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Estimation of current population status of the Alcon large blue *Phengaris alcon* (Denis & Schiffermüller, 1775) (Lepidoptera: Lycaenidae) in Bela krajina (SE Slovenia) based on egg counts

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Abstract. An estimate of population size of the endangered Alcon large blue (*Phengaris alcon*) in Bela krajina (SE Slovenia) was performed based on egg counts. The presence of eggs has been confirmed in all seven currently known populations of the larval host plant *Gentiana pneumonanthe* in the area. Owing to the short distances between the populations it is probable that the species forms a single metapopulation with an estimated population size of about 190 individuals. One local extinction is documented on a site where *P. alcon* was present in 2008. We propose conservation measures to preserve the existence of *P. alcon* in Bela krajina.

Key words: *Phengaris alcon*, *Gentiana pneumonanthe*, Papilionoidea, Bela krajina, Slovenia, oviposition, population size, species conservation

Izvleček. Ocena stanja populacije sviščevega mravljiščarja *Phengaris alcon* (Denis & Schiffermüller, 1775) (Lepidoptera: Lycaenidae) v Beli krajini (JV Slovenija) na podlagi štetja jajčec – V članku je podana ocena velikosti populacije ogrožene vrste metulja sviščevega mravljiščarja (*Phengaris alcon*) v Beli krajini z uporabo metode štetja jajčec. Jajčeca so bila potrjena na vseh sedmih znanih rastiščih hranilne rastline močvirskega svišča (*Gentiana pneumonanthe*) na območju raziskave. Zaradi majhnih razdalj med posameznimi najdišči je verjeten obstoj ene metapopulacije z ocenjeno velikostjo okoli 190 osebkov. Zabeležili smo izgin sviščevega mravljiščarja iz habitatne krpe, kjer se je še pojavljal leta 2008. Predlagamo varstvene ukrepe za ohranjanje habitata sviščevega mravljiščarja v Beli krajini.

Ključne besede: *Phengaris alcon*, *Gentiana pneumonanthe*, Papilionoidea, Bela krajina, Slovenija, ovipozicija, velikost populacije, ohranitev vrste

Introduction

Butterflies of the genus Phengaris (Lepidoptera: Lycaenidae) in Slovenia are represented by four species: *Phengaris arion* (Linnaeus, 1758), *P. teleius* (Bergsträsser, 1779), *P. nausithous* (Bergsträsser, 1779) and *P. alcon* (Denis & Schiffermüller, 1775) (Verovnik et al. 2012). Species from the genus *Phengaris* have been intensively studied due to their interesting and unique life cycle (Als et al. 2002, Mihoci et al. 2007, Czekes et al. 2014). The availability of both specific host plants and specific *Myrmica* ant hosts is essential for the survival of *Phengaris* butterflies species (Nowicki et al. 2005).

Caterpillars spend their early larval stages feeding inside the flower buds of their host plants. After reaching their fourth instar, the larva falls to the ground, waiting for a foraging *Myrmica* ant to adopt it and take it into the nest (Thomas et al. 2010, Czekes et al. 2014). In ant colonies they either prey on ant brood in the case of predatory species (*P. arion*, *P. nausithous* and *P. teleius*) or are fed by nurse ants in the case of the cuckoo species (*P. alcon*) (Nowicki et al. 2005, Czekes et al. 2014). After spending at least 10–11 months in the ants' nest, gaining about 98% of their final biomass, they pupate during late spring (Als et al. 2002, Czekes et al. 2014).

