ABS

ACTA BIOLOGICA SLOVENICA

VOL. 55 ŠT. 2 LJUBLJANA 2012

prej/formerly BIOLOŠKI VESTNIK

ISSN 1408-3671
UDK 57(497.4)

izdajatelj/publisher
Društvo biologov Slovenije

ACTA BIOLOGICA SLOVENICA

VOL. 55 ŠT. 2 LJUBLJANA 2012

prej/formerly BIOLOŠKI VESTNIK

ISSN 1408-3671
UDK 57(497.4)

izdajatelj/publisher
Društvo biologov Slovenije

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2011 Vol. 55, Št. 2: 1–80

Acta Biologica Slovenica
Glasilo Društva biologov Slovenije – Journal of Biological Society of Slovenia

Izdaja – Published by
Društvo biologov Slovenije – Biological Society of Slovenia

Glavna in odgovorna urednica – Editor in Chief
Alenka Gaberščik, e-mail: alenka.gaberscik@bf.uni-lj.si

Tehnični urednik – Managing Editor
Gregor Zupančič, e-mail: gregor.zupancic@bf.uni-lj.si

Uredniški odbor – Editorial Board
Robert Zorec (SLO), Matija Gogala (SLO), Nada Gogala (SLO), Alenka Malej (SLO), Livio Poldini (I), Mark Tester (AUS), Nejc Jogan (SLO), Mihael J. Toman (SLO), Franc Janžekovič (SLO), Branko Vreš (SLO), Boris Sket (SLO), Franc Batič (SLO), Hubert Potočnik (SLO), Georg A. Janauer (A), Doekele G. Stavenga (NL)

Naslov uredništva – Adress of Editorial Office
Acta Biologica Slovenica, Večna pot 111, SI-1001 Ljubljana, Slovenija
http://bijh.zrc-sazu.si/abs/

Oblikovanje – Design
Žare Vrezec

ISSN 1408-3671 UDK 57(497.4)

Natisnjeno – Printed on: 2012
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 knjigo Republike Slovenije

Acta Biologica Slovenica je indeksirana v – is indexed in: CAB Abstracts, Ulrichsweb

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 3–11

The advantages of flow cytometry in comparison to fluorometric measurement in algal toxicity test

Prednosti merjenja s pretočno citometrijo v primerjavi s fluorimetričnim merjenjem v strupenostnih testih z algami

Tina Eleršek

National Institute of Biology, Večna pot 111, 10001 Ljubljana, Slovenia
correspondence: tina.elersek@nib.si

Abstract: Fluorometric measurements in algal toxicity tests are very commonly used as surrogate parameters for algal biomass. Although, fluorometry is a powerful technique, we have demonstrated that it is not suitable for determination of toxic effects of chemicals, which alter the fluorescence spectra. We recommend the flow cytometry as the best technique for detecting algal and/or cyanobacterial cell count and fluorescence per cell. Flow cytometry has many advantages: little volume of algal/cyanobacterial sample required, suitable also for little algal/cyanobacterial cells, distinction between live and dead cells. Furthermore, flow cytometry reveals the early changes in fluorescence spectra as a consequence of the specific chemicals presence or stress, even though the cell count is not yet affected (an early marker for ecotoxicology testing).

Keywords: algae, cyanobacteria, ecotoxicity test, fluorescence, flow cytometry

Izvleček: V strupenostih testih z algami se zelo pogosto izvajajo fluorometrične meritve kot nadomestni parameter za biomaso alg. Čeprav je fluorimetrija zelo uporabna tehnika, smo pokazali, da ni primerna za določanje strupenih učinkov kemikalij, ki spremenijo spekter fluorescence. Za določanje števila alg in/ali cianobakterij in njihove fluorescence na celico priporočamo uporabo pretočne citometrije. Uporaba pretočne citometrije za zaznavanje alg in/ali cianobakterij ima mnogo prednosti: majhni volumni vzorca alg/cianobakterij, primerno tudi za majhne celice alg/cianobakterij, razlikovanje med živimi in mrtvimi celicami. Poleg tega pretočna citometrija razkrije zgodnje spremembe v spektru fluorescence, ki so posledica prisotnosti specifičnih kemikalij oz. stresa, čeprav število celic še ni spremenjeno (zgodnji marker v ekotoksikoloških testiranjih).

Ključne besede: alge, cianobakterije, ekotoksikološki test, fluorescenca, pretočna citometrija

Introduction

Many toxicity tests and standards are used to describe the water quality. Frequently algal toxicity tests are used because of their relative toxicity simplicity and low price (ISO 8692, 2012; OECD TG 201, 2011). The purpose of these tests is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria. It is not used strictly for toxicity testing of pure substances but also for mixtures of chemicals, rapid screening of waste water, etc. The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure time period. From the average specific growth rate recorded in a series of test solutions, the concentration bringing a specified inhibition of growth rate (e.g. 50%) is determined and expressed as the ECx (e.g. EC50). In ecotoxicity tests the EC10 is used very often to replace LOEC/NOEC values (Shieh at al. 2001, Warne and Rick 2008).

The principle of this test is based on exponentially growing algae and/or cyanobacteria exposed to the test substance in batch cultures over a period of normally 72 hours. In spite of the relatively brief test duration, effects over several generations can be assessed. As a result the reduction of growth in a series of algal cultures exposed to various concentrations of a test substance is observed. The response is evaluated as a function of the exposure concentration in comparison with the unexposed control cultures. For full expression of the system response to toxic effects, unlimited exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time is provided to the cultures (OECD TG 201, 2011).

Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. Algal biomass is defined as the dry weight per volume, e.g. mg algae/litre test solution. The algal biomass in each flask is determined at least daily during the test period. However, dry weight is difficult to measure and therefore surrogate parameters are used: cell counts (most often), cell volume, fluorescence, optical density, etc. A conversion factor between the measured surrogate parameter and biomass should be known (OECD TG 201, 2011). Measurement of biomass is done by manual cell counting by microscope or an electronic particle counter (by cell counts and/or biovolume). Alternative techniques e.g. flow cytometry, *in vitro* or *in vivo* chlorophyll fluorescence (Mayer at al. 1997; Slovacey and Hanna 1997), or optical density can be used if a suitable correlation with biomass can be demonstrated over the range of biomass occurring in the test.

There are many occasions where microscope counting for biomass measurements is very complicated because of the shape, granularity or other specific features of the cells; e.g. small cells of cyanobacteria *Synechococcus leopoliensis* are distributed on many different vertical levels in the counting chamber. Therefore it is impossible to count them accurately under the light microscope. Standards recommend electronic particle counters equipped for counting particles down to a size of approximately 1 µm, but also *in vitro* fluorometric measurements are applicable.

Fluorimetric measurements in algal toxicity test can be very useful, since algae and cyanobacteria possess photosynthetic pigments. The fluorometer emits an excitation light at a particular wavelength (approximately 430 to 470 nm) that causes the chlorophyll (CHL) *a* to fluoresce at another wavelength (approximately 650 to 700 nm). The concentration of CHL *a* is proportional to the amount of CHL *a* fluorescence emitted. All algae contain CHL *a*, but there are many kinds of algae and especially cyanobacteria with distinctly different accessory pigments that fluoresce at different wavelengths. Some accessory pigments such as CHL *b* and CHL *c* fluoresce within the same wavelength and may influence the CHL *a* determination (Hambrook-Berkman and Canova 2007).

In this article we demonstrate the method for accessing the biomass with flow cytometry, which gives us even more information than "classic" fluorescence measurements or electronic particle counter. In our study only 70 µl of algal/cyanobacterial sample is used for flow cytometry measurement and we get information about two parametrs simultaneously: cell count and fluorescence spectra. Furthermore, we demonstrate that some chemicals alter the fluorescence spectra of algae. In these cases only flow citometry measurements give us accurate estimation of biomass in contrast to fluorometric measurements, which are not applicable at all.

Materials and methods

Cyanobacterial and algal species

Algal growth inhibition test were conducted according to the OECD TG 201 (2011). Two phytoplankton species were selected for all the experiments: green algae *Pseudokirchneriella subcapitata* SAG 61.81 algae collection and cyanobacteria *Synechococcus leopoliensis* SAG 1402-1 (Fig. 1), from the SAG (Sammlung von Algenkulturen Univerziätt Göttingen).

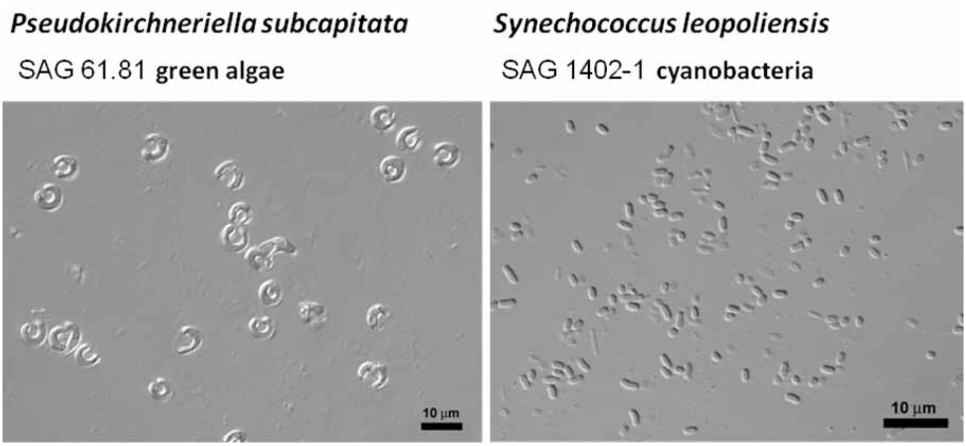


Figure 1: Green algae (left) and cyanobacteria (right) used for the algal toxicity tests in this study.
Slika 1: Zelene alge (levo) in cianobakterije (desno), ki smo jih uporabljali za strupenostne teste z algami v tej študiji.

Test design

Algal or cyanobacterial strains were cultivated for several generations in a defined medium containing a range of 5–6 concentrations of the test sample and inoculums of exponentially growing cells. The test batches were incubated for a period of 72 h during which the cell density in each test solution was measured at least every 24 h. Inhibition was measured as a reduction in specific growth rate relative to control cultures grown under identical conditions.

Test was performed in glass Erlenmeyer flasks at least three times in triplicates. The culture volume at the beginning was set to 20 ml. Biomass density at the beginning of the test was within the interval $5*10^3$-$5*10^4$ cells/ml for *Pseudokirchneriella subcapitata* and $5*10^4$-$5*10^5$ cells/ml for *Synechococcus leopoliensis*, in order to achieve stabile exponential growth. The composition of the growth medium is defined in OECD TG 201, Annex 3 (2011). The duration of all tests was 72 h. The flasks were incubated at 24°C under cooled lamps Sylvania GRO-Lux F 18 W/GRO-T8 with continuous light (24h) and shaking (GFL 3017, Germany). Light intensity at 80-120 $\mu Em^{-2}s^{-1}$ was determined with Delta-T Logger (Delta-T Devices Ltd., UK) equipped with QS Quantum sensor. Small fractions were taken away daily for microscopic examination, fluorescence measurement or flow cytometry (not more than 1 ml).

Test substances

Three cytostatics were tested: cisplatin (CP) from Sigma-Aldrich (Seelze, Germany), etoposide (ET) and imatinib mesylate (IM) from Santa Cruz Biotechnology, Inc., US. All cytostatics and their containers were disposed as hazardous waste. Concentrated stock solutions were prepared, stored in refrigerator and used within 2 months. Stock solution for IM was aliquoted and stored in a freezer for maximum 2 months.

Biomass determination

Microscopic examination of algae and/or cyanobacteria samples, closely observing the cell morphology changes, was done at the beginning and at the end of each experiment. For the determination of biomass samples of algae and/or cyanobacteria were analysed with: (a) cell counting under inverted light microscope, (b) flow cytometry and (c) *in vitro* fluorescence determination:

(a) Cells were closely examined for visual cell abnormalities and counted under a light inverted microscope (Nikon Eclipse TE300) using Bürker Türk haemocytometer. The microscope was equipped with a digital camera. Cells were measured with software Lucia (System for Image Processing and Analysis LUCIA 4.6, Laboratory Imaging Ltd.).

(b) Flow cytometer (BD FACSCalibur, USA) was used for the assessment of cell count. Laser sensors FL3 (with Band Pass Filter at 620nm) and FL4 (with Band Pass Filter at 675nm) were used for *P. subcapitata* and FL4 for *S. leopolinesis*. The volume of sample was 70 µl. Flow rate was set to 65 µl/min. The evidence of correlation between cell concentration using different methods was evident from the calibration curve – "flow cytometer cell count" vs. "microscopically determined cell count" for more than 100 simultaneous measurements/counts (data not shown). Furthermore, we have simultaneously count the external control (red and blue microsphaeres from EQAT – External quality assessment trials Phytoplankton, red = external control for cyanobacteria *S. leopoliensis*, blue = external control for green algae *P. subcapitata*).

(c) Changes in chlorophyll fluorescence were followed in the red (l_{em} 680 nm) using l_{ex} 440 nm, where the chlorophyll *a* has a significant absorption peak (Lee at al. 1994).

Before measuring fluorescence, samples were dark-adapted for approx. two hours. The fluorescence was measured using Synergy Mx Monochromator-Based Multi-Mode Microplate Reader; excitation and emission slit widths were 20 nm, shaking before measurement was: 5 sec at 24°C. The measurement were normalised to the control at the beginning of the experiment, since the cell count in controls varies between different experiments.

Statistical evaluations

Data were analysed (software Prism 5, GraphPad Inc.) as measurement from each individual flask (pooled together) rather than means of replicates, in order to extract as much information from the data as possible (suggested in OECD TG 201, 2011). For dose-response graphs the nonlinear regression model was used ("log(inhibitor) vs. response – variable slope", no constrains). For graphs with % of inhibition vs. concentration of cytostatic (and EC10, EC20 and EC50) assessment, the nonlinear regression model was used ("log (inhibitor) vs. response – variable slope", with constrains for shared value for bottom and top).

Results and discussion

The fluorometric measurements are based on properly selected excitation and emission wavelength to detect the fluorochromes of algae and/or cyanobacteria. Green alga *P. subcapitata* and cyanobacterium *S. leopoliensis* demonstrated weak correlation between fluorometric measurements and microscopically determined cell count for more than 160 simultaneous measurements/counts (calibration curves on Fig. 2). The average correlation factor was quite low 0.68. Since the cell density may influence the calibration curve, we have divided results into "low and high density" cultures (compare A and B on Fig. 2). There is no significant influence of cell density on fluorescence in the frame of exponential growth phase.

On the other hand, flow cytometry uses the principles of light scattering, light excitation, and emission of fluorochrome molecules to generate specific multi-parameter data from particles and cells in the size range of 0.5 µm to 40 µm diameter. Flow cytometry in our study showed very consistent results with the "standard" red and blue microsphaeres, when compared to microscope counting (Fig. 3).

One unique feature of flow cytometry is that it measures fluorescence per cell or particle. This is in contrast with spectrophotometry in which the percent of specific wavelengths of light is

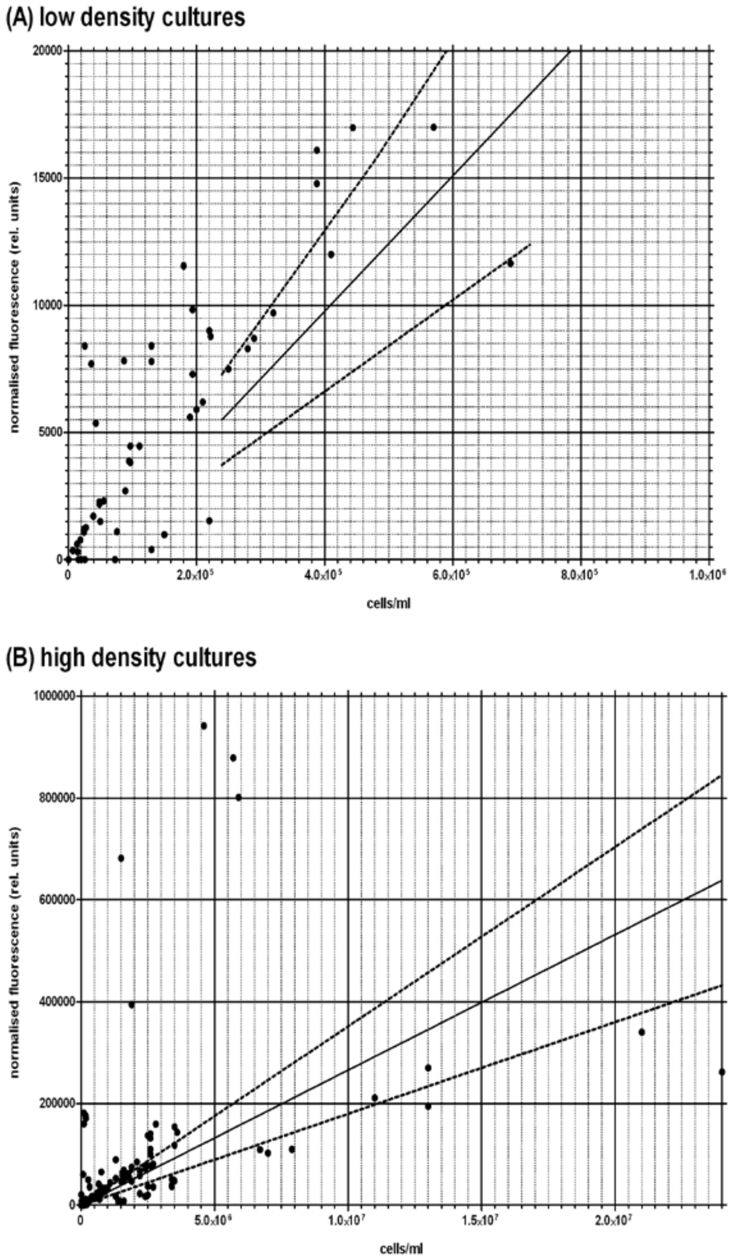


Figure 2: The cell concentration on the calibration curve - the fluorimetric measurements (axis y) and microscopically determined cell count (axis x) show no evident correlation in low (A) or high (B) density cultures of P. subcapitata.

Slika 2: Koncentracija celic na umeritveni krivulji – fluorometrične meritve (os y) in mikroskopsko določeno število celic (os x) ne kaže očitne korelacije niti pri nizki (A) niti pri visoki (B) gostoti kultur P. subcapitata.

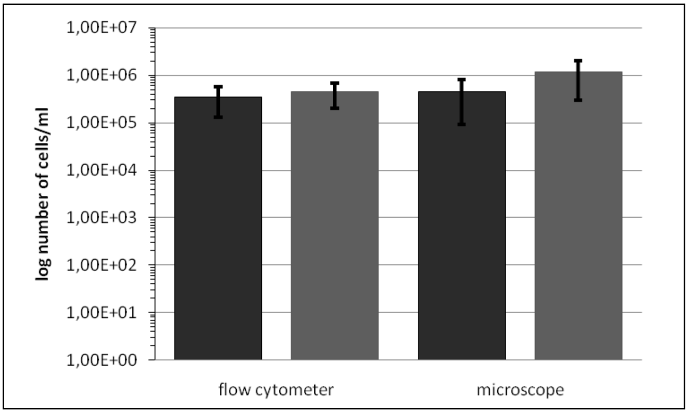


Figure 3: The comparison of counting method with flow cytometer vs. microscope counting of the external control (microsphaeres, EQUAT, black colour = control for cyanobacteria *S. leopoliensis*, gray colour = control for green algae *P. subcapitata*).

Slika 3: Primerjava števne metode s pretočnim citometrom vs. mikroskopsko štetje zunanje kontrole (kroglice EQUAT, črna barva = kontrola za cianobakterijo *S. leopoliensis*, siva barva = kontrola za zeleno algo *P. subcapitata*).

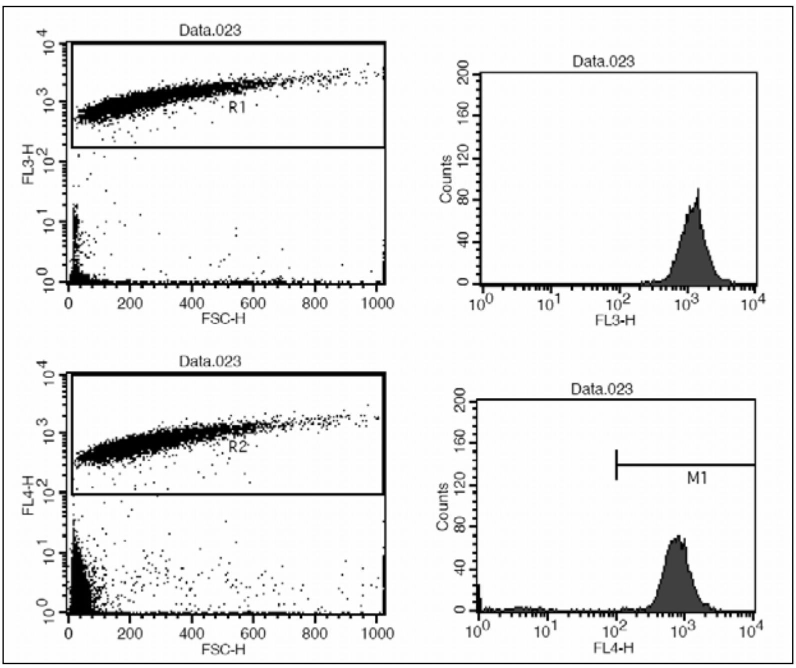


Figure 4: Flow cytometry dot-blot histogram (left) of green algae P. subcapitata and fluorescence spectra (right) of gated events (for gate R1 we used laser sensor FL3 = top and for gate R2 laser sensor FL4 = bottom, counting interval = M1).

Slika 4: Točkovni histogram pretočnega citometra (levo) zelene alge P. subcapitata in spekter fluorescence (desno) uokvirjenih dogodkov (za okvir R1 smo uporabili laserski senzor FL3 = zgoraj in za okvir R2 laserski senzor FL4 = spodaj, interval štetja = M1).

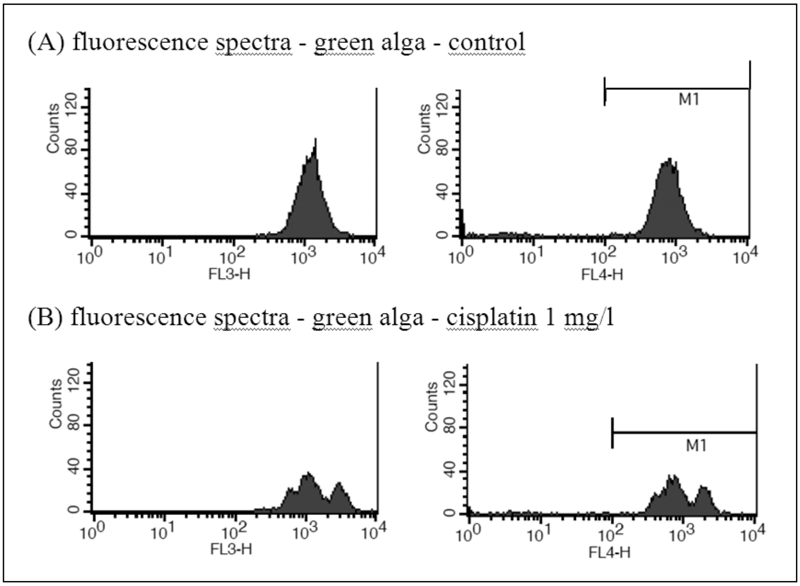


Figure 5: Flow cytometry histogram (with laser sensor FL3 = left and FL4 = right, counting interval = M1) showing shift in fluorescence spectra of green algae P. subcapitata 24 hours after the addition of cisplatin at 1 mg/l (B) in comparison to control (A), even though the cell count is not yet affected.

Slika 5: Histogram pretočnega citometra (z laserskim senzorjem FL3 = levo in FL4 = desno, interval štetja = M1) kaže spremembo v spektru fluorescence zelene alge P. subcapitata 24 ur po dodatku cisplatina v koncentraciji 1 mg/l (B) v primerjavi s kontrolo (A), kljub temu, da učinek na koncentracijo celic še ni bil opazen.

measured for a bulk volume of sample. To study toxic changes in *P. subcapitata* and *S. leopoliensis* with flow cytometry we have detected the size and granularity (two parameter histogram or Dot Plot, Fig. 4 – left) and fluorescence spectra of gated events (Fig. 4 – right, Fig. 5). Flow cytometer revealed that tested cytostatic cisplatin (CP) altered the fluorescence spectra in green alga, even though the cell count was not affected. Under the same conditions no changes in fluorescence spectra were found in the case of imatinib (IM) or etoposide (ET), data not shown. The fluorescence spectra of selected algae and cyanobacteria may change as a consequence of chemical action, as proved with the flow cytometry in our study with cytostatic cisplatin (Fig. 5) and other chemicals (e.g. Regel at al. 2002). Therefore, we considered fluorometric technique not precise enough and for this reason all the subsequent testing was done with the use of flow cytometry, which is becoming more and more popular (Hashemi at al. 2011). Also fluorometric measurements are quite popular in algal toxicity test (Mayer at al. 1997, Berden-Zrimec at al. 2007, Nguyen-Ngoc at al. 2009), but some chemicals may shift or move the fluorescence peak and one should be very cautious with the interpretation of fluorometric measurement at specific wavelength(s).

There are many advantages of the use of flow cytometer for algal/cyanobacterial detection. As little as 70 µl of algal/cyanobacterial suspension (+70 µl of filtrated distilled water) is enough to accurately determine the cell count and fluorescence spectra in our exponentially growing cultures. Flow cytometry is suitable also for little algal/cyanobacterial cells, which can not be precisely counted under the microscope. It counts only cells which possess active chlorophyll pigments, so it can be used to distinguish between live and dead cells. Flow cytometry reveals the changes in fluorescence spectra as a consequence of specific chemical presence or stress, even though the cell count is not yet affected. This is the reason why this method can be used for early detection of chemicals which alter the fluorescence spectra as an early marker for ecotoxicology testing.

Povzetek

Fluorometrične meritve v strupenostnih testih z algami se zelo veliko uporabljajo kot nadomestni parameter za biomaso alg. Kljub temu, da je fluorimetrija zelo uporabna tehnika, bi radi izpostavili pomanjkljivost pri uporabi za določanje strupenih učinkov kemikalij, ki spremenijo spekter fluorescence. Vrh fluorescence se namreč spremeni oz. premakne v druge valovne dolžine, zato detekcija pri določenih valovnih dolžinah (s »klasično fluorometrično metodo«) ni dovolj natančna in posledično ne moremo izračunati pretvorbenega faktorja med merjenim nadomestnim parametrom in biomaso. Pretočno citometrijo priporočamo za detekcijo biomase alg, saj ima mnogo prednosti kot npr. sočasno merjenje števila celic in fluorescence na celico.

Summary

Fluorometric measurement in algal toxicity tests are very commonly used as surrogate parameters for algal biomass. Although, fluorometry is a powerful technique, we would like to point out a disadvantage, when used for determination of toxic effects of chemicals, which alter the fluorescence spectra. Since the fluorescent peak is shifted or moved to other wavelength, detection at specific wavelength (with “classic fluorimetric methods”) is not precise enough and the conversion factor between the measured surrogate parameter and biomass can not be determined. We recommend the flow cytometry for detecting algal biomass, since it has many advantages, including synchronous measurement of cell count and fluorescence per cell.

Acknowledgements

The work was done in the frame of FP7/2007–2013 project under grant agreement number 265264 – CytoThreat (Fate and effects of cytostatic pharmaceuticals in the environment and the identification of biomarkers for and improved risk assessment on environmental exposure). Special acknowledgement goes to Dr. Mihael Bricelj for the leadership and valuable ideas. Many thanks to Katja Kološa, for professional and technical assistance with the flow cytometer.

References

Berden-Zrimec, M., Drinovec, L., Zrimec, A., Tišler, T., 2007. Delayed fluorescence in algal growth inhibition tests. Central European Journal of Biology, 2 (2), 169–181.

Franklin, N.M., Stauber, J.L., Lim, R.P., 2004. Development of multispecies algal bioassays using flow cytometry. Environ. Toxicol. Chem., 23, 1452–1462.

Hambrook-Berkman, J.A., Canova, M.G., 2007. Chapter 7 Algal biomass indicators, version 1.0 (8/2007) U.S. Geological Survey TWRI Book 9, pp 1–86.

Hashemi, N., Erickson, J.S., Golden, J.P., Jackson, K.M., Ligler F.S., 2011. Microflow cytometer for optical analysis of phytoplankton. Biosensors and Bioelectronics, 26, 4263– 4269.

ISO 8692 Water quality – Fresh water algal growth inhibition test with unicellular green algae, ISO 2012

Lee, T., Tsuzuki, M., Takeuchi, T., Yokoyama, K., Karube, I., 1994. *In vivo* fluorimetric method for early detection of cyanobacterial waterblooms. Journal of Applied Phycology, 6, 489–495.

Mayer, P., Cuhel, R., Nyholm, N., 1997. A simple *in vitro* fluorescence method for biomass measurements in algal growth inhibition tests. Water Research**,** 31 (10), 2525–2531.

Nguyen-Ngoc, H., Durrieu, C., Tran-Minh, C., 2009. Synchronous scan fluorescence of algal cells for toxicity assessment of heavy metals and herbicides. Ecotoxicology and Environmental Safety, 72 (2), 316–320.

OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test OECD. Published by OECD Publishing, 2011.

Regel, R.H., Ferris, J.M., Ganf, G.G., Brookes, J.D., 2002. Algal esterase activity as a biomeasure of environmental degradation in a freshwater creek. Aquatic Toxicology, 59, 209–223.

Shieh, J.N., Chao, M.R., Chen, C.Y., 2001. Statistical comparisons of the no-observed-effect concentration and the effective concentration at 10% inhibition (EC10) in algal toxicity tests. Water Science and Technology, 43 (2), 141–146.

Slovacey, R.E., Hanna, P.J., 1997. In vivo fluorescence determinations of phytoplancton chlorophyll. Limnology and Oceanography, 22 (5), 919–925.

Stauber, J.L., Franklin, N.M., Adams M.S., 2002. Applications of flow cytometry to ecotoxicity testing using microalgae. Trends in Biotechnology, 20 (4), 141–143.

Warne, M.S.J., Rick, V.D., 2008. NOEC and LOEC data should no longer be generated or used. Australasian Journal of Ecotoxicology, 14, 1–5.

