of water for agricultural irrigation and intense cotton production. Salinity increased and most of invertebrate and fish species disappeared. A significant drop of water level has been recorded in the past 20 years and Aral Lake is presently divided into a small northern lake basin and a larger south basin. Kokaral dam construction resulted in increased water level and decreased salinity. Many invertebrate species reappeared in Small Aral and fish returned from Syr Darya river. Ecological situation in Large Aral is different, eastern part of this basin is completely dried out. The data on salinity levels, some chemical characteristics and above all the data about zooplankton, zoobenthos and fish in Small Aral have been recorded and presented in the article. Salinity ranges between 1 and 8 g/L, the lowest is near the river inlet. Five species of zooplankton (*Keratella quadrata*, *Brachionus plicatilis*, *Evadne anonyx*, *Calanipeda aquaedulcis*, *Cyclops vicinus*) and rotifers from the genus *Synchaeta* are very abundant, ten species are less numerous and seven summer species very rare. Different zoobenthos species are present, but only four abundant (*Hediste diversicolor*, *Chironomus plumosus*, *Syndosmya segmentum* and *Cyprideis torosa*). Zoobenthos mainly consist of Polychaeta, Mollusca, Crustacea and Diptera. The highest diversity was found near the Kokaral dam. Many fish species are commercially important: 14 of them are abundant, including endemic bream *Abramis brama orientalis*, *Chalcalburnus chalcoides aralensis*, carp *Cyprinus carpio aralensis*, and Aral roach *Rutilus rutilus aralensis*. White-eye bream *Abramis sapa aralensis*, silver carp *Hypophthalmichthys molitrix*, orfe *Leuciscus idus oxianus*, and snakehead *Channa argus warpachowskii* are less numerous. Aral barbel *Barbus brachycephalus brachycephalus* and Turkistan barbel *Barbus capito conocephalus* remain very rare. It can be concluded that significant positive changes occurred after Kokaral dam construction. Particularly, biocenoses and the Aral lake environment have been improved and fisheries returned. Today Kazakhstan Government is discussing an idea to improve this dam and dike

and we support this discussion and advise to make it higher. All can lead to improve ecological state of the Small Aral.

Keywords: Aral Sea, biodiversity, ecological state, zooplankton, zoobenthos, fish

Izvleček: Aralsko jezero je bilo četrto največje jezero na svetu. Njegovo prispevno območje je zelo veliko, dve glavni reki sta pritekali v jezero, Amu Darja in Sir Darja. Hidrološko stanje jezera se je drastično spremenilo po letu 1960 po regulaciji in preusmeritvi obeh glavnih rek za namakanje bombažnih nasadov. Povečala se je slanost, številne vrste nevretenčarjev in rib so izginile. V 20 letih se je gladina vode v jezeru opazno znižala in jezero se je razdelilo na dva dela, manjši severni bazen in večji južni bazen. Po izgradnji jezua Kokaral se je gladina vode zvišala in slanost znižala. Mnoge nevretenčarske vrste so se vrstile v Mali Aral, iz Sir Darje so prišle tudi ribe. Ekološko stanje v Velikem Aralu je drugačno, vzhodni del tega bazena je popolnoma suh. V članku so zbrani podatki o slanosti, nekateri kemijski parametri in predvsem združbe zooplanktona, zoobentosa in rib v Malem Aralu. Slanost variira med 1 g/L in 8 g/L, najnižja pri rečnem vtoku. Zelo pogostih je pet zooplanktonskih vrst (*Keratella quadrata*, *Brachionus plicatilis*, *Evadne anonyx*, *Calanipeda aquaedulcis*, *Cyclops vicinus*), ena nedoločena vrsta kotačnika *Synchaeta*. Deset vrst je manj pogostih, zelo redkih pa je šest vrst pomladnih zooplantontov. Prisotnih je tudi več različnih vrst zoobentosa, le štiri vrste pa so pogoste (*Hediste diversicolor*, *Chironomus plumosus*, *Syndosmya segmentum*, and *Cyprideis torosa*). Zoobentos sestavljajo Polychaeta, Mollusca, Crustacea in Diptera. Največja pestrost je bila ugotovljena ob jezu Kokaral. Mnoge ribje vrste so gospodarsko pomembne, 14 od njih je pogostih, vključno z endemnimi taksoni *Abramis brama orientalis*, *Chalcalburnus chalcoideus aralensis*, *Cyprinus carpio aralensis*, *Rutilus rutilus aralensis*. Manj številčne so *Abramis sapa aralensis*, *Hypophthalmichthys molitrix*, *Leuciscus idus oxianus*, *Channa argus warpachowskii*. Zelo redki sta dve vrsti mrene *Barbus brachycephalus brachycephalus* in *Barbus capito conocephalus*. Ugotavljamo, da so se opazne in pozitivne spremembe zgodile po izgradnji jezua Kokaral. Zlasti se je izboljšala vrstna pestrost združb in jezersko okolje nasploh, zato se je vrnilo ribištvo. Danes Kazahstanska vlada razmišlja o izboljšanju jezua in nasipa. To razmišljanje podpiramo in obenem svetujemo povišanje jezua, kar bi prineslo izboljšanje ekološkega stanja Malega Arala.

Ključne besede: Aralsko jezero, vrstna pestrost, ekološko stanje, zooplankton, zoobentos, ribe

Introduction

The Aral Sea is a terminal lake, lying amidst the vast deserts of Central Asia. From the 1600's to the 1960's, the hydrological regime of the Aral Sea was in reasonable balance. This lake is Transboundary Lake and 7 countries (Afghanistan, Iran, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan) are contributing water into it. Since 1960 the anthropogenic regression and salinization of the Aral Sea has begun. This has resulted in the disappearance of most of invertebrates and

fish species. Some of them have become extinct. At the end of the 1980's due to the fall of water level, the Aral Sea was divided into northern Small Aral and the southern Large Aral having different hydrological regimes.

Regression and salinization of Large Aral continues. After the construction of the Kokaral dam (Aladin, 2014) Small Aral Sea level has increased and a gradual decline in its salinity has begun. Because of this dam lake restoration is possible. To date, salinity of the Small Aral Sea has become lower than it was before the 1960's.

There is a process of restoration of former biodiversity. Many invertebrate species are reappearing due to salinity decrease. Commercial freshwater fish species returned into the Small Aral from Syr Darya River and lakes in its lower reaches where they survived. Their populations are in good state. Fisheries are restoring and catches are growing (Aladin and Plotnikov, 2012; Aladin et al., 2012; Plotnikov et al., 2012).

At present Aral Sea is divided to the number residual parts (lotic and lentic). Large Aral Sea is currently the most suffering part of the lake. In the beginning of 21st century it was divided in 3 parts: Western Large Aral Sea, Eastern Large Aral Sea and Tsche-Bas Bay. Since last few years appeared a new fourth part of Large Aral Sea – New Central Aral Sea.

Summer 2014 marked another milestone for the Large Aral Sea. For the first time in modern history, the Eastern Large Aral Sea has completely dried. So we have again currently only 3 parts Western Large Aral Sea, Tsche-Bas Bay and New Central Aral Sea.

