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# Za vedno je odšel Tone Wraber

V letu, ko je Botanični vrt obhajal 200-letnico delovanja, ko smo praznovali leto biodiverzitete, in v času, ki so ga vsako leto generacije študentov biologije preživljale na terenskih vajah na Komni, nas je zapustil profesor dr. Tone Wraber.

Kdorkoli je v zadnjih nekaj desetletjih vsaj malo pokukal med slovensko floristično literaturo, mu je to ime gotovo ostalo v spominu, saj je bil Tone s svojim vsestranskim delovanjem in čez 1000 objavljenimi deli gotovo botanik, kakršnega slovenska narava še ni doživela.

Tone se je rodil 4. marca 1938. Po materi Sabini, klasični filologinji, je podedoval ljubezen do jezikov, oče Maks, eden prvih slovenskih fitocenologov, pa mu je vcepil ljubezen do rastlinstva in slovenskih gora. Taborništvo je spoznaval pri Pavlu Kunaverju, Sivem volku, in Tonetova pot med svečenike gorskega cvetja je bila s tem začrtana. A čeprav je vse življenje odločno in z ljubeznijo sledil tej poti, se ni mogel upreti niti številnim odcepom, vzporednicam in bližnjicam, ter je s svojo vsestransko aktivnostjo zaznamoval poleg botanike še kar nekaj drugih področij, bil je med utemeljitelji slovenskega naravovarstva, neumorno je preučeval zgodovino stroke in se uspešno spoprijemal z lingvističnimi izzivi.

Končal je študij biologije na Univerzi v Ljubljani in še kot študent sodeloval na tedanjem Zavodu za spomeniško varstvo. Z diplomskim delom »Rastlinstvo melišča pri Črnem jezeru nad Komarčo« pod mentorstvom prof. dr. Gabrijela Tomažiča se je začelo prvo obdobje Tonetove fitocenološke aktivnosti, ki se ji je posvečal intenzivneje do doktorata leta 1972 v Trstu pod mentorstvom prof. dr. Sandra Pignattija z delom »Contributo alla conoscenza della vegetazione pionere (Asplenietea rupestra e Thlaspeetea rotundifolii)«. Vzporedno s fitocenološko aktivnostjo je bil aktiven predvsem na področju floristike in taksonomije. Konec šestdesetih let se je tako pod njegovo koordinacijo pričelo sistematično kartiranje slovenske flore po vzorcu, ki je bil v rabi drugod v Srednji Evropi. To je po eni strani pomenilo ustrezno ureditev starejših floristčnih podatkov in pripravo za vnos v računalnik, po drugi strani se je s pomočjo novih popisnih listov začelo s sistematičnim kartiranjem flore na tere-

nu. V okviru teh aktivnosti je bilo pod Tonetovim mentorstvom izvedenih veliko diplomskih nalog, ki so ne le dale pomembne floristične rezultate iz dotlej manj raziskanih predelov Slovenije, ampak tudi izobrazile številne mlade poznavalce flore.

Prva službena leta (1960-1970) je preživel v Prirodoslovnem muzeju in tu sodeloval s Cirilom Jegličem pri obnavljanju in urejanju alpskega botaničnega vrta Julijana v Trenti. Tudi kasneje je zavzeto skrbel za razvoj Julijane. Februarja 1968 je bil imenovan za asistenta v Botaničnem vrtu Univerze v Ljubljani in tedaj postal tudi kurator herbarijske zbirke LJU pri katedri za botaniko biološkega oddelka. Na Oddelku za biologijo Biotehniške fakultete je bil zaposlen vse do upokojitve 2003 in se v teh letih od asistenta povzpel do rednega profesorja ter bil prav zadnje dni svojega življenja odlikovan z nazivom zaslužnega profesorja Univerze v Ljubljani.

S svojimi bogato ilustriranimi predavanji je približal rastlinsko sistematiko in floro številnim študentom biologije. Predaval je predmete Sistematska botanika, Osnove sistematske botanike, Biogeografija (rastlinski del), Osnove latinščine za biologe ter Pestrost in ogroženost rastlinstva. S posebnim žarom in predanostjo je predaval bo-

tanično latinščino. Skrbno in ciljno izbrane teme predavanj so mladim generacijam pomagale razumeti smisel in način uporabe njemu tako ljube latinščine v biologiji in tudi v vsakdanjem življenju. Predmet, čeprav neobvezen, je vsako leto pritegnil in navdušil mnoge študente biologije.

Znanstvene članke je objavljal v domačih revijah, predvsem v Biološkem vestniku, reviji Acta biologica Slovenica in Hladnikia, pa tudi v tujih revijah, kot na primer Candollea in Acta botanica croatica. Med znanstvenimi in strokovnimi monografijami je gotovo strokovno najbolj opazno soavtorstvo pri vseh štirih izdajah Male flore Slovenije (1969, 1984, 1999 in 2007). S področja naravovarstva je najpomembnejše in najbolj vplivno delo Rdeči seznam praprotnic in semenk (1989) v soavtorstvu s Petrom Skobernetom. Pri oblikovanju temeljev varstva narave v Sloveniji je Tone sodeloval tudi z desetinami naravovarstvenih prispevkov, objavljenih v revijah Varstvo narave, Biološki vestnik in Proteus. Njegove ostale pomembnejše strokovne monografije so Sto znamenitih rastlin na Slovenskem (1990), Rože na Slovenskem (besedila k fotografijam Luke Pintarja, 1990), 2 x sto alpskih rastlin na Slovenskem (2006), Travniške rastline na Slovenskem (skupaj z Andrejem Seliškarjem, 1986), s katerimi je dosegel in navdušil tudi bralce zunaj ozkega strokovnega kroga.

Naravoslovno javnost je s strokovnimi in poljudno-strokovnimi članki redno seznanjal s florističnimi odkritji, opozarjal na naravovarstvene probleme, podajal vtise s svojih botaničnih popotovanj, razpravljal o botaničnih zanimivostih pri nas in na tujem. S tem je širil naravoslovno kulturo in vzpodbujal mlade k raziskovalnemu delu, bistveno pa je tudi prispeval k razvoju in uveljavljanju slovenskega biološkega izrazja.

Do narave je bil vedno iskreno in nesentimentalno pozoren. Na rastline ni gledal le kot na objekte preučevanja, bil je občudovalec njihove lepote, imenoval jih je 'cvetoče prijateljice' in poskušal razumeti tudi njihovo vpetost v kulturno-zgodovinski okvir. Rad je pripovedoval njihove zgodbe in z veseljem smo mu prisluhnili tako botaniki kot širša javnost. Večkrat je povedal in tudi napisal, da ima posebej rad ljudi, ki imajo radi rože, posebej, če to počno ob sicer drugačnem življenjskem poklicu. Ob vseh svojih obveznostih je za take ljubitelje vedno našel čas - s predavanji v lokalnih skupnostih, z vodenjem botaničnih izletov, aktivnim sodelovanjem v planinskih društvih in podobno. S poljudnimi članki v revijah kot so npr. Moj mali svet in Naš vrt, s članki in diskusijami v dnevnem časopisju, intervjuji, televizijskimi in radijskimi oddajami ter številnimi predavanji v širši javnosti ob raznih priložnostih je izjemno prispeval k popularizaciji botanike in naravoslovja nasploh v širši javnosti.

Kot področni urednik in s poldrugo stotino prispevkov je sodeloval pri nastanku Enciklopedije Slovenije, ki jo je v letih 1987-2002 izdala založba Mladinska knjiga v sodelovanju s Slovensko akademijo znanosti in umetnosti.

Poleg članstva v številnih domačih in tujih strokovnih združenjih, je bil ustanovni član Botaničnega društva Slovenije in kasneje tudi urednik glasila Hladnikia, predsednik društva in njegov častni član. Vsa leta pa je bil aktiven v Društvu biologov Slovenije, ki ga je vrsto let tudi vodil. Bil je tudi član uredniškega odbora društvenega glasila Acta Biologica Slovenica.

Profesor dr. Tone Wraber je bil najbolj vpliven ter pisno daleč najbolj plodovit botanik delujoč na območju Slovenije v 20. stoletju, v svojem delovanju pa je bil vsestranski. V pogovorih, dopisovanjih, objavah in med skupnimi botaničnimi tereni je svoje bogato enciklopedično znanje in izkušnje vedno rad delil z nami, svojimi botaničnimi kolegi. Iskreno smo mu hvaležni. Vrzeli, ki jih je s svojim odhodom pustil med nami, še dolgo ne bodo zapolnjene.

*Requiescat in pace*!

N. Jogan in T. Bačič

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# *Juncus atratus* Krock. (*Juncaceae*) rediscovered in Slovenia

## Temnocvetni loček (*Juncus atratus* Krock., *Juncaceae*) ponovno najden v Sloveniji

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**Abstract:** *Juncus atratus* Krock. of the *Juncaceae* family is a Central European- Southern Siberian wet-meadow species. In Central Europe, the species is very rare and threatened. In Slovenian Red Data List, it is listed among unsufficiently known species (K). The only record of the species in Slovenian territory (Prem near Ilirska Bistrica, SW Slovenia) is over 100 years old and has never been confirmed afterwards. In May 2010, the species was found in Goričko (NE Slovenia), were it thrives on a wet meadow near Kobilje village. The article discusses the recent finding of the species in Slovenia in broader context, its distribution, habitat and threat factors. The aim of the study was also to provide bases for nature-conservation of the species in Slovenia. According to recent finding and the results of the study, the authors suggest *Juncus atratus* to be regarded as 'endangered' (E) in the national red data list.

**Key words:** *Juncus atratus, Juncaceae,* nature-conservation, Slovenian flora, Goričko, Red Data List, wet meadows

**Izvleček:** Temnocvetni loček (*Juncus atratus* Krock.) iz družine ločkovk (*Juncaceae*) je srednjeevropsko-južnosibirska vrsta vlažnih travnikov. V Srednji Evropi je vrsta zelo redka in ogrožena. Na slovenskem rdečem seznamu je navedena med nezadostno znanimi vrstami (K). Edini podatek o uspevanju vrste za Slovenijo (Prem pri Ilirski Bistrici, Jugozahodna Slovenija) je star več kot 100 let in ga kasnejše raziskave niso uspele potrditi. Maja 2010 je bila vrsta najdena na Goričkem (Severovzhodna Slovenija), kjer raste na vlažnem travniku pri vasi Kobilje. Članek obravnava nedavno najdbo vrste v Sloveniji v širšem kontekstu, razširjenost vrste, habitat in faktorje ogrožanja. Namen raziskave je postaviti temelje za ohranjanje vrste v Sloveniji. Glede na nedavno najdbo in rezultate naše raziskave, avtorji predlagajo, da se vrsto *Juncus atratus* v slovenskem rdečem seznamu uvrsti med prizadete vrste slovenske flore (E).

**Ključne besede:** *Juncus atratus, Juncaceae*, naravovarstvo, slovenska flora, Goričko, rdeči seznam, vlažni travniki.

## Introduction

*Juncus atratus* Krock. is a member of *Juncaceae* family. It is 40-120 cm tall perennial with a creeping, sparingly branched rhizome with 7- to 11-angled leaves and inflorescence of 15-50 clusters, each with distinctly dark brown flowers. It is a Central European - Southern Siberian wet-meadow species (Pignatti 1982), distributed in C and E Europe, northwards to Latvia (Snogerup 1980). In Central Europe, the species is very rare and also listed among Central European vascular plants requiring priority

conservation measures (Schnittler and Günther 1999).

Until recently, there was only one published (over 100 years old) record of the species from Slovenia of Pospichal (1897-99) from Submediterranean region (in upper valley of Reka river at Prem near Ilirska Bistrica, SW Slovenia) under synonim *J. melananthos* Rchb., with his remark, that the species probably thrives also in Brkini area nearby. The Pospichal's record (in 0351/4 MTB quadrant) is the only dot in distribution map of *Juncus atratus*, published in 'Materials for the Atlas of Flora of Slovenia' (Jogan et al. 2001), which is a key reference for distributional data of Slovenian vascular plants and where the distribution maps of most taxa are shown. However, the presence of the species on Pospichal's locality was not confirmed by other botanists afterwards, although the attempts were made to find *Juncus atratus* in this area in the last decade (Rozman B., personal communication). As seen in general distribution map of this species in

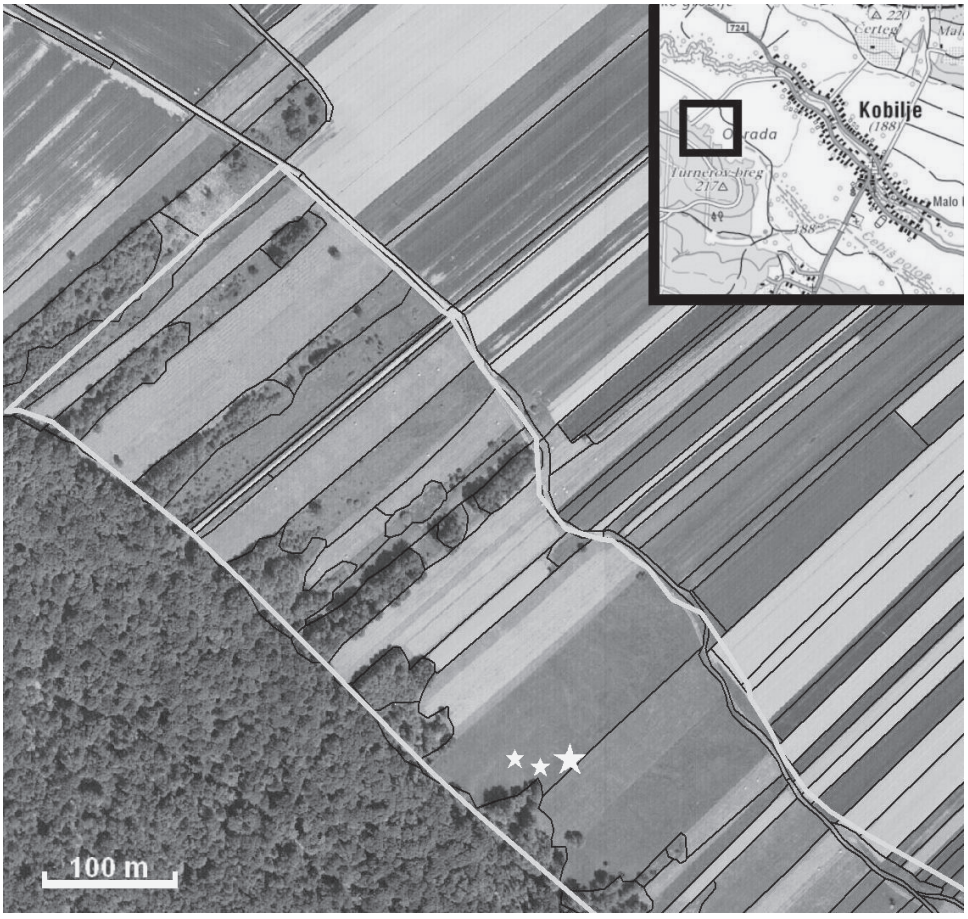


Figure 1: The locality of *Juncus atratus* near Kobilje (Goričko, Slovenia). Top righ corner: map of the vicinity of Kobilje with marked area, which is presented in bigger scale. Rest of the picture: aerial photo of the locality, depressions with *J. atratus* marked with white stars.

Slika 1: Nahajališče temnocvetnega ločka *Juncus atratus* pri Kobilju (Goričko, Slovenija). Zgoraj desno: zemljevid okolice Kobilja z označenim izsekom, povečanim na letalskem posnetku. Ostali del slike: zračni posnetek nahajališča, populacije *J. atratus* označene z belimi zvezdicami.

Meusel et al. (1965), the mentioned Pospichal's localities would lie on the western border of species' distribution range. Populations here could have been destroyed by severe changes of wetland habitat types in the last century but without available voucher material also a misdetermination could not be excluded.

During a field excursion in Goričko (Subpannonian phytogeografical region), in May 2010 first author of the present paper discovered the dark-flowered *Juncus* plants, somewhat similar to *Juncus articulatus*, but distinctly taller, in wet-meadow near Kobilje village. Later, the plants were unambiguously determined as *Juncus atratus*.

The article discusses the recent finding of the species in Slovenia. Since deficient knowledge about distribution, ecology and threat factors of species can cause serious problems in considerations and judgments when proposing nature-conservation strategies and composing red data lists, the article focuses on the issues mentioned above and discusses the broader context of the finding. The aim of the study was also to provide bases for nature conservation of the species in Slovenia.

## Methods

The study included 3 field-work days during vegetation season, on 30th May, 2nd July and 17th August 2010. The field-work was carried out in Kobilje in Goričko region (Fig. 1). In the locality of *Juncus atratus* the approximate size of the population was assesed from number of flowering shoots and vascular plants on the locality were mapped. They were determined using standard floristic literature (mostly Martinčič et al. 2007 and Fischer et al. 2008).

After the field work, revision of the available herbarium material was done in LJU (Department of Biology, University of Ljubljana), which is the biggest herbarium collection with Slovenian plants. The revision included also related *Juncus* taxa to detect possible misdeterminations. Where necessary, characters were observed and measured using a stereomicroscope.

For the preparation of the distribution map we checked all available distribution data for Slovenia: literature records, herbarium data from LJU, unpublished data stored in the database 'Flora Slovenije' at the Centre for Cartography of Fauna and Flora (CKFF), where also the distribution maps were produced. Distribution patterns are presented in MTB grid (Niklfeld 1971). In distribution map, we used the biogeographic division of Slovenia by M. Wraber (1969), which was also adopted by the authors of the Slovenian floristic reference work *Mala flora Slovenije* (Martinčič et al. 2007).

## Results

The newly discovered locality of *J. atratus* is situated about 700 m southwest from Kobilje village, 190 a.s.l.. It lies in Regional park Goričko. NE Goričko was recognized as important for the conservation of Natura 2000 habitat types with qualificatory vegetion types of Molinion (6410) and Ranunculo-Alopecuretum (6510) (Jogan et al. 2004) and as such included in SI3000221 SCI with 7 qualificatory habitat types including the mentioned two (Naravovarstveni atlas, http://www.naravovarstveni-atlas.si/ISN2KJ/)

The area of extensively maintained meadows at Kobilje is also 'natural value of state importance' (Kobilje - ekstenzivni travniki: 7298). Despite that, land owners' management is not completely under nature-conservation control, which results in several negative impacts as digging ditches, applying fertilizers, filling up wet depressions with rubbish, earth or waste building materials.

In the locality, the population thrives in 3 shallow depressions of the wet meadow, the larger beeing about 400 $m^2$ large, while the two smaller depressions of cca. 200 $m^2$. The maximum depth of the depressions is cca. 0.5 m. The distances between the three depressions are approximately 30 m. The depressions are much damper than the adjoining parts of the meadow and after rainfall the water can persist in them for several consequent days.

The population of *J. atratus* in the largest depression counts about 30 - 40 flowering individuals, while the approximate number of flowering shoots in the two smaller depressions is up to 10.


Figure 2: *Juncus atratus* in the recently found locality near Kobilje (Goričko, Slovenia). **a**: flowering cluster detail with distinctly blackish tepals; **b**: compound inflorescence; **c**: locality with *J. atratus.* Photos: T. Lainšček.
Slika 2: Temnocvetni loček (*Juncus atratus*) na novoodkritem nahajališču pri Kobilju (Goričko, Slovenija). **a**: delno socvetje z razločno črnorjavimi cvetnimi listi; **b**: celotno socvetje; **c**: rastišče *J. atratus*. Posnetki: T. Lainšček.

The meadow is mowed twice to three times in vegetation period, in June, August and October.

At the locality (Fig. 2), besides *J. atratus*, the following vascular plants were recorded: *Agrostis stolonifera, Agrostis canina, Alopecurus pratensis, Carex vulpina, Eleocharis carniolica, Gratiola officinalis, Holcus lanatus, Iris pseudacorus, Juncus articulatus, Juncus effusus, Lysimachia nummularia, Lythrum salicaria, Myosotis scorpioides, Peplis portula, Polygonum minus, Ranunculus flammula*. That type of vegetation fits well into Natura 2000 qualifying habitat type *Ranunculo-Alopecuretum* (6510). For more precise description of the site ecological conditions a phyto-sociological analysis should be done, but that was beyond our scope.

Of the above mentioned species, *Eleocharis carniolica, Gratiola officinalis* and *Peplis portula* are wetland species, considered vulnarable (V) in Slovenian Red Data List (Anon. 2002). From nature-conservation point of view, *Eleocharis carniolica* is particularly important, as it is one of the Natura 2000 plants, listed among strictly protected plant species included in Appendix 1 of the Bern Convention on the conservation of European wildlife and natural habitats (Bern, 19th September 1979) and in Annex 2 of Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

The revision of *Juncus* material in LJU revealed that at present there is no available material for *Juncus atratus* in Slovenia. The distribution map (Fig. 3) includes only two records for

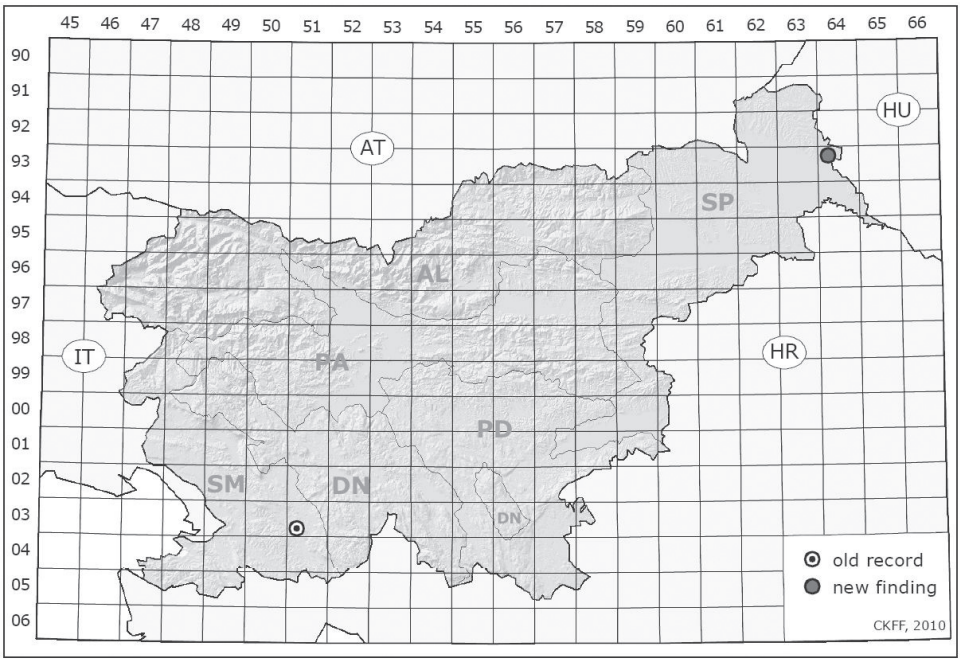


Figure 3: The known distribution of *Juncus atratus* in Slovenia.
Slika 3: Znana razširjenost vrste *Juncus atratus* v Sloveniji.

Slovenia: Pospichal's record for Prem (0351/4) and first author's record for Goričko (9364/1).

## Discussion

*J. atratus* is a well characterised species, but can be somewhat similar to *J. articulatus*, since the jointed rush is very variable in habit. In the field, the two can be easily distinguished, since *J. atratus* is usually much higher, up to 120 cm, it has characteristically blackishbrown tepals (not just brown as in *J. articulatus,* but character not easily comparable and quite variable). Leaves are (5-) 7- to 11-angled and this character is apperent in fresh or dry state of the material. In contrary, the leaves of *J. articulatus* (as well as *J. acutiflorus* and *J. alpinoarticulatus*) are not angled, just slightly ridged when dry. However, there is another character which should be taken into account: the leaves of *J. articulatus, J. alpinoarticulatus and J. acutiflorus* have very distinctive transverse septa, while in *J. atratus* leaves, the septa are present, but inconspicuous. Descriptions followed Fischer et al. (2008) and Kirschner (2002).

As mentioned in Introduction, in Central Europe, *Juncus atratus* is very rare and also endangered (Schnittler and Günther 1999). The species is threatened also in neighbouring Austria. It is considered 'vom Aussterben bedroht' - 'in risk of extinction' by Niklfeld and Schratt-Ehrendorfer (1999) and is known to thrive in only one locality in Niederössterreich (Marchfeld) (Fischer et al. 2008). In Italy, the species is considered to be very rare, with only one known locality (near Verona) (Pignatti 1982). There are no records for Croatia. In Hungarian Red Data List the species is listed among 'near threatened' (NT) (Király 2007), distributed in several localities, but scattered (Simon 2002). In Czech, *J. atratus* is considered 'criticaly endangered taxon' (CR) (Kubát, 2002) and in Germany 'strongly endangered' (Haeupler and Muer, 2000).

Besides our record, there are no recent reports on the species for Slovenia and Pos-

pichal's observation remained unconfirmed. The fact, that there are no other records of *J. atratus* in Slovenia can not be attributed to misdeterminations or neglect by botanists during the fieldwork, as we saw, that the species is easily recognizable in the field. Therefore we can conclude, that *J. atratus* is indeed extremely rare in Slovene territory, as in Europe. According to Oberdorfer (1990) the species is charasteristic of Cnidio-Violetum of Cnidion alliance, which is subcontinental vegetation of temporarily inundated meadows. In Slovenia this type of vegetation is not known, but we can expect its occurence in most continental parts of Slovenia, which is Goričko region.

The habitat of *Juncus atratus* is endangered not only due to abandonment of mowing, which leads to gradual spontaneous reforestation, but particularly due to human influence on wet habitats, such as changing water regime, direct filling of depression or fertilizing. Maintaining of this type of vegetation should encounter regular mowing at least one per year with removing of the mown grass and enabling of regular inundation (Chytry et al. 2001).

In the Slovene red data book, *J. atratus* is categorized as unsufficiently known species (K; Wraber and Skoberne 1989, Anonymous 2002), but Bačič (2006) suggested *Juncus atratus* to be recategorized in Ex?, since the only, over 100 years old record was never confirmed, despite the attempts to find the plant in the locality. According to recent finding and the results of the study, the authors suggest *Juncus atratus* to be regarded as 'endangered' (E) in the national red list, since it is rare and its habitats are threatened because of human activity and natural succession.

## Acknowledgement

The authors are grateful to dr. Peter Schönswetter and dr. Božo Frajman for confirmation of the determination of *Juncus* plants and for the valuable remarks on the finding and to Centre for Cartography of Fauna and Flora for producing the distribution map.

## Povzetek

Temnocvetni loček *Juncus atratus* Krock. je predstavnik družine ločkovk (*Juncaceae)*. Je 40 -120 cm visoka trajnica s plazečim, le malo razvejeno koreniko, s 7- to 11-robimi listi and socvetjem, sestavljnim iz 15-50 klobk z izrazito črnorjavimi cvetovi. Ta srednjeevropsko-južnosibirska vrsta vlažnih travnikov (Pignatti 1982) je razširjena v Srednji in Vzhodni Evropi, severno pa sega njen areal do Latvije (Snogerup 1980). V Srednji Evropi je vrsta zelo redka in navedena med 'srednjeevropskimi vaskularnimi rastlinami, ki potrebujejo prednostna merila za ohranitev' (Schnittler and Günther 1999).

Do nedavnega edini podatek o uspevanju vrste v Sloveniji je star več kot 100 let. Gre za Pospichalovo (1897-99) navedbo za submediteransko fitogeografsko območje (zgornji del doline Reke pri Premu pri Ilirski Bistrici), avtor pa piše, naj bi vrsta verjetno uspevala tudi v bližnjih Brkinih. Prisotnost vrste na tej lokaliteti botaniki kasneje kljub trudu niso uspeli potrditi (Rozman B., osebno posvetovanje). Med terensko ekskurzijo na Goričko maja 2010 je prvi avtor na vlažnem travniku pri Kobiljem naletel na izrazito temnocvetne rastline ločka, ki so bile nekoliko podobne bleščečeplodnem ločku (*J. articulatus*), a precej višje. Kasneje so bile rastline določene za *J. atratus*.

Terenski del raziskave je potekal med vegetacijsko sezono 2010, v maju, juliju in avgustu. Na nahajališču smo ocenili velikost populacije ter popisali najpogostejše rastline, ki uspevajo ob temnocvetnem ločku. Sledila je revizija herbarijskega materiala v herbarijski zbirki LJU na Oddelku za biologijo Biotehniške fakultete Univerze v Ljubljani.

Novo odkrito nahajališče temnocvetnega ločka leži približno 700 m jugozahodno od vasi Kobilje na nadmorski višini 190 m. Območje je del Regijskega parka Goričko in predvsem del omrežja Natura 2000 (Goričko, SI5000009). Kljub temu pa upravljanje z zemljišči ni povsem pod nadzorom naravovarstva, kar ima lahko za posledico negativne vplive na habitate (kopanje izsuševalnih jarkov, uporaba gnojil, zasipanje mokrotnih depresij s smetmi, zemljo ali odpadnim gradbenim materialom itd.).

Na lokaliteti uspeva populacija v treh plitvih, med seboj približno 30 m oddaljenih depresijah na vlažnem travniku. Največja depresija je velika približno 400 $m^2$, drugi dve pa sta manjši, obsegata približno 200 $m^2$. Največja globina depresij je približno 0,5 m. Depresije so precej bolj mokrotne kot okolica in po deževjih v njih še nekaj dni zastaja voda.

Populacija v največji depresiji šteje okoli 30-40 cvetočih primerkov, v manjših dveh pa do 10.

Travnik kosijo dva do trikrat v vegetacijski sezoni, junija, avgusta in včasih tudi oktobra.

Na nahajališču smo zabeležili poleg temnocvetnega ločka še naslednje vrste: *Agrostis stolonifera, Agrostis canina, Alopecurus pratensis, Carex vulpina, Eleocharis carniolica, Gratiola officinalis, Holcus lanatus, Iris pseudacorus, Juncus articulatus, Juncus effusus, Lysimachia nummularia, Lythrum salicaria, Mysotis scorpioides, Peplis portula, Polygonum minus, Ranunculus flamula.*

Revizija ločkov v LJU je pokazala, da poleg naših primerkov zaenkrat ni na voljo nobenega herbarijskega materiala te vrste.

Na terenu je temnocvetni loček načeloma lahko prepoznaven, le nekoliko podoben vrsti *J. articulatus*. Od te se dobro loči po višini (do 120 cm visok, *J. articulatus* je navadno znatno nižji), po izrazito in značilno črnorjavih perigonovih listih (pri *J. articulatus* so ti le svetlejše rjavi), v času cvetenja pa iz cvetov molijo značilno škrlatne brazde (pri *J. articulatus* so belkaste). Pomemben razlikovalni znak je tudi oglatost listov. Ti so (5) 7- do 11-oglati, kar je očitno tako pri svežih kot pri posušenih rastlinah. Listi vrste *J. articulatus* (kot tudi bližnjih, a redkejših *J. acutiflorus* in *J. alpinoarticulatus*) v svežem stanju niso robati in le nekoliko izbrazdani, ko so suhi. V listih vrste *J. articulatus, J. alpinoarticulatus* in *J. acutiflorus* so izrazite prečne pregrade, vidne v posušenem stanju, ne da bi list vzdolžno prerezali. Tudi v listih *J. atratus* so prečne pregrade, vendar ne tako izrazite. Slednji znak je za opazovanje nekoliko neroden, če s temi vrstami nimamo predhodnih izkušenj.

Temnocvetni loček je v Sredni Evropi izjemno redek, še največ navedb je za Madžarsko (Simon 2002), a še tu se pojavlja le raztreseno. Kritično je ogrožen v sosednji Avstriji (Fischer et al. 2008), za Italijo obstala le ena sama navedba (Pignatti 1982), na Hrvaškem pa vrsta ne uspeva. V Sloveniji potrjeno uspeva le na enem samem nahajališču. Glede na to, da je vrsta dobro prepoznavna, je malo verjetno, da bi jo botanično oko na terenu spregledalo in bi bilo poznavanje njene razširjenosti iz tega razloga pomanjkljivo. Vrsta je kot kaže v resnici izjemno redka, poleg tega pa so njeni habitati zelo ogroženi. Po eni strani jih ogroža naravna sukcesija - spontano zaraščanje zaradi opuščanja košnje, posebej nevaren pa je vpliv človeka - spreminjanje vodnega režima, zasipanje vlažnih depresij in gnojenje. Za ohranjanje takšne vegetacije je treba na območjih uvesti oz. ohraniti košnjo vsaj enkrat letno, z odstranjevanjem pokošenega materiala in dopustiti redno poplavljanje (Chytry et al. 2001).

V slovenskem rdečem seznamu (Wraber in Skoberne 1989, Anonymous 2002) je temnocvetni loček obravnavan med nezadostno znanimi vrstami (K), pred leti pa je bila že predlagana uvrstitev med domnevno izumrle vrste - Ex? (Bačič 2006). Glede na nedavno najdbo in rezultate te raziskave, avtorji predlagajo, da se vrsto uvrsti v kategorijo ‚prizadeta vrsta' (E).

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# Damage by Pests in Herbarium LJU

## Škoda zaradi herbarijskih škodljivcev v Herbariju LJU

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**Abstract:** The article discusses the damage caused by herbarium pests in Herbarium LJU. The aim of the study was to determine the damage in the herbarium, to find out, which herbarium-pests are present, and to investigate their food-preference by means of checking the extent of damage on a selection of plant families *Alliaceae, Apiaceae, Araceae, Asteraceae*, *Brassicaceae*, *Chenopodiaceae*, *Cichoriaceae, Fabaceae*, *Lamiaceae*, *Poaceae*, *Polypodiaceae*, *Ranunculaceae*, *Rosaceae* and *Scrophulariaceae.* Since the *Asteraceae* and *Cichoriaceae* are known to be among the most attractive families for herbarium-pests, we examined them in detail. In the study about 7500 herbarium sheets were examined, which represents 5% of all the sheets in Herbarium LJU. In addition to the most frequent pest tobacco beetle (*Lasioderma sericorne),* we also found beetles *Stegobium paniceum* and *Attagenus piceus*, booklice (*Psocoptera*), moulds and Pharaoh's ants (cf. *Monomorium pharaonis*). Pest-damage was observed in 18 % of the examined herbarium sheets. The study confirmed that the pests are prone to attack certain families over others: the greatest damage (about 25 % - 40 % of the damaged sheets) was observed in *Apiaceae*, *Asteraceae s. lat.*, *Brassicaceae*, *Fabaceae*, *Alliaceae*, *Araceae*, *Rosaceae* and *Chenopodiaceae,* while the other investigated families ranked among less damaged (about less then 10 % of the damaged sheets). Among *Asteraceae*, tribus *Cardueae* proved to be the most attractive tribe for the pests.

**Keywords:** Herbarium LJU, herbarium-pests, dry plants, *Lasioderma serricorne, Stegobium paniceum, Attagenus piceus, Psocoptera.*

**Izvleček:** Članek obravnava škodo, ki jo povzročajo herbarijski škodljivci v herbarijski zbirki LJU. Namen raziskave je bil ugotoviti obseg poškodovanosti rastlinskega materiala, določiti, kateri herbarijski škodljivci so prisotni v LJU in raziskati njihovo prehrambeno preferenco z ugotavljanjem obsega škode na izboru rastlinskih družin *Alliaceae, Apiaceae, Araceae, Asteraceae*, *Brassicaceae*, *Chenopodiaceae*, *Cichoriaceae, Fabaceae*, *Lamiaceae*, *Poaceae*, *Polypodiaceae*, *Ranunculaceae*, *Rosaceae* in *Scrophulariaceae.* Ker družini *Asteraceae in Cichoriaceae* veljata za najbolj privlačne za herbarijske škodljivce, smo ju še posebej podrobno obravnavali. V raziskavi smo pregledali okoli 7500 herbarijskih pol, kar predstavlja 5 % vseh pol v herbariju LJU. Ugotovili smo, da je v LJU najpogostejši škodljivec tobačni hrošč (*Lasioderma serricorne),* poleg njega pa smo naleteli tudi na hrošča *Stegobium paniceum* in *Attagenus piceus*, prašne uši (*Psocoptera*), molje and faraonske mravlje (cf. *Monomorium pharaonis*). Škodo zaradi herbarijskih škodljivcev smo opazili v 18 % pregledanih herbarijskih pol. Raziskava je potrdila, da herbarijski škodljivci jedo herbarijski material določenih rastlinskih družin raje kot drugih. Največjo poškodovanost herbariziranih rastlin (25 do 40 % pregledanih pol s poškodovanih materialom) smo tako opazili pri družinah *Apiaceae*, *Asteraceae s. lat.*, *Brassicaceae*, *Fabaceae*, *Alliaceae*, *Araceae*, *Rosaceae* in *Chenopodiaceae.* Ostale pregledane družine so bile znatno manj poškodovane (pod cca. 10 % poškodovanih pol). Znotraj družine *Asteraceae* se je kot najbolj privlačen za herbarijske škodljivce izkazal tribus *Cardueae*.

**Ključne besede:** herbarjska zbirka LJU, herbarijski škodljivci, posušene rastline, *Lasioderma serricorne*, *Stegobium paniceum, Attagenus piceus, Psocoptera.*

## Introduction

Herbaria are permanent collections only if preserved in dry, airy, dark place and protected against pests (Podobnik 1993). The problem of damage by pests is common problem of many herbaria. Most significant herbarium pests are some insects, moulds and sometimes even some rodents. In temperate regions herbivore insects grazing plants in nature can not survive in heated buildings and so they are not considered a threat to herbarium material. The infestation, caused by herbarium pests, occurs within storehouses, buildings, cabinets and herbaria (Skvorcov 1977). Therefore fresh plant material is not infected by herbarium pests yet, and the other pests, brought with the fresh material from the field, can not survive the drying procedure.

There are two common features of herbarium pests (Stein 1986): they feed on dry herbal material and they can survive under severe conditions of temperature and humidity in herbarium. Unlike the fresh-plant eating insects, being usually stenophagous, the herbarium pests are commonly euryphagous. They feed on the preserved plant material as well as on any other plant products like paper, textile, dry food etc. Provisionally deposed herbarium specimens in uncontrolled conditions are very likely to become infected (Skvorcov 1977). Most serious threat to herbarium material is by insects, particularly some beetle species (*Coleoptera*). They can also be found in storehouses, causing damage on cereals, flour, tea, tobacco, as well as in museum collections. The most frequent pest, causing great damage in herbaria as well as in tobacco storehouses, is tobacco beetle *Lasioderma serricorne* (*Anobiidae*), which is distributed worldwide (Ryan 1995, Zuska 1994). Some other significant beetle pests are: *Stegobium paniceum* (*Anobiidae*), *Ptinus fur* (*Ptinidae*), *Anobium punctatum* (*Anobiidae*), *Hylopteres bajulus* (*Cerambycidae*), *Xestobium rufovilllosum* (*Anobiidae*), *Lyctus brunneus* (*Lyctidae*), *Mycrobium castaneum* (*Anobiidae)*, *Attagenus piceu*s (*Dermestidae*) (Valentin 1993, Stein 1986). Some herbarium pests can also be found among other invertebrate groups: various booklices (*Psocoptera*) and silverfish (*Lepisma saccharina*, *Thysanura*).

Long-lasting high humidity in herbarium room often results in fungal damage of plant material. Fungal attack causes decomposition of the plant tissues and may cause plant features to be obscured and unsuitable for study (Bridson 1992). Even if the hyphae are destroyed, their spores may still persist for a longer period of time and germinate, when the conditions change (Rode 1989). Naphthalene and lauryl pentachlorophenate (LPCP) are believed to have fungicidal properties, however, Thymol is quite effective as fungicide (Maden 2004). There are many different chemical methods for desinsection and mould control in herbaria, but at present time there does not seem to be an ideal chemical to protect specimens from mould or insect attack (Clark 1986). Of the physical methods deep freezing for the incoming material is advisable (Clark 1986) and even microwave treatment is used in some herbaria (Hall 1981, Hill 1983).

Interesting feature of herbarium pests is their food-preference: insect pests are known to be naturally attracted to herbarium material of certain plant families like *Asteraceae* s. lat., *Brassicaceae, Capparaceae* and petaloid monocotyledons (*Liliaceae* s. lat.). If *Stegobium paniceum* is present, the damage occurs on *Asteraceae* s. lat*., Apiaceae*, *Ericaceae* and plant families that contain latex, e.g. *Apocynaceae*, and *Asclepiadaceae*. (Bridson 1992). Families *Betulaceae*, *Fagaceae*, *Caryophyllaceae*, *Convolvulaceae*, *Poaceae* and *Polypodiaceae* are considered to be less attractive to pests and therefore less threatened (Skvorcov 1977).

Since the important prevention measure against pests is a constant look-out for insects, Skvorcov (1977) recommends to bait the herbarium-pest traps with their favorite plant material to monitor the eventual infestation of the herbarium. He also suggests regular checks of *Tragopogon*, *Angelica* and *Vicia faba* herbarium sheets.

In the last decade, a considerable pest damage was observed in Herbarium LJU, at the Department of Biology, Biotechnical Faculty of University of Ljubljana. The aim of the study was to determine the damage in the herbarium, to find out, which herbarium-pests are present, and to investigate their food-preference by

means of checking the extent of damage on a set of certain plant families. The results of the research in Herbarium LJU should be comparable to other smaller herbaria in the temperate regions. They should also represent a basis for pest-control strategy in Herbarium LJU and comparable herbaria.

## Materials and Methods

*Herbarium LJU*

Herbarium LJU is a typical local herbarium, covering mostly Slovenian flora. It comprises about 160.000 herbarium sheets. Though it is relatively small, it represents the largest herbarium collection in Slovenia and has a history of over 100 years. It includes several important exsiccate collections, like Flora exsiccata Carniolica and Flora exsiccata Styriaca, and relatively many type specimens.

Before 1995 the herbarium was regularly fumigated with various gases, like carbon disulfide and – later – hydrogen cyanide. In 1995 the herbarium was moved to a new location. The first fumigation of the herbarium in the new locality was performed in 2000; phosphine gas was used. Until 2009 three additional fumigations with phosphine gas were performed and re-infestation with insects has not been observed so far. A common practice for prevention of infestation in LJU is decontamination of the incoming dried material before it enters the herbarium room. The sheets are subjected to deep freezing (at least -18°C) for a few days.

Until recently, the temperature and humidity in the herbarium were not controlled and the conditions somewhat vary during the year. During our investigation, the temperature and humidity was measured. The air temperature was found to be 19°C and the humidity 42 %. Since the investigation was performed during heating season, the measured temperature was probably a bit higher then otherwise.

*The Examined Plant Material*

The following set of plant families was chosen for the examination: *Alliaceae, Apiaceae, Araceae, Asteraceae, Cichoriaceae*, *Brassicaceae*, *Chenopodiaceae*, *Fabaceae*, *Lamiaceae*, *Poaceae*, *Polypodiaceae*, *Ranunculaceae*, *Rosaceae* and *Scrophulariaceae*.

For each of the studied families (with an exception of *Asterales*) in a random sample of genera a satisfactory amount of herbarium sheets has been chosen. In small plant families (*Araceae, Alliaceae*) a sample of over 30 herbarium sheets of various genera was chosen and in larger families (*Apiaceae, Brassicaceae, Chenopodiaceae, Asteraceae, Cichoriaceae, Fabaceae, Lamiaceae*, *Poaceae, Rosaceae, Scrophulariaceae, Ranunculaceae* and *Polypodiaceae*) the size of the sample was over 100 sheets. The criterion for the random sampling of genera was set according to alphabetical order. For the inclusion of a certain genus in a sample, the genus had to be represented by a minimum of 10 sheets (smaller genera) or 30 sheets (larger genera) of at least one species: the examined genera and the sample sizes are listed in

Table 1: Percentages of the damaged sheets in the examined genera.
Tabela 1: Odstotek poškodovanih herbarijskih pol pri pregledanih rodovih.

| | % damaged sheets | inspected sheets | | % damaged sheets | inspected sheets |
|---|---|---|---|---|---|
| ***Alliaceae*** | | | *Anthriscus* | 34,0 | 50 |
| *Allium* | 24,1 | 170 | *Aethusa* | 33,3 | 21 |
| ***Araceae*** | | | ***Compositae*** | | |
| *Arum* | 25 | 24 | *Achilea* | 90,1 | 223 |
| *Acorus* | 5 | 2 | *Adenostyles* | 13,9 | 36 |
| ***Apiaceae*** | | | *Ambrosia* | 33,3 | 15 |
| *Aegopodium* | 28,6 | 21 | *Antennaria* | 8,6 | 58 |
| *Angelica* | 51,5 | 33 | *Anthemis* | 25,0 | 44 |

| | % damaged sheets | inspected sheets |
|---|---|---|
| *Aposeris* | 11,8 | 34 |
| *Arctium* | 72,7 | 44 |
| *Arnica* | 42,9 | 35 |
| *Artemisia* | 46,8 | 79 |
| *Aster* | 17,4 | 167 |
| ***Brassicaceae*** | | |
| *Aethionema* | 11,5 | 26 |
| *Alliaria* | 6,7 | 15 |
| *Alyssum* | 36,7 | 79 |
| *Arabidopsis* | 12,5 | 16 |
| *Arabis* | 22,7 | 255 |
| ***Chenopodiaceae*** | | |
| *Atriplex* | 17,2 | 29 |
| ***Fabaceae*** | | |
| *Anthyllis* | 41,5 | 130 |
| *Astragalus* | 1,0 | 103 |
| ***Lamiaceae*** | | |
| *Acinos* | 2,3 | 130 |
| *Ajuga* | 0,0 | 96 |
| *Ballota* | 0,0 | 16 |
| *Betonica* | 3,8 | 79 |
| ***Poaceae*** | | |
| *Achnatherum* | 0,0 | 20 |
| *Agropyron* | 0,0 | 62 |
| *Agrostis* | 0,0 | 39 |
| *Alopecurus* | 2,5 | 40 |
| *Anthoxanthum* | 1,8 | 56 |
| *Apera* | 16,7 | 12 |
| *Arrhenatherum* | 0,0 | 30 |
| ***Polypodiaceae*** | | |
| *Adianthum* | 14,3 | 21 |
| *Asplenium* | 3,7 | 242 |
| *Athyrium* | 4,8 | 104 |
| *Telypteris* | 0,0 | 73 |
| *Pteridium* | 3,0 | 33 |
| ***Ranunculaceae*** | | |
| *Actaea* | 5,0 | 40 |
| *Anemone* | 0,6 | 181 |
| *Batrachium* | 3,1 | 127 |
| *Pulsatilla* | 13,0 | 100 |
| ***Rosaceae*** | | |
| *Agrimonia* | 0,0 | 39 |
| *Alchemilla* | 34,6 | 347 |
| *Aphanes* | 0,0 | 10 |
| *Aremonia* | 4,5 | 44 |
| *Aruncus* | 32,0 | 25 |
| *Amelanchir* | 25,0 | 44 |
| ***Scrophulariaceae*** | | |
| *Bartsia* | 0,0 | 28 |
| *Digitalis* | 11,4 | 44 |
| *Scrophularia* | 40,9 | 44 |

Since ordo *Asterales* is known to be one of the most attractive families for herbarium pests, we examined it in detail, checking all the available genera. In this separate analysis the following genera were included: *Achilea, Adenostyles, Ambrosia, Anthemis, Antennaria, Aposeris, Arctium, Arnica, Artemisia, Aster, Bellis, Carduus, Carlina, Centaurea, Cirsium, Conyza, Crepis, Doronicum, Echinops, Erigeron, Eupatorium, Filago, Gnaphalium, Hieracium, Homogyne, Hypochoeris, Inula, Lactuca, Leontodon, Leucanthemum, Petasites, Picris, Pulicaria, Saussurea, Scorzonera, Senecio, Serratula, Solidago, Sonchus, Tanacetum, Taraxacum, Tragopogon* and *Tussilago.* For each of the investigated genera all the available material was examined, with an exception of *Hieracium, Crepis, Leontodon* and *Centaurea*, where only a satisfactory large random sample was chosen.