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 13–24

Elemental composition of wheat, common buckwheat, and tartary buckwheat grains under conventional production

Vsebnosti elementov v zrnju pšenice, navadne in tatarske ajde s polja s konvencionalno pridelavo

Lea Orožen[a], Katarina Vogel-Mikuš[a], Matevž Likar[a], Marijan Nečemer[b], Peter Kump[b], Marjana Regvar[a]*

[a]Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 SI-Ljubljana, Slovenia
[b]Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia
*correspondence: marjana.regvar@bf.uni-lj.si

Abstract: The elemental composition of cereal and pseudocereal grain is believed to significantly affect the portions of the minerals supplied for particular human populations. Therefore, care needs to be taken to improve the availability of the essential elements and to decrease unwanted metal accumulation in edible plant parts. In the present study, we have investigated the element accumulation in the grain of wheat (*Triticum aestivum* L.), common buckwheat *(Fagopyrum esculentum* Moench), and tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.), harvested from the same field under conventional grain production. Soil and grain element compositions were analysed using energy dispersive X-ray fluorescence spectrometry and total reflection X-ray fluorescence spectrometry. The wheat grain shows significantly higher ($p < 0.05$) higher element concentrations than both of the buckwheat species tested. The contents of elements in 100 g grain were higher than the concentrations listed in the literature for wheat and buckwheat flours, which indicates significant losses of elements during milling and polishing. Concerns are raised due to the high and unwanted metal accumulation in wheat and buckwheat. The data indicate that both of these buckwheat species accumulate less metal contaminants when compared to wheat.

Keywords: dietary reference intake; energy dispersive X-ray fluorescence spectrometry, *Fagopyrum esculentum*, *Fagopyrum tataricum*, metals, minerals, trace elements, *Triticum aestivum*; total reflection X-ray fluorescence spectrometry.

Izvleček: Žita in psevdožita so pomemben vir mineralnih elementov v prehrani določenih svetovnih populacij, zato je pomembno izboljšati elementno sestavo žit in zmanjšati vnos neželenih kovin v užitne dele rastlin. V raziskavi smo proučevali akumulacijo mineralnih elementov v zrnju pšenice (*Triticum aestivum* L.), navadne ajde (*Fagopyrum esculentum* Moench) in tatarske ajde (*Fagopyrum tataricum* (L.) Gaertn.) s polja s konvencionalno pridelavo. Elemente v tleh in zrnju smo analizirali z energijsko disperzijsko rentgensko fluorescenčno spektroskopijo, oziroma rentgensko fluorescenčno spektroskopijo s popolnim odbojem. Zrnje pšenice je imelo višje ($p < 0.05$) koncentracije elementov od zrnja navadne in tatarske ajde. Vsebnosti mineralnih elementov v 100 g zrnja pšenice in ajde so bile višje od vsebnosti navedenih v literaturi za moko pšenice in ajde, kar kaže na izgubo elementov med postopki za pripravo moke. Zaskrbljujoče so visoke vsebnosti nekaterih nezaželenih kovin v pšenici in ajdi. Iz rezultatov je razvidno, da navadna in tatarska ajda v zrnju akumulirata manj nezaželenih kovin kot pšenica.

Ključne besede: Energijska disperzijska rentgenska fluorescenčna spektroskopija, *Fagopyrum esculentum*, *Fagopyrum tataricum*, elementi v sledeh, kovine, minerali, prehranski referenčni vnosi za odrasle, *Triticum aestivum*, rentgenska fluorescenčna spektroskopija s popolnim odbojem.

Introduction

Wheat is one of most widely cultivated crops in developing countries, where up to 70% of the daily energy demands of the people are covered by its products (Cakmak et al. 2010). The biochemical characteristics of cereal grain tissues (e.g., starch , ferulic, coumaric and phytic acids, alkylresorcinols) are the major determinants of the quality of the products that are to be prepared from these ingredients (Hemery et al. 2009). Furthermore, grain represents an important source for the supply of the 22 mineral microelements in particular populations, and therefore their mineral content and element bioavailability are of paramount importance (White and Broadley 2005). Slovenian wheat and buckwheat flours do not differ significantly in their total starch composition (Kreft et al. 1998). Significant differences between wheat and buckwheat flours have, however, been reported for essential mineral nutrient concentrations (Ikeda et al. 2006).

Only the bioavailable soil fraction of the essential elements in soil can be accessed by plants. As well as their natural deposition and bedrock weathering, the concentrations of bioavailable elements in the soil are also influenced by soil pH, organic matter content, and cationic exchange capacity, which control the solubility, and consequentially the availability, of elements and their uptake into plants (Marschner 1995; Moreno et al. 1996). Mineral nutrient concentrations in grain are successively determined by physiological processes, including nutrient uptake, xylem loading, remobilisation from leaves, and deposition in the seed structures. The rates of mineral nutrient uptake and remobilisation primarily depend on the presence and activity of particular element transporters, which results from the expression patterns of their genes (Waters and Sankaran 2011). Consequently, the partitioning of mineral nutrients between plant organs (with the exception of P) is typically characterised by lower mineral nutrient concentrations in seeds, when compared to leaves (Tyler and Zohlen 1998). Significant variations have also been observed between and within plant species. Recently, a genetic improvement of yield that resulted in mineral micronutrient dilution was recognised as one of the factors driving the variability in nutrient concentrations of wheat grain (McKevith 2004; Zhao et al. 2009).

Large quantities of fertilisers are routinely applied to crops, to supply adequate N, P and K levels for optimal plant growth and yield. The commercially available products used for this purpose, however, frequently contain heavy-metal contaminants. Furthermore, the use of pesticides adds to the unintentionally supplied metals in agricultural crops (Adriano 2001). It is estimated that crop production of 60% of the cultivated soils worldwide is hampered by either nutrient deficiency or toxicity (Cakmak 2002). Consequently, the accumulation and bioavailability of heavy metals in crop plants is of increasing concern, due to food safety issues and potential health risks (Wang et al.2009). Legislative government acts and guidelines (Ur. list RS 68/1996; US EPA 2002; NSF/ANSI 2003) have been aimed at determining the acceptable levels of elements in soils and plant parts. Thus, as well as a growing demand for sustainable crop production with optimised element contents, systematic monitoring procedures are needed to correctly address the constant threat of unwanted metal accumulation in the food chain.

The main goals of the present study were therefore: (i) to assess the biomass and element composition of selected wheat, common and tartary buckwheat grain produced in Slovenia; (ii) to estimate the soil element availability for selected species (grown in the same field) using bioconcentration factors (BCFs); (iii) to evaluate the element concentrations in grain with regard to the Dietary Reference Intake (USDA DRI 2004) levels for the intake of mineral nutrients; and (iv) to screen for potentially hazardous element accumulation in wheat and buckwheat grain and to compare these with the maximal tolerable levels of elements in plants used for food, according to the literature.

Materials and Methods

Experimental design

Wheat (*Triticum aestivum* L.) cv. Remus, common buckwheat (*Fagopyrum esculentum* Moench) cv. Darja, and tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) cv. domestic population of Luxembourg, were sown on two plots (6 m × 24 m) in an experimental field within a wheat and buckwheat producing agricultural area in Moravče, near Ljubljana, Slovenia (382.6 m above sea level, 46°8’34.75”N, 14°41’54.71”E). For each of the plots, the grain were sown in a 6 × 3 factorial experimental design (4 parallels/species). The individual seeding beds of 2 m × 2 m were separated by 1-m-wide belts. On average, 200 grains/m^2 were sown for wheat, and 400 grains/m^2 for buckwheat. The wheat grain were sown in April 2011 and harvested when they reached maturity, which was 94 days after sowing. The common and tartary buckwheat varieties were sown in May 2011, and harvested at maturity at 136 days and 128 days after sowing, respectively. Grain yield estimates calculated from the average biomass of grain per plots were: 112.5 kg/ha for wheat, 969.7 kg/ha for common buckwheat, and 751.1 kg/ha for tartary buckwheat.

Soil samples collected prior to the experiment were examined at the Agricultural Institute of Slovenia, and these demonstrated that the soil was moderately acidic (pH 6.4; ISO 10390), with an organic matter content of 4.9% (ISO 14235). The concentrations of the biologically available nutrients were as follows: 89 mg/100 g P_2O_5 (MET/Z/016); 70 mg/100 g K_2O (MET/Z/017); 21 mg/100g Mg (MET/Z/018); and 7.7 mg/kg NO_3-N (RQ – flex).

Rhizosphere soils

At harvest, the rhizosphere soil of each plant was collected, passed through a 1-mm sieve, and dried at 50 °C for 24 h. Energy dispersive X-ray fluorescence spectrometry (EDXRF) was used to determine the total element concentrations of the soil. The concentrations of the biologically available elements were determined using total reflection X-ray fluorescence spectrometry (TXRF), at the Jožef Stefan Institute.

For the determination of the total soil element concentrations, 250 mg soil per sample was powdered and compressed into pellets using a pellet die and a hydraulic press. These pellets were analysed using a EDXRF spectrometer. An annular radioisotope excitation source of Fe-55 and Cd-109 from Isotope Products Laboratories U.S.A. was used as the primary excitation source. The emitted fluorescence radiation was measured by an energy dispersive X-ray spectrometer composed of a Si(Li) detector (Canberra), a spectroscopy amplifier (Canberra M2024), an analog-to-digital converter (Canberra M8075), and a PC-based multichannel analyser card (S-100, Canberra). The energy resolution of the spectrometer was 175 eV at 5.9 keV. The estimated uncertainty of the analysis was from 5% to 10% (Nečemer et al. 2008).

Analysis of the bioavailable elements was performed according to Baker et al. (1994). In brief, 5 g dried soil was suspended in 25 ml 1 M ammonium acetate solution (pH 7) and shaken for 2 h at 23 °C. The extracts were filtered through 0.4 μm Millipore membrane filters. For TXRF analysis, the 10 ml soil extracts were spiked with 100 μl Ga standard solution (Sigma-Aldrich), as an internal standard. From these spiked solutions, 10 μl was applied twice to a quartz sample carrier plate and dried in a desiccator. The X-ray spectrometer was based on a Si(Li) detector (Princeton Gamma Tech.), with a resolution of approximately 145 eV at 5.9 keV, an integrated signal processor (M 1510, Canberra), and a PC-based multichannel analyser card (S-100, Canberra). A Seifert X-ray generator (Rich Seifert & Co) model ISO-DEBYFLEX 3003 (60 kV, 80 mA), and a Mo anode fine focus

X-ray tube (FK 60-04, Rich. Seifert & Co.) were used. The estimated uncertainty of the element analysis was between 5% and 10% (Nečemer et al. 2008). The sensitivity level, however, strongly depended on the atomic number of the element; although it extended down to a few ppb (1 ng/g dry weight) for the TXRF (Schwenke and Knoth 1993), compared to a few ppm (1 mg/g dry weight) for the heavier elements using EDXRF (Vogel-Mikuš et al. 2010).

Grain analysis

The mature dry harvested grain of the wheat and common and tartary buckwheat were weighed and ground in liquid nitrogen using a porcelain mortar and pestle. The element concentrations were then determined using TXRF. In brief, 100 mg grain powder was placed in Teflon vessels and spiked with 3 ml 65% HNO_3. A CEM MARS 5 microwave oven (Matthews, NC, USA) was used for chemical digestion of the grain samples. The vessels were gently shaken to wet the samples with the acid, and then covered using vessel caps and put onto the rotor plate. The digestion procedure was performed using the following temperature programme: ramp up to 180 °C over 20 min, hold at 180 °C for 20 min, and cool over 20 min. Upon cooling, the Teflon vessels were vented and the vessel caps removed (Nečemer et al. 2008). The element compositions in these grain samples were then determined by TXRF.

The BCFs of the elements were calculated as [(total grain concentration)/(bioavailable soil concentration)] (Baker 1981; Mensch et al. 2010).

To define the contributions of the elements to the daily recommended dietary allowances, the relative proportions of the elements in 100 g grain with respect to the Dietary Reference Intakes (DRIs) were calculated as follows: Element content in 100 g grain (mg/100g)/DRI (mg/d). These data are expressed as percentages, in terms of the Recommended Dietary Allowances (RDAs), the Adequate Intake Levels (AIs) and/or the Tolerable Upper Intake Levels (ULs) (USDA DRI 2004).

The total concentrations of phosphorous, were measured using a UV-VIS spectrophotometer (Shimadzu UV-1800) at the Biotechnical Faculty of the University of Ljubljana (Olsen et al. 1982). The grain were mineralised by microwave-assisted wet digestion, as described above. After digestion, 1 ml digest was spiked with 2 ml MoV reagent and 7 ml 0.2% HNO_3. Absorption of the samples was measured at 400 nm. A standard phosphorous solution (Sigma-Aldrich) was used to prepare the calibration curve.

Statistical analysis

Statistical analysis of the element concentrations was performed using Statistica Statsoft 8.0 software. One-way ANOVA and *post-hoc* Duncan's tests were used to calculate the differences in the grain biomass, the concentrations of the elements in the soil and grain, and the BCFs ($p < 0.05$).

Results

Nutrients in soil

The soil nutrient concentrations were determined at the Agricultural Institute of Slovenia prior to the experiments (see Materials and Methods). These were compared to the recommended values of the elements in soil and the norms for fertilising (Mihelič et al. 2010), which showed that the nutrient supply was in general good or in excess. The element composition of the rhizosphere soil of each of the plants was determined after the harvest, using EDXRF (Tab. 1), and these data confirmed the high total element concentrations. For the total soil concentrations, Zn (122 mg/kg) was within the range of acceptable levels (Ur. list RS 68/1996), Cu (80 mg/kg) and Pb (91 mg/kg) were close to the alert concentrations, Ni (84.4 mg/kg) was in the range of the alert levels, and Cr (366 mg/kg) was close to the critical values. With the soil ammonium acetate extractable fraction analysis, however, only Cr (1.94 mg/kg) exceeded the values defined in the guidelines for field, horticulture and homeowner soil tests for heavy metals (Grubinger and Ross 2011).

Plant growth and nutrient accumulation

The grain biomass of the wheat (0.023 g) and common buckwheat (0.024 g) did not differ significantly, whereas the biomass of the tartary buckwheat grain was significantly lower (0.017 g;

Table 1: Total and biologically available element concentrations in the rhizosphere soil determined by EDXRF, and recommended rates of supply[a,b] or soil pollution classification concentrations[c].

Tabela 1: Skupne in biološko razpoložljive koncentracije elementov v rizosfernih tleh določene z EDXRF, priporočene vsebnosti[a,b] in klasifikacija koncentracij za onesnažena tla[c].

Element	Soil concentrations (mg/kg)		Rates of supply of the soil[a,b] Levels in soils[c] (mg/kg)		
	Total	Available	Poor[a,b] Limit[c]	Adequate[a,b] Alert[c]	Extreme[a,b] Critical[c]
K	17325 ± 1126	nd	nd	nd	nd
Ca	7318 ± 301	1529 ± 31.1	<1000[b]	nd	>2000[b]
Cr	366 ± 62.8	1.94 ± 0.08	100[c]	150[c]	380[c]
Mn	2200 ± 68.9	9.76 ± 0.92	30[a]	45[a]	60[a]
Fe	44600 ± 204	5.93 ± 1.39	nd	nd	nd
Ni	84.4 ± 10.6	0.43 ± 0.06	50[c]	70[c]	210[c]
Cu	80 ± 7.4	0.46 ± 0.05	<3[a] 60[c]	5.5[a] 100[c]	8[a] 300[c]
Zn	122 ± 4.2	1.18 ± 0.56	<1.1[a] 200[c]	2.05[a] 300[c]	>3.0[a] 720[c]
Ti	10600 ± 227	3.25 ± 0.17	nd	nd	nd
Br	nd	0.27 ± 0.02	nd	nd	nd
Pb	91 ± 2.7	0.39 ±0.18	85[c]	100[c]	530[c]

[a](Mihelič et al. 2010); [b](Marx et al. 1999); [c](Ur. list RS št. 68/1996).
nd –not determined.
Data are means ± SE (n = 4)
Srednja vrednost ± SN (n = 4)

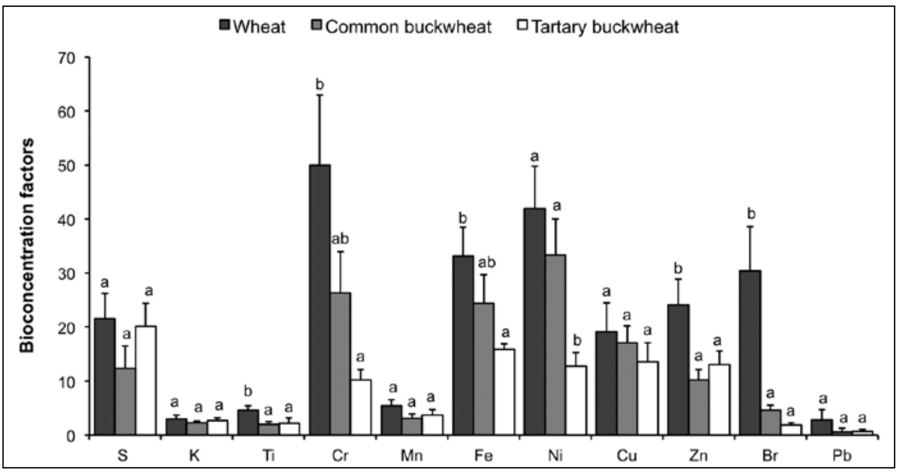


Figure 1: Bioconcentration factors for the elements in the wheat, common and tartary buckwheat grain (as indicated), as calculated from (total grain concentration)/(bioavailable soil concentration). Data are means ± SE, ($n_{\text{Wheat}} = 7$; $n_{\text{Common buckwheat}} = 7$; $n_{\text{Tartary buckwheat}} = 6$). Different letters indicate statistically significant differences in the one-way ANOVA and Duncan's *post-hoc* tests ($p < 0.05$).

Slika 1: Biokoncentracijski faktorji elementov pšenice, navadne in tatarske ajde [(celotne koncentracije v semenih) /(biološko razpoložljive koncentracije v tleh)] (srednja vrednost ± SN, $n_{\text{Pšenica}} = 7$, $n_{\text{Navadna ajda}} = 7$, $n_{\text{Tatarska ajda}} = 6$). Črke nad stolpci povprečnih vrednosti označujejo statistično značilne razlike testa enosmerne ANOVA in Duncanovega *post-hoc* testa ($p < 0.05$).

$p < 0.05$; one-way ANOVA and Duncan's *post-hoc* tests).

The concentrations of the measured macronutrients (P, S, K) were higher in the wheat than in the common and tartary buckwheat, whereas the highest concentration of Ca was in the tartary buckwheat (Tab. 2). The wheat also contained the highest concentrations of the microelements Cr, Fe, Ni, Cu and Zn, and the highest concentration of Br.

The grain BCFs (as [total grain concentration]/[bioavailable soil concentration]; Baker 1981; Mensch et al. 2010) for S, K, Mn and Pb did not differ significantly between these species, whereas the BCFs of Ti, Zn and Br were higher in the wheat than in the common and tartary buckwheat (Fig. 1). In addition, the BCFs for Cr, Fe and Ni were significantly lower in the tartary buckwheat, as compared to the wheat.

The nutritive values of the grain were compared on the basis of the grain element contents in a sample of 100 g grain. The results show that the contents of the macroelements in wheat are in general higher than in both of the buckwheat species. The amounts of the daily needs of adults that a portion of grain covers were calculated for each of the elements through a comparison of the grain contents (mg/100 g) with the DRI (mg/d) for adults (considering the RDAs and the AIs (USDA DRI 2004) (Tab. 2)). If all of the minerals in the consumable seed tissues were in a form available for absorption, a portion of the wheat grain (100 g) would almost cover the daily needs

Table 2: Element concentrations in the grain of the wheat, common and tartary buckwheat, and relevant Dietary Reference Intakes for adults, where available.

Tabela 2: Koncentracije elementov v zrnju pšenice, navadne in tatarske ajde in prehranski referenčni vnosi za odrasle (DRI).

Element		Concentration (µg/g)			DRI		
		Wheat	Common buckwheat	Tartary buckwheat	RDA (mg/d)	AI (mg/d)	UL (mg/d)
Macro	P	620 ± 6.0 a	315 ± 28.0 b	263 ± 14.0 c	700	nd	4000
	S	322 ± 23.0 a	170 ± 33.0 b	326 ± 32.0 a	nd	nd	nd
	K	1663 ± 96.0 a	1182 ± 102.0 b	1356 ± 94.0 b	nd	4700	nd
	Ca	314 ± 31.0 b	233 ± 16.0 b	440 ± 37.0 a	nd	1000	2500
Micro	Cr	40.5 ± 2.8 a	16.8 ± 1.6 b	10.8 ± 1.3 b	nd	0.030	0.2*
	Mn	12.5 ± 1.1 a	9.52 ± 0.57 a	10.9 ± 1.3 a	2.05	nd	11
	Fe	115 ± 6.0 a	56.6 ± 3.2 b	52.6 ± 5.2 b	nd	13	45
	Ni	12 ± 0.4 a	6.46 ± 0.32 b	3.9 ± 0.12 c	nd	0.0275	1.0
	Cu	5.08 ± 0.19 a	4.24 ± 0.37 b	2.69 ± 0.27 c	0.90	nd	10
	Zn	25.4 ± 1.5 a	15.7 ±1.0 b	17.6 ± 2.3 b	9.5	nd	40
Trace	Ti	6.07 ± 0.85 a	4.48 ± 0.35 ab	2.73 ± 0.66 b	nd	0.35**	nd
	Br	8.34 ± 1.13 a	1.16 ± 0.04 b	0.39 ± 0.05 b	nd	nd	nd
	Pb	1.33 ± 0.33 a	1.45 ± 0.12 a	0.83 ± 0.17 a	nd	0.27*	1.75*

DRI, Dietary Reference Intakes for adults (USDA DRI 2004); RDA, Recommended Dietary Allowance; AI, Adequate Intake Level; UL, Tolerable Upper Intake Level; *(NSF/ANSI 173 2003); **(WHO 1982).
nd, not determined.
Data are means ± SE ($n_{Wheat} = 15$; $n_{Common\ buckwheat} = 7$; $n_{Tartary\ buckwheat} = 9$).
Different letters indicate statistically significant differences across the columns of one-way ANOVA and Duncan's *post-hoc* tests ($p < 0.05$) between plant species.
DRI, prehranski referenčni vnosi za odrasle (USDA DRI 2004); RDA, priporočeni dnevni prehranski referenčni vnosi; AI, primerni dnevni prehranski referenčni vnosi; UL, zgornje dopustne vrednosti dnevnega vnosa; *(NSF/ANSI 173 2003); **(WHO 1982).
nd, ni določeno.
Srednja vrednost ± SN; ($n_{Pšenica} = 15$, $n_{Navadna\ ajda} = 7$, $n_{Tatarska\ ajda} = 9$)
Črke ob povprečnih vrednostih po stolpcih označujejo statistično značilne razlike testa enosmerne ANOVA in Duncanovega *post- hoc* testa ($p < 0,05$) med rastlinskimi vrstami.

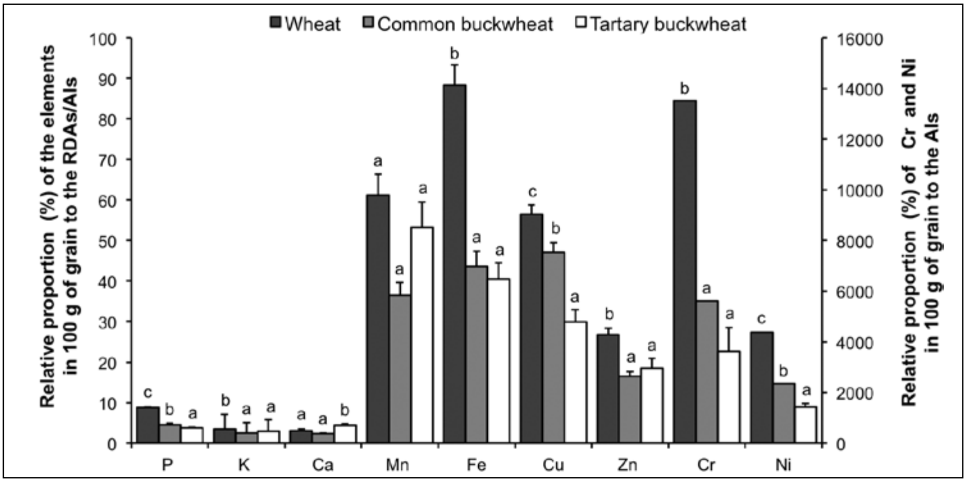


Figure 2: Elements in 100 g of the wheat, common and tartary buckwheat grain relative [%] to the RDAs or AIs (USDA DRI 2004) (see Materials and methods for details). Data are means ± SE ($n_{Wheat} = 15$; $n_{Common\ buckwheat} = 7$; $n_{Tartary\ buckwheat} = 9$). Different letters indicate statistically significant differences in the one-way ANOVA and Duncan's *post-hoc* tests ($p < 0.05$).

Slika 2: Elementi v 100 g zrnja pšenice, navadne in tatarske ajde v odstotnih deležih [%] priporočenih dnevnih vnosov (RDAs) oziroma vrednosti primernih dnevnih vnosov (AIs; USDA DRI 2004) (Glej Materiale in metode). (Srednja vrednost ± SN ($n_{Pšenica} = 15$, $n_{Navadna\ ajda} = 7$, $n_{Tatarska\ ajda} = 9$). Črke nad stolpci povprečnih vrednosti označujejo statistično značilne razlike testa enosmerne ANOVA in Duncanovega *post-hoc* testa ($p < 0.05$).

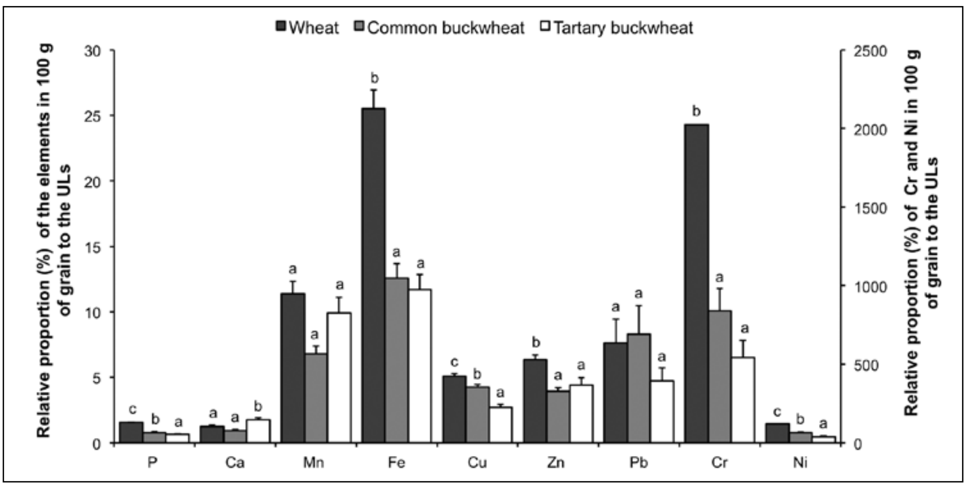


Figure 3: Elements in 100 g of the wheat, common and tartary buckwheat grain relative [%] to the ULs (USDA DRI 2004) (see Materials and methods for details). Data are means ± SE ($n_{Wheat} = 15$; $n_{Common\ buckwheat} = 7$; $n_{Tartary\ buckwheat} = 9$). Different letters indicate statistically significant differences in the one-way ANOVA and Duncan's *post-hoc* tests ($p < 0.05$).

Slika 3: Elementi v 100 g zrnja pšenice, navadne in tatarske ajde v odstotnih deležih [%] zgornjih dopustnih vrednosti (ULs (USDA DRI 2004) (Glej Materiale in metode). (Srednja vrednost ± SN, $n_{Pšenica} = 15$, $n_{Navadna\ ajda} = 7$, $n_{Tatarska\ ajda} = 9$). Črke nad stolpci povprečnih vrednosti označujejo statistično značilne razlike testa enosmerne ANOVA in Duncanovega *post-hoc* testa ($p < 0.05$).

of adults for Fe, with 60% for Mn, and 50% for Cu (Fig. 2). In comparison, a portion of common buckwheat (100 g) would cover 50% of the daily requirement for Fe and Cu, whereas for tartary buckwheat grain it would cover 50% of the daily needs for Mn and 40% for Fe.

It is also of importance that the concentrations of Cr and Ni in wheat critically exceeded the DRI AIs, and the recommended concentrations were also exceeded in the grain of both of the buckwheats (Fig. 2). Therefore, the contents in 100 g of grain are compared to the ULs (USDA DRI 2004) (Tab. 2). These data show that the contents of Cr in 100 g of wheat and common and tartary buckwheat exceed the upper limits by 20.3-fold, 8.5-fold and 5.5-fold, respectively, whereas the contents of Ni only slightly exceed the upper limits (1.2-fold) in wheat, but not in common and tartary buckwheat (Fig. 3).

Discussion

Large variations in the mineral element compositions of the edible portions have been reported between different crop species (White and Broadley 2005), and efforts to improve the element composition of wheat have resulted in the selection of crop cultivars with significantly improved use of Fe and Zn (Cakmak et al. 2010). In the present study, higher BCFs are seen in wheat for Cr, Fe, Ni, Cu, Zn and Br when compared to both of the buckwheat species, which has been identified as one of the most important factors that drives the higher efficiency of wheat element bioextraction from the same soluble soil mineral nutrient pool. As a consequence, wheat shows higher concentrations of these elements in the grain when compared to both of the buckwheat species. These differences can be attributed to differences in the physiology of nutrient uptake, xylem loading, element remobilisation from the leaves, and seed deposition processes in wheat, when compared to buckwheat.

The total grain element concentrations in the whole grain of these analysed species are significantly higher than the concentrations of macronutrients and micronutrients that have been commonly reported for wheat and buckwheat flours (Czerniejewski et al. 1964; Ikeda et al. 2000; 2006). Element localisation studies within the grain have demonstrated that the majority of the essential nutrients in the grain of wheat and buckwheat are stored in the embryo and aleurone tissues in specialised cellular and subcellular compartments, while their concentrations in the endosperm are generally very low (Mazzolini et al. 1985; Vogel-Mikuš et al. 2009; Lombi et al. 2011; Pongrac et al. 2011; Regvar et al. 2011). As a consequence of these structurally related localisation patterns, large amounts of mineral nutrients are readily lost by milling and polishing of the grain. It has therefore been suggested that different flour fractions that are prepared by successive milling can be successfully reintroduced as a dietary source of essential elements (Ikeda et al. 2000). Thus, as well as the need for optimisation of the grain element accumulation properties, refinements in flour production technology should prove useful in future attempts to resolve the problem of mineral malnutrition in humans.