In autumn 2009 some people without any calculations and without direct observations reported that Eastern Large Aral Sea dry up completely (http://earthobservatory.nasa.gov/Features/WorldOfChange/aral_sea.php). Next year 2010 Eastern Large Aral Sea came back because it was a very wet year and a lot of water came from Amu Darya River delta.

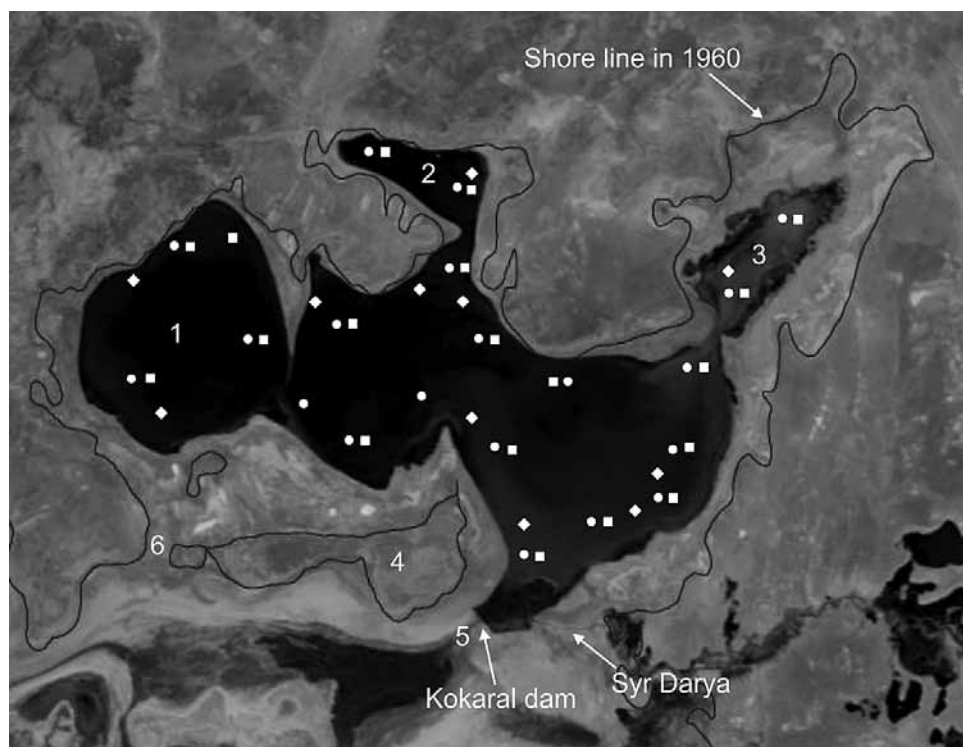


Figure 1. Map of sampling places in Aral Sea. 1 – Saryshiganak Bay; 2 – Butakov Bay; 3 – Shevchenko Bay; 4 – Kokaral island; 5 – Berg Strait; 6 – Auzy-Kokaral Strait. Standard stations: ◆ – ichthyological, ● – hydrobiological, ■ – hydrochemical.

Slika 1. Označena vzorčna mesta v Aralskem jezeru. 1 – zaliv Saryshiganak, 2 – zaliv Butakov, 3 – zaliv Shevchenko, 4 – otok Kokaral, 5 – ožina Berg, 6 – ožina Auzy_Kokaral. Standardne postaje: ◆ – ihtiološka, ● – hidrobiološka, ■ – hidrokemijska.

Material and Methods

Number of field trips around Aral Sea were made. We studied the following places: Small Aral Sea, Tsche-Bas Bay; Western Large Aral Sea (Only Chernyshov Bay); New Central Aral Sea. Small Aral Sea level is 42.0 m a.s.l. with a volume of 27.1 km³. Water covers an area of 3288 km². Basin has maximal depth of 15.5 m (average depth of 8.2 m).

In our studies of Aral Sea we are using IL²BM platform (Integrated Lotic/Lentic Basin Management) (<http://www.ilec.or.jp/en/>).

Salinity was measured using YSI-85 and conductometer LF-330. Other chemical parameters were determined using standard methods. Oxygen concentration and pH were measured at the sampling points. Parameters such as COD (permanganate method), NH₄⁺, NO₂⁻, NO₃⁻ and PO₄³⁻ were analysed in the laboratory.

Plankton samples were collected according to standard methodology using plankton net (mesh size 60µm). Invertebrates (zoobenthos) were sampled using Petersen bottom sampler (0.025 m²) and sediments were washed through the sieve No 36. All samples were fixed with formalin (4%) and investigated using stereo microscope (MBS-10). All collected animals were taken to the Aral branch of Kazakh Research Institute of Fishery and Zoological institute of RAS. For species identification Atlas of the Aral Sea invertebrates was used.

Fish were sampled several years. They were caught with fixed fishing nets (mesh size 18-65 mm).

Results and discussion

Chemical data

Oxygen concentrations, COD, concentrations of NH₄⁺, NO₂⁻, NO₃⁻, PO₄³⁻ and pH were measured in different years and very little changes were found. More changes were detected in salinity (Table 1).

Salinity in different parts of Small Aral Sea was from 1 g/L up to 8 g/L. The lowest level of salinity was observed in May 2014 near the Kokaral dam (near Syr Darya delta) and the highest in August in Butakov Bay near Akespe village. The level of Small Aral in investigated period was from 42.1 to 42.6 m a.s.l. The lowest was in August, the highest in May (unpublished data by Ermakhanov from institutional report).

Much higher salinity was measured in Tsche-Bas Bay, ranging from 78 g/L up to 89 g/L. The lowest level of salinity was observed in May near connection to New Central Aral Sea and the highest – in August near the northern coast of Tsche-Bas Bay. The level of Tsche-Bas Bay was from 28.7 to 29.1 m a.s.l. The lowest was in August, the highest was in May.

In Western Large Aral Sea (Only Chernyshov Bay) salinity was very different compared to Small

Table 1: Selected chemical data from Small Aral Sea in the years 2007 – 2013.

Tabela 1: Izbrani kemijski podatki o malem Aralu v letih 2007 – 2013.

Year	pH	O ₂ , mg/dm ³	COD mg/dm ³	Salinity, ‰	Biogenes, mg/dm ³			
					NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	PO ₄ ³⁻
2007	7.90-8.30	6.71-13.63	4.3-12.0	6.3	0.65-1.25	0.003-0.055	0.00-0.60	0.005-0.068
2008	6.85-7.20	5.32-11.42	1.5-12.2	12.1	0.05-1.23	0.002-0.135	0.02-0.59	0.008-0.050
2009	6.93-7.32	6.20-12.51	1.4-11.7	12.9	0.38-0.83	0.012-0.101	0.05-0.43	0.010-0.025
2010	7.20-7.30	7.84-12.30	5.8-7.0	11.0	0.23-0.37	0.003-0.039	4.17-5.94	0.000-0.040
2011	7.20-7.45	7.62-12.74	2.4-4.6	9.9	0.36-0.39	0.028-0.032	4.35-5.30	0.022-0.036
2012	7.20-7.25	7.74-9.28	3.2-3.9	5.7	0.37-0.39	0.022-0.029	3.99-4.97	0.017-0.025
2013	7.10-8.15	7.70-9.20	3.1-3.5	5.3	0.12-0.24	0.009-0.027	3.22-3.33	0.015-0.019

Aral with very high values, from 143 g/L up to 169 g/L. The lowest level of salinity was observed in May 2014 near sampling camp of Mangistau Bioresource Company and the highest in August near the northern coast of Chernyshov Bay. The level of Western Large Aral Sea in investigated period was from 24.9 to 25.5 m a.s.l. The lowest was in August, the highest was in May.