Very old sheets (over 100 years) were not taken into account, since most of the them were at that time protected with mercuric chloride, which provides a long-lasting immunity and may therefore blur the results. Non-slovenian material was also excluded from the examination.

## Examining the Extent of Damage

The examination of the extent of damage included visual inspection and counting the damaged and intact herbarium sheets. The counts obtained were used to calculate percentage of

the damaged sheets for the examined families and genera.

The sheets were characterized as 'damaged', if the following evidences of herbarium pests were present:

– deposits of fine granular droppings on the plant specimens and paper,
– missing parts of plants, holes in sheets, leaves, stems, roots and rhizomes, inflorescence and/or flowers as a result of larval forage,
– ruined inflorescence and/or flowers,
– a presence of crawling or dead adult pests.

## Results and Discussion

*The herbarium pests*

In the last few years and during our examination the following *Coleoptera* species were found: *Lasioderma serricorne*, *Stegobium paniceum* in *Attagenus piceus.* Most of the found individuals were determined as *Lasioderma serricorne*, while the last two species were represented each by only a single adult. All the adult beetles were found dead. The results partly correspond to the list of coleopteran pests, found in herbarium material in nature-history department of National museum in Ljubljana (Kos 1944). Among the listed species in Kos (1944) there are *Attagenus piceus* and *Sitodrepa panicea* (syn.: *Stegobium paniceum*), but *Lasioderma serricorne*, the main pest in LJU 50 years latter, was missing. The other species from the museum herbarium are *Anthrenus verbasci*, *Ptinus fur*, *Cartodere filiformis*, *C. filum* (most common) (Kos 1944).

Other observed pests belong to *Psocoptera*: the booklices were alive and very numerous. They are known to attack tenderer plant organs, like petals and anthers (Skvorcov 1977). The damage we observed was rather heavier: leaves, stems and peduncles were also attacked.

Fungal damage was observed, too. Considering the fact that in LJU there had been no humidity control, the fungal infection is not surprising.

In the working room of herbarium, where a considerable amount of unsorted material is tem-

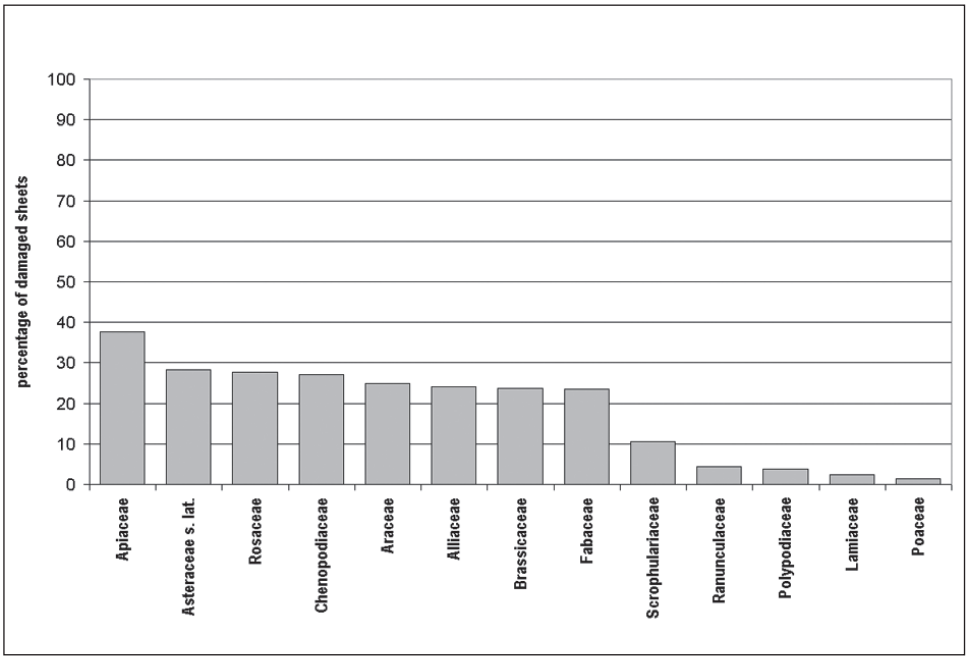


Figure 1: Percentages of the damaged sheets in the examined families.
Slika 1: Odstotek poškodovanih herbarijskih pol po družinah.

porarily deposed and the temperature is a few degrees higher, Pharaoh's ants (cf. *Monomorium pharaonis*) were found in some sheets. Bridson and Forman (1992) report that these pests are rather common in heated herbaria of temperate regions. They are also common pests in storehouses in Ljubljana (Hržič and Urek 1989).

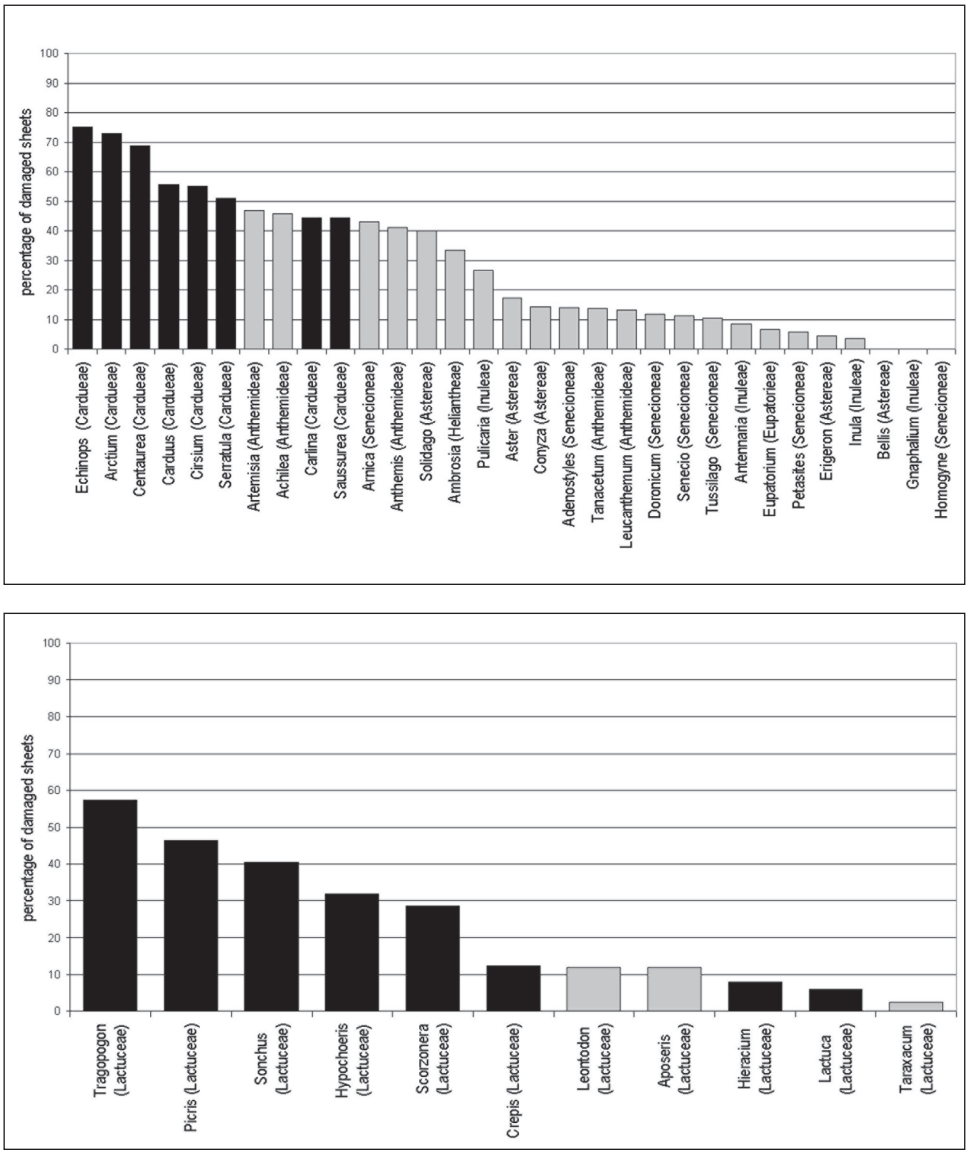


Figure 2: Percentages of the damaged sheets in the examined Asteraceae genera grouped by 2 subfamilies (upper graph: *Asteroideae*, lower graph: *Cichorioideae*). Tribus *Cardueae* shown by black coloumns.

Slika 2: Odstotek poškodovanih herbarijskih pol pri košarnicah združenih po poddružinah (zgornji graf: *Asteroideae*, spodnji graf: *Cichorioideae*), tribus *Cardueae* obarvan črno.

*The damage*

In the study there were about 7500 herbarium sheets examined, which roughly represent 5 % of all the sheets in Herbarium LJU. Pest-damage was observed in 18 % of the examined herbarium sheets. This percentage can not be applied to all the material in LJU, since the family sample was not chosen randomly.

The results fully confirm the fact, that the pests are prone to attack certain families over others: in accordance with the literature ( Skvorcov 1977, Hall 1988, Bridson 1992, Nikolić 1996,) the greatest damage was observed in *Apiaceae*, *Asteraceae, Cichoriaceae*, *Brassicaceae*, *Fabaceae* and *Alliaceae* (Fig. 1). The study showed approximately the same high level of damage in *Araceae*, *Rosaceae* and *Chenopodiaceae* (Fig.1).

The high percentage of the damaged sheets in *Chenopodiaceae* was rather unexpected since Skvorcov (1977) reports the family to be among less threatened. However, the damage on *Chenopodiaceae* specimens was far less extreme than for instance on *Apiaceae* and *Asteraceae*. In the column chart in Fig. 1 a gap between the 10 % and 20 % of the damaged sheets can be observed: it represents the gap between the 'threatened' and the 'less threatened' families. *Poaceae* and *Polypodiaceae* proved to be among the latter. Family *Lamiaceae*, commonly reported to be attractive to pests (Skvorcov 1977), also appeared among the less damaged. Considering the great content of essential oils, which are known to act as repellent substances, this result can be explained. Families *Ranunculaceae* and *Scrophulariaceae* also ranked among the less damaged. The two are known for their poisonous and repellent secondary substances. Tab. 1 shows the sample sizes and percentages of the damaged sheets in all of the examined genera.

With over 20 000 species, family *Asteraceae s.lat.* is one of the largest plant families. According to some authors (Cronquist 1981) one of the reasons for it's evolutionary successfulness is also the presence of poisonous and repellent substances, for instance polyacetylene, sesquiterpene lactones, alkaloids (*Senecioneae*), latex (*Lactuceae*) and further some malodoriferous essential substances (*Heliantheae* in *Anthemideae*). According to the study, the poisons and repellents are not very effective in the case of herbarium-pest attack.

Among *Asteraceae, Cardueae* proved to be the most attractive tribe. The damage increases from about 50 % of the damaged sheets in genera *Saussurea*, *Carlina*, *Cirsium* and *Carduus*, reaches 70 % in genus *Centaurea* and the maximum damage of 75 % in *Echinops* (Fig. 2).

The damage is generally limited to the inflorescence, but sometimes leaves are also eaten. The tribe *Cardueae* is known for it's ambiguous taxonomical status: it's (sub)family alliance is yet uncertain (Heywood 1993). The position of the *Senecioneae* tribe, one of the less damaged, is also doubtful: some authors consider it as a third subfamily (Heywood 1993). As expected (Skvorcov 1977), among *Cichoriaceae, Tragopogon* is the most attractive (almost 60 % of the damaged sheets). All the investigated genera belong to *Lactuceae*, which is also mentioned by Hall (1988) as one of the most attractive for the herbarium pests. The column chart of the two families in Fig. 2 reveals a gap approximately between the 15 % and 30 % of the damaged sheets, the 'less threatened' genera being under the lower value and the 'threatened' above the upper. Probably the pests eat the 'less threatened' material only by chance or when their population is sufficiently large.

## Conclusions

As a result of our study, a list of most threatened families and genera by herbarium pests is produced. For the herbaria with predominantly northern hemisphere temperate region plant material, keepers should regularly monitor sheets of *Apiaceae*, *Asteraceae s. lat.*, *Brassicaceae*, *Fabaceae*, *Alliaceae*, *Araceae*, *Rosaceae* and *Chenopodiaceae* for the presence of common herbarium-pests. In addition to that, for *Stegobium paniceum* a pheromone trap is commercially available and can be used specially in herbaria after infestation of *Lasioderma serricorne* to monitor efficiency of pest control and early detect surviving population.

## Povzetek

Težave s škodljivci imajo v mnogih herbarijskih zbirkah. Najbolj značilni herbarijski škodljivci so nekatere žuželke (posebej hrošči - *Coleoptera*, pa tudi prašne uši - *Psocoptera* in srebrne ribice - *Thysanura*), plesni in celo nekateri glodalci. Herbarijski škodljivci se hranijo s posušenim rastlinskim materialom, kot tudi z izdelki iz rastlin, kot so papir, tkanine, suha hrana, tobak ...

Žuželke v vlogi herbarijskih škodljivcev naravno privlačijo herborizirane rastline določenih družin pred drugimi. Tako so na primer posebej ogrožene družine *Asteraceae* s. lat., *Brassicaceae, Capparaceae* in petaloidne enokaličnice (*Liliaceae* s. lat.), po drugi strani pa so družine kot *Betulaceae*, *Fagaceae*, *Caryophyllaceae*, *Convolvulaceae*, *Poaceae* in *Polypodiaceae* škodljivcem manj privlačne (Skvorcov 1977).

Herbarij LJU na Oddelku za biologijo Biotehniške fakultete Univerze v Ljubljani obsega približno 160 000 herbarijskih pol in kljub relativni majhnosti predstavlja največjo urejeno herbarijsko zbirko v Sloveniji. V zadnjem desetletju je bila v herbariju LJU opažena znatna škoda zaradi harbarijskih škodljivcev. Namen raziskave je bil ugotoviti obseg škode, dognati, kateri herbarijski škodljivci se tu pojavljajo in ugotoviti njihovo prehrambeno preferenco s primerjanjem obsega poškodovanosti herbarijskih pol različnih rastlinskih družin. Rezultati raziskave lahko služijo kot osnova za pripravo strategije nadzora nad herbarijskimi škodljivci tako v zbirki LJU, kot tudi v primerljivih manjših herbarijih v zmernem pasu.

V raziskavo smo vključili naslednje rastlinske družine: *Alliaceae, Apiaceae, Araceae, Asteraceae, Cichoriaceae*, *Brassicaceae*, *Chenopodiaceae*, *Fabaceae*, *Lamiaceae*, *Poaceae*, *Polypodiaceae*, *Ranunculaceae*, *Rosaceae* in *Scrophulariaceae*. Za vsako od raziskovanih družin (razen *Asteralceae* s. lat.) smo znotraj naključno izbranega niza rodov pregledovali herbarijske pole. Pri manjših družinah (*Araceae, Alliaceae*) je bil vzorec velik preko 30 pol iz različnih rodov, pri velikih družinah (*Apiaceae, Brassicaceae, Chenopodiaceae, Asteraceae, Cichoriaceae, Fabaceae, Lamiaceae*, *Poaceae, Rosaceae, Scrophulariaceae, Ranunculaceae* in *Polypodiaceae*) pa je bilo število pregledanih pol čez 100. Košarnice (*Asterales*) smo pregledali podrobneje, in sicer v glavnem vse pole vseh razpoložljivih rodov: *Achilea, Adenostyles, Ambrosia, Anthemis, Antennaria, Aposeris, Arctium, Arnica, Artemisia, Aster, Bellis, Carduus, Carlina, Centaurea, Cirsium, Conyza, Crepis, Doronicum, Echinops, Erigeron, Eupatorium, Filago, Gnaphalium, Hieracium, Homogyne, Hypochoeris, Inula, Lactuca, Leontodon, Leucanthemum, Petasites, Picris, Pulicaria, Saussurea, Scorzonera, Senecio, Serratula, Solidago, Sonchus, Tanacetum, Taraxacum, Tragopogon* in *Tussilago.*

Za vsako polo smo ugotovili, ali herborizirane rastline kažejo znake poškodovanosti zaradi herbarijskih škodljivcev, kot so odloženi drobni zrnati iztrebki na rastlini ali papirju, požrti deli rastlin, luknje v papirju, listih, steblih, podzemnih delih, socvetju ali cvetovih kot posledica pašnje larv, uničenost socvetij ali cvetov in prisotnost živih ali mrtvih odraslih škodljivcev. Poškodovane pole smo prešteli in iz števil poškodovanih in vseh pregledanih pol izračunali odstotek poškodovanih pol za posamezne družine oz. rodove. Opazili smo naslednje škodljivce: hrošče *Lasioderma sericorne*, *Stegobium paniceum* in *Attagenus piceus,* prašne uši - *Psocoptera* in plesni, v herbarijskem predprostoru pa tudi faraonske mravlje *(Monomorium pharaonis).* Med hrošči nismo našli živih primerkov, prašne uši pa so bile žive in zelo številne. Napadajo predvsem nežnejše rastlinske dele, kot so cvetni listi in prašniki (Skvorcov 1977).

Med raziskavo smo pregledali okoli 7500 pol, kar predstavlja približno 5 % vseh pol v LJU. Škodo zaradi herbarijskih škodljivcev smo opazili pri 18 % pregledanih pol.

Rezultati povsem podpirajo navedbe, da imajo herbarijski škodljivci preferenco do določenih družin pred drugimi. Največja škoda je tako bila opažena pri družinah *Apiaceae*, *Asteraceae, Cichoriaceae*, *Brassicaceae*, *Fabaceae* in *Alliaceae* (Fig. 1)*,* ki jih tudi literatura navaja za posebej privlačne (Bridson 1992, Skvorcov 1977, Nikolić 1996, Hall 1988). Približno enak (visok) delež poškodovanosti pa je bil ugotovljen tudi za družine *Araceae*, *Rosaceae* in *Chenopodiaceae* (Fig.1). Visoka stopnja poškodovanost družine *Chenopodiaceae* je v

nasprotju z literaturnimi navedbami, po katerih naj bi bila ta družina manj privlačna (Skvorcov, 1977). Družini *Poaceae* in *Polypodiaceae* sta se izkazali za manj privlačne. Tudi *Lamiaceae*, ki jih sicer navajajo za bolj privlačne (Skvorcov 1977), so se v naši raziskavi uvrščale med manj poškodovane. To si lahko razlagamo z veliko vsebnostjo eteričnih olj, ki imajo repelentno delovanje. Tudi družini *Ranunculaceae* in *Scrophulariaceae* se uvrščata med manj privlačne, obe sta tudi znani po strupenih in repelentnih snoveh. Znotraj družine *Asteraceae* so se za najprivlačnejše izkazali predstavniki tribusa *Cardueae.* Odstotek poškodovanosti pol je naraščal od približno 50 % poškodovanih pol pri rodu *Saussurea*, *Carlina*, *Cirsium* in *Carduus*, dosegel 70 % pri rodu *Centaurea*, največja poškodovanost pol (75 %) pa se je izkazala pri rodu *Echinops* (Fig. 2). Najpogosteje so bila poškodovana socvetja, sem in tja pa tudi listi. Po pričakovanjih (glej Skvorcov 1977) je znotraj družine *Cichoriaceae* najprivlačnejši rod *Tragopogon* (skoraj 60 % poškodovanih pol).

## Acknowledgement

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# Vegetation of the depressions with *Eleocharis quinqueflora* in spring fens in Slovenia

## Vegetacija uleknin z vrsto *Eleocharis quinqueflora* na povirnih barjih v Sloveniji

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**Abstract:** During the investigations of wetlands in Slovenia over the last decade specific plant communities in spring fens were found. Stands with species *Eleocharis quinqueflora* occurring in depressions inundated with standing and/or running water were found in the Alpine, pre-Alpine and Dinaric phytogeographic regions of Slovenia. Standard Central European method for vegetation research was used and multivariate analyses were performed using Syn-Tax program. Stands were classified in two different species-poor, small-scale plant communities, most of them into association *Eleocharitetum pauciflorae* Lüdi 1921. This rare plant community occurs in the Alpine and Carpathian regions and in northern Europe and has not been recorded in Slovenia before. The association *Eleocharitetum pauciflorae* is a two-layered plant community of calcium-rich fens. It thrives in shallow temporary paddies and on the sandy or stony slopes with seeping water. Smaller group of relevés was classified into association *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960. This association thrives in permanent paddies, where the water is deeper as in a case of the first association. Since the dominating species *Eleocharis quinqueflora* and *Utricularia minor*, respectively, have the status of a vulnerable species according to Red List of Slovenia, the stands of the studied communities, which represent vital populations, should be preserved as well as the corresponding habitat types.

**Key words:** wetland, fen, plant community, *Eleocharis quinqueflora, Utricularia minor*, vulnerable species.

**Izvleček:** V sklopu preučevanja mokrišč v Sloveniji v zadnjem desetletju, smo v povirnih barjih našli specifične rastlinske združbe. V Alpskem, Predalpskem in Dinarskem fitogeografskem območju smo našli sestoje z vrsto *Eleocharis quinqueflora*, ki se pojavljajo v ulekninah poplavljenih s stoječo ali tekočo vodo. Pri preučevanju vegetacije smo uporabili standardno srednjeevropsko metodo, multivariatne analize pa so bi le opravljene s programom Syn-Tax. Popise smo uvrstili v dve različni vrstnorevni miniaturni rastlinski združbi – večino v asociacijo *Eleocharitetum pauciflorae* Lüdi 1921. Ta redka rastlinska združba se pojavlja v Alpski in Karpatski regiji ter v severni Evropi in v Sloveniji še ni bila popisana. Asociacija *Eleocharitetum pauciflorae je dvoslojna* rastlinska združba s kalcijem bogatih nizkih barij, uspeva v plitvih občasnih lužah in na peščenih ali kamnitih brežinah z mezečo vodo. Manjšo skupino popisov smo uvrstili v asosciacijo *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960. Ta asociacija uspeva v stalnih lužah, kjer je voda globlja kot pri prvi asociaciji. Ker imata dominantni vrsti *Eleocharis quinqueflora* in *Utricularia minor* status ranljivih vrst v skladu z Rdečim seznamom Republike Slovenije, bi morali sestoje preučevanih združb, ki predstavljajo njihove vitalne populacije zavarovati, kakor tudi odgovarjajoč habitatni tip.

**Ključne besede:** mokrišče, nizko barje, rastlinska združba, *Eleocharis quinqueflora*, *Utricularia minor,* ranljive vrste.

## Introduction

Almost 100 years ago Rübel (1911) recorded the stands of the characteristic species *Eleocharis quinqueflora* on the sandy and wet bank of a dike on the altitude of 1715 m above the sea in Switzerland. Ten years later the association with the name *Eleocharitetum pauciflorae* Lüdi 1921 was described. The syntaxonomic classification of the analysed type of vegetation is questionable (Martinčič and coworkers 1994, Pott 1995) and it depends on the authors.

Koch (1926) and Tüxen (1937) treated community *Eleocharitetum pauciflorae* as subassociation of the association *Schoenetum nigricantis*. Both authors classified this community into class *Scheuchzerio-Caricetea fuscae* which unifies plant communities of the fens. Passarge (1964) classified this community named *Triglochin-Eleocharis pauciflora* into the mentioned class and order *Caricetalia davallianae,* but into special alliance *Eleocharition pauciflorae*. Braun (1968) classified this type of vegetation into class *Utricularietea intermedio-minoris,* order *Utricularietalia intermedio-minoris* and alliance S*phagno-Utricularion.* Similarly Oberdorfer (1977) classified such stands into *Scorpidio-Utricularietum minoris scorpidietosum* var. *Eleocharis quinqueflora.* Dierssen and Dierssen (1985), Steiner (1993), Pott (1995), Hájek and Háberová (2001) classified this plant community into class *Scheuchzerio-Caricetea fuscae*, order *Caricetalia davallianae*, alliance *Caricion davallianae* and association *Eleocharitetum pauciflorae* Lüdi 1921.

The classification of vegetation with *Utricularia minor* is studied and explained in detail in Dite and coworkers (2006). The association *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960 is classified into class *Isoëto-Nanojuncetea (*Hájek and Háberová 2001, Dite et al. 2006).

Wetlands, especially fens are supposed to be highly endangered ecosystems and so are the fen plant-communities and specific plant taxa as well. Ecology and vegetation of fens were studied in Slovenia by different authors: Leskovar 1990, 1996, Leskovar-Štamcar 1996, 1991, 1996, 2001, Martinčič and coworkers 1994, Zelnik. This type of plant communities, which thrives in inunda-ted depressions and shallow small lakes within different fen-vegetation types have not been studied before.

Thus the aim of the paper is to present original data on floristic composition, the distribution and ecology of this type of vegetation in Slovenia, to point out vulnerable species thriving in these stands and to emphasize the need to protect the fens.

## Materials and methods

The standard Central European phytosociological method (Braun-Blanquet 1964) was used for vegetation sampling. Cover-abundance estimation values were used, which were transformed in accordance with van der Maarel (1979). Relevés were made in summer months, when the majority of plant species are in optimal phase and determinable. The size of the plots ranges from 1 to 15 $m^2$ and depends on the microrelief. Multivariate statistical analyses of vegetation relevés were performed using SYNTAX 2000 (Podani 2001) programme. We used ordination method - Principal Coordinates Analysis (PCoA) and complement of Similarity ratio.

The nomenclature of vascular plants follows Martinčič et al. (2007); the nomenclature of mosses follows Martinčič (2003). The nomenclature of syntaxa is in accordance with Steiner (1993) and Valachovič and Ot'ahel'ová (2001).

## Results and discussion

During the survey of fens in Slovenia the stands of these small-scale plant communities were also found and 16 stands were recorded. Analytical table (Tab. 1) shows the floristic composition of these species-poor stands.

Table 1. Analytical table of associations: *Eleocharitetum pauciflorae* Lüdi 1921 and *Scorpidio-Utricularietum minoris* Ilschner ex. T.Müller et Görs 1960. V – vulnerable species, on the Red List of Ferns and Flowering plants in Republic Slovenia.

Tabela 1. Analitična tabela asociacij *Eleocharitetum pauciflorae* Lüdi 1921 in *Scorpidio-Utricularietum minoris* Ilschner ex. T.Müller et Görs 1960. V – ranljiva vrsta, na Rdečem seznamu praprotnic in semenk republike Slovenije.

| RL of RS | column number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **relevé number** | **2** | **3** | **4** | **5** | **7** | **8** | **13** | **6** | **9** | **10** | **1** | **14** | **15** | **11** | **12** | **16** |
| | **No. of vascular taxa** | 16 | 13 | 15 | 18 | 16 | 14 | 7 | 10 | 9 | 13 | 7 | 6 | 7 | 4 | 6 | 5 |
| | **No. of plant taxa** | 18 | 15 | 17 | 20 | 19 | 17 | 9 | 13 | 13 | 16 | 8 | 9 | 11 | 8 | 9 | 6 |
| **ass. *Scorpidio-Utricularietum minoris*, alliance *Scorpidio-Utricularion minoris*** | | | | | | | | | | | | | | | | | |
| and **class Utricularietea intermedio-minoris char. taxa** | | | | | | | | | | | | | | | | | |
| V | *Utricularia minor* | . | . | . | . | . | . | . | . | . | . | . | + | 1 | **3** | **2** | **1** |
| . | *Scorpidium scorpioides* | . | . | . | . | . | . | . | . | . | . | . | . | . | **.** | **2** | **3** |
| V | *Triglochin palustre* | . | . | . | . | . | . | . | . | . | . | + | . | . | . | + | . |
| **ass. *Eleocharitetum pauciflorae* char. taxon** | | | | | | | | | | | | | | | | | |
| V | *Eleocharis quinqueflora* | **5** | **5** | **4** | **5** | **5** | **4** | **3** | **4** | **4** | **4** | **4** | **3** | **2** | 1 | 2 | 1 |
| **subass. *drepanocladetosum* diff. taxon** | | | | | | | | | | | | | | | | | |
| . | *Drepanocladus cossonii* | 4 | 4 | + | + | + | + | + | 1 | + | + | . | + | + | 2 | 1 | . |
| **subass. *charetosum* diff. taxon** | | | | | | | | | | | | | | | | | |
| . | *Chara sp.* | . | . | . | . | . | . | . | **2** | **2** | **3** | **1** | **+** | **+** | . | . | . |
| **Caricion davallianae** | | | | | | | | | | | | | | | | | |
| . | *Carex lepidocarpa* | + | 1 | + | 1 | 1 | + | . | 1 | + | 1 | . | . | + | . | + | . |
| V | *Eriophorum latifolium* | r | . | . | + | + | + | . | . | + | + | . | . | . | 1 | + | + |
| V | *Carex davalliana* | 1 | + | + | 1 | . | . | . | 1 | 1 | + | + | + | . | . | . | . |
| V | *Carex hostiana* | . | . | + | + | + | + | . | + | . | . | . | . | . | . | . | . |
| . | *Valeriana saxatilis* | . | . | . | + | + | + | . | . | + | + | . | . | . | . | . | . |
| V | *Epipactis palustris* | . | . | + | . | . | . | + | . | . | . | . | . | . | . | . | . |
| V | *Schoenus nigricans* | . | . | . | . | . | . | . | . | 1 | 1 | . | . | . | . | . | . |
| . | *Equisetum telmateia* | r | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| **Caricetalia davallianae** | | | | | | | | | | | | | | | | | |
| . | *Pinguicula alpina* | 2 | 2 | + | + | . | + | + | + | . | + | . | + | + | . | . | . |
| . | *Tofieldia calyculata* | 1 | + | + | + | + | . | . | . | . | + | . | . | + | . | . | . |
| . | *Parnassia palustris* | . | + | + | + | + | + | + | . | + | . | . | . | . | . | . | . |
| V | *Equisetum variegatum* | . | . | . | . | + | + | . | . | . | . | . | . | . | . | . | . |
| . | *Juncus alpinoarticulatus* | . | . | . | . | . | . | . | . | . | . | + | + | . | . | . | . |
| . | *Campylium stellatum* | . | . | . | . | . | . | . | . | . | . | . | . | + | + | . | . |
| . | *Carex flava* | . | . | . | . | . | . | . | . | . | . | . | . | . | + | . | . |
| **Scheuchzerio-Caricetea fuscae** | | | | | | | | | | | | | | | | | |
| . | *Carex panicea* | 2 | + | 2 | 2 | + | 2 | + | 1 | 3 | 3 | + | . | . | . | . | . |
| . | *Juncus articulatus* | . | . | . | + | + | 1 | . | 1 | + | . | . | . | . | . | . | + |
| . | *Calliergon trifarium* | . | . | . | . | . | . | + | . | . | . | . | + | + | 2 | + | . |
| V | *Equisetum fluviatile* | . | . | . | . | . | . | . | . | . | . | . | + | + | . | . | . |
| V | *Drosera anglica* | . | . | . | . | . | . | . | . | . | . | . | . | + | . | . | . |
| **Molinio-Arrhenatheretea** | | | | | | | | | | | | | | | | | |
| . | *Equisetum palustre* | + | 1 | + | + | + | 1 | + | + | . | + | . | . | . | . | + | + |
| . | *Molinia caerulea* | 1 | 1 | + | + | 2 | 1 | + | + | . | + | . | . | . | . | . | . |
| . | *Cirsium oleraceum* | . | . | . | . | + | . | . | . | . | . | . | . | . | . | . | . |
| . | *Juncus acutiflorus* | . | . | . | . | . | . | . | . | . | . | + | . | . | . | . | . |
| **other phanerophytes** | | | | | | | | | | | | | | | | | |
| . | *Pinus sylvestris (juv.)* | + | + | + | + | + | . | . | r | . | . | . | . | . | . | . | . |
| . | *Fraxinus ornus (juv.)* | + | + | + | + | . | . | . | . | . | + | . | . | . | . | . | . |
| . | *Equisetum arvense* | r | . | . | + | + | + | . | . | . | . | + | . | . | . | . | . |
| . | *Acer pseudoplatanus (juv.)* | + | 1 | . | + | + | . | . | . | . | . | . | . | . | . | . | . |
| . | *Picea abies (juv.)* | + | + | + | + | . | . | . | . | . | . | . | . | . | . | . | . |
| . | *Potentilla erecta* | + | . | + | . | . | + | . | . | . | . | . | . | . | . | . | . |
| . | *Eupatorium cannabinum* | . | . | . | . | . | . | . | . | . | + | . | . | . | . | . | . |
| **other mosses** | | | | | | | | | | | | | | | | | |
| . | *Bryum pseudotriquetrum* | 1 | 1 | 4 | 4 | 1 | + | . | + | + | + | . | . | . | + | . | . |
| . | *Aneura pinguis* | . | . | . | . | + | + | . | . | + | . | . | . | . | . | . | . |

On the base of the results of multivariate analysis of the relevés and their aggregation in two distinct groups (Fig. 1) these two relevé groups can be undoubtedly classified in two mentioned plant communities. According to the presence and abundance of characteristic species bigger group was classified into association *Eleocharitetum pauciflorae* Lüdi 1921, while the second group was classified into association *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960.

*Floristic composition*

Beside the dominant species *Eleocharis quinqueflora* ten other species have the status of vulnerable species according to Red list of republic of Slovenia (Wraber et al 2002) (Table 1). That means that over one third of present species of vascular plants (11 out of 32) are considered as vulnerable.

The community *Eleocharitetum pauciflorae* (syn.: *Scirpus pauciflorus* ass. Osvald 1923, *Eleocharitetum quinqueflorae* (Zobrist 1935) Braun 1968 is a two-layered, species-poor, association of calcium-rich fens. Recorded relevés contain 6-18 species of vascular plants (Table 1).

The association is defined by the absence of character species of other associations of the alliance *Caricion davallianae,* but most of all, by dominance of *Eleocharis quinqueflora* (Hájek and Háberová 2001). Steiner (1992, 1993) has defined the following diagnostic species combination:

Character and dominant species: *Eleocharis quinqueflora*

Dominant and/or most common species: *Drepanocladus revolvens agg.* (dom.)*, Carex flava* (subdom.)*, C. panicea, Eriophorum latifolium, E. angustifolium, Molinia caerulea, Parnassia palustris, Pinguicula vulgaris, Tofieldia calyculata.*

On the base of the presence and abundance of the cryptogams, we can divide the association *Eleocharitetum pauciflorae* Lüdi 1921 in two subassociations (Hájek and Háberová 2001). Steiner (1993) defined two subassociations - one with taxon *Chara* thriving on flooded sites and the other with *Drepanocladus revolvens* thriving on the calcium-rich stony or sandy sites with thin-layered peat. Since the species *Drepanocladus revolvens* thrives on acidic peat bogs, this subassociation could not be defined with the mentioned moss species but with species *Drepanocladus cossonii,* which was actually found in our fens. Both of the subassociations were found in the researched area (Tab. 1). Subassociation with *Chara* on average contains nine species of vascular plants, while the subassociation with *Drepanocladus* characteristic for less wet conditions contains 14 species on average. The reason is in anoxic conditions in inundated substrate, which often occur in the sites of the first subassociation, while the sites of the second subassociations can dry up during the periods of summer droughts.

On some of the sites of the same habitat type, but with slightly deeper and permanent water the community *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960 was found. This plant community is characterized, above all, by the dominance of free floating macrophyte *Utricularia minor*. Three character species occur in research area: *Utricularia minor, Scorpidium scorpidioides* and *Triglochin palustre*. Diferential species *Eleocharis quinqueflora,* which is subdominant in stands of this association (Valachovič and Ot'ahel'ová 2001) is present in all relevés (Tab. 1).

Community *Scorpidio-Utricularietum* consists of 3-7 species on average only (Valachovič and Ot'ahel'ová 2001). Recorded relevés contain 4-6 species of vascular plants (Tab. 1), but 6-9 species in total, what is slightly higher than stands recorded in Slovakia. Possible reasons are warmer climate and a greater species pool of Dinaric floral province.

*Syntaxonomic position and distribution*

The distribution of the species *Eleocharis quinqueflora* and the distribution of the association *Eleocharitetum pauciflorae* in Slovenia are shown on the map (Fig. 2). This rare association reaches here the southeast border of its distribution. It was found in the Alpine, pre-Alpine and Dinaric phytogeographic regions of Slovenia in fens on dolomite limestone substrate in mountain belt. The altitude of those fens ranges between 500 and 755 m above the sea level.

Association *Scorpidio-Utricularietum minoris* has even narrower distribution in Slovenia due to narrow distribution of its dominant species *Utricularia minor*. It was found on three localities only, namely on the Bloke plateau in Dinaric and near Hotedršica in pre-Alpine phytogeographic region. In all three localities *Eleocharitetum pauciflorae* was also found. These stands were found within fens, the altitude ranged from 570 to 775 m.

According to the floristic composition, presence of the characteristic species and location within the communities from the mentioned fen vegetation types the association *Eleocharitetum pauciflorae* Lüdi 1921 was classified into alliance *Caricion davallianae*, order *Caricetalia davallianae* and class *Scheuchzerio-Caricetea fuscae*. The mentioned order and a class unify the vegetation of fens.

Association *Eleocharitetum pauciflorae* Lüdi 1921 occurs from SW France across the Alpine and Carpathian regions to the Northern Europe and Baltic region, respectively (Balátová-Tuláčková and Venanzoni 1990, Steiner 1992). Pott (1995) reports its occurring in pre-Alpine region, in fens and sites with seeping water in the mountains of the middle belt of Central Germany and on the shores of the islands in the North Sea. This community was also found in the alpine region in Austria, the closest in South Carinthia and Styria (Steiner 1992). Community is very rare in Slovakia and southern Poland (Hájek and Háberová 2001).

We have classified association *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960 into alliance *Scorpidio-Utricularion minoris* Pietsch 1964, order *Utricularietalia intermedio-minoris* Pietsch 1965 and class *Isoëto-Nanojuncetea* Br.-Bl. et R.Tx. ex Westhoff et al. 1946, according to the floristic composition and presence of the characteristic species. The order *Utricularietalia intermedio-minoris* contains floating plant communities of the fen and peat lakes.

Stands of the association *Scorpidio-Utric-*

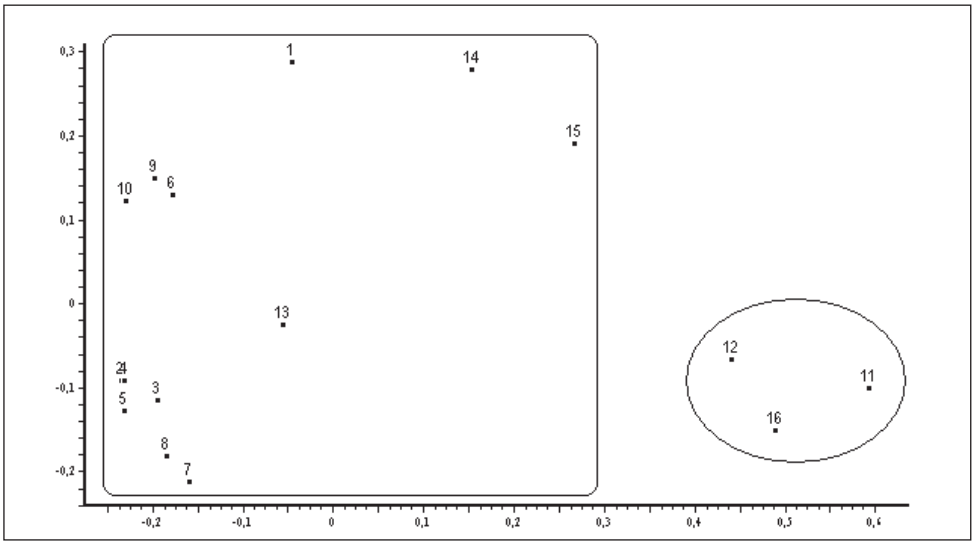


Figure 1. Ordination diagram of the relevés obtained as a result of Principal Coordinate analysis. Relevés on the right-hand side (11, 12, 16 – in ellipse) were classified into association *Scorpidio-Utricularietum minoris*. All other relevés belong to the association *Eleocharitetum pauciflorae* (in rectangle). Relevés 14 and 15 represent a transition since they contain species characteristic for both associations.

Slika 1. Ordinacijski diagram popisov kot rezultat analize glavnih koordinat (PCoA). Popisi na desni strani (11, 12, 16 – v elipsi) so bili uvrščeni v asociacijo *Scorpidio-Utricularietum minoris*. Vsi ostali popisi spadajo v asociacijo *Eleocharitetum pauciflorae* (v pravokotniku). Popisa 14 in 15 predstavljata prehod, saj vsebujeta značilne vrste obeh asociacij.

*ularietum minoris* have the centre of distribution in boreal-suboceanic region in Alpine region stands are species-poorer (Valachovič and Ot'ahel'ová 2001). Association is common in Germany (Pott 1995) and in Austria (Wallnöfer 1993), but rarely found in Slovakia, Czech Republic and southern Poland (Valachovič and Ot'ahel'ová 2001).

The subassociation *Scorpidio-Utricularietum minoris charetosum* is very similar to *Eleocharitetum pauciflorae charetosum*. Oberdorfer (1977) even treats both associations as *Scorpidio-Utricularietum minoris*. Some other authors also consider both associations as one.

*Ecology*

Both associations are pioneer plant communities, which are sometimes only fragmentally developed (Valachovič and Ot'ahel'ová 2001) and we found them on disturbed sites within different fen communities, mostly of the alliance *Caricion davallianae*.

*Eleocharitetum pauciflorae* is a community of calcium-rich fens. It is always occurring in small patches on sites where the substrate is saturated with basic ions (Pott 1995). The permeable sandy soil is wet due to seepage or high groundwater rich in carbonate (Steiner 1993). This community also thrives in coastal area in wet depressions of halophile grasslands (Pott 1995).

In Slovenia this rare fen community can be found on limestone-rich sites. Dominant species *Eleocharis quinqueflora* forms low and loose stands. These stands thrive on open and mostly wet sandy or silty substrate. This community thrives in spring areas in shallow paddies and on the sandy or stony slopes with seeping water. Water is alkaline and rich in $Ca^{2+}$ (>20 mg/l), electric conductivity is always higher than 300

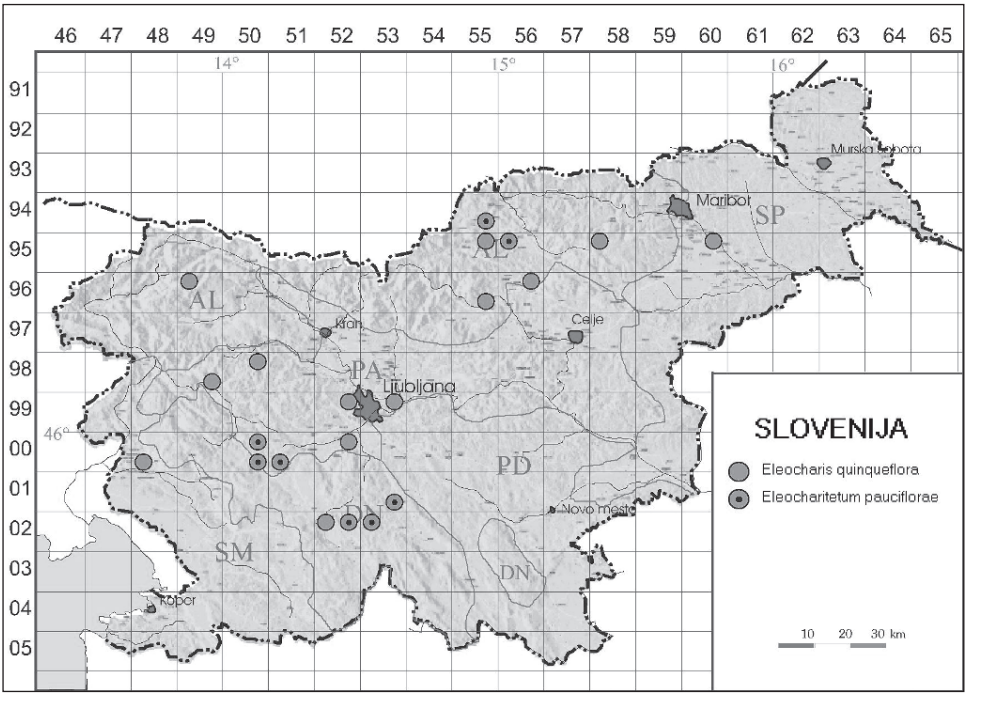


Figure 2. Distribution of the species *Eleocharis quinqueflora* in Slovenia and of the association *Eleocharitetum pauciflorae*. Localities are presented in the grid of central European mapping.