Based on the comparison of the mineral compositions with the intake of minerals (the AIs), we show here that on average 50% of the needed daily intake of Mn can be covered by 100 g of wheat or tartary buckwheat grain. All three species are also good sources of Fe, and also of Cu. It should be noted, however, that although the total amounts of elements might be the same as in other food sources, Fe and Zn from vegetable sources are likely to be less available for absorption due to the differences in their chemical forms and/or to the presence of phytic acid and other constituents that can reduce the absorption (Hunt 2003), thus further diminishing the nutritive value of grain.

Intensive agricultural approaches can easily result in unintentional increases in the accumulation of various heavy metals in soils. The application of multi-element analytical techniques for analysis and screening of soils and edible plant parts is therefore particularly rewarding in contemporary monitoring programmes that are aimed at the detection of unintended contaminant metal accumulation. Application of EDXRF and TXRF analyses here shows that the field soils from conventional grain production and the grain produced on these soils can contain a wide range of unwanted metals that can easily remain overlooked in the majority of the classical soil analyses. A particularly disturbing aspect is the total soil concentrations of Cr, Ni, Cu and Pb, with Cr reaching critical levels (Ur. list RS 68/1996). In addition to these, the soil extractable Cr levels exceed those values defined in the guidelines for field, horticulture and homeowner soil tests for heavy metals (Grubinger and Ross 2011), which confirms that soil Cr concentrations in particular are of considerable concern. As a consequence, the grain of all three species here accumulated Cr in concentrations that exceeded the ULs (USDA DRI 2004). The same was also true for the accumulation of Ni in wheat. Wheat grains are known to be prone to Cu and Ni accumulation when grown in sludge-enriched soils (Wang et al. 2009). With the buckwheat grain, Ni is localised in the embryonic axis and the aleurone (Pongrac et al. 2011) as a result of the specific physiology of the grain-filling process. During milling and polishing of durum wheat, however, up to a 61% reduction in Ni and a 65% reduction in Cr have been shown in the milling product (semolina), compared to dry grain (Cubadda et al. 2005). It is therefore reasonable to expect that the metal concentrations in the milling products will be considerable lower than those found in the grain. In addition, it is of interest that this unwanted element accumulation is less severe in the buckwheat grain. Taken together, these data indicate the need for careful monitoring of such unintentional metal accumulation in grain, and the greater applicability of common and tartary buckwheat for growth in moderately polluted soils through their lower accumulation of these unwanted metals, when compared to wheat.

Conclusions

1. The higher bioconcentration capacity of wheat, when compared to the buckwheats, results from differences in the physiology of the element uptake and partitioning. This is identified as one of the most important factors that drive the greater potential for bioextraction in wheat from the same bioavailable pool of the mineral element in the soil in the field for conventional grain production. As a consequence, this results in higher concentrations of these elements at the whole grain level.
2. The low content of the nutrient elements reported for wheat and buckwheat flours compared to the grain imply that as well as optimisation of the plant accumulation properties, further changes in the flour production technology should prove useful to successfully address the problem of mineral malnutrition in humans.
3. The data indicate an accumulation of Ni and Cr in grain from soils under conventional grain production, which will primarily result from unintentional soil deposition of these elements due to agricultural activities. The application of multi-elemental analytical techniques in contemporary screening and monitoring programmes for the detection of unwanted metal accumulation is therefore suggested.
4. The lower accumulation of unwanted metals in the common and tartary buckwheat grains, when compared to wheat, indicates the greater applicability of both of these buckwheat species for grain production in metal-enriched soils that have resulted from conventional field management practices.

Povzetek

Žita in psevdožita so pomemben vir mineralov v prehrani nekaterih populacij, zato bi bilo pomembno izboljšati elementno sestavo žit in zmanjšati vnos neželenih kovin v užitne dele rastlin. Rastline lahko absorbirajo samo biološko razpoložljive elemente, ki so določene z naravno depozicijo v tleh, pH vrednostjo tal, vsebnostjo organske snovi in sposobnostjo kationske izmenjave (Marschner 1995; Moreno et al. 1996). Poleg teh dejavnikov je pomembna tudi sposobnost rastlin za privzem, transport in razporejanje esencialnih elementov v rastlinskih organih (Waters and Sankaran 2011). V tleh s konvencionalno pridelavo rastlin, pa tkiva lahko vsebujejo tudi presežne vrednosti nezaželenih elementov, ki se posledično nalagajo v rastlinah.

Izvedli smo raziskavo akumulacijske sposobnosti pšenice (*Triticum aestivum*), navadne ajde (*Fagopyrum esculentum*) in tatarske ajde (*Fagopyrum tataricum*) s polja s konvencionalno pridelavo semen. Elementno sestavo rizosfernih tal rastlin smo določili z EDXRF, in potrdili dobro preskrbljenost tal. Skupne koncentracije Zn v tleh (122 mg/kg) so bile pod mejnimi vrednostmi,

koncentracije Cu in Pb (80 in 91 mg/kg) so bile blizu opozorilnih vrednosti, koncentracije Ni (84.4 mg/kg) so presegale opozorilne vrednosti, koncentracije Cr (366 mg/kg) pa so bile blizu kritičnih vrednosti (Ur. list RS, 68/1996; Tab. 1).

Biomasa zrnja pšenice (0.023 g) in navadne ajde (0.024 g) se ni značilno razlikovala, biomasa tatarske ajde (0.017 g) pa je bila statistično značilno nižja ($p < 0.05$; enosmerna ANOVA in Duncanov *post-hoc* test). Koncentracije izmerjenih makroelementov (P, S, K) in mikroelementov Cr, Fe, Ni, Cu, Zn in Br v zrnju izbranih rastlinskih vrst, določene s TXRF, so bile najvišje v zrnju pšenice, najvišje koncentracije Ca pa je imela tatarska ajda (Tab. 2). Vzorci pšenice so imeli višje biokoncentracijske indekse (BCF) za Cr, Fe, Ni, Zn in Br od tatarske ajde (Sl. 1). Biokoncentracijske faktorje smo izračunali iz razmerja med skupno koncentracijo elementov v zrnju in koncentracijo biološko razpoložljivih elementov v tleh in so pomemben pokazatelj ekstrakcijske sposobnosti izbranih rastlinskih vrst za posamezne mineralne nutriente.

Vsebnosti elementov v 100 g zrnja smo primerjali s prehranskimi referenčnimi vnosi za odrasle (USDA DRI 2004). Če bi bili vsi elementi v zrnju biološko dostopni, bi z uživanjem 100 g pšenice dnevno lahko pokrili priporočene dnevne vnose Fe 80%, dnevne vnose Mn 60%, in polovico priporočenih dnevnih vnosov Cu. Z uživanjem 100 g navadne ajde bi lahko pokrili polovico dnevnih potreb po Fe in Cu, medtem ko bi s 100 g tatarske ajde pokrili polovico dnevnih potreb po Mn in 40% priporočene vrednosti za Fe. Vendar je pri teh ocenah potrebno upoštevati, da imajo elementi v zrnju zmanjšano biološko dostopnost za absorpcijo zaradi vezanosti na fitnsko kislino in nekatere strukturne komponente semen (Hunt 2003). Nizke vsebnosti elementov v moki pšenice in ajde navedene v literaturnih virih (Czerniejewski et al. 1964; Ikeda et al. 2000; 2006) v primerjavi s koncentracijami v zrnju nakazujejo, da bi bilo poleg povečevanja privzema esencialnih elementov v zrnje koristno prilagoditi tudi tehnološke postopke priprave mlevskih izdelkov, če želimo v njih povečati količino mineralnih nutrientov.

Vsebnosti Cr in Ni so presegle primerne dnevne prehranske referenčne vnose (Adequate Intake Levels – AIs; USDA DRI 2004; Tab. 2). Primerni dnevni prehranski referenčni vnosi za Cr in Ni so bili najbolj preseženi v pšenici, vendar so bili le-ti preseženi tudi pri navadni in tatarski ajdi (Sl. 2). S primerjavo vsebnosti elementov v zrnju z zgornjimi dopustnimi vrednostmi dnevnega vnosa (Tolerable Upper Intake Levels -ULs; USDA DRI 2004; Sl. 3) smo ugotovili, da vsebnosti Cr v zrnju pšenice, navadne in tatarske ajde presegajo zgornje dopustne vrednosti za 20.3, 8.5 oziroma 5.5 krat, presežena pa je bila tudi meja dopustnega dnevnega vnosa za Ni pri pšenici. Rezultati kažejo na potrebo po sistematičnem spremljanju vnosa nezaželenih kovin v zrnje izbranih rastnskih vrst in večjo uporabnost navadne in tatarske ajde za vzgojo v tleh s konvencionalno pridelavo.

Acknowledgements

The present work was carried out in the framework of ARRS projects (J1-4117 Research of the ionome of selected mycorrhizal plants; J4-4224 Sustainable land use in relation to soil and crop quality, J4-3618 Tartary buckwheat as a new source for functional food), the programme P1-0212 Plant Biology, and the Ministry of Higher Education, Science and Technology Grant No. 373-694. The authors would like to thank Milena Kubelj for technical assistance.

References

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals. Springer-Verlag, New York, pp. 867.

Baker, A.J.M, 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3, 643–654.

Baker, A.J.M., Reeves, R.D., Hajar, A.S.M., 1994. Heavy metal accumulation and tolerance in British population of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae) New Phytologist, 127, 61–68.

Cakmak, I., 2002. Plant nutrition research: Priorities to meet human needs for food in sustainable ways. Plant and Soil, 247, 3–24.
Cakmak, I., Pfeiffer, W.H., McClafferty, B., 2010. Biofortification of durum wheat with zinc and iron. Cereal Chemistry, 87, 10–20.
Cubadda, F., Raggi, A., Marconi, E., 2005. Effects of processing on five selected metals in the durum wheat food chain. Microchemical Journal, 79, 97–102.
Czerniejewski, C.P., Shank, C.W., Bechtel, W.G., Bradley, W.B., 1964. The minerals of wheat, flour and bread. Cereal Chemistry, 41, 65–72.
Grubinger, V., Ross, D., 2011. Interpreting the results of soil tests for heavy metals. Cooperative Extension work, USDA and University of Vermont, Berlington, 4 pp.
Hemery Y., Lullien-Pellerin, V., Rouau, X., Abecassis, J., Samson, M.-F., Aman, P., von Reding, W., Spoerndli, C., Barron, C., 2009. Biochemical markers: Efficient tools for the assessment of wheat grain tissue proportions in milling fractions. Journal of Cereal Science, 49, 55–64.
Hunt, J.R., 2003. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. The American Journal of Clinical Nutrition, 78, 633S–639S.
Ikeda, S., Yamashita, Y., Kreft, I., 2000. Essential mineral composition of buckwheat flour fractions. Fagopyrum, 17, 57–61.
Ikeda, S., Yamashita, Y., Tomura, K., Kreft, I., 2006. Nutritional comparison in mineral characteristics between buckwheat and cereals. Fagopyrum, 23, 61–65.
Kreft, I., Jost, M., Skrabanja, V., 1998. Genetic basis of wheat ideotype for special blended bread. Proc. 9th International Wheat Genetics Symposium, Saskatoon, 4, pp. 184–186.
Lombi, E., Smith, E., Hansen, T.H., Paterson, D., de Jonge, M.D., Howard, D.L., Persson, D.P., Husted, S., Ryan, C., Schjoering, J.K., 2011. Megapixel imaging of (macro)nutrients in mature barley grains. Journal of Experimental Botany, 62, 273–282.
Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London, UK.
Marx, E.S., Hart, J., Stevens, R.G., 1999. Soil Test Interpretation Guide EC 1478. Oregon State University extension service, 8 pp.
Mazzolini, A.P., Pallaghy, C.K., Legge, G.J.F. 1985. Quantitative microanalysis of Mn, Zn and other elements in mature wheat seed. New Phytologist, 100, 483–509.
McKevith, B., 2004. Nutritional aspects of cereal. Final report to the Home Grown Cereal Authority. British Nutrition Foundation, pp 62. http://www.hgca.com/publications/documents/cereals.pdf (27. 7. 2012)
Mensch, M., Lepp, N., Bert, V., Schwitzguébel, J.-P., Gawronski, S.W., Schröder, P., Vangronsveld, J., 2010. Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. Journal of Soils and Sediments, 10, 1039–1070.
Mihelič, R., Čop, J., Jakše, M., Štampar, F., Majer, D., Tojnko, S., Vršič, S., 2010. Smernice za strokovno utemeljeno gnojenje. Ministrstvo za kmetijstvo, gozdarstvo in prehrano, Ljubljana, 185 pp.
Moreno, J.L., Hernández, C.G., Pascual, J.A.,1996. Transference of heavy metals from a calcareous soil amended with sewage–sludge compost to barley plants. Bioresource Technology, 55, 251–258.
Nečemer, M., Kump, P., Ščančar, J., Jaćimović R., Simčič, J., Pelicon, P, Budnar, M, Jeran, Z., Pongrac, P., Regvar, M., Vogel-Mikuš, K., 2008. Application of X-ray fluorescence analytical techniques in phytoremediation and plant biology studies. Spectrochimica Acta Part B: Atomic Spectroscopy, 63, 1240–1247.
NSF/ANSI 173, 2003. American National Standard for Nutritional/Dietary Supplements 173 (NSF international), Metal contaminant acceptance levels, 21 pp.
Olsen, S.R., Sommers, L.E. 1982. Phosphorus. In: A.L. Page, R.H. Miller (Eds). Methods of Soil Analysis. Part 2. 2nd ed. Agronomy Monograph 9, ASA and SSSA, Madison, WI, pp. 403–430.
Pongrac, P., Vogel-Mikuš, K., Regvar, M., Vavpetič, P., Pelicon, P., Kreft, I., 2011. Improved lateral discrimination in screening the elemental composition of buckwheat grain by micro-PIXE. Journal of Agricultural and Food Chemistry, 59, 1275–1280.

Regvar, M., Eichert, D., Kaulich, B., Gianoncelli, A., Pongrac, P., Vogel-Mikuš, K., Kreft, I., 2011. New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron soft X-ray microscopy. Journal of Experimental Botany, 62, 3929–3939.

Schwenke, H., Knoth, J., 1993. Total reflection XRF. In: Van Grieken RE, Markowicz AA (eds) Handbook of X-Ray Spectronomy. Marcell Dekker, New York, pp 453–490.

Tyler, G., Zohlen, A., 1998. Plant seeds as mineral nutrient resource for seedlings – a comparison from calcareous and silicate soils. Annals of Botany, 81, 455–459.

Ur. list RS, 68/1996. Decree on limit values, alert thresholds and critical levels of dangerous substances into the soil, p 5773.

US EPA, 2002. Supplemental guidance for developing soil screening levels for superfund sites. Office of Solid Waste and Emergency Response, Washington, D.C. http://www.epa.gov/superfund/health/conmedia/soil/index.htm (28. 8. 2012)

USDA DRI Dietary Reference Intakes, 2004: Food and Nutrition Board, Institute of Medicine, USDA, National Agricultural Library. http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables (28. 8. 2012).

Vogel-Mikuš, K., Pelicon, P., Vavpetič, P., Kreft, I., Regvar, M., 2009. Elemental analysis of edible grains by micro-PIXE: Common buckwheat case study. Nuclear Instruments and Methods in Physics Research B, 267, 2884–2889.

Vogel-Mikuš, K., Kump, P., Nečemer, M., Pelicon, P., Arčon, I., Pongrac, P., Povh, B., Regvar, M., 2010. Quantitative analyses of trace elements in environmental samples: options and (im)possibilities. In: Sherameti I., Varma A. (eds.): Soil heavy metals. Soil Biology, Berlin, Springer-Verlag, 19, 113–138.

Wang, S., Nan, Z., Liu, X., Li, Y., Qin, S., Ding, H., 2009. Accumulation and bioavailability of copper and nickel in wheat plants grown in contaminated soils from the oasis, northwest China. Geoderma, 152, 290–295.

Waters, B.M., Sankaran, R.P., 2011. Moving micronutrients from the soil to the seeds: Genes and physiological processes from a biofortification perspective. Plant Science, 180, 562–574.

White, P.J., Broadley, M.R., 2005. Biofortifying crops with essential mineral elements. Trends in Plant Science, 10, 586–592.

WHO World Health Organisation, 1982. International programme on chemical safety. Environmental health criteria 24. Titanium. Geneva., 68 pp

Zhao, F.J., Su, Y.H., Dunham, S.J., Rakszegi, M., Bedo, Z., McGrath, S.P., Shewry, P.R., 2009. Variation in mineral micronutrient concentrations in grain of wheat of diverse origin. Journal of Cereal Science, 49, 290–295.

ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 25–38

The presence of invasive alien plant species in different habitats: case study from Slovenia

Razširjenost tujerodnih invazivnih vrst rastlin v različnih habitatih: primer iz Slovenije

Igor Zelnik

University of Ljubljana, Biotechnical Faculty, Department of Biology,
Večna pot 111, 1000 SI-Ljubljana, Slovenia
correspondence: igor.zelnik@bf.uni-lj.si

Abstract: Invasive alien plants present a threat to diversity of native species. An attempt to evaluation of potential endangerment of specific habitats due to the presence of invasive alien plant species was made and results are presented in this paper. Data about the presence of invasive alien plants in specific habitats were extracted from the database Flora of Slovenia (at the Centre for Cartography of Fauna and Flora). The list of the most frequent invasive alien plant taxa in natural and semi-natural habitats is presented as well as the lists of invasive alien plants with potentially most negative influence on the biodiversity in different habitats. In general, taxa with potentially most negative influence on the biodiversity of natural habitats in Slovenia are: *Robinia pseudacacia*, *Solidago gigantea, Fallopia japonica* and *F.* × *bohemica, Rudbeckia laciniata, Helianthus tuberosus*. In the dataset the highest number of data about presence of invasive alien plants referred to riparian zones (44%). The second highly infected group of habitats was floodplain woods. According to the Ellenberg indicator values the most of the invasive alien plants prefer nutrient-rich and sunny sites. The negative effect of *Fallopia* × *bohemica* on light conditions in established stands and consequently on the species richness of native plants was also detected.

Keywords: invasive alien plants, habitats, biodiversity, Slovenia, riparian zones.

Izvleček: Invazivne tujerodne vrste rastlin ogrožajo pestrost domorodnih vrst. V članku so prikazani rezultati poskusa ovrednotenja potencialne ogroženosti posameznih habitatov zaradi prisotnosti invazivnih tujerodnih vrst rastlin. Podatki o prisotnosti invazivnih tujerodnih rastlin v posameznih habitatih, so pridobljeni iz podatkovne zbirke Flora Slovenije (na Centru za Kartografijo Favne in Flore). Predstavljen je seznam najbolj pogostih invazivnih tujerodnih vrst rastlin v naravnih in sonaravnih habitatih kot tudi seznam invazivnih tujerodnih vrst rastlin, ki imajo potencialno najbolj negativen učinek na biodiverziteto v različnih habitatih. Na splošno so taksoni s potencialno najbolj negativnim učinkom na biodiverziteto v naravnih habitatih Slovenije naslednji: *Robinia pseudacacia*, *Solidago gigantea, Fallopia japonica* in *F.* × *bohemica, Rudbeckia laciniata* ter *Helianthus tuberosus*. V podatkovni bazi se je največ tovrstnih podatkov nanašalo na obrežne pasove (44%). Druga najbolj okužena skupina habitatov so bili poplavni in močvirni gozdovi. Glede na Ellenbergove indikatorske vrednosti večini invazivnih tujerodnih vrst ustrezajo s hranili bogata sončna rastišča.

Ugotovili smo tudi negativni učinek taksona *Fallopia* × *bohemica* na svetlobne razmere in posledično na vrstno pestrost v dobro razvitih sestojih omenjenega taksona.

Ključne besede: invazivne tujerodne rastline, habitati, biodiverziteta, Slovenija, obrežni pasovi.

Introduction

The spreading of invasive alien plant species affects the diversity of native plants and animals in infected ecosystems (Essl and Rabitsch 2002). Invasive alien species are considered to be the second largest reason for biodiversity loss worldwide (Vitousek 1996). When invasive alien plant species dominate in the community, they alter the conditions in the ecosystem in a way that it becomes unsuitable for thriving of native plants (Hejda and Pyšek 2008). Main reasons for negative impacts on biodiversity are competitive exclusion of native species, alteration of ecosystems structure and availability of resources, as well as change of microclimate (Essl and Rabitsch 2002).

Vilá et al. (2011) found out that invasive alien plant species significantly reduced fitness and growth of native plant species by 41.7 and 22.1%, respectively, and changed plant community structure by decreasing species abundance (43.5%) and diversity (50.7%).

The most vulnerable areas are those with high number of endemic species. It is expected that invasive alien species would affect the biodiversity of aquatic ecosystems, especially standing waters, to the greatest extent, while among terrestrial ecosystems the biodiversity of Mediterranean ecosystems is under the greatest pressure (Essl and Rabitsch 2002).

In Central Europe neophytes occur predominately in ecosystems with frequent human or natural disturbances (Pyšek 1998, Kowarik 1999). Their colonization is possible especially in places where different disturbance have caused damage or clearances in closed stands.

Several authors (e.g. Rejmánek et al. 2005, Simonová and Lososová 2008, Šilc 2010) claim, that in general the antropogenic vegetation is most infected with invasive alien plants due to strong and frequent disturbances. However, in Central Europe in the majority of habitat types the endangerment of native flora and fauna is relatively low (Essl and Rabitsch 2002). From the aspect of biodiversity maintenance the most problematic is the impact of invasive alien species in natural and semi-natural habitats, especially in floodplain woods, riparian zones along the streams and in Pannonian lowland forests (Essl and Rabitsch 2002).

The greatest changes in structure and above all in functioning of ecosystems are expected for those invasive alien plant species that represent the new growth forms in infected ecosystem or growth forms that used to be rare in such ecosystem. That causes changes in density and coverage of the vegetation and may also cause a total change of the structure of such vegetation (Kowarik 1999). Species with greater life span – e.g. perennials instead of annuals or woody species instead of the herbs and grasses – often use the resources more efficiently that leads into significant changes in the availability of resources (light, nutrient contents, litter production) as well as in the production of the ecosystems (Gurevitch et al. 2002, Larcher 2003). Invasive alien plants also change the speed and direction of the succession (Řehounková and Prach 2008).

Therefore, the species like *Impatiens glandulifera*, *Fallopia japonica*, *F.* x *bohemica*, which are much taller than native species in the herb layer of riparian zones and floodplain woods, cause the increase of the thickness of the herb layer (Kowarik 1999). On the other hand the species *Acer negundo* builds additional lower tree layer in floodplain woods. Additional shading might negatively influence the growth and establishment of native species.

Invasive alien tall-herbs, such as *Fallopia japonica, Fallopia × bohemica, Solidago gigantea, Solidago canadensis, Helianthus tuberosus* and other similar species can greatly reduce the speed of the succession of forest communities, since the conditions for germination and growth of the woody species are very poor in such stands (Essl and Rabitsch 2002). Mentioned fast growing species can significantly increase the productivity of infected ecosystems.

Species *Fallopia japonica* has established in coastal and inland wetlands, riparian zones, ruderal sites, settlements and along the roads (Roufied et al. 2011).

Black-locust (*Robinia pseudacacia*) is in general, very invasive species in Europe. Its threat to the biodiversity was proved several times (Somodi et al. 2012, Benesperi et al. 2012). It prevents the regeneration of native plant species and changes the ecosystems, mostly due to enrichment of soil with nitrogen with the help of symbiotic N-fixing bacteria. Because of this reason black-locust stands contain mostly nitrophilous species and ruderal generalists instead of a local flora (Řehounková and Prach 2008). If this species occurs during secondary succession, it changes the site essentially because of N-fixing and so does the direction of the succession leading into creation of ecosystem and/or community which is not expected in natural conditions (Řehounková and Prach 2008). Results published by Kleinbauer et al. (2010) show that climate changes would favour the distribution and establishment of this species in central Europe even more. Beside the threat to diversity of plant species its negative influence to diversity of bird species was detected as well.

The share of invasive alien species is the highest in areas with warmer climate. In Slovenia, the frequency of invasive alien plant species decreases with decreasing mean annual temperature (Šilc et al. 2012). The threat to diversity due to invasive alien plant species is mostly limited to the lowlands and to the zone up to the 600 m a.s.l., respectively.

The aim of present research was to examine the presence of invasive alien plant species in different habitats in Slovenia and to estimate their potential threat to biodiversity.

Methods and materials

Distribution of invasive alien plant species in specific habitats was surveyed using the data from the database Flora of Slovenia of the Centre for Cartography of Fauna and Flora (CCFF), which gathers the highest number of data needed for the performance of such analyses for the territory of Slovenia. Data from the mentioned database were used for all of the analyses in present paper. The only data about presence of invasive alien plant species that could be used from this database were those that also contain the information about the habitat where they had been found. This database of floristic relevés was made with very low bias towards the certain taxa or just selected habitats. About 3.500 suitable data were extracted from the database that met the mentioned criteria and were considered in this survey.

For the assessment of potentially negative influence of invasive alien plant species to the habitats, which is likely to occur due to their distribution and capabilities to compete with native species for light, space and nutrients in the habitat, we tried to adequately evaluate these taxa on the basis of their characteristics, using the equation (a+b)/2) where:

- a stands for life span of the taxon was determined in accordance with its life form and data in determination key (Martinčič et al. 2007): 1 – annual, 2 – biennial, 3 – perennial herb, 4 – woody species.
- b presents a potential influence on the availability of resources (light, nutrients) at the site, which bases on data about habitus of the taxon. This parameter was calculated as the average value following the equation: (b1+b2)/2 where:
  - b1 stands for the height of the taxon: 1 – lower herb layer in the stands (mostly <1 m); 2 – tall-growing herbs and shrubs (1–3 m); 3 – tall shrubs and lianas (>3 m); 4- trees;
  - b2 presents the estimate of the shading ability, which includes data about the size and shape of the laminas as well as the density and distribution of the leaves on the shoots (that influences the shading ability of plants). The shading ability was expressed as a quantity gradient (1–4; 1 – low shading ability, 2 – moderate shading ability, 3 – high shading ability, 4 – very high shading ability).

Considering the frequency of occurring in specific habitats based on the mentioned database, we have multiplied the characteristics of the taxon that were supposed to represent the potential impact on the diversity of native species with the frequency rank of invasive alien plant species in specific habitat. On the basis of these semi-quantitative total estimates we ranked invasive alien plant taxa.

In this way we selected taxa from the list of invasive alien plants that were supposed to have the greatest potentially negative effect to the biodiversity in natural and semi-natural habitats (extensively managed) with considerably high diversity of native species: forests and other forest habitats, floodplain woods and swamps, riparian zones, dry grasslands, wetlands.

On the basis of the Ellenberg indicator values (Ellenberg et al. 1992) ecological preferences of some of the most invasive alien plant species were also analysed in relation to: light intensity (L), temperature (T), moisture (F), soil reaction (R) and available nutrients (N).

In the riparian zone of Glinščica stream the intensity of photosynthetically active radiation (PAR) was measured on the sites with the taxon *Fallopia* × *bohemica* as well as on the sites without the mentioned taxon. In the same sites/plots all present species of vascular plants were recorded and their coverage was estimated.

Results and discussion

Most frequent invasive alien plant species according to the data from the mentioned data base (CCFF) are listed in Table 1 (according to the number of data about invasive alien plant species in all habitats from floristic records). Only the taxa which are present in at least 10 localities are presented.

Beside some exceptions, this list (Tab. 1) includes the same taxa as the list of the experts from Austria (Essl and Rabitsch 2002) who have included the most problematic invasive species regarding the conservation of biodiversity in natural and semi-natural habitats. Although most frequent invasive alien plant species *Erigeron annuus* is less important from the aspect of nature conservation, since it occurs mostly in sites under strong human influence, having minor effects on natural plant communities due to its habitus and short life span.

Beside the species in Table 1, two invasive alien plants should be mentioned, namely *Pistia stratiotes* L. and *Spiraea japonica* L. f., which have only local distribution.

Table 2 presents the most frequent invasive alien plants in natural and semi-natural habitats (extensively managed) with considerably high diversity of native species: forests and other forest habitats, floodplain woods and swamps, riparian zones, dry meadows, wetlands.

Table 1: The most common invasive alien plant species.
Tabela 1: Najpogostejše invazivne tujerodne vrste rastlin.

		No. of data
1.	*Erigeron annuus* (L.) Pers.	462
2.	*Solidago gigantea* Aiton	447
3.	*Robinia pseudacacia* L.	403
4.	*Impatiens glandulifera* Royle	338
5.	*Elodea canadensis* Michx.	228
6.	*Fallopia japonica* (Houtt.) Ronse Decr. and *F.* × *bohemica* (Chrtek & Chrtkova) J.P. Bailey	185
7.	*Rudbeckia laciniata* L.	175
8.	*Echinocystis lobata* (Michx.) Torr. & A. Gray	139
9.	*Impatiens parviflora* DC.	135
10.	*Solidago canadensis* L.	130
11.	*Ambrosia artemisiifolia* L.	129
12.	*Juncus tenuis* Willd.	127
13.	*Helianthus tuberosus* L.	111
14.	*Acer negundo* L.	63
15.	*Parthenocissus quinquefolia* (L.) Planch.	46
16.	*Ailanthus altissima* Desf.	44
17.	*Bidens frondosa* L.	25
18.	*Commelina communis* L.	18
19.	*Parthenocissus inserta* (Kerner) Fritsch	18
20.	*Pinus strobus* L.	18
21.	*Aster squamatus* (Spreng.) Hieron.	16
22.	*Physocarpus opulifolius* (L.) Maxim.	15
23.	*Quercus rubra* L.	15
24.	*Duchesnea indica* (Andrews) Focke	14
25.	*Telekia speciosa* (Schreb.) Baumg.	12
26.	*Asclepias syriaca* L.	11
27.	*Cuscuta campestris* Yunck.	11

The taxa present in this table (Tab. 2) are the same as in the previous case (Tab. 1), where all data from the mentioned database were considered, the difference occur only in the order of the taxa. In some taxa, the number of the data / localities is much lower here as well. In these ecosystems 1st and 3rd position belonged to tall-growing perennials, tree species black-locust took 2nd place while annuals with lower habitus *Erigeron annuus* and *Ambrosia artemisifolia* occurred less frequently in natural habitats (42 and 43% lower frequency). Their low-growing form and life strategy do not enable their establishment in the stands resulting in their lower invasiveness.