Salinity in New Central Aral Sea varied from 6 g/L up to 77 g/L. The lowest level was observed in May near the Kokaral dam and the highest in August near the connection to Tsche-Bas Bay. The level of New Central Aral Sea at the place of planned southern dike was from 28.5 to 28.9 m a.s.l. and at Kokaral dam from 29.5 to 31.6 m a.s.l. The lowest level was in August, the highest was in May.

Zooplankton

Zooplankton biocenoses in Small Aral Sea mainly consist of three Rotifera, Cladocera and Copepoda (Table 2). Only few species of freshwater and brackish planktonic Protozoa were detected (Plotnikov et al., 2014; Smurov, 1995).

Five species of zooplankton were numerous: *Keratella quadrata* (Müller), *Brachionus plicatilis* Müller, *Evadne anonyx* Sars, *Calanipeda aquaedulcis* Kritchagin, *Cyclops vicinus* Uljanin and one undetermined species of rotifers from the genus *Synchaeta* (unpublished data by Ermakhanov from institutional report).

Less numerous were ten species: *Brachionus quadridentatus* Hermann, *Brachionus calyciflorus* Pallas, *Hexarthra oxyuris* (Zernov), *Bosmina longirostris* Müller, *Chydorus sphaericus* Müller, *Ceriodaphnia reticulata* (Jurine), *Podonevadne camptonyx* (Sars), *Phyllodiaptomus blanci* (Guerne et Richard), *Mesocyclops leuckarti* (Claus), *Acanthocyclops viridis* (Jurine). Copepod taxa from order Harpacticoida were also less numerous (unpublished data by Ermakhanov from institutional report).

Seven species of zooplankton mainly from rotifers group were very rare and all of them were observed only in summer time: *Asplanchna priodonta* Gosse, *Keratella cochlearis* (Gosse), *Notholca acuminata* (Ehrenberg), *Filinia longiseta* (Ehrenberg), *Moina mongolica* Daday, *Diaphanosoma brachyurum* Lievin, *Podonevadne angusta*

groups: (Sars) (unpublished data by Ermakhanov from institutional report).

In Tsche-Bas Bay only two species of zooplankton were numerous: brine shrimp *Artemia parthenogenetica* Bowen et Sterling from May till September, and halophilic ciliate *Fabrea salina* Henneguy during all summer months from June till August (Plotnikov et al., 2014).

Zooplankton species *Moina mongolica* was very rare and only few individuals (parthenogenetic females) were observed only in summer time.

In Western Large Aral Sea (Only Chernyshov Bay) only one species of zooplankton brine shrimp *Artemia parthenogenetica* was numerous due to high salinity. It occurs from May till September. One species of halophilic ciliate *Fabrea salina* was observed only at the end of summer in August and was very rare (Plotnikov et al. 2014).

Near the Kokaral dam the biodiversity of zooplankton is the highest. Three species were numerous: *Keratella quadrata*, *Brachionus plicatilis*, *Calanipeda aquaedulcis*, seven species were less numerous: *Brachionus quadridentatus*, *Bosmina longirostris*, *Chydorus sphaericus*, *Ceriodaphnia reticulata*, *Phyllodiaptomus blanci*, *Mesocyclops leuckarti*, *Acanthocyclops viridis*.

Near connection to Tsche-Bas Bay the biodiversity of zooplankton are the lowest. Only three species of zooplankton were numerous: brine shrimp *Artemia parthenogenetica* Plotnikov et al., 2014, euryhaline *Moina mongolica* from May till September, and halophilic ciliate *Fabrea salina* during all summer months from June till August.

Zoobenthos

Zoobenthic biocenosis in Small Aral Sea mainly consists of groups: Polychaeta, Mollusca, Crustacea, Insecta/Diptera (Table 3). Only few species of Foraminifera and Nematoda were found in samples (Filippov et al. 1993; Plotnikov et al. 2014).

Four species of zoobenthos were numerous: *Hediste diversicolor* (Müller), *Chironomus plumosus* (L.), *Syndosmya segmentum* Récluz, *Cyprideis torosa* (Jones) (Plotnikov et al., 2014; unpublished data by Ermakhanov from institutional report).

Six species of zoobenthos were less numerous: *Chironomus behningi* Goetghebuer, *Glyptotendipes gripekoveni* Kieffer, *Limnocythere aralensis* Schornikov, *Limnocythere inopinata*

Table 2: Taxonomic composition of zooplankton in the Small Aral Sea in the years 2011 – 2013 (species only found in the samples are indicated).

Tabela 2: Taksonomska sestava zooplanktona v Malem Aralu v letih 2011 – 2013 (naštete so le vrste, ki so bile najdene v vzorcih).

Taxon	Occurrence, %		
	2011	2012	2013
Rotifera			
<i>Asplanchna priodonta</i> Gosse	-	5	5
<i>Synchaeta</i> spp.	50	60	74
<i>Keratella quadrata</i> (Gosse)	68	77	74
<i>Keratella cochlearis</i> (Gosse)	-	-	5
<i>Brachionus quadridentatus</i> Hermann	41	40	26
<i>B. calyciflorus</i> Pallas	-	9	26
<i>B. plicatilis</i> Müller	23	40	68
<i>Notholca acuminata</i> Ehrenberg	5	5	5
<i>Filinia longiseta</i> (Ehrenberg)	23	27	-
<i>Hexarthra oxyuris</i> (Zernov)	5	36	37
Cladocera			
<i>Bosmina longirostris</i> (Müller)	10	18	21
<i>Chydorus sphaericus</i> (Müller)	15	23	26
<i>Moina mongolica</i> Daday	10	9	-
<i>Ceriodaphnia reticulata</i> (Jurine)	15	36	21
<i>Diaphanosoma brachyurum</i> (Lievin)	-	-	5
<i>Podonevadne angusta</i> (Sars)	5	5	-
<i>P. camptonyx</i> (Sars)	10	5	37
<i>Evadne anonyx</i> Sars	73	73	79
Copepoda			
<i>Phyllodiaptomus blanci</i> (Guerne et Richard)	14	14	11
<i>Calanipeda aquaedulcis</i> Kritschagin	100	100	100
<i>Cyclops vicinus</i> Uljanin	60	86	79
<i>Mesocyclops leuckarti</i> (Claus)	20	23	26
<i>Acanthocyclops viridis</i> (Jurine)	28	36	32
Harpacticoida gen. sp.	25	14	26

(Baird), *Tyrrenocythere amnicola donetziensis* (Dubowsky),

Amnicythere cymbula (Livental), unspecified species from genera *Chironomus*, *Candona* and family Ceratopogonidae were also less numerous (Plotnikov et al. 2014; unpublished data by Ermakhanov from institutional report).