Slika 2. Razširjenost vrste *Eleocharis quinqueflora* in asociacije *Eleocharitetum pauciflorae* v Sloveniji. Lokalitete so predstavljene v mreži srednjeevropskega kartiranja.

μS/cm (Martinčič 1994).

In similar sites with slightly deeper water the community *Scorpidio-Utricularietum minoris* was also found. This community thrives mostly in paddies with 5-20 cm deep water. Water is mesotrophic to oligotrophic, pH being neutral to alkaline.

Alkaline fens are classified onto the List of a Decree on Habitat Types which are being preferentially maintained in good condition and are rare in Republic of Slovenia, vulnerable or they have small natural distribution area.

## Conclusions

Both studied plant communities have been alternatively classified as a subunit of the other one, by different authors. On the other hand they belong to different types of vegetation, even into different classes – *Eleocharitetum pauciflorae* belongs into the class of fens and *Scorpidio-Utricularietum minoris* belongs into the class of fen and/or peat lakes, that indicates the evident differences in ecological conditions and species composition. The most probable reason for such ambiguity is a low number of characteristic species in both associations, what could make classification difficult especially in the presence of species that both have in common. However the main difference in ecological conditions, which forms two vegetation types, is the duration of water phase. Water phase is more or less permanent in case of the association with *Utricularia* (*Scorpidio-Utricularietum minoris),* while in the sites of other association (*Eleocharitetum pauciflorae*) the water dries up regularly.

The common fact in both plant communities is the high share of vulnerable Red List species and their rarity in Slovenia and in Central Europe. The presence of the studied plant communities would give additional reasons for protection of the fens, which have already been recognized as endangered habitat types. Stands with *Eleocharis quinqueflora* are also vulnerable due to their dependence on hydrological regime in the landscape, which is changing nowadays. The studied stands are on the southeast margin of their distribution. Marginal areas are most sensitive to the changes, so these data represent important material for the estimation of consequences of the local as well as global changes.

## Povzetek

Sestoji z vrsto *Eleocharis quinqueflora* so bili popisani že pred skoraj 100 leti, vendar so še več desetletij po tem različni avtorji tovrstne sestoje uvrščali v različne vegetacijske tipe. Nekateri avtorji so sestoje s prevladujočima vrstama *Eleocharis quinqueflora* in *Utricularia minor* uvrstili v isto rastlinsko združbo. Vrstna sestava, ekologija in razširjenost preučevane vegetacije je v nekaterih evropskih državah dobro preučena, v Sloveniji pa o tem še ni bilo objavljenih del. V Sloveniji smo v povirnih barjih našli specifične rastlinske združbe. V Alpskem, Predalpskem in Dinarskem fitogeografskem območju smo našli sestoje z vrsto *Eleocharis quinqueflora,* ki se pojavljajo v stalno ali občasno poplavljenih ulekninah.

Pri preučevanju vegetacije smo uporabili standardno srednjeevropsko metodo, multivariatne analize pa so bile opravljene s programom Syn-Tax. Pri tem smo uporabili analizo glavnih koordinat (PCoA). Popise smo uvrstili v dve različni vrstnorevni miniaturni rastlinski združbi – večino v asociacijo *Eleocharitetum pauciflorae* Lüdi 1921. Manjšo skupino popisov smo uvrstili v asosciacijo *Scorpidio-Utricularietum minoris* Ilschner ex T.Müller et Görs 1960.

Asociacija *Eleocharitetum pauciflorae* je dvoslojna rastlinska združba s kalcijem bogatih nizkih barij, uspeva v plitvih občasnih lužah in na peščenih ali kamnitih brežinah z mezečo vodo. Je redka rastlinska združba, ki se pojavlja v Alpski in Karpatski regiji ter v severni Evropi. Asociacija je definirana predvsem z dominanco vrste *Eleocharis quinqueflora.*

Asociacija *Scorpidio-Utricularietum minoris* uspeva v stalnih lužah, kjer je voda globlja kot pri prvi asociaciji. V vodi na teh rastiščih je visoka koncentracija kalcija in ima bazično reakcijo. Asociacija je definirana predvsem z dominanco prosto plavajoče vrste *Utricularia minor.*

Ker imata dominantni vrsti *Eleocharis quinqueflora* in *Utricularia minor* status ranljivih vrst v skladu z Rdečim seznamom Republike Slovenije, bi morali sestoje preučevanih združb,

ki predstavljajo njihove vitalne populacije zavarovati, kakor tudi odgovarjajoč habitatni tip.

Poleg omenjenih dominantnih vrst ima še devet drugih vrst status ranljive vrste in so uvrščene na Rdeči seznam RS, kar predstavlja več kot tretjino vseh popisanih vrst cvetnic.

Na podlagi prisotnosti in pogostosti mahov in alg, lahko asociacijo *Eleocharitetum pauciflorae* razdelimo na dve subasociaciji, ki smo jih tudi našli na preučevanem območju. Subasociacija s taksonom *Chara* uspeva na poplavljenih rastiščih, druga s taksonom *Drepanocladus revolvens,* pa na kamnitih ali peščenih rastiščih. Ker vrsta *Drepanocladus revolvens* iz istoimenskega agregata uspeva le na kislih visokih barjih, gre tukaj za vrsto *Drepanocladus cossonii,* ki smo jo v naših sestojih tudi določili in je značilna za bazična rastišča.

Izpostavljene so majhne okoljske razlike, ki pogojujejo razlike v vegetaciji. Sestoji z vrsto *Eleocharis quinqueflora* so še posebno ranljivi, saj so odvisni od hidrološkega režima v krajini, ki pa se danes spreminja. Sestoji v Sloveniji se nahajajo na južni meji razširjenosti. Robni predeli arealov so najbolj občutljivi na spremembe, zato tovrstni zapisi predstavljajo pomemben dokument, za ugotavljanje posledic, ne samo lokalnih, temveč tudi globalnih sprememb.

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**Appendix:**

**List of the relevé localities (from Table 1):**

**1:** 9556/1 Raduše, Smrčun; **2:** 9556/1 Raduše, Smrčun; **3:** 9556/1 Sele, Blatnik; **4:** 9556/1 Sele, Blatnik; **5:** 9556/1 Sele, Blatnik; **6:** 9455/4 Kot pri Prevaljah; **7:** 9455/4 Kot pri Prevaljah; **8:** 9455/4 Kot pri Prevaljah; **9:** 9455/4 Kot pri Prevaljah; **10:** 9556/14 Raduše, Smrčun; **11:** 0050/4 Hotedršica, Žejna dolina; **12:** 0050/4 Hotedršica, Žejna dolina; **13:** 0253/1 Volčje (Bloke); **14:** 0050/2 Žibrše, Žejna dolina; **15:** 0050/2 Žibrše, Žejna dolina; **16:** 0252/2 Ulaka (Bloke);

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# Environmental assessment and macrophytes of the watercourses Bloščica and Cerkniščica

## Okoljska ocena in makrofiti vodotokov Bloščice in Cerkniščice

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**Abstract:** The aim of the present work was to determine the abundance and distribution of macrophytes in streams Bloščica and Cerkniščica and to establish the relation between environment characteristics and abundance of macrophytes. The environmental and macrophytes' inventory was made on the whole length of the watercourse. We determined a presence, abundance and growth form of macrophytes and environmental parameters according to modified RCE Inventory. Nineteen taxa were found in the watercourse Bloščica and 20 taxa in the watercourse Cerkniščica. Canonical correspondence analysis revealed that six environmental parameters significantly affected macrophyte community, the most influential being bottom structure, the width of riparian zone, retention devices in a channel and the land use beyond the riparian zone.

**Key words:** environmental assessment, macrophytes, watercourses

**Izvleček:** V prispevku podajamo rezultate raziskav pojavljanja, razporeditve in pogostosti makrofitov v vodotokih Bloščica in Cerkniščica ter ugotavljamo povezavo med okoljskimi razmerami in pojavljanjem makrofitov. Makrofite, njihovo pogostost, rastno obliko in stanje širšega vodnega okolja po prirejeni RCE metodi, smo popisali na celotni dolžini izbranih vodotokov. V vodotoku Bloščica smo popisali 19 taksonov, v vodotoku Cerkniščica pa 20 taksonov. Kanonična korespondenčna analiza je pokazala, da šest okoljskih parametrov značilno vpliva na pojavljanje in pogostost makrofitov. Največji vpliv imajo struktura dna, širina obrežnega pasu, zadrževalne strukture v strugi in zaledje.

**Ključne besede:** okoljska ocena, makrofiti, vodotoki

## Introduction

Rivers are diverse and dynamic systems that play an important role in the complexity of the landscape (Chovanec et al. 2000). Macrophytes are fundamental to the structure and functioning of lowland river habitats (Baatrup-Pedersen and Riis 1999). Distribution and abundance of macrophytes are affected by several environmental and antropogenic factors and their interactions (Lacoul and Freedman 2006). Parameters exerting impact on macrophyte's growth and abundance in running waters are the following: climate, hydrology, geomorphology, nutrients and other chemical factors, biological interactions and human activities (Onaindia et al. 1996, Bernez et al. 2004, Hrivnàk et al. 2007). Ecological status of rivers is influenced by human activities that affect the physical properties of the riverbed, riparian vegetation and land beyond the riparian zone (Petersen 1992). These changes worsened the conditions of the river ecosystem and water quality as well as altered communities of aquatic organisms, including macrophytes, which play important roles in energy flow, nutrient cycling and sedimentation processes (Holmes 1999, Gaberščik et al. 2003). Macrophytes improve water quality, both directly through oxygenation and nutrient recycling, and

indirectly by providing surface for water-purifying algae, fungi and bacteria (Holmes 1999). Species composition of macrophytes and their abundance reflect the quality of an ecosystem as a whole. For that reason macrophytes are included in the EU Water Framework Directive (Council of the European Communities 2000), presenting one of the four indispensable biological elements, which determine the ecological status of rivers (Dodkins et al. 2005).

The aims of the present study were to determine the presence, abundance and distribution of macrophytes in watercourses Bloščica and Cerkniščica and to find out the relation between the environmental parameters and the occurrence of macrophytes.

## Materials and Methods

*Study area*

Watercourses Bloščica and Cerkniščica are located in Notranjska region (Slovenia) in karst area. Almost half of the catchment of both watercourses is covered by forest.

The watercourse Bloščica is intermittent watercourse flowing on Bloke plateau being a part of the watershed of the river Ljubljanica. Elevation of its flow ranged from 720 and 750 m. Catchment comprises of small tributaries (Runarščica, Blatni potok, Krajič, Ribjek) covering about 25 km$^2$. Due to its low slope, the watercourse Bloščica flows slowly and makes many meanders. It flows on dolomites first 6 km of its length. At Velike Bloke it cut its bed into limestone and dolomite and finnaly sinks underground. The upper part of the watercourse Bloščica flows mainly through preserved landscape, while the lower part from Ulake downstreams is more affected by human activity.

Spring of the about 17 km long watercourse Cerkniščica is located in a hilly area of Sveti Vid and Cajnarji. The catchment comprises 50 km$^2$. It is the biggest surface tributary of the lake Cerknica. The water level changes very quickly in the case of strong rain, so it can be designated as a torrent watercourse. The watercourse Cerkniščica

Table 1: List of taxa, determined in the watercourse Bloščica.
Tabela 1: Seznam vrst, prisotnih v Bloščici.

| Taxa | Abbreviation | Growth form |
|---|---|---|
| *Alisma plantago-aquatica* L. | Ali pla | am |
| Bryophyta | Bryophy | sa |
| *Chara* sp. | Cha sp. | sp |
| *Equisetum palustre* L. | Equ pal | he |
| *Lythrum salicaria* L. | Lyt sal | he |
| *Mentha aquatica* L. | Men aqu | am |
| *Mentha longifolia* (L.) Hudson | Men lon | am |
| *Menyanthes trifoliata* L. | Men tri | he |
| *Myosotis scorpioides* L. | Myo sco | am |
| *Myriophyllum spicatum* L. | Myr spi | sa |
| *Nasturtium officinale* R. Br. In Aiton | Nas off | he |
| *Petasites hybridus* (L.) Gaertner | Pet hyb | he |
| *Phragmithes australis* (Cav.) Trin ex Steud. | Phr aus | he |
| *Plantago altissima* L. | Pla alt | he |
| *Potamogeton lucens* L. | Pot luc | sa |
| *Potamogeton nodosus* Poir. | Pot nod | fl |
| *Schoenoplectus lacustris* (L.) Palla | Sch lac | he |
| *Sparganium erectum* L. | Spa ere | he, sa |
| *Typha latifolia* L. | Typ lat | he |

Legend: ap = plants floating on the water surface, sp = submerged pleustophytes, sa = submerged anchored plants, fl = floating leaf rooted plants, am = amphiphytes, he = helophytes

flows in its upper part in narrow and deep valley. From Cajnarji to Begunje by Cerknica, its bottom becomes wider and steeper, covered by fluvial deposits. At Begunje by Cerknica it flows on karst area. The watercourse Cerkniščica is regulated in the settlements Cerknica and Dolenja vas. It sinks underground at Cerkniško polje.

The catchments of both studied watercourses are part of the Natura 2000 network.

*Riparian, Channel, and Environment Inventory (RCE)*

Studied watercourses were divided to stretches from 360 to 1030 m long. The start of the new stretch was determined where presence or abundance of macrophytes changed, when we observed changes in land use type, channel characteristics or riparian zone. Every stretch was assessed according to the modified RCE Inventory (Petersen 1992, Germ et al. 2003). RCE Inventory was developed for the assessment of physical condition of the riparian zone and the stream channel in lowland streams, flowing through agricultural landscape. Modified RCE Inventory consisted from 12 parameters, each describing 4 levels of environmental gradient. The parameters include land-use type beyond the riparian zone, characteristics of the riparian zone (width, completeness and vegetation type), and morphology of the stream channel (channel structure, bank structure and undercutting, occurrence of retention structures and sediment accumulation, type of stream bottom and detritus and dynamics of the flow).

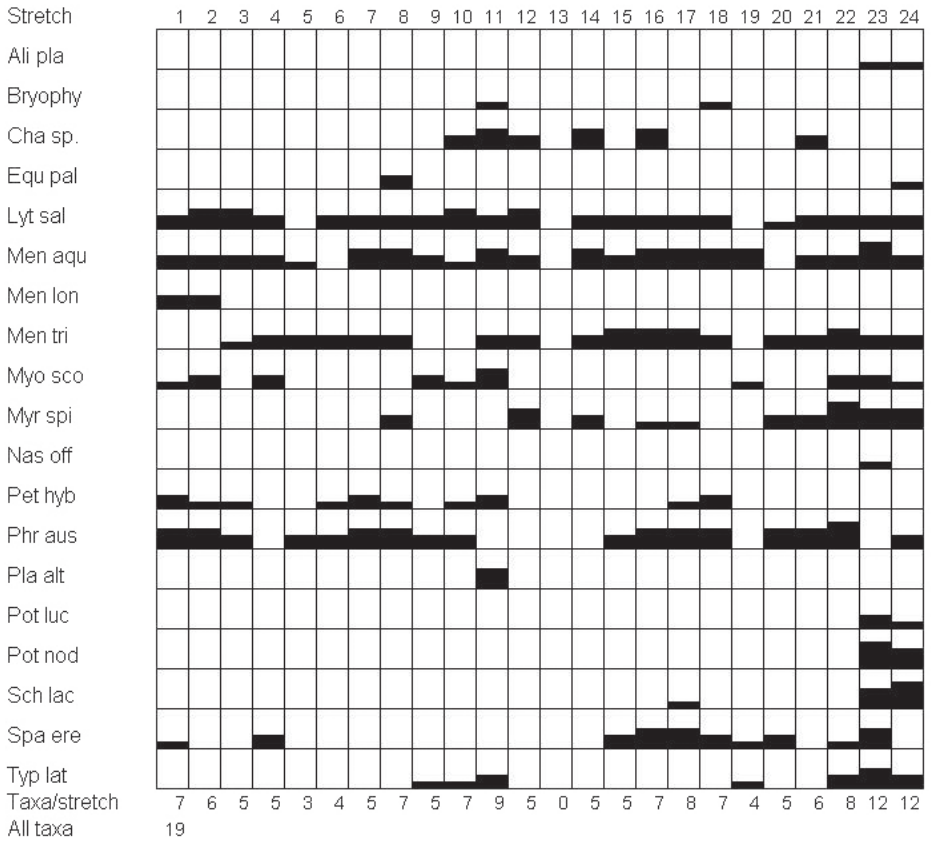


Figure 1: Distribution of macrophytes in the watercourse Bloščica. Stretch 10 was not surveyed.
Slika 1: Razporeditev in pogostost makrofitov v Bloščici. Odsek 10 ni bil pregledan.

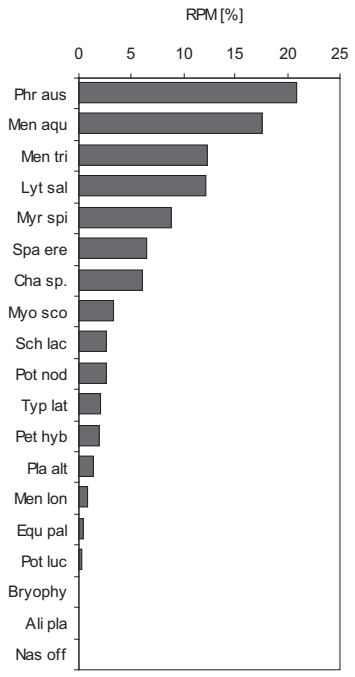


Figure 2: Relative plant mass (RPM) of macrophytes in the watercourse Bloščica.
Slika 2: Relativna rastlinska masa (RPM) makrofitov v Bloščici.

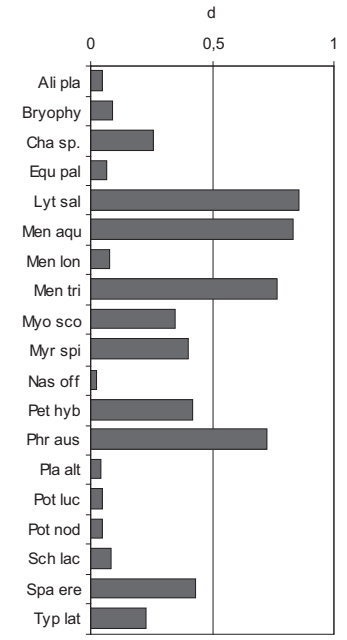


Figure 3: The ratio of the length of the watercourse Bloščica overgrown by certain species of macrophytes »d« value; 0.5 for example means that 50 % of watercourse in owergrown with macrophytes.
Slika 3: Delež dolžine vodotoka, poraslega z določeno vrsto makrofitov »d« vrednost; 0,5 npr. pomeni, da je 50 % vototoka porastlega z določeno vrsto.

*Macrophyte survey*

The distribution and abundance of macrophytes in studied watercourses were assessed from the source to the outflow, using a boat and a rake with hooks.

The abundance was evaluated using a five degree scale as follows (Kohler and Janauer 1995): 1 = very rare; 2 = infrequent; 3 = common; 4 = frequent; 5 = abundant, predominant. Plants were identified using the keys by Preston (1995), Casper and Krausch (1980) and Martinčič et al. (1999).

*Statistical analysis*

On the basis of plant abundance, a relative plant mass was calculated (RPM) that is related to true biomass with function $x^3$ (Pall and Janauer 1995, Kohler and Janauer 1995). Canonical correspondence analysis (CCA) (Canoco for Windows Version 4.5) was used to assess the relationship between the composition and abundance of macrophytes, and environmental parameters. Environmental parameters were coded numerically from 1 (the most modified or degraded condition) to 4 (the natural or near natural condition).

## Results

*Presence and abundance of macrophytes*

In the watercourse Bloščica 19 taxa of macrophytes was detected on the 17.800 m length (Tab. 1 and Fig. 1). Three species found are listed on the Slovenian Red list of Pteridophyta and Spermatophyta (Ur. l. RS 82/2002) as vulnera-

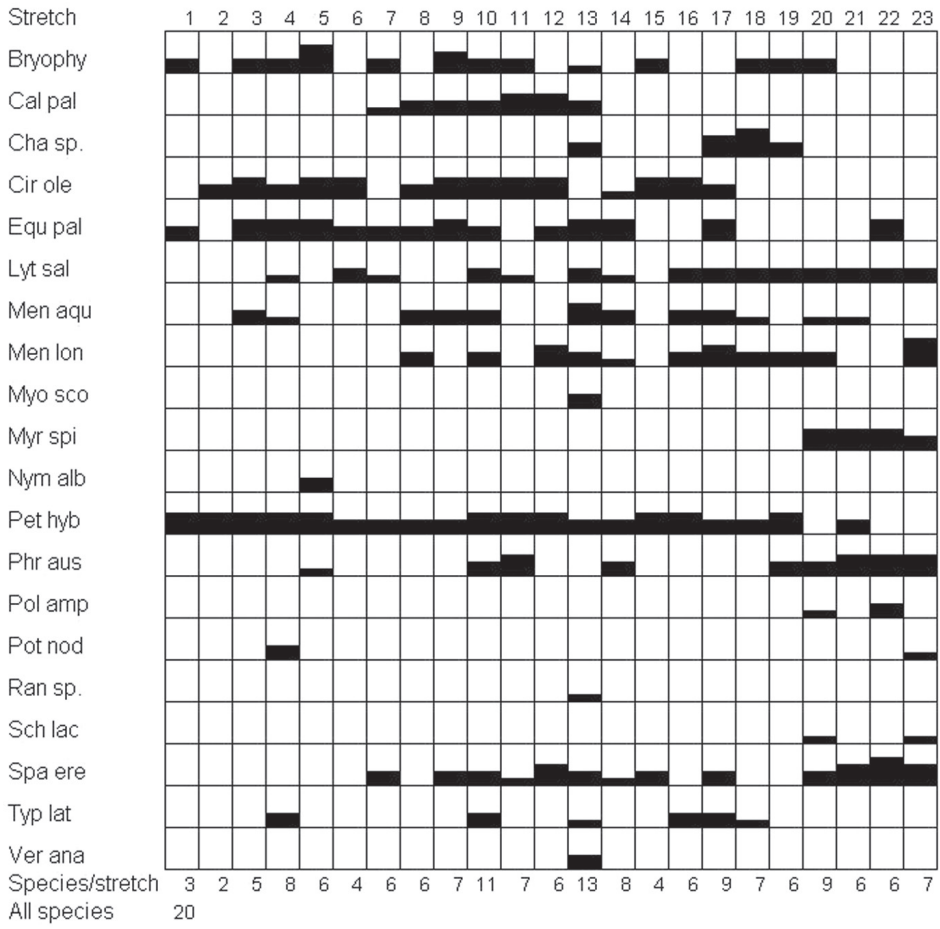


Figure 4: Distribution of macrophytes in the watercourse Cerkniščica.
Slika 4: Razporeditev in pogostost makrofitov v Cerkniščici.

ble (*Menyanthes trifoliata*, *Potamogeton lucens* and *P. nodosus*). The highest number of species was found in the stretches 18 (12) and 19 (12).

The highest RPM reached *Phragmites australis* (20.9 %), followed by *Mentha aquatica* (17.6 %), *Menyanthes trifoliata* (12.4 %) and *Lythrum salicaria* (12.2 %) (Fig. 2). *Lythrum salicaria* and *Mentha aquatica* occured in more than 80 % of the watercourse, followed by *Menyanthes trifoliata* (d = 0.77) and *Phragmithes australis* (d = 0.73) (Fig. 3). The majority of species in the both watercourses had amphibious or helophitic growth form, while submerged species were relatively scarce.

In the watercourse Cerkniščica 20 taxa of macrophytes were found (Tab. 2 and Fig. 4). Three of them are listed on the Slovenian Red list of Pteridophyta and Spermatophyta (Ur. l. RS 82/2002) determined as vulnerable: *Nymphaea alba*, *Potamogeton nodosus* and *Polygonum amphibium*.

The highest RPM reached *Petasites hybridus* (17.5 %), followed by *Cirsium oleraceum* (13.5 %) and *Equisetum palustre* (12.4 %) (Fig. 5). *Petasites hybridus* occured in 85 % of the watercourse, followed by *Lythrum salicaria* (d = 0.67), *Equisetum palustre* and *Cirsium oleraceum* (d = 0.62) (Fig. 6).

Table 2: List of taxa, determined in the watercourse Cerkniščica.
Tabela 2: Seznam vrst, prisotnih v Cerkniščici.

| Taxa | Abbreviation | Growth form |
|---|---|---|
| Bryophyta | Bryophy | sa |
| *Caltha palustris* L. | Cal pal | he |
| *Chara* sp. | Cha sp. | sp |
| *Cirsium oleraceum* (L.) Scop | Cir ole | he |
| *Equisetum palustre* L. | Equ pal | he |
| *Lythrum salicaria* L. | Lyt sal | he |
| *Mentha aquatica* L. | Men aqu | am |
| *Mentha longifolia* (L.) Hudson | Men lon | am |
| *Myosotis scorpioides* L. | Myo sco | am |
| *Myriophyllum spicatum* L. | Myr spi | sa |
| *Nymphaea alba* L. | Nym alb | ap |
| *Petasites hybridus* (L.) Gaertner | Pet hyb | he |
| *Phragmithes australis* (Cav.) Trin ex Steud. | Phr aus | he |
| *Polygonum amphibium* L. | Pol amp | am |
| *Potamogeton nodosus* Poir. | Pot nod | fl |
| *Ranunculus* sp. | Ran sp. | sa |
| *Schoenoplectus lacustris* (L.) Palla | Sch lac | he |
| *Sparganium erectum* L. | Spa ere | he, sa |
| *Typha latifolia* L. | Typ lat | he |
| *Veronica anagallis-aquatica* L. | Ver ana | sa |

Legend: ap = plants floating on the water surface, sp = submerged pleustophytes, sa = submerged anchored plants, fl = floating leaf rooted plants, am = amphiphytes, he = helophytes

*Environmental parameters and distribution of macrophytes*

Canonical correspondence analysis (CCA) (Canoco for Windows Version 4.5) was used to assess the relationship between environmental parameters and the composition and abundance of macrophytes in the watercourses Bloščica and Cerkniščica. Six examined parameters significantly affected the variability within the macrophyte community, the most influential were stream bottom type, width and completeness of the riparian zone, occurrence of retention devices, land use beyond the riparian zone and bank undercutting (Fig. 7).

The stretches are arranged in the ordination diagram according to the characteristics of environmental parameters in individual stretch. The quality of environmental parameters increases in the direction of the arrows. The stretches of Bloščica and streaches of Cerkniščica were present at different parts of the ordination diagram.

Rocky bottom was colonised by taxa *Cirsium oleraceum*, *Equisetum palustre* and *Petasites hibridus*, while the mixture of slime and sand was overgrown by *Menyanthes trifoliata*, *Mentha aquatica* and *Phragmites australis*. The taxon *Ranunculus* sp. and the species *V. anagalis-aquatica* occurred in stretches surrounded by wetland and forests, while the species *S. lacustris*, *P. lucens*, *Alisma plantago-aquatica* and *P. nodosus* preferred open locations.

## Discussion

The Riparian, Channel and Environmental (RCE) Inventory has been developed to assess the physical and biological conditions of small, lowland streams in agricultural areas (Petersen 1992). The modified RCE inventory consists of twelve characteristics, which define the structure of the riparian zone, stream channel mor-

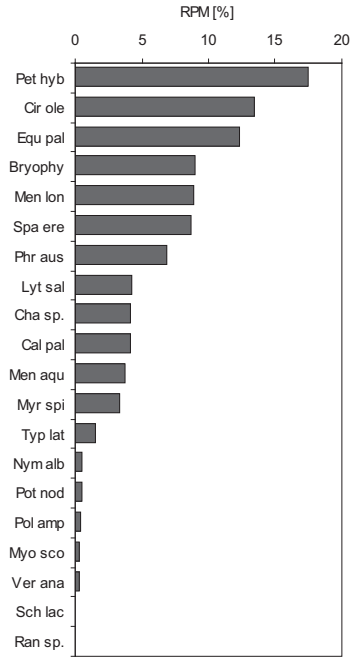


Figure 5: Relative plant mass (RPM) of macrophytes in the watercourse Cerkniščica.
Slika 5: Relativna rastlinska masa (RPM) makrofitov v Cerkniščici.

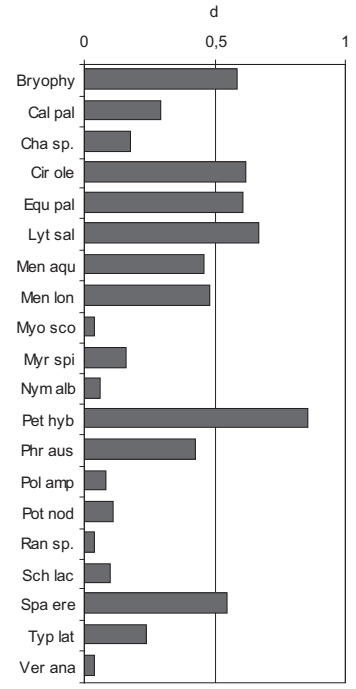


Figure 6: The ratio of the length of the watercourse Cerkniščica overgrown by certain species of macrophytes »d« value; 0.5 for example means that 50 % of watercourse in owergrown with macrophytes.
Slika 6: Delež dolžine vodotoka, poraslega z določeno vrsto makrofitov »d« vrednost; 0,5 npr. pomeni, da je 50 % vototoka porastlega z določeno vrsto.

phology and the biological condition in both habitats.

Numerous agricultural point discharges from field present a serious problem threating a good ecological status of watercourses. Wide and complete riparian vegetation has key role in prevention of erosion and retention of organic and even toxic substances (Johnston et al. 1990). Prevailed land use type of the watercourse Cerkniščica in upper part was forest and wet grassland and in lower parts agricultural and urban areas prevailed. Mosses were frequent, because of shading of the channel due to riparian vegetation and water level fluctuations. The river-bed of the watercourse Cerkniščica was channelized in the settlements Cerknica and Dolenja vas and therefore riparian vegetation was scarce or absent.

The bottom of the watercourse Bloščica consisted from fine, anaerobic sediment. Prevailing land use was wood and wet grassland. Riparian vegetation was removed at certain sites, that increased the vulnerability of the watercourse. This is also confirmed with canonical correspondence analysis that revealed that most influential environmental parameters shaping macrophyte community were bottom structure, width of riparian zone, retention devices in the channel and land use beyond the riparian zone.

Macrophyte species diversity was relatively low in either of the studied watercourses. 19 taxa were detected in the watercourse Bloščica and 20 in the watercourse Cerkniščica. 14 taxa were present in both watercourses. Important parameter, affecting the growth of macrophytes is light (Hut-

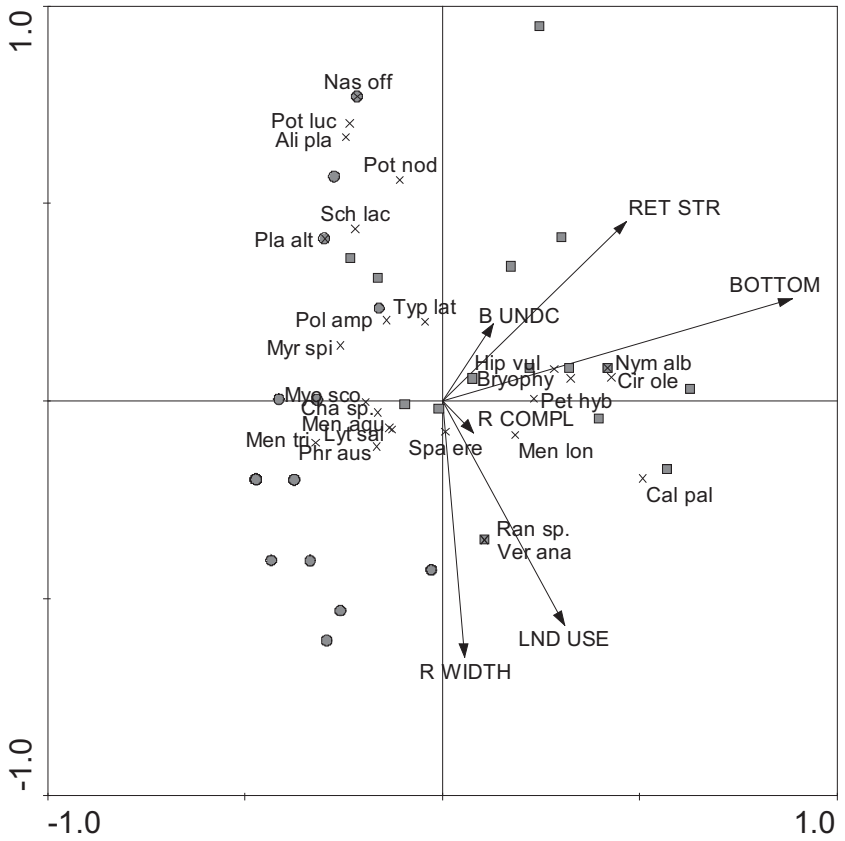


Figure 7: CCA ordination diagram showing the relationship between the macrophytes and environmental parameters. Lnd use - land use pattern beyond the riparian zone; R width - width of riparian zone; R compl - completeness of riparian zone; Ret str - retention structures; B undc - bank undercutting; Bottom - stream bottom; ● - Bloščica; ■ - Cerkniščica. Codes for macrophyte taxa are given in Table 1 and Table 2.

Slika 7: CCA ordinacijski diagram s taksoni makrofitov in spremenljivkami okolja. Lnd use - raba tal v zaledju struge; R width - širina obrežnega pasu; R compl - sklenjenost vegetacije v obrežnem pasu; Ret str - zadrževalne strukture v strugi; B undc - spodjedanje brega; Bottom - dno; ● - Bloščica; ■ - Cerkniščica. Oznake za taksone makrofitov so v Tabeli 1 in Tabeli 2.

cinson 1975). Shaded parts of the watercourses were scarcely colonised with vascular macrophytes. Mosses were dominant group of macrophytes in that stretches. Diversity and distribution of macrophytes in lowland rivers depend on the concentration of nutrients, current velocity and anthropogenic impact (Hrivnàk et al. 2007). Human impact on water ecosystem was less evident in the case of the watercourse Bloščica, where wet grasslands colonising the areas along the watercourse were less appropriate for agricultural use. Similarly, human impact was not prominent in the upper parts of watercourse Cerkniščica, since the area was covered by forests and wet meadows. Six species, listed on the Slovenian Red list (Ur. l. RS 82/2002) as vulnerable, thrive in the sites, where watercourses flow in the natural or little altered landscape with relatively wide riparian zone, and mixed silty and sandy sediment with organic matter as also reveals from previous researches (Kuhar et al. 2009).

The decrease of heterogeneity of habitats induces lower diversity of macrophytes (O'Hare et al. 2006). In the watercourse Bloščica fine mate-

rial and detritus was the prevailing sediment type, banks predominantly consisted from fine inorganic material. Lower number of macrophytes thereafter reflected the homogeneity of the habitat. Relatively homogeneous habitat was found also in the watercourse Cerkniščica. Flow velocity influences the occurrence of macrophytes (Janauer et al. 2010). Upper part of the watercourse Bloščica had higher slope and fast current velocity. Watercourse became wider downstream, current velocity was slower. In that part of the Bloščica, the variety of macrophytes species was higher.

Riis et al. (2000) stated that water chemistry and different tolerance of species to nutrient load determine the distribution of macrophytes. Thus, on the basis on the presence of the certain species of macrophytes, the loading of watercourses with nutrients can be assessed. Acceleration of eutrophication is a consequence of human activity, especially urbanization, agriculture and industry (Germ et al. 2008). *Potamogeton lucens* grows in eutrophic, relatively deep and on calcareous bedrock flowing lowland streams (Preston 1995) as holds true for the last part of the watercourse Bloščica. Genus *Chara* was found in both watercourses only in stretches located far from agricultural areas. Species richness of the genus *Chara* drops when amount of nutrient arises. Bornette and Arens (2002) stated that species in genus *Chara* are pioneer species in habitats, where disturbance appears very often. In the case of both watercourses water-level fluctuations presented the main disturbance. *Myriophillum spicatum* avoids fast flowing and oligotrophic waters (Germ and Gaberščik 1999); it was found in the lower parts of studied watercourses.

## Conclusions

1. In the watercourse Bloščica 19 taxa of macrophytes were detected. Three species found are listed on the Slovenian Red list of Pteridophyta and Spermatophyta as vulnerable namely *Menyanthes trifoliata*, *Potamogeton lucens* and *P. nodosus*.
2. In the watercourse Cerkniščica 20 taxa of macrophytes were found. Three of them are listed on the Slovenian Red list of Pteridophyta and Spermatophyta as vulnerable namely *Nymphaea alba*, *Potamogeton nodosus* and *Polygonum amphibium*.
3. The majority of species in the both watercourses had amphibious and helophytic growth form, while submerged species were relatively scarce.
4. Presence and abundance of macrophytes changed along the watercourses mainly due to land use type, width of the riparian zone and bottom properties. Abundance of macrophytes in both watercourses was the highest in unshaded stretches with the middle current velocity.

## Povzetek

Namen raziskave je bil ugotoviti pojavljanje, razporeditev in pogostost makrofitov v Bloščici in Cerkniščici. Opisali smo stanje širšega vodnega okolja obeh vodotokov ter povezavo med okoljskimi razmerami in pojavljanjem makrofitov. Vodotoka smo razdelili na odseke (24 odsekov na Bloščici, 23 odsekov na Cerkniščici), v katerih smo popisali makrofite. Hkrati s popisom makrofitov smo ocenili širše okolje s po Petersenu prirejeno RCE metodo (Germ et al. 2003) in habitatne parametre. V obeh vodotokih smo skupno popisali 25 taksonov makrofitov. Večjo pestrost makrofitov smo zasledili na mestih, kjer je vodni tok počasnejši in kjer je vpliv človeka zmanjšan (npr. urbanizirana območja, kmetijske površine). Vodotoka se razlikujeta v habitatnih parametrih in prisotnosti makrofitov. Pomemben je tip sedimenta, saj se rastline lažje ukoreninjajo v bolj trdnem substratu kot v rahlih, finih delcih. V Bloščici se je večinoma pojavljal detrit, v Cerkniščici pa so se ob detritu pojavljali še pesek in skale. Kanonična korespondenčna analiza je pokazala, da šest okoljskih parametrov značilno vpliva na pojavljanje in pogostost makrofitov. Največji vpliv imajo struktura dna, širina obrežnega pasu, zadrževalne strukture v strugi in zaledje.

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ABS ACTA BIOLOGICA SLOVENICA
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# A hydro-acoustics approach of accessing macrophyte biomass data

## Hidro-akustično pridobivanje podatkov o biomasi makrofitov

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**Abstract:** Hydro-acoustic methods are commonly used to estimate the abundance and distribution pattern of fish in aquatic environments, while studies on the assessment of the biomass of submerged macrophytes in the littoral zones are still rare. In the present study we provide first results showing that indeed this method is a useful tool to estimate the aquatic plant stands in lakes. The aim of the recent presentation is to show an initial data evaluation by graphs describing hydro-acoustic signals at three distinct layers in a small shallow lake: the solid sediment, the fine or muddy sediment, and the 'plant canopy' of submerse macrophytes. The most difficulties of data processing and assessment of biomass were for hydro-acoustic records close to the water surface where the echo-signal is interfered by reflectance. Methodological details and progress in evaluating hydro-acoustic records will be discussed.

**Key words:** aquatic macrophytes, biomass, hydro-acoustics

**Izvleček:** Hidro-akustične metode se navadno uporabljajo za oceno pogostosti in zastopanosti rib v vodnem okolju, medtem ko so tovrstne raziskave biomase makrofitov v litoralu razmeroma redke. Raziskava podaja rezultate, ki kažejo na uporabnost metode za oceno sestojev makrofitov v jezerih. Namen raziskave je prikazati začetno vrednotenje podatkov hidro-akustičnih signalov na treh različnih plasteh majhnega plitvega jezera: trdnih usedlin, finih oziroma rahlih usedlin in sestojev potopljenih makrofitov. Največja težava pri obdelavi podatkov in oceni biomase so bili signali v bližini vodne površine, ki so interferirali z odbojem. V prispevku je podan metodološki pristop in potek ocenitve hidro-akustičnih signalov.

**Ključne besede:** vodni makrofiti, biomasa, hidro-akustika

## Introduction

Assessing aquatic plant stands in freshwater and marine systems might become of even more interest as macrophyte belts are known to be important to inhabit young fish, as they play an important role to share nutrient sources with planktonic primary producers by alternative stable states and are hence of decisive importance for a good ecological status of an aquatic ecosystem (Scheffer 2002). Beside the need of more quantitative information about macrophytes, studies about macrophyte records are still rare compared with other aquatic assemblages as e.g. studies of fish or plankton. One reason could be the effort used for recording macrophyte data. The state of art of assessing and processing biomass information of aquatic plants is based traditionally on harvesting methods, or on field surveys focusing on visual plant mass estimates. The effort of estimating standing crop of macrophytes becomes even higher in case that recording can be done only by divers. Different from that approach is the application of hydro-acoustics. This method that is usually applied to fish-relevant research and commonly used in practice (Brandt 1996, Rakowitz et al. 2009), has also been used for macrophyte research for

over two decades in some water bodies, with sufficient success in freshwater and marine science (e.g. Komatsu et al. 2002), too. Some trials on macrophytes were done with dual frequency echo sounder (Dumfart and Pall 2003, Jäger et al. 2004), and some other trials detected biomass in an experimental set-up by means of horizontal echo sounding (Hohausová et al. 2008). Trials with the same low-cost instrument we were using have also been applied by the Portland State University (USA; Litz 2007). A widely applicable method for estimating macrophyte biomass, however, is missing.

Echo sounding reveals only numerical information on depth. The advantage of the method, however, is, that many systems can store the original signal and can hence provide high resolution measurements by trials on both, temporal and spatial scales. Aside from calculating information on fish types, little effort has been put into differentiating a typical macrophyte signal, and a simplification of data processing and display of results is also needed. The aim of the study is to show graphically first results of quantitative recording of macrophyte stands by using a low-sophistication standard sonar equipment. This method allows to detect macrophyte stands and to calculate water plant-filled volumes leading to a semi-automatic assessment of biomass.

## Materials and methods

Our approach is based on low-sophistication standard sonar equipment normally used in fishery. The sonar generates a standard echogram with 256 bit resolution, which will be used for detection of ground, sediment and macrophyte height. This signal can be used for automatic height detection of macrophyte stands. The respective water volume covering the plants refers to a semiautomatic biomass estimate.

LMS-480M Sonarviewer (Lowrance Inc.) was used in our research which is standard sonar

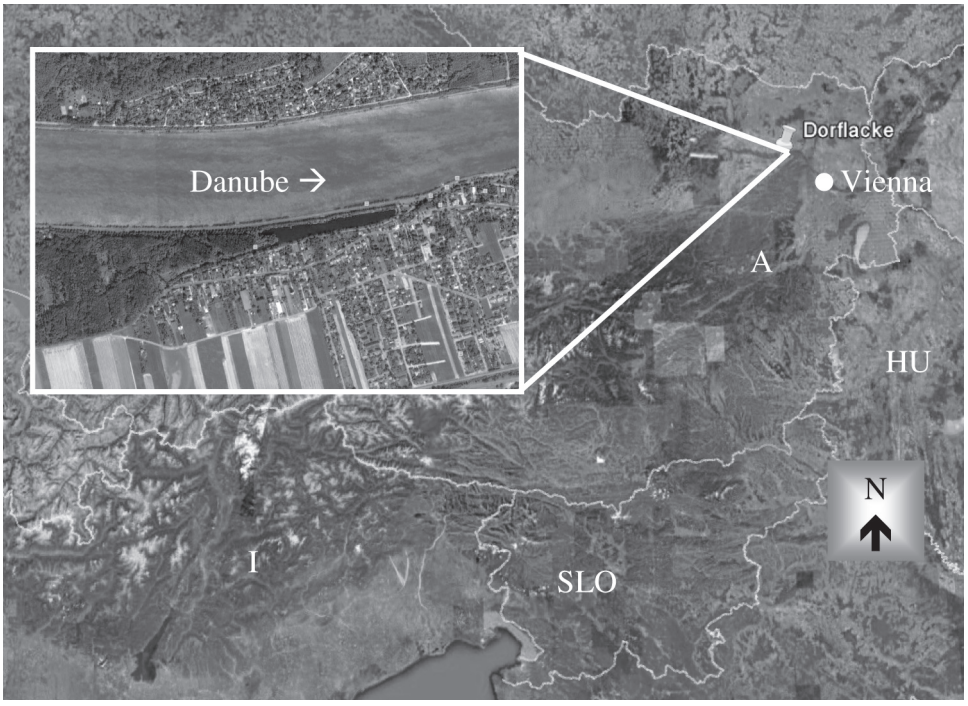


Figure 1: Survey site “Dorflacke”, a part of former side channels of the Danube River.
Slika 1: Preiskovano območje “Dorflacke”, del stare stranske struge reke Donave.