In case of aquatic habitats data for *Elodea canadensis* Michx. (228 data) and *Pistia stratiotes* L were included in the database. The latter species was not considered in further analysis, since all of the data (21) are confined to a relatively small area.

Based on the frequency, life-span and influence on the availability of resources (light, nutrients) 18 invasive alien plant taxa (from Tab. 2) which are supposed to have the most negative influence on the biodiversity of natural and most preserved habitats, were chosen using the mentioned estimates (see Tab. 3). To provide the representative list, only taxa that were supported with the data from at least 20 locations in the database were considered.

Table 2: The most common invasive alien plant species in natural and extensively managed habitats. Taxa with at least 10 data / localities in the data-base are listed only.

Tabela 2: Najpogostejše invazivne tujerodne vrste rastlin v naravnih in ekstenzivno gospodarjenih habitatih. Seznam vključuje le tiste taksone, za katere so v podatkovni bazi podatki iz vsaj 10 lokalitet.

		No. of data
1.	*Solidago gigantea* Aiton	390
2.	*Robinia pseudacacia* L.	311
3.	*Impatiens glandulifera* Royle	305
4.	*Erigeron annuus* (L.) Pers.	267
5.	*Fallopia japonica* (Houtt.) Ronse Decr. and *F. × bohemica* (Chrtek & Chrtkova) J.P. Bailey	152
6.	*Rudbeckia laciniata* L.	149
7.	*Echinocystis lobata* (Michx.) Torr. & A. Gray	127
8.	*Impatiens parviflora* DC.	99
9.	*Helianthus tuberosus* L.	97
10.	*Juncus tenuis* Willd.	93
11.	*Solidago canadensis* L.	92
12.	*Ambrosia artemisiifolia* L.	73
13.	*Acer negundo* L.	55
14.	*Parthenocissus quinquefolia* (L.) Planch.	36
15.	*Ailanthus altissima* Desf.	29
16.	*Bidens frondosa* L.	21
17.	*Aster squamatus* (Spreng.) Hieron.	16
18.	*Physocarpus opulifolius* (L.) Maxim.	14
19.	*Pinus strobus* L.	12
20.	*Quercus rubra* L.	11

Based on the data from the used database Flora of Slovenia (CCFF) the species *Robinia pseudacacia* is the invasive alien plant species with potentially most negative influence on the biodiversity. It is followed by tall-growing perennial herbs with rhizomes: *Solidago gigantea, Fallopia japonica* and taxon *Fallopia × bohemica*.

Species with potentially strong negative influence to biodiversity are also tall-growing perennial herbs *Rudbeckia laciniata* and *Helianthus tuberosus*, which often form dense up to 3m high stands. Such stands are mostly found in riparian zones of the streams.

Tree species *Ailanthus altissima* and *Acer negundo* also belong to the invasive alien plants with most negative influence on the biodiversity.

The species *Solidago canadensis* tends to have slightly less harmful effect on biodiversity like *S. gigantea.* Vast stands in natural habitats are found in the riparian zones along the streams and man-made standing waters as well as on the edge of floodplain woods.

The next pair of species from the list (Tab. 4) presents the lianas *Parthenocissus quinquefolia* and *Echinocystis lobata* where the latter is much more frequent, especially in the riparian zones along streams and man-made standing waters as well as on the edge of floodplain woods.

The species *Elodea canadensis* is most widespread invasive alien plant among the aquatic macrophytes in the territory of Slovenia. This species seemed to be invasive only in reservoirs and degraded streams in lowlands.

Species with potentially negative influence on the biodiversity in wet habitats are also: *Impatiens glandulifera, Juncus tenuis, Impatiens parviflora* and *Bidens frondosa*. Species from this list that thrive in dry sites are *Erigeron annuus* and *Ambrosia artemisiifolia.*

Based on our estimates other invasive alien plant species represent minor threat to biodiversity due to their local / limited distribution, or due to

Table 3: The estimate of the characteristics of plants related to competitive ability of the taxon, which were the basis for estimation of potential negative influence in certain habitats.
Tabela 3: Ocena značilnosti rastlin, ki vplivajo na kompetitivne sposobnosti taksona in so bile osnova za oceno potencialno negativnega učinka v določenih habitatih.

Taxon	Life span	Plant height	Shading ability	Frequency rank of the taxon	Total estimate
	a	b1	b2		
Robinia pseudacacia L.	4	4	4	4	4.0
Solidago gigantea Aiton	3	2	2	4	2.5
Fallopia japonica (Houtt.) Ronse Decr.	3	3	4	3	2.4
Rudbeckia laciniata L.	3	2	3	3	2.1
Helianthus tuberosus L.	3	2	3	3	2.1
Ailanthus altissima Desf.	4	4	4	2	2.0
Acer negundo L.	4	4	4	2	2.0
Solidago canadensis L.	3	2	2	3	1.9
Parthenocissus quinquefolia (L.) Planch.	4	3	4	2	1.9
Echinocystis lobata (Michx.) Torr. & A. Gray	1	3	4	3	1.7
Elodea canadensis Michx.	3	1	2	3	1.7
Impatiens glandulifera Royle	1	2	2	4	1.5
Juncus tenuis Willd.	3	1	1	3	1.5
Erigeron annuus (L.) Pers.	1	1	1	4	1.0
Pinus strobus L.	4	4	4	1	1.0
Quercus rubra L.	4	4	4	1	1.0
Ambrosia artemisiifolia L.	1	1	2	3	0.9
Impatiens parviflora DC.	1	1	2	3	0.9
Physocarpus opulifolius (L.) Maxim.	4	2	3	1	0.8
Bidens frondosa L.	1	2	2	2	0.8
Pistia stratiotes L.	3	1	4	1	0.7
Aster squamatus (Spreng.) Hieron.	1	1	1	1	0.3

the fact that characteristics of those plant species reflect in their lower invasiveness.

Forest habitats

There are few established neophytes in Central European zonal forest communities (Kowarik 1999), moreover their frequency is also decreasing with increasing altitude. Situation is different in lowland forests, especially in areas with higher mean annual temperature, like the major part of sub-Mediterranean and sub-Pannonian phytogeographic area. Especially in these areas black-locust and tree of heaven seemed to be very invasive tree species. In some areas of Lower Carniola the species *Pinus strobus* is frequent, since it was massively planted in the past.

About 5.1% of the data about presence of invasive alien plant species refers to the forest habitats (Tab. 5). These data include also forest edges, where especially invasive alien herbaceous species occur more often than deeper in the forest stand, where the light intensity in under-storey is low. Considering also hedges, forest clearings and shrublands (together 3.7%), which are more outstanding habitats due to frequent disturbances, this number is higher – i.e. 8.8%. Floodplain woods and swamps, which are subjected to more frequent disturbances and therefore higher possibility of spreading of invasive alien plants along the streams, are presented in a special part. List of invasive alien plant species with potentially most negative influence on the biodiversity in forest habitats are presented in Table 5.

Table 4: List of invasive alien plant taxa with potentially most negative influence on the biodiversity in natural and extensively managed habitats.
Tabela 4: Seznam invazivnih tujerodnih rastlinskih taksonov s potencialno najbolj negativnim učinkom na biodiverziteto v naravnih in ekstenzivno gospodarjenih habitatih.

	No. of data
1. *Robinia pseudacacia* L.	311
2. *Solidago gigantea* Aiton	390
3. *Fallopia japonica* (Houtt.) Ronse Decr. and *F.* × *bohemica* (Chrtek & Chrtkova) J.P. Bailey	152
4. *Rudbeckia laciniata* L.	149
5. *Helianthus tuberosus* L.	97
6. *Ailanthus altissima* Desf.	29
7. *Acer negundo* L.	55
8. *Solidago canadensis* L.	92
9. *Parthenocissus quinquefolia* (L.) Planch.	36
10. *Echinocystis lobata* (Michx.) Torr. & A. Gray	127
11. *Elodea canadensis* Michx.	228
12. *Impatiens glandulifera* Royle	305
13. *Juncus tenuis* Willd.	93
14. *Erigeron annuus* (L.) Pers.	267
15. *Ambrosia artemisiifolia* L.	73
16. *Impatiens parviflora* DC.	99
17. *Bidens frondosa* L.	21

Table 5: Invasive alien plant species with potentially most negative influence on the biodiversity in forest habitats.
Tabela 5: Invazivne tujerodne vrste rastlin s potencialno najbolj negativnim učinkom na biodiverziteto v gozdnih habitatih.

	No. of data
Robinia pseudacacia	28
Solidago gigantea	23
Juncus tenuis	14
Rudbeckia laciniata	10
Erigeron annuus	25
Impatiens glandulifera	12
Impatiens parviflora	13

Species *Robinia pseudacacia* is frequent in hedges, while in shrublands and afforesting areas species like *Solidago gigantea, Robinia pseudacacia* and *Solidago canadensis* are common. Species *Impatiens glandulifera, Rudbeckia laciniata, Solidago gigantea* and *Solidago canadensis* are found in forest clearings.

Floodplain woods and swamps

Neophytes and invasive species among them have great influence on the ecosystem structure and biodiversity in floodplain woods and swamps in the alluvial plains. The establishment of invasive alien plants in these ecosystems is easier due to outstanding impacts of human- or nature-induced disturbances (Kowarik 1999). The highest number of invasive alien plant species can be found in lowland floodplain woods in sub-Pannonian phytogeographic area.

In mentioned forest types 11.8% of the data about presence of invasive alien plant species in the database are found, that is much more than in other forest types (5.1%). Considering also the fact that 60% of Slovenia is covered in forests among which these floodplain woods represent only a small share, the biodiversity in these habitats is probably much more threatened. According to our data invasive alien plants with potentially most negative influence on biodiversity in floodplain woods and swamps are listed in Table 6.

Species *Robinia pseudacacia* and *Acer negundo* are frequent in the tree layer of floodplain

Table 6: Invasive alien plant species with potentially most negative influence on biodiversity in floodplain woods and swamps.
Tabela 6: Invazivne tujerodne vrste rastlin s potencialno najbolj negativnim učinkom na biodiverziteto v logih in močvirnih gozdovih.

	No. of data
Robinia pseudacacia	53
Solidago gigantea	69
Fallopia japonica and F. × *bohemica*	37
Impatiens glandulifera	60
Echinocystis lobata	31
Rudbeckia laciniata	20
Helianthus tuberosus	18
Acer negundo	11
Solidago canadensis	13
Impatiens parviflora	25
Erigeron annuus	27

woods. Liana *Echinocystis lobata* is also frequent. Among the herb invasive alien plant species most frequent are the following species: *Solidago gigantea, Fallopia japonica, Impatiens glandulifera*.

Ruderal habitats

Since these habitats are greatly exposed to antropogenic disturbances they host a high share of neophytes and invasive species, respectively. In general, settlements are starting-points for the spreading of mentioned species as well as the environment where they are most frequent. The share of neophytes in the flora of a settlement increases with the size of a settlement.

Since these man-made habitats host mostly antropogenic vegetation types, the threat to biodiversity is far less important than in natural habitats. The aspects that are problematic are the facts that these habitats (Tab. 7) are the starting-points for the spreading of mentioned species into the natural environment and that traffic infrastructure represents a very important corridor for spreading of invasive alien plants.

Waters

In the mentioned data-base (CCFF), 7.3% of data about invasive alien plant species refers to the two hydrophytes, namely *Elodea canadensis* (92 % of these data) and *Pistia stratiotes*.

Elodea canadensis

According to the data set this species is more frequent in streams (162 data) than in standing waters (66 data) like ponds, oxbow-lakes and various reservoirs.

Kuhar et al. (2010) surveyed 785 km of reaches from 39 Slovenian streams. Mentioned species was found in 12 streams, in 132 reaches (of 1227 investigated) and in 99 km of those streams. Well developed stands of *E. canadensis* were found in 47 km of streams (6% of total surveyed length). Relative biomass of this species was low, what points to the fact, that this species in not invasive in the streams of Slovenia. It was not the only species in any reach, only rarely occurred as dominant species. This species mostly thrives in diverse communities.

The species *E. canadensis* is rarely found in naturally preserved streams (Šraj-Kržič et al. 2007). It prefers the streams running through agricultural landscape and have narrow, more or less disturbed riparian zone with moderate presence of retention structures (enable the growth of such stands). It prefers fine sediment that is a mixture of pebbles, sand, silt and detritus. Species was not found in reaches with higher current velocity and in streams in karst area (Kuhar et al. 2010) due to the frequent and strong changes in water level.

Martinčič et al. (2007) claim that more suitable habitat for this species are eutrophic standing waters with fine and nutrient-rich sediments (e.g.

Table 7: The most frequent invasive alien plant species in the settlements and traffic infrastructure.
Tabela 7: Najpogostejše invazivne tujerodne vrste rastlin v naseljih in prometni infrastrukturi.

	No. of data	
	Settlements including parks	Traffic infrastructure
% of data in data-base	9.2	5.3
Erigeron annuus	68	53
Robinia pseudacacia	45	17
Juncus tenuis	22	*
Solidago gigantea	18	14
Fallopia japonica and F. × bohemica	17	*
Impatiens glandulifera	16	*
Impatiens parviflora	14	13
Rudbeckia laciniata	14	*
Solidago canadensis	12	12
Ambrosia artemisifolia	10	25

accumulation lakes). Important characteristic is the allelopathic potential of the plants against epiphytes and phytoplankton that increase the transparency of water around the *Elodea* stand and contribute to their survival in more productive water (Erhard and Gross 2006).

However, *E. canadensis* did not express its invasive character in Slovenian running waters. In spite of numerous competitive advantages *E. canadensis* could be substituted by other, more successful invasive species *E. nuttallii* what occurred in France (Thiebaut, 2007). *E. nuttallii* was more competitive than *E. canadensis* and vegetative fragments of *E. nuttallii* also had higher survival rates following artificial disturbances than those of *E. canadensis*. *E. nuttallii* occurs in Slovenia in the stands of *E. canadensis*, and it is likely that it becomes a successful alternative for *E. canadensis* in the case of nutrient enrichment. So far this species was found in accumulation lakes on the Drava River (Mazej and Germ, unpublished) and in the Ledava River (Kiraly et al. 2007).

<u>*Pistia stratiotes*</u>

Species was discovered for the first time in Slovenia in the year 2001. It was found in the oxbow-lake near the village Prilipe, which is conditioned with water from the permanent thermal spring. Šajna et al. (2007) noticed a decrease in presence of native submerged macrophytes in this previously species-rich ecosystem. In the year 2004 a thick layer of this species completely covered the water surface all year long. Despite the removal every autumn, this species overgrow the lake every spring (Šajna et al. 2007). The Topla Stream is a potential source for further spreading of this species.

Riparian zones

Similarly like in northern Italy (Assini et al. 2009), Austria (Essl and Rabitsch 2002) or elsewhere in central Europe (e.g. Pyšek et al. 2012), neophytes are very widespread and abundant in the riparian zones along the streams and standing waters in Slovenia. More than 44% of all data about invasive alien plant species in used database (CCFF) refer to riparian zones. Like in several other parts of central Europe invasive alien plants are very frequent in riparian zones along the streams (Essl and Rabitsch 2002, Assini et al. 2009, Pyšek et al. 2012).

Woody or wetland vegetation in riparian zone has exceptional role in maintenance and increase of biodiversity in terrestrial and aquatic ecosystems. Riparian zone is transition between terrestrial and aquatic ecosystems and it is subjected to many unfavourable influences that reflect in its specific structure (Richardson et. al 2007). Riparian zone:

- maintains biodiversity of the landscape and increases the biodiversity of the aquatic ecosystem and the adjacent habitats,
- is the habitat and the corridor for native species,
- influences the quality and amount of organic matter in water ecosystem,
- is a buffer zone, which decreases the pollution from farmlands, prevents bank erosion and has a favourable influence on the ecological condition of the aquatic ecosystem.

Many invasive alien plants are very successful in colonization of the riparian zone. This is a serious problem in Slovenia as well as in Europe. Stream ecosystem is very prone to the spreading of invasive alien plants, especially due to its dynamic hydrology and its role as a corridor for their spreading (Richardson et al. 2007). Invasive alien species spread also to adjacent natural habitats.

<u>*Riparian zones along the streams*</u>

In the riparian vegetation along the streams (riparian reeds and sedges, tall-forbes, willow and alder communities, pioneer vegetation of gravel-beds and sands) stands consisting of invasive alien plants are common. In comparison to other considerably natural plant communities the highest share of alien species can be found here, among them several species are invasive. Invasive alien plants often form dominant stands. In general their share is the highest in riparian zones along the nutrient-rich lowland reaches of the streams. In such reaches the substrate consists mostly of finer particles like sand, clay or detritus.

The high frequency of invasive alien plant species in the riparian zones along the streams could be explained mostly with frequent and strong natural disturbances, which are the consequences of strong alternation of water level (contemporary flooding and sediment deposition as well as bank erosion). Additionally there is also contribution of simple way of spreading along the streams and human influence like eutrophication of stream and catchment area, hydro-technical measures on the banks (bank reinforcements, channel regulations...). Settlements and traffic infrastructure are also common along the streams, facilitating the spreading of invasive alien plants.

28.5% of the data about presence of invasive alien plant species from our data-base refer to their presence in the riparian zones along the streams, which is the highest number among all surveyed habitats. Invasive alien plant species with potentially most negative influence on biodiversity in riparian zones along the streams are listed in Table 8.

Gravel-beds:

Alien species are common in gravel-beds along the middle part of the watercourses in many parts of Central Europe. Among them ruderal plant species like *Erigeron annuus* occur frequently. With increasing altitude along the headwaters, the number of invasive alien species decreases.

Presence of invasive alien plants on gravel-beds slightly differs from the general pattern found in riparian zones along the streams (data from gravel-beds represent 11% of the mentioned data). Taxa with potentially most negative influence on biodiversity on gravel-beds are listed in Table 8.

Riparian zones around the standing waters

Invasive alien plants occur less frequently in the riparian zones or littoral around the standing waters than along the streams for several afore mentioned reasons. These riparian zones contain 15.7% of the data about invasive alien plant species from data-base. The most frequent species are: *Solidago gigantea, Impatiens glandulifera, Robinia pseudacacia, Erigeron annuus, Rudbeckia laciniata, Juncus tenuis, Acer negundo, Solidago canadensis, Echinocystis lobata, Ambrosia artemisifolia, Bidens frondosa, Fallopia japonica, Helianthus tuberosus*.

Riparian zones around the standing waters of human origin:

Invasive alien plants occur in littoral of different types of man-made lentic water bodies which vary considerably in their size. This group of water bodies consists of different types of the ponds (karst ponds, fish-ponds, pools or puddles), accumulation lakes, abandoned clay-pits

Table 8: Invasive alien plant species with potentially most negative influence on biodiversity in riparian zones along the streams.
Tabela 8: Invazivne tujerodne vrste rastlin s potencialno najbolj negativnim učinkom na biodiverziteto v obrežnih pasovih vzdolž vodotokov.

	No. of data	
	riparian zones along watercourses	gravel-beds only
Robinia pseudacacia	101	*
Solidago gigantea	139	10
Fallopia japonica and F. × bohemica	84	10
Impatiens glandulifera	125	14
Echinocystis lobata	63	*
Rudbeckia laciniata	48	*
Helianthus tuberosus	41	12
Solidago canadensis	33	*
Acer negundo	20	*
Erigeron annuus	75	11
Ailanthus altissima	12	*
Impatiens parviflora	36	*
Juncus tenuis	19	*
Parthenocissus quinquefolia	10	*
Ambrosia artemisiifolia	22	*

and gravel-pits filled with water. These water bodies are most common type of lentic waters in Slovenia and together represent nearly one half of the total surface of all standing waters. Invasive alien plant species with potentially most negative influence on biodiversity in riparian zones around the man-made standing waters are listed in Table 9.

Table 9: Invasive alien plant species with potentially most negative influence on biodiversity in riparian zones around the standing waters.
Tabela 9: Invazivne tujerodne vrste rastlin s potencialno najbolj negativnim učinkom na biodiverziteto v obrežnih pasovih stoječih voda.

	No. of data	
	Man-made	Oxbow-lakes
Robinia pseudacacia	66	10
Solidago gigantea	69	31
Rudbeckia laciniata	34	20
Impatiens glandulifera	50	28
Juncus tenuis	33	*
Erigeron annuus	52	10
Acer negundo	10	*
Echinocystis lobata	15	*
Solidago canadensis	11	*

<u>Riparian zones around the oxbow-lakes:</u>

Oxbow-lakes are a special type of lentic ecosystems found also in Slovenia that are being formed in alluvial plains of lowland reaches of the rivers. They are mostly found along the Mura and Sava Rivers, only some cases are found along the Kolpa and Vipava Rivers. The majority of the data about presence of invasive alien plants in these habitats refer to the oxbow-lakes from lower parts of the Mura and Sava Rivers. Invasive alien plant species with potentially most negative influence on biodiversity in riparian zones around the oxbow-lakes are listed in Table 9.

Agricultural land

About 6.3% of the data on invasive alien plant species is connected with agricultural land. Smaller part of these data can be found on the arable land (1.3%), while major part of the data (5%) refers to different grassland types.

Most common invasive alien plant species on arable land are ruderals *Erigeron annuus* and *Ambrosia artemisifolia*.

Most common invasive alien plant species in grasslands are: *Erigeron annuus, Robinia pseudacacia, Solidago gigantea, Solidago canadensis, Rudbeckia laciniata.* Species *Erigeron annuus* was most common in intensively cultivated meadows as well as in dry-meadows. The mentioned ruderal species *Erigeron annuus* and tree species *Robinia pseudacacia* are most frequent invasive alien plant species in grasslands on the territory of Austria (Essl and Rabitsch 2002), especially in drier sites.

Wetlands

<u>Inland wetlands</u>

About 3% of the data on presence of invasive alien plant species come from inland wetlands (swamps, intermittent lakes, wet grasslands). None of those species seemed to be problematic in these habitats. As the species with potentially most negative influence on biodiversity *Solidago gigantea* and *Erigeron annuus* were selected.

<u>Coastal wetlands</u>

Only 1% of the data on presence of invasive alien plant species come from the coastal wetlands. The most common invasive alien plant species is *Aster squamatus*. Besides, widespread black-locust (*Robinia pseudacacia*) is found in these habitats as well.

Ecological preferences of some of the most invasive alien plant taxa

The greatest deviations from moderate Ellenberg indicator values (value 5 is in the middle on the ordinal scale 1-9 according to Ellenberg et al. 1992) were detected in the case of N values that indicate preference for nutrient-rich sites. N values of invasive alien species ranged from 3 to 9 (mean = 7.3), while L values that indicate the preference for open non-shaded sites ranged from 4 to 9 (mean = 7.2). Both deviations were more outstanding in the case of herb species. Predominantly higher values than average were detected also for soil reaction (6.9), temperature (6.4) and moisture of the site (6.3).

Based on these results it can be confirmed that invasive alien plants prefer nutrient-rich, open (e.g. non-forest) sites on soils with basic reaction as well that they thrive better in warm and moist sites. Their invasiveness is potentially the highest in such conditions. Ecosystems where such condition are found are those, where high contents of nutrients are available to plants (fertilization, alluvial deposits) and primary woody vegetation was removed (e.g. degraded riparian zones, degraded floodplain woods, abandoned arable land and intensively fertilized meadows).

The influence of taxon Fallopia × bohemica on the biodiversity

According to the data from literature (e.g. Child et al. 1992, Gerber et al. 2008, Strgulc-Krajšek and Jogan 2011) and our results from previous vegetation periods, we can affirm the known negative influence of invasive plant species on the biodiversity.

Light intensity was measured in three vegetation types / plant communities found in riparian zone along the Glinščica stream. About 20 cm above the grounds the measured values differed significantly. Light intensity in the flood meadows was on average 1200 μmol/m^2 s, in tall-forbs it was 460, while in the stands consisting of the taxon *Fallopia × bohemica* light intensity reached only 20 μmol/m^2 s, that presents less than 1% of full day-light measured during this analysis.

However, unification of site conditions in the stands with dominant taxon *Fallopia × bohemica* and deterioration of light conditions in different habitat types was detected.

Conclusions

Data about the presence of invasive alien plants in specific habitats were extracted from the database Flora of Slovenia (at the Centre for Cartography of Fauna and Flora). The list of the most frequent invasive alien plant taxa in the mentioned database is presented (Tab. 1) as well as the list of invasive alien plants in natural and semi-natural habitats (Tab. 2). In general, the most frequent invasive alien plant species is *Erigeron annuus,* while in natural and extensively managed habitats the species *Solidago gigantea, Robinia pseudacacia* and *Impatiens glandulifera* are more frequent as the afore mentioned species.

An attempt to evaluation of potential endangerment of specific habitats due to the presence of invasive alien plant species was also made (Tabs. 3-4). The lists of invasive alien plants with potentially most negative influence on the biodiversity in different habitats are presented (Tabs. 5-9). In general, taxa with potentially most negative influence on the biodiversity of natural habitats in Slovenia are the following: *Robinia pseudacacia*, *Solidago gigantea, Fallopia japonica* and *F. × bohemica, Rudbeckia laciniata, Helianthus tuberosus*. In the dataset 44% of data about presence of invasive alien plants referred to riparian zones and among these two thirds referred to riparian zones along the streams. These habitats are most infected in Slovenia according to our data. The second highly infected group of natural habitats were floodplain woods. According to Ellenberg Indicator Values the most of the invasive alien plants prefer nutrient-rich and sunny sites.

To get more representative results about the presence and establishment of invasive alien plant species in specific habitats, additional systematic surveys should be done all over the territory of Slovenia. For total estimate of the potential negative effect of invasive alien plant species some other plant characteristics could be included that would better reflect the invasiveness of analysed plant taxa. However, an example of evaluation of potential endangerment of specific habitats due to the presence of invasive alien plant species was made. Further research in this field is needed since these plants present a threat to diversity of native species.

Acknowledgments

This survey was done in the framework of the project Neobiota of Slovenia. The work was possible on the basis of data collected by the members of the Centre for Cartography of Fauna and Flora (Slovenia) who supplied the data extracted from the database Flora of Slovenia that is gratefully acknowledged.

References

Assini, S., Bracco, F., Sartori, F., 2009. Plant invasion in riparian habitats in Northern Italy. In: Celesti-Grapow L., Pretto F., Brundu G., Carli E., Blasi C. (eds.) Plant invasions in Italy, Ministry for the Environment Land and Sea Protection, Rome, pp. 15.

Benesperi, R., Giuliani, C., Zanetti, S., Gennai, M., Mariotti Lippi, M., Guidi, T., Nascimbene, J., Foggi, B., 2012. Forest plant diversity is threatened by *Robinia pseudoacacia* (black-locust) invasion. Biodiversity and Conservation, DOI 10.1007/s10531-012-0380-5

Child, L.E., De Waal, L.C., Wade, P.M., 1992. Control and management of *Reynoutria* species (knotweed). Aspects of Applied Biology, 29, 295-307.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D., 1992. Zeigerwerte von Pflanzen in Mitteleuropa, 2. ed. Scripta Geobotanica, 18, 1–258.

Erhard, D., Gross, E.M., 2006. Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. Aquatic Botany, 85, 203–211.

Essl, F., Rabitsch, W., 2002. Neobiota in Österreich. Umweltbundesamt, Wien, 432 pp.

Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R., Schaffner, U., 2008. Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. Biological Conservation, 141, 646–654.

Gurevitch, J., Scheiner, S.M., Fox, G., 2002. The Ecology of Plants. Sinauer Associates, Sunderland.

Hejda, M., Pyšek, P., 2006. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biological Conservation, 132, 143–152.

Kiraly, G., Mesterhazy, A., Bakan, B., 2007. *Elodea nuttallii* (Planch.) H. St. John, *Myosotis laxa* Lehm. and *Pyrus austriaca* Kern., new for Slovenia, as well as other floristic records. Hladnikia, 20, 11–15.

Kleinbauer, I., Dullinger, S., Peterseil, J., Essl, F., 2010. Climate change might drive the invasive tree *Robinia pseudacacia* into nature reserves and endangered habitats. Biological Conservation, 143, 382–390.

Kowarik, I., 1999. Neophytes in Germany: Quantitative Overview, Introduction and Dispersal Pathways, Ecological Consequences and Open Questions. Texte des Umweltbundesamtes Berlin 18/99, 12–36.

Kuhar, U., Germ, M., Gaberščik, A., 2010. Habitat characteristics of the alien species *Elodea canadensis* in Slovenian watercourses. Hydrobiologia (Den Haag), 656, 205–212.

Larcher, W., 2003. Physiological plant ecology. 4th ed. Springer, Berlin, 513 pp.

Martinčič, A., Wraber, T., Jogan, N., Podobnik, A., Turk, B., Vreš, B., Ravnik, V., Frajman, B., Strgulc Krajšek, S., Trčak, B., Bačič, T., Fischer, M.A., Eler, K., Surina, B., 2007. Mala flora Slovenije. Tehniška založba Slovenije, Ljubljana.

Mazej, Z., Germ, M., 2012. The introduction of invasive alien species *Elodea nuttallii* has influenced the spatial pattern of macrophytes in two impoundments in the Drava River (Slovenia) (submitted).

Pyšek, P., 1998. Alien and native species in Central European urban floras: a quantitative comparison. Journal of Biogeography, 25, 155–163.

Pyšek, P., Chytrý, M., Pergl, J., Sádlo, J., Wild, J., 2012. Plant invasions in the Czech Republic: current state, introduction dynamics, invasive species and invaded habitats. Preslia, 84, 575–629.

Řehounková, K., Prach, K., 2008. Spontaneous vegetation succession in gravel–sand pits: a potential for restoration. Restoration Ecology, 16, 305–312.

Rejmánek, M., Richardson, D.M., Pyšek, P., 2005. Plant invasions and invasibility of plant communities. In: van der Maarel E. (ed.), Vegetation Ecology. Blackwell Science, Oxford.

Richardson, D.M., Holmes, P.M., Esler, K.J., & al., 2007. Riparian vegetation: degradation, alien plant invasions, and restoration prospects. Diversity and Distributions, 13, 126–139.

Roufied, S., Puijalon, S., Viricel, M.R., Piola, F., 2011. Achene buoyancy and germinability of the terrestrial invasive *Fallopia ×bohemica* in aquatic environment: a new vector of dispersion? Ecoscience, 18, 79–84.

Simonová, D., Lososová, Z., 2008. Which factors determine plant invasions in man-made habitats in the Czech Republic? Perspectives in Plant Ecology, 10, 89–100.