Four species of zoobenthos were very rare and all of them were observed from May till September: *Dreissena polymorpha aralensis* (Andrusov), *Cerastoderma isthmicum* Issel, *Paramysis inter-*

media (Czerniavsky), *Palaemon elegans* Rathke. Unspecified species from genus *Chironomus* and family Ceratopogonidae were also less numerous (Plotnikov et al., 2014; unpublished data by Ermakhanov from institutional report).

Unspecified species from genera *Cryptochironomus*, *Cladotanytarsus*, *Tanytarsus*, *Caspiohydrobia* were very rare and all of them were observed from May till September (unpublished data by Ermakhanov from institutional report).

Table 3: Taxonomic composition of zoobenthos in Small Aral Sea in in the years 2012 – 2013 (species only found in the samples are indicated).

Tabella 3: Taksonomska sestava zoobentosa v Malem Aralu v letih 2012 – 2013 (naštete so le vrste, ki so bile najdene v vzorcih).

Taxa	Occurrence, %	
	2012	2013
Polychaeta		
<i>Hediste diversicolor</i> (Müller)	86	80
Insecta: Diptera		
<i>Chironomus behningi</i> (Goetghebuer)	25	18
<i>Chironomus</i> sp.	-	36
<i>Ch. plumosus</i> (Linne)	50	50
<i>Glyptotendipes gripekoveni</i> (Kieffer)	-	10
<i>Cryptochironomus</i> sp.	30	5
<i>Cladotanytarsus</i> sp.	-	8
<i>Tanytus villipennis</i> (Kieffer)	-	8
<i>Tanytarsus</i> sp.	17	8
Ceratopogonidae gen. sp.	-	18
Mollusca: Bivalvia		
<i>Syndosmya segmentum</i> Récluz	60	46
<i>Dreissena polymorpha aralensis</i> (Andrusov)	5	4
<i>Cerastoderma isthmicum</i> Issel	25	4
Mollusca: Gastropoda		
<i>Caspiohydrobia</i> spp.	5	-
Crustacea		
<i>Paramysis intermedia</i> (Czerniavsky)	-	4
<i>Palaemon elegans</i> Rathke	5	-

In Tsche-Bas Bay three species of zoobenthos were numerous from May till September: salt tolerant halophilic ostracod *Eucypris inflata* (Sars), euryhaline ostracod *Cyprideis torosa* (Jones) and halophilic larvae of *Chironomus salinarius* Kieffer (Plotnikov et al. 2014).

Two species of zoobenthos were very rare: euryhaline Turbellaria *Mecynostomum agile* (Beklemischev) and large ciliate *Frontonia marina* Fabre-Domergue. Unspecified species of foraminifers and nematodes were also were very rare (Plotnikov et al. 2014).

In Western Large Aral Sea (Only Chernyshov Bay) only one species of zoobenthos halophilic larvae of *Chironomus salinarius* were numerous and present from May till September. One rare species of zoobenthos was found: large ciliate *Frontonia marina*. Very rare were unspecified species of foraminifers and nematodes (Plotnikov et al. 2014).

Near the Kokaral dam the biodiversity of zoobenthos is the highest. Four species were numerous: *Hediste diversicolor* (Müller), *Chironomus plumosus* (L.), *Syndosmya segmentum*, *Cyprideis torosa*. The following four species such as *Chironomus behningi* Goetghebuer, *Limnocythere aralensis*, *Tyrrenocythere amnicola donetziensis*, *Amnicocythere cymbula* were less numerous, while species *Cerastoderma isthmicum* and *Palaemon elegans* were very rare.

Near the connection to Tsche-Bas Bay the biodiversity of zoobenthos was the lowest. Three species of zoobenthos from May till September were numerous and occurred from May till September: salt tolerant halophilic ostracod *Eucypris inflata* (Sars), euryhaline ostracod *Cyprideis torosa* and halophilic larvae of *Chironomus salinarius*. Euryhaline large ciliate species *Frontonia marina* was very rare. Unspecified species of foraminifers and nematodes were also were very rare (Plotnikov et al. 2014).

Fish

In Small Aral Sea 14 species of commercial fish were numerous: pike *Esox lucius* Linnaeus, bream *Abramis brama orientalis* Berg, asp (zherekh) *Aspius aspius iblioides* (Kessler), crucian carp *Carassius carassius gibelio* Bloch, Aral shemaya *Chalcalburnus chalcoides aralensis* (Berg), carp *Cyprinus carpio aralensis* Spitshakow, grass

carp *Ctenopharyngodon idella* (Valenciennes), sabrefish *Pelecus cultratus* (Linnaeus), Aral roach *Rutilus rutilus aralensis* Berg, rudd *Scardinius erythrophthalmus* (Linnaeus), wels *Silurus glanis* Linnaeus, perch *Perca fluviatilis* (Linnaeus), pike perch or zander *Stizostedion lucioperca* (Linnaeus), Black Sea flounder *Platichthys flesus* (Linnaeus) (Ermakhanov et al. 2012) (Table 4).

Less numerous were 4 species: white-eye bream *Abramis sapa aralensis* Tjapkin, silver carp *Hypophthalmichthys molitrix* (Valenciennes), orfe *Leuciscus idus oxianus* (Kessler), snakehead *Channa argus warpachowskii* Berg (Ermakhanov et al. 2012).

Only 5 species of commercial fish were very rare: Baltic herring *Clupea harengus membras* (Linnaeus), spotted silver carp *Aristichthys nobilis* (Richardson), black carp *Mylopharyngodon piceus* (Richardson), Aral barbel *Barbus brachycephalus brachycephalus* Kessler, Turkestan barbel *Barbus capito conocephalus* Kessler (Ermakhanov et al. 2012). Fishery is under control by authorities.

Nine fish species in the lake are not commercial. Six of them are numerous: ruff *Gymnocephalus cernuus* (Linnaeus), nine-spined stickleback *Pungitius platygaster aralensis* (Kessler), Caspian atherine *Atherina boyeri caspia* Eichwald, bubyry goby, transcaucasian goby *Pomatoschistus caucasicus* Berg [= *Knipowitschia caucasica* (Berg)], sand goby *Neogobius fluviatilis pallasi* (Berg), round goby *Neogobius melanostomus affinis* (Eichwald) (Ermakhanov et al., 2012). Three species of not commercial fish are rare: syrman goby *Neogobius syrman eurytomus* (Kessler), tubenose goby *Proterorhynchus marmoratus* (Pallas), bighead goby *Neogobius kessleri gorlap* Iljin (Ermakhanov et al. 2012).

In Tsche-Bas Bay and in Western Large Aral Sea (Only Chernyshov Bay) fish are not living now due to high salinity (Ermakhanov et al. 2012).

The highest biodiversity of fish was found near the Kokaral dam. Nine species of commercial fish were numerous: pike *Esox lucius* Linnaeus, bream *Abramis brama orientalis* Berg, Aral shemaya *Chalcalburnus chalcoides aralensis* (Berg), carp *Cyprinus carpio aralensis* Spitshakow, Aral roach *Rutilus rutilus aralensis* Berg, wels *Silurus glanis* Linnaeus, perch *Perca fluviatilis* (Linnaeus), pike perch or zander *Stizostedion lucioperca* (Linnaeus), Black Sea flounder *Platichthys flesus* (Linnaeus).