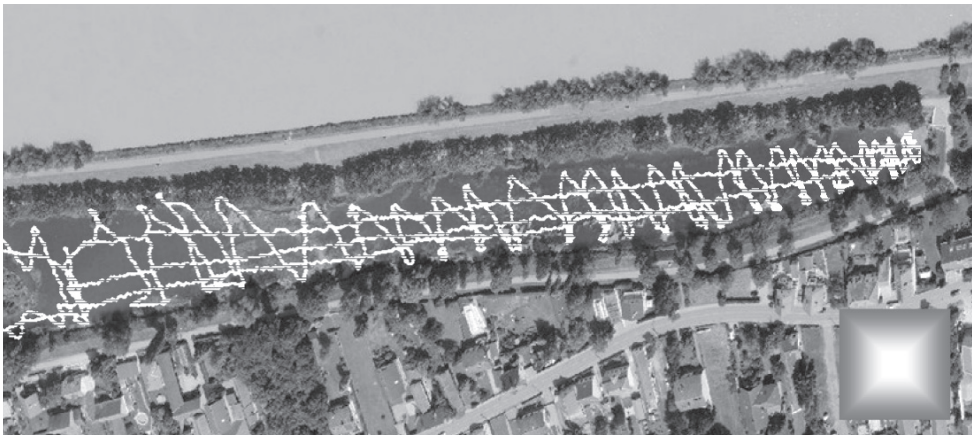

Figure 2: Track of hydro-acoustic run.
Slika 2: Potek hidro-akustičnega pregleda dna.

and usually used for detecting fish. This instrument features single frequency (50/200 kHz depending on the detected depth) with 500 watts of RMS power and displays depth from approximately 50 cm to 400 m with a resolution of 5 cm on its 5" diagonal high resolution, 256-bit monochrome display. The high speed skimmer transducer has a built-in temperature sensor. The instrument is equipped with a 12 channel WAAS/EGNOS compatible GPS receiver and provides accuracy up to 3 meters. The sonar has a built-in SDRAM card slot and stores the original sonar data for further processing in an internal raw data format. This internal data format was reverse engineered by means of Matlab version 7.4. This program was used for reading and processing the raw data and further post processing and visualisation. Google Earth (Google Inc.) and ArcGIS 9.3 (ESRI) are used for defining the shore line of the water body under survey. When using our equipment in areas under nature protection the instrument is used from a conventional rubber boat propelled by an electric outboard motor.

Data processing for the calculation of macrophyte biomass yield is described in detail in Janauer and Wychera (2000). According to an empirical data set, the biomass yield was calculated by the significant relationship between the volume of the plant stands and the plant material harvested from patches of defined area of usually 0.25 to 0.5 $m^2$. This relationship was tested for macrophyte assemblages at different height in the respective water layer. Further a yield index for specific macrophyte dominance pattern was developed (Janauer et Wychera 2000, Kohlbauer 2008). Such calibration data set and biomass calculations are not shown in the recent study for Dorflacke. We focused here on the graphical presentation of hydro-acoustic measurements.

The hydro-acoustics run was performed in zigzag pattern once during summer, on June 30, 2010. The track is shown in Fig. 1. Where plants reached the surface the transducer was lifted for a short moment to avoid getting entangled in plant stems. This procedure did not interfere with either the reception of the acoustic signal nor with the GPS reading.

*Study Area*

Dorflacke is a relict part of the lower reach of the small river 'Kleine Tulln', which merged with Danube River side channels before the construction of the Greifenstein run-of-river hydroelectric power plant. Today this water body is located in parallel to the longitudinal levee of the power plant reservoir. The Dorflacke receives most of its water from the groundwater and seepage from the reservoir (Fig. 2). Its length and width is about 500 and 40 m, respectively. The maximum depth is 5.5 m.

## Results

Results of data processing of hydro-acoustic records in shallow Dorflacke are presented by graphical illustration shown in Figs. 3 and 4. Our measurements comprise a data set of 65 transversal transects and 3 longitudinal runs on the summer trial. The results of the summer macrophyte situation are exemplified here by a single transversal transect (Fig. 3). This figure shows a typical echogram obtaining three zones of diffe-rent acoustic properties. The lowest line delineates the zone of the bottom layer at the ground and refers to solid sediment at the bottom of the lake. The area between the ground and the middle line indicates the zone of muddy sediment (fine sediment). The upper line refers to the 'underwater canopy of macrophytes'. The area between the lines of fine sediment and of the canopy relates to water layers inhabiting macrophyte biomass. The dotted grey line in Fig. 3 might indicate the top layer where hydro-acoustic data do not provide reliable information on macrophyte biomass as explained in detail in the discussion. This shallow depth is electronically attenuated and hence indicated a recorded signal which is invalid describing a hydro-acoustic pattern.

The morphometry of the water basin of Dorflacke is shown in Fig. 4. This contour map is automatically generated by the sonar diagram and can be linked to transect information exemplified in Fig. 4. The map shows that even a shallow water body can vary by spatial heterogeneity. Even if most area is indicated by a depth of two meters only, some limited areas refer to a water depth deeper than 4-5 m. Further, this high resolution measurement shows that a littoral zone shallower than 1 m is more common at the south shore line than compared to the northern part.

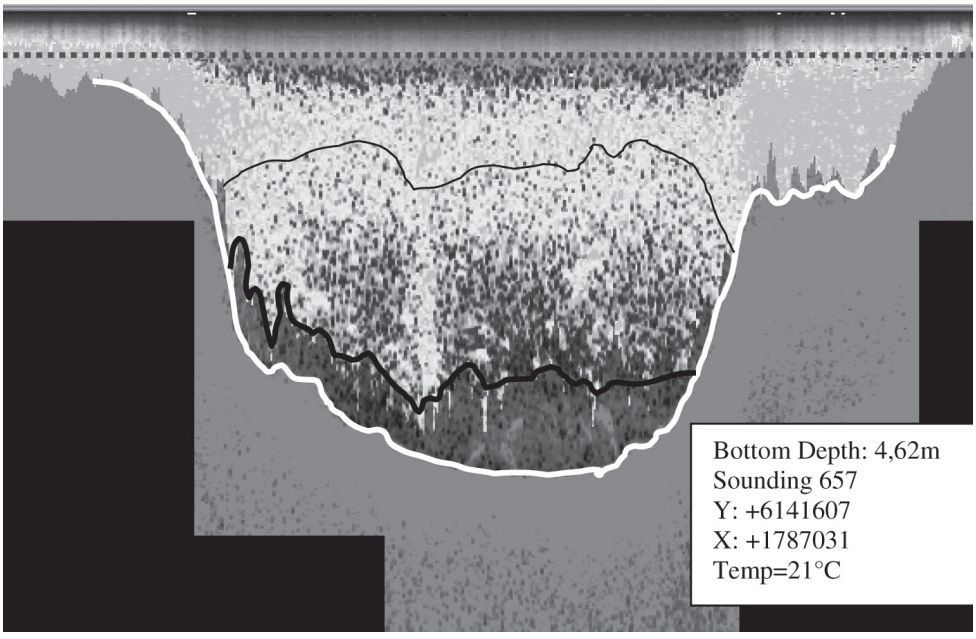


Figure 3: Echogram of a transect, with ground line of solid sediment (white), sediment line of fine/muddy sediment (bold black), smoothed macrophyte line of plant canopy (thin black) and dotted grey line minimum depth of detection.

Slika 3: Ehogram transekta z mejo trdne usedline (belo), mejo fine / rahle usedline (odebeljeno-črno), izravnano mejo makrofitske vegetacije (tanka-bela) in minimalno mejo detekcije (pikčasta siva črta).

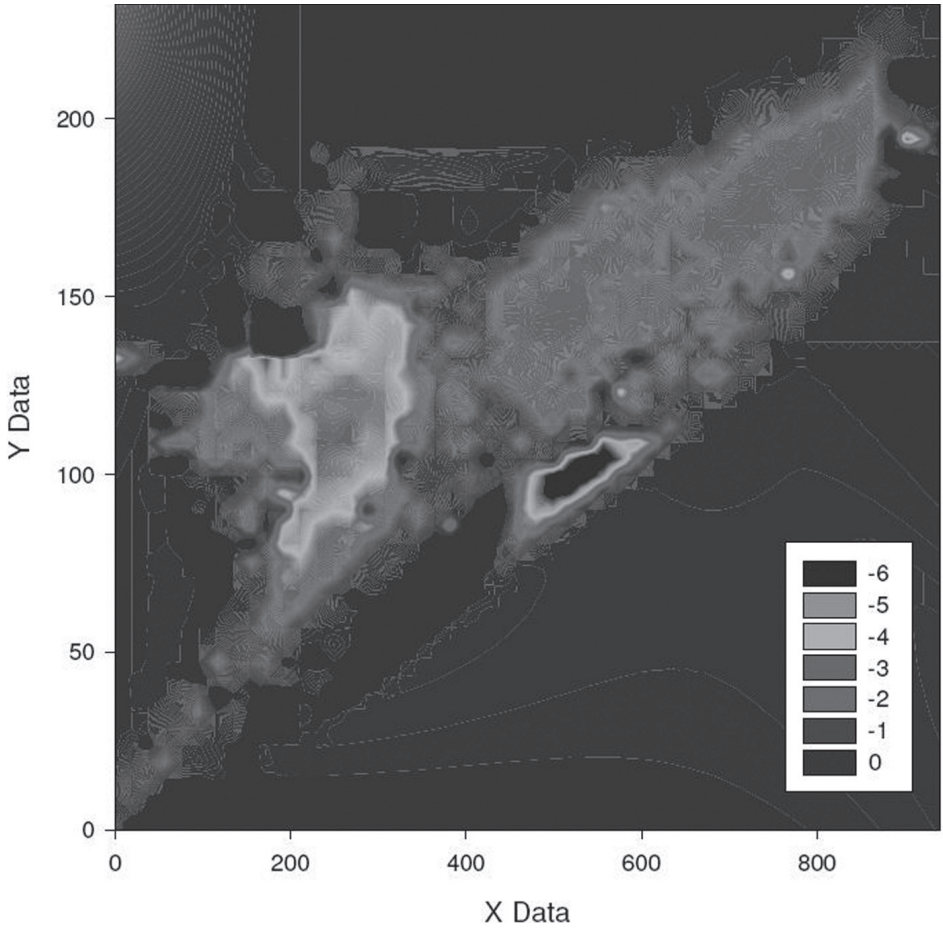


Figure 4: Depth contour map of Dorflacke, colour coded. For better visualisation the y-axis has been stretched, banks are in white, some artefacts located outside the water body are due to mathematical interpolation. Both axes indicate the spatial distance in m.

Slika 4: Globinske črte proste vode v vodnem telesu Dorflacke (različno obarvano). Zaradi boljše preglednosti je merilo na y osi povečano, bregovi pa so belo obarvani. Artefakti zunaj vodnega telesa so posledica matematične interpolacije.

## Discussion

The results by our hydro-acoustic measurements sound promising as both, the water depth and the water plant canopy, were recorded with excellent accuracy. Based on this information a 3-D-model of the plant mass will be calculated for Dorflacke in a further study relating to an estimation of total macrophyte biomass.

The analysis of the acoustic data shown in this study allowed an automatic separation and detection of three distinct ‘surfaces’ as regards filtering and edge detection of the echo sounding signal of solid sediment, the fine and/or muddy sediment, and the ‘plant canopy’ indicating the height of the aquatic plant stands. The graphical presentation refers to a calculation of triangulated irregular networks which allow a three dimensional description required for estimating macrophyte biomass yield in water bodies.

A semiautomatic evaluation was carried out following the procedure described by Kohlbauer (2008), in a process of validating our approach. A problem in automatically assessing the total volume of plant stands is due a low reliability of data at the near surface layer. In the uppermost strata of the water body, usually at a range of less than 50 cm, the acoustic signal is interfered by the reflectance of the water surface. As this shallow layer at water surface provides an invalid hydro-acoustic signal, plant stands reaching all the way up to the water surface are not accurately recorded in these parts of the water body by standard data processing. Therefore data need to be corrected manually in an additional post processing step. The areas can be digitized by means of GIS and marked as 'macrophytes-to-surface' along the survey track or can be simply estimated by recording the perimeter of these areas by means of normal GPS.

In case of calculating biomass of submerse macrophytes, aside the hydro-acoustic data the information about the macrophyte composition along transects is needed. Therefore, a hydro-acoustic run might be accompanied by a botanical survey of the macrophyte beds in the field. Processing the data in the lab, the signal of hydro-acoustics needs to be linked to the specific species composition at the respective measured area. In an earlier study by Janauer and Wichera (2000), a calibration data set for more than 20 macrophyte yield patterns is developed to provide specific yield indices. Such index refers to the ratio of the macrophyte biomass and the water volume covered by the submerse plants for each macrophyte taxon. In a next step of data processing, the recent hydro-acoustic data of Dorflacke presented in this study, will linked to this calibration index calculating finally the species-specific biomass.

## Conclusion

Our results show first promising results as graphical mapping provide in principle reliable data for macrophyte canopy and contour plots for water basin morphometry.

In a next step a user-friendly access will be developed for digitizing the shoreline, and for a convenient user interface enabling post-processing the plant stand volumes, as well as the calculation of biomass will be developed. The automatic differentiation of the different readings of solid and muddy sediment, and of the macrophytes by means of Kalman filter as well as an automated detection of 'macrophytes-to-surface' areas will be included.

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# *In vitro* propagation of *Lilium martagon* L. var. *cattaniae* Vis. and evaluation of genotoxic potential of its leaves and bulbs extracts

## *In vitro* razmnoževanje in ocena genotoksičnosti izvlečkov listov in čebul taksona *Lilium martagon* L. var. *cattaniae* Vis.

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**Abstract:** *Lilium martagon* L. var. *cattaniae* Vis. (*Liliaceae*) is endemic plant of Dinaridi mountain. In this work we established protocol for fast in vitro propagation and multiplication of *Lilium martagon* var. *cattaniae*. The aim was to enable fast production of plant material as potential source of pharmaceutically valuable secondary metabolites. Seeds of *L. martagon* var. *cattaniae* were germinated on a Murashige and Skoog basal medium with a supplement of 0.15 mg/l gibberellic acid ($GA_3$), and multiplication was performed on MS medium supplemented with 0.1 mg/l gibberellic acid ($GA_3$), 0.2 mg/l indole-3-butyric acid (IBA) and 0.5 mg/l 6-benzylaminopurine (BAP). We used ultrasound assisted extraction to prepare extracts of leaves and bulbs of *Lilium martagon* var. *cattaniae*, which were evaluated for their genotoxic potential using Allium test and cytokinesis-block micronucleus test in human lymphocytes culture. There was statistically significant difference between all used concentrations of lilium extracts and control on proliferation of cells of root tip of onion (*Allium cepa*). In cytokinesis-block micronucleus test no statistically significant difference between frequencies of analyzed parameters in samples treated with tested concentrations of extracts and control was obtained.

**Keywords:** *in vitro* culture, ultrasound assisted extraction, micronuclei, genotoxic, *Lilium martagon* L. var. *cattaniae* Vis.

**Izvleček:** *Lilium martagon* L. var. *cattaniae* Vis. (*Liliaceae*) je endemični takson Dinaridov. Članek podaja načrt za hitro in vitro propagacijo in multiplikacijo taksona *Lilium martagon* L. var. *cattaniae*. Cilj je bil omogočiti hitro propagacijo rastlinskega materiala, ki je potencialni izvor metabolitov, pomembnih za farmacijo. Semena taksona *Lilium martagon* var. *cattaniae* smo kalili na Murashige in Skoog (MS) hranljivi podlagi, kateri smo dodali 0,15 mg/l giberelinske kisline ($GA_3$). Multiplikacija je prav tako potekala na MS podlagi, ki smo ji dodali 0,1 mg/l giberelinske kisline ($GA_3$), 0,2 mg/l indol-3-maslene kisline (IBA) in 0,5 mg/l 6-benzilaminopurina (BAP). Za pripravo izvlečkov listov in korenin taksona *Lilium martagon* var. *cattaniae* smo uporabili ultrazvočno ekstrakcijo. Za ugotavljanje genotoksičnega potenciala teh izvlečkov je bil uporabljen Allium test in citokinetski blok mikronukleus test na humanih limfocitih. Različne koncentracije izvlečkov lilij so imele značilen vpliv na proliferacijo celic korenin čebule (*Allium cepa*) glede na kontrolo. Pri citokinetskem blok mikronukleus testu ni bilo značilnih razlik med frekvencami analiziranih parametrov pri vzorcih obravnavanih z različnimi koncentracijami izvlečkov in kontrolo.

**Ključne besede:** *in vitro* kultura, ultrazvočna ekstrakcija, mikronukleusi, genotoksičnost, *Lilium martagon* L. var. *cattaniae* Vis.

## Introduction

*Lilium martagon* L. var. *cattaniae* Vis. *Liliaceae* is an endemic plant species of Bosnia and Herzegovina (Šilić 2007). According to Flora Europaea, (Matthews 1980) Turk's-cap lilies in Europe are represented by: *L. martagon* L., *L. chalcedonicum* L., *L. pomponium* L., *L. pyrenaicum* Gouan. and *L. carniolicum* Bernh. ex Koch. In addition there are also endemic European taxa with unclear taxonomic status: *L. albanicum* Griseb., *L. bosniacum* (G. Beck) Beck ex Fritsch and *L. jankae* A. Kerner from the *L. carniolicum* complex, and *L. cattaniae* (Vis.) Vis. from the *L. martagon* complex (Matthews 1980).

This species is named after Maria Cattani Selebam who showed differences between *Lilium cattaniae* Vis. (Vis.) and *Lilium martagon* L. to R. Visiani so he published in year 1872 that it is new species (Šilić 2007). This species is used as medicinal plant in Mediterranean area. It represents an important resource both for phytochemical and pharmacological research (Redžić 2010). Bulbs of its closest species *Lilium martagon* L. posses cardiotonic properties and are used in the treatment of dysmenorrhoea (Khare 2007), liver diseases in both humans and animals in Northern Albania (Pieroni et al. 2005). Bulbs of *Lilium martagon* L. are used externaly for ulcers (Khare 2007). There are studies confirming presence of anticarcinogenic components, such as jatropham which is antileucemic agent, in *Lilium martagon* L. The presence of kaempferol, quercetin and isorhamnetin was identified in *L. martagon* (Eisenreichová et al. 2004). HPLC analysis should be done to characterise crude leaf and bulb extracts of *Lilium martagon* L. var. *cattaniae* Vis. for major secondary metabolites.

Culture *in vitro* is revolutionary methodology useful in development of synthesis and accumulation of natural products and possible method for modification of products (Remington 2005). This is especially important when endemic and rare plants are used as a source of medically active substances. Bulbous plants, like lilies, have proved to be ideal for tissue culture, as their regeneration potential is usually high. Tissue culture has been applied to the propagation of lilies since the late 1950's. Nowadays, lilies are one of the most important bulbous crops produced in tissue culture also in an industrial scale. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products (Pelkonen 2005).

When preparing herbal extracts, method of extraction plays very important role. In history many different methods of extraction have been developed. Non-conventional extraction techniques have gained more attention recently, and one of these techniques is ultrasonically assisted extraction. In the area of inter-phase mass transfer, solid-liquid extractron appears to be most greatly enhanced by the application of ultrasonic waves. The mechanism believed to be primarily responsible for the larger increases is the cell disruption brought about by cavitation. Cavitation can result when high-intensity acoustic waves are passed through liquids producing small bubbles in the liquid. On collapse, the contents of the bubbles are compressed to very high temperatures and are capable of producing shock waves (Chendke et al. 1975).

Ultrasonic extraction is simple, low cost in terms of solvent used and less time consumed. This method promotes better penetration of solvent into plant particles and uses low extraction temperature which affects the stability of active components (Rouhani et al. 2009). Ultrasonic processing is still in its infancy and requires a great deal of future research (Dolatowski et al. 2007).

It is very important that herbal extracts used in traditional medicine, are not toxic, or that its toxicity is under defined limits. Remarkable aspect of toxicity is genotoxicity. Numerous tests can be used in studying genotoxicity. In this study we used Allium test and cytokinesis-block micronucleus test in human lymphocytes culture, to evaluate genotoxicity potential of *L. martagon var. cattaniae* leaves and bulbs extracts.

## Materials and methods

### *Plant material*

Seeds of *Lilium martagon* L. var. *cattaniae* Vis. used in this study as starting material were provided by dr.sci. Edina Muratovic. Seeds were collected on Borova Glava, 1100 m, Bosnia and

Herzegovina. Voucher specimens are deposit at Department of Biology, Faculty of Science, University in Sarajevo.

*In vitro* culture of *Lilium martagon* L. var. *cattaniae* Vis.

For *L. martagon var. cattaniae* seed germination, commercial MS (Murashige and Skoog, 1962) medium (*Duchefa*, *Netherlands*) was used. Medium pH was adjusted to 6.5. Seeds were sterilized and germinated according to protocol given by Parić et al. 2008. Five to six cm long lilies explants were cut into smaller which contained 1-3 bulbs. Explants were inoculated on MS medium supplemented with 0.1mg/l $GA_3$, 0.2 mg/l IBA (indole butiric acid) and 0.5 mg/l BAP (benzyl amino purine). Explants were cultivated for three weeks, subcultivated for three times and then collected for extraction.

*Extraction*

Bulbs and leaves were separated and plant material was dried in a flow of hot air, chopped and extracted with water. First, plant material was soaked in distilled water (1:20) for 2 hours. After that mixture was put in ultrasound bath (Iskra UZ4R) for 25 minutes. The mixture was put in a dark place for 22 hours with frequent shaking, filtrated and vacuum dried on temperature 30 ºC and pressure 50 mbar. Dried extracts were held on room temperature above silica gel until they were used.

*Testing of genotoxic potential*

Allium test

Fresh, healthy, equal-sized bulbs of a commercial variety of *Allium cepa* L. were selected. Just before use, the outer scales of the bulbs were carefully removed and the brownish bottom plates were scraped away without destroying the root primordia. Four concentrations (0.1mg/ml, 0.5mg/ml, 1mg/ml and 5mg/ml) of dried extracts of leaves and bulbs were used in the experiment. For every concentration and for negative control two bulbs were taken. They were put in water on room temperature and in shadow for 48 hours. After that, bulbs were put in extracts for 24 hours (control bulbs were left in water). Then root tips were fixated in ice-cold acetic acid: ethanol 1:3 on 4 ºC for few hours. Root tips were hydrolyzed with 1 N HCl at 60 ºC for 7 min and after that washed in distilled water. For every concentration two slides were prepared. Three root tips were taken for every slide, the meristematic cell region was removed by cutting 2 mm from the root cap, this section was set on a clean slide, macerated with metal stick, immersed in drop of 1% lacto-propion orcein and squashed under a cover glass, excess color was removed with filter paper and after that, borders of cover glass were paraffinated. Slides were observed under 40× magnification. On every slide 1000 cells were analyzed for frequency of cells in interphase, prophase, methaphase, anaphase and telophase. Mitotic index (MI) was calculated as percentage of mitotic cells in all analyzed cells.

Cytokinesis-block micronucleus test

According to results of Allium test two concentrations of extracts (0,1 mg/ml and 1 mg/ml) were chosen for micronucleus test in human lymphocytes. Micronucleus test was performed on blood samples from four persons. Donors of blood were not on therapy with medicines and did not suffer any chronic disease in past 6 months, they were not smokers and age was between 24 and 27 years. Two donors were female and two donors were male. The study was conducted in accordance to ethical principals.

Cytokinesis-block micronucleus assay was performed according to protocol and scoring criteria given by Fenech and coworkers (2003) and Fenech (2000). Blood was cultivated for 72 hours on RPMI medium. After 24 hours of cultivation extracts were added. After 45 hours of cultivation citohalazin B was added (4.5 µg/ml).

Estimation of lymphocytes proliferation was done by calculating nuclear division index (NDI) according to Eastmond and Tucker (1989). The calculation was made according a formula: NDI= [M1 + 2(M2) + 3(M3) + 4(M4)]/N, where M1-M4 represent cells with one to four nucleus, N is total number of viable cells analyzed.

*Statistical analysis*

Z-test and ANOVA followed by pair-wise comparisons with Newmans-Keuls Multiple

comparison test were calculated for statistical analysis, using Winks 4.5 Professional software (TexaSoft, Cedar Hill, TX, USA). Level of significance was $p \leq 0.05$.

## Results and discussion

*In vitro propagation and multiplication of Lilium martagon L. var. cattaniae Vis.*

*In vitro* cultivation is very important tehnique for production of significant natural products in pharmacy. One of the aims is to produce big amount of plant material in the shortest time. If the starting material is seed, germination is a very important factor, and it can be improved using appropriate protocol for *in vitro* germination. Seeds of *Lilium* species generally have deep dormancy. Removing of seed coats and cutting seeds allowed germination, showing that dormancy of *L. bosniacum* and *L. martagon* var. *cattaniae* was induced by the presence of the testa (Parić et al. 2008). On different species of *Lillium* sp. it is determined that removal of seed testa increases germination. It is assumed that, besides it is physical barrier, testa contains physiological system that maintains dormancy of seed (Pelkonen 2005). In this work sterilized seeds were germinated on MS medium with $GA_3$. After 21 day of incubation all seeds germinated.

Optimal combination and concentrations of growth regulators are necessary to achieve wanted processes on cellular level and wanted growth of the whole plant. There are many works about which combinations of $GA_3$, BAP and IBA are good for growth and development of plants *in vitro*. For example Petrović and Jačimović-Plavšic (1992) proved that the best development and propagation of axillary buds of *Aronia melanocarpa Elliot* was achieved on MS medium supplemented with 0.1 mg/l GA3, 0.1 mg/l IBA and 0.5 mg/l BAP. In this work thirty days after germination plants were transferred to MS medium containing of $GA_3$, IBA and BAP. Three multiplications lasting 20 days were performed and 86 g of fresh material was obtained.

Selected method of extraction significantly

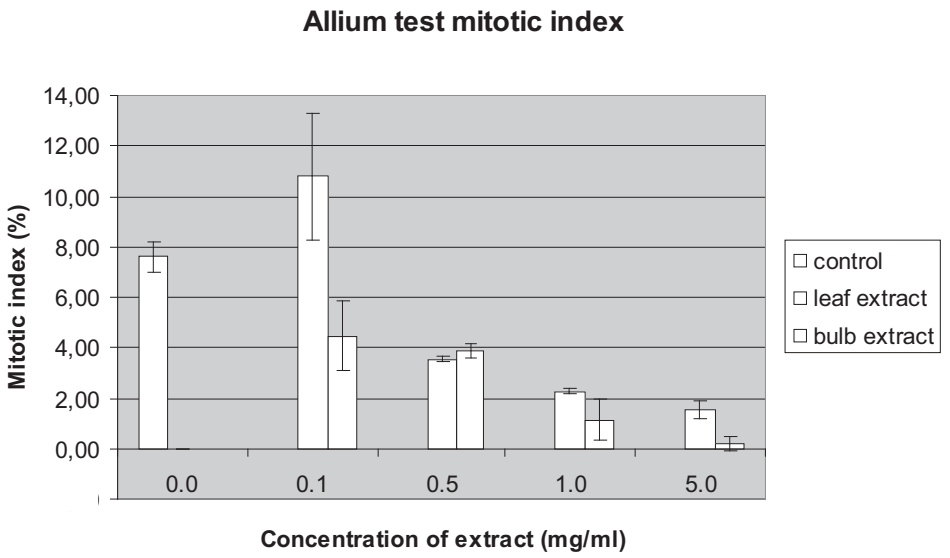


Figure 1: Mitotic index of cells of root tip of onion after treatment with extracts of *L. martagon* var. *cattaniae* and control. The data represent the means ± SD.

Slika 1: Mitotski indeks celic korenin čebule po obravnavanju z izvlečki taksona *L. martagon* var. *cattaniae* v primerjavi s kontrolo. Rezultati so predstavljeni kot povprečje ± SD.

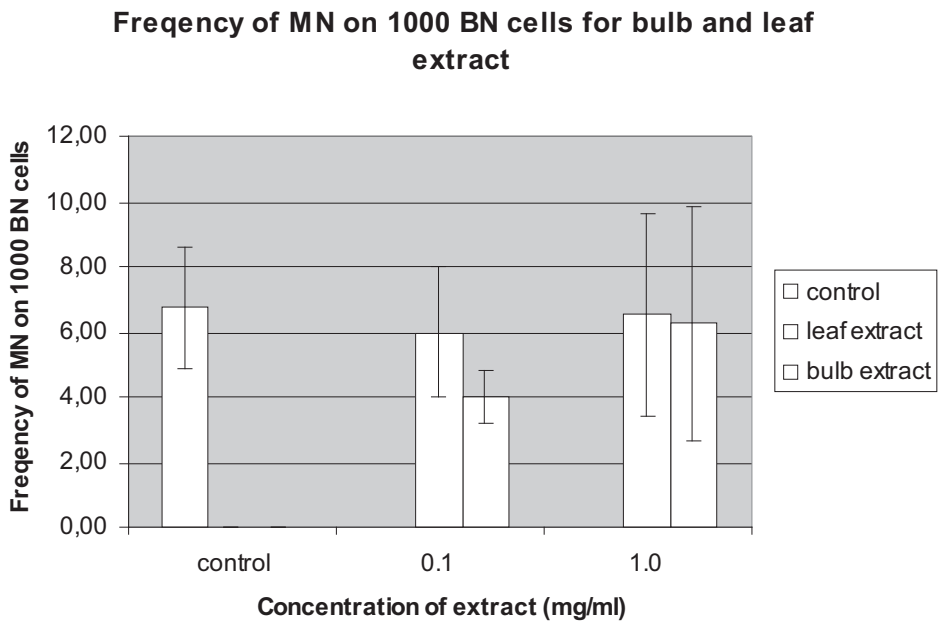


Figure 2: Frequency of MN on 1000 BN cells after treatment with extracts of *L. martagon* var. *cattaniae* and control. The data represent the means ± SD.

Slika 2: Frekvenca MN na 1000 BN celic po obravnavanju z izvlečki taksona *L. martagon* var. *cattaniae* v primerjavi z kontrolo. Rezultati so prikazani kot povprečje ± SD.

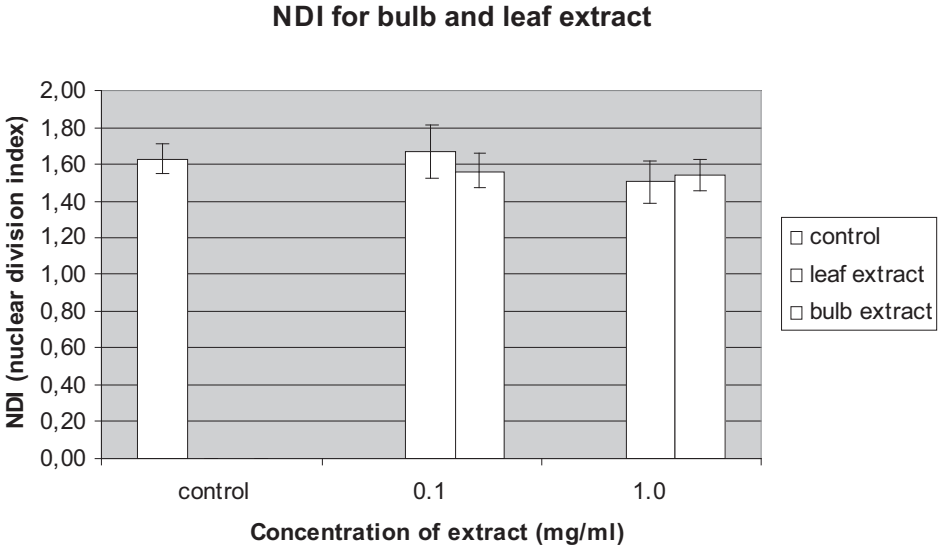


Figure 3: Nuclear division index after treatment with extracts of *L. martagon* var. *cattaniae* and control. The data represent the means ± SD.

Slika 3: Indeks delitve nukleusa po obravnavanju z izvlečki taksona *L. martagon* var. *cattaniae* v primerjavi z kontrolo. Rezultati so predstavljeni kot povprečje ± SD.

affects composition of obtained extracts. Ultrasound assisted extraction attracts more attention recently because of many benefits, primarily better penetration of solvent into plant particles and low extraction temperature which affects the stability of active components (Rouhani et al. 2009).

Weng and coworkers (2004) were doing extraction of loganin in wine on room temperature and they achieved concentration of 48 mg/l after 30 days, while using ultrasound assisted extraction the concentration of 50 mg/l was achieved after 2 days.

In this work total 9.85g of dried material was obtained (4.88g from leaves and 4.97g from bulbs). After extraction, 1.54 g of leaf powder and 1.12 g of bulbs powder was obtained.

Allium test

Four concentrations of extracts (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml and 5 mg/ml) were examined. Mitotic index (MI), presenting percentage of cells in mitosis in total number of analyzed cells, was determined (Figure 1)

It was established that there were statistically significant differences between mitotic index in control and all tested samples.

In vitro micronucleus test on human lymphocytes

Binucleated cells (BN) with one and two micronuclei (MN) were observed. Frequency of MN on 1000 BN cells are presented in Figure 2.

Statistical evaluation of data using Z-test has shown that there is no statistically significant difference in frequency of micronuclei (MN) on 1000 binucleated (BN) cells among control and examined concentrations of leaf and bulb extracts.

Results of calculating NDI for controls and treated cultures are presented in Figure 3.

All extracts induced reduction of NDI comparing to control, except leaf extract with concentration 0.1 mg/ml which in two blood samples induced growth of NDI. After statistical evaluation of data using Independent Group Analysis ANOVA test it was determined that there was no statistical significance in NDI values between control and examined concentrations of leaf and bulb extracts. Using Newman-Keuls multiple comparison it was determined that there was no significant difference among all examined samples.

## Conclusions

- Sterile germination of *Lilium martagon* var. *cattaniae* seed on MS medium with addition of 0.15 mg/l GA3 with removal of testa and border parts of endosperm was successful so in this work 100% germination was achieved.
- In this work multiplication was done on MS medium with 0.1mg/l GA3, 0.2mg/l IBA and 0.5mg/l BAP and satisfying results were achieved.
- Results of ultrasound assisted extraction were very good because from 4.88g of dried leaf 1.536g of dried extract was achieved, and from 4.97g of dried bulbs 1.117g of dried extract was achieved.
- There was statistically significant difference between all used concentrations of lilium extracts and negative control (water) on proliferation of cells of root tip of onion (*Allium cepa*). Leaf extract of 0.1 mg/ml significantly increased proliferation was significantly decreased. All concentrations of leaf extract significantly decreased proliferation, and effect was bigger with higher concentrations.
- Results of proliferation in micronucleus test on human lymphocytes are similar to those in allium test, but in this case there is no statistically significant difference between used extracts and control. To explain mechanisms with which extracts change proliferation it would be necessary to do chemical analysis of extracts and do some more investigations.
- Used extracts didn't show genotoxic properties under experimental conditions.

## Povzetek

*Lilium martagon* L. var. *cattaniae* Vis. (*Liliaceae*) je endemični takson Dinaridov

(Šilić 2007). Čebula se največ uporablja v ljudski medicini na območju Mediterana. Pomembna je za fitokemijske in farmakološke raziskave. Čebula vrste *Lilium martagon* L. ima kardiotonične lastnosti in se uporablja pri obravnavanjih dismenoreje (Khare 2007) in za zdravljenje jetrenih bolezni pri ljudeh in živalih v Severni Albaniji (Pieroni et al. 2005). Čebula vrste *Lilium martagon* se uporablja za zunanje rane (Khare 2007), nekatere raziskave pa so pokazale prisotnost antikancerogenih komponent (Eisenreichová et al. 2004). *In vitro* kultura se uporablja za masovno produkcijo in tako za ohranjanje endemičnih sort lilij. Kaljenje semen taksona *L. martagon* var. *cattaniae* je potekalo na MS hranljivi podlagi, kateri 0,15 mg/l giberelinske kisline ($GA_3$). Kalitev je bila 100%. Za multiplikacijo smo uporabili MS podlago ter dodali 0,1 mg/l giberelinske kisline ($GA_3$), 0,2 mg/l indol-3-maslene kisline (IBA) ter 0,5 mg/l 6-benzilaminopurina (BAP). Po treh tednih kultivacije smo rastline posušili in material uporabili za ekstracijo. Uporabljena je bila ultrazvočna ekstrakcija. Rezultati ultrazvočne ekstrakcije so bili odlični, saj smo iz 4,85 g suhih listov dobili 1,54 g suhega izvlečka, iz 4,9 g suhe čebule pa 1,12 g suhega izvlečka. Za ugotavljanje genotoksičnega potenciala izvlečkov lista in čebul taksona *L. martagon* var. *cattaniae* smo uporabili Allium test in citokinetski-blok mikronukleus test na humanih limfocitih *in vitro*. V primerjavi s kontrolo so vse koncentracije izvlečkov lilij statistično značilno vplivale na proliferacijo celic korenin čebule (*Allium cepa*). Izvleček listov v koncentraciji 0,1 mg/ml je značilno povečal proliferacijo, medtem ko so imele vse druge koncentracije negativne vplive. Učinek se je povečeval z višjanjem koncentracije. Izračun NDI (nucelar division index) mikronukleus testa ni pokazal značilnih razlik.

## Acknowledgements

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# Respiration and ingestion rate of different sized *Daphnia pulex* fed on four algal species

## Dihanje in prehranjevanje različno velikih osebkov vrste *Daphnia pulex* s štirimi vrstami alg

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**Abstract:** Respiration rate and ingestion rate for four different algal species (*Scenedesmus quadricauda, Asterionella formosa, Aphanizomenon flos-aquae* and *Planktotrix rubescens*) of different sized *Daphnia pulex* were measured in the laboratory. Population of *D. pulex* grew maximally when it fed *S. quadricauda*, but the presence of *P. rubescens* and *A. flos-aquae* caused negative population growth rate. Ingestion rates increased with increasing body size for all investigated algae; the lowest *b* value was obtained for *S. quadricauda* and the highest one for *P. rubescens*. The amount of ingested carbon exceeded the required amount for standard metabolism in both small and large sized individuals fed all four algal species. Relatively higher amount of ingested *A. flos-aquae* and *P. rubescens* in comparison with *A. formosa* and *S. quadricauda* and the results of the growth experiments indicate that the inhibitory effect of filamentous blue-green algae on *D. pulex* is more due to toxicity, low assimilation efficiency or/and inadequate composition than incapability of ingestion due to mechanical interference with filaments.

**Key words:** ingestion rate, respiration, *Daphnia pulex,* algae, growth scope

**Izvleček:** V laboratoriju smo pri različno velikih osebkih *Daphnia pulex* merili dihanje in stopnjo hranjenja s štirimi različnimi vrstami alg (*Scenedesmus quadricauda, Asterionella formosa, Aphanizomenon flos-aquae* and *Planktotrix rubescens*). Populacija *D. pulex* je najbolje uspevala pri hranjenju z algo *S. quadricauda*, v prisotnosti vrst *A. flos-aquae* in *P. rubescens* pa smo opazili negativno rast populacije. Stopnja hranjenja se je povečevala z naraščajočo telesno velikostjo pri vseh vrstah alg; najnižjo vrednost *b* smo dobili pri hranjenju s *S. quadricauda*, najvišjo pa s *P. rubescens*. Količina zaužitega ogljika je presegala porabo za standardni metabolizem pri hranjenju z vsemi štirimi vrstami alg. Večje količine zaužitih vrst *A. flos-aquae* in *P. rubescens* v primerjavi z vrstama *A. formosa* in *S. quadricauda* in rezultati rastnih poskusov kažejo, da je inhibitorni vpliv nitastih modro-zelenih alg na osebke vrste *D. pulex* bolj posledica strupenosti, nizke asimilacijske učinkovitosti ali/in neustrezne sestave kot pa nezmožnosti zaužitja zaradi težav, ki bi jih povzročala nitasta oblika alg.

**Ključne besede:** stopnja hranjenja, dihanje, *Daphnia pulex*, alge, obseg rasti

## Introduction

Herbivorous zooplankton is functionally important in aquatic webs. They constitute a link between primary producers and higher trophic levels. A number of studies have investigated the effect of food quality (e.g. Knisley and Geller 1986, Fulton III 1988, Butler et al. 1989, Hawkins and Lampert 1989, Gulati and DeMott 1997, Kilham et al. 1997, Wagner and

Kamjunke 2001) and food quantity (e.g. Porter et al. 1982, Urabe and Watanabe 1991) on feeding and/or on growth, survival and reproduction of *Daphnia*.

As eutrophication often results in a proliferation of blue-green algae (Arnold 1971), the blue-green algae had been studied as food for *Daphnia* in several studies (e.g. Arnold 1971, Gliwicz 1977, Richman and Dodson 1983, Fulton III 1988, Gilbert and Durand 1990, Gliwicz 1990, DeMott 1999, Trabeau et al. 2004). Blue-greens are usually found to be an inadequate food for *Daphnia* due to mechanical interference of colonies of filaments with food collection, low digestibility or poor nutritive quality. Many genera of blue-greens produce either hepatotoxic or neurotoxic secondary metabolites (Trabeau et al. 2004). In the recent years, food-quality research has increasingly focused on the biochemical nutrient requirements of *Daphnia*. It has been shown that fatty acid and phosphorous content of food affect the growth and reproduction of *Daphnia* (Sundbom and Vrede 1997, Park et al. 2002, Ferrão-Filho et al. 2003, Gladyshev et al. 2008, Martin-Creuzburg and Von Elert 2009).

*Daphnia* is filter feeders, having appendages specialized for respiration and food gathering. Food is rejected when the collected amount is greater than it can be ingested, when it is physically unacceptable (i.e., colonies or filaments too large) or if it is chemically unacceptable (see Lampert 1987). The dependence of filtering or ingestion rate (IR) on the body length (L) of *Daphnia* can be described by power equation of the form $IR = a L^b$ (Lampert 1987). One important factor influencing *b* is the size of the food. Although large particles can be better handled by large daphnids than by small ones (Lampert 1987), some studies showed that the feeding of larger *Daphnia* species and larger individuals of single species is more affected by the presence of filaments than the feeding of smaller ones (Hawkins and Lampert 1989, Gilbert and Durand 1990). As filamentous and colonial algae are differently consumed by different body sized *Daphnia* (Hawkins and Lampert 1989), different ingestion of food particles in juveniles and adults was expected. The effect of food quality on both feeding and respiration of *Daphnia* were investigated in few studies (Richman and Dodson 1983, Trabeau et al. 2004) where the effect of different blue-greens on respiration rate of adult stages of *Daphnia* in the presence of food was measured. However, information about the capability of different sized *Daphnia* to ingest enough food to meet their carbon demands, required for standard metabolism, is still lacking. Such studies are important to obtain the basic information on growth scope of different sized animals.

The aim of the present study was to determine an ingestion rate of different sized individuals of *D. pulex* that were fed on four different algal species, i.e. *Scenedesmus quadricauda, Asterionella formosa, Aphanizomenon flos-aquae* and *Planktotrix rubescens*. Respiration rates were measured in different sized developmental stages in order to estimate carbon demands for standard metabolism. It was hypothesized that ingestion rates of different algal species and respiration rates differ in different sized animals. Growth experiments were carried out to test nutritional value of a single alga and possible toxicity of blue-green algae used in the experiments.

## Material and methods

### *Cultures*

*Daphnia pulex* Leydig originated from a permanent laboratory culture in National Institute of Biology (Ljubljana, Slovenia). Animals were kept in 10 L aquaria and some hundred specimens were there all the time. The water temperature was 24.0 ± 1.5 °C. The animals were fed every second day with suspension of *Scenedesmus* sp. and yeast. For feeding experiments adult females without eggs were selected, but for growth experiments ovigerous females of similar size were selected. Single animal were transferred using a narrow glass pipette.

Algal cultures of *Scenedesmus quadricauda, Asterionella formosa, Aphanizomenon flos-aquae* and *Planktotrix rubescens* were obtained from the National Institute of Biology collection (Ljubljana, Slovenia). Algae were cultured in Jaworski medium. Algal cultures were maintained in log growth phase. Algal characteris-

Table 1: Morphological characteristics of algae used in experiments. Mean ± SD (n -number measured).
Tabela 1: Morfološke značilnosti alg, uporabljenih v poskusih. Povprečje ± SD (n - število meritev).

| | Width of particles (μm) | Length of particles (μm) | Max dimension of colony (μm) | Dry weight (μg cell $^{-1}$) |
|---|---|---|---|---|
| *Aphanizomenon flos-aquae* | 3.54 ± 0.73 (25) | 42.47 ± 25.90 (64) | - | $5.33 * 10^{-4}$ |
| *Planktotrix rubescens* | 4.96 ± 0.53 (31) | 103.96 ± 52.75 (31) | - | $1.07 * 10^{-3}$ |
| *Asterionella formosa* | 3.98 ± 0.67 (37) | 46.96 ± 14.4 (43) | 88.45 ± 25.1 (20) | $2.36 * 10^{-4}$ |
| *Scenedesmus quadricauda* | 5.17 ± 0.59 (20) | 8.70 ± 1.1 (20) | 12.6 ± 3.51 (20) | $6.83 * 10^{-5}$ |

tics are given in Table 1. Cells were counted and measured by Soft Imaging System, GmbH, analySIS 3.0, Münster, Germany. Suspension of a single alga was filtered through pre-weighted filter (glass microfibrile filter Whatman GF/C) and dried for 24 h at 60°C. Filters were weighted on 10 μg electrobalance (Sartorius). Dry weight of single cell or filament was calculated from the concentration and volume of filtered suspension.