The genus *Phengaris* is thought to have evolved in the steppes of central Asia (Sibatani et al. 1994, Als et al. 2002). The European *Phengaris* species were pre-adapted to survive and disperse in traditional European agricultural landscapes (Als et al. 2002). Today, all the *Phengaris* species in Europe are threatened and are declining due to habitat fragmentation, habitat loss, habitat degradation and changes in agricultural management (Als et al. 2002, Maes et al. 2004). As a result, all *Phenagris* species were included in the red lists of most European countries (Czekes et al. 2014). In Slovenia, all four species from the genus *Phenagris* are on the Red List of Slovenian Butterflies (Ur. l. RS 2002) and are also protected at the national level with the Decree on protected wild animal species (Ur. l. RS2004a). Two species (*P. teleius* and *P. nausithous*) are listed on Annex II and Annex IV of The EU Habitats Directive 92/43/EEC, while *P. arion* is listed in Annex IV. In Slovenia, *P. alcon* is listed as 'endangered' (EN) species and is one of the fastest declining butterflies in Slovenia (Verovnik et al. 2012). It has disappeared from large parts of its range, becoming possibly extinct in the Koroška region and having only a handful of isolated populations left in the Štajerska, Gorenjska and Bela krajina regions (Verovnik et al. 2012).

P. alcon has two ecotypes based on larval host plant and habitat type (f. *alcon* uses *G. pneumonanthe* and f. *rebeli* uses *G. cruciata*). Here, we focused on f. *alcon* which lives on humid grasslands, but its populations are very local and rare (Verovnik et al. 2012, Rebeušek 2006). The only regions where it can be locally abundant are in the karst poljes – flat depressions in the Dinarides, the edges of the Ljubljansko barje south of Ljubljana, and in the eastern part of Goričko (Verovnik 2000, Verovnik et al. 2012). The distribution of *P. alcon* in Bela krajina (study area) is well known (Verovnik & Škvarč 2002).

The aim of our study was to assess the population status of *P. alcon* in Bela krajina (SE Slovenia). We counted the eggs of *P. alcon* and the genets of *G. pneumonanthe* at locations with known presence of *P. alcon* or *G. pneumonanthe* from the literature. In addition, localities with suitable habitat were checked for possible new findings of larval host plant (*G. pneumonanthe*) or *P. alcon*. The results of the study are important for the conservation of *P. alcon* in Bela krajina.

Materials and methods

Study area

Our study was conducted in the central part of Bela krajina (SE Slovenia) around the village of Dragatuš with existing old data on occurrence of *P. alcon* or *G. pneumonanthe*. The central part is a flat karst plain (150–200 m a.s.l.) bordered to the north by the Gorjanci Hills, westwards by the karst plateau Kočevski rog, while in the south and towards the east it borders to the Kolpa River, which also forms the border with Croatia.

Bela krajina is a region of temperate continental (sub-Pannonian) climate with annual precipitation of 1200–1300 mm. The types of soil are a reflection of an overwhelming carbonate bedrock. The karst landscape of Bela krajina is characterized by scarce superficial water flows and an intense underground water connection. The Lahinja is the largest river in Bela krajina and flows into the Kolpa (Štangelj & Ivanovič 2013). The Lahinja meanders through the plain forming wetlands that are partly conserved, for example Lahinjske luge and Nerajske luge which are complex wetlands consisting of wet meadows, marshes and reed beds. Of the few existing tributaries, it is worth mentioning the Podturščica, which is app. 3.5 km long, mainly regulated stream. The only part left with meanders are the first 500 m near the spring. Until the beginning of the 20th century the landscape of Bela krajina was agricultural. After mass human emigration in the 20th century and changes in economic activities, the landscape has become heterogeneous with a substantial proportion of land being overgrown with forest (Paušič & Čarni 2012). Compared with the 20th century, the agriculture is today intensive and present in the flat lowlands. The streams were mainly regulated and the agricultural land drained in the 1980s.

The study area is partly protected by law. Lahinjske luge and Nerajske luge are two natural reserves within Lahinja Regional Park (Ur. l. RS 1998). The Lahinja and Podturščica rivers with the pertaining wet meadows are listed as valuable natural features (Ur. l. RS 2004b).

Field work

Field work was carried out on 28. and 29. 7. 2015. Flight period of *P. alcon* in Slovenia begins in June and lasts until early September; however, it depends on the region and on the season. At our study sites we assumed that the flying period was at its end, although we still saw some egg-laying females (at three locations). The egg count is