Somodi, I., Čarni, A., Ribeiro, D., Podobnikar, T., 2012. Recognition of the invasive species *Robinia pseudacacia* from combined remote sensing and GIS sources. Biological Conservation, 150, 59–67.

Strgulc Krajšek, S., Jogan, N., 2011. Rod *Fallopia* Adans. v Sloveniji. Hladnikia 28, 17–40.

Šajna, N., Haler, M., Škornik, S., Kaligarič, M., 2007. Survival and expansion of *Pistia stratiotes* L. in a thermal stream in Slovenia. Aquatic Botany, 87, 75–79.

Šilc, U., 2010. Synanthropic vegetation: pattern of various disturbances on life history traits. Acta Botanica Croatica, 69, 215–227.

Šilc, U., Čarni, A., Vrbničanin, S., Božić, D., Dajić Stevanović, Z., 2012. Alien plant species and factors of invasiveness of anthropogenic vegetation in the Northwestern Balkans – a phytosociological approach. Central European Journal of Biology, 7, 720–730.

Šraj-Kržič, N., Germ, M., Urbanc-Berčič, O., Kuhar, U., Janauer, G.A., Gaberščik, A., 2007. The quality of the aquatic environment and macrophytes of karstic water-courses. Plant Ecology, 192, 107–118.

Thiebaut, G., 2007. Invasion success of non-indigenous aquatic and semi-aquatic plants in their native and introduced ranges. A comparison between their invasiveness in North America and in France. Biological Invasions, 9, 237–251.

Vilá, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek., P., 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters, 14, 702–708.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R., 1996. Biological invasions as global environmental change. American Scientific, 84, 468–478.

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 39–48

The structure of fish community in the river Mirna

Struktura ribje združbe v reki Mirni

Jasmina Jakopin, Mihael Jožef Toman*

University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenija;
*correspondence: mihael.toman@bf.uni-lj.si

Abstract: Natural perturbations and human pressure increasingly affect the river ecosystems and their biological communities. Fish species are a good indicator of the river status and one of the factors for the assessment of ecological condition by the Water Framework Directive (Directive 2000/60/EC). River Mirna, one of the larger right tributaries of the river Sava, is a good example of anthropogenic pressure on the running water ecosystem. Fish community species structure was analyzed at selected eight sampling sites located over the 28 km length of the river. Abiotic conditions were determined by measuring the physical, chemical and hydro-morphological parameters and the influence of measured parameters on present fish community was analyzed. It has been determined, that the river Mirna is loaded with organic matter and physically changed by regulations, river beds canalizing and bank stabilization. Fish were sampled with standard electro-fishing method to determine species diversity, biomass of the specimens and their size. 29 fish species from seven families (26 native and 3 allochthonous species) were recorded along with one type of the Danubian brook lamprey (*Eudontomyzon vladykovi*). Only two species, *Telestes souffia* and *Cottus gobio*, were present at all eight sampling sites. The largest number of specimens (890) and the largest biomass (5697 g) per 100 m^2 was recorded at the sampling site located after the town as a result of the flow of purified water from the water treatment plant and a large number of invertebrates, which are an important food source. The minimum number of specimens per 100 m^2 (86) and the minimum biomass of fish per 100 m^2 (457 g) was measured at the relatively natural part of the channel. The most common causes for this are low temperatures, less food and a fast, turbulent flow. The study confirms that the changes in abiotic factors induced by anthropogenic activities significantly influence the structure of fish species community.

Keywords: running waters, loading, anthropogenic pressure, fish community, fish biodiversity

Izvleček: Naravne motnje in naraščajoči vplivi človeka vedno močneje vplivajo na rečne ekosisteme in njihove življenjske združbe. Ribje vrste so dober pokazatelj stanja reke in eden od elementov za oceno ekološkega stanja po okvirni vodni direktivi (Direktiva 2000/60/ES). Reka Mirna, eden večjih desnih pritokov reke Save, je dober primer antropogenega vpliva na ekosisteme tekoče vode. Vrstno strukturo ribje združbe smo analizirali na izbranih osmih vzorčnih mestih na dolžini 28 km reke. Z meritvami fizikalnih, kemijskih in hidromorfoloških parametrov smo ugotavljali abiotske razmere in analizirali vplive merjenih parametrov na prisotno združbo rib. Ugotovili smo, da je reka Mirna obremenjena z organskimi snovmi in fizično spremenjena z regulacijami, kanaliziranjem struge in utrjevanjem brežin. Ribe smo vzorčili z elektroagregatom, določili vrstno pestrost, biomaso osebkov in njihovo velikost. Evidentirali smo 29 vrst rib iz sedmih družin (26 avtohtonih in 3 alohtone vrste) in eno vrsto donavskega potočnega piškurja (*Eudontomyzon vladykovi*). Le dve vrsti, *Cottus gobio* in *Telestes souffia*, sta bili zastopani na vseh osmih vzorčnih mestih. Največje število osebkov na 100 m^2 je bilo na vzorčnem mestu za naseljem (890), z največjo ocenjeno biomaso na 100 m^2 (5697 g), kar je posledica dotoka očiščenih vod iz čistilne naprave naselja in večje število nevretenčarjev, ki so pomemben vir hrane. Najmanjše število osebkov na 100 m^2 (86) in najmanjšo biomaso rib na 100 m^2 (457 g) smo ugotovili na izvirnem delu struge. Najpogostejši vzroki za takšno stanje so nizka temperatura, manj hrane in hiter, turbulenten tok. Potrdili smo, da spremembe abiotskih dejavnikov, ki jih sprožijo antropogene aktivnosti, signifikantno vplivajo na vrstno strukturo ribje združbe.

Ključne besede: tekoče vode, obremenjevanje, antropogeni vplivi, združba rib, diverziteta ribjih vrst

Introduction

Aquatic ecosystems are complex systems of living and non-living components, which are governed by homeostatic mechanisms. Human activities reduce the flexibility of homeostatic processes consequently affecting the stability of the natural ecosystem (Tarman 1992). These changes impair the ecological conditions in the river ecosystem. The altered water quality and sediment structure impact the communities of aquatic organisms, including fish.

Fish are good bioindicators of the quality status of a river system. They respond to natural and anthropogenic-induced changes, and are one of the biological elements which help evaluate the ecological status of surface waters according to the Water Framework Directive (Directive 2000/60/EC).

Fish communities in aquatic ecosystems are being affected by several hydro-morphological, physical and chemical factors that vary spatially among different habitats and on a time scale among different seasons and hours within the day itself (Jackson et al. 2001). Specific conditions thus greatly influence the fish community in both the abundance of individual species as well as in the length and biomass of individual specimens (Cattanéo et al. 2002).

The Mirna River flows through the Mirna valley in central Dolenjska region (Slovenia) and has been regulated in several locations due to frequent flooding in the past. Its catchment area is settlements, agricultural land and major industrial plants. The effects of pollution are occasionally acute and result in fish mortality. The structure of fish community of the Mirna River and the effect of human activities on the fish are poorly known.

Materials and Methods

The analyzed location

Research was conducted on the 28.30 km long section of the Mirna River. Eight sampling sites were determined, which contrasted according to different effects of the catchment area. The first two sampling sites (Zagrad and Mirna1) were representative – on these sections no significant anthropogenic influences are present. The third site (Mirna 2) represents a regulated section of the Mirna River and the fourth site (Mirna 3) is located just after the wastewater treatment plant in the city. Three sampling sites (Puščava, Pijavica and Gabrje) were hydrologically diverse and had a different periphyton community and rate of bank regulation. The eighth sampling site (Zapuže) is located 2.6 km before the outflow to the Sava River, where the Mirna River is slow flowing and meandering (Fig. 1).

Research was conducted in August and September. Measurements of physical and chemical parameters (temperature, conductivity, pH level) were performed on a 100 meter section of the riverbed using EUTECH PCD 650 and HACH HQD equipment. The width and depth of the riv-

Figure 1: The Mirna River basin from its source to its mouth with marked sampling sites (scale 1: 121856; Interactive Atlas of the environment, 2006).
Slika 1: Porečje reke Mirne od izvira do izliva z označenimi vzorčnimi mesti (merilo 1: 121856; Interaktivni Atlas okolja, 2006).

erbed were measured and the fish harvesting area was calculated per 100 m^2. Different flow types, shoreline vegetation proportion, shading-regulation of the banks and catchment area were estimated at each sampling site. The AQEM method (2002) (Urbanič and Toman 2003) was used for the quantification of the categories of inorganic substrate.

Fish sampling

Fish were sampled using a standardized electro-fishing method (EN 14011, CEN 2003) with two back-mounted electro aggregates with the power of 0.6 to 1.5 kW. The stunned fish were collected with a net and transferred to vessel containing water and narcotics (5 mL ethyleneglycolmonophenyl ether per 10 L of water). The species of each stunned fish was determined and the weight and total body length were measured. The analyzed fish were returned to the river. To estimate the size of fish populations at each sampling site the procedure was repeated twice.

Statistical analysis

The fish population of the Mirna River was compared among different sampling sites according to species diversity, number of fish per 100 m^2 and biomass (g per 100 m^2).

Species diversity at individual sampling sites were confirmed by Margalef index of species diversity (R_1) and the Shannon-Wiener diversity index (H'), which was calculated using the program Ecological Methodology (Krebs 1999). The value of the Bray-Curtis similarity index (S_{BC}) for each sampling site was calculated with the PAST (http://folk.uio.no/ohammer/past) program and a dendrogram depicting the similarities among sampling sites was prepared.

Results

Analysis of the hydro-morphological, physical and chemical parameters

Hydro-morphological conditions changed longitudinally from the first to the eighth sampling site. The channel expands increasing the water flow. Substrate at the first two sites can be grouped to the category of macro- and mesolital but in the central and lower part acal and psamal prevail. At the last sampling site (Zapuže) the substrate is again grouped in the category of macro- and mesolital. At all analyzed sites slow flow predominates. The riverbed is natural at most of the sampling sites with the exception of Mirna 2, where the river is regulated. At the Pijavica sampling site the embankment is fortified with large stones. The total riparian part of the river is sporadically planted with trees.

Differences in water temperature, pH level and conductivity were minimal among sampling sites and within the expected values for this type of river (Tab. 1).

Table 1: Water temperature (T [°C]), pH and conductivity (G [μS/cm]) at each sampling site.
Tabela 1: Temperatura vode (T [ºC]), pH in elektroprevodnost (G [μS/cm]) na posameznih vzorčnih mestih.

Sampling site	T [ºC]	pH level	G [μS/cm]
Zagrad	16,7	8,4	561
Mirna 1	15,8	8,5	455
Mirna 2	18,0	6,9	551
Mirna 3	16,7	8,3	571
Puščava	20,9	8,3	494
Pijavica	21,8	8,5	502
Gabrje	19,0	8,5	505
Zapuže	19,3	8,7	483

Fish population analysis

29 species of fish from seven families along with one type of the Danubian brook lamprey (*Eudontomyzon vladykovi*), (larvae and adults were present) were determined. The majority of fish species (26) were indigenous only three (3) types of allochthonous (*Oncorhynchus mykiss*, *Carassius gibelio* and *Lepomis gibbosus*) were found (Tab. 2). The biggest diversity of fish species was recorded at Puščava sampling site (24 species) (moderate flow), and the poorest diversity at the first sampling sites (only 6 species). This has further been confirmed by the calculated value of the Margalef index of species diversity (Fig. 2A) and Shannon – Wiener diversity index (Fig. 2B) which were greatest for the sampling site Puščava (R_1 = 7.251 and H' = 3.182) and lowest for the sampling site Zagrad (R_1 = 1.917 and H' = 0.458). The comparison of fish communities according to Bray – Curtis similarity index revealed greatest similarity between the sampling site Pijavica and Gabrje (0.769) (Fig. 3), which shared 14 species. Only two species (*Telestes souffia* and *Cottus gobio*) were present at all eight sampling sites (Tab. 2).

The number of fish at each sampling site was estimated as the number of individuals per 100 m^2. The highest number of fish (890) was recorded at the Mirna 3 sampling site, and the lowest number of specimens (86) at the Zagrad site (the natural riverbed) (Fig. 4A). At all sampling sites individuals from the *Alburnoides bipunctatus*, *Cottus gobio* and *Telestes souffia* species prevailed. The biomass of fish at individual sampling sites is presented in g per 100 m^2. The highest biomass was measured at Mirna 3 site (5697 g/100 m^2) (located after the treatment plant in the city), the lowest at Zagrad (457 g/100 m^2) and Mirna 2 (708 g/100 m^2) sites, located at the start of the river (Fig. 4B).

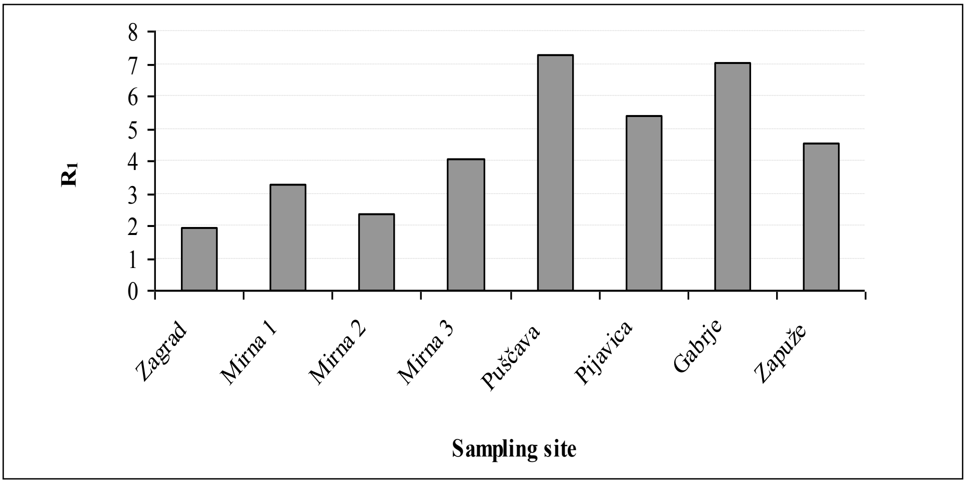
Discussion

Fish communities are suitable bioindicators of the quality of aquatic ecosystems (Feyrer and Healey 2003), since they are affected by various abiotic and anthropogenic factors. Among the abiotic factors, hydro-morphological, physical and chemical factors are extremely important (Jackson et al. 2001). The key anthropogenic factors influencing aquatic ecosystems are river engineering interventions, the type and degree of pollution and the introduction of non-native fish species (Trontelj 2005). All of these elements affects the conditions in aquatic ecosystems and consequently alter the fish communities (Giller and Malmqvist 1998).

Throughout the hydro-morphologically, physically and chemically diverse flow of the Mirna River 29 species of fish were determined. Great species diversity was recorded at sites with a calm, meandring flow (such as Puščava), and only a few fish species were caught at the start of the river (Zagrad). This can be explained by the habitat heterogeneity at the Puščava site where different substrates, meanders and riverbank vegetation were found. Similar conclusions have been reported by Grossman et al. (1998). In contrast, the Zagrad site is characterized by highly homogeneous and fast water flow, high riverbank shading rate (90–100%), which resulted in the lowest fish species diversity.

Low species diversity was also recorded at the regulated channel (Mirna 2). Povž and Sket (1990), Cattanéo et al. (2002), Dekar and Magoulick (2007) observed that river regulation negatively affects the diversity of micro habitats leading to reduced species diversity. Fish are considerably more vulnerable to predators (eg. fish-eating birds) in regulated rivers, because there is no convenient hiding place available (Jackson et al. 2001). Therefore, if hydrological conditions permit the fish will move to other parts of the riverbed with suitable hiding places and spawning (Povž and Sket 1990). The largest number of specimens (890) and the largest biomass (5697 g) per 100 m^2

A



B

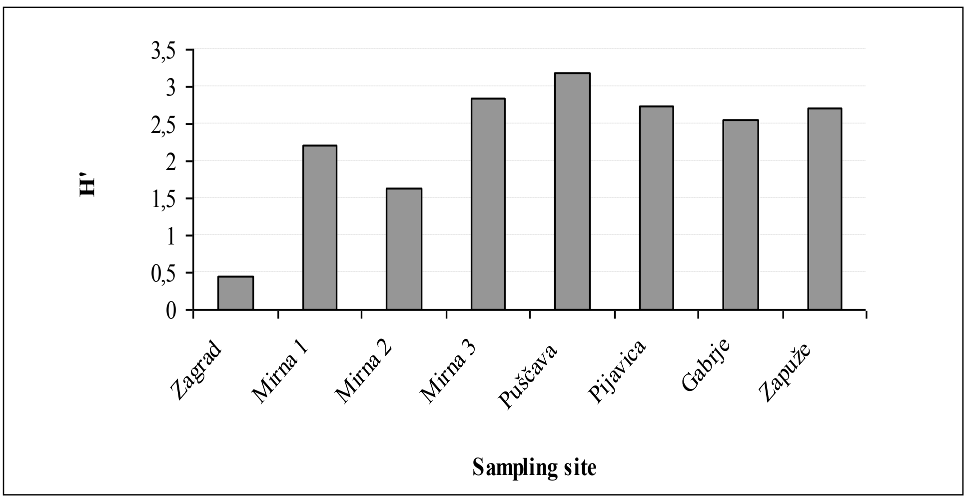


Figure 2: Margalef index (R_1) (A) and Shannon – Wiener index (H') (B) at each sampling site.
Slika 2: Margalefov indeks vrstnega bogastva (R_1) (A) in Shannon – Wienerjev diverzitetni indeks (H') (B) na posameznih vzorčnih mestih.

Table 2: List of fish species found in the Mirna River at each sampling site.
Tabela 2: Seznam ribjih vrst najdenih v reki Mirni na posameznih vzorčnih mestih.

Family	Species	Sampling site SS 1	SS 2	SS 3	SS 4	SS 5	SS 6	SS 7	SS 8
Salmonidae	*Salmo trutta m. fario* (L., 1758)	+	+	+	+	+		+	+
	Oncorhynchus mykiss (Walbaum, 1792)							++	
Cyprinidae	*Carassius carassius* (L., 1758)					+			
	C. gibelio (Bloch, 1782)					++			
	Cyprinus carpio (L., 1758)					+			
	Barbus barbus (L., 1758)					+	+	+	+
	B. balcanicus (Kotlík et al., 2002)		+	+	+	+	+	+	+
	Gobio gobio (L., 1758)				+	+	+	+	+
	Romanogobio vladykovi (Fang, 1943)							+	+
	R. kessleri (Dybowski, 1862)					+	+	+	+
	R. uranoscopus (Agassiz, 1828)								+
	Squalius cephalus (L., 1758)	+	+		+	+	+	+	+
	Rutilus rutilus (L., 1758)		+			+		+	
	R. virgo (Heckel, 1852)					+		+	+
	Telestes souffia (Risso, 1826)	+	+	+	+	+	+	+	+
	Chondrostoma nasus (L., 1758)				+	+		+	
	Vimba vimba (L., 1758)					+			
	Abramis brama (L., 1758)				+		+		
	Alburnoides bipunctatus (Bloch, 1782)		+	+	+	+	+	+	+
	Alburnus alburnus (L., 1758)					+	+	+	
	Rhodeus amarus (Bloch, 1782)				+	+	+	+	
	Phoxinus phoxinus (L., 1758)		+	+	+		+		
Cobitidae	*Cobitis elongatoides* (Bacescu et Maier, 1969)				+	+	+		
	C. elongata (Heckel et Kner, 1858)					+	+	+	+
	Sabanejewia balcanica (Karaman, 1922)					+	+	+	+
Balitoridae	*Barbatula barbatula* (L., 1758)	+		+	+	+	+	+	+
Centrarchidae	*Lepomis gibbosus* (L., 1758)							++	
Percidae	*Perca fluviatilis* (L., 1758)		+		+	+	+	+	
Cottidae	*Cottus gobio* (L., 1758)	+	+	+	+	+	+	+	+
Petromyzontidae	*Eudontomyzon vladykovi* (Oliva & Zanandrea, 1959)	+	+	+	+	+	+	+	

Legend: SS 1 = sampling site Zagrad, SS 2 = sampling site Mirna 1, SS 3 = sampling site Mirna 2, SS 4 = sampling site Mirna 3, SS 5 = sampling site Puščava, SS 6 = sampling site Pijavica, SS 7 = sampling site Gabrje, SS 8 = sampling site Zapuže, + = present native species, + + = present allochthonous species

was recorded at the sampling site located after the town as a result of the flow of purified water from the water treatment plant and a large number of invertebrates, which are an important food source. The minimum number of specimens per 100 m^2 (86) and the minimum biomass of fish per 100 m^2 (457 g) was measured at the relatively natural part of the channel. The most common causes for this are low temperatures, less food and a fast, turbulent flow. The same applies to the regulated

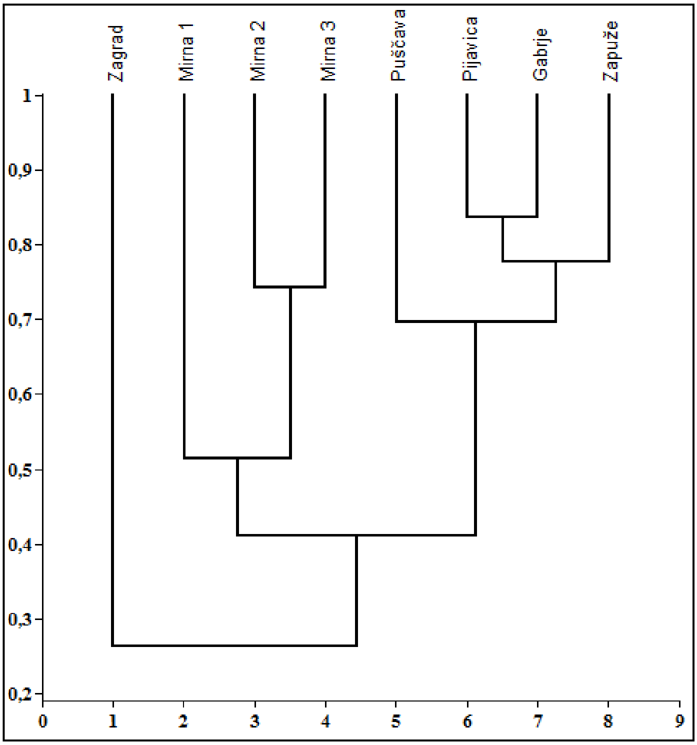


Figure 3: Comparison of different sampling sites based on Bray – Curtis similarity index.
Slika 3: Primerjava različnih vzorčnih mest na osnovi Bray – Curtisovega indeksa podobnosti.

part of the channel, which is also in accordance with the results of other researchers (Pollux et al. 2006, Dauwalter et al. 2007).

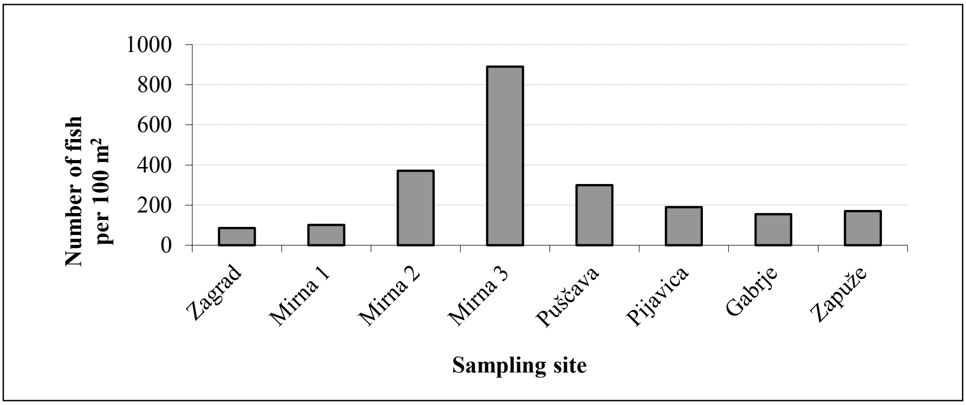
Conclusions

The Mirna river system has a typical longitudinal alteration of abiotic conditions such as stream width, water depth, type of inorganic substrate, type of water flow, embankment regulation and vegetation and fairly similar temperature, pH and conductivity levels. The fish community was analyzed at selected sites along the river and 29 fish species along with one species of Danubian brook lamprey (*Eudontomyzon vladykovi*) were confirmed. 26 species were native and three allochthonous (*Oncorhynchus mykiss*, *Carassius gibelio* and *Lepomis gibbosus*).

According to the Slovenian "Regulations on the classification of endangered plant and animal species on the Red List" (Official Gazette of RS, no. 82/02) 10 endangered species can be found in the Mirna River, namely: *Salmo trutta m. fario, Cyprinus carpio*, *Barbus barbus*, *Rutilus virgo*, *Telestes souffia*, *Chondrostoma nasus*, *Vimba vimba*, *Rhodeus amarus*, *Cobitis elongata*, *Sabanejewia balcanica* and 5 vulnerable fish species: *Romanogobio vladykovi*, *R. kessleri*, *R. uranoscopus*, *Cobitis elongatoides*, *Cottus gobio*. Among the endangered species the Danubian brook lamprey (*Eudontomyzon vladykovi*) has been detected in Mirna River.

The analyzed sites varied according to the number of species present, and the number of specimens and biomass per 100 m^2. The maximum species diversity was determined at Puščava site, where the morphologic parameters were the most heterogeneous. The highest mean number of fish per 100 m^2 and maximum average biomass of fish per 100 m^2 were determined at Mirna 3 sampling site. We assume that this is due to the partially cleaned water from the biological wastewater treatment plant being released to the Mirna River, providing greater nutrient intake, and consequently more fish food.

A



B

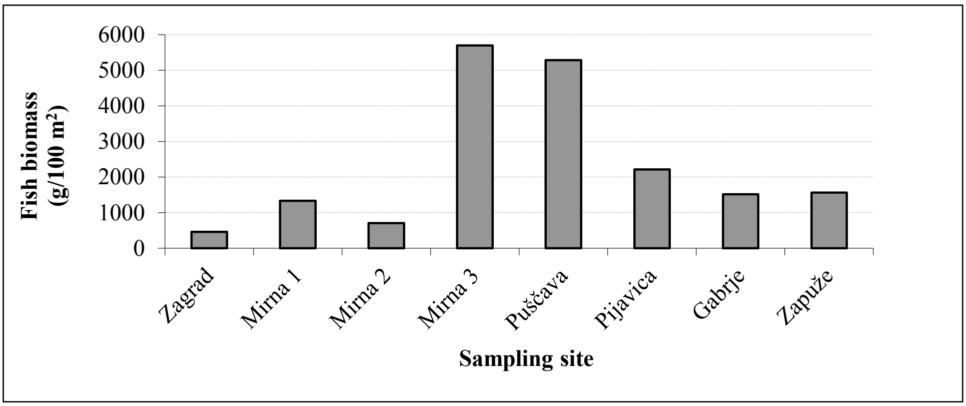


Figure 4: Number of fish per 100 m^2 (A) and fish biomass (g/100 m^2) (B) at each sampling site.
Slika 4: Število rib na 100 m^2 (A) in biomasa rib (g/100 m^2) (B) na posameznih vzorčnih mestih.

Povzetek

Članek govori o vrstni pestrosti strukture ribje združbe na odsekih reke Mirne in podaja ugotovitve o vplivih nekaterih abiotskih in antropogenih dejavnikov na njihovo združbo. Na izbranih delih reke od izvira do izliva smo izmerili in ocenili določene hidromorfološke, fizikalne in kemijske parametre, ki so nam omogočili oceno vplivov na prisotno združbo rib. V reki Mirni smo ugotovili prisotnost 29 vrst rib, od tega 26 avtohtonih in 3 alohtone vrste ter vrsto donavskega potočnega piškurja (*Eudontomyzon vladykovi*). Ugotovljene vrednosti, kot so število vrst, število osebkov na 100 m^2 in biomasa na 100 m^2, so bile na različnih odsekih reke različne in v veliki meri odvisne od abiotskih dejavnikov in oblikovanosti struge. Največje vrstno bogastvo smo ugotovili na srednjem delu toka (Puščava), kjer so bili hidromorfološki parametri najbolj heterogeni, meandrirana struga z mirnim tokom. Pričakovano najmanjše število vrst je bilo na izvirnem delu s homogenim substratom, hitrim vodnim tokom in 100 % zasenčenostjo struge. Vrstno bogastvo na posameznih vzorčnih delih struge smo prikazali z Margalefovim indeksom vrstnega bogastva in s Shannon-Wienerjevim diverzitetnim indeksom. Na podlagi Bray-Curtisovega indeksa podobnosti smo največjo podobnost združb rib ugotovili na štirih vzorčnih mestih pred izlivom Mirne v

reko Savo in na treh vzorčnih mestih gornjega dela struge. Združba rib izvirnega dela (Zagrad) se je od preostalih združb najbolj razlikovala. Največje povprečno število rib na 100 m^2 in največjo povprečno biomaso na 100 m^2 smo določili na delu reke za naseljem, na katerega vplivajo delno očiščene vode iz biološke čistilne naprave. Posledično je v reki večja vsebnost hranil, intenzivnejša obrast in več nevretenčarjev, pomembne hrane za ribe. Najmanjšo povprečno število rib na 100 m^2 in najmanjšo povprečno biomaso rib na 100 m^2 smo pričakovano ugotovili na izvirnem delu struge v Zagradu. Na takšno stanje najpogosteje vplivajo nizka temperatura, pomanjkanje hrane in hiter tok vode.

Acknowledgements

The authors are thankful to Samo Podgornik Ph.D. for the support and assistance in field work and data analysis and to professional colleagues of Fisheries Research Institute of Slovenia (SRC).

References

Cattanéo, F., Lamouroux, N., Breil, P., Capra, H., 2002. The influence of hydrological and biotic processes on brown trout (*Salmo trutta*) population dynamics. Canadian Journal of Fisheries and Aquatic Sciences, 59, 1, 12–22.

CEN, 2003. Water quality – Sampling of fish with electricity, EN 14011.

Councile of the European Communities, 2000. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. Official Journal of the European Communities L327, 1–73.

Dauwalter, D. C., Splinter, D. K., Fusher, W. L., Marston, R. A., 2007. Geomorphology and stream habitat relationships with smallmouth bass (*Micropterus dolomieu*) abundance at multiple spatial scales in eastern Oklahoma. Canadian Journal of Fisheries and Aquatic Sciences, 64, 8, 1116–1129.

Dekar, M. P., Magoulick, D. D., 2007. Factors affecting fish assemblage structure during seasonal stream drying. Ecology of Freshwater Fish, 16, 3, 335–342.