*Population growth experiments*

Fifteen ovigerous females of similar size (2.20 ± 0.23 mm; average eggs number per female was 2.2) were placed in bottles, each containing 600 mL of synthetic medium (ISO standard) with algal concentration of $1*10^4$ cells (or filaments in the case of blue-green algae) per mL. These animals were collected from the same container, in order to assure that they had similar age and number of eggs. Three replicate bottles for each alga as food were started at the same time to avoid other factors that might affect on experimental conditions. Experimental bottles were kept at 25°C. Before feeding half of water was changed every second day. Relatively constant food level was kept during the experiments. Population growth experiments lasted for 14 days. At the end, animals were killed in formalin solution. Body length was measured from the top of the helmet to the base of the spine using Soft Imaging System, GmbH, analySIS 3.0, Münster, Germany. Population growth rates (r) were estimated as:

$$r = (\ln N_2 - \ln N_1)/(t_2 - t_1),$$

with N1 and N2 being the population sizes of sampling days $t_1$ and $t_2$.

*Feeding experiments*

At the beginning of the experiments, animals were piped into tubes filled with 2 mL suspensions of algae. One animal was placed in each test tube. The initial algal cell concentration was $1 * 10^4$ cells mL$^{-1}$. Animals were fed for 3 hours. Animals and algae were then killed with formalin solution. In each tube, the ingestion rate (IR) were determined as

$$IR = (c_0 - c_1) * V / t$$

where c is the concentration of algae at the beginning ($c_0$) and the end ($c_1$) of the feeding time (t), and V is volume of suspension (2 mL). Animals were starved for 3 hours before being used in feeding experiments.

Respiration rate was estimated by the closed bottle method (Lampert 1984). 150 mL ground glass stoppered bottles were filled with synthetic medium and aerated water from the same, well-mixed, container. Ten bottles received animals (50 similar sized animals were placed in a single bottle), while three bottles served as final con-

Table 2: Population growth rates ($d^{-1}$) for population of *Daphnia pulex* fed different species of algae (n = 3).

Tabela 2: Stopnja rasti populacij ($d^{-1}$) pri vrsti *Daphnia pulex* pri hranjenju z različnimi vrstami alg (n = 3).

| | Mean ± SD |
|---|---|
| *Aphanizomenon flos aquae* | -0.481 ± 0.052 |
| *Planktotrix rubescens* | -0.040 ± 0.021 |
| *Asterionella formosa* | 0.006 ± 0.001 |
| *Scenedesmus quadricauda* | 0.115 ± 0.010 |

trols. All bottles were kept at 25°C. After 24 h the concentration of dissolved oxygen in the experimental and control bottles was measured by polarographic oxygen electrode (OXI 96, WTW). The difference between the oxygen of each experimental as well as mean oxygen concentration of control bottles was taken as the amount of oxygen consumed by animals. The amount of oxygen consumed was then converted to respiration rate per individum (µL $O_2$ /ind/h). Respiratory carbon loss was calculated using the conversion factor 1 mL $O_2$ = 0.5 mg $C_{org}$ (Lampert 1984). In con-

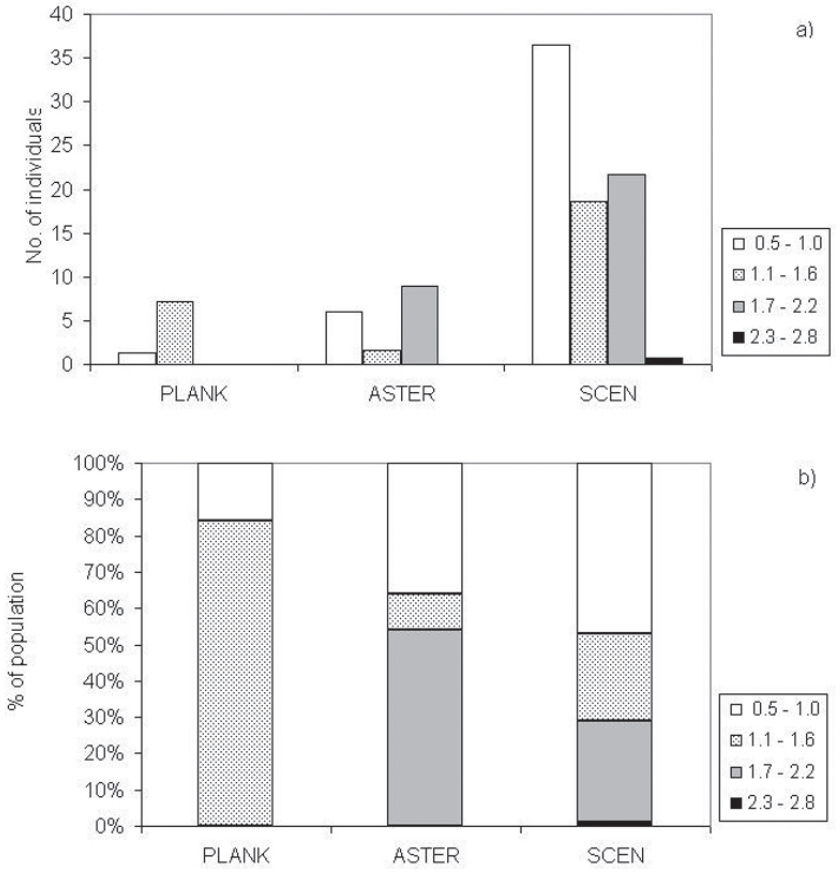


Figure 1: a) Number of individuals and b) percentage of populations for different size classes (mm) of *Daphnia pulex* fed *Planktotrix rubescens* (PLANK), *Asterionella formosa* (ASTER) and *Scenedesmus quadricauda* (SCEN).

Slika 1: a) Število osebkov in b) odstotki populacij po velikostnih razredih (mm) pri vrsti *Daphnia pulex*, ki se je prehranjevala s *Planktotrix rubescens* (PLANK), *Asterionella formosa* (ASTER) in *Scenedesmus quadricauda* (SCEN).

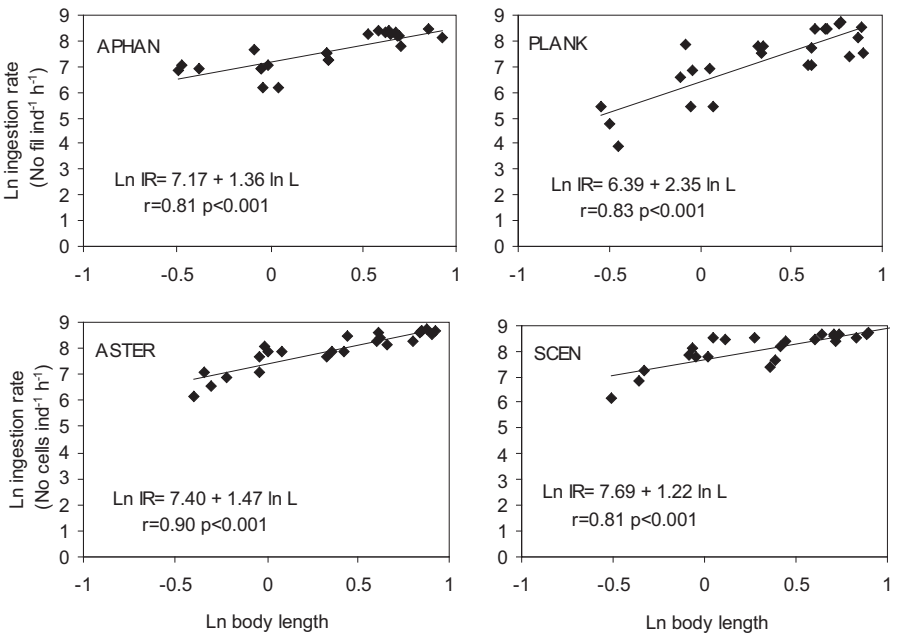


Figure 2: Relationships between ingestion rates and body length of *Daphnia pulex* fed *Aphanizomenon flos-aquae* (APHAN), *Planktotrix rubescens* (PLANK), *Asterionella formosa* (ASTER) and *Scenedesmus quadricauda* (SCEN).

Slika 2: Razmerje med stopnjo hranjenja in telesno velikostjo osebkov *Daphnia pulex* pri prehranjevanju s *Aphanizomenon flos-aquae* (APHAN), *Planktotrix rubescens* (PLANK), *Asterionella formosa* (ASTER) in *Scenedesmus quadricauda* (SCEN).

verting respiration to carbon units, a respiratory quotient (RQ) of 1.0 was assumed. Growth scope was calculated as a subtraction of respiratory carbon loss ($C_{res}$) from ingested carbon ($C_{ing}$).

*Statistical analyses*

One-way analysis of variance (ANOVA) on population growth rates was performed to test differences between different algae. Linear regressions between body length and ingestion rate, and between body length and respiration rate were calculated using Microsoft Excel.

## Results and discussion

*Growth experiments*

Population growth experiments showed that population growth rates differed between food sources (ANOVA, $p<0.001$). Population of *D. pulex* grew maximally when it fed *S. quadricauda* (Table 2).

Populations that fed *A. formosa* had positive growth rates, but the presence of *P. rubescens* caused negative population growth rate. Structure of populations revealed that all individuals of populations that fed *P. rubescens* belonged to two the smallest size classes (Figure 1). Populations that fed *A. formosa* had larger individuals in comparison with *P. rubescens* as a food source, but the largest animals were observed in diet with *S. quadricauda*. Feeding on *A. flos-aquae* resulted in the collapse of populations during six days.

These results are in accord with those previous studies where blue-greens are considered as poor-quality food for *Daphnia*, due to the interference of the filaments with the collection of

available food, toxicity, and a low nutritional quality (Arnold 1971, Gliwicz 1977, Richman and Dodson 1983, Fulton III, 1988, Gilbert and Durand 1990, Gliwicz 1990, DeMott 1999, Trabeau et al. 2004). Arnold (1971) reported that there were differences among the blue-greens in their effects on animals. In the present study *A. flos-aquae* showed toxicity towards *D. pulex*, but *P. rubescens* probably did not provide sufficient nutrition to maintain a population of *D. pulex* (Table 2). DeMott (1999) reported that *D. pulex* exhibited stronger inhibition than *D. magna, D. pulicaria,* and *D. galeata*, when it fed a mixture of *Scenedesmus acutus* and *Microcystis aeruginosa*. Sharp decline in gross growth efficiency showed on growth inhibition as a result of both feeding inhibition and direct toxicity (DeMott 1999). The results of the present study revealed that *S. quadricauda* was high quality food for *D. pulex* as the number and body size of animals exceeded those of animals fed *P. rubescens* or *A. formosa* (Figure 1). High quality of *Scenedesmus* sp. was also reported by Hawkins and Lampert (1989) and Vijverberg (1989). Size of particles of this species is convenient to be high quality food for all life stages of the crustaceans (Vijverberg 1989). As *Planktotrix* sp. has the lowest assimilation efficiency among investigated algae and also, *Asterionella* sp. has lower assimilation efficiency than *Scenedesmus* sp. (cited in Lampert 1987), dissimilar growth of populations could be probably partly explained by differences in assimilation rates.

*Feeding experiments*

Ingestion rate increased with body size for all investigated algae (Figure 2). Increasing of ingestion rates with increasing body length of cladocerans was also observed in DeMott (1982) and Mourelatos and Lacroix (1990). Regression showed that larger individuals consumed significantly more food than smaller ones ($p<0.001$). The *b* value ranged from 1.22 for *S. quadricauda* to 2.35 for *P. rubescens*. One important factor influencing *b* is the size of the food. Large particles can be better handled by large daphnids than small ones. On the other hand, small daphnids have finer filters and can retain smaller particles (Lampert 1987). Therefore, low *b* values which were obtained in the presence of small food particles and high *b* values for large particles (i.e. *P. rubescens*) are in accord with expectations.

Previous studies showed that ingestion rate depends also on food concentration (DeMott

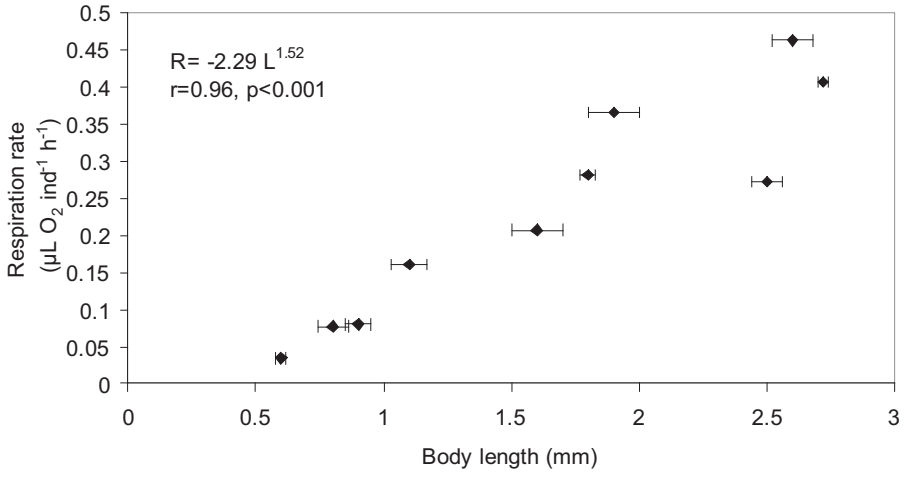


Figure 3: Relationship between respiration rate and mean body length of *Daphnia pulex* present in single experimental bottle. Bars represent ± 1SD.

Slika 3: Razmerje med stopnjo dihanja in povprečno velikostjo osebkov *Daphnia pulex* v posamezni poskusni steklenici. Odkloni predstavljajo ± 1SD.

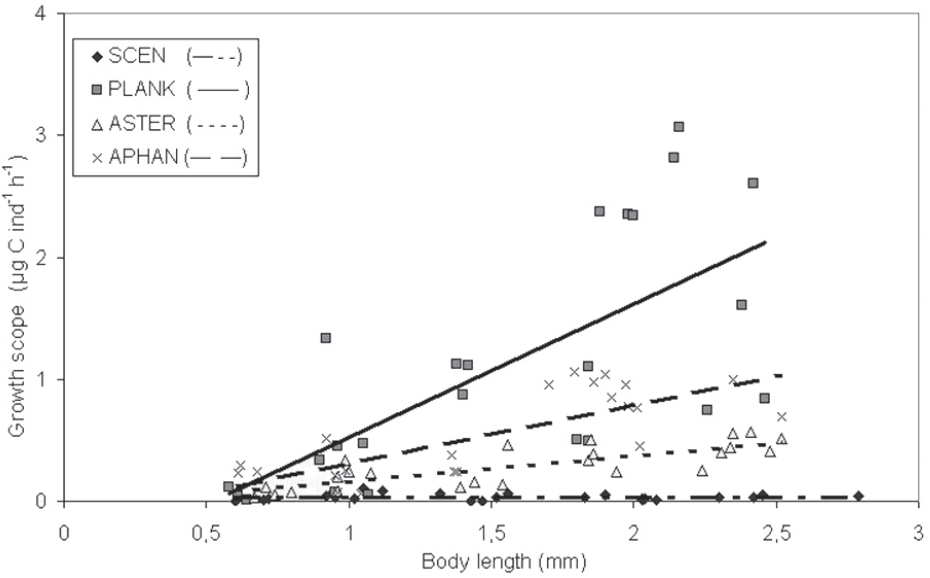


Figure 4: Relationship between the growth scope and body length of *Daphnia pulex* fed *Aphanizomenon flos-aquae* (APHAN), *Planktotrix rubescens* (PLANK), *Asterionella formosa* (ASTER) and *Scenedesmus quadricauda* (SCEN).

Slika 4: Razmerje med obsegom rasti in velikostjo osebkov *Daphnia pulex* pri prehranjevanju s *Aphanizomenon flos-aquae* (APHAN), *Planktotrix rubescens* (PLANK), *Asterionella formosa* (ASTER) in *Scenedesmus quadricauda* (SCEN).

1982, Porter et al. 1982, Gilbert and Durand 1990, Mourelatos and Lacroix 1990). In general, ingestion rates increased with increasing food concentration until incipient limiting level (ILL) was reached. Above the ILL the ingestion rate remains constant. Animals control the ingestion rate by rejecting of superfluous food from the food groove with abdominal claw. The rate of rejection remains constant below the ILL, but it increases at high concentration (Porter et al. 1982). In the present study, both growth and feeding experiments were performed at concentration that was considered as ILL (i.e., $1*10^4$ cell mL$^{-1}$) (Porter et al. 1982). This food concentration should provide optimal feeding conditions for animals.

*Respiration rate measurements*

Respiration rates (R) increased with increasing body length (L) of *D. pulex* according regression equation: ln R= -2.28 + 1.52 ln L (r= 0.96; p<0.001) (Figure 3). In the present experiments, the standard metabolism and expenditure on locomotion were measured. The expenditure on feeding and specific dynamic action (SDA) was minimal (Philippova and Postnov 1988) because the animals were not fed just prior to or during the experiments. Therefore, measured respiration rates represent minimal maintenance costs of metabolism in different sized animals. Feeding and processing of the food increase respiration rates (Philippova and Postnov 1988) so higher respiratory carbon demands can be expected in the presence of food.

*Relation between respiratory carbon demands and ingested amount of food*

The amount of ingested carbon exceeded the amount of that required for standard metabolism and locomotion in both small and large sized individuals for all four algal species (Figure 4). Therefore, animals of all sizes were capable of consumption sufficient amount of *S. quadricauda* and *A. formosa* as well as filamenous algae *A.*

*flos-aquae* and *P. rubescens* to meet needs related to minimal metabolic demands. The amount of ingested carbon was the lowest for *S. quadricauda*, while the highest values were observed for *P. rubescens*. The reason is probably different sized algal particles that were offered to animals, as a passive filtering of similar number of particles resulted in different amount of collected food.

The results of the present study indicate that although *P. rubescens* can be ingested by *D. pulex*, it cannot provide sufficient nutrition to support a population that does not have other food available. Arnold (1971) also found that some blue-green algae are inadequate food source for *D. pulex.* Animals can ingest food in large amounts but assimilate it poorly, or allocate most of the quantity assimilated to maintenance costs. Thus, those animals which fed on low quality food are unable to increase or even maintain the existent population.

## Conclusions

It is concluded that both juvenile and adult *D. pulex* can ingest relatively large amount of blue-green algae as well as green algae and diatoms. Thus, these results indicate that the inhibitory effect of filamentous blue-green algae *A. flos-aquae* and *P. rubescens* is more due to toxicity, low assimilation efficiency or/and inadequate composition than incapability of ingestion due to mechanical interference with filaments. Also, relatively small amount of ingested *S. quadricauda* showed that this alga is high quality food for *D. pulex*. *A. formosa* probably should be considered as an adequate, but less qualitative food source in comparison with *S. quadricauda*.

## Acknowledgments

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## Povzetek

Rastlinojedi zooplankton ima v prehranjevalni mreži ključno vlogo, saj predstavlja povezavo med primarnimi producenti in višjimi trofičnimi nivoji. Številni raziskovalci, ki so preučevali vpliv količine in kvalitete hrane na prehranjevanje, rast, preživetje in razmnoževanje osebkov iz rodu *Daphnia*, poročajo, da so modro-zelene alge zaradi velikosti kolonij, slabe prebavljivosti, nizke hranilne vrednost in celo strupenosti pogosto neustrezna hrana. V večini raziskav so preučevali vpliv hrane na odrasle osebke, manj pa je znanega o prehranjevanju mladičev oz. različno velikih osebkov. Ker je količina zaužite hrane odvisna od velikosti osebkov in velikosti delcev hrane, smo ugotavljali, ali različno veliki osebki lahko zaužijejo zadostno količino hrane, ki je potrebna za vzdrževanje osnovnih fizioloških potreb. Tako smo ugotavljali stopnjo hranjenja pri različno velikih osebkih *Daphnia pulex*, ki smo jih hranili s štirimi različnimi vrstami alg (*Scenedesmus quadricauda, Asterionella formosa, Aphanizomenon flos-aquae* in *Planktotrix rubescens*). Z merjenjem dihanja pri različno velikih osebkih smo ocenili potrebo po energiji za standardni metabolizem. Hranilno vrednost in strupenost posamezne vrste alge smo ugotavljali z rastnimi poskusi. Največja je bila rast populacije *D. pulex* pri hranjenju s *S. quadricauda*, nato sledi *A. formosa*. V prisotnosti *P. rubescens* smo opazili negativno rast populacije, pri *A. flos-aquae* pa je prišlo do njenega propada že v nekaj dneh. Rezultati rastnih poskusov so pokazali strupenost vrste *A. flos-aquae,* nizko hranilno vrednost pri vrsti *P. rubescens* in najvišjo kvaliteto za vrsto *S. quadricauda.* V skladu s pričakovanji se je stopnja hranjenja povečevala z naraščajočo telesno velikostjo pri vseh vrstah alg. Najnižjo vrednost *b* smo dobili pri hranjenju vodnih bolh s *S. quadricauda,* najvišjo pa s *P. rubescens*. Količina zaužitega ogljika je presegala potrebe po energiji za standardni metabolizem pri hranjenju z vsemi štirimi vrstami alg. Raziskava je pokazala, da tako mladiči, kot tudi odrasli osebki *D. pulex* lahko zaužijejo dokaj veliko količino modrozelenih, zelenih in kremenastih alg. To pomeni, da je inhibitorni vpliv nitastih modro-

zelenih alg vrste *A. flos-aquae* in *P. rubescens* na *D. pulex* bolj posledica strupenosti, nizke asimilacijske učinkovitosti ali/in neustrezne sestave, kot pa nezmožnosti zaužitja zaradi težav, ki bi jih povzročala nitasta oblika alg. Vrsta *A. formosa* se je sicer izkazala kot zadosten vir hrane, a je bila v primerjavi z vrsto *S. quadricauda* manj hranilna.

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# Conservation assessment of the butterfly fauna along the River Sava between Krško and the state border

## Naravovarstveno vrednotenje favne dnevnih metuljev ob reki Savi med Krškim in državno mejo

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**Abstract:** An inventory of butterfly fauna was carried out in 2008 within the southern part of the Ecological Important Area »the Sava River between Radeče and the state border with Croatia« with the aim of evaluating the most important areas for butterflies. Butterfly fauna was surveyed within a study area of 32 km$^2$, using the transect method. Twenty-one combined habitat types were included in the transect lines, with a total length of 19.2 km. The following parameters were used to evaluate the conservation importance of the combined habitat types: species richness of the combined habitat type, population density of species in combined habitat type, total population density of combined habitat type, maximum population density of species in combined habitat type, number of species with maximum population density in combined habitat type, number of threatened species in combined habitat type and number of threatened species with maximum population density in combined habitat type. A total of 69 species of butterflies (38% of Slovene butterfly fauna) were recorded, 10 of which are threatened on national or European level. The most important combined habitat types for butterflies are extensively managed dry grasslands, abandoned dry grasslands, some types of semi-intensively used grasslands, some types of ruderal communities, and dry woodland rides and edges. On the basis of the distribution of the most important combined habitat types in the study area, four important areas for butterflies were designated, with a total area of 6.6 km$^2$. They are important for preserving threatened species, ecological specialists and other rare or locally distributed species in the sub-pannonian part of SE Slovenia.

**Key words:** the River Sava between Krško and the state border, Ecological Important Area, butterflies, population density, species richness, index of distribution, threatened species, Habitats Directive

**Izvleček:** V južnem delu Ekološko pomembnega območja »Sava od Radeč do državne meje« (EPO Id: 63700), med Krškim in državno mejo s Hrvaško, smo v letu 2008 izvedli inventarizacijo favne dnevnih metuljev z namenom opredeliti naravovarstveno najpomembnejša območja za dnevne metulje v EPO. V raziskovanem območju s površino 32 km$^2$ smo na transektih dolžine 19,2 km z metodo transektnega popisa inventarizirali relativno številčnost vrst v 21 zbirnih habitatnih tipih. Za naravovarstveno ovrednotenje zbirnih habitatnih tipov smo uporabili naslednje parametre: vrstno bogastvo zbirnega habitatnega tipa, populacijska gostota vrste v zbirnem habitatnem tipu, celokupna populacijska gostota v zbirnem habitatnem tipu, maksimalna populacijska gostota vrste v zbirnem habitatnem tipu, število vrst z maksimalno populacijsko gostoto v zbirnem habitatnem tipu in število ogroženih vrst v zbirnem habitatnem tipu. Zabeležili smo 69 vrst dnevnih metuljev (38 % favne dnevnih metuljev Slovenije), med njimi 10 v nacionalnem ali evropskem merilu ogroženih vrst. Naravovarstveno najpomembnejši habitatni tipi za dnevne metulje so ekstenzivno gospodarjeni suhi travniki, zaraščajoča suha travišča, nekateri polintenzivno gospodarjeni travniki, nekatere ruderalne združbe ter gozdne poti

in robovi na suhih rastiščih. Na podlagi razširjenosti najpomembnejših zbirnih habitatnih tipov v raziskovanem območju smo opredelili štiri naravovarstveno pomembna območja za dnevne metulje s skupno površino 6,6 km2. Območja so pomembna za ohranjanje ogroženih in drugih ekološko specializiranih, redkih ali lokalno razširjenih vrst v območju subpanonske JV Slovenije.

**Ključne besede:** reka Sava med Krškim in državno mejo s Hrvaško, ekološko pomembno območje, dnevni metulji, populacijska gostota, vrstno bogastvo, indeks razširjenosti, ogrožene vrste, Direktiva o habitatih

## Introduction

Butterflies are one of the most important invertebrate bioindicator groups (see, e.g., Kudrna 1986, Oostermeijer and Van Swaay 1998, Ricketts et al. 2002; Maes and Van Dyck 2005, Thomas 2005, Settele et al. 2009). Data on their distribution and numbers have been collected in Europe at least 20 years within the framework of the project »Butterfly Monitoring Schemes« (Van Sway and Van Strien 2008, Van Sway et al. 2008). The method for monitoring butterfly populations is well described, extensively tested and scientifically sound (Pollard and Yates 1993). Butterflies are the only invertebrate taxon for which it is currently possible to estimate rates of decline among terrestrial insects (De Heer et al. 2005, Thomas 2005). They are also representative indicators of trends observed in most other terrestrial insects, which together represent approximately two-thirds of the world's species (Thomas 2005). In 2007 as a result of the project »**S**treamlining **E**uropean 2010 **B**iodiversity **I**ndicators«, the European Environment Agency proposed 26 indicators for inclusion in the set of European biodiversity indicators (i.e. SEBI 2010 Indicators) (Van Sway and Van Strien 2008, Van Sway 2010). The biodiversity indicators offer a quick and easy tool for presenting general trends on the state of biodiversity (European Commission 2010). Butterflies were proposed as indicators for assessment of the state and trends in European grasslands (European Grassland Butterfly Indicator), which are the most important habitat of European species of butterflies (Van Sway 2010), since they are occupied by more than half of European species (280 species, 57%) (Van Sway et al. 2006).

The main goal of this research was inventory of butterfly fauna and, on the basis of the distribution and relative numbers of adults, to define from a nature conservation point of view important populations, habitat types and areas for butterflies along the lower course of the River Sava between Krško and the state border, i.e., in the southern part of the ecologically important area of the »Sava from Radeče to the state border« (EPO Id: 63700, http://www.naravovarstveni-atlas.si). In the analysis, we included other habitat types, such as those on the basis of which EPO was defined (i.e., river with gravel bars, river branches, oxbows, floodplain groves, fragments of floodplain forest; http://www.naravovarstveni-atlas.si), above all various types of grassland, woodland edges, tall herb communities and ruderal areas. We thus at the same time supplemented the currently knowledge of the fauna of EPO »Sava from Radeče to the state border«.

### *Review of existing data*

There is relatively little published data about the butterfly fauna of the study area. The only planned research of butterfly fauna in the area of the lower Sava downstream from Krško was done by Rebeušek (2001). Older publications on the presence of some species of butterflies refer to the surroundings of Krško (e.g., Hafner 1909, Lorković 1993, 1996) and Bregana (e.g., Lorković and Mladinov 1971, Čelik et al. 2004). More numerous data have been published for the wider hinterland of the research area in the sub-Pannonian zoogeographic region (e.g., Kostanjevica, Novo Mesto, Tolsti vrh, Gorjanci, Kozje etc.) (e.g., Hafner 1909 – manuscript, Lorković 1927, Carnelutti and Michieli 1955, 1960, Sijarić 1991, Čelik et al. 2004, Čelik et al. 2005).

## Material and Methods

*Study area*

The study area is situated in the south-eastern part of Slovenia (45° 54' 35" N, 15° 34' 32" E; WGS 84), at an altitude of 150 m. It belongs to the sub-Pannonian region (Carnelutti 1992), the northern part borders on the pre-Alpine region. It embraces the lowlands on both banks of the River Sava, from Krško in the north to the state border with Croatia by the settlement of Ribnica in the south; in the east it extends to the road Krško–Zgornji Obrež–Brežice–Dobova, and in the west to the road Žadovinek–Mrtvice–Krška vas–Čatež ob Savi, at first towards the south to the motorway Čatež ob Savi–Podgračeno (Fig. 1). It extends over an area of 32 km$^2$. The study area is mainly agricultural landscape. It is consisted of five landscape types (Denac et al. 2009): agricultural landscape (66%; arable fields, differently managed grasslands), mosaic of hedges, scrub, small farmland areas and small forest fragments where no habitat type predominates (14%), woodland (10%; mainly fragments of floodplain forest), river (8%; riverbed with banks, river branches, gravel bars) and other water bodies (2%; gravel pits, oxbows). In agricultural landscape, where arable fields and intensively used grasslands prevail (46% of the study area; cf. Trčak et al. 2008), the abandoned dry grasslands in early succession stages and the

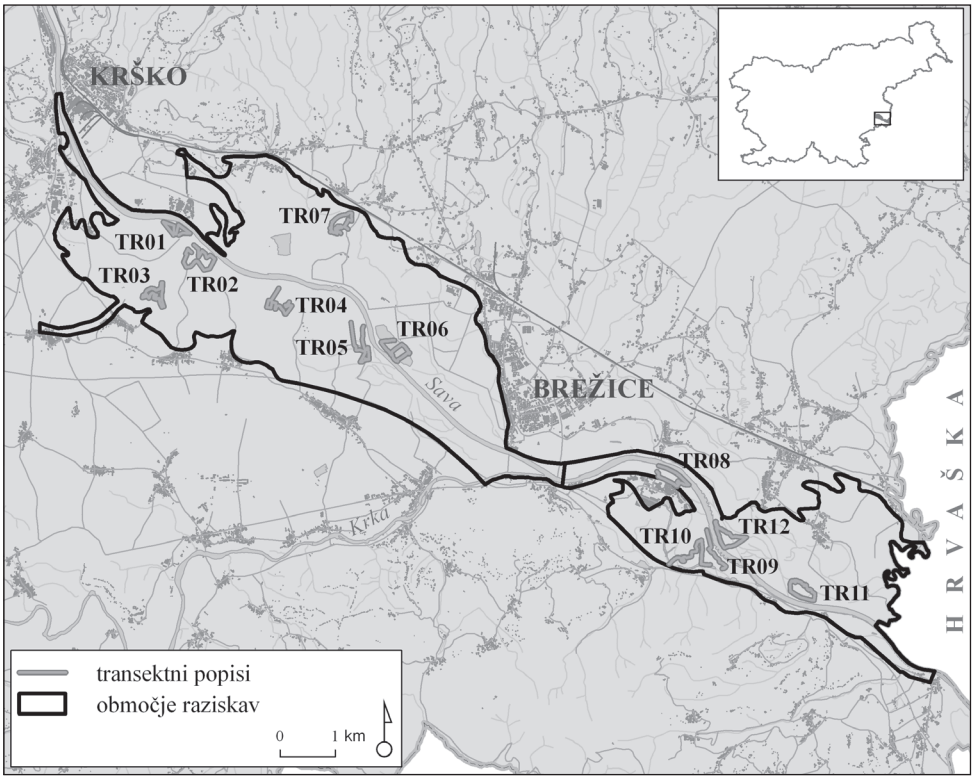


Figure 1: Study area and transect lines (TR) for inventory of butterflies along the River Sava between Krško and state border with Croatia in 2008.

Slika 1: Raziskovano območje in transekti (TR) za inventarizacijo dnevnih metuljev ob reki Savi med Krškim in državno mejo s Hrvaško v letu 2008.

extensively managed dry grasslands represent only a small part of the area (7%; cf. Trčak et al. 2008). The most extensive areas of these two types of grasslands extend in the NW part of the study area on the right bank of the River Sava. Minor areas are also in the northern part of the study area on the left bank of the River Sava, and in the SE part of the study area on the right and left bank of the River Sava. The important characteristic of the study area is also the presence of invasive allochthonous plant species (e.g. *Solidago gigantea*, *Solidago canadensis*, *Impatiens glandulifera*, *Rudbeckia laciniata*, *Echinocystis lobata*, *Fallopia japonica*, *Robina pseudoacacia*, *Ailanthus altissima*), widely distributed on moist ground of woodland areas, on river banks and near other water bodies. As pure stands they represent 2% of the study area, but in combination with other habitat types they actually extend over a much larger area (Trčak et al. 2008).

*Field methods*

We surveyed the butterfly fauna of the study area from March to September 2008, at 12 sampling sites (Fig. 1). At each sampling site, butterflies were monitored using the line transect method (Pollard and Yates 1993, Thomas 2005) – 1 transect line/sampling site. Transect counts were conducted at a time interval of approximately 14 days (1 count in April, 2 counts in May, 1 count in June, 2 counts in July, 1 count in September). The entire length of the transect lines was 19,157 metres. Each transect line was divided into sections, each section represented a specific habitat type. The habitat types were identified exactly, to the 3rd and 4th levels of the methodology determined by Jogan et al. (2004). The 12 transect lines contained 53 sections.

In the field, it is not possible to distinguish between *Colias alfacariensis* Ribbe, 1905 and *Colias hyale* (Linnaeus, 1758), and also between *Leptidea sinapis* (Linnaeus, 1758) and *Leptidea reali* Reissinger, 1989. Therefore, the both *Colias* species were treated as a complex *C. alfacariensis*/*hyale*, and both *Leptidea* species as a complex *L. sinapis*/*reali* for the analysis.

*Data analysis*

Because of the large number of habitat types (40), we merged the 40 habitat types into 21 combined habitat types (Tab. 1) that we used in further analysis. A combined habitat type is a higher and more general defined category, which includes habitat types that are similar in terms of floristic composition, structure of vegetation and management. Therefore, **the length of the combined habitat type** is the sum of the lengths of all transect sections with habitat type belonging to those combined habitat type.

For data analysis we used the following parameters: population density, total population density, maximum population density, species richness, index of distribution of species in the study area, number of species which had maximum population density in specific combined habitat type and number of threatened species in specific combined habitat type.

The **population density** of each butterfly species in each combined habitat type was calculated according to Kitahara et al. (2008) as follows. The monthly count was determined as the mean of twice-monthly counts conducted in May-July or as the value of single counts in April, June and September. The mean monthly count over the season was then calculated using only those months when the species was observed to minimize the effect of variable voltinism between species. Finally, the population density (number of adults/month/100 m of combined habitat type) was obtained by dividing the mean monthly count by the length of the combined habitat type (in metres x 100). The population density of each threatened species in each combined habitat type in proposed important areas for butterflies was calculated in the same manner, except that we included in the length of the combined habitat type only sections in transects that lay within the proposed important area.

The **total population density** in each combined habitat type was the sum of population densities of all species observed in each combined habitat type.

The **maximum population density** of a species was the population density of species in the combined habitat type in which it was most abundant.

The **species richness** in each combined habitat type/important area for butterflies was the total number of butterfly species observed in each combined habitat type/important area for butterflies during the study period.

The **index of distribution of a species in the study area** was expressed as the number of transect lines in which the species was observed. We treated as a generally widespread species, those species with an index of distribution > 9 (> 75% of all transect lines), as rare/locally distributed species those with an index of distribution < 4 (< 25% of all transect lines).

Kendall rank correlation coefficient was used to test whether a relationship exists between the length of the combined habitat type and the following parameters: species richness of the combined habitat type, total population density, number of species with maximum population density in the combined habitat type, number of threatened species in the combined habitat type and number of threatened species with maximum population density in the combined habitat type. In the interpretation of species richness of the combined habitat types in relation to the length of the combined habitat type, we treated as "not different in terms of the length" those combined habitat types that differ in length by less than 50 m, and as "not different in terms of species richness" those combined habitat types that differ in number of species by less than 3.

To define the important areas for butterflies in the study area, first the conservation importance of the combined habitat types for butterflies was assessed. For the conservation evaluation of each combined habitat type, six parameters were used: species richness, median of population densities of species observed in the treated combined habitat type, total population density, number of species with maximum population density in the treated combined habitat type, number of threatened species in the combined habitat type and number of threatened species with maximum population density in the treated combined habitat type. The non-paramteric pairwise correlations were used to test the independence between parameters. Because of the small data set (21 combined habitat types) with a large number of tied ranks, Kendall's tau was applied. In the cases where the significant relationship between parameters was established, the actual values of Kendall's correlation coeficient shown only weak or moderate correlation. Therefore, all six parameters were used in the conservation assesment. They were applied as criteria in further conservation evaluation of the combined habitat types. For each criterion the combined habitat types were ranked according to parameter value. The rank 1 was assigned to the combined habitat type with the highest parameter value, and the rank 21 (in the case of data set without tied ranks) to the combined habitat type with the lowest value of parameter. The nature conservation value of each combined habitat type was determined as the median of ranks assigned to each combined habitat type within the scope off all criteria. The combined habitat types with the median value lower then 10,5 were defined as the most important for butterflies in the study area from the conservation point of view. Finally, the important areas for butterfly species were outlined on the base of the distribution of the most important combined habitat types within the study area. The distribution of all habitat types which were similar, in terms of floristic composition, structure of vegetation and management to the combined habitat types of transect lines was established from the map of habitat types of the study area (Trčak et al. 2008).

Statistical calculations were performed by SPSS 13.0 (SPSS Inc. 1989–2004). We used the program Arc Map 9.2 (ESRI Inc. 1999–2006) for outlining transects and important areas for butterfly species. The nomenclature of species of butterflies is taken from Van Sway et al. (2010), plant species from Martinčič et al. (2007), the typology of habitat types according to Jogan et al. (2004), threatened species according to the Red list of Lepidoptera of Slovenia (Uradni list RS 82/2002), the Decree on protected wild animal species (Uradni list RS 46/2004), the European Red list of Butterflies (Van Swaay et al. 2010), the Directive on the conservation of natural habitats and of wild fauna and flora (Directive 92/43/EEC) (hereinafter: Habitats Directive) and the Convention on the Conservation of European Wildlife and Natural Habitats (Uradni list RS 17/1999) (hereinafter: Bern Convention).

## Results and discussion

*Species richness and distribution of species in the study area*

In the period from March to September 2008, we recorded 7396 individuals from 69 species in the transect lines (Tab. 1), which represents 38% of all butterfly species living in Slovenia.

In the only previous planned inventory of butterflies, which was carried out in the study area in 2001, 60 species were recorded (Rebeušek 2001). They included four species that we did not record in 2008: *Cyaniris semiargus* (Rottemburg, 1775), *Argynnis niobe* (Linnaeus, 1758), *Brentis ino* (Rottemburg, 1775) and *Neptis sappho* (Pallas, 1771). In view of the fact that all four species were observed in 2001 in areas in which we carried out transect counts, the reasons that we did not register these species in 2008 could be: (i) very low numbers of the populations, which reduces the probability of detection of the species in a transect line; (ii) the species do not live in the areas of the transect lines. In 2008, we found 13 species that had not been observed in 2001: *Aphantopus hyperantus* (Linnaeus, 1758), *Argynnis paphia* (Linnaeus, 1758), *Carcharodus alceae* (Esper, 1780), *Coenonympha arcania* (Linnaeus, 1761), *Leptotes pirithous* (Linnaeus, 1767), *Limenitis populi* (Linnaeus, 1758), *Lycaena hippothoe* (Linnaeus, 1761), *Melitaea didyma* (Esper, 1778), *Pieris mannii* (Mayer, 1851), *Satyrium acaciae* (Fabricius, 1787), *S. spini* (Dennis & Schiffermüller, 1775), *S. w-album* (Knoch, 1782) and *Thymelicus lineola* (Ochsenheimer, 1808).

Mostly intensively managed anthropogenic landscape of the study area is reflected in the species richness of butterfly fauna (69 species/32 km$^2$). It is lower than in Natura 2000 sites and some other important conservation areas in Slovenia, in which inventories of butterfly fauna are already performed. It is evident from the comparisons with the species richness of the following areas: Škocjanske jame Regional Park: 90 species/4 km$^2$ (Čelik 2004); Natura 2000 site "Radensko polje" – SI3000171: 68 species/7 km$^2$ (Rebeušek and Verovnik 2000); Natura 2000 site "Planinsko polje" – SI5000016: 78 species/10 km$^2$ (Čelik 2007); Natura 2000 site "Banjšice" – SI3000034: 78 species/12 km$^2$ (Čelik 2009).

Generally widespread species (= index of distribution > 9) in the study area are species that are not ecologically specialised, of which we recorded 19: *Aglais io* (Linnaeus, 1758), *Aricia agestis* (Dennis & Schiffermüller, 1775), *Boloria dia* (Linnaeus, 1767), *Brenthis daphne* (Bergsträsser, 1780), *Coenonympha glycerion* (Borkhausen, 1788), *C. pamphilus* (Linnaeus, 1758), *Colias crocea* (Geoffroy, 1785), *Cupido argiades* (Pallas, 1771), *Erynnis tages* (Linnaeus, 1758), *Gonepteryx rhamni* (Linnaeus, 1758), *Leptidea sinapis/reali* (Linnaeus, 1758/ Reissinger, 1989), *Maniola jurtina* (Linnaeus, 1758), *Melitaea athalia* (Rottemburg, 1775), *M. phoebe* (Dennis & Schiffermüller, 1775), *Ochlodes sylvanus* (Esper, 1777), *Pieris napi* (Linnaeus, 1758), *P. rapae* (Linnaeus, 1758), *Plebejus argus* (Linnaeus, 1758) and *Polyommatus icarus* (Rottemburg, 1775) (Tab. 1). Rare/ locally distributed species (index of distribution < 4; 21 species) occupy woodland rides (11 species), abandoned dry and semi-dry grasslands (9 species), extensively managed dry grasslands (8 species), semi-intensively managed grasslands (7 species), woodland edges (7 species), ruderal areas (5 species) and scrub (1 species) (Tab. 1).

*Species richness of the combined habitat types*

The species richest combined habitat types in the study area are extensive dry grasslands with erect brome (48 species), abandoned dry and semi-dry grasslands (STzS: 46, STzT: 41 species), semi-intensively managed dry grasslands with erect brome and tall oat-grass (40 species) and woodland rides on moist grund overgrown with autochthonous and allochthonous plant species (36 species) (Tab. 1).

The significant strong positive correlation exists between the length of the combined habitat type and its species richness ($\tau = 0.657$, $P<0.001$). From a comparison between combined habitat types that differ in length by less than 50 m and in number of species by more than 2 (Fig. 2a) and combined habitat types of different lengths that do not differ in terms of the number of species (Fig. 2b), we can conclude

Table 1: List of recorded species with population densities in combined habitat types in which a species appeared and index of distribution of species recorded in transect lines along the River Sava between Krško and the state border in 2008.