Two species of commercial fish were less numerous: silver carp *Hypophthalmichthys molitrix* (Valenciennes) and snakehead *Channa argus warchowskii* Berg.

Near connection to Tsche-Bas Bay the biodiversity of fish is the lowest. Only one species of commercial fish was very rare in this part of the New Central Aral Sea. Black Sea flounder *Platichthys flesus* was caught several times from May to June (Ermakhanov et al. 2012).

Table 4: Species composition of ichthyofauna in the Small Aral Sea.

Tabela 4: Vrsta sestava ihtiofavne v Malem Aralu.

Taxa	Status
Esocidae	
<i>Esox lucius</i> Linnaeus (Pike)	A, C-
Cyprinidae	
<i>Rutilus rutilus aralensis</i> Berg (Aral roach)	A, C
<i>Leuciscus idus oxianus</i> (Kessler) (Orfe)	A, C-
<i>Aspius aspius iblioides</i> (Kessler) (Asp, zherekh)	A, C
<i>Scardinius erythrophthalmus</i> (Linnaeus) (Rudd)	A, C-
<i>Barbus capito conocephalus</i> Kessler (Turkestan barbell)	A, C-, RB
<i>Barbus brachycephalus brachycephalus</i> Kessler (Aral barbell)	A, C-, RB
<i>Abramis brama orientalis</i> Berg (Bream)	A, C
<i>Abramis sapa aralensis</i> Tjapkin (White-eye bream)	A, C-
<i>Chalcalburnus chalcoides aralensis</i> (Berg) (Aral shemaya)	A, C-
<i>Pelecus cultratus</i> (Linnaeus) (Sabrefish)	A, C-
<i>Carassius carassius gibelio</i> Bloch (Crucian carp)	A, C-
<i>Cyprinus carpio aralensis</i> Spitshakow (Carp)	A, C
<i>Ctenopharyngodon idella</i> (Valenciennes) (Grass carp)	I, C-
<i>Hypophthalmichthys molitrix</i> (Valenciennes) (Silver carp)	I, C-
<i>Aristichthys nobilis</i> (Richardson) (Spotted silver carp)	I, C-
<i>Mylopharyngodon piceus</i> (Richardson) (Black carp)	I, C-
Siluridae	
<i>Silurus glanis</i> Linnaeus (Wels)	A, C-
Gasterostidae	
<i>Pungitius platygaster aralensis</i> (Kessler) (Nine-spined stickleback)	A, NC
Percidae	

<i>Stizostedion lucioperca</i> (Linnaeus) (Pike perch, zander)	A, C
<i>Perca fluviatilis</i> Linnaeus (Perch)	A, C-
<i>Gymnocephalus cernuus</i> (Linnaeus) (Ruff)	A, NC
Clupeidae	
<i>Clupea harengus membras</i> (Linnaeus) (Baltic herring)	I, C--
Atherinidae	
<i>Atherina boyeri caspia</i> Eichwald (Caspian atherine)	I, NC
Gobiidae	
<i>Pomatoschistus caucasicus</i> Berg (Bubyr goby, transcaucasian goby) [= <i>Knipowitschia caucasica</i> (Berg)]	I, NC
<i>Neogobius fluviatilis pallasii</i> (Berg) (Sand goby)	I, NC
<i>Neogobius melanostomus affinis</i> (Eichwald) (Round goby)	I, NC
<i>Neogobius syrman eurystomus</i> (Kessler) (Syrman goby)	I, NC
<i>Proterorhynchus marmoratus</i> (Pallas) (Tubenose goby)	I, NC
<i>Neogobius kessleri gorlap</i> Iljin (Bighead goby)	I, NC
Channidae	
<i>Channa argus warpachowskii</i> (Berg) (Snakehead)	I, C
Pleuronectidae	
<i>Platichthys flesus</i> (Linnaeus) (Black Sea flounder)	I, C

Abbreviations: A – aboriginal; I – introduced; C – commercial; C- – commercial but low stocks C-- – while commercial but stocks very low for fishery; NC – not commercial; RB – in Red Book.

All above mentioned data collected from May till September is the evidence of great practical and commercial importance of all four Aral Sea Areas in Republic of Kazakhstan: Small Aral Sea, Tsche-Bas Bay, Western Large Aral Sea (Only Chernyshov Bay), New Central Aral Sea.

As it is said in the introduction to this paper big positive changes in Aral Sea environment and in Aral Sea fisheries happened immediately after construction of a Kokaral dam in the Berg strait. Today local people and Kazakhstan Government are discussing an idea to improve this dike. We are supporting this discussion and we advise to make it higher from 42-43 meters above ocean level up to 46 - 48 meters as it was advised by us in 1992.

We also propose to build two more dams in addition to this Central dam:

1) The Northern dam could be build in the entrance to the Bolshoy Sarychaganak Bay near Trekhgorka place (three-headed mountain place in English). The canal from Kamyslibash Lake to Bolshoy Sarychaganak Bay should be build too. The dam should be as high as 49-50 meters a.s.l. Geographical coordinates of future dike could be N 49°29'16", E 61°15'51".

2) The Southern dam could be build at the southern edge of New Central Aral Sea where it unites with Tsche-Bas Bay. This dam will enable keeping the water that is running away from Kokaral dam in Republic of Kazakhstan via its spillway. Geographical coordinates of future dike could be N 45°55'37", E 59°40'15".

New Central Aral Sea which appeared in 2005 - 2006 after new Kokaral dam was built in

comparison to the other three parts of the Aral Sea described in the article is studied very poor. Fauna is not studied yet so more studies should be done as soon as possible.

Povzetek

Usoda četrtega največjega jezera na svetu do leta 1980, je sicer poznana tudi svetovni javnosti, veliko manj pa je bilo objav o spremembah ekološkega stanja, življenjskih združb in posebno gospodarsko pomembnih rib. Članek govori o kemizmu in slanosti, biodiverziteti planktonskih, bentoških in ribjih združb v različnih letih, ekološkem stanju in hidrološkem režimu ter možnosti ohranjanja in izboljšanja trenutnega stanja predvsem v severnem delu nekdanjega jezera, danes imenovanega Mali Aral.

Hidrološki režim jezera se je drastično začel spreminjati že kmalu po letu 1960, ko so Sovjeti z regulacijami preusmerili dve veliki reki, Syr Darjo in Amu Darjo, ki sta sicer polnili veliko Aralsko jezero. Razlog preusmeritve rek je bilo namakanje velikih površin posajenih z bombažem v nekdanjih sovjetskih republikah Uzbekistanu in Kazahstanu. Vode je jezeru je pričelo primanjkovati, gladina se je hitro zmanjševala in že v 80. letih prejšnjega stoletja se je veliko jezero razdelilo v dva bazena, severni Mali Aral in južni Veliki Aral s povsem drugim hidrološkim režimom. Slanost v teh bazenih se je izjemno povečala, marsikje je dosegla vrednost prek 100 g/L soli. To je bil začetek vrstnega siromašenja življenjskih združb, izumiranja nekaterih vrst, med njimi tudi gospodarsko pomembnih rib. Jezersko dno je postalo puščavsko območje, ki je vsebovalo tudi različne toksične snovi, posledice kemijskih in bioloških poskusov v času hladne vojne. Biotsko izjemno diverzitetni otok Barsakelmes je postal puščavski.