Feyrer, F., Healey, M. P., 2003. Fish community structure and environmental correlates in the highly altered southern Sacramento-San Joaquin Delta. Environmental Biology of Fishes, 66, 123–132.

Giller, P. S., Malmqvist, B., 1998. The biology of streams and rivers. Oxford, Oxford University Press: 296 pp.

Grossman, G. D., Ratajezak, R. E., Crawford, Jr. M., Freeman, M. C., 1998. Assemblage Organization in Stream Fishes: Effects of enviromental Variation and Interspecific Interactions, Ecological Monographs, 68, 3, 395–420.

Interaktivni Atlas okolja, 2006. Agencija Republike Slovenije za okolje

http://gis.arso.gov.si/atlasokolja/profile.aspx?id=Atlas_Okolja_AXL@Arso

Jackson, D. A., Peres – Neto, P. R., Olden, J. D., 2001. What controls who is where in freshwater fish communities the roles of biotic, abiotic, and spatial factors. Canadian Journal of Fisheries and Aquatic Sciences, 58, 1, 157–170.

Krebs C.J., 1999. Ecological methodology. 2nd ed., A. Wesley Longman, NY, USA.

PAST (http://folk.uio.no/ohammer/past)

Pollux, B. J. A., Korosi, A., Verberk, W., Pollux, P., Velde, G., 2006. Reproduction, Growth and Migration of Fishes in a regulated Lowland Tributary: Potencial Recruitment to the River Meuse. Hydrobiologia, 565, 1, 105–120.

Povž, M., Sket, B., 1990. Naše sladkovodne ribe. Ljubljana, Mladinska knjiga: 370 pp.

Tarman, K., 1992. Osnove ekologije in ekologija živali. Ljubljana, Državna založba Slovenije, 547 pp.

Trontelj, P., 2005. Vretenčarji Slovenije: gradivo za vaje iz zoologije strunarjev. Ljubljana, Študentska založba, 95 pp.

Uradni list RS, 2002. Pravilnik o uvrstitvi ogroženih rastlinskih in živalskih vrst v rdeči seznam. Uradni list RS, 82–24. 9. 2002.
Urbanič, G., Toman, M. J., 2003. Varstvo celinskih voda. Ljubljana, Študentska založba, 94 pp.

ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 49–63

Status and distribution of Eurasian lynx (*Lynx lynx*) in Slovenia from 2005 to 2009

Stanje in razširjenost evrazijskega risa (*Lynx lynx*) v Sloveniji v obdobju 2005–2009

Ivan Kos [a]*, Iztok Koren[b], Hubert Potočnik[a], Miha Krofel[a]

[a] Biotechnical Faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
[b] Slovenia Forest Service, OE Tolmin, Tumov drevored 17, 5220 Tolmin, Slovenia
*correspondence: ivan.kos@bf.uni-lj.si

Abstract: In Slovenia, the status of the re-introduced Eurasian lynx population is monitored using the SCALP (Status and Conservation of Alpine Lynx Populations) methodology. Monitoring is organized by the Slovenia Forest Service in cooperation with other institutions and individuals. We analysed the data for the 2005–2009 monitoring period and compared it with the previous periods to explore population status and trends for the northern part of the Dinaric population. During this last pentad we recorded six C1 category data points, 832 data points of category C2, and 96 points of category C3. These numbers are comparable to the previously reported period of 2000–2004. The spatial distribution of signs of lynx presence remained similar compared to the previous period. Presence and status of the lynx are easier to interpret because of additional telemetry data and a habitat suitability that has been produced since the last report. We assume that this lynx population is critically endangered, because of demographic as well as genetic reasons. To prevent local extinction, an active approach is needed for revitalization of the population which would address demographic factors as well as improve the depleted gene pool.

Keywords: Eurasian lynx, *Lynx lynx*, Dinaric population, monitoring, distribution, Slovenia.

Izvleček: V Sloveniji poteka monitoring prisotnosti introducirane populacije evrazijskega risa na osnovi SCALP metodologije v organizaciji Zavoda za Gozdove Slovenije ob sodelovanju drugih inštitucij in posameznikov. Z analizo podatkov in primerjavo s preteklimi obdobji podajamo stanje in trend severnega dela dinarske populacije. V petletnem obdobju 2005–2009 je bilo zabeleženih 6 podatkov C1; 832 C2 in 96 C3 kategorije, kar je podobno kot v preteklem obdobju 2000–2004. Tudi prostorsko so podatki podobno razporejeni po Sloveniji. Predstava o prisotnosti in statusu risa se dopolnjuje s podatki radiotelemetričnega spremljanja in modelom primernega prostora, ki je bil izdelan v zadnjem petletnem obdobju. Predvidevamo, da je populacija kritično ogrožena tako zaradi demografskih kakor tudi genetskih razlogov. Za uspešno varstvo bo treba aktivno pristopiti k revitalizaciji populacije tako s popolnjevanjem in varovanjem demografske in spolne strukture kakor tudi reševanjem osiromašenega genskega sklada.

Ključne besede: Evrazijski ris, *Lynx lynx*, dinarska populacija, monitoring, razširjenost, Slovenija.

Introduction

Eurasian lynx (*Lynx lynx*) was exterminated in Slovenia in the early 20th century. The current population originates from a 1973 reintroduction, when three pairs of animals have been released from quarantine enclosures. These animals were captured for reintroduction in Rudogorje (Slovakia). The newly established population showed incredible dynamics of population growth and spatial expansion (Čop 1994). Relatively intensive hunting was introduced already in 1978 as a part of the population management. Lynx hunting reached its peak in 1990 when 13 animals were legally shot in Slovenia (Kos et al. 2004, Potočnik et al. 2009). The lynx now present in Slovenia belong to the north-western part of the Dinaric population, which is sometimes considered to be divided into Dinaric and Alpine subpopulations (Koren et al. 2006). It is a part of the population that extends to the south-eastern Alps and presents an important source for potential recolonization of the Alps from East. It is therefore crucial for establishment of the potential future pan-Alpine metapopulation. The southern (Dinaric) and northern (Alpine) subpopulations are separated by Ljubljana–Koper highway and additionally by areas of unsuitable habitat (Skrbinšek 2004). Therefore it is important to maintain the connectivity between the subpopulations and adapt conservation efforts accordingly.

Since the reintroduction, there was a considerable effort applied in intensive monitoring of signs of presence and registration of all detected lynx mortalities (Čop 1994, Čop and Frković 1998, Staniša et al. 2001, Koren et al. 2006). However, the estimations of population size that followed were based on misunderstood, often anecdotal data, lack of knowledge and individual (often erroneous) opinions of individual managers, which caused considerable overestimations of the population size with estimates of up to 200 animals (data Slovenian Hunting Association, State`s Hunting Grounds, unpublished). These estimates were the basis for approval of high hunting quotas, and possibly also a promoter of illegal killings, especially after the protection of lynx in 1993 when only exceptional harvest was allowed based on the decision of the competent minister. Systematic, science-based, fine-scale monitoring is a prerequisite for successful conservation, especially if it includes management actions such as hunting, as well as for establishing positive attitudes of different interest groups (Treves 2009, Treves and Naughton – Treves 1999). Uncertainty in population size estimates can cause extinctions in exploited lynx populations (Saether et al. 2010). Lynx monitoring frequently includes high proportion of uncertain data that can give biased results on distribution and abundances (Molinari-Jobin et al. 2012a).

Lynx habitat in Slovenia is fragmented into several habitat patches (Skrbinšek 2004). With exception of Snežnik–Javorniki and Kočevje forest habitat patches, other areas are fragmented by linear barriers or agricultural/urban landscape, with fragments that are frequently the size of only a single average lynx territory, or even smaller. Dispersal and distribution of lynx in such fragmented landscape is very unpredictable. Habitat fragmentation can have considerable effect on occupancy of the patches as well as on adequate sex structure within the patches (Niedzialkowska et al. 2006, Molinari-Jobin et al. 2010, Samelius et al. 2011). Only through systematic and intensive recording of signs of lynx presence categorized according to reliability we can track dynamics and spatial pattern of lynx distribution (Molinari-Jobin et al. 2012a,b). In this paper we present the results of the monitoring of signs of lynx presence and determination of Dinaric lynx population status in Slovenia between 2005 and 2009.

Methods

Data collection

Data on signs of lynx presence have been collected following methodology described by Koren et al. (2006). Data on signs of lynx presence, dead lynx and attacks on domestic animals have been continuously recorded since the population was established population in 1973 (Čop 1994). Since 1994 lynx monitoring has been coordinated by Slovenia Forest Service (SFS) (Staniša 2001, Jonozovič 2004, Koren et al. 2006,). The data are classified into three reliability levels in accordance with the SCALP guidelines (Molinari-Jobin et al. 2012a) and the possibility to verify the collected data: C1: Confirmed "hard facts", verified and undisputable records of lynx presence such as (1) dead lynx, (2) live captured lynx, (3) good-quality and geo-referenced lynx photos (e.g., from camera traps), and (4) samples (e.g. excrements, hair) attributed to lynx by means of scientifically reliable analyses, such as genetic analysis. C2: Records confirmed by a lynx expert (e.g. trained member of the SFS or Slovenian Hunting Society) such as (1) killed livestock or (2) wild prey, and (3) lynx tracks or other assessable signs of lynx presence. C3: Unconfirmed category: observations (kills, tracks, other signs of lynx presence too old or not thoroughly documented, but with description indicating lynx presence) and all unverified observations such as sightings and vocalizations.

Research activities were intensified during the 2005–2009 period with live capturing of lynx, GPS telemetry, monitoring of lynx using hair-traps and photo-traps, prey analysis and habitat and population modelling (Potočnik 2004, Skrbinšek 2004, Krofel 2006, Krofel et al. 2006, Krofel 2008, Skrbinšek and Krofel 2008, Potočnik et al. 2009, Kos et al. 2011, Krofel et al. 2012). Some of these data, particularly from Snežnik–Javorniki area are also included in the report. All reported damages on domestic animals have been inspected by authorized SFS personnel.

Data analysis

All recorded data of lynx signs of presence were entered into the monitoring databases, kept and analysed at the regional unit of SFS in Tolmin. The number of lynx presence data according to their category, including mortality data and damages attributed to lynx, were graphically presented and compared to previous pentads. Lynx population ranges for particular pentads were calculated using geo-referenced lynx presence data of all three categories and fixed kernels, using least square cross-validation (LSCV) approach at 50, 75 and 90% probability area, were prepared (ESRI, ArcGIS 10.0).

Results

Monitoring of lynx distribution

During the 15-year period of collecting and recording signs of lynx presence, coordinated by SFS, 2357 records have been collected according to the SCALP methodology. The number of recorded data have been increasing over the pentads (1995–99: 505; 2000–04: 908; 2005–09: 944). The increase is mainly due to an increasing proportion of C2 data (Tab.1). Majority of the data (71%) were collected in the southern (Dinaric) subpopulation, others in northern (Alpine) subpopulation.

During the last pentad (2005–09) the number of signs of lynx presence has been constantly decreasing from 249 in 2005 to 158 in 2009 (Fig. 1). The decline is most pronounced for C2 data (Fig. 2). In the last pentad there were seven C1 data from photographed or collared lynx and nine data from non-invasive genetic samples (scats, urine, hair), collected during that period. The majority of C1 data from the previous periods referred to killed (shot) lynx. On the other hand, during our study period no dead lynx were reliably recorded (Fig. 3).

In State`s Hunting Reserves (LPNs) that are managed by SFS, daily monitoring of large carnivores has been implemented since 1991. Among them, the highest number of signs of lynx presence during the last pentad was recorded in LPN Jelen (278 km^2), however there has been substantial decline in the last three years (Fig. 4). Similarly, in LPN Medved (379 km^2) the number of records was low compared to the previous periods (Fig. 4). In other LPNs such intensive monitoring was started in 2008.

Distribution of lynx presence

In Slovenia, lynx are present in two areas: (1) Alpine area and (2) Dinaric area. – Sporadic and mostly unconfirmed (C3) data have been reported also from Kamnik–Savinja Alps in the north of Slovenia.

In the last three pentads we did not observe any major change in the range where signs of lynx presence have been recorded. Lynx population ranges (95% fixed kernel) over pentads were 5820, 6270 and 5530 km^2 (Figs. 5–7). The concentrations of the signs remained more or less constant

Data category	Southern subpopulation			Northern subpopulation			Total/country		
	1995 - 1999	2000 - 2004	2005 - 2009	1995 - 1999	2000 - 2004	2005 - 2009	1995 - 1999	2000 - 2004	2005 - 2009
C1	12	7	6	1	0		13	7	16
C2	230	674	770	77	93	62	307	767	832
C3	61	48	7	124	86	89	185	134	96
Total/period	303	729	783	202	179	151	505	908	944

Table 1: Number of data of lynx presence by different SCALP categories.
Tabela 1: Število znakov prisotnosti risa po različnih SCALP kategorijah.

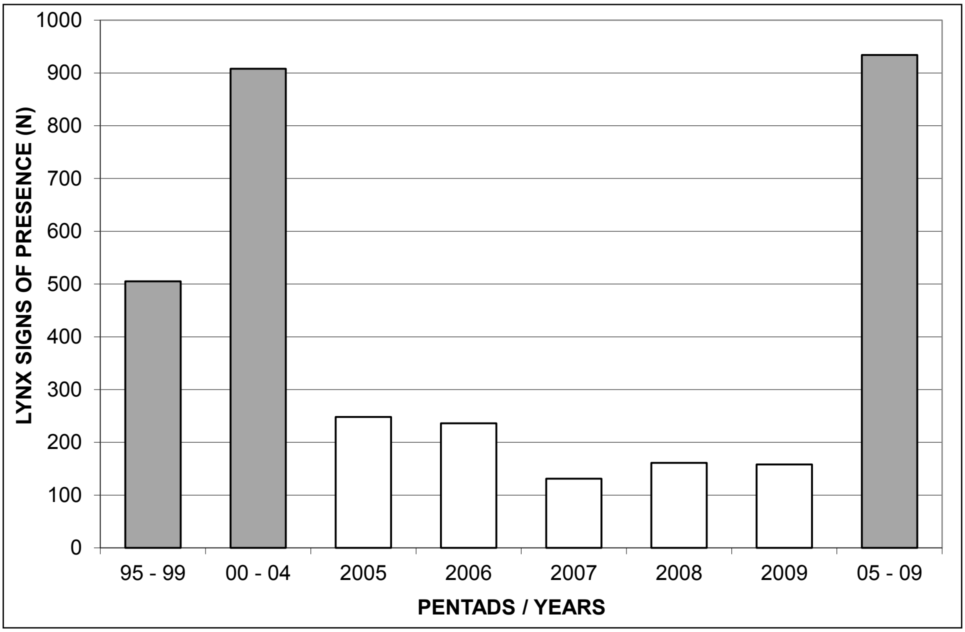


Figure 1: Dynamics of recorded lynx presence data over the pentads between 1995–2009. Lynx presence data in the last pentad – white bars.
Slika 1: Dinamika vseh podatkov monitoringa med petletnimi obdobji v letih 1995–2009. Podatki monitoringa v zadnjem petletnem obdobju – beli stolpci.

only in the area of the Snežnik plateau, whereas in the other areas signs have declined. In the last pentad, compared to the previous monitoring periods, substantial decline of the presence data was detected in south-eastern Slovenia (Kočevsko region) and in the Alps (Figs. 5–7).

Damages attributed to lynx

Inspected livestock damages attributed to lynx during the entire 15 year monitoring ranged between 2 (1996) and 34 (2001) cases and estimated annual value of damages ranged between 400 € and 35,800 € (Tab. 2). Number of reported cases of damages attributed to lynx in last pentad was smaller than in previous pentads and number of killed animals declined for more than 80% compared to the previous period (Tab. 2, Fig. 3).

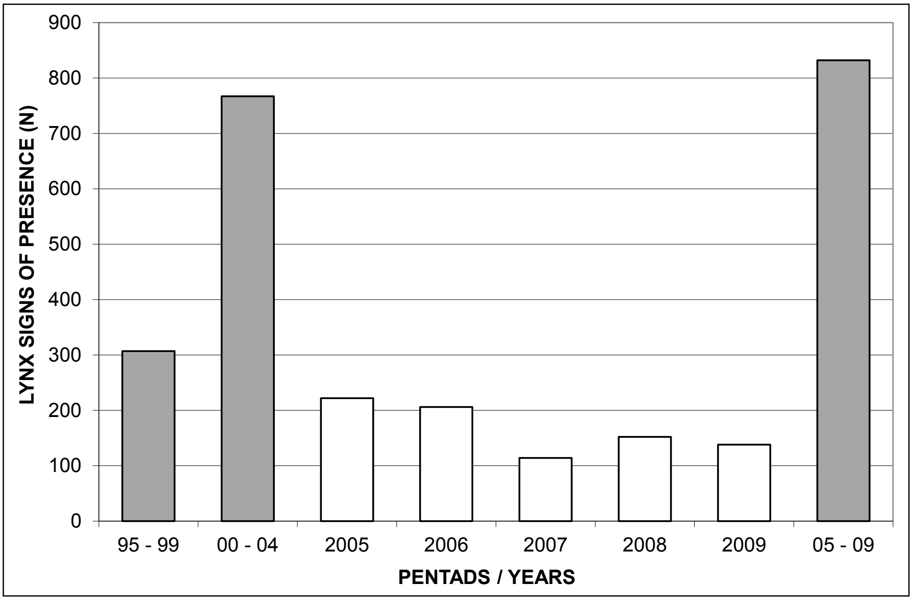


Figure 2: Dynamics of C2-category data collected over the pentads between 1995–2009. Lynx presence data for the last pentad – white bars.
Slika 2: Dinamika zbiranja C2 podatkov med petletnimi obdobji v letih 1995–2009. Podatki monitoringa v zadnjem petletnem obdobju – beli stolpci.

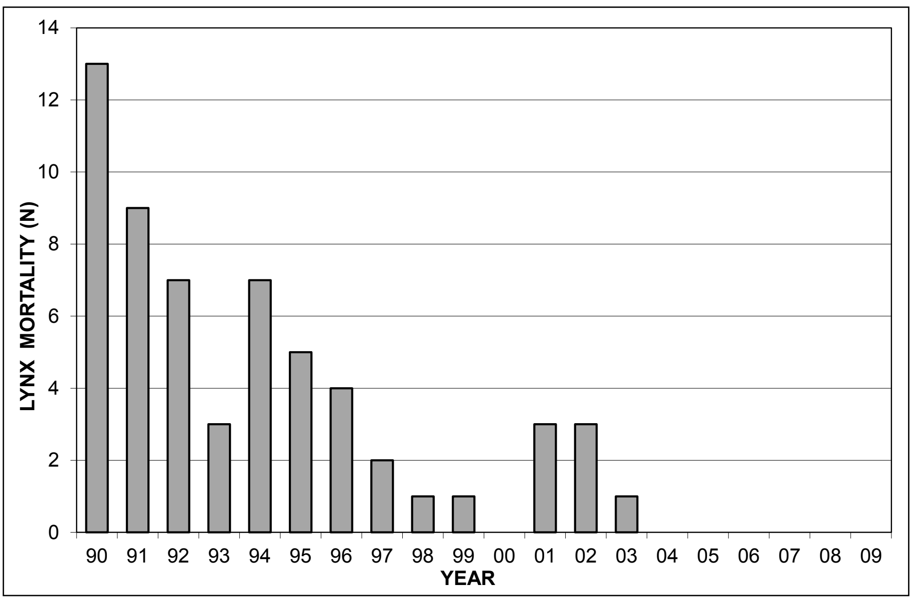


Figure 3: Number of recorded lynx mortality (killed or found dead) in Slovenia from 1990 to 2009.
Slika 3: Število zabeleženih mrtvih (odstreljenih ali drugače poginulih) risov v Sloveniji od 1990 do 2009.

Discussion

Continuous monitoring according to the SCALP methodology (Molinari-Jobin et al. 2012a) provides a basic insight into the lynx population in Slovenia. In the period 2005–2009 the distribution of signs of lynx presence indicates a similar distribution range as in the previous periods. However, the results also indicate that the population size is declining.

It is shown that during the 15 years of monitoring the number and proportion of C2 category data was constantly increasing, while the number of C3 category data declined. Increasing number of experts from SFS and other institutions were getting involved in the lynx monitoring during this period. Also research projects on lynx with intensive field work in the last pentad additionally increased the monitoring effort. Thus the number of the monitoring data over the entire period is not reflecting the lynx population density; however the relative spatial distribution of the data is still a good indicator of the population's spatial distribution. During the last pentad there was only one unverified case of lynx mortality on the Ljubljana–Zagreb highway in 2008 (Poličnik et al. 2010). It was registered in reports of the Slovenian motorway company DARS, but this data is not reliable since the carcass was not preserved, checked or photographed, and it was determined to be a lynx only by an unidentified DARS employee (B. Pokorny, personal communication). During the last pentad the competent Ministry issued no hunting quota. As the lynx population size was estimated to be substantially declining, the decision of the Ministry was that hunting to regulate the population and to maintain higher tolerance for lynx is not eligible anymore (Marn 2008). In the period between 1978 and 1993 lynx were hunted, similarly to Norway for recreational hunting and for other reasons (Herfindal et al. 2005).

Only C3 category data are available in the area of Trnovski gozd in the south-west and Kamnik–Savinja Alps in the north. Although lynx presence in this region currently cannot be confirmed, C3 category data can be useful to indicate potential

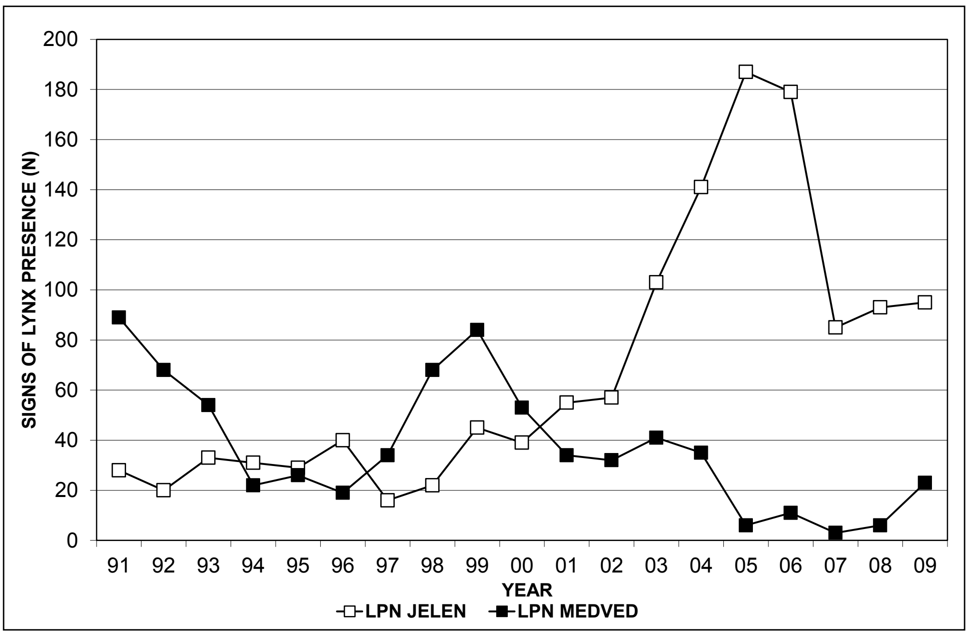


Figure 4: Dynamics of recorded lynx presence data within the monitoring programme in state hunting reserves LPN Jelen and LPN Medved.

Slika 4: Dinamika zbranih podatkov znakov prisotnosti v okviru monitoringa v LPN Jelen in LPN Medved.

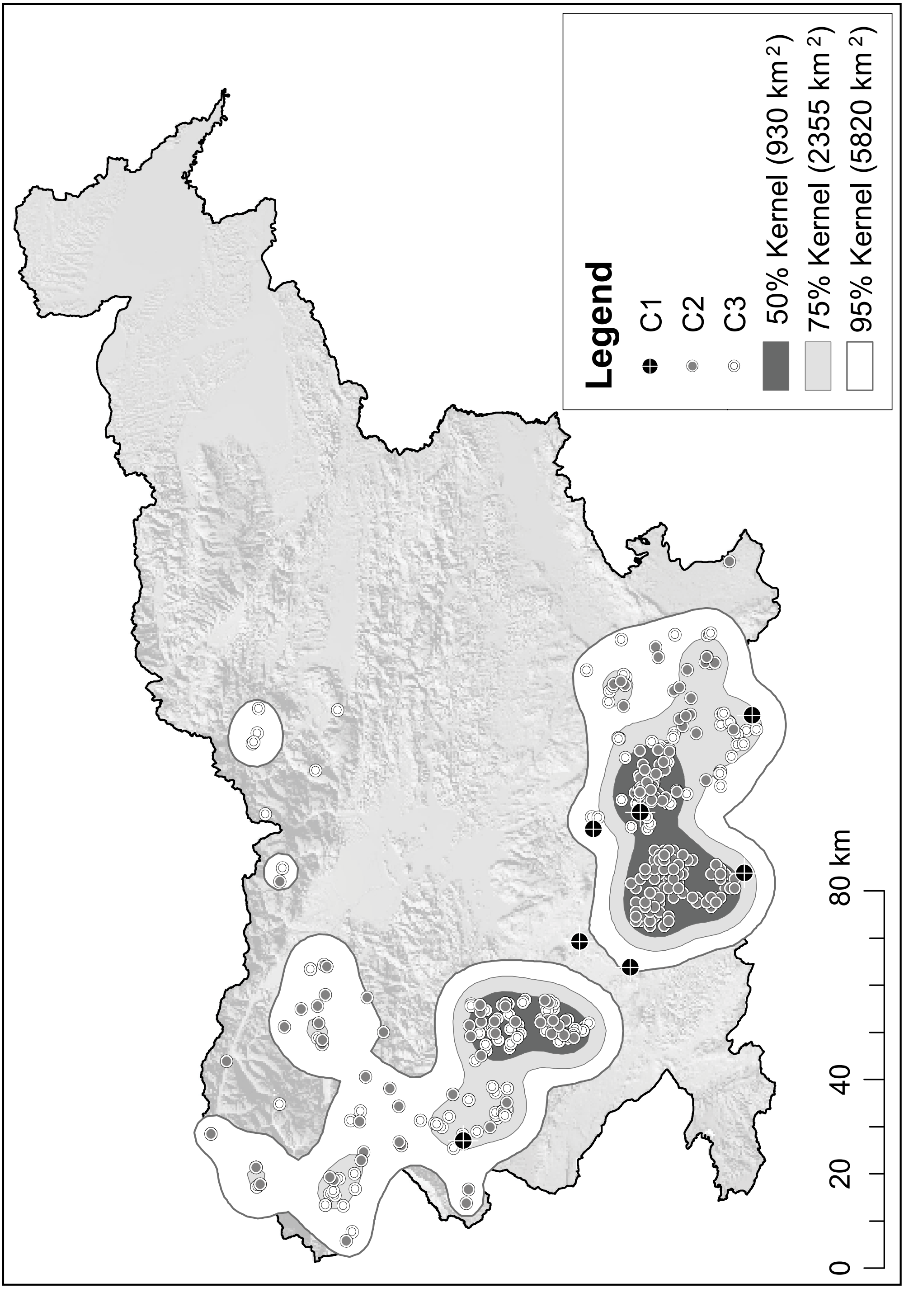


Figure 5: Lynx population range in Slovenia 1995–1999.
Slika 5: Populacijsko območje risa v Sloveniji 1995–1999.

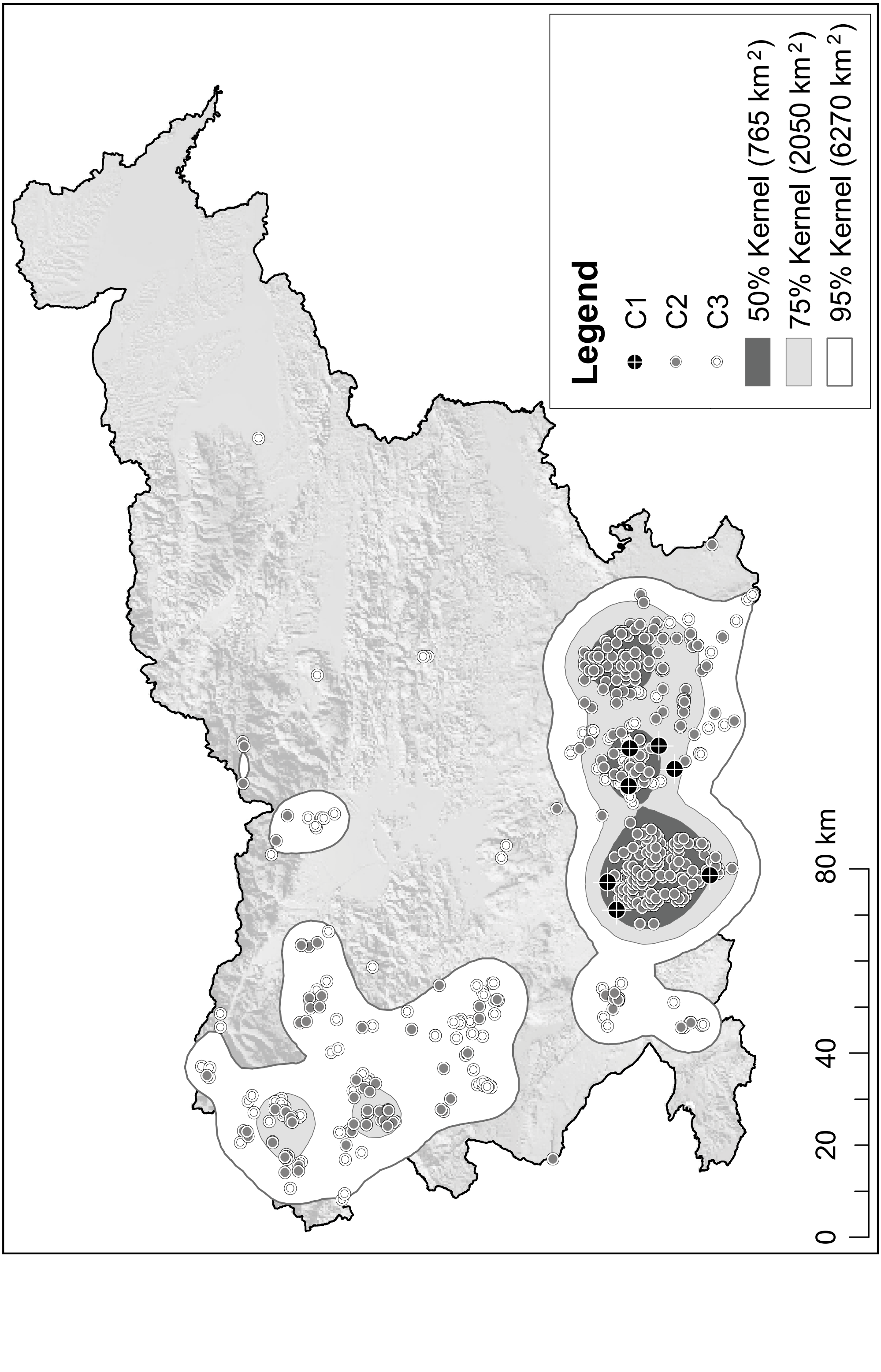


Figure 6: Lynx population range in Slovenia 2000–2004.
Slika 6: Populacijsko območje risa v Sloveniji 2000–2004.