Tabela 1: Seznam registriranih vrst s populacijskimi gostotami v zbirnih habitatnih tipih in indeks razširjenosti vrst, zabeleženih na transektih ob reki Savi med Krškim in državno mejo v letu 2008.

| Combined habitat type | GMvT | GPsS | GPsT | GPvT | GPvVs | GRsS | GRsT | GRvT | GRvVs | MTiA | MTpA | N | Rg | Rgm | Rgmz | STeB | STpA | STpBA | STzS | STzT | TVTp | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of combined habitat type (m) | 549 | 501 | 381 | 2068 | 686 | 202 | 642 | 492 | 1246 | 1999 | 1243 | 50 | 448 | 476 | 287 | 2838 | 972 | 1057 | 1923 | 928 | 169 | Index of distribution |
| Species | Population density | | | | | | | | | | | | | | | | | | | | | |
| *Aglais io* | 0,05 | 0,05 | 0,33 | 0,02 | | | 0,12 | 0,20 | 0,17 | 0,04 | 0,02 | | | 0,05 | 0,17 | 0,01 | 0,05 | | 0,01 | 0,03 | 0,07 | 12 |
| *Aglais urticae* | | 0,07 | 0,04 | | | | 0,05 | | | 0,01 | 0,04 | | 0,07 | 0,07 | | 0,01 | | | | 0,02 | | 8 |
| *Anthocharis cardamines* | | 0,20 | 0,13 | 0,16 | 0,07 | | 0,08 | 0,05 | 0,04 | 0,03 | | | 0,11 | 0,16 | 0,35 | | 0,03 | | 0,04 | | 0,30 | 9 |
| *Apatura ilia* | | | | 0,08 | | | | 0,14 | | | | | 0,04 | 0,04 | 0,23 | | | | | | | 3 |
| *Aphantopus hyperantus* | 0,05 | 0,10 | 0,46 | 0,01 | | | | | | | | | | | | 0,03 | 0,03 | | 0,49 | 0,24 | | 5 |
| *Araschnia levana* | | | 0,07 | 0,09 | | | 0,04 | 0,05 | | 0,01 | | | | 0,11 | 0,26 | | | | | | | 5 |
| *Argynnis paphia* | | 0,10 | | 0,01 | | | | | | 0,03 | | | | | | 0,02 | | | | | | 4 |
| *Aricia agestis* | | 0,02 | | 0,01 | | 0,19 | | | | 0,03 | 0,06 | | 0,08 | | 0,09 | 0,22 | 0,03 | 0,20 | 0,40 | 0,44 | | 11 |
| *Boloria dia* | 0,02 | | | 0,01 | | | 0,12 | | | 0,28 | 0,28 | | | | | 0,48 | 0,72 | 0,56 | 0,46 | 0,10 | | 10 |
| *Brenthis daphne* | 0,18 | | | 0,15 | 0,44 | | 0,16 | 0,41 | 0,72 | | 0,16 | | | 0,21 | 0,35 | | | 0,47 | 0,05 | 0,22 | | 10 |
| *Callophrys rubi* | | | 0,13 | | | | | | | | | | | | | | | | 0,05 | 0,11 | | 3 |
| *Carcharodus alceae* | 0,05 | | | | | | | | | | | | | | 0,09 | | | | 0,01 | | | 3 |
| *Celastrina argiolus* | | | 0,07 | 0,05 | | | | 0,05 | | | | | | | | | | | | | | 5 |
| *Coenonympha arcania* | | | 0,59 | 0,02 | | | 0,08 | | | 0,05 | | | | | | 0,10 | | 0,07 | 1,39 | 3,18 | | 5 |
| *Coenonympha glycerion* | 0,39 | | | 0,01 | | 0,37 | 0,21 | 0,03 | 0,14 | 0,27 | 0,44 | 0,50 | | | | 0,60 | 0,45 | 0,93 | 1,14 | 0,84 | | 11 |
| *Coenonympha pamphilus* | 0,66 | | | 0,02 | 0,09 | 0,43 | 0,31 | 0,03 | 0,27 | 0,42 | 0,40 | 1,00 | 0,03 | | 1,70 | 0,60 | 0,78 | 0,48 | 0,57 | 0,32 | 0,15 | 12 |
| *Colias alfacariensis/ hyale* | | | | | 0,02 | | | | 0,03 | 0,02 | 0,01 | | | | | 0,06 | 0,02 | 0,13 | 0,05 | 0,02 | | 8 |
| *Colias croca* | 0,39 | 0,22 | 0,03 | 0,04 | 0,07 | 0,25 | 0,16 | 0,30 | 0,13 | 0,31 | 0,83 | 0,75 | 0,81 | 0,18 | 2,31 | 0,88 | 0,32 | 0,79 | 0,42 | 0,40 | | 12 |
| *Cupido argiades* | 0,87 | 0,22 | 0,39 | 0,05 | 0,02 | | 0,23 | 0,28 | 0,08 | 0,10 | 0,24 | | 0,95 | 0,18 | 1,31 | 0,36 | 0,15 | 0,19 | 0,45 | 0,36 | | 12 |
| *Cupido minimus* | | | | | | | | | | | | | | | | 0,02 | | | 0,05 | | | 2 |

| Combined habitat type | GMvT | GPsS | GPsT | GPvT | GPvVs | GRsS | GRsT | GRvT | GRvVs | MTiA | MTpA | N | Rg | Rgm | Rgmz | STeB | STpA | STpBA | STzS | STzT | TVTp | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of combined habitat type (m) | 549 | 501 | 381 | 2068 | 686 | 202 | 642 | 492 | 1246 | 1999 | 1243 | 50 | 448 | 476 | 287 | 2838 | 972 | 1057 | 1923 | 928 | 169 | Index of distribution |
| Species | Population density | | | | | | | | | | | | | | | | | | | | | |
| *Erynnis tages* | 0,09 | 0,07 | 0,26 | | 0,10 | | 0,05 | 0,07 | 0,03 | 0,03 | | | | | 0,17 | 0,21 | 0,02 | 0,27 | 0,29 | 0,45 | | 10 |
| *Glaucopsyche alexis* | | | 0,13 | | | | | | | | | | | | | 0,02 | | 0,05 | | | | 3 |
| *Gonepteryx rhamni* | 0,09 | 0,05 | 0,56 | 0,10 | 0,02 | 0,06 | 0,27 | 0,03 | 0,03 | 0,01 | 0,02 | | 0,03 | 0,03 | 0,13 | 0,04 | 0,03 | 0,09 | 0,14 | 0,08 | 0,15 | 12 |
| *Hesperia comma* | | | | | | | | | | | | | | | | 0,04 | | 0,14 | 0,07 | 0,05 | | 4 |
| *Heteropterus morpheus* | | | | 0,02 | | | | | | | | | | | | 0,04 | | 0,09 | 0,18 | 0,65 | | 4 |
| *Iphiclides podalirius* | | | 0,04 | 0,02 | | | | | | | | | | | | | | | 0,02 | | | 3 |
| *Issoria lathonia* | | | | | | | | | | 0,01 | | | | | | 0,02 | 0,03 | 0,02 | | 0,02 | | 5 |
| *Lasiommata megera* | 0,12 | | | | 0,02 | 0,08 | | | 0,05 | | | | | | | 0,01 | | | | | | 4 |
| *Leptidea sinapis/reali* | 0,11 | 0,20 | 0,45 | 0,16 | 0,16 | 0,20 | 0,34 | 0,22 | 0,26 | 0,19 | 0,39 | 0,40 | 0,04 | 0,04 | 0,21 | 0,22 | 0,22 | 0,33 | 0,58 | 0,74 | 0,53 | 12 |
| *Leptotes pirithous* | | | | | | | | | 0,08 | | | | | | | | | | | | | 1 |
| *Limenitis populi* | | | | | 0,07 | | | | | | | | | | | | | | | | | 1 |
| *Limenitis reducta* | | 0,03 | 0,31 | 0,01 | | | 0,05 | | | | | | | | | | | 0,02 | 0,01 | 0,05 | 0,10 | 5 |
| *Lycaena dispar* | | | | | | | | | | | | | 0,15 | | 0,06 | | 0,03 | | | | | 2 |
| *Lycaena hippothoe* | | | | | | | | | | | 0,04 | | | | | | | | | | | 1 |
| *Lycaena phlaeas* | | | | | | | 0,16 | | 0,08 | | | | | | | | | 0,09 | 0,10 | | | 4 |
| *Lycaena tityrus* | 0,03 | | | | | 0,17 | | | 0,01 | | 0,04 | | | | | 0,01 | 0,02 | 0,03 | 0,02 | 0,02 | | 6 |
| *Maniola jurtina* | 0,32 | 0,37 | 2,26 | 0,18 | | 2,72 | 1,21 | 0,10 | 0,10 | 0,66 | 0,30 | | | | 0,17 | 0,86 | 0,27 | 1,40 | 1,20 | 0,70 | 0,07 | 12 |
| *Melanargia galathea* | 0,05 | 0,50 | 0,39 | | | 3,47 | 4,91 | | | 0,26 | 0,04 | | | 0,11 | | 3,10 | 0,15 | 4,92 | 8,07 | 3,61 | | 9 |
| *Melitaea athalia* | 0,06 | | | 0,05 | 0,02 | | 0,31 | | 0,51 | 0,21 | 1,73 | | | | 0,41 | 0,38 | 0,82 | 0,65 | 0,09 | | 0,49 | 10 |
| *Melitaea aurelia* | | | 0,13 | | | 0,50 | 0,08 | | | | 0,10 | | | | | 1,39 | 0,05 | 0,19 | 0,59 | 0,38 | | 7 |
| *Melitaea britomartis* | | | | | | | | | | | | | | | | 0,07 | 0,05 | | 0,07 | | | 4 |
| *Melitaea cinxia* | | | | | | 0,12 | | | | | | | | | | 0,19 | | 0,19 | | | | 4 |
| *Melitaea didyma* | | | | | | | | | 0,01 | | | | | | | 0,11 | | 0,01 | | | | 3 |
| *Melitaea phoebe* | | | | 0,02 | | | 0,08 | | 0,02 | 0,04 | 0,22 | | | | 0,35 | 0,11 | 0,05 | 0,14 | | 0,03 | | 10 |
| *Minois dryas* | | 0,40 | 0,59 | 0,05 | | | 1,13 | | | | | 0,50 | | | | 0,24 | 0,03 | 0,26 | 2,65 | 2,45 | | 5 |
| *Ochlodes sylvanus* | 0,03 | 0,03 | 0,22 | 0,03 | 0,05 | 0,17 | 0,13 | | 0,17 | 0,10 | 0,12 | | | 0,04 | 0,06 | 0,04 | 0,10 | 0,17 | 0,10 | 0,07 | 0,69 | 12 |
| *Papilio machaon* | | | | | | | | | | 0,01 | 0,06 | | | | 0,09 | 0,02 | | 0,06 | 0,02 | | | 8 |
| *Pararge aegeria* | | | | | 0,15 | | | | | | | | | 0,21 | | | | | | | | 2 |
| *Pieris brassicae* | | | | 0,02 | | | 0,05 | | | 0,05 | | | | | | 0,03 | 0,03 | 0,03 | 0,02 | | | 8 |

| Combined habitat type | GMvT | GPsS | GPsT | GPvT | GPvVs | GRsS | GRsT | GRvT | GRvVs | MTiA | MTpA | N | Rg | Rgm | Rgmz | STeB | STpA | STpBA | STzS | STzT | TVTp | Index of distribution |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| Species | Population density | | | | | | | | | | | | | | | | | | | | | |
| *Pieris mannii* | | | | | | | | | | 0,05 | 0,12 | | | | | | | 0,05 | 0,03 | | | 5 |
| *Pieris napi* | 0,02 | 0,10 | 0,31 | 0,20 | 0,09 | 0,20 | 0,03 | 0,04 | 0,05 | 0,07 | 0,02 | | 0,02 | 0,29 | 0,42 | 0,00 | 0,01 | | 0,04 | 0,08 | | 12 |
| *Pieris rapae* | 0,22 | 0,36 | 0,39 | 0,13 | 0,28 | 0,59 | 0,56 | 0,26 | 0,14 | 0,36 | 0,47 | 0,40 | 1,05 | 0,32 | 0,38 | 0,54 | 0,27 | 0,43 | 0,22 | 0,22 | 0,12 | 12 |
| *Plebejus argus* | 0,20 | 0,12 | | 0,01 | | | 0,23 | 0,03 | 0,33 | 0,19 | 1,01 | | | | 0,04 | 0,23 | 0,84 | 0,88 | 0,44 | 0,32 | 0,15 | 10 |
| *Plebejus argyrognomon* | | | | | | | | | | | | | | | | 0,04 | | | | | | 1 |
| *Plebejus idas* | | | | | | | | | | | | | | | | 0,07 | | 0,05 | 0,16 | 0,05 | | 3 |
| *Polygonia c-album* | | 0,14 | 0,05 | 0,04 | | | | 0,02 | 0,02 | | | | | 0,06 | | | | | 0,03 | | | 7 |
| *Polyommatus bellargus* | | | | | | | | | | | | | | | | 0,02 | | | 0,05 | 0,16 | | 3 |
| *Polyommatus icarus* | 0,55 | 0,02 | | 0,02 | 0,04 | | 0,31 | 0,30 | 0,01 | 0,22 | 0,32 | | 0,59 | 0,18 | 1,22 | 0,71 | 0,37 | 0,53 | 0,57 | 0,61 | | 12 |
| *Pontia daplidice* | | | | | | | | | | | | | 0,39 | | | 0,03 | 0,03 | | | | | 3 |
| *Pyrgus malvae* | 0,11 | | | | | | | 0,05 | 0,01 | | | | | 0,03 | | 0,03 | 0,01 | 0,02 | 0,14 | 0,07 | | 8 |
| *Satyrium acaciae* | | | | 0,34 | | | 0,16 | | | | | | | | | 0,04 | | 0,28 | 0,31 | 0,43 | | 3 |
| *Satyrium pruni* | | 0,10 | 0,20 | | | | | | | | | | | | | | | | | 0,05 | | 3 |
| *Satyrium spini* | | | 0,66 | | | | 0,08 | | 0,02 | | | | | | | | | | | | | 2 |
| *Satyrium w-album* | | | 1,05 | 0,01 | | | 0,08 | | | | | | | | | | | | | | | 2 |
| *Thymelicus lineola* | 1,64 | | | 0,48 | | | | 2,03 | 0,16 | 0,40 | 0,48 | | | | 2,44 | 0,21 | 1,03 | 1,14 | 0,47 | 0,54 | | 9 |
| *Thymelicus sylvestris* | | | 0,26 | | | | 0,16 | | | | | | | | | | | | 0,16 | 0,32 | | 2 |
| *Vanessa atalanta* | | 0,04 | 0,08 | 0,00 | 0,03 | | | | 0,02 | | | | 0,07 | 0,08 | | 0,00 | 0,01 | | | 0,01 | 0,06 | 8 |
| *Vanessa cardui* | | | | 0,01 | | | 0,04 | | | | | | | 0,03 | | 0,00 | | 0,01 | | 0,01 | | 6 |
| *Zerynthia polyxena* | 0,05 | | | | | | 0,58 | | 0,02 | | 0,08 | | | | | 0,05 | 0,08 | 0,02 | 0,13 | 0,22 | | 7 |
| Number of species | 25 | 23 | 29 | 36 | 18 | 15 | 34 | 21 | 30 | 30 | 28 | 6 | 15 | 20 | 24 | 48 | 34 | 40 | 46 | 41 | 12 | |
| Total population density | 6,32 | 3,53 | 10,59 | 2,64 | 1,74 | 9,51 | 12,55 | 4,69 | 3,73 | 4,42 | 8,06 | 3,55 | 4,43 | 2,41 | 13,00 | 12,47 | 7,13 | 16,39 | 22,53 | 18,67 | 2,88 | |
| Median of population densities | 0,25 | 0,15 | 0.37 | 0,07 | 0,10 | 0,63 | 0,37 | 0,22 | 0,12 | 0,15 | 0,29 | 0,59 | 0,30 | 0,12 | 0,54 | 0,26 | 0,21 | 0,41 | 0,49 | 0,46 | 0,24 | |
| No. of species with maximum population density | 1 | 2 | 10 | 0 | 1 | 2 | 4 | 0 | 2 | 0 | 4 | 0 | 4 | 2 | 12 | 5 | 2 | 2 | 7 | 8 | 1 | |

Legend

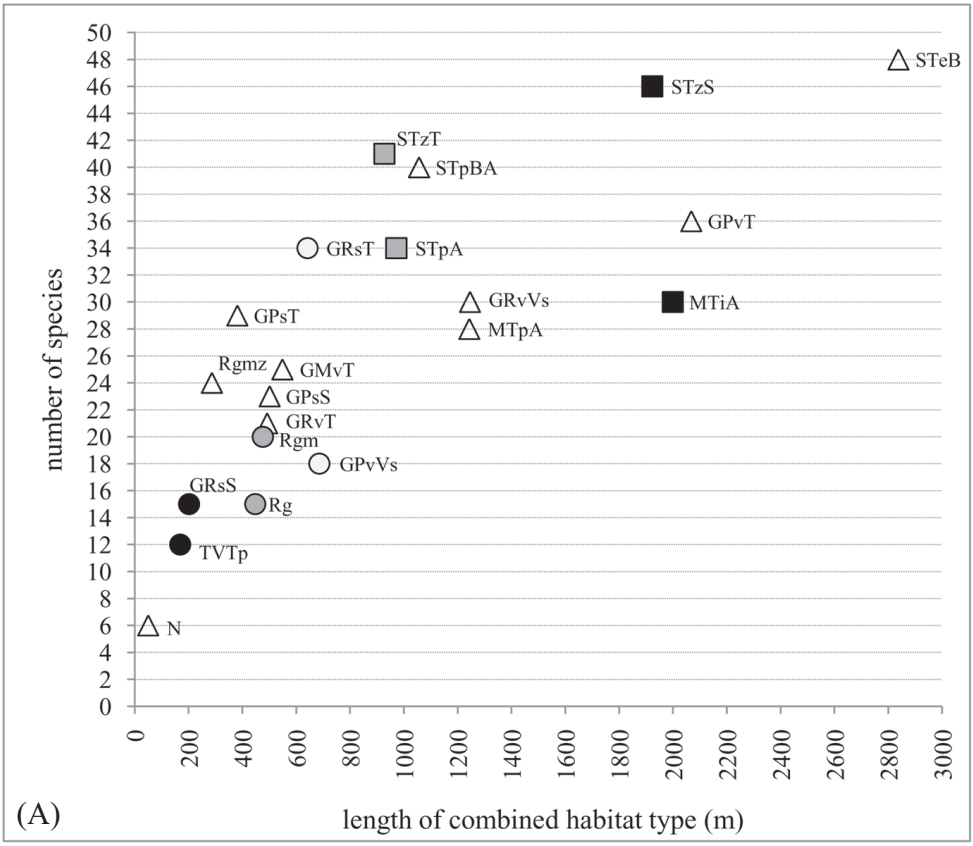
| | |
|---|---|
| GMvT | Small shrubs on moist ground; presence of allochtonous and autochtonous plant species |
| GPsS | Woodland ride on dry ground; autochtonous plant species predominate |
| GPsT | Woodland ride on dry ground; presence of allochtonous and autochtonous plant species |
| GPvT | Woodland ride on moist ground; presence of allochtonous and autochtonous plant species |
| GPvVs | Woodland ride with tall herb communities on moist ground; presence of allochtonous and autochtonous plant species |
| GRsS | Woodland edge on dry ground; autochtonous plant species predominate |
| GRsT | Woodland edge on dry ground; presence of allochtonous and autochtonous plant species |
| GRvT | Woodland edge on moist ground; presence of allochtonous and autochtonous plant species |
| GRvVs | Woodland edge with tall herb communities on moist ground; presence of allochtonous and autochtonous plant species |
| MTiA | Mesophilous mesotrophic and eutrophic grasslands with tall oatgrass (*Arrhenathereum elatius* L.); intensively managed |
| MTpA | Mesophilous mesotrophic and eutrophic grasslands with tall oatgrass (*Arrhenathereum elatius* L.); semi-intensively managed |
| N | Arable fields |
| Rg | Area with ruderal vegetation; mostly bare ground, only some herb species |
| Rgm | Area with ruderal vegetation; mostly shrub species |
| Rgmz | Area with ruderal vegetation; shrub and herb species |
| STeB | Dry and semi-dry grasslands with erect brome (*Bromopsis erecta* Huds.); extensively managed |
| STpA | Dry and semi-dry grasslands with tall oatgrass (*Arrhenathereum elatius* L.); semi-intensively managed |
| STpBA | Dry and semi-dry grasslands with erect brome (*Bromopsis erecta* Huds.) and tall oatgrass (*Arrhenathereum elatius* L.); semi-intensively managed |
| STzS | Abandoned dry and semi-dry grasslands; autochtonous shrub and tree species predominate |
| STzT | Abandoned dry and semi-dry grasslands; presence of allochtonous and autochtonous shrub and tree species |
| TVTp | Common reed bed along semi-intensively managed moist mesotrophic grassland |

that the type of vegetation or/and type of management in the combined habitat type are more important factors affecting species richness than the length of the combined habitat type. The influence of type of vegetation on the number of species in the combined habitat type is evident from the following comparisons: despite differences in length < 50 m, (i) dry woodland edges in which autochtonous plant species predominate (GRsS) are more species rich than common reed bed along semi-intensively managed moist mesotrophic grassland (TVTp) (Fig. 2a), and (ii) ruderal areas overgrown predominantly with shrubs (Rgm) are more species rich than mostly bare ruderal areas (Rg) (Fig. 2a), which are only a habitat for the thermoregulation of adults, and (iii) dry woodland edges (GRsT) are more species rich than woodland rides on moist ground overgrown with tall herb communities (GPvVs), among which predominate invasive allochthonous species that are not larval food plants or nectar plants of butterfly species that live in Slovenia (Fig. 2a); despite the shorter length (difference in length is more than 50 m; see Data analysis), (iv) ruderal areas overgrown with shrub and herb species (Rgmz) not differ in species richness from scrub (GMvT) and dry woodland rides (GPsS) (Fig. 2b), and (v) ruderal areas overgrown predominantly with shrub (Rgm) not differ in species richness from woodland rides on moist ground in which invasive allochthonous tall herb species predominate (GPvVs) (Fig. 2b), and (vi) dry woodland edges (GRsS) have the same number of species as bare ruderal

areas (Rg) (Fig 2b). That the management of the combined habitat type is a more important factor that influences species richness than its length is evident from the following comparisons: despite differences in length < 50 m, (i) abandoned dry grasslands in which autochtonous shrub and tree species predominate (STzS) are more species rich than intensively managed grasslands (MTiA) (Fig. 2a), and (ii) abandoned dry grasslands with approximately the same proportion of allochtonous and autochtonous shrub and tree species (STzT) are more species rich than semi-intensively managed grasslands with tall oat-grass (STpA) (Fig. 2a); despite the shorter length (difference in length is more than 50 m; see Data analysis), (iii) abandoned dry grasslands with approximately the same proportion of allochtonous and autochtonous shrub and tree species (STzT) not differ in species richness from semi-intensively managed dry grasslands with erect brome and tall oat-grass (STpBA) (Fig. 2b), and (iv) woodland edges (GRsT, GRvVs) and dry woodland rides (GPsT) not differ in species richness from semi-intensively and intensively managed grasslands with tall oat-grass (STpA, MTpA, MTiA) (Fig. 2b). Namely, woodland edges and dry woodland rides overgrown with autochthonous richly flowering species of herbs and shrubs are also feeding habitats of adults at the time when semi-intensively and intensively cultivated grasslands have already been mown.

*Total population density, median of population densities of species, maximum population density of species*

The total population density is not in correlation with the length of the combined habitat type



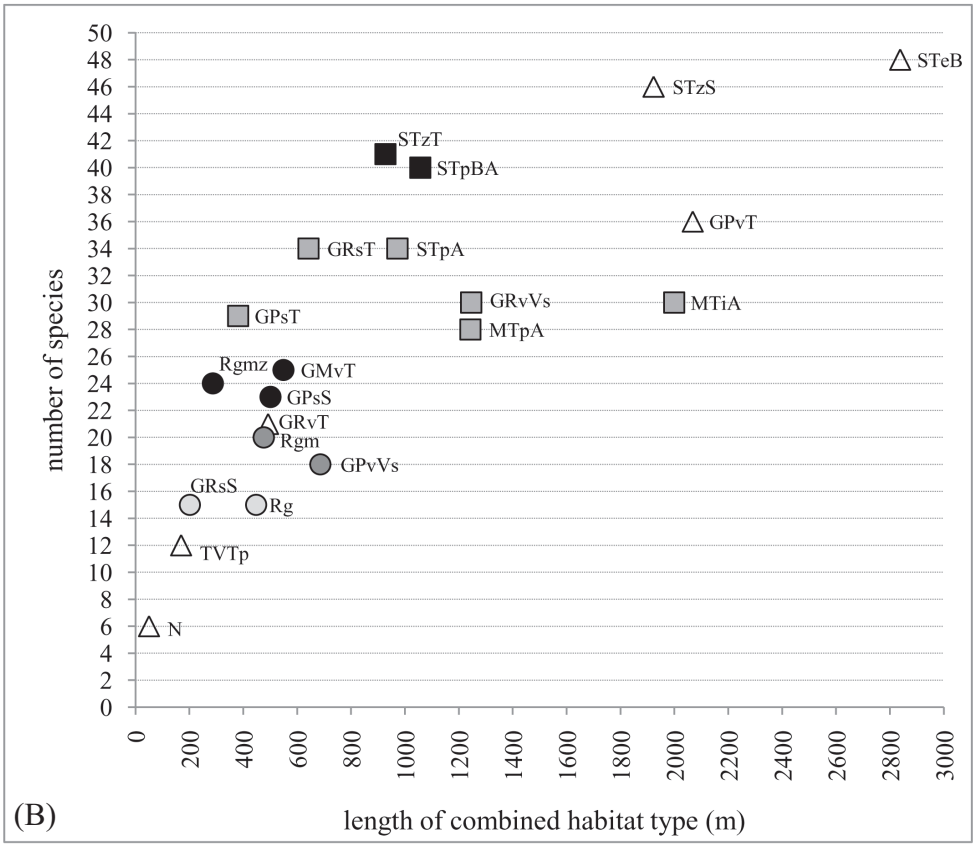



Figure 2: Species richness of the combined habitat type in relation to the length of the combined habitat type along the River Sava between Krško and the state border in 2008 (for abbreviations of the combined habitat types, see Tab. 1).
(A) – Comparison between combined habitat types that differ in length by less than 50 m and in the number of species by more than two. (B) – Comparison between combined habitat types that differ in length by more than 50 m and do not differ in terms of the number of species (for explanation, see Data analysis). ○ - combined habitat types that reflect the influence of type of vegetation, □ - combined habitat types that reflect the influence of management, △ - combined habitat types that are not included in comparison

Slika 2: Število vrst v zbirnih habitatnih tipih glede na dolžino zbirnega habitatnega tipa ob reki Savi med Krškim in državno mejo v letu 2008. (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)
(A) – Primerjava med zbirnimi habitatnimi tipi, ki se v dolžini razlikujejo za manj kot 50 m in v številu vrst za več kot dve. (B) – Primerjava med zbirnimi habitatnimi ttipi, ki se v dolžini razlikujejo za več kot 50 m in v številu vrst za manj kot 3 (za razlago glej poglavje Data analysis). ○ - zbirni habitatni tipi, ki odražajo vpliv tipa vegetacije, □ - zbirni habitatni tipi, ki odražajo vpliv načina gospodarjenja, △ - zbirni habitatni tipi, ki niso vključeni v primerjavo

($\tau = 0.124$, $P>0.4$), despite the fact that there is a strong positive correlation between the species richness and the length of the combined habitat type (see above) and a moderate positive correlation between the species richness and the total population density ($\tau = 0.446$, $P<0.01$). Concerning the finding that there is no correlation between the total population density and the length of combined habitat type, we can conclude that the combined habitat types with the

highest total population densities are abandoned dry and semi-dry grasslands (STzS: 22,53; STzT: 18,67), semi-intensively managed dry grasslands with erect brome and tall oat-grass (STpBA: 16,39), shrub and herb-overgrown ruderal areas (Rgmz: 13,00), dry woodland edges (GRsT: 12,55) and extensively managed dry grasslands (STeB: 12,47) (Tab. 1). In all other combined habitat types, the total population densities are at least twice (up to 13x in GPvVs) lower than in combined habitat type with the highest total population density (STzS).

Total population density of the combined habitat type is also affected by the population densities of species that inhabit it, since the weak positive correlation between total population density and median value of population densities of species that occupy the combined habitat type is significant ($\tau = 0.313$, $P<0.01$). There is also a significant moderate positive correlation between total population density and the number of species with maximum population density in the combined habitat type ($\tau = 0.536$, $P<0.05$) (Fig. 3). The actual values of Kendall's correlation coeficient show that the number of species with maximum population density in a combined habitat type is a more important factor influencing total population density than the species richness of the combined habitat type and population densities of species that inhabit it.

Generally widespread grassland species (*M. galathea*, *C. arcania*, *M. jurtina*, *M. dryas*, *T. lineola*, *C. crocea*, *M. athalia*, *C. pamphilus*) have the highest population densities in the study area (Fig. 4), which are also often found to be markedly more abundant in grassland habitats (grasslands, abandoned grasslands) elsewhere in Slovenia than other species (own observation); the species *M. dryas* is an exception. It is not widely distributed in Slovenia because it

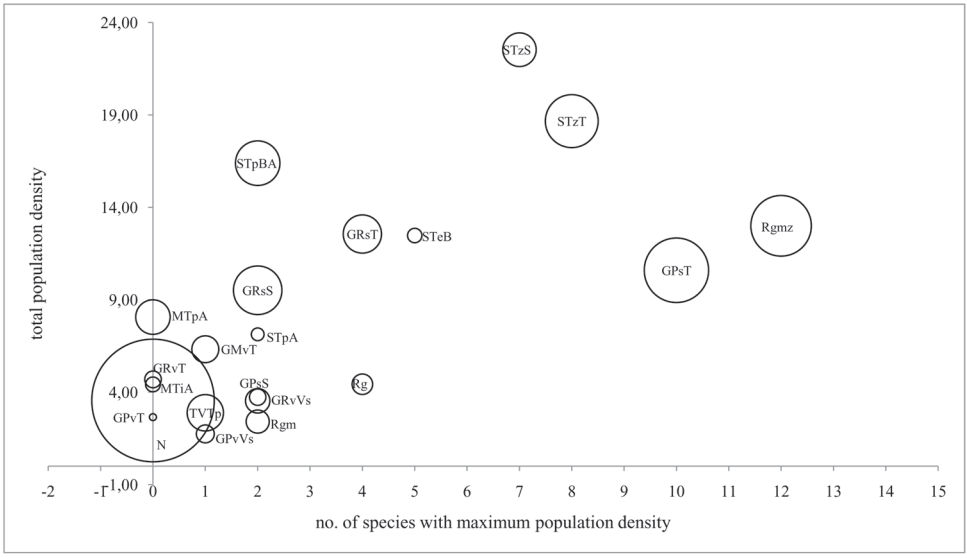



Figure 3: Total population density in relation to the number of species with the maximum population density in combined habitat type and the median value of population densities of species that inhabit combined habitat type in the study area along the River Sava between Krško and the state border in 2008 (median value of population densities of species is given by the diameter of the circle). (for abbreviations of the combined habitat types, see Tab. 1)

Slika 3: Celokupna populacijska gostota zbirnega habitatnega tipa glede na število vrst z maksimalno populacijsko gostoto v zbirnem habitatnem tipu in mediano vrednostjo populacijskih gostot vrst, ki poseljujejo zbirni habitatni tip za zbirne habitatne tipe ob reki Savi med Krškim in državno mejo v letu 2008 (mediano vrednost populacijskih gostot vrst v zbirnem habitatnem tipu določa premer kroga). (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)

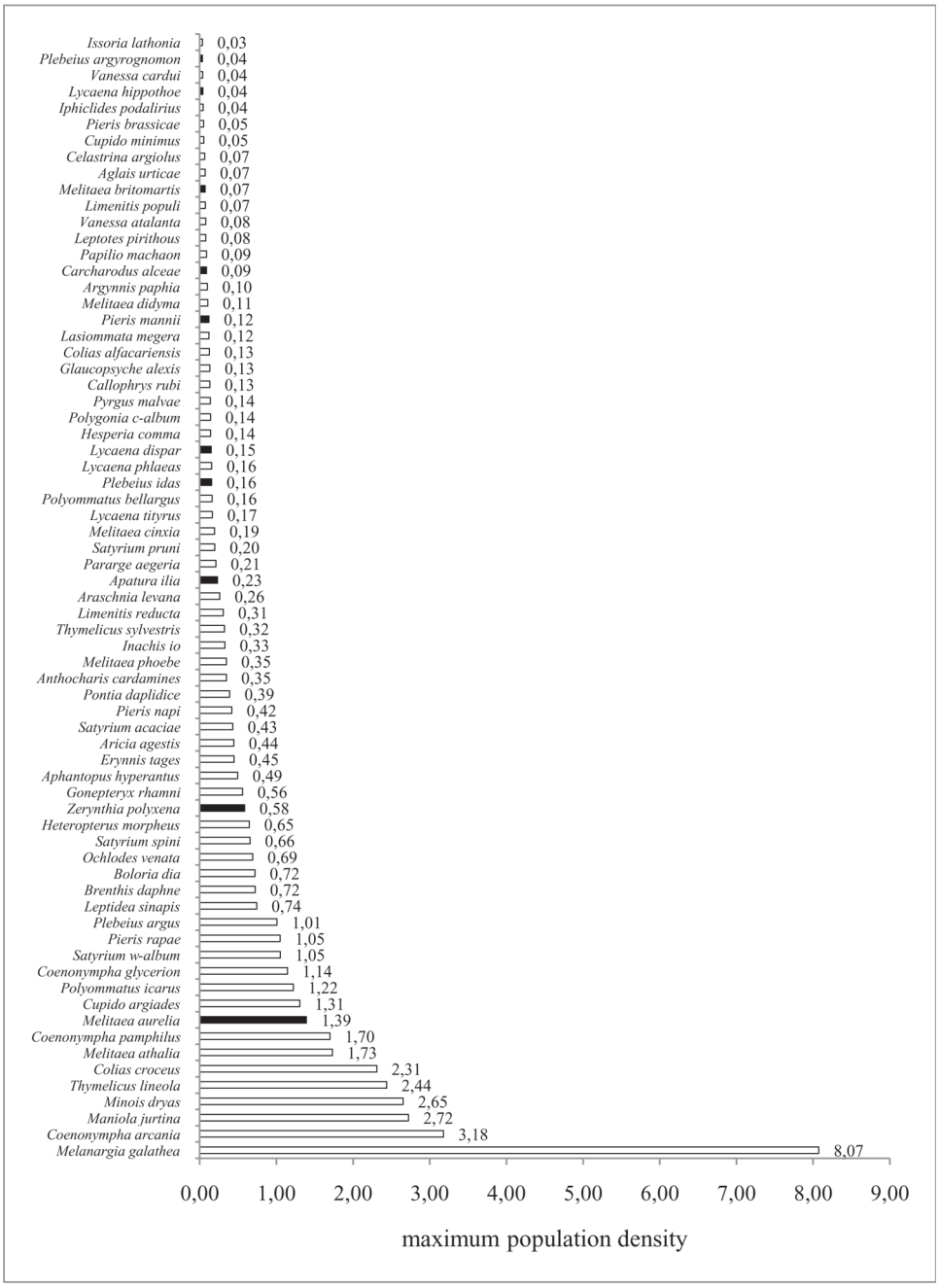



Figure 4: Comparison of maximum population densities of species recorded along the River Sava between Krško and the state border in 2008. The columns of threatened species are marked in black.

Slika 4: Primerjava maksimalnih populacijskih gostot vrst, zabeleženih ob reki Savi med Krškim in državno mejo v letu 2008. Črno obarvani stolpci označujejo ogrožene vrste.

mostly inhabits xerothermophilous grasslands. The caterpillars of the mentioned species feed on grasses (*Poaceae*), with the exception of the ubiquitous species *C. crocea*, of which the larval food plants are various low growing legume species (*Fabaceae*).

It can be concluded from comparison of maximum population densities the relation between the sizes of populations of recorded species. The maximum population density of the most abundant species, *M. galathea* e.g., is 2.5–5x greater than the maximum population density of other of the aforementioned grassland species and 50–250x greater than a third of the species with the lowest maximum population densities. In the first third of species (Fig. 4), two are threatened, *Melitaea aurelia* and *Zerynthia polyxena*, with maximum population densities of 14 and 6 adults/month/1 km of combined habitat type, which means that their populations are relatively abundant in comparison with other species. The finding that ecological generalists predominate in the first third of species, while the two threatened species are ecological specialists relying on dry or semi-dry abandoned and extensively or semi-intensively managed grasslands and dry forest edges, also indicates the importance of the study area for both mentioned species. Ecologically specialised species in the first third include also the monofagous species *Satyrium w-album*, whose caterpillars feed on the leaves of elm (*Ulmus* spp.). This is an arboreal species, which means that, because of the behaviour of the adults (staying in the crowns), it is difficult to detect in a transect counts. The high assessed maximum population density of the species indicates that the study area, primarily dry woodland rides, is important for maintain-

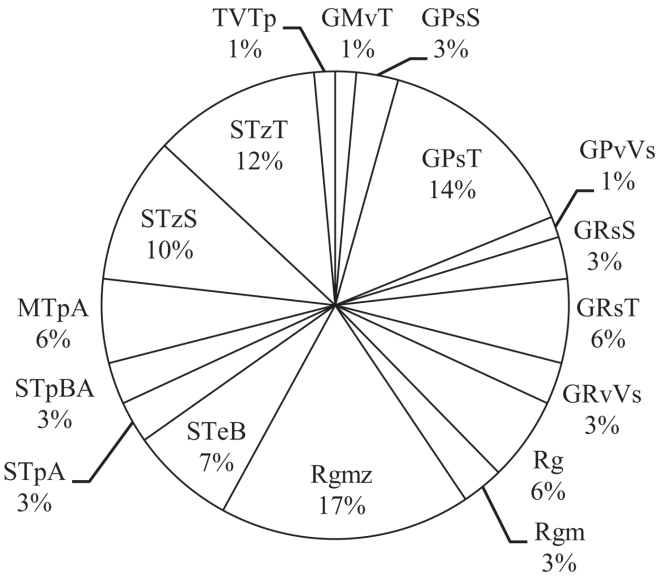



Figure 5: Distribution of the number of species with maximum population densities by combined habitat type for the species recorded along the River Sava between Krško and the state border in 2008. (for abbreviations of the combined habitat types, see Tab. 1)

Slika 5: Porazdelitev števila vrst z maksimalnimi populacijskimi gostotami po zbirnih habitatnih tipih za vrste zabeležene ob reki Savi med Krškim in državno mejo v letu 2008. (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)

ing its populations. The main reason for high number of observed adults on the dry woodland rides were the extensive stands of annual fleabane (*Erigeron annus*) serving as nectar plant. Three threatened species (*Apatura ilia*, *Plebejus idas*, *Lycaena dispar*), with maximum population densities of 1-2 adults/month/1 km of combined habitat type belong in the second third of recorded species, and 5 threatened species (*Pieris mannii*, *Carcharodus alceae*, *Melitaea britomartis*, *Lycaena hippothoe*, *Plebejus argyrognomon*), with maximum population densities ≤ 1 adult/month/1 km of combined habitat type in the last third (Fig. 4).

The number of species with maximum population density in combined habitat type is not in correlation with the length of the combined habitat type ($\tau = 0.015$, $P>0.9$). Considering this finding, the distribution of maximum population densities of species by combined habitat types (Fig. 5) directly indicates the importance of individual combined habitat types for the existence of populations of butterflies in the study area. The recorded species achieved the highest population densities in 17 combined habitat types. Four combined habitat types in which none of the recorded species had the highest density are intensively cultivated grasslands, arable fields and woodland edges and rides on moist ground (Tab. 1). On dry grasslands (extensive, semi-intensive, abandoned) 24 (35%) species had highest population densities, 18 (26 %) species in ruderal communities, 13 (18 %) species on woodland rides, 8 (12 %) species on woodland edges, 4 (6 %) of species on mesophilous semi-intensively managed grasslands and one species each on common reed bed along semi-intensively managed moist mesotrophic grassland, on damp scrub and on woodland rides on moist ground overgrown with riverine shrubs and tall herb communities (Fig. 5).

*Threatened species*

We recorded 10 threatened species in the study area in 2008 (Tab. 2), which is 18% of the threatened butterfly species in Slovenia. The species *Lycaena dispar* and *Zerynthia polyxena* are on the list of Annexes of the Habitats Directive and the Bern Convention, which include 14 (Habitats Directive) and 13 (Bern Convention) of the butterfly species of Slovenia; on the Red List of the Lepidoptera of Slovenia, they are classified in the category of vulnerable species (36 butterfly species); they are among protected animal species of Slovenia, whereby the animals (27 species of butterfly) and their habitats (26 species of butterfly) are protected. The remaining eight threatened species on the Red List of the Lepidoptera of Slovenia are in the category of vulnerable species. Two (*Melitaea aurelia*, *M. britomartis*) of them are on the European Red List of Butterflies in the category of potentially threatened species (NT) and the remaining six among species for which there is a low risk of extinction (LC).

The index of distribution indicates (Tab. 2) that 6 threatened species (*A. ilia*, *C. alceae*, *L. dispar*, *L. hippothoe*, *P. argyrognomon*, *P. idas*) are rare or very locally distributed in the study area. Threatened species occupy 17 combined habitat types (Fig. 6). Four combined habitat types in which none of the threatened species were recorded are arable fields, common red bed, woodland rides on moist ground overgrown with riverine shrubs and tall herb communities and dry woodland rides.

The number of threatened species in combined habitat type is in significant positive correlation with the length of the combine habitat type ($\tau = 0.406$, $P<0.05$), the species richness of the combined habitat type ($\tau = 0.566$, $P<0.01$), total population density ($\tau = 0.645$, $P<0.001$) and the number of species with the maximum population density in the combined habitat type ($\tau = 0.479$, $P<0.01$). The number of threatened species with maximum population densities in combined habitat type is not in correlation with the length of the combine habitat type ($\tau = 0.158$, $P>0.4$) or the species richness of the combined habitat type ($\tau = 0.214$, $P>0.2$).

Seven threatened species were recorded on extensively managed dry grasslands (STeB) and on abandoned dry grasslands with tall oatgrass overgrown with autochthonous woody species (STzS). These are xerothermophilous or mesophilous thermophilous species: *C. alceae*, *M. aurelia*, *M. britomartis*, *P. mannii*, *P. argyrognomon*, *P. idas* and *Z. polyxena*. Four xerothermophilous species are the most abundant in these habitats (Tab. 2). The species ***M. aurelia***

Table 2: List of threatened species recorded in transect lines along the River Sava between Krško and the state border in 2008. Threat categories, number of habitat types in which species appeared, index of distribution of species, maximum population density of species and combined habitat types in which a species achieves maximum population density are shown. (for abbreviations of the combined habitat types, see Tab. 1)

Tabela 2: Seznam ogroženih vrst, zabeleženih na transektih ob reki Savi med Krškim in državno mejo v letu 2008. Prikazane so kategorije ogroženosti vrst, število zbirnih habitatnih tipov, v katerih je bila vrsta opažena, indeks razširjenosti vrst, maksimalna populacijska gostota vrst ter zbirni habitatni tip, v katerem vrsta dosega maksimalno populacijsko gostoto. (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)

| Species | [1] RS | [2] RSE | [3] UZŽV | [4] FFH | [5] BERN | No. of [6] HT | Index of distribution | Maximum population density | HT in which a species achieves maximum population density |
|---|---|---|---|---|---|---|---|---|---|
| *Apatura ilia* | V | LC | | | | 5 | 3 | 0,23 | Rgmz |
| *Carcharodus alceae* | V | LC | | | | 3 | 3 | 0,09 | Rgmz |
| *Lycaena dispar* | V | LC | 1, 2 | II, IV | II | 3 | 2 | 0,15 | Rg |
| *Lycaena hippothoe* | V | LC | | | | 1 | 1 | 0,04 | MTpA |
| *Melitaea aurelia* | V | NT | | | | 9 | 7 | 1,39 | STeB |
| *Melitaea britomartis* | V | NT | | | | 3 | 4 | 0,07 | STeB |
| *Pieris mannii* | V | LC | | | | 4 | 5 | 0,12 | MTpA |
| *Plebejus argyrognomon* | V | LC | | | | 1 | 1 | 0,04 | STeB |
| *Plebejus idas* | V | LC | | | | 4 | 3 | 0,16 | STzS |
| *Zerynthia polyxena* | V | LC | 1, 2 | IV | II | 9 | 7 | 0,58 | GRsT |

[1] RS = Red list of Lepidoptera of Slovenia (Uradni list RS 82/2002)
[2] RSE = European Red list of Butterflies (Van Swaay s sod. 2010)
[3] UZŽV = Decree on protected wild animal species (Uradni list RS 46/2004)
[4] FFH = Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora
[5] BERN = Convention on the Conservation of European Wildlife and Natural Habitats
[6] HT = combine habitat type

has the most numerous populations on extensive, semi-intensive and abandoned dry grasslands with erect brome and dry woodland edges, where autochthonous plant species predominate. The food plants of the caterpillars are ribwort plantain (*Plantago lanceolata*), various species of speedwell (*Veronica* spp.) and cow-wheat (*Melampyrum* spp.) (Beneš and Konvička 2002a). In the northern part of the study area (northeast of the settlement of Brege, southwest of the settlement of Zgornji Obrež), where there are extensive mosaics of extensive and abandoned dry grasslands in early succession stages, there are very good living conditions for the species, so their populations are relatively numerous in comparison with other butterfly species (Fig. 4). The species ***M. britomartis*** appears mainly on extensive and abandoned dry grasslands with erect brome. The food plants of the caterpillars are large speedwell (*Veronica teucrium*), yellow rattle (*Rhinantus minor*) (Ebert and Rennwald 1993a, Beneš and Konvička 2002b) and ribwort plantain (*Plantago*

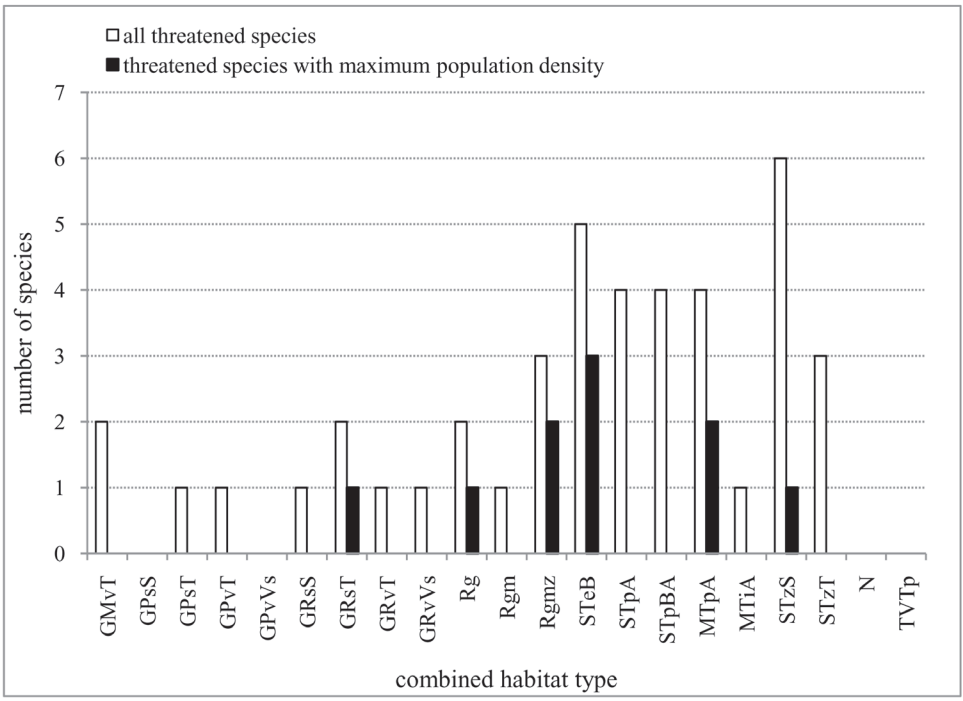



Figure 6: Importance of combined habitat types for threatened species recorded in transect lines along the River Sava between Krško and the state border in 2008. Combined habitat types inhabited by threatened species (white columns) and combined habitat types in which threatened species have maximum population densities (black columns) are shown. (for abbreviations of the combined habitat types, see Tab. 1)

Slika 6: Število ogroženih vrst po zbirnih habitatnih tipih v transektnih linijah ob reki Savi med Krškim in državno mejo v letu 2008. Belo obarvani stolpci označujejo zbirne habitatne tipe, ki jih poseljujejo ogrožene vrste, črno obarvani stolpci pa zbirne habitatne tipe v katerih imajo ogrožene vrste maksimalne populacijske gostote. (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)

*lanceolata*) (SBN 1991, Tolman and Lewington 1997). The species ***P. argyrognomon*** was recorded in 2008 in only one location, in the southern part of the area, south of the settlement of Mihalovec, on extensive dry grassland with erect brome. The food plants of the caterpillars are crown vetch (*Coronilla varia*) and liquorice milkvetch (*Astragalus glycyphyllos*) (Ebert and Rennwald 1993b, Beneš 2002a). In research in 2001 (Rebeušek 2001), the species was found only in the northern part of the area, on the right bank of the Sava, east of the settlement of Brege. The species ***P. idas*** appears on extensive, semi-intensive and abandoned dry grasslands with erect brome. The caterpillars feed on various species of the legume family (Fabaceae), above all black medic (*Medicago lupulina*), red clover (*Trifolium pratense*), silky leaf woadwaxen (*Genista pilosa*), bird's-foot trefoil (*Lotus corniculatus*), honey clover (*Melilotus albus*), common kidney vetch (*Anthyllis vulneraria*), common broom (*Sarothamnus scoparius*) (Beneš 2002b), and common sea-buckthorn (*Hippophaë rhamnoides*) from the oleaster family (Elaeagnaceae) (Huemer 2004).