Raziskovalci so skušali rešiti preostanek severnega dela iz izgradnjo večjega nasipa in jezu imenovanega Kokaral v bližini delte Syr Darje, ki je preprečeval odtokanje vode v puščavo. Nivo vode se je nekoliko zvišal, predvsem pa se ni več zmanjševal. Največji učinki so se pokazali v slanosti, ta se je zmanjšala pod 10 g/L, zato so se vrnili mnogi nevretenčarji v planktonu in bentosu in z njimi tudi nekatere ribje vrste predvsem iz porečja in manjših jezer prispevnega območja.

Ribje populacije v Malem Aralu so danes v dobrem stanju in omogočajo tudi kontroliran in za nekatere vasi gospodarsko pomemben ribolov. Usoda južnega Velikega Arala pa je še naprej negotova, v suhem letu 2014 je v del te kotanje povsem presušil.

Planktonska združba je danes v Malem Aralu zmerno pestra, prevladujejo vrste iz skupin Rotifera, Cladocera in Copepoda, nekaj je protozojskih vrst. Pet vrst je zelo pogostih, med njimi rotatorija *Keratella quadrata* in *Brachionus plicatilis*, vodna bolha *Evdane anonyx* in dve vrsti kopepodov *Calanipeda aquaedulcis* in *Cyclops vicinus*. Poleg teh je manj pogostih še deset vrst in zelo redkih, ter še vedno ogroženih sedem vrst, med njimi nekatere, v drugih jezerih sicer zelo pogoste vrste, npr. *Keratella cochlearis*, *Filinia longistea* ter *Moina mongolica*.

Podobno pestra je tudi združba nevretenčarjev v bentosu, sestavljena predvsem iz skupin Polychaeta, Mollusca, Crustacea in Diptera. Zelo pogosta vrsta je *Chironomus plumosus*, ki je značilen predstavnik občasno anoksičnih jezerskih sedimentov in obenem dobro prilagojen na večjo slanost. Zelo pogost je tudi polihet *Hediste diversicolor*, ki kaže na slan tip celinskega vodnega telesa. Marsikje invazivna vrsta *Dreissena polymorpha aralensis* se v Malem Aralu sporadično pojavlja in je zelo redka.

Posebna pozornost je bila v naših raziskavah dana ribjim združbam. Kar 14 vrst gospodarsko pomembnih rib je danes v Malem Aralu, med njimi bi izpostavili ščuko *Esox lucius*, krapa *Carassius carassius gibelio*, aralsko vrsto rdečeočke *Rutilus rutilus aralensis* in *Platichthys flesus*, ki velja za eno najbolj okusnih rib. Manj pogosta je vrsta aralskega ploščiča *Abramis sapa aralensis*. Izlov dveh redih, sicer gospodarsko pomembnih vrst poher, aralske pohre *Barbus brachycephalus brachycephalus* in turkestanske pohre *B. capito conecephalus*, je strogo kontroliran. V jezeru je kar devet vrst gospodarsko nepomembnih rib, med njimi tudi endemne. Njihov obstoj ni vezan na ribištvo, ampak na ekološke razmere v malem Aralu.

Kazahstanska vlada si močno prizadeva vzdrževati pridobljeno ekološke stanje v Malem Aralu in ga celo izboljšati z nadgradnjo in obnovo jezu Kokaral ter gradnjo dveh novih pregrad. Veliko bolj negotova je usoda Velikega Arala, kjer

nižanje vodostaja in slanstostne razmere omogočajo preživetje le nekaterim zelo specializiranim nevretenčarskim vrstam.

Acknowledgements

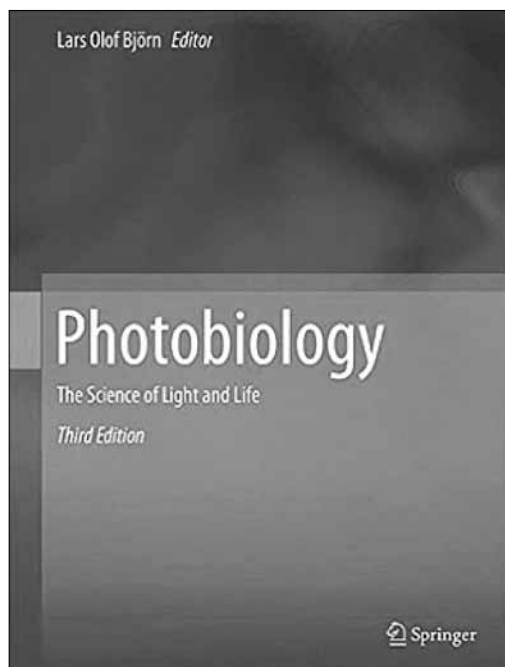
This paper is dedicated to the memory of Dr. Sandeep Joshi, Director of SERI (Shristi Eco-Research Institute) who passed away on 23rd September 2014 in Delhi.

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Photobiology, The Science of Light and Life

Fotobiologija, Znanost o svetlobi in življenju



Björn, L.O., 2015. Photobiology - The Science of Light and Life, 3rd ed. Springer, New York, Heidelberg, Dordrecht, London.

Svetloba, ki prihaja od Sonca, je ključnega pomena za življenje. Je gonilna sila procesov v biosferi, služi kot vir energije za organizme in jim zagotavlja informacije o okolju, v katerem živijo. Za rastline je svetloba pomembna iz več vidikov; svetlobo vežejo v procesu fotosinteze, svetloba tudi usmerja rast in razvoj rastlin od kalitve do cvetenja. Svetloba paima tudi toplotne in škodljive učinke na rastlinsko tkivo. Rastline zaznavajo kakovost, jakost, trajanje in smer svetlobe s pomočjo različnih molekul pigmenta, medtem ko pri živalih, svetloba večinoma omogoča informiranje preko

vida. Različne valovne dolžine svetlobe imajo različne učinke na organizme.

Knjigo Fotobiologija: Znanost o svetlobi in življenju je uredil Lars Olof Björn, profesor na Oddelku za biologijo Univerze v Lundu, in na Fakulteti za znanosti o življenju (South China Normal University). Lars Olof Björn ima dolgotletne izkušnje na področju fotobioloških raziskav. Njegovo raziskovalno delo je usmerjeno v fotobiologijo rastlin, zanima pa ga tudi vid živali, fotobiologija kože in bioluminiscenca. Profesor Björn ni samo urednik knjige, je tudi avtor in

soavtor 22 od 29 skupno poglavij. Ta, že tretja izdaja te knjige, se bistveno razlikuje od prvih dveh izdaj, predvsem glede slikovnega gradiva in obsega vsebine. Slikovni material je bogatejši kot v prejšnjih izdajah in vključuje tudi barvne fotografije in risbe. Dodanih je tudi nekaj novih poglavij: »Fotoaktivne beljakovine«, »Proteinski fotoreceptorji in njihov razvoj«, »Privzem svetlobe v procesu fotosinteze«, »Okužbe, odvisne od svetlobe«, in »Pomen ultravijoličnega sevanja v evoluciji«. Knjiga celovito obravnava naravo svetlobe, strukturne in funkcionalne prilagoditve organizmov ter njihove interakcije s svetlobo.