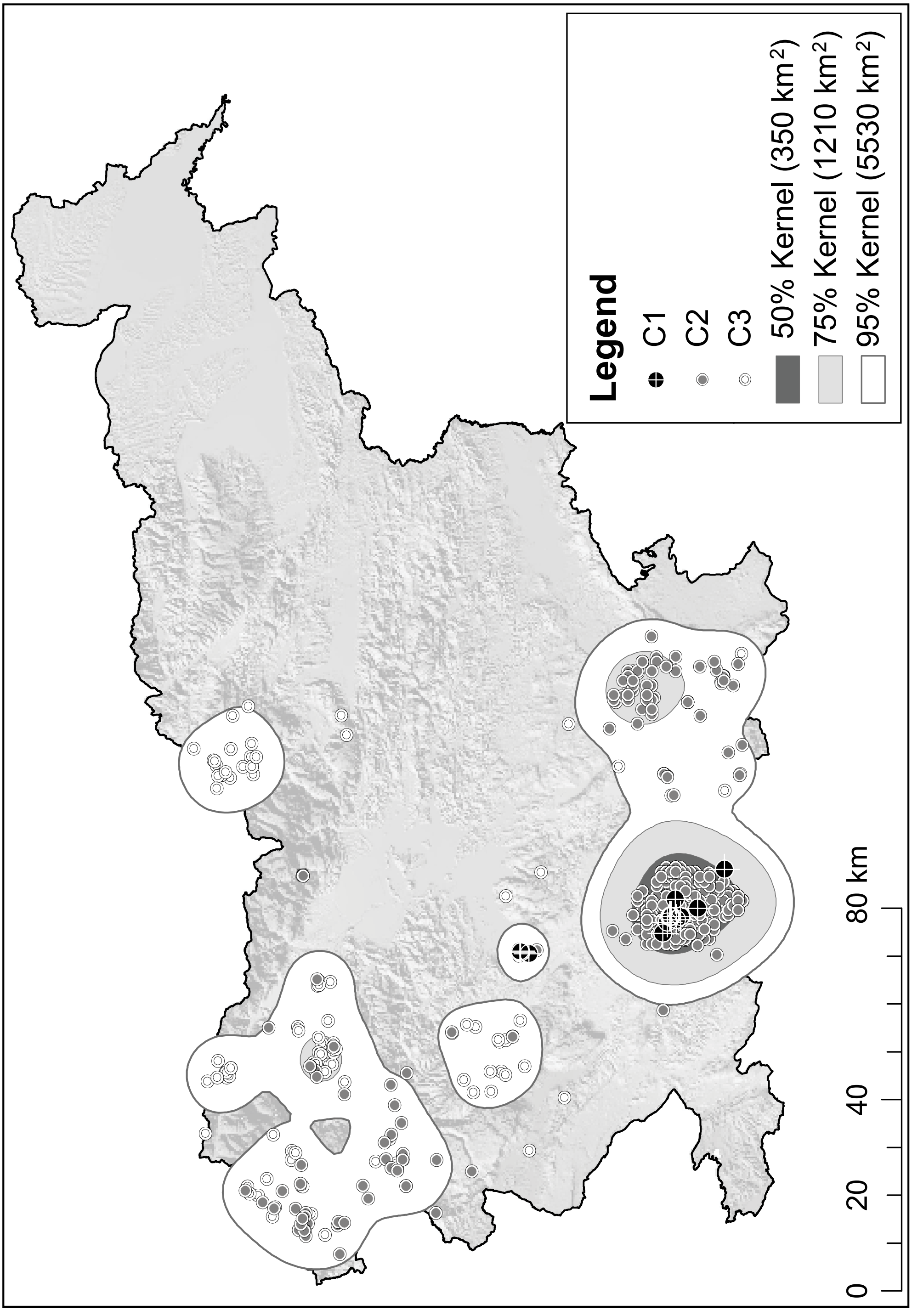


Figure 7: Lynx population range in Slovenia 2005–2009.
Slika 7: Populacijsko območje risa v Sloveniji 2005–2009.

lynx presence (Molinari-Jobin et al. 2012a) and identify the areas where the monitoring effort should be intensified.

In the period 2005–2009 we observed the strongest decrease in the number of lynx records in Kočevsko region. Since the re-introduction there were regularly two reproductive pairs present in forest plateaus Velika gora and Goteniška gora with more or less constant reproduction until 2003 (Huber et al. 1995, I. Kos et al. unpublished data), but only sporadic single animals have been detected in the last pentad. In 2012 one unsuccessful attempt of reproduction which ended with the death of the young GPS-collared female and her three kittens was observed (unpublished data). High numbers of C2 category data as well as C1 data in Snežnik region coincide with telemetry study of two females with kittens and two males (Krofel et al. 2012). Their neighbouring territories extended partially over the border to Croatia. According to reports on lynx sightings in Snežnik–Javorniki region there was probably another reproductive pair in Javorniki area. Snow-tracking, telemetry data (Krofel et al. 2006; Krofel et al. 2012), genetic monitoring (Polanc 2012), hair-trapping (Krofel 2008), photo- and video-trapping (unpublished data), and sightings of lynx in Slovenia indicate regular reproduction only in the Snežnik–Javorniki forest complex that is probably the last permanent reproductive stronghold in Slovenia, and probably inhabited by three reproductive pairs. This region represents also the largest habitat patch suitable for the lynx in Slovenia (Skrbinšek 2004) and continues across border to Croatia. In other regions, especially in the eastern and northern Slovenia, the suitable habitat is more fragmented due to human settlements and agriculture or due to linear barriers like rivers, highways and other infrastructure. In Europe lynx occurrence was predominantly in regions (grid size 50x50 km) with forest cover over 50% (Mikusinski and Angelstam 2004). Lynx was negatively associated to human settlements and transportation infrastructure (Niedzilkowska et al. 2006). Prey and direct human disturbance probably play an important role as well.

There is a potential threat of further lynx habitat fragmentation in Slovenia associated with development of transportation infrastructure (e.g. highway Ljubljana – Zagreb) or with establishing new industrial zones (e.g. Podskrajnik – Rakek) at corridors between forest patches. According to the lynx habitat model in Slovenia (Skrbinšek 2004) there are currently only two large suitable habitat patches (Pohorje and Zasavje) with no signs of lynx presence.

We can expect the data that are collected within a particular habitat patch to frequently refer to only one or very few animals, predominantly males. Among 65 genotyped non-invasive samples (scats, urine), males in the analysed sample represented more than 78% (Polanc, 2012). Males disperse more frequently and over larger distances (Molinari-Jobin et al. 2010a; Samelius et al. 2011). This partially explains sexually biased population structure obtained from the genetic analyses on the population edges and slower colonization rate of lynx toward the south-eastern Alps. Low reproduction due to absence of potential mates associated with fragmentation and possible decreased survival due to human activities is besides unfavourable genetic burden another possible reason for empty habitat patches or patches occupied by only a single lynx.

This re-introduced, bottlenecked population (only three pairs with some already related animals; mother – son (Štrumbelj 1996)) is evidently experiencing a decline, has a very low effective population size and carries a weak genetic legacy. Genetic analyses of Dinaric population showed the lowest genetic diversity of all studied European populations (He = 0.482 and on average 3.11 alleles per locus) (Sindičić et al. submitted). Genetic diversity is dissolving through genetic drift, and is lower for samples collected after the year 2000 (He = 0.42 and average allelic diversity = 2.5). Effective inbreeding in comparison with the source population is 0.22, but raises up to 0.30 in the 2000–2010 period (Polanc 2012, Sindičić et al. submitted). Since lynx appear in low densities, are rarely found dead or handled by researchers, and the amount of research done on the Dinaric population is limited, no signs of inbreeding depression were yet detected. However, inbreeding depression has been proven in captive bred lynx and in some wild lynx in Switzerland (Ryser-Degiorgis 2001, Ryser-Degiorgis et al. 2004), and can be reasonably expected in the Dinaric lynx. Therefore we should assume that there is a need to augment the Dinaric population with animals from another population in the near future. When selecting the animals for

YEAR	Nr.of cases	Nr. animals killed	SIT	EUR
TOTAL 1995-1999	71	116*	11,154,000	25,805
1995	25	?	1,250,000	5,750
1996	2	3	93,000	400
1997	8	13	310,000	1,450
1998	21	66	7,770,000	10,205
1999	15	34	1,731,000	8,000
TOTAL 2000-2004	126	375	16,298,200	71,522
2000	14	49	2,159,000	9,600
2001	34	128	6,502,700	29,100
2002	19	47	2,975,000	13,400
2003	28	58	2,637,000	10,987
2004	31	93	2,024,500	8,435
TOTAL 2005-2009	86	169	5,598,175	23,325
2005	25	67	2,039,803	8,499
2006	15	29	780,132	3,250
2007	8	11	337,440	1,406
2008	18	29	1,021,200	4,255
2009	20	33	1,419,600	5,915

** without year 1995*

Table 2: Damages to livestock estimated to be caused by lynx.
Tabela 2: Ocenjene škode na domačih živalih, pripisane risu.

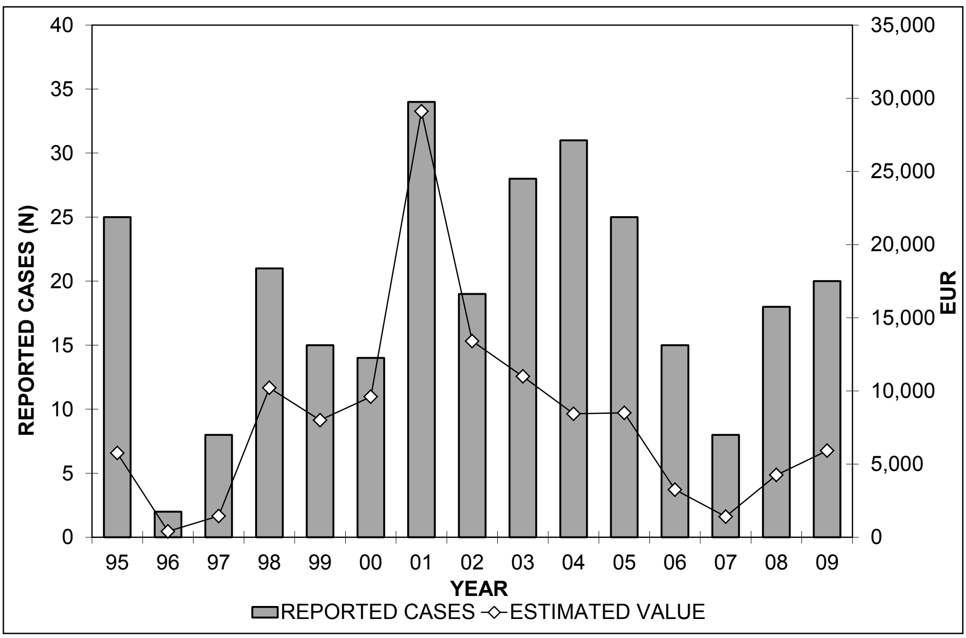


Figure 8: Number of reported cases and estimated value of damages attributed to lynx during the 1995–2009 period.
Slika 8: Število ocenjenih škodnih dogodkov in ocenjena nominalna vrednost škod pripisanih risu v obdobju 1995–2009.

population augmentation, genetic and ecological factors of potential donor populations should be considered (Schmidt et al. 2011, Skrbinšek et al. 2012). Successful conservation of lynx as well as other large carnivores depends mainly on the positive attitudes of the general public and specific interest groups (Røskaft et al. 2007). The only way to ensure survival of the lynx population in a human-dominated landscape is to ensure its coexistence with humans. According to research of public attitudes toward large carnivores, the general public as well as hunters strongly support the idea of augmenting the lynx population in Slovenia (Slana 2010).

Monitoring intensity varies between different regions of Slovenia. For example, the south-western part of the population range in Slovenia (Primorska region) rarely gets snow cover. We can expect a lower detection rate of lynx presence in this areas since a substantial part of the data in other regions comes from snow-tracking. Data from snow-tracking are especially important for detection of females with kittens (Linnel et al. 2007, Andren et al. 2012), thus other methods, like photo-trapping (Zimmermann et al. 2010) or intensive hair-trapping (Demšar 2005, Krofel 2008, Schmidt and Kowalcyk 2006) should be implemented for lynx monitoring in the regions without snow.

When we took into the account the monitoring data presented in this paper, the data on reproduction events (four to five per year), available lynx habitat (as predicted with the habitat model) in the areas where lynx presence was confirmed (manuscript in preparation, 4490 km^2 of suitable habitat in 10 habitat patches of sufficient size) and knowledge about spatial requirements of lynx (eg. Kos et al. 2004), we can conclude that the number of lynx in Slovenia during the 2005–2009 period has declined compared to the previous pentads. On basis of this analysis we can also estimate that 15 to 25 resident lynx may have been still remaining in the population during the reported period (manuscript in preparation). A decrease was detected in the Alpine subpopulation and in the south-eastern part of the Dinaric subpopulation.

Conclusions

(1) In the period 2005–2009 a larger proportion of lynx presence data were registered within the higher quality categories compared to the previous pentads.
(2) No verified lynx mortality has been recorded during the last pentad.
(3) The lynx habitat in Slovenia is characterized by four areas, of which two are areas of (1) southern (Dinaric) and (2) northern (Alpine) subpopulation, one area represents an isolated patch with occasional lynx presence (3) Kamnik–Savinja Alps), and (4) the area of suitable habitat without lynx presence.
(4) During this last 2005–2009 monitoring pentad the population range of the lynx remained approximately the same as in the previous five-year period; however a decrease in relative population density has been detected during the last years of this period.
(5) Compared to the previous five-year periods, there was a decrease in the number of registered attacks of lynx on small livestock.
(6) During the 2005–2009 period the number of lynx in Slovenia seems to have been decreasing. This decrease seems to be the strongest in south-eastern part of the lynx distribution.
(7) The survival of lynx population in Slovenia without an intensive and pro-active conservation program is uncertain.

Acknowledgements

The data for this report were contributed by the personnel of several departments and professional hunting grounds of Slovenia Forest Service, and University of Ljubljana. Some data were collected during research projects funded by the European Union through INTERREG IIIA Neighbourhood Program Slovenia/Hungary/Croatia2004–2006 (project "DinaRis"), by Slovenian Research Agency (project P1-0184), Ministry of Agriculture, Forestry and Food (project V4-0497) and Slovenian Environmental Agency (projects no. 2523-09-100075). We are grateful also to Anja Molinari-Jobin and Tomaž Skrbinšek for review, useful comments and suggestions on the manuscript.

References

Andren, H. Linnell, J.D.C., Liberg, O., Ahlqvist, P., Andersen, R., Danell, A., Franzen, R., Kvam, T., Odden, J, Segerstrom, P., 2012. Estimating total lynx (*Lynx lynx*) population size from censuses of family groups. Wildlife Biol., 8, 299–306.

Čop, J., 1994. Spremljanje naselitve risa (*Lynx lynx* L.) v Sloveniji (1973–1993). Raziskovalna naloga, IGLG Ljubljana, 256 pp.

Čop, J., Frković, A., 1998. The re-introduction of lynx in Slovenia and its present status in Slovenia and Croatia. Hystrix, 10, 65–76

Demšar, P., 2005. Monitoring of carnivores with scent-stations and hair snares in the area of ribniška Velika gora. University of Ljubljana, Biotechnical faculty, Graduation thesis. Ljubljana, 86 pp.

Herfindal, I., Linnell, J.D.C., Moa, P.F., Odden, J., Austmo, L.B., Andersen, R., 2005. Does recreational hunting of lynx reduce depredation loses of domestic sheep. J. Wildlife Manage., 69, 1034–1042.

Huber, T., Kaczensky, P., Staniša, C., Čop, J., Gossow, H., 1995. Luchs – Telemetrieprojekt Kocevska, Slowenien. Abschlussbericht des Projektes, 24 pp.

Jonozovič, M., 2004. Monitoring pojavljanja risa v Sloveniji. In Kos I. (ed.): Lynx in Slovenia, background documents for conservation and management. University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, pp. 133–140. (in Slovenian with English summary)

Koren, I., Jonozovič, M., Kos, I., 2006. Status and distribution of the Eurasian lynx (*Lynx lynx* L.) in Slovenia in 2000–2004 and comparison with the year 1995–1999. Acta Biol. Slov., 49(1), 27–41.

Kos, I., Potočnik, H., Skrbinšek, T., Skrbinšek Majić, A., Jonozovič, M., Krofel, M., 2004. Lynx in Slovenia, background documents for conservation and management. University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, pp. 1–239. (in Slovenian with English summary)

Kos, I., Potočnik, H., Krofel, M., Kljun, F., Skrbinšek, T., Jelenčič, M., Marinčič, A., Ražen, N., 2011. Ugotavljanje stanja populacije evrazijskega risa (*Lynx lynx*) v Sloveniji z uporabo GPS telemetrije. Univerza v Ljubljani, Biotehniška fakulteta, neobjavljeno poročilo za Agencijo Republike Slovenije za Okolje, 32 pp.

Krofel, M., 2006. Predation and feeding habits of Eurasian lynx (*Lynx lynx*) in Slovenian Dinaric mountains. Graduation thesis. Ljubljana, Oddelek za biologijo, Biotehniška fakulteta. Univerza v Ljubljani: 100 pp. (in Slovenian)

Krofel, M., 2008. Možnosti monitoringa s pomočjo vonjalnih količkov. Projekt DinaRis, končno poročilo. Ljubljana, Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani: 16 pp.

Krofel, M., Potočnik, H., Skrbinšek, T., Kos, I., 2006. Movement and predation patterns of eurasian Lynx (*Lynx lynx*) on Menišija and Logatec plateau (Slovenia). Vet. nov. 32 (1/2), 11–17. (in Slovenian)

Krofel, M., Skrbinsek, T., Kos, I., 2012. Use of GPS location clusters analysis to study predation, feeding, and maternal behavior of the Eurasian lynx. Ecol. Res. , on line: DOI10.1007/s11284-012-1005-x, 1–14.

Linnel, J.D.C., Fiske, P., Herfindal, I., Odden, J., Broseth, H., Andersen, R., 2007. An evaluation of structured snow-track surveys to monitor Eurasian lynx (*Lynx lynx*) populations. Wildlife Biol. 13, 456–466.

Marn, A., 2008. Zapisnik seje Strokovne komisije za podporo pri načrtovanju upravljanja z velikimi zvermi v zvezi z izdelavo Strokovnega mnenje za odstrel velikih zveri v letu 2008. Ministrstvo za okolje in prostor; http://www.mko.gov.si/fileadmin/mko.gov.si/pageuploads/podrocja/velike_zveri/zapisnik_komisije_odstrel_zveri_08.pdf

Mikusiński, G., Angelstam, P., 2004. Occurrence of mammals and birds with different ecological characteristics in relation to forest cover in Europe – do macroecological data make sense? Ecol. Bull., 51, 265–275.

Molinari-Jobin, A., Kos, I., Marboutin, E., Molinari, P., Wölfl, S., Fasel, M., Blažič, M., Breitenmoser- Würsten, Ch., Fuxjäger , Ch., Huber, T., Koren, I., 2010a. Expansion of lynx in the Alps. KORA-Ber., 50, pp. 1–17.

Molinari-Jobin, A., Marboutin, E., Wölf, S., Wölf, M., Molinari. P., Fasel, M., Kos, I., Blažič, M., Breitenmoser-Würsten, Ch., Fuxjäger, Ch., Huber, T., Koren, I., Breitenmoser, U., 2010b. Recovery of the Alpine lynx (*Lynx lynx*) metapopulation. Oryx, 44 (2), 267–275.

Molinari-Jobin, A., Kéry, M., Marboutin, E., Molinari, P., Koren, I., Fuxjäger, C., Breitenmoser-Würsten, Ch., Wölf, S., Fasel, M., Kos, I., Wölf, M., Breitenmoser, U., 2012a. Monitoring in the presence of species misidentification: the case of the Eurasian lynx in the Alps. Animal Conserv., 15, 266–273.

Molinari-Jobin, A., Wölfl, S., Marboutin, E., Molinari, P., Wölfl, M., Kos, I., Fasel, M., Koren, I., Fuxjäger, Ch., Breitenmoser-Würsten, Ch., Huber, T., Blažič, M., Breitenmoser, U., 2012b. Monitoring the Lynx in the Alps. Hystrix, 23(1), 49–53.

Niedziałkowskaa, M., Jedrzejewski, W.. Mysłajek, R.W, Nowak, S., Jedrzejewska, B., Schmidt, K., 2006. Environmental correlates of Eurasian lynx occurrence in Poland–Large scale census and GIS mapping. Biol. Conserv., 133, 63– 69.

Poličnik, H., Triglav Brežnik, G., Al Sayegh Petkovšek, S., Flis, J., Zaluberšek, M., Vidmar, M., Pokorny, B., 2010. Assessment of effectiveness of achieved measures for the crossing of wildlife on the Motorway Kronovo–Smednik. 10. slovenski kongres o cestah in prometu, Portorož, 20.–22. oktobra 2010, 907–919.

Polanc, P., 2012. Population genetic of Eurasian lynx (*Lynx* lynx L.) from Slovenia. Dissertation, University of Ljubljana, Biotechnical faculty, 155 pp. (in Slovenian with English summary)

Potočnik, H., 2004. Modeli razvoja dinarske populacije risa. In: Kos I. (ed.): Lynx in Slovenia, background documents for conservation and management. University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, pp. 118–129. (in Slovenian with English summary)

Potočnik, H., Skrbinšek, T., Kos, I., 2009. The reintroduced Dinaric lynx population in PVA simulation: the 30 years retrospection and the future viability. Acta Biol. Slov., 52(1), 3–18.

Røskaft, E., Händel, B., Bjerke, T., Kaltenborn, B.P. 2007: Humanattitudes towards large carnivores in Norway. Wildlife Biol., 13, 172–185.

Ryser-Degiorgis, M.P., 2001. Todesursachen und Krankheiten beim Luchs – eine Übersicht. KORA Bericht Nr, 8, pp. 1–19.

Ryser-Degiorgis, M.-P., Ryser, A., Obexer-Ruf, G., Breitenmoser-Wuersten, Ch., Breitenmoser, U., Lang, J., 2004. Emergence of congenital malformations in free-ranging Lynx from Switzerland: first evidence of inbreeding depression? European Association of Zoo- and Wildlife Veterinarians (EAZWY), 307–311.

Saether B. E., Engen S., Odden J., Linnell J.D.C., Grotan V., Andren H., 2011. Sustainable harvest strategies forage-structured Eurasian lynx populations: the use of reproductive value. Biol. Conserv., 143, 1970–1979.

Samelius, G., H. Andrén, O.Liberg, J.D.C.Linnell, J.Odden, P. Ahlqvist, P.Segerström, K.Sköld, 2011. Spatial and temporal variation in natal dispersal by Eurasian lynx in Scandinavia. J. Zool., on line doi: 10.1111/j.1469-7998.2011.00857.x, 1–11.

Schmidt, K., Kowalcyk, R., 2006. Using scent marking stations to collect hair samples to monitor Eurasian lynx populations. Wildlife Soc. B., 34, 462–466.

Schmidt, K., Ratkiewicz, M., Konopin M., 2011. The importance of genetic variability and population differentiation in the Eurasian lynx (*Lynx lynx*) for conservation in the context of habitat and climate change. Mammal Rev., on line: doi:10.1111/j.1365-2907.2010.00180.x, 1–13.

Skrbinšek, T., 2004. Model primernega prostora za risa v Sloveniji. In Kos I. (ed.): Lynx in Slovenia, background documents for conservation and management. University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, pp. 122–147 (in Slovenian with English summary).

Skrbinšek, T., Krofel, M., 2008. Analiza kvalitete habitata, hrana in kompeticija. Projekt DinaRis, končno poročilo. Ljubljana, Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani: 17 pp.

Skrbinšek, T., Jelenčič, M., Waits, L.P., Potočnik, H., Kos, I., Trontelj, P., 2012. Using a reference population yardstick to calibrate and compare genetic diversity reported in different studies : an example from the brown bear. Heredity, 2012, 109(5), 299–305.

Slana, D., 2010. Aspects of hunters and the general public towards the possible additional settlement of the Eurasian lynx (*Lynx lynx*) in Slovenia. University of Ljubljana, Biotechnical faculty, Graduation thesis, 89 pp.

Staniša, C., 2004. Lynx Survey Europe 2001 – Slovenia. In: VonArx, M,, Breitenmoser-Würsten, C., Zimmermann, F, Breitenmoser, U., (ed.): Status and conservation of the Eurasian lynx (Lynx lynx) in 2001. KORA Bericht no.19, pp. 184–190.

Staniša, C., Koren, I., Adamič, M., 2001. Situation and distribution of the Lynx (*Lynx lynx* L.) in Slovenia from 1995–1999. Hystrix, 12(2), 43– 51.

Štrumbelj, C., 1996. Zvečer smo jih spustili na svobodo... Lovec, 79(4), 145–149.

Treves, A., 2009. Hunting for large carnivore conservation. J. Appl. Ecol., 46(6), 1350–1356.

Treves, A., Naughton-Treves, L., 1999. Risk and opportunity for humans coexisting with large carnivores. J. Hum. Evol., 36 (3), 275–282.

Zimmermann, F., Vogt, K., Ryser, A., Theus, M., Breitenmoser- Würsten, C., Breitenmoser, U., 2010. Abundanc und Dichte des Luchses im nördlichen Schweizer Jura: Fang-Wiederfang- Schätzung mittels Fotofallen im K-I. KORA Bericht Nr. 51, 1–10.

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 65–69

Eurasian lynx (*Lynx lynx*) in the Austrian Alps in period 2005–2009

Evrazijski ris (*Lynx lynx*) v avstrijskih Alpah v obdobju 2005 do 2009

Christian Fuxjäger[a], Jens Laass[b], Anja Molinari-Jobin[c]*

[a]Paracelsusstrasse 10, 4540 Bad Hall, Austria
[b]Färbergasse 2, 3233 Kilb, Austria
[c]KORA, Oltreacqua 2A, 33018 Tarvisio, Italy
*correspondence: a.molinari@kora.ch

Abstract: To assess the status of Eurasian lynx (*Lynx lynx*) in the Austrian Alps we evaluated signs of lynx presence collected from 2005 to 2009. The spatial distribution and the number of records collected (228 versus 225) remained stable compared to the 5-year period 2000–2004. The distribution of the signs of presence showed three clusters: (1) the clearest in Upper Austria (Kalkalpen National Park), (2) in Styria (Niedere Tauern), and (3) in southern Carinthia (Carnic Alps). From other regions, only isolated or unverified records are reported. In fact, based on an analysis of the spatial and temporal distribution of the information we conclude that there is no population established, presumably even reproductive units are lacking. Instead, the scattered observations rather indicate the presence of single individuals only.

Keywords: Alps, Austria, distribution, *Lynx lynx*, status, lynx

Izvleček: Za oceno stanja populacije evrazijskega risa (*Lynx lynx*) v avstrijskih Alpah smo ocenili podatke o znakih prisotnosti risa, zbrane od leta 2005 do 2009. Prostorska razporeditev in število zbranih podatkov je ostalo nespremenjeno v primerjavi s predhodnim petletnim obdobjem 2000–2004 (228 v primerjavi s 225). Razporeditev znakov prisotnosti risa kaže na zgostitve v treh območjih: (1) najbolj očitne zgostitve v Zgornji Avstriji (Narodni park Kalkalpen), (2) na Štajerskem (Nizke Ture) in (3) na južnem Koroškem (Karnijske Alpe). Iz drugih regij, so poročali le posameznih ali nepreverjenih znakih. Na osnovi prostorske in časovne analize porazdelitve podatkov o znakih prisotnosti risa sklepamo, da na teh območjih ni vzpostavljene populacije, verjetno so odsotna tudi razmnoževalna jedra. Sporadična opažanja verjetno kažejo le na pojavljanje posameznih živali.

Ključne besede: Alpe, Avstrija, distribucija, *Lynx lynx*, status, ris

Introduction

In the Austrian Alps all larger, contiguous forest areas can in principle be regarded as suitable lynx habitat. In the eastern part of the Austrian Alps mountain peaks are rather low and rarely reach an altitude of 3000 m and forest cover is high. Even compared to all the Alps this area represents one of the most suitable and most extensive habitats for the lynx.

The origin of the lynx can be attributed to several sources. In the years 1977–1979, a total of nine lynx were reintroduced in the Turrach region (Styria). Furthermore, some lynx are assumed to have immigrated from the then rapidly growing Slovenian population since the end of the 1980s. Rumors about clandestine releases and escaped zoo animals could not be verified. Whether lynx from the Bohemian population have already crossed the Danube and immigrated into the Alps has not been proven yet.

In the frame of SCALP (Status and Conservation of the Alpine Lynx Population, Molinari-Jobin et al. 2010), each Alpine country updates the status and distribution of lynx in the respective territory in a five-year interval. The first status reports for Austria summarized the data from the reintroductions until 1995 (Huber and Kaczensky 1998). The data from 1995 to 1999 were analysed by Huber et al. (2001), and those from 2000 to 2004 by Laass et al. (2006). Here, we give an overview on the development of the status and distribution of lynx in Austria summarizing data from 2005 to 2009.

Methods

The monitoring of lynx in the Austrian Alps consisted of a passive collection of reports on lynx observations and indirect signs of presence. The information is collected by the regional hunting associations and local institutions (e.g. IWJ University of Natural Resources and Applied Life Sciences Vienna, Kalkalpen National Park, Nature Centre Bruck/Mur). Until 2007, the data was collected and evaluated at the IWJ, thereafter the examination of the data was privately organized.