On semi-intensively managed grasslands seven threatened species were recorded, 2 of which achieve maximum population density on mesophilous grasslands (MTpA) (Tab. 2). The hygromesophilous species ***L. hippothoe*** was only recorded on mesophilous grassland with tall oat-grass south of the settlement of Loče.

The food plants of the caterpillars are common sorrel (*Rumex acetosa*) (Ebert and Rennwald 1993b, Weidenhoffer and Fric 2002), sheep's sorrel (*R. acetosella*) and bistort (*Polygonum bistorta*) (SBN 1991). The xerothermophilous species ***P. mannii*** appears on semi-intensive and abandoned dry grasslands and on mesophilous semi-intensive grasslands – such as are a mosaic of central European xerophilous lowland grasslands on relatively dry soils with erect brome and intensively cultivated grasslands. The caterpillars feed on garlic cress (*Peltaria alliacea*), candytuft species (*Iberis* spp.) as well as other species from the crucifer family (Brassicaceae) (SBN 1991, Beneš 2002c, Huemer 2004).

Ruderal areas are inhabited by 3 threatened species, which also achieve maximum population density in these habitats (Tab. 2). The mesophilous species ***A. ilia*** lives on light rides of riverine forests and on abandoned ruderal areas near the woody riverine vegetation. The food plants of the caterpillars are aspen (*Populus tremula*), black (*P. nigra*) and white (*P. alba*) poplar and willow, above all goat willow (*Salix caprea*) and white willow (*S. alba*) (Ebert and Rennwald 1993a, Vrabec 2002, Huemer 2004). The xerothermophilous species ***C. alceae*** inhabits abandoned dry grasslands and ruderal areas that are overgrown with shrubs and herb species, among which are the food plants of caterpillars. These are species from the mallow genus, mainly greater musk-mallow (*Malvea alcea*), musk mallow (*M. moschata*) and common mallow (*M. neglecta*) and common hollyhock (*Alcea rosea*) (Ebert and Rennwald 1993b, Beneš 2002d). The hygrophilous species ***L. dispar*** appears mainly in ruderal areas that are either predominantly bare and only partially overgrown with tall herbs or overgrown with shrubs and herbs, and on semi-intensively managed dry grasslands with tall oat-grass. The food plants of the caterpillars are water dock (*Rumex hydrolapathum*), Scottish dock (*R. aquaticus*), broad-leaved dock (*R. obtusifolius*), curled dock (*R. crispus*) and common sorrel (*R. acetosa*) (Ebert and Rennwald 1993b; SBN 1991).

On woodland edges three threatened species were recorded, the species ***Z. polyxena*** has the most numerous populations in these habitats (Tab. 2). It inhabits sunny woodland edges on both dry and moist ground, abandoned dry grasslands, semi-intensive mesophilous grasslands and extensive or semi-intensive dry grasslands, in which there are sunny hedges, which are favourable habitats for the food plants of the caterpillars - birthwort (*Aristolochia clematitis*).

*Important areas for butterflies*

The conservation evaluation of each combined habitat type based on the ranking of the combined habitat types according to six criteria (i.e. species richness, median of population densities of species observed in the treated combined habitat type, total population density, number of species with maximum population density in the treated combined habitat type, number of threatened species in the combined habitat type, number of threatened species with maximum population density in the treated combined habitat type) shows (Fig. 7) that 8 combined habitat types have median of ranks lower than 10,5. These combined habitat types are defined as most important for butterfly species in the study area. The difference in ranks between the combined habitat types with median of ranks lower than 10,5 and combined habitat with median of ranks higher than 10,5 is highly significant (Mann–Whitney $Z = -7.34$; $P<0.001$). The most important combined habitat types for butterflies in the study area are extensively managed dry grasslands (STeB), abandoned dry grasslands in early succession stages (STzS, STzT), semi-intensively managed dry grasslands with erect brome (*Bromopsis erecta*) and tall oatgrass (*Arrhenathereum elatius*) (STpBA), semi-intensively managed mesophilous grasslands with tall oatgrass (MTpA), ruderal areas overgrown with shrub and herb species (Rgmz) and dry woodland rides and edges in which allochtonous and autochtonous plant species are present (GPsT, GRsT).

On the basis of the distribution of the most important combined habitat types we identified four important areas for butterfly species in the study area: (1) Žadovinek–Brege, (2) Čateške Toplice–Prilipe, (3) Loče–Mihalovec and (4) Zgornji Obrež (Fig. 8). The number of species in important areas ranges between 57% (Loče–Mihalovec) to 84% (Žadovinek–Brege) of the species richness of the study area (Tab. 3). Considering the maximum population densities of

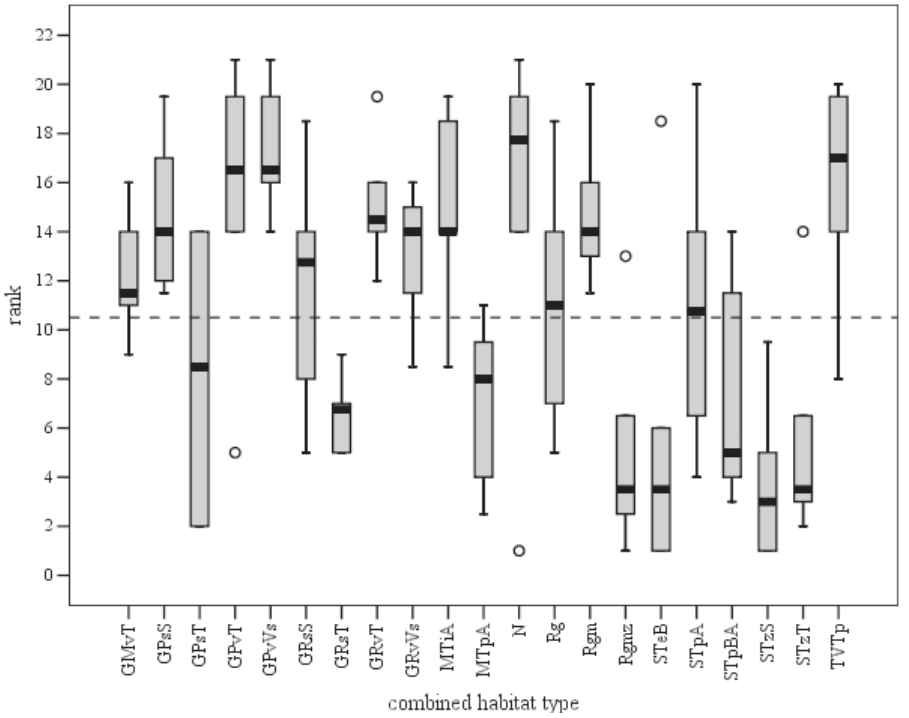



Figure 7: Nature conservation value of each combined habitat type in the study area along the River Sava between Krško and the state border, based on the ranking of the combined habitat types according to six criteria. (for explanation, see Data analysis; for abbreviations of the combined habitat types, see Tab. 1)

Slika 7: Naravovarstvena vrednost zbirnih habitatnih tipov v raziskovanem območju ob reki Savi med Krškim in državno mejo, ocenjena z rangiranjem zbirnih habitatnih tipov po šest kriterijih (za razlago glej poglavje Data analysis). (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)

threatened species, the area Žadovinek–Brege is most important for two species (*M. aurelia*, *P. idas*), the area Čateške Toplice–Prilipe for four species (*A. ilia*, *C. alceae*, *P. mannii*, *L. dispar*), the area Loče–Mihalovec for two species (*L. hippothoe*, *P. argygnomon*), and the area Zgornji Obrež also for two species (*M. britomartis*, *Z. polyxena*) (Tab. 3).

Designated areas for butterflies are important (i) for preserving high butterfly species richness in the mainly intensively used agricultural landscape along the lower course of the River Sava between Krško and the state border with Croatia, and (ii) for preserving viable populations of threatened and other ecologically specialised, rare or locally distributed species (Tab. 3) and preventing their isolation in the region of sub-pannonian southeast Slovenia.

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Table 3: Important areas for butterfly species along the River Sava between Krško and the state border with Croatia. Species richness, number of threatened species, population densities and combined habitat types of threatened species, and other rare/local distributed species or ecological specialists are shown for each important area. Bold text indicates the maximum population density of threatened species in the important area. (for abbreviations of the combined habitat types, see Tab. 1)

Tabela 3: Vrstno bogastvo, število ogroženih vrst, populacijske gostote in zbirni habitatni tipi ogroženih vrst ter druge redke/lokalno razširjene/ekološko specializirane vrste v naravovarstveno pomembnih območjih za dnevne metulje ob reki Savi med Krškim in državno mejo s Hrvaško. S krepkim tiskom je označena maksimalna populacijska gostota ogrožene vrste v območju. (za razlago okrajšav zbirnih habitatnih tipov glej Tab. 1)

| Name of important area | No. of species | No. of threatened species | Threatened species | Population density | Combined habitat type | Rare / local distrubuted species / ecological specialists |
|---|---|---|---|---|---|---|
| Žadovinek – Brege (3.9 km$^2$) | 58 | 6 | *Carcharodus alceae* | **0,02** | STzS | *Iphiclides podalirius, Melitaea didyma, Satyrium pruni, S. acaciae, Glaucopsyche alexis, Polyommatus bellargus, Aphantopus hyperantus, Heteropterus morpheus* |
| | | | *Melitaea aurelia* | **1,70** | STeB | |
| | | | *Melitaea aurelia* | 0,50 | GRsS | |
| | | | *Melitaea aurelia* | 0,26 | STzS | |
| | | | *Melitaea aurelia* | 0,09 | STzT | |
| | | | *Melitaea aurelia* | 0,07 | STpBA | |
| | | | *Melitaea britomartis* | **0,06** | STeB | |
| | | | *Pieris mannii* | **0,03** | STzS | |
| | | | *Plebeius idas* | **0,21** | STzS | |
| | | | *Plebeius idas* | 0,09 | STzT | |
| | | | *Plebeius idas* | 0,09 | STeB | |
| | | | *Plebeius idas* | 0,07 | STpBA | |
| | | | *Zerynthia polyxena* | **0,31** | STzT | |
| | | | *Zerynthia polyxena* | 0,05 | STeB | |
| Čateške Toplice - Prilipe (1.2 km$^2$) | 48 | 7 | *Apatura ilia* | **0,23** | Rgmz | *Iphiclides podalirius, Melitaea didyma, Satyrium spini, S. w-album, S. acaciae* |
| | | | *Apatura ilia* | 0,15 | GPvT | |
| | | | *Apatura ilia* | 0,14 | GRvT | |
| | | | *Carcharodus alceae* | **0,09** | Rgmz | |
| | | | *Carcharodus alceae* | 0,06 | GMvT | |
| | | | *Lycaena dispar* | **0,07** | STpA | |
| | | | *Lycaena dispar* | 0,06 | Rgmz | |
| | | | *Melitaea aurelia* | **0,48** | STpBA | |
| | | | *Melitaea aurelia* | 0,26 | MTpA | |
| | | | *Melitaea aurelia* | 0,10 | STpA | |
| | | | *Melitaea britomartis* | **0,10** | STpA | |
| | | | *Pieris mannii* | **0,16** | STpBA | |
| | | | *Pieris mannii* | 0,11 | MTpA | |
| | | | *Zerynthia polyxena* | **0,16** | MTpA | |
| | | | *Zerynthia polyxena* | 0,15 | STpA | |
| | | | *Zerynthia polyxena* | 0,08 | STpBA | |
| | | | *Zerynthia polyxena* | 0,06 | GMvT | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Loče - Mihalovec (1.1 km$^2$) | 39 | 6 | *Lycaena dispar* | **0,02** | MTpA | *Limenitis populi, Melitaea didyma* |
| | | | *Lycaena hippothoe* | **0,07** | MTpA | |
| | | | *Melitaea britomartis* | **0,10** | STeB | |
| | | | *Pieris mannii* | **0,13** | MTpA | |
| | | | *Plebeius argyrognomon* | **0,19** | STeB | |
| | | | *Zerynthia polyxena* | **0,05** | STeB | |
| | | | *Zerynthia polyxena* | 0,03 | MTpA | |
| | | | *Zerynthia polyxena* | 0,03 | GRvVs | |
| Zgornji Obrež (0.4 km$^2$) | 49 | 3 | *Melitaea aurelia* | **1,54** | STzS | *Iphiclides podalirius, Satyrium pruni, S. spini, S. w-album, Polyommatus bellargus, Aphantopus hyperantus, Heteropterus morpheus* |
| | | | *Melitaea aurelia* | 0,84 | STzT | |
| | | | *Melitaea aurelia* | 0,17 | GPsT | |
| | | | *Melitaea aurelia* | 0,08 | GRsT | |
| | | | *Melitaea britomartis* | **0,26** | STzS | |
| | | | *Zerynthia polyxena* | **0,58** | GRsT | |
| | | | *Zerynthia polyxena* | 0,51 | STzS | |
| | | | *Zerynthia polyxena* | 0,07 | STzT | |

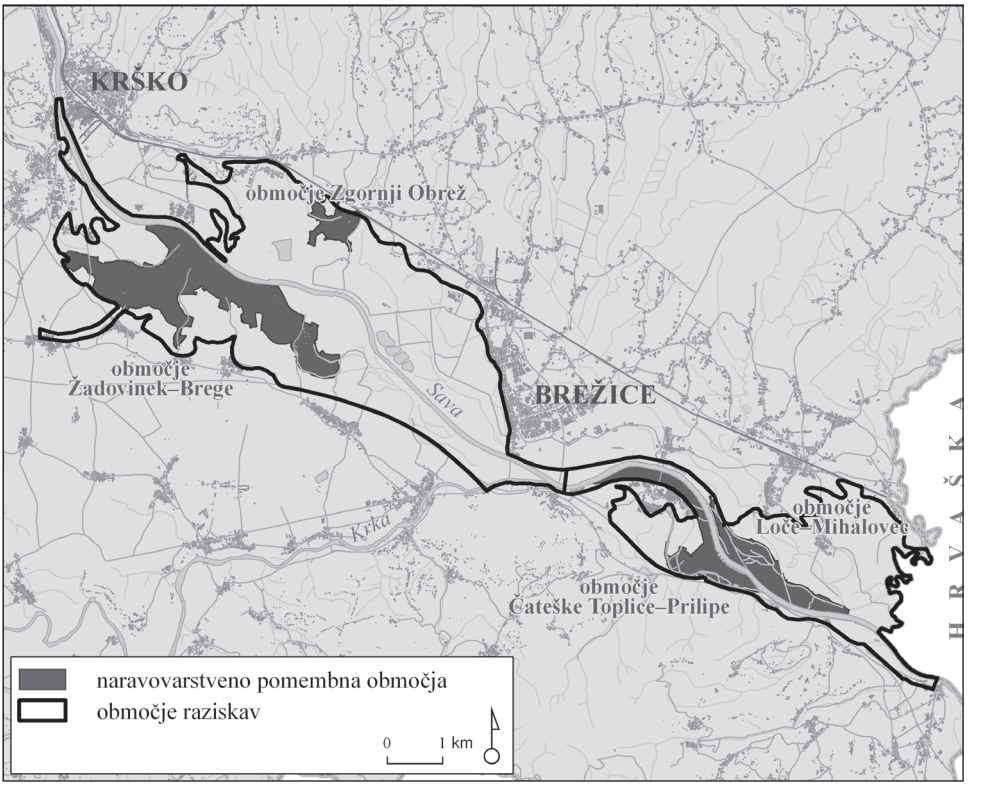


Figure 8: Important areas for butterfly species in the study area along the lower course of the River Sava between Krško and the state border with Croatia.

Slika 8: Naravovarstveno pomembna območja za dnevne metulje v raziskovanem območju ob spodnjem toku reke Save med Krškim in državno mejo s Hrvaško.

## Povzetek

Dnevni metulji so ena izmed najpomembnejših nevretenčarskih bioindikatorskih skupin. So edini nevretenčarski takson za katerega je trenutno možno oceniti stopnjo upadanja številčnosti populacij in so reprezentativni indikatorji trendov opaženih pri večini kopenskih žuželk, ki predstavljajo približno dve tretjini vseh svetovnih vrst. Evropska okoljska agencija jih je, z namenom zmanjšanja upadanje biodiverzitete do leta 2010, v okviru projekta »**S**treamlining **E**uropean 2010 **B**iodiversity **I**ndicators« leta 2007 uvrstila med evropske biodiverzitetne indikatorje (SEBI 2010 Indicators), s katerimi se vrednoti splošne trende v stanju biodiverzitete v Evropi. Dnevni metulji so bili izbrani kot indikatorji za oceno stanja in trendov evropskih travišč (European Grassland Butterfly Indicator), ki so najpomembnejši habitati evropskih vrst dnevnih metuljev, saj jih poseljuje več kot polovica (280 vrst, 57%) evropskih vrst.

V južnem delu Ekološko pomembnega območja »Sava od Radeč do državne meje« (EPO Id: 63700), med Krškim in državno mejo s Hrvaško, smo v letu 2008 izvedli inventarizacijo favne dnevnih metuljev z namenom opredeliti naravovarstveno najpomembnejša območja za dnevne metulje v EPO. Raziskovano območje s površino 33 km$^2$ je obsegalo približno 1 km širok pas na obeh bregovih reke Save. Območje je pretežno kmetijska krajina, saj 66 % površine zavzemajo njive in različno gospodarjeni travniki. Prevladujejo njive in intenzivno gospodarjeni travniki (46 % površine raziskovanega območja), medtem ko ekstenzivno gospodarjeni suhi travniki in opuščena suha travišča v zgodnjih fazah zaraščanja zavzemajo le 7 % površine območja. Predvsem na vlažnih rastiščih (npr. gozdni robovi in poti, obrežja reke, gramoznic in mrtvic), so priostne invazivne alohtone rastlinske vrste, ki kot samostojni sestoji predstavljajo kar 2 % površine območja, v kombinaciji z drugimi habitatnimi tipi po so razširjene na veliko večji površini.

Favno dnevnih metuljev smo inventarizirali s standardizirano metodo transektnega popisa na 12 transektih. Na vsakem transektu smo šteli odrasle osebke v časovnem intervalu pribl. 14 dni (1x v aprilu, 2x v maju, 1x v juniju, 2x v juliju, 1x v septembru). Vsak transekt je bil razdeljen na odseke, vsak odsek je predstavljal določen zbirni habitatni tip. Na transektih s skupno dolžino 19,2 km smo opredelili 21 zbirnih habitatnih tipov. Z indeksom razširjenosti smo identificirali splošno razširjene ter redke/lokalno razširjene vrste v raziskovanem območju. V procesu opredelitve naravovarstveno pomembnih območij za dnevne metulje smo najprej ovrednotili zbirne habitatne tipe v transektih. Za določitev naravovarstvene vrednosti vsakega zbirnega habitatnega tipa smo uporabili naslednje parametre: vrstno bogastvo zbirnega habitatnega tipa, populacijska gostota vrste v zbirnem habitatnem tipu, celokupna populacijska gostota v zbirnem habitatnem tipu, število vrst z maksimalno populacijsko gostoto v zbirnem habitatnem tipu, število ogroženih vrst v zbirnem habitatnem tipu in število ogroženih vrst z maksimalmo populacijsko gostoto v zbirnem habitatnem tipu. S Kendallovim korelacijskim koeficientom smo ugotavljali povezanost med uporabljenimi parametri. Parametre smo uporabili kot kriterije v nadaljnem postopku evalvacije tako, da smo v okviru vsakega kriterija (parametra) zbirne habitatne tipe rangirali (od 1 do 21) glede na vrednost parametra. Rang 1 smo pripisali zbirnemu habitatnemu tipu z največjo in rang 21 (v primeru različnih rangov) zbirnemu habitatnemu tipu z najmanjšo vrednostjo parametra. Zbirne habitatne tipe z mediano vrednostjo rangov nižjo od 10, 5 smo definirali kot najpomembnejše za dnevne metulje. Naravovarstveno pomembna območja za dnevne metulje smo nato opredelili na podlagi razširjenosti najpomembnejših zbirnih habitatnih tipov v raziskovanem območju, pri čemer smo vključili tudi habitatne tipe (podobne po floristični sestavi in strukturi vegetacije ter gospodarjenju najpomembnejšim zbirnim habitatnim tipom) izven transektnih linij.

Zabeležili smo 69 vrst dnevnih metuljev (38 % favne dnevnih metuljev Slovenije), med njimi 10 v nacionalnem ali evropskem merilu ogroženih vrst (*A. ilia*, *C. alceae*, *L. dispar*, *L. hippothoe*, *M. aurelia*, *M. britomartis*, *P. manni*, *P. argyrognomon*, *P. idas*, *Z. polyxena*). 19 vrst je splošno razširjenih (ekološko nespecializirane vrste), 21 vrst je redkih/lokalno razširjenih, med njimi je 6 ogroženih vrst. Vrstno najbogatejši zbirni habitatni tipi so ekstenzivno gospodarjeni suhi travniki (48 vrst), opuščena suha travišča

(46 oz. 41 vrst), polintenzivno gospodarjeni suhi travniki (40 vrst) in vlažne gozdne poti (36 vrst). Registrirane vrste imajo največje populacijske gostote na ekstenzivnih, polintenzivnih in zaraščajočih se suhih travnikih (24 vrst), na ruderalnih površinah (18 vrst), na gozdnih poteh (13 vrst) in gozdnih robovih (8 vrst). Na intenzivno gospodarjenih travnikih, njivah ter vlažnih gozdnih robovih in poteh nobena vrsta nima najštevilčnejših populacij. Največje populacijske gostote imajo splošno razširjene travniške vrste (*M. galathea*, *C. arcania*, *M. jurtina*, *M. dryas*, *T. lineola*, *C. crocea*, *M. athalia*, *C. pamphilus*). Primerjava maksimalnih populacijskih gostot med vrstami kaže, da v raziskovanem območju živijo pomembne populacije dveh ogroženih vrst, *M. aurelia* in *Z. polyxena*, ter ekološko specializirane vrste *S. w-album*. Ogrožene vrste imajo največje populacijske gostote na ekstenzivnih in zaraščajočih se suhih travnikih, mezofilnih polintenzivno gospodarjenih travnikih, ruderalnih združbah in na suhih gozdnih robovih.

Osem zbirnih habitatnih tipov je z naravovarstvenega vidika najpomembnejših za dnevne metulje v raziskovanem območju, to so: ekstenzivno gospodarjeni suhi travniki, opuščena suha travišča v zgodnjih fazah zaraščanja, polintenzivno gospodarjeni travniki s pokončmim stoklascem in visoko pahovko, polintenzivno gospodarjeni mezofilni travniki z visoko pahovko, ruderalne površine zaraščajoče se z grmovjem in zeliščnimi vrstami ter gozdni robovi in poti na suhih rastiščih. Na podlagi njihove razširjenosti smo v raziskovanem območju opredelili 4 naravovarstveno pomembna območja za dnevne metulje (Žadovinek–Brege, Čateške Toplice–Prilipe, Loče–Mihalovec in Zgornji Obrež), s skupno površino 6,6 km$^2$. Število vrst v predlaganih območjih dosega 57 % (Loče–Mihalovec) do 84 % (Žadovinek–Brege) vrstnega bogastva raziskovanega območja. Upoštevaje maksimalno populacijsko gostoto ogroženih vrst v predlaganih območjih, je območje Žadovinek–Brege najpomembnejše za dve vrsti (*M. aurelia*, *P. idas*), območje Čateške Toplice–Prilipe za štiri vrste (*A. ilia*, *C. alceae*, *P. mannii*, *L. dispar*), območje Loče–Mihalovec za dve (*L. hippothoe*, *P. argygnomon*) in območje Zgornji Obrež za dve ogroženi vrsti (*M. britomartis*, *Z. polyxena*). Predlagana območja so pomembna za ohranjanje razmeroma velikega vrstnega bogastva dnevnih metuljev v pretežno intenzivno obdelani kmetijski krajini ob reki Savi med Krškim in državno mejo, in za ohranjanje viabilnih populacij ogroženih in drugih ekološko specializiranih, redkih ali lokalno razširjenih vrst ter preprečevanje njihove izoliranosti v območju subpanonske JV Slovenije.

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# Biological knowledge of Slovenian students in the living systems content area in PISA 2006

## Znanje slovenskih učencev na vsebinskem področju Živi sistemi v raziskavi PISA 2006

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**Abstract:** In the PISA 2006 program Slovenian students exceeded the international average of scientific achievements, while in the field of living systems performed below the national average. The purpose of our analysis was to determine in which areas of biology Slovenian students are weak and in which they are strong, and to determine their biological knowledge in comparison to 23 other European countries, which, by various criteria, are the most comparable to Slovenia and interesting for us. We analysed 24 tasks that tested the biological knowledge in PISA 2006. We found that student achievement in Slovenia wasn't poor in any of the tested biological topics. There are two factors that can explain lower achievements resolving individual tasks: (1) Question type: Students poorly answered the open-constructed response questions that require independent formulation of coherent responses. They were more successful with complex multiple choice questions and multiple choice questions, where they had to choose the correct answer from several given suggestions; (2) Difficulty: Students didn't perform as well with higher cognitive level tasks which required using knowledge. This suggests that in the period when these students were receiving elementary education, their biology teaching focused on developing knowledge of biological content (knowledge of science) and competence explaining phenomena scientifically, while the development of other knowledge (knowledge about science) and the competence to draw evidence-based conclusions (using knowledge) was inadequate. It is therefore in the hands of all in Slovenia involved in biological education, especially teachers of biology, to give students more opportunities for problem-solving, rather than only focusing on content.

**Key words:** PISA 2006, biology, science, knowledge, competencies, Slovenia

**Izvleček:** Slovenski učenci so v raziskavi PISA 2006 presegli mednarodno povprečje naravoslovnih dosežkov, vendar so bili na področju biologije slabši kot na drugih naravoslovnih področjih. Namen naše analize je bil ugotoviti, na katerih področjih biologije imajo slovenski učenci šibko in na katerih močno znanje ter kakšno je njihovo biološko znanje v primerjavi s 23 drugimi evropskimi državami, ki so po različnih kriterij najbolj primerljive s Slovenijo oziroma zanimive za nas. Analizirali smo 24 nalog, ki so v PISA 2006 preverjale biološko znanje. Ugotovili smo, da so bili dosežki učencev v Sloveniji pri vseh preverjenih bioloških temah dobri. Dejavnika, s katerima je bilo mogoče pojasniti slabše dosežke pri posameznih nalogah, sta bila tip naloge in njena težavnost. Učenci so slabše reševali naloge odprtega tipa, pri katerih so morali samostojno oblikovati smiseln odgovor. Uspešnejši so bili pri nalogah kompleksnega izbirnega tipa in izbirnega tipa, kjer so morali med danimi odgovori izbrati pravilnega. Učenci so slabše reševali naloge višje kognitivne stopnje, ki so zahtevale uporabo znanja. Iz tega sklepamo, da je bil v obdobju, ko so bili ti učenci vključeni v osnovnošolsko izobraževanje, pri biologiji poudarek na razvijanju poznavanja biološke vsebine (knowledge of science) in kompetence znanstveno razlaganje pojavov (explaining phenomena scientifically), pomanjkljivo pa je bilo

razvijanje znanja o naravoslovnih znanostih (knowledge about science) ter kompetence uporaba naravoslovno-znanstvenih podatkov in preverjenih dejstev (using scientific evidence). V rokah vseh, ki se v Sloveniji ukvarjajo z biološkim izobraževanjem, predvsem pa učiteljev biologije, je, da usposobijo učence tudi s tega vidika, ne samo z vidika vsebinskega znanja.

**Ključne besede:** PISA 2006, biologija, naravoslovje, znanje, kompetence, Slovenija

## Introduction

In 2006 Slovenia participated for the first time in the Program for International Student Assessment (PISA). The first PISA study in 2000 focused on reading literacy, the second in 2003 on mathematical literacy, and the third in 2006 on scientific literacy, which means the proportion of science tasks was significantly larger than the proportion of mathematical and reading tasks. The result was the first extensive and complete collection of internationally comparable data on the science competencies of students in 57 countries from all over the world.

The PISA differs from other assessments (for example from TIMSS, which is also international) in that it is not directly linked to the science curricula of any of the participating countries, but collects data about the competences that students will need for effective functioning in their adult professional and personal life, and are important both for individuals and for the whole society. According to the OECD definition (PISA 2006 2007), scientific literacy is "a capacity to use scientific knowledge, to identify questions, explain scientific phenomena, and to draw evidence-based conclusions in order to understand and help make decisions about science-related issues." The term 'literacy' indicates focusing on the application of knowledge and abilities (Bybee 2008). The tasks are structured in a manner that enables the assessment of the ability to solve tasks related to life situations, and hence not limited to knowledge of a specific subject (Štraus et al. 2007).

The PISA includes students who, in most participating countries, are approaching the end of compulsory education. The age of participants is precisely specified, ranging from 15 years and 3 months to 16 years and 2 months. This means that the PISA 2006 students were born in 1990. In the study 6,595 Slovenian students took part (Štraus et al. 2007).

### *Science achievements of Slovenian students in PISA 2006*

Slovenian students in PISA 2006 scored the average science achievement of 519 points (Tab. 1), therefore exceeding the average achievement of 500 points of all the participating students by 19 points (Achievements of students in PISA 2006).

The PISA 2006 science assessment evaluated students' knowledge in two areas: knowledge component and competency component. Knowledge component comprises two categories - knowledge *about* science (which includes scientific inquiry and scientific explanations) and knowledge *of* science (which includes Earth and space systems, living systems, and physical systems). The results of Slovenian students in the category of knowledge *about* science was as many as 9 points below the national average, while in the category of knowledge *of* science they showed a good knowledge in the areas of Earth and space systems (15 points above average), and physical systems (12 points above average). In the area of living systems, covering biology, Slovenian students ranked lower than the national average by 2 points (Tab. 1). On the international scale the biggest difference between knowledge *about* science and knowledge *of* science in PISA 2006 was 29 points. In the case of Slovenian students this difference was 17 points in favour of knowledge *of* science. These results are not a clear indicator of whether one or the other of these two categories of knowledge leads to higher overall scientific achievements (Štraus et al. 2007).

In the competency component (explaining phenomena scientifically) our students achieved 4 points above the international average (Tab. 1). The other two competences are less developed, but the differences are not significant: in the competence of identifying scientific questions they rank 2 points below the international

average, and in the competence of drawing evidence-based conclusions 3 points below the international average. In many countries with the highest achievements the students are particularly successful in the competence of drawing evidence-based conclusions. It turns out that in all of the successful countries the students are quite strong in this competence. The conclusion is that the ability to draw evidence-based conclusions, i.e. interpret and apply scientific data and verified facts, is particularly characteristic of highly developed science literacy (Štraus et al. 2007).

Table 1: Comparison of the overall achievements of Slovenian students in science with the achievements of individual scientific competencies and in the various science areas (adapted from Štraus et al., p. 51).
Tabela 1: Primerjava skupnega dosežka slovenskih učencev pri naravoslovju z dosežki pri posameznih naravoslovnih kompetencah in na posameznih naravoslovnih področjih (povzeto po Štraus et al., p. 51).

| | Overall achievement | The difference between the overall achievement and the achievements on individual rankings |
|---|---|---|
| Science | 519 | |
| Competency component | | |
| 1. Identifying scientific questions | | -2 |
| 2. Explaining phenomena scientifically | | 4 |
| 3. Drawing evidence-based conclusions | | -3 |
| Knowledge component | | -9 |
| 1. Knowledge *about* science | | 15 |
| 2. Knowledge *of* science | | -2 |
| - Earth and space systems | | 12 |
| - Living systems | | |
| - Physical systems | | |

*Objective analysis*

The purpose of our analysis was to determine the strong and weak areas of biological knowledge of Slovenian students, and where this knowledge ranks in comparison with other countries. These results allow us to extract the characteristics of biology teaching in Slovenian primary schools during the period when these students were in elementary school, and identify any necessary changes.

## Material and methods

*Tasks included in the analysis*

Of the total set of tasks in the area of living systems, i.e. tasks related to biological topics in PISA 2006, one part did not test the knowledge *of* (biology) science, but verified other components of scientific literacy (knowledge *about* science, identifying scientific questions, explaining phenomena scientifically, drawing evidence-based conclusions) based on biological content. These tasks are not included in our analysis because in this case biology was just a basis from which students demonstrated their knowledge and competencies not linked specifically to biology, but to any scientific topic. Students had to show knowledge *of* science in the content area of living systems in 27 tasks, of which 24 were used for our detailed analysis.

*Countries selected for comparison with Slovenian achievements*

There were 57 countries participating in PISA 2006, while for the purpose of our analy-

sis we presented the comparative performance of Slovenia and 23 other European countries, which are, according to various criteria (similar cultural background and history), most comparable with Slovenia and interesting for us. These were: Austria, Belgium, Switzerland, Czech Republic, Germany, Denmark, Spain, Estonia, Finland, France, Great Britain, Hungary, Ireland, Iceland, Italy, Lithuania, Latvia, Nederland, Norway, Poland, Portugal, Slovakia, and Sweden.

*Data analysis*

The overall achievements of the selected 23 countries in each of the 24 questions that tested the knowledge *of* (biology) science were calculated. The significant differences between the achievements of Slovenian students with regard to biological topic and the type of question were determined by one way ANOVA and Chi-square test ($P < 0.05$).

## Results and discussion

*1. General achievements of Slovenian students in the area of living systems*

The achievements of Slovenian students in 24 biological questions ranged from 22.4% to 89.5% (Fig. 1). Within this, the majority of tasks placed between 45.0% and 77.9%. The upward deviation was demonstrated in one task with 89.5% and downward in a group of five tasks where the achievement was less than 37%.

*2. Achievements of Slovenian students in the area of living systems in terms of content (biological topic)*

The 24 analysed tasks tested the knowledge of six areas of biology. The tasks were classified into four groups that contained 4 to 8 tasks each. We formed thematic groups: (1) ecology with 7 tasks; (2) physiology with 4 tasks; and (3) health with 8 tasks. The remaining three biological ar-

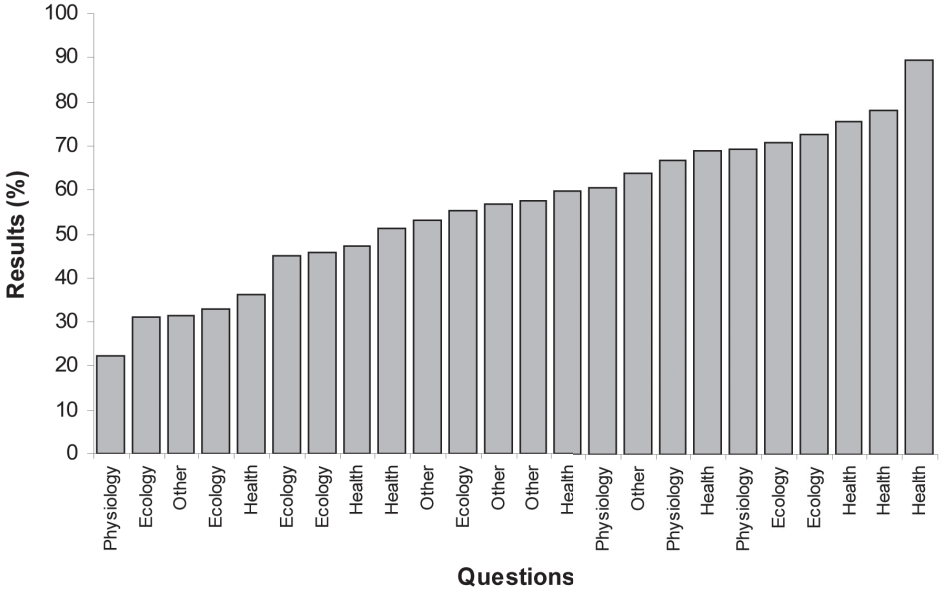


Figure 1: Achievements of Slovenian students in 24 tasks testing competency knowledge of science based on the content area of living systems

Slika 1: Dosežki slovenskih učencev pri 24 nalogah, ki so preverjale kompetenco znanje naravoslovja z vsebinskega področja živi sistemi.

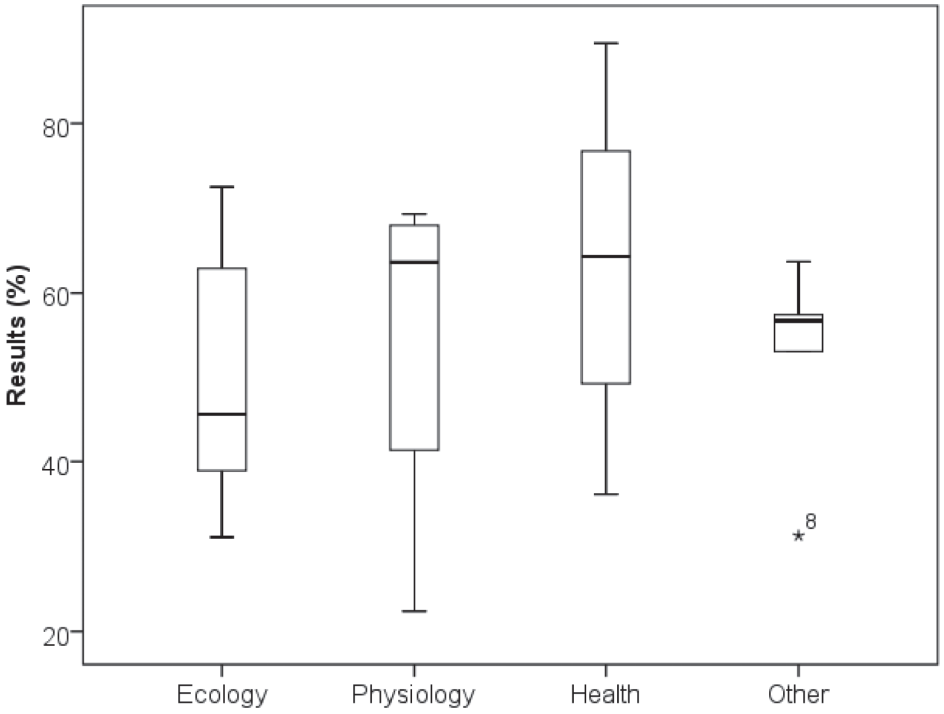


Figure 2: Medians, quartiles, and extreme values of the achievements of Slovenian students in tasks testing the knowledge of science based on the content area of living systems.
Slika 2: Mediane, kvartili in skrajne vrednosti dosežkov slovenskih učencev pri nalogah, ki so preverjale znanje naravoslovja z vsebinskega področja živi sistemi. Tematska področja: ekologija, fiziologija, zdravje, drugo.

eas were combined with the fourth group of varied content with 5 tasks. Such a division of tasks is not fully in line with the official topics of the PISA study in the area of living systems, which are: Health, Natural Resources, Environmental Quality, Hazards, and Frontiers of Science and Technology (PISA 2006 2007), but it seemed sensible from the standpoint of biological content, and, additionally, it gave us the minimum number of tasks for statistical data processing in each group.

The achievements of Slovenian students are considerably dispersed (Fig. 2) in all three subject areas (ecology, physiology and health). In the fourth group, which includes tasks from various other areas of biology, the achievements are more level, which is attributed to the fact that all the tasks in this group were of the same type, in this case complex multiple choice questions. Students gave the best answers in the areas of physiology and health (median = 60.5 and 59.8), while the achievement in ecology is lower (median = 45.7), but the differences among the achievements in individual thematic areas are not statistically significant (ANOVA, $P > 0.05$)

*3. Achievements of Slovenian students in the area of living systems with regard to the type of question*

The 24 tasks used in our analysis were presented in three different ways (8 tasks each): in the form of the complex multiple choice questions (a set of options from which the student chooses one); in the form of multiple choice questions (4 or 5 suggested answers of which

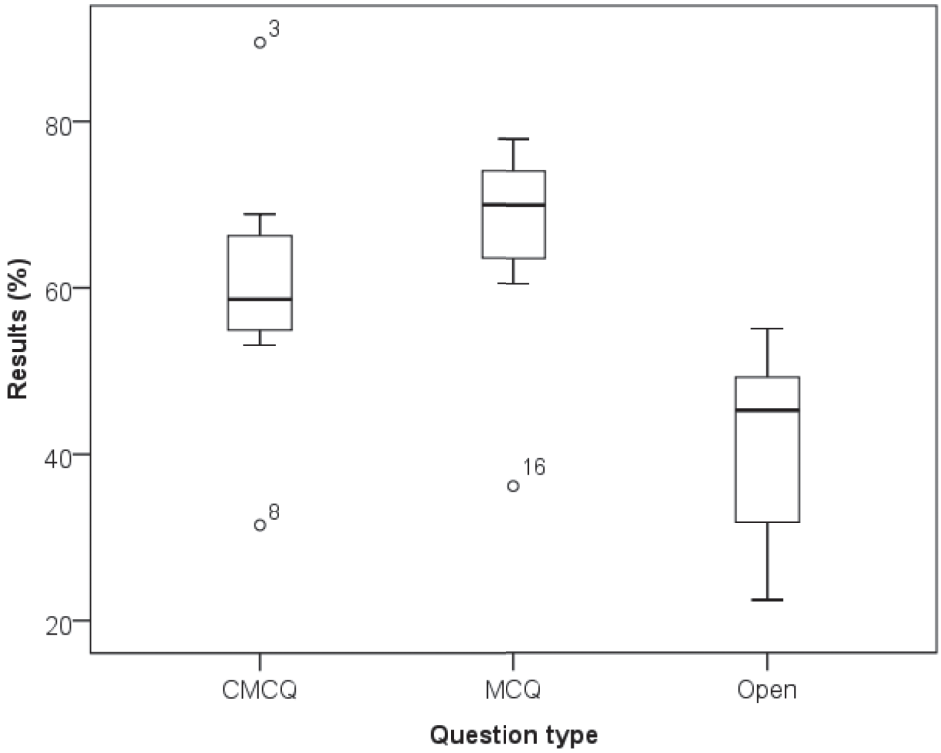


Figure 3: Medians, quartiles, and extreme values of the achievements of Slovenian students in tasks testing the knowledge of science based on the content area of living systems. CMCQ – complex multiple choice questions, MCQ – multiple choice questions, Open – open-constructed response questions.

Slika 3: Mediane, kvartili in skrajne vrednosti dosežkov slovenskih učencev pri nalogah, ki so preverjale znanje naravoslovja z vsebinskega področja živi sistemi. Tipi nalog: C – naloge kompleksnega izbirnega tipa, M – naloge izbirnega tipa, O – naloge odprtega tipa.

only one is correct); and in the form of open-constructed response questions (which require a longer written answer).

Achievements of Slovenian students with regard to the type of task are not so dispersed (Fig. 3) as the achievement with regard to biological topic. Students gave the best answers to multiple choice questions and slightly less appropriate answers to the complex multiple choice questions (median = 69.3 and 57.5), but this difference is not statistically significant (ANOVA; $P > 0.05$). Students showed significantly lower achievements answering open-constructed response questions (median = 45.1; ANOVA; $P = 0.004$).

Other research, for example National Assessment of Knowledge in Slovenia, showed that students have difficulties in formulating responses to open-constructed response questions, which applies not only to biology, but to all areas (Jagodnik et al. 2009). Poor performance on these questions thus indicates lack of ability to formulate the knowledge into sensible short answers rather than lack of content knowledge.