Uvodna poglavja knjige (od 1 do 7) povzemajo splošne vidike svetlobe: interakcije svetlobe s snovjo, merjenje svetlobe, svetloba kot orodje za znanstvene raziskave, ter lastnosti svetlobe v kopenskih in vodnih okoljih.

Drugi del knjige se ukvarja s strukturnimi in funkcionalnimi lastnostmi živih organizmov v odnosu do svetlobe. Vključuje različne ravni organizacije, od molekul do organizmov. Naslov osmega poglavja je »Akcijska spektroskopija v biologiji«. Akcijska spektroskopija je metoda, ki služi za identifikacijo vrste molekul, ki absorbirajo aktivno svetlobo. Akcijski spekter nam pove stopnjo fiziološke aktivnosti v odvisnosti od valovne dolžine svetlobe in je uporaben za določanje učinkov določenih valovnih dolžin na fiziološko aktivnost. To znanje je še posebej pomembno pri načrtovanju poskusov s svetlobnimi viri pod nadzorovanimi razmerami, vključno z raziskavami UV sevanja. Deveto in deseto poglavje podajata izsledke na področju bioluminiscence ter vsebujeta vsebine o pomenu svetlobnih spektrov v biologiji, predvsem za fotosintezo, za vid živali ter za interakcije med rastlinami in njihovimi opraševalci. Ti dve poglavji odgovarjata na vprašanja, zakaj so rastline zelene, kaj določa spektre pigmentov, kako so usklajeni pigmenti in vid in kako različne biotske strukture odbijajo in pršijo svetlobo, kot tudi kako nastane strukturna obarvanost, bele lise in presevnost pri listih.

Enajsto poglavje se osredotoča na fotoaktivne proteine, ki so odgovorni za vrsto različnih procesov v rastlinah kot je uravnavanje encimske aktivnosti, svetlobno regulirano delovanje ionske črpalke in ionskih kanalov, privzem svetlobe pri fotosintezi, fotorecepcija in bioluminiscenca. Dvanajsto in trinajsto poglavje govorita o zaz-

navanju svetlobne pri organizmih. V zoologiji se izraz fotoreceptor nanaša na celice, ki se odzivajo na svetlobo (na primer čepki v očeh), medtem ko se pri rastlinah beseda fotoreceptor nanaša na molekule pigmenta, ki absorbirajo svetlobo in povzročajo zaporedje različnih reakcij in s tem povezanih »informacij«. Posebna pozornost je namenjena reševanju posebnih »težav«, ki so se pojavljale v evoluciji živali, kot so oči v vodi, kromatična aberacija, oči dvoživk, oči insektov, oči z zrcalno optiko ter oči, ki imajo sposobnost skeniranja. Šestnajsto in sedemnajsto poglavje povzemata pomembne informacije o fotosintezi, procesu, v katerem rastline privzemajo sončno energijo. Šesnajsto poglavje podaja izsledke o razvoju fotosinteze in njenem vplivu na okolje, kar je pomembno z vidika človekovega vpliva na okolje ter z vidika rastlinske pridelave. Mehanizem privzema svetlobe v procesu fotosinteze je predstavljen v sedemnajstem poglavju. Naslov osemnajstega poglavja je: »Kako svetloba spremeni cirkadiani ritem?« To poglavje vsebuje pregled cirkadianih ritmov različnih organizmov, od gliv, cianobakterij, alg, semen rastlin, živali in ljudi in razlaga, kako uporabiti dnevne in letne cikle v svojo korist. Posebna pozornost je namenjena reševanju praktičnih težav, kot so izmensko delo, »jet lag«, in motnje spanja in njihovim vplivom na cirkadiani ritem pri človeku. V tem poglavju avtorji navajajo več kot 700 referenc. Osemnajsto poglavje ponuja vpogled v procese, povezane s fotomorfogenzo in fotoperiodizmom pri rastlinah. Rastline, kot sesilni organizmi, uporabljajo informacije o svojem okolju, ki temeljijo tudi na svetlobnih razmerah. To je zelo pomembno, saj svetloba ne vpliva le na fotosintezo, ampak tudi na kalitev, apikalno rast, rast internodijev in listov, proizvodnjo barvil, delovanje listnih rež, dormanco popkov, razvejanost in cvetenje. Svetloba služi tudi kot pomembna informacija v kombinaciji z magnetnim poljem. Dvajseto poglavje z naslovom »Svetlobno odvisni magnetni kompas«, razpravlja o magnetorecepciji, ki je odvisna od svetlobe z vedenjskega, fiziološkega, nevrobiološkega in biofizikalnega vidika. Enaindvajseto poglavje povzema mehanizme fototoksičnosti v povezavi s svetlobo. Fototoksičnost označuje spremembo lastnosti snovi, ki prvotno niso strupene, ampak postanejo strupene po izpostavljenosti svetlobi. To poglavje vključuje različna podpodročja, ki

razpravljajo o fototoksičnosti v rastlinski obrambi, o fototoksičnih zdravilih in kozmetiki ter o metabolnih procesih, ki lahko pripeljejo do fototoksičnih učinkov. Pomemben del tega poglavja je namenjen policikličnim aromatskim ogljikovodikom kot fototoksičnim snovem v vodnih okoljih, saj njihova strupenost nastopi po izpostavljenosti UV-B sevanju. Čeprav številna poglavja omenjajo tudi različne vidike UV-B sevanja, se dvainvajseto poglavje osredotoča na posledice tanjšanja ozonske plasti za življenje, medtem ko je sedemindvajseto poglavje zanimivo branje o vlogi UV sevanja pri razvoju življenja. Trindvajseto in štiriindvajseto poglavje sta tudi povezani z učinki UV-B sevanja. Triindvajseto poglavje poroča o fotobioloških in ekoloških vidikih vitamina D, medtem ko štiriindvajseto obravnava fotobiologijo človeške kože, vključno z imunosupresijo in nekaterimi fotosenzitivnostnimi motnjami. Splošno je znano, da lahko UV sevanje uničuje mikroorganizme, vendar je manj dokazov o njegovih pozitivnih učinkih na življenje. Kratko poglavje (poglavje številka 25) z naslovom »Svetloba poveča možnost

okužbe« navaja primere pri različnih organizmih. Šestindvajseto poglavje govori o bioluminiscenci, ki se pojavlja pri različnih skupinah organizmov, predvsem tistih, ki živijo v morju. Bioluminiscenca ima različne vloge kot so razmnoževanje, zaščita plena pred plenilci, pridobivanje hrane, zaščita pred reaktivnimi kisikovimi spojinami in popravljanje poškodb DNA. Poleg bioluminiscence to poglavje obravnava mehanizme nastajanja svetlobe, nadzora oddajanja svetlobe in načine človekovega izkoriščanja bioluminiscence. Zadnje poglavje povzema nekatere praktične vidike delovanja svetlobe, ki jih lahko uporabimo v procesu izobraževanja.