Additionally, the Kalkalpen National Park (Upper Austria) organized a systematic monitoring within the Park boundaries using camera traps and effectuating transects in winter. A minimum of six camera trap stations were active year-round, covering an area of 200 km^2, and seven transects (60 km) were covered simultaneously searching for lynx tracks. All signs of presence are evaluated based on their reliability. The classification was jointly developed by lynx experts from the Alpine countries (Molinari-Jobin et al. 2012):

C1: Confirmed "hard facts", verified and undisputable records of lynx presence such as (1) dead lynx, (2) captured lynx, (3) good-quality and geo-referenced lynx photos (e.g., from camera traps), and (4) samples (e.g. excrements, hair) attributed to lynx by means of scientifically reliable analyses.

C2: Records confirmed by a lynx expert (e.g. trained member of the network) such as (1) killed livestock or (2) wild prey, and (3) lynx tracks or other assessable field signs.

C3: Unconfirmed observations (kills, tracks, other field signs too old or badly documented, where however the description conforms to a lynx sign) and all observations such as sightings and calls which by their nature cannot be verified. Reports that did not seem plausible were rejected from the dataset when we re-examined all records for the analysis.

Results

For the period January 2005 to December 2009, we were able to collect 228 records of lynx presence in the Austrian Alps. Sixty-one percent (140) of these records were classified as C3 data, signs of lynx presence (Table 1). Fifty-six reports (25%) of trained members of the network on prey-remains and tracks were classified as C2 data, 4 of the kills concerned livestock depredation events (1 killed sheep, 1 goat and 2 fallow deer). Additionally, 32 (14%) camera track photos represented hard-facts (C1). During this five-year period no lynx carcass was recovered and no signs of reproduction were reported, as well. Rumours about lynx that escaped from enclosures were reported from Upper Austria and Salzburg, but could not be verified.

The spatial distribution (Fig. 1) and the number of records collected remained stable compared to the 5-year period 2000–2004 (228 versus 225, Table 2). However, the number of C1 data collected increased five-fold while only half as many C2 data were collected. Half of all the records and 85% of C1 and C2 records were collected in and around the Kalkalpen National Park. With the exception of two photos all lynx photos were taken within the National Park Kalkalpen and show the same individual that was photographed the first time in 2000 in the Kalkalpen National Park (Laass et al. 2006). In 2009, two other lynx were photographed in the Pinzgau and in the Tennengau (Salzburg), respectively by two different hunters (Fig. 1). From the Salzburg region no other signs

DATA CATEGORY		2005	2006	2007	2008	2009	SUM
C1	cam.-trap photo	1	6	14	2	9	32
	TOTAL	1	6	14	2	9	32
C2	prey remains	5	4	5		3	17
	tracks	9	7	15	3	5	39
	TOTAL	14	11	20	3	8	56
C3	prey remains	15	13	9	11	5	53
	tracks	6	3	3	2	9	23
	sightings	4	10	18	12	14	58
	vocalisations		2	1	1		4
	markings	1					1
	hair				1		1
	TOTAL	26	28	31	27	28	140
TOTAL		41	45	65	32	45	228

Table 1: Number of data of lynx presence recorded in the Austrian Alps in period 2005–2009 by different SCALP categories.
Tabela 1: Število znakov prisotnosti risa zbranih v avstrijskih Alpah v obdobju 2005 do 2009 po različnih SCALP kategorijah.

DATA CATEGORY		1995-1999	2000-2004	2005-2009
C1	dead lynx	1	1	
	cam.-trap photo		2	32
	analysed scats		3	
	TOTAL	1	6	32
C2	prey remains	7	56	17
	tracks	5	48	39
	TOTAL	12	104	56
C3	prey remains	55	56	53
	tracks	35	23	23
	sightings	34	31	58
	vocalisations		4	4
	markings		1	1
	hair			1
	TOTAL	124	115	140
TOTAL		137	225	228

Table 2: Number of data of lynx presence recorded in the Austrian Alps by different SCALP categories compared by pentads since 1995.
Tabela 2: Število znakov prisotnosti risa zbranih v avstrijskih Alpah po različnih SCALP kategorijah po petletjih od leta 1995.

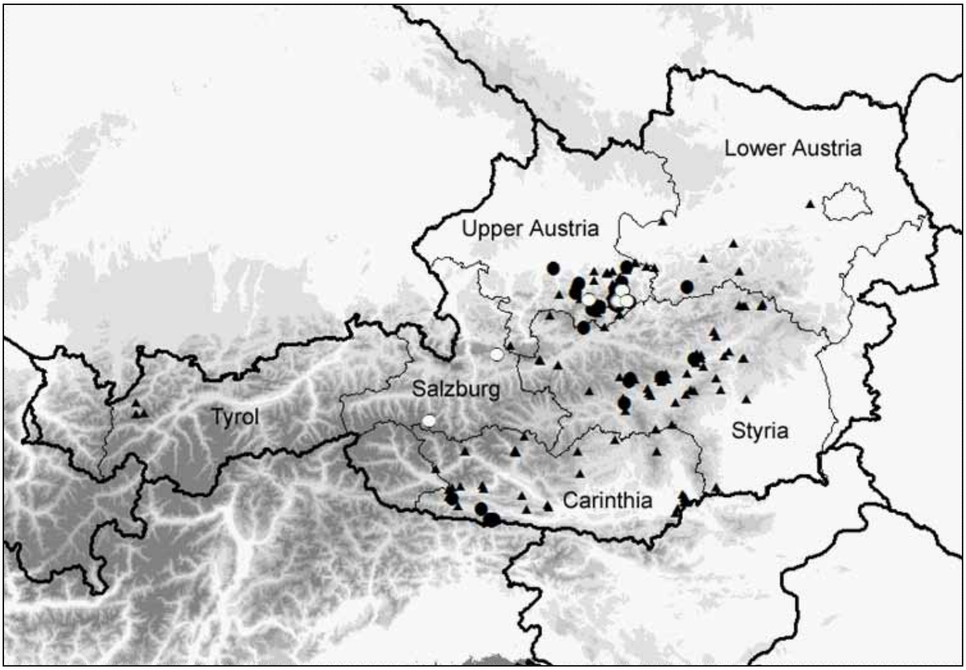


Figure 1: Distribution of lynx signs of presence in the Austrian Alps for the five-year period 2005–2009 (white points = confirmed hard fact data C1; black points = confirmed data C2; black triangles = unconfirmed data C3).

Slika 1: Razporeditev znakov prisotnosti risa v avstrijskih Alpah v petletnem obdobju 2005 do 2009 (beli krožci = potrjeni dokazi C1; črni krožci = potrjeni podatki C2; črni trikotniki = nepotrjeni podatki C3).

of presence were reported in this 5-year period. Isolated C3 observations were reported from Tyrol, Carinthia, Styria and Lower Austria (Fig. 1).

Discussion

In the Austrian Alps, the presence of lynx was ascertained by hard fact data (C1) in Upper Austria and Salzburg, and by confirmed data (C2) in Upper Austria, Lower Austria, Styria and Carinthia; only unverified records (C3) were available for Tyrol (Fig. 1). However, only three clusters are apparent: the clearest in Upper Austria (Kalkalpen National Park), the second in Styria (Niedere Tauern), and the third in southern Carinthia (Carnic Alps). In fact, based on an analysis of the spatial and temporal distribution of the information we conclude that there is no population established, presumably even reproductive units are lacking. Instead, the scattered observations rather indicate the presence of single individuals only.

The uneven density of point observations may not only reflect differences in the presence (or absence) of lynx but also in monitoring effort. With the exception of the National Park Kalkalpen the collection and confirmation of lynx signs of presence in the Austrian Alps depended on private initiatives of a small number of interested individuals. This mostly opportunistic collection of data allowed the estimation of distribution and abundance of lynx in the Austrian Alps only with great difficulty and limitation. Based on the distribution of all observations, we estimate the number of lynx in the Austrian Alps for the reporting period 2005–2009 at 5–10 individuals. The two lynx pictures from camera traps in the Salzburg region give indication of a growing use of camera traps by hunters. We hope that this source will provide valuable information in the future, if it can be collected from the hunters on a regular basis. Still this will not be able to substitute a systematic and active monitoring of the lynx in the Austrian Alps, which would be especially necessary for areas of continuous presence or new occurrences like in the Salzburg area.

Acknowledgements

This report is based on reports of lynx presence collected and submitted by the hunter's associations of Upper Austria, Styria, Carinthia, Vorarlberg and Salzburg, the National Park Kalkalpen, Johannes Rüdisser and Heimo Kienzl. We are thankful for every effort taken to examine reports to confirm signs of lynx presence and for their reporting. We additionally thank Georg Rauer and Peter Gerngross for their valuable comments. Financial and logistical support was granted by the National Park Kalkalpen and until 2006 the University of Natural Resources and Applied Life Sciences, Vienna.

References

Huber, T., Kaczensky, P., 1998. The situation of the lynx (*Lynx lynx*) in Austria. Hystrix, 10, 43–54.

Huber, T., Laass, J., Engleder, T., 2001. Present knowledge on the distribution of the lynx (*Lynx lynx*) in Austria. Hystrix, 12, 31–37.

Laass, J., Fuxjäger, C., Huber, T., Gerstl, N., 2006. Lynx in the Austrian Alps 2000 to 2004. Acta Biol. Slov., 49, 43–49.

Molinari-Jobin, A., Marboutin, E., Wölfl, S., Wölfl, M., Molinari, P., Fasel, M., Kos, I., Blažič, M., Breitenmoser, C., Fuxjäger, C., Huber, T., Koren, I., Breitenmoser, U., 2010. Recovery of the Alpine lynx metapopulation. Oryx, 44, 267–275.

Molinari-Jobin, A., Kéry, M., Marboutin, E., Molinari, P., Koren, I., Fuxjäger, C., Breitenmoser-Würsten, Ch., Wölfl, S., Fasel, M., Kos, I., Wölfl, M., Breitenmoser, U., 2011. Monitoring in the presence of species misidentification: the case of the Eurasian lynx in the Alps. Animal Conserv., 15, 266–273.

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2012 Vol. 55, Št. 2: 71–76

Merozoon vestigatum g. n., sp. n., a new freshwater subterranean isopod (Isopoda: Sphaeromatidae) from a cave in Croatia.

Merozoon vestigatum g. n., sp. n., nov sladkovoden, podzemeljski rak enakonožec (Isopoda: Sphaeromatidae) iz jame na Hrvaškem.

Boris Sket

Department of Biology, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia
Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, Ljubljana, Slovenia.
correspondence: boris.sket@bf.uni-lj.si

Abstract: A pleon of a new freshwater isopod, provisionally attributed to the family Sphaeromatidae (Monolistrini *sensu* Racovitza, 1910) was found in a cave in Dalmacija (Croatia). Although known from such a small part of the body, *Merozoon vestigatum* g. n., sp. n. is easily recognizable. It is doubtlessly a new species which can only be attributed to a new genus in agreement with the criteria up to now used for this genera-group. All pleonites are fused with the pleotelson, the free epimera of the anterior pleonites are very poorly developed, not reaching the pleotelson lateral borders; uropods are strongly reduced, uniarticulate, inserted in the middle of the pleotelson lateral sides. It could be shown that its inclusion into Sphaeromatidae can hardly be challenged, and that its only alternative, Cirolanidae, is very unlikely.

Keywords: Isopoda, Sphaeromatidae, taxonomy, subterranean, Croatia.

Izvleček: Pleon novega sladkovodnega izopoda, ki ga pripisujemo družini Sphaeromatidae (skupini Monolistrini *sensu* Racovitza, 1910) je bil najden v jami v Dalmaciji (Hrvaška). Čeprav poznamo le tako majhen del njegovega telesa, je *Merozoon vestigatum* g. n., sp. n. zlahka prepopznaven. Je nedvomno nova vrsta, ki jo lahko v skladu z znanimi lastnostmi te skupine rodov pripišemo le novemu rodu. Vsi pleoniti so zliti s pleotelzonom; proste epimere sprednjih pleonitov so zelo slabo razvite in ne dosegajo zunanjih robov pleotelzona; uropodi so močno pokrneli, enočlenski, izraščajo iz sredine bočnih strani pleotelzona. Izkaže se, da bi le stežka osporavali pripadnost novega taksona družini Sphaeromatidae, edina alternativa, družina Cirolanidae, je skrajno neverjetna.

Ključne besede: Isopoda, Sphaeromatidae, taksonomija, podzemeljski, Hrvaška.

Introduction

Beside two genera and more than 30 taxa of the species category (Sket 1986a), a number of undescribed taxa of the *Monolistra*-group (Monolistrini *sensu* Racovitza 1910) are present in the department's collections. Here is described a putative monolistrine, representing a new, biogeographically very intriguing genus. It was decided to describe this new taxon although only a pleotelson with uropods is available, since the remote locality was visited and thoroughly sampled more than fifteen times without success, during more than 35 years. Further ignorance of taxonomically and biogeographically so intriguing animal would not be reasonable. Its novelty and uniqueness is out of doubt and even its taxonomical position can only hardly be challenged. The genus-value of the pleon articulation within the actual sphaeromatid system is well established and the position of uropods could only hardly be attributed to a non-sphaeromatid isopod. So, the taxonomic name will persist and can only be attributed to the same taxon when an entire animal will be discovered in next decades, without arising confusion in taxonomy.

Merozoon gen. n.

Diagnosis

Monolistra-shaped pleotelson (Figs 1-2) vaulted (half-hemispherical), without caudal foramen or ventral channel. No complete dorsal articulation within the entire pleon-pleotelson region. First pleonite with epimeral parts poorly developed (weaker than in *Caecosphaeroma* or *Monolistra*); second (pseudo)pleonite with epimeral parts only slightly longer than in the first one, apically rounded, not elaborated as in the above mentioned genera, not reaching ventro-lateral pleotelson margins. Uropods strongly reduced, uniarticulate, attached in the middle of pleotelson lateral parts. Other body parts unknown.

Type and the only species

Merozoon vestigatum sp. n.

Etimology

'Meros' in Greek, a part (of); zoon, in new Latin, 'animal'.

Merozoon vestigatum sp. n.

Material examined (holotype): A complete pleon with pleotelson and uropods, without pleopods. Collected in cave Šipun špilja, Cavtat near Dubrovnik, Croatia, September 1975, coll. B. Sket; deposited in the invertebrate collection of Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani.

Diagnosis

Characters of the genus. The milimeter long and wide pleotelson smooth, without any ornamentation. Uropods strongly reduced, uniarticulate, their length approximately 35% of pleotelson length, with some setae in their apical part.

Etimology

'Vestigatum' (vestigatus) in Latin, 'searched (for)'- due to the author's 35 years of searching for a more complete specimen.

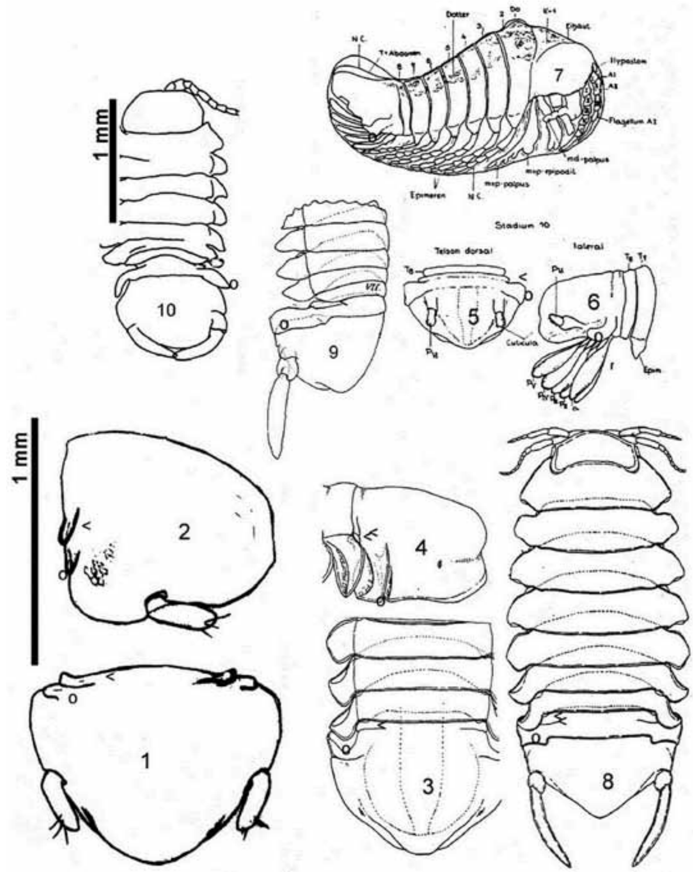
Distribution and Ecology

The fragment was found in the anchihaline lake of the cave Šipun in southern Dalmacija (Dalmatia). It was found together with the freshwater *Proasellus anophthalmus* ssp., in the limnic layer of the mixohaline water body.

Remarks

The pleotelson in concern matches the characters of Sphaeromatidae as described by Harrison and Ellis (1991) and by Poore (1994), but with a progression in the fusion as described for the *Monolistra*-group (Monolistrini) by Racovitza (1910) and Sket (1965). The pleotelson is very variably fused also in some Australian marine sphaeromatids (Bruce 1993, 2003; Poore 1994). All Australian species with fused pleons are very different from the "northern" sphaeromatids in many other morphological characters. Being also geographically so remote, the similarity in the pleon architecture is unquestionably a homoplasy.

Among the Northern Hemisphaere taxa, regarding the fusion of the pleonal regions, the new taxon resembles most closely the genus *Caecosphaeroma*, and less *Monolistra* (Figs 3-4, 8). The two pairs of epimera, however, in both are the latter, however, are much better developed, they reach the pleotelson margins and are included into its anterior ventro-lateral angle. The pleon-pleotelson in *Caecosphaeroma* equals in its shape the same in the related (Racovitza 1910, unpublished molecular data) *Monolistra*, except for the lack of any suture.



Figures 1-10: figs 1-2, *Merozoon vestigatum* sp. n., g. n., pleotelson dorsal and left lateral aspect (with scale bar); figs 3-4, adult *Caecosphaeroma burgundum* Dollfus, pleotelson with posterior pereonites; figs 5-6, stad. 10 intramarsupial stages of *C. burgundum*; fig. 7, stad. 9 of the same; fig. 8, adult *Monolistra caeca* Gerstaecker; figs 9-10, intramarsupial manca stad. of *Monolistra caeca* (10 with scale bar); < – points to the epimeral part of the (pseudo)pleonite I; 0 – of the (pseudo)pleonite II. 3–4, 8 – from Racovitza, 1910; 5-7 – from Daum, 1954; 10 – from Sket, 1965.

Slike 1-10: sl. 1-2, *Merozoon vestigatum* sp. n., g. n., pleotelzon od zgoraj in z leve (z merilom); sl. 3-4, odrasla *Caecosphaeroma burgundum* Dollfus, pleotelzon z zadnjimi pereoniti; sl. 5-6, *C. burgundum,* intramarsupialni stadij 10; sl. 7, stadij 9 iste; sl. 8, odrasla *Monolistra caeca* Gerstaecker; sl. 9–10, *Monolistra caeca*, intramarsupialni stadij manca (10 z merilom); < – kaže na epimere (psevdo)pleonita I; 0 – ob (psevdo)pleonitu II. 3-4, 8 – po Racovitza, 1910; 5-7 – po Daum, 1954; 10 – po Sket, 1965.

Vestigial epimera can hardly be either a juvenile character or a paedomorphy, as in supposedly related *Monolistra* spp. and *Caecosphaeroma burgundum* Dollfus, the epimera are well developed already in the intramarsupial *manca* stages, or even in the embryo (Figs 5-7, 9-10; see Sket 1965: Plate 26, Fig. 4, Plate 27, Figs. 1–4; Daum 1954: Table 8, Figs. 1, 3). Also the genus specific final degree of the pleon-pleotelson fusion is reached at the mentioned stages. The available *Merozoon* pleotelson evidently belonged to a free (not marsupial) young or to a mature individual, since it is well sclerotized, while intra-marsupial animals exhibit a soft cuticle and their pleotelsons are less vaulted. The uniarticulated uropod of the new taxon resembles the uropods of *Caecosphaeroma* *virei* Dollfus, 1896, in its position as well as in its size, although it is less reduced.

The degree of fusion of pleonites within the pleotelsonal region proved to be a useful character for genus definitions in the monolistrines. It characterizes two geographically well delimited groups of species which appeared to be also molecularly related, but very distinct (unpublished data).

The anterior pleonites in Sphaeromatidae are evidently generally partly reduced (pleonite I) and partly fused. In the second pleonite, sutures indicate a fusion of 2-3 somites (Racovitza 1910, Kusakin 1979), therefore Racovitza (1910) calls them warily pseudo-somites. Probably, more than just one pleonite is fused into the pleotelson without a track (without a residual suture). This is most likely the reason why uropods in Sphaeromatidae are only exceptionally inserted at the anterior angle of the pleotelson (see Kusakin 1979), they are usually placed away from it. Such is the situation in the *Monolistra*-group as well as in our new taxon.

While all other sphaeromatid genera with progressively fused pleon are marine and distributed in the Southern Hemisphere, the south-European *Monolistra*-group is biogeographically and morphologically an acceptable candidate for the inclusion of this new taxon.

Another possible candidate for the inclusion of the new taxon would be the family Cirolanidae. In most cirolanids, the pleonites I-V are free while only pleonite VI is fused with the telson and therefore uropods are attached at the antero-lateral angles of the pleotelson, immediately behind the last pleonal epimera (compare Racovitza, 1912, Monod 1972, Botosaneanu et al.1986). Such is the position of uropods also in *Kensleylana* Bruce and Herrando-Perez (2005) in which the pleon articulation is extremely reduced, but the epimera of the last free pleonite are normally developed, reaching the body margin.

Particularly the insertion points of uropods make the cirolanid affinities with the new taxon very unlikely.

The genus status of this taxon can hardly be challenged. In several tens of taxa of the genus *Monolistra* as well as in both species of *Caecosphaeroma* the pleon is characteristically uniformly structured. However, our sample might belong to a deformed specimen; but among several hundreds of *Monolistra* specimens we never met anything similar. Another problem might present the phylogenetic position of the new taxon. It could be phylogenetically nested within the genus *Monolistra* and thus render *Monolistra* paraphyletic. Although – considering the kind of the difference – *Monolistra* and *Merozoon* are more probably sister taxa, the above mentioned case is impossible to be excluded without a DNA analysis. But, there are only very few genera which have been verified such a way, and this has nothing to do with the deficiency of the specimen.

Ecology and Biogeography

The nature of the collection locality leaves little doubt that the pleotelson belonged to a freshwater cave animal. The anchihaline cave water in Šipun is mixohaline, i. e. brackish and even euhaline in deeper layers, with a rich anchihaline fauna (see Sket 1986b: Fig. 6; 2004: p. 64). Its surface layer is usually highly diluted and after heavy rains probably absolutely limnic. Together with this fragment of a supposed subterranean sphaeromatid, a living specimen of the freshwater asellid *Proasellus anophthalmus* ssp. was found, also at this occasion only. Very likely, both animals had been drifted into the lake's shallow from the fissure system after a strong rainfall. In other localities of the Dinaric karst, numerous other cave animals were found in similar circumstances, including *Monolistra* spp. (Sket et al. 2004).

The collection site is close to the southeastern border of the distribution for *Monolistra* (Sket 1986a). It has been emphasized that a number of *Monolistra* spp. had invaded continental waters independently from the sea, and that some species invaded caves independently from the surface (Sket 1986c). The morphologically closest genus, *Caecosphaeroma*, is geographically remote from the new taxon, its area in western Europe is separated from the new taxon locality by an area not inhabited by monolistrines, followed by a wide belt of the less similar *Monolistra* species. Therefore, it may be supposed that all three genera (*Monolistra, Caecosphaeroma, Merozoon*) originated from separate freshwater invasions. The highly apomorphic character of the only facultatively anchihaline *Monolistra* species, *M. radjai* Prevorčnik and Sket (2007), most probably represents a secondary adaptation to the saline water. If this supposition is correct, no (very) close relative of the subterranean *Monolistra* group is living in the sea recently.

Povzetek

Iz evropskih sladkih voda sta znana dva rodova mokric krogličark (družina Sphaeromatidae). V zahodni Evropi sta doma dve vrsti rodu *Caecosphaeroma*, v Južnih apneniških Alpah in Dinaridih pa je kar nekaj deset vrst rodu *Monolistra*. Vse so troglobiotske. V dalmatinski jami je bil najden le pleotelzon drugačne vrste, a kljub več obiskom v tej jami skozi 35 let, nismo našli popolnejšega osebka. Ker gre za dokaj svojevrstno žival, menim, da ne moremo več odlašati z objavo tega podatka in s taksonomsko definicijo te živali.

Rakca opisujem pod imenom ***Merozoon vestigatum*** g. n., sp. n. in ga uvrščam v družino Sphaeromatidae, rodovno skupino *Monolistra* (Monolistrini *sensu* Racovitza). Za novi rod je značilen obokan pleotelzon, z vsemi pleoniti zlitimi vanj; proste so le epimere sprednjih pleonitov, ki pa so slabo razvite in ne segajo do stranskega roba pleotelzona; uropodi so močno reducirani, enočlenski, izraščajo iz sredine pleotelzonovih bočnih strani. Dolžina uropodov je pri tej vrsti le 35% dolžine pleotelzona. Pri obeh doslej znanih rodovih te skupine je zgradba pleona-pleotelzona nespremenljiva. Uropodi so vedno poenostavljeni vsaj do enovejnate oblike, sicer pa je njihova razvitost od vrste do vrste različna, lahko tudi manjkajo.

O pripadnosti taksona družini Sphaeromatidae priča prav namestitev uropodov daleč za sprednjim robom pleotelzona. Takšno namestitev si lahko razlagamo tako, da je v pleotelzon brez sledu zlit še pleonit ali dva pred zadnjim, ki nosi uropode. Edina druga možnost bi bila pripadnost družini Cirolanidae, a pri njenih članih izraščajo uropodi vedno tik za sprednjim robom pleotelzona; to verjetno pomeni, da nečlenjeni del pleotelzona začne z zadnjim pleonitom.

Dvome o reprezentativnosti tega majhnega dela telesa odklanjam. Slaba razvitost pleonitov ne more biti posledica mladosti, saj imajo mladiči te skupine že v zadnjih stadijih v valilniku enako razvit pleon in pleotelzon, kot odrasli. Sklerotiziranost tega kosa pa priča, da je celo pripadal odraslemu ali mlademu osebku zunaj valilnika. Členjenost pleona, kot je značilna za eden ali drugi rod monolistrinov, je povsem stabilna. Med stotinami osebkov nismo našli spačka, ki bi bil podoben tukaj opisani živali.

Deli pleona-pleotelzona so nekoliko zliti pri rodu *Monolistra*, bolj pri zahodnoevropskemu rodu *Caecosphaeroma*, najbolj pa pri novemu rodu *Merozoon*, čeprav je bil ta najden pri južnem koncu areala monolistre. Kaže, da je nova žival tudi filogenetsko-biogeografsko zanimiva, a za postavitev kakršne koli hipoteze jo bo treba raziskati ne le popolneje morfološko, temveč tudi molekulsko-filogenetsko.

References

Botosaneanu, L., Bruce, N., Notenboom, J., 1986. Isopoda: Cirolanidae. In: Botosaneanu, L. (Ed.), Stygofauna Mundi, pp. 423–427.

Bruce, N. L., 2003. New genera and species of sphaeromatid isopod crustaceans from Australian marine coastal waters. Memoirs of Museum Victoria, 60, 309–369.

Bruce, N.L., 1993. Two new genera of marine isopod crustaceans (Flabellifera: Sphaeromatidae) from Southern Australia, with a reappraisal of the Sphaeromatidae. Invertebrate Taxonomy, 7, 151–171.

Bruce, N. L., Herrando-Perez, S., 2005. *Kensleylana briani*, a new genus and species of freshwater cave-dwelling cirolanid (Crustacea: Isopoda) from Spain. Proceedings of the Biological Society of Washington, 118, 74–83.

Daum, J., 1954. Zur Biologie einer Isopodenart unterirdischer Gewaesser: *Caecosphaeroma (Vireia) burgundum* Dollfus. Annales Universitatis saraviensis. Naturwissenschaften, 3, 104-160, tab. 1–11.

Harrison, K., Ellis, J.P., 1991. The genera of the Sphaeromatidae (Crustacea: Isopoda): a key and distribution list. Invertebrate Taxonomy, 5, 915–952.

Kusakin, O. G., 1979. Morskie i solonovatovodnye ravnonogie rakoobraznye (Isopoda) holodnyh i umerennyh vod severnogo polušarija. Podotrjad Flabellifera. Nauka, Leningrad.

Prevorčnik, S., Sket, B., 2008. An ecologically peculiar new species of *Monolistra* (Crustacea: Isopoda: Sphaeromatidae) from cave waters in the Dinaric karst of Croatia. Subterranean Biology, 5, 23–28.

Monod, T., 1972. Contribution a l'étude de la Grotte de Sof Omar (Ethiopie Méridionale). No 3 – Sur une espèce nouvelle de Cirolanidé cavernicole, *Skotobaena mortoni* (Crusst., Isopoda). Annales de Speleologie, 27, 205–220.

Poore, G.C.B., 1994. *Maricoccus brucei*, an unusual new genus and species of Sphaeromatidae from Southern Australia (Crustacea: Isopoda). Memoirs of the Museum of Victoria, 54, 171–178.

Racovitza, E.-G., 1910. Sphéromiens (premiere série) et révision des Monolistrini. Archives de Zoologie experimentale et generale, 5e Ser., 4, 625–758, tab. 18–31.

Racovitza, E.-G., 1912. Cirolanide (premiere serie). Archives de Zoologie experimentale et generale, 5e Ser., 5, 203-329, tab. 15-28.

Sket, B., 1965. Östliche Gruppe der Monolistrini. II. Biologischer Teil. International Journal Speleology, 1, 249–267, tab. 60–66.

Sket, B., 1986a. Isopoda: Sphaeromatidae. In: Botosaneanu, L. (Ed.), Stygofauna Mundi, pp. 423–427.

Sket, B., 1986b. Ecology of the mixohaline hypogean fauna along the Yugoslav coast. Stygologia, 2, 317–338.

Sket, B., 1986c. Evaluation of some taxonomically, zoogeographically, or ecologically interesting finds in the hypogean waters of Yugoslavia (in the last decades). Comunicacions, 9. Congreso Internacional de Espeleologia, 1, 126–128.

Sket, B., Trontelj, P., Žagar, C., 2004. Speleobiological characterization of the epikarst and its hydrological neighborhood: its role in dispersion of biota, its ecology and vulnerability. Epikarst. Karst Water Institute Special Publication 9, Washington, pp. 104–113.

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)

15. Peer Review

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