*4. Comparison of Slovenian students' achievements depending on topic and type, and deviations from the average of 24 countries*

A detailed analysis showed that the achievements of Slovenian students were mainly influ-

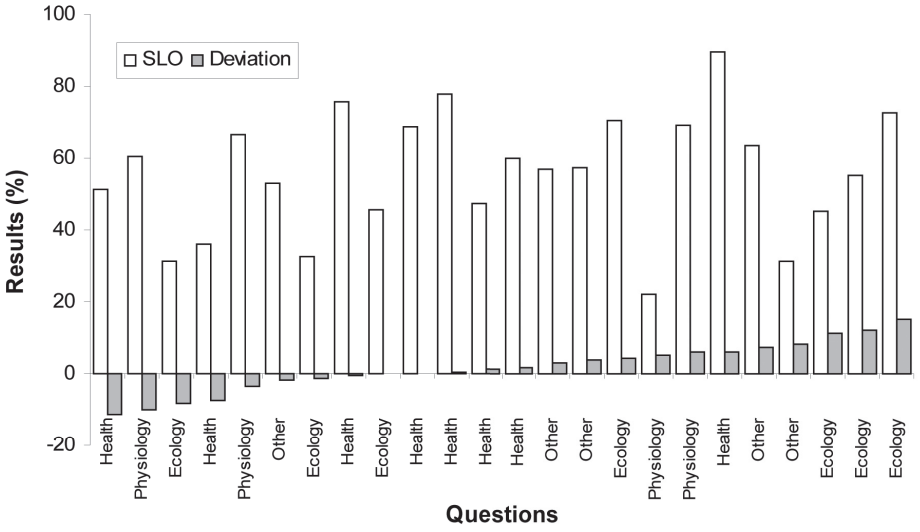


Figure 4: Achievements of Slovenian students in 24 tasks testing competency knowledge of science based on the content area of living systems, and relative deviations of these achievements compared to students of 23 European countries. Shown according to the relative deviation value.

Slika 4: Dosežki slovenskih učencev pri 24 nalogah, ki so preverjale kompetenco znanje naravoslovja na vsebinskem področju živi sistemi in relativno odstopanje dosežkov slovenskih učencev v primerjavi z učenci 23 evropskih držav. Prikaz po velikosti relativnega odstopanja.

enced by the type of task, and not by the biological content that a task tested ($\chi^2$ test, P = 0.03). Some tasks deviated from these findings, in other words, their result was influenced by biological content and not by the task type. Only from those tasks it is therefore possible to identify the strong and weak areas and other characteristics of biological knowledge of Slovenian students.

With regard to the biological topic, students were less successful in resolving four tasks: two in ecology, and one in physiology and health. The biological content of these tasks can be found in the Slovenian education curricula for primary school, which means that our students must have been exposed to the content. In our view the content should not have been too difficult for the students. But the difficulty increased as a result of the way the questions were presented. All four questions the Slovenian students had trouble with required the utilisation of knowledge. We concluded that students in Slovenia don't lack biological knowledge (knowledge *of* science), but other knowledge and competencies, namely knowledge *about* science and the competence to draw evidence-based conclusions.

We also arrived at the same conclusion when we analysed the relative deviations of the achievements of Slovenian students comparing to 23 European countries comparable to Slovenia for all 24 tasks testing the knowledge *of* science in the content area of living systems (Fig. 4). None of the tasks in which the Slovenian students exceeded the average (right side of the graph) was, in our opinion, cognitively challenging. This means that upward deviations of achievements of Slovenian students can be largely explained by lower levels of cognitive difficulty of tasks. On the other hand, it is our view that the tasks Slovenian students performed below average (left side of the graph) are cognitively more demanding, though one of them was impossible for Slovenian students to answer since Slovenian schools don't address the content in the way the question anticipated. Our finding that students in Slovenia don't lack biological knowledge (knowledge *of* science),

but other knowledge and competencies, is consistent with what Štraus et al. (2007) wrote in the PISA 2006 National report for the entire area of science. They say that in order to effectively solve science tasks a sequence of three processes is required: (1) students must first recognize the problem; (2) use their knowledge to explain phenomena scientifically; (3) and finally interpret and apply the results. Traditional teaching of science is often directed at another process, namely the scientific interpretation of phenomena or, in other words, the acquisition of key science knowledge. From the achievements of Slovenian students Štraus et al. (2007) concluded that the Slovenian elementary schools focus precisely on these work methods. Such teaching doesn't lead to comprehensive science literacy because later in life the student's ability to successfully use these data and arguments will be limited despite good command of the theory.

In Slovenia, the current elementary school curriculum for the area of living systems, at least at the primary school level, considers the need for students to develop both content knowledge and the process of science, as they followed the recommendations in Benchmarks (American Association for the Advancement of Science 1993). But the curricula for biology at higher levels of primary school do not. They are instead content-focused, with few process goals, and it is therefore not surprising that our students in PISA 2006 showed relatively good content knowledge of biology (knowledge *of* science and competence explaining phenomena scientifically), and, on the other hand, a considerable lack of other competences and knowledge *about* science.

The PISA 2006 results not only offer insights into the current status, but also allow for predictions of how today's 15 year olds will function as adults citizens who will need to use their knowledge and abilities in new situations and make decisions on issues related to, for example, the common good of humankind, wellness, the environment, human ecology, and other applications of biology (Bybee 2008). Education in the 21st century should consider the changing circumstances. DeHard Hurd (2001) claims that today's data is more qualitative than quantitative in nature. The intellectual skills required to do research in today's biology are mostly those of problem-solving. It is therefore in the hands of all in Slovenia involved in biological education, especially teachers of biology, to give students opportunities to utilise their knowledge and not only focus on content.

## Conclusions

The analysis of Slovenian students' achievements in 24 questions in the area of living systems in PISA 2006 showed that:

1. The achievements of Slovenian students were good in all of the tested biological topics.
2. There are two factors that could explain the poor performance in specific tasks:
   - Question type: Students were less successful in answering open-constructed response questions where they had to independently formulate a coherent response. They were better with the complex multiple choice questions and multiple choice questions, where they had to pick a correct answer among several suggestions
   - Difficulty: Students were less successful with higher cognitive level tasks that required the use of knowledge. This suggests that in the period, during which the students were given elementary education, biology teaching focused mainly on developing knowledge of biological content (knowledge *of* science) and competencies in explaining phenomena scientifically, and less on developing other knowledge (knowledge *about* science) and competences in drawing evidence-based conclusions (use of knowledge).

The sample of tasks that we were able to include in the analysis was predetermined by the PISA 2006 structure. Because it's so small, our findings should be viewed with caution. In addition, due to a small number of available tasks the achievements of the students were analysed only with regard to the content and type of the task, not taking into account a third possible aspect - proficiency level. Each task was in fact classified in one of six proficiency levels on the basis of substantive considerations relating to the nature of the underlying competencies (PISA 2006 2007).

From the standpoint of biology, a very important issue remains unresolved: what is the reason that Slovenian students achieved significantly lower results in the area of living systems than in the areas of Earth and space systems, and physical systems. This information could be obtained by an overall interdisciplinary analysis aimed at discovering the essential characteristics of biology, physics, and chemistry teaching in Slovenian elementary schools. A comparison would point to the aspects of teaching that strongly deviate in biology and are the likely reason for poorer performance of the students.

## Povzetek

Slovenski učenci so v raziskavi PISA 2006 dosegli povprečni naravoslovni dosežek 519 točk, kar presega povprečje vseh sodelujočih držav (500 točk). Znotraj naravoslovja so bili slovenski učenci nadpovprečni na področju fizike, kemije in geografije, medtem ko so bili na področju biologije rahlo podpovprečni. Namen naše analize je bil ugotoviti, na katerih področjih biologije imajo slovenski učenci šibko in na katerih močno znanje ter kakšno je njihovo biološko znanje v primerjavi s 23 drugimi evropskimi državami, ki so po različnih kriterij najbolj primerljive s Slovenijo oziroma zanimive za nas.

Iz celotnega nabora nalog, ki so bile v raziskavi PISA 2006 povezane z biološko tematiko, je bil del takih, ki so na bioloških vsebinah preverjale znanje o naravoslovnih znanostih (knowledge about science) in ne naravoslovnega znanja (knowledge of science) samega. Teh nalog nismo analizirali, ker je bila biologija v tem primeru samo tema, na kateri so morali učenci pokazati kompetenci znanstveno razlaganje (scientific explanations) ali znanstveno raziskovanje (scientific enquiry), ki nista vezani samo na biologijo, temveč na katerokoli naravoslovno znanost. Kompetenco znanja (knowledge of science) na področju biologije so učenci morali pokazati pri 27 nalogah, od katerih smo jih 24 uporabili za našo analizo. Naloge smo razvrstili v tri tematske skupine: ekologija s 7 nalogami, fiziologija s 4 nalogami in zdravje z 8 nalogami. Preostalih pet nalog smo združili v vsebinsko raznoliko četrto skupino.

Ugotovili smo, da dosežki učencev v Sloveniji pri vseh preverjenih bioloških temah dobri. Dejavnika, s katerima je bilo mogoče pojasniti slabše dosežke pri posameznih nalogah, sta bila tip naloge in njena težavnost. Učenci so slabše reševali naloge odprtega tipa, pri katerih so morali samostojno oblikovati smiseln odgovor. Uspešnejši so bili pri nalogah kompleksnega izbirnega tipa in izbirnega tipa, kjer so morali med danimi odgovori izbrati pravilnega. Učenci so slabše reševali naloge višje kognitivne stopnje, ki so zahtevale uporabo znanja. Iz tega sklepamo, da je bil v obdobju, ko so bili ti učenci vključeni v osnovnošolsko izobraževanje, pri biologiji poudarek na razvijanju poznavanja biološke vsebine (knowledge of science) in kompetence znanstveno razlaganje pojavov (explaining phenomena scientifically), pomanjkljivo pa je bilo razvijanje znanja o naravoslovnih znanostih (knowledge about science) ter kompetence uporaba naravoslovno-znanstvenih podatkov in preverjenih dejstev (using scientific evidence). V rokah vseh, ki se v Sloveniji ukvarjajo z biološkim izobraževanjem, predvsem pa učiteljev biologije, je, da usposobijo učence tudi s tega vidika, ne samo z vidika vsebinskega znanja.

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# *Science goes to school:* A new model for introduction of modern biology teaching strategies to Slovene schools

## *Znanost gre v šolo:* nov pristop k uvajanju sodobnih metod poučevanja bioloških vsebin v slovenske šole

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**Abstract:** In the framework of the project *Science Goes to School*, we developed and tested a new model for introduction of modern biology teaching strategies to Slovene schools. The project focused around a close university-school partnership, bringing together the expertise of scientists from the University of Ljubljana and the experiences of teachers from 22 Slovene secondary schools (grades 9-12, age of students 15-19). The project comprised three phases. During the introductory workshop, project scientists and partner teachers identified curriculum topics with an acute lack of good-quality teaching materials. During the second phase, university scientists developed new practical activities for students and prepared comprehensive teaching materials. Each new activity was tested in partner schools, with a scientist acting as a visiting teacher. Partner teachers were present in the class during testing and were hence trained in the authentic environment of their own classrooms. Both teachers and students contributed their comments and suggestions for improvement of new activities. The visiting scientist also acted as a role model motivating the students to consider science careers. During the third phase, the new teaching materials were published in a handbook for teachers and on the internet. In addition, the new activities were presented to a wider community of teachers and school laboratory assistants during a training workshop. The project was favourably received among the teachers, the project scientists and the students in partner schools. To efficiently improve biology education in Slovene schools, such activities require long-term, stable funding from national sources.

**Keywords:** science education, biology, teaching, effective learning, university-school partnership

**Izvleček:** V okviru projekta *Znanost gre v šolo* smo razvili in preizkusili nov pristop k uvajanju sodobnih metod poučevanja bioloških vsebin v slovenske šole. V središču projekta je bilo tesno partnerstvo univerze in šol, s katerim smo povezali strokovno znanje znanstvenikov z Univerze v Ljubljani in izkušnje učiteljev z 22 partnerskih srednjih šol. Projekt je obsegal tri faze. Med uvodno delavnico so znanstveniki in partnerski učitelji opredelili vsebine v učnem načrtu, pri katerih močno primanjkuje kakovostnih učnih gradiv. Med drugo fazo so znanstveniki razvijali nove praktične aktivnosti za pouk biologije in pripravili izčrpna učna gradiva. Vsako novo aktivnost smo preizkusili v partnerskih šolah, pri čemer je eden od znanstvenikov obiskal šolo kot gostujoči učitelj. Med testiranjem so bili partnerski učitelji prisotni v razredu in so se tako strokovno usposabljali v avtentičnem okolju lastnih učilnic. Tako učitelji kot dijaki so prispevali pripombe in predloge za izboljšanje novih aktivnosti. Gostujoči znanstvenik je bil hkrati vzornik pri spodbujanju dijakov, da bi se odločili za naravoslovne študije. Med tretjo fazo projekta smo nova učna gradiva objavili v priročniku za učitelje in na spletnih straneh. Poleg tega smo organizirali zaključno delavnico za učitelje, na kateri se je širši krog učiteljev in šolskih laborantov usposabljal za izvedbo novih aktivnosti. Projekt so z navdušenjem podprli učitelji, sodelujoči znanstveniki in dijaki s partnerskih

šol. Za učinkovito izboljšanje kakovosti biološkega izobraževanja v slovenskih šolah bi morali tovrstne dejavnosti dolgoročno in stabilno financirati iz nacionalnih virov.

**Ključne besede:** naravoslovno izobraževanje, biologija, poučevanje, učinkovito učenje, partnerstvo univerz in šol

## Introduction

Recently, biology became the most rapidly developing natural science. In addition, topics such as biodiversity, global warming, invasive species, genetically modified organisms, stem cells and gene therapy have gained a high social importance. Consequently, a modern citizen needs biological knowledge to cope with everyday problems, such as understanding the news in the media and deciding about health issues. For young people, the main source of up-to-date biological knowledge is their biology teacher.

In the context of the increasing importance of biological literacy for personal and social decision-making, biology teachers face the challenge of updating the teaching content and changing their practices from teaching factual knowledge to conveying conceptual understanding of living systems. However, biology teachers have a high teaching load and have to cover a wide range of biology topics. They also lack time and expertise to convert important scientific discoveries into classroom activities.

For the teacher to cope with emerging new biology topics and the increasingly interdisciplinary and systemic approach to teaching biology, he/she needs an excellent education and continuous in-service training (Moore 2003, 2007). In many schools, teaching lags far behind new scientific findings. Many countries have reported problems with overloaded and outdated curricula, outdated textbooks, insufficient »real« practical work, the perception of biology as a »soft« scientific subject, inappropriate pedagogy, lack of teacher and student enthusiasm and lack of continuous teacher training (Moore 2007, Tunniclife and Ueckert 2007).

Introduction of new approaches to teaching science as an exciting and dynamic topic is a long-term process (Mervis 2002, Vilhar 2007). It comprises development of new curricula and changes in teacher education, and has to be supported with new textbooks and teaching materials. One of the problems is development of scientific thinking in science class. Presently, the phylosophy of science is often wrongly presented as a collection of recipes for experiments (Mervis 2002, Bonner 2004, National Research Council 2002, 2005, Moore 2007). Empirical evidence shows that active learning works (Michael 2006), motivating students to become active learners and problem solvers (Lujan and DiCarlo 2006). As stated in the review with a meaningful title *Too much teaching, not enough learning: What is the solution*, extensive curricular changes are required to achieve effective learning (Lujan and DiCarlo 2006).

Modernisation of science teaching in schools has to be supported by active involvement of university scientists. Scientists are competent to select and suggest new science topics and help to develop new approaches to teaching biology in schools, conveying the true spirit of science (Bhattacharjee 2005, Moore 2007). However, these new ideas need to be adequately fitted into the curriculum, since learning will only occur after teaching if students are given enough time to process the new information and connect it to their previous knowledge and conceptions (Tunniclife and Ueckert 2007). To achieve this, expertise of scientists has to be complemented with experience of teachers, who know well the capabilities of their students and real-life situations in the classroom (McDiarmid et al. 1989, Tanner et al. 2003).

The above mentioned problems are also present in Slovene schools. In the past, biology teachers frequently complained about the lack of systematic support from scientists. Students also felt that changes are needed in secondary school biology. More than 71% of students thought that the curriculum should be more connected to everyday life, 66% would like to have more experiments and 59% more excursions (field work) during biology lessons (Gabrščček et al. 2005; see Tab. 1).

The project *Science Goes to School* connected scientists, teachers and students from secondary schools with the aim to improve science teaching. We tested a new model for introduction of modern teaching strategies to our secondary schools. The project was a university-school partnership

based on experiences of similar projects in other countries (e.g. Mervis 2002, Tanner et al. 2003), but taking into account the specific circumstances in Slovene schools and universities.

## Methods

*Survey of teachers' perceptions of problems in biology education*

In order to investigate teachers' perceptions of the major problems in biology education, we prepared a questionnaire for teachers in general secondary schools. The questionnaire was handed out to teachers who participated at a training seminar in January 2006. Participation in the survey was voluntary. 35 out of 78 participants returned a filled-in questionnaire.

*The project* Science Goes to School

The idea for the project *Science Goes to School* was based on previous similar projects in other countries (e.g. Mervis 2002, Tanner et al. 2003), in particular the program Graduate STEM Fellows in K-12 Education in the USA (National Science Foundation 2010). This program supports fellowships and training for graduate students in science, technology, engineering, and mathematics (STEM). We adapted the project activities to specific circumstances in Slovenia, as explained in the Results. The project duration was 1.5 years (project budget: 62 600 €).

The project activities were evaluated using questionnaires. The first questionnaire was handed out to biology teachers participating at the introductory workshop in May 2006. 18 out of 20 teachers returned a filled-in questionnaire. The second questionnaire was handed out to 72 participants at the training workshop for teachers (53 biology teachers and 19 school laboratory assistants) in September 2007. 46 teachers and 13 laboratory assistants returned a filled-in questionnaire.

*Statistical methods*

Standard statistical methods were used to analyse questionnaires. Data were analysed with the software package Prism 5 for Windows (Graph Pad Software). Average values are expressed as mean ± standard error of the mean.

## Results

*Teachers' perceptions of problems in biology education*

The questionnaire for teachers about the main problems in biology education, which we

Table 1: Perception of biology teachers and students about the main problems of biology education in secondary school. Teachers' perceptions were evaluated in January 2006 on the basis of a questionnaire. The open-ended question was: List three main problems in biology education. Answers were grouped to categories. Data about students' opinions are from Gaberšček et al. 2005.

Tabela 1: Mnenja učiteljev biologije in bivših dijakov o problemih na področju biološkega izobraževanja v srednji šoli. Mnenja učiteljev so iz vprašalnika, ki so ga gimnazijski učitelji izpolnili januarja 2006. Vprašanje odprtega tipa je bilo: Napišite tri ključne probleme na področju biološkega izobraževanja. Odgovori so razvrščeni v kategorije. Podatki o mnenju bivših dijakov so iz Gaberšček in sod. 2005.

| **Problem** | **Fraction of teachers (%)** N = 35 | **Fraction of students (%)** N = 862 |
|---|---|---|
| curriculum | 71 | 71 |
| in-service teacher training | 69 | --- |
| laboratory and field work | 51 | 62 |
| teaching materials (in Slovene) | 46 | --- |
| textbooks and workbooks | 40 | 19 |
| general circumstances at school | 37 | --- |

Table 2: **Expectations of biology teachers about activities of university scientists in the field of biological education.** Teachers' perceptions were evaluated in January 2006 on the basis of a questionnaire. The open-ended question was: *What are your expectations from the Department of Biology [Biotechnical Faculty, University of Ljubljana] in this field?* Answers were grouped to categories.

Tabela 2: **Pričakovanja učiteljev biologije o aktivnostih matične stroke na področju biološkega izobraževanja.** Mnenja učiteljev so iz vprašalnika, ki so ga gimnazijski učitelji izpolnili januarja 2006. Prikazana je analiza odgovorov učiteljev biologije na vprašanje odprtega tipa: *Kakšna so vaša pričakovanja od Oddelka za biologijo na tem področju?* Odgovori so razvrščeni v kategorije.

| Activity | Fraction of teachers (%) N = 35 |
|---|---|
| introduction of novelties to school | 77 |
| in-service teacher training | 71 |
| collaboration with teachers | 71 |
| participation in development of curricula | 29 |
| increased impact in wider society | 29 |

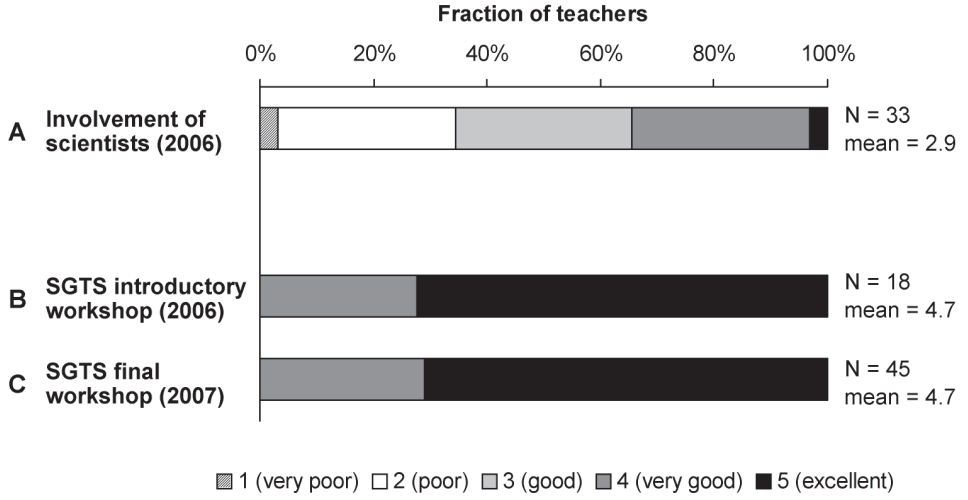


Figure 1: **Teachers' evaluation of activities in the field of biological education.** Teachers used "school" grades from 1 (very poor) to 5 (excellent) to evaluate activities. **A** - Involvement of scientists (2006): questionnaire handed out at a workshop for biology teachers in secondary school in January 2006 (question: *Use school grades from 1 to 5 to evaluate current activities of the Department of Biology [Biotechnical Faculty, University of Ljubljana] in the field of biology education.*) **B** - SGTS introductory workshop (2007): questionnaire handed out at the introductory workshop of the project Science Goes to School in May 2006 (question: Use school grades from 1 to 5 to evaluate today's workshop.). **C** - SGTS final workshop (2007): questionnaire handed out at the final workshop of the project Science Goes to School in September 2007 (question: *Use school grades from 1 to 5 to evaluate today's workshop.*).

Slika 1: **Mnenje učiteljev o dejavnostih na področju biološkega izobraževanja.** Učitelji so dejavnosti ocenjevali s "šolskimi" ocenami od 1 do 5. **A** - Involvement of scientists (2006): anketa izvedena na seminarju za gimnazijske učitelje biologije januarja 2006 (vprašanje: *Ocenite s šolsko oceno od 1 (nezadostno) do 5 (odlično) trenutno delovanje Oddelka za biologijo na področju biološkega izobraževanja.*). **B** - SGTS introductory workshop (2007): anketa izvedena na uvodni delavnici projekta Znanost gre v šolo maja 2006 (vprašanje: S šolsko oceno od 1 do 5 ocenite delo na današnji delavnici.). **C** - SGTS final workshop (2007): anketa izvedena na zaključni delavnici projekta Znanost gre v šolo septembra 2007 (vprašanje: *S šolsko oceno od 1 do 5 ocenite današnji seminar.*).

prepared in January 2006, contained open-ended questions. Teachers' answers were sorted into categories. The most frequently mentioned problems were related to inappropriate and outdated curriculum, lack of good-quality teacher training seminars and workshops, problems with laboratory and field work and lack of good-quality teaching materials in Slovene language (Tab. 1).

We also investigated what teachers expected from university scientists working in the field of biology. Teachers most frequently listed support in relation to introduction of novelties to school, involvement in in-service teacher training activities and general collaboration with teachers (Tab. 2). Teachers were also asked to evaluate past activities of university scientists in the field of biology education using the scale of school grades from 1 (very poor) to 5 (excellent). The average grade was 2.9 ± 0.2 (Fig. 1A).

*The project* Science Goes to School

The project *Science Goes to School* specifically aimed to address three problems that teachers identified as the main problems in biology education: lack of appropriate teacher training, lack of new materials for practical activities of students and lack of teaching materials in Slovene language (Tab. 1). The project was based on an intense partnership between university and schools, aiming to overcome the previous discontent of teachers with insufficient involvement of university scientists in biology teaching in schools (Tab. 2, Fig. 1A). In particular, project scientists helped teachers introduce novelties to school and offered additional teacher training. The project collaborators included scientists from the Department of Biology, Biotechnical Faculty, University of Ljubljana and partner teachers from 22 secondary schools (grades 9-12, age of students 15-19).

The project activities comprised three phases (Fig.2). During the one-day introductory workshop, four project scientists and 20 partner teachers participated in brainstorming sessions, aiming to identify the topics in the secondary-school biology curriculum for which there was an acute lack of useful teaching materials. Possible teaching strategies for these topics were also discussed. We decided to use new approaches to teaching biology that support effective teaching for long-lasting knowledge, such as experiment-based learning and educational games (Lujan and DiCarlo 2006). Teachers evaluated the introductory workshop with an average grade 4.7 ± 0.1 (Fig. 1B).

During the second phase, university scientists developed new activities and tested them in partner schools. The new practical activities for students were based on our own ideas or modified from materials developed by other authors (Vilhar et al. 2007). Development of materials included testing of experiments in laboratories and writing supporting materials for teachers and students. We paid special attention to possible errors that students could do during execution of the practical activity at school and possible unexpected results of experiments. We also reviewed relevant textbooks for secondary schools in Slovene language and exposed the main sources of misconceptions and problems with understanding of selected topics.

We tested all new activities in partner schools. One of the project scientists came to the classroom during regular biology lessons and taught the subject using newly developed methods. The partner teacher was present in the classroom and was thus trained in the authentic environment of his/her own classroom. We collected the opinions about the new practical activity from the teachers and the students and used their ideas and comments to improve the teaching material. Partner teachers and students in visited schools thus actively contributed to the quality of newly developed practical activity. The improved version of the practical activity was tested again in another school. In some cases, new practical activities were tested prior to the first visit to school with first-year university students of biology and pre-service biology teachers, who volunteered to participate in the project.

Within the framework of the project, we developed and tested eight new activities, which can be directly used for teaching biology in Slovene secondary schools (Vilhar et al. 2007):

– Diffusion and osmosis (experiment-based learning with educational role-playing game and a computer simulation; Strgulc Krajšek and Vilhar 2010)
– Describing and naming in biology (discovery-

based learning)
– Determination keys (discovery-based learning)
– How to grow fern gametophytes? (discovery-based learning)
– Respiration (experiment-based learning using computer-linked measurement instruments)
– Muscle fatigue (experiment-based learning using computer-linked measurement instruments)
– The plant game (educational computer game)
– Bio impro-league (educational card game).

The prepared teaching materials include references to the relevant curriculum topics, duration of the activity, theoretical background for teachers, detailed instructions for preparing and teaching the activity, worksheets for students with solutions and comments for teachers (including the expected results of experiments), links to websites with additional materials, lists of books and other relevant literature, safety warnings, explanations about common misconceptions and how to overcome them, and some interesting stories linked to the topic that teachers can use to motivate the students. We paid special attention to include references to the history of science in the teaching activities (e.g. Strgulc Krajšek and Vilhar 2010), thus emphasising the importance of the largely neglected aspect of science education, namely explaining to students the nature of science.

During the third phase, at the end of the project, we made the new teaching activities available to a wider community of teachers. We published the teaching materials in a handbook for teachers (Vilhar et al. 2007), which was sent to all secondary schools. Supporting material was published on the project website (http://znanost-gre-v-solo.biologija.org/). While some supporting material is publicly available, specific comments for teachers are accessible with a password for registered teachers. The worksheets for students were published in a format that allows teachers to modify the text and thus adapt the teaching materials to conform to their teaching strategies and the time they allocate to each activity.

We also organised a one-day training workshop for teachers and school laboratory assistants, where project scientists and partner teachers acted together as instructors. Participants (53 teachers and 19 school laboratory assistants) were divided into groups, so that each participant was trained in three of the new activities. The seminar was closed with a general discussion of all participants and scientists, where impressions, questions and comments about the new activities were shared.

Participants evaluated the quality of the training workshop with a questionnaire. Teachers evaluated the workshop with an average grade 4.7 ± 0.1 (Fig. 1C). They thought that the model for introduction of novelties to schools used in our project was very appropriate (average grade 4.8 ± 0.1; Fig. 3A). They also evaluated five of the eight new activities. The average grades ranged from 4.3 to 4.8 (Fig. 3B-F). For the five evaluated activities, the fraction of teachers who thought that they would use the new activity in school was 95% to 100%. Teachers also expressed a strong support for follow-up projects similar to the project *Science Goes to School* (average grade 4.91 ± 0.04; Fig. 3G).

## Discussion

Our survey conducted in January 2006 clearly showed that teachers need and expect support from university scientists (Tab. 2; Fig. 1A). Prior to the project *Science Goes to School*, there was no close collaboration between scientists and teachers with the goal to develop new biology teaching strategies and introduce them to Slovene schools. While partner teachers were somewhat sceptical at the very beginning of the project *Science Goes to School*, the close university-school partnership later lead to enthusiasm among partner teachers and project scientists (Figs. 1B, 1C) and sparked valuable exchange of ideas and experiences. The cooperation of teachers and scientists thus turned out to be beneficial for both partners. Laursen et al. (2007), who worked in the USA with K-12 students and their teachers, came to similar conclusions.

The teachers liked the comprehensive approach in the new teaching materials of the project *Science Goes to School*, with extensive theoretical background, explanation of common misconceptions, comments on possible mistakes

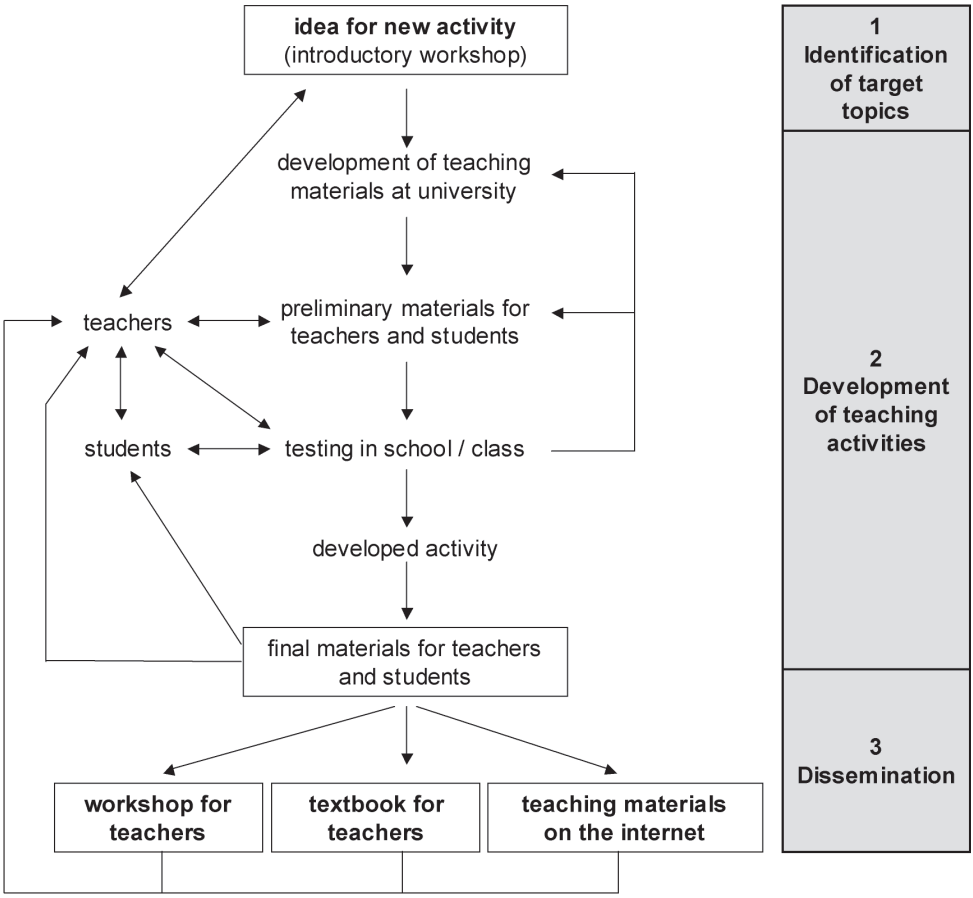


Figure 2: **Outline of activities of the project *Science Goes to School.*** See the main text for details.
Slika 2: **Pregled aktivnosti v okviru projekta *Znanost gre v šolo.*** Pri razvoju novih učnih gradiv za praktične aktivnosti so sodelovali univerzitetni znanstveniki, učitelji in dijaki s partnerskih šol.

that students can make in class and references to the history of science. The active involvement of university scientists reassured the teachers that the new teaching materials were of good quality and contained correct scientific information. The teachers appreciated the fact that the new practical activities for students were focused around specific teaching goals (subject content knowledge) and were thus a constructive part of the overall learning process in biology class. Their support for the outcomes of the project is reflected in their answers to questionnaires (Figs. 1B, 1C, 3). In addition, inclusion of school laboratory assistants in project training activities was perceived as important for improvement of biology education (analysis of questionnaires not shown).

We paid special attention to tightly link the new activities to curriculum topics, and to instruct the teachers to give their students adequate guidance. Results of previous empirical investigations show that learning on the basis of students' own previous experiences alone is less efficient than teaching methods which include proper guidance of the students during the learning process (Klahr and Nigram 2004, Mayer 2004, Novak and Cañas 2006, Kirshner et al. 2006, Sweller et al. 2007). If students have no prior conceptual understand-

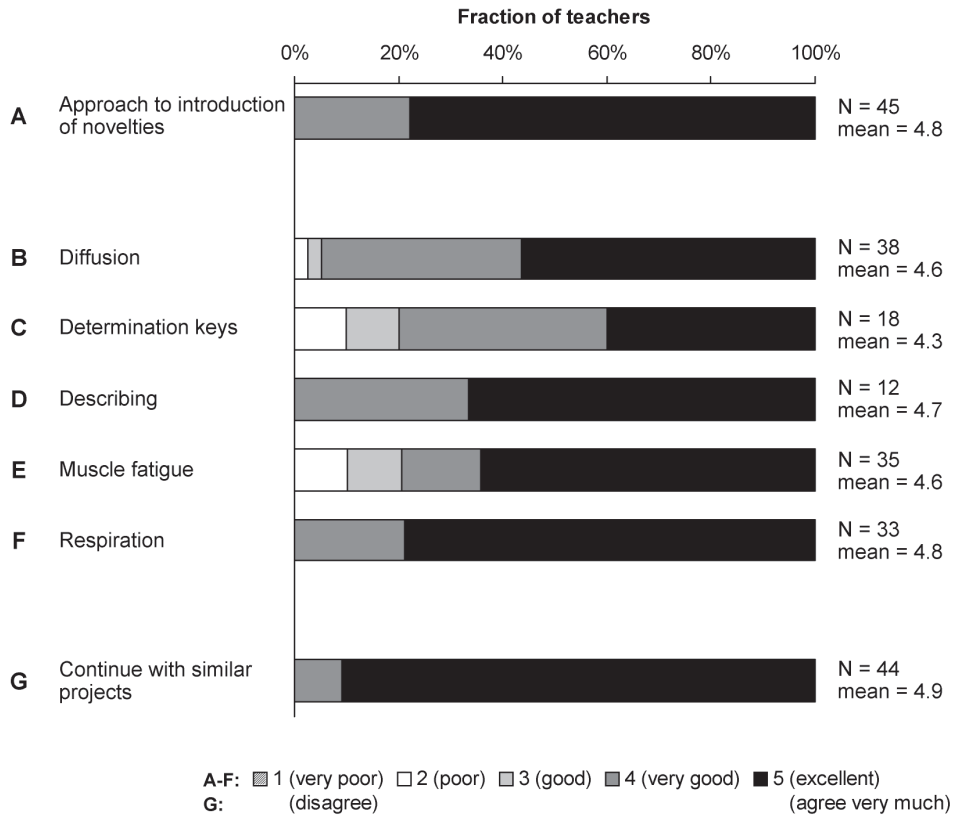


Figure 3: **Teachers' evaluation of the training workshop of the project *Science Goes to School.*** The questionnaire was handed out at the final training workshop for teachers in the framework of the project *Science Goes to School* in September 2007 The grading system is shown in the legend. **A** – question: *Do you find the approach to development of new activities which we used in the project* Science Goes to School *appropriate?* **B-F** – general evaluation of the new school activity using school grading system from 1 to 5. **F** – question: Do you think it is reasonable to continue with activities similar to the project Science Goes to School?

Slika 3: **Mnenje učiteljev o zaključne delavnice za učitelje na projektu *Znanost gre v šolo.*** Anketa je bila izvedena na zaključni delavnici za učitelje v okviru projekta *Znanost gre v šolo* septembra 2007. Način ocenjevanja je prikazan v legendi: A do F – "šolske ocene" od 1 do 5; G – 1 – sploh ne, 5 – zelo. **A** – *Ali se vam zdi način razvoja aktivnosti, ki smo ga uporabili v projektu Znanost gre v šolo, primeren?* **B do F** – Splošna ocena nove vaje: "šolska" ocena od 1 do 5. G – vprašanje: Ali se vam zdi smiselno nadaljevanje s programi, podobnimi projektu Znanost gre v šolo?

ing of a natural phenomenon that they are investigating, they often acquire no new conceptual knowledge during their practical activities (Novak and Cañas 2006). Likewise, development of skills such as learning to learn and searching for relevant information cannot replace the need for understanding science concepts, in particular not in the 21st century, when science knowledge needs to be upgradeable (Sweller et al. 2007). In this respect, the experiences in Norway are particularly interesting. After a curricular reform that focused on acquiring skills through various activities and neglected subject content knowledge, the achievements of students in mathematics and science, evaluated in international studies PISA and TIMSS, dropped considerably

(Institute for Teacher Education and School Development 2006). These examples demonstrate that science teaching should focus around understanding of science concepts, with a balanced use of different teaching methods

A particular feature of the project *Science Goes to School* was a "science ambassador" – a scientist who visited schools during regular biology lessons. While the main goal of these visits was testing of new activities and teacher training, the visiting scientist also served as a role model to increase students' interest in science and motivate them to consider science careers. The students in partner schools responded with enthusiasm. Examples of their comments in questionnaires are: *I wish we had more such lessons, this way we learn more*; *I liked the lesson, it was fun and instructive*; *Come back again*; *Keep delivering such lessons in the future* (see students' comments in Strgulc Krajšek and Vilhar 2010). These experiences support previous observations that active learning works (Abrahams and Millar 2008) and that universities can promote change towards more efficient science teaching (Tanner et al. 2003). Different models of scientists visiting schools have been used in other countries, with encouraging results (e.g. Peplow 2004, Beck et al. 2006, Brodie 2006, Laursen 2007).

The project *Science Goes to School* was a pilot project, introducing and testing a new model for development of teaching materials and for teacher training in Slovenia. While the target subject of the project was biology, the same model is applicable for introduction of novelties in other science subjects (science, physics and chemistry). The strong support from the teachers (Fig. 3G) clearly shows that such activities should be continued on a regular basis. However, these activities are only financed in Slovenia on a short-term basis (in particular with financial support from the EU), which greatly diminishes the long-term impact on improvement of biology education. Notably, centres for biology teacher training with full-time staff exist in many EU countries, but there is no such centre in Slovenia. In 2009, The European Network of Academies on Science Education stated that the use of limited EU seed funds must be followed up by substantial investments nationally, from ministries of education, Academies of Sciences, research institutions and industry (ALLEA 2009). The long-term strategy for improvement of science education in Slovenia should follow these guidelines.

## Conclusions

- Biology teachers need and expect support from university scientists.
- The new approach to university-school partnership developed and tested during the project *Science Goes to School* was favourably received among the teachers, the project scientists and the students in partner schools.
- Long-term funding, in particular from the national sources, is needed for such activities to have a long-lasting effect on improvement of biology education in Slovenia.

## Povzetek

Ob hitrem napredku bioznanosti ter naraščajočem pomenu biološkega znanja za osebne in družbene odločitve se precej spreminjajo tudi pristopi k biološkemu izobraževanju. Pri posodobitvi pouka učitelji biologije potrebujejo strokovno podporo in ustrezno strokovno izpopolnjevanje (tab. 1), pri čemer pričakujejo tudi pomoč znanstvenikov z univerz (tab. 2, sl. 1A).

V okviru projekta *Znanost gre v šolo* smo razvili in preizkusili nov pristop k uvajanju sodobnih metod poučevanja bioloških vsebin v slovenske šole. V središču projekta je bilo tesno partnerstvo univerze in šol, s katerim smo povezali strokovno znanje znanstvenikov z Univerze v Ljubljani in izkušnje učiteljev z 22 partnerskih srednjih šol. Zamisel za projekt smo oblikovali na temelju podobnih projektov v drugih državah (Mervis 2002, Tanner in sod. 2003, National Science Foundation 2010), pri čemer smo projektne dejavnosti prilagodili specifičnim razmeram na slovenskih šolah in univerzah. Projekt je trajal leto in pol (vrednost projekta: 62 600 €).

Projekt je obsegal tri faze (sl. 2). Med uvodno delavnico so znanstveniki in partnerski učitelji opredelili vsebine v učnem načrtu, pri katerih močno primanjkuje kakovostnih učnih gradiv,

ter razpravljali o možnih didaktičnih pristopih k poučevanju teh tem.

Med drugo fazo projekta so znanstveniki razvijali nove praktične aktivnosti za pouk biologije in pripravili izčrpna učna gradiva. Vsako novo aktivnost smo preizkusili v partnerskih šolah, pri čemer je eden od znanstvenikov obiskal šolo kot gostujoči učitelj. Med testiranjem so bili partnerski učitelji prisotni v razredu in so se tako strokovno usposabljali v avtentičnem okolju lastnih učilnic. Tako učitelji kot dijaki so prispevali pripombe in predloge za izboljšanje novih aktivnosti. Gostujoči znanstvenik je bil hkrati »znanstveni ambasador« - vzornik pri spodbujanju zanimanja dijakov za naravoslovne znanosti in naravoslovne študije.

V okviru projekta smo pripravili gradiva za osem novih praktičnih aktivnosti (Vilhar in sod. 2007). Učna gradiva vsebujejo navedbo ustreznih tem in ciljev v učnem načrtu, trajanje aktivnosti, teoretično razlago za učitelje, navodila za pripravo in izvedbo aktivnosti, delovne liste za dijake z rešitvami in komentarji za učitelje (vključno s pričakovanimi rezultati poskusov), povezave na spletne strani z dodatnimi gradivi, seznam strokovne literature, varnostna opozorila, razlago o pogostih napačnih predstavah ter zanimivosti, s katerimi lahko učitelj motivira učence. V učna gradiva smo vključevali tudi zgodbe iz zgodovine znanosti, s katerimi lahko dijakom predstavimo naravo znanosti.

Med tretjo fazo projekta smo nova učna gradiva objavili v priročniku za učitelje (Vilhar in sod. 2007) in na spletnih straneh (http://znanost-gre-v-solo.biologija.org/). Organizirali smo tudi zaključno delavnico za učitelje, na kateri se je širši krog učiteljev in šolskih laborantov usposabljal za izvedbo novih aktivnosti. Projekt so z navdušenjem podprli učitelji (sl. 1B, 1C, 3), sodelujoči znanstveniki in dijaki s partnerskih šol.

Projekt Znanost gre v šolo je bil pilotski projekt, s katerim smo v slovenski prostor uvedli nov model za razvoj učnih gradiv in usposabljanje učiteljev. Čeprav je projekt obravnaval poučevanje biologije, je enak model uporaben tudi za posodobitev drugih naravoslovnih predmetov (naravoslovja, kemije in fizike). Za učinkovito izboljšanje kakovosti biološkega izobraževanja v slovenskih šolah bi morali tovrstne dejavnosti dolgoročno in stabilno financirati iz nacionalnih virov (ALLEA 2009).

## Acknowledgements

We thank Dr Gregor Zupančič and Dr Nejc Jogan for constructive discussions and collaboration on the project *Science Goes to School* / *Znanost gre v šolo*. The project was supported by the European Union (the European Social Fund) and The Ministry of Education and Sport of the Republic of Slovenia (http://znanost-gre-v-solo.biologija.org/).

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**1. Types of Articles**

a) 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.

b) 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.

c) 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.

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

e) ASSOCIATION NEWS reports on the work of Slovene biology associations.

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

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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.

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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.

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

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

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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.

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Starting with the first issue of the 53rd volume the ABS will be processing the graphic material only electronically. All the figures have to be submitted in the electronic form. The ABS publishes figures either in pure black and white or in halftones. 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. 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).

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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.

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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 1994 a,b). 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.

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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):

1. English title .....
   (Times New Roman 14, bold)
2. Slovene title .....
   (Times New Roman 14, bold)
3. Names of authors with clearly indicated addresses, affiliations and the name of the

corresponding author.....
(Times New Roman 12)

4. Author(s) address(es) / institutional addresses.....
(Times New Roman 12)
5. Fax and/or e-mail of the corresponding author.....
(Times New Roman 12)
6. Keywords in English.....
(Times New Roman 12)
7. Keywords in Slovene.....
(Times New Roman 12)
8. Running title.....
(Times New Roman 12)
9. Abstract in English.....
(Times New Roman 12, title – Times New Roman 14 bold)
10. Abstract in Slovene.....
(Times New Roman 12, title – Times New Roman 14 bold)
11. Introduction.....
(Times New Roman 12, title – Times New Roman 14 bold)
12. Material and methods.....
(Times New Roman 12, title – Times New Roman 14 bold)
13. Results.....
(Times New Roman 12, title – Times New Roman 14 bold)
14. Discussion.....
(Times New Roman 12, title – Times New Roman 14 bold)
15. Summary in Slovene.....
(Times New Roman 12, title – Times New Roman 14 bold)
16. Figure captions; each in English and in Slovene.....
(Times New Roman 12, figure designation and figure title – Times New Roman 12 bold)
17. Table captions; each in English and in Slovene.....
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18. Acknowledgements.....
(Times New Roman 12, title – Times New Roman 14 bold)
19. Literature.....
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