Ta obsežna knjiga je edinstven nabor znanja o svetlobi in življenju in prinaša obilico novosti na področju fotobiologije. Vsebuje številne primere z različnih ravni biološke organizacije. Knjiga je nepogrešljiv pripomoček za različne bralce, od študentov in univerzitetnih učiteljev, do znanstvenikov s področja biologije pa tudi iz drugih znanstvenih področij.

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1. slovensko posvetovanje mikroskopistov
Piran 2015

V organizaciji Slovenskega društva za mikroskopijo (SDM) je 18. in 19. maja 2015 v Piranu potekalo 1. slovensko posvetovanje mikroskopistov. Srečanja se je udeležilo 107 udeležencev, predavateljev, gostov in razstavljalcev, večina iz Slovenije in tudi nekaj iz drugih držav. V otvoritvenem nagovoru je predsednik SDM, prof. dr. Sašo Šturm, izpostavil ključna namena posveta - predstavitev različnih mikroskopskih metod in njihove uporabe na področju naravoslovnih znanosti, znanosti o materialih in v industriji ter oblikovanje priložnosti za boljše povezovanje mikroskopistov v Sloveniji. V plenarnem predavanju sta prof. dr. Jasna Štrus z Univerze v Ljubljani in prof. dr. Miran Čeh z Inštituta Jožef Stefan predstavila pregled razvoja mikroskopije v Sloveniji, zlasti elektronske mikroskopije. Tega so zaznamovali dosežki različnih slovenskih strokovnjakov, ki so delovali na področju razvoja opreme in tehnik priprave vzorcev za mikroskopske analize. Med ključnimi mejniki sta izpostavila pionirsko delo prof. dr. Aleša Strojnika, ki je leta 1955 v Ljubljani skonstruiral presevalni elektronski mikroskop. Za raziskave materialov je bil l. 1954 v Sloveniji nameščen prvi elektronski mikroskop, ki mu je l. 1965 sledila postavitve presevalnega elektronskega mikroskopa za biološke in medicinske raziskave.

V dvodnevem programu smo v nadaljevanju spremljali 5 vabljenih predstavitev, ki so odražala izrazito interdisciplinarnost srečanja. V okviru vabljenih predavanj so bile predstavljene naslednje vsebine: (i) vrstična tunelska mikroskopija in uporaba te metode za vizualizacijo površin z atomsko ločljivostjo ter za kontrolirano manipulacijo osnovnih gradnikov v raziskavah materialov in v nanotehnologiji; (ii) kombinirana uporaba fluorescenčne in elektronske mikroskopije za lokalizacijo membranskih proteinov *in vivo* ter na ultrastrukturnem nivoju, s poudarkom na študiju urotelijskih celic; (iii) avtomatska analiza neko-

vinskih vključkov v jeklu z vrstično elektronsko mikroskopijo, ki je bila razvita za sistematično sledenje proizvodnih procesov; (iv) napredna kvantitativna mikroanaliza z valovnodolžinsko disperzijsko spektroskopijo, prikazana na primeru študija kemijske sestave kristalov in (v) superločljivostna fluorescenčna mikroskopija, s poudarkom na mikroskopiji z vzbujenim praznjenjem emisije (mikroskopija STED: STimulated Emission Depletion), pri razvoju katere so sodelovali tudi raziskovalci iz Slovenije in jo uspešno uporabili v različnih raziskavah v celični fiziologiji.

Novosti na področju razvoja mikroskopije, raznolikost mikroskopskih metod in možnosti njihove uporabe v raziskavah, diagnostiki in industriji so predstavili udeleženci v 24 krajših predavanjih, ki so jim sledile zanimive razprave. Širok spekter predavateljev iz vrst študentov, uveljavljenih raziskovalcev in strokovnjakov je omogočal izmenjavo različnih izkušenj in znanj. Poleg tega je bilo na srečanje uvrščenih 31 posterskih predstavitev, ki so jih pripravili avtorji iz različnih univerz, inštitutov in drugih organizacij, pogosto v okviru medinstitucionalnih sodelovanj. Vsi prispevki so objavljeni v zborniku Slovensko posvetovanje mikroskopistov - Knjiga povzetkov (COBISS.SI-ID 3463247). K celovitejšemu pregledu tehnoloških novosti so prispevali tudi strokovni predstavniki proizvajalcev mikroskopov ter instrumentov za pripravo vzorcev, ki so organizirali informativne predstavitve in demonstracijo opreme na razstavnem prostoru.

Udeleženci smo se strinjali, da je tovrstno srečanje dobra priložnost za izmenjavo znanj med različnimi področji znanosti in dobrodošla spodbuda za interdisciplinarno sodelovanje na področju razvoja in uporabe mikroskopije v raziskavah, storitvenih dejavnostih, industriji in izobraževanju.

Nada Žnidaršič

Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani

INSTRUCTIONS FOR AUTHORS

1. Types of Articles

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

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

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

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

ASSOCIATION NEWS reports on the work of Slovene biology associations.

2. Originality of Articles

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

3. Language

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

4. Titles of Articles

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

5. Abstract

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

6. Keywords

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

7. Running title

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

8. Introduction

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

9. Illustrations and Tables

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

10. The quality of graphic material

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

11. Conclusions

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

12. Summary

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

13. Literature

References shall be cited in the text. If a reference work by one author is cited, we write Allan (1995) or (Allan 1995); if a work by two authors is cited, (Trinajstić and Franjić 1994); if a work by three or more authors is cited, (Pullin et al. 1995); and if the reference appears in several works, (Honsig-Erlenburg et al. 1992, Ward 1994a, Allan 1995, Pullin et al. 1995). If several works by the same author

published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc.: (Ward 1994a,b). If direct quotations are used, the page numbers should be included: Toman (1992: 5) or (Toman 1992: 5–6). The bibliography shall be arranged in alphabetical order beginning with the surname of the first author, comma, the initials of the name(s) and continued in the same way with the rest of the authors, separated by commas. The names are followed by the year of publication, the title of the article, the international abbreviation for the journal (periodical), the volume, the number in parenthesis (optional), and the pages. Example:

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

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

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

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

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

14. Format and Form of Articles

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

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

English title – (Times New Roman 14, bold)

Slovene title – (Times New Roman 14, bold)

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

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

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

Keywords in English – (Times New Roman 12)

Keywords in Slovene – (Times New Roman 12)

Running title – (Times New Roman 12)

Abstract in English (Times New Roman 12, title – Times New Roman 14 bold)

Abstract in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Introduction – (Times New Roman 12, title – Times New Roman 14 bold)
Material and methods – (Times New Roman 12, title – Times New Roman 14 bold)
Results – (Times New Roman 12, title – Times New Roman 14 bold)
Discussion – (Times New Roman 12, title – Times New Roman 14 bold)
Summary in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Figure legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, figure designation and figure title – Times New Roman 12 bold)
Table legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, table designation and table title – Times New Roman 12 bold)
Acknowledgements – (Times New Roman 12, title – Times New Roman 14 bold)
Literature – (Times New Roman 12, title – Times New Roman 14 bold)
Figures, one per page; figure designation indicated top left – (Times New Roman 12 bold)
Tables, one per page; table designation indicated top left – (Times New Roman 12 bold)
Page numbering – bottom right – (Times New Roman 12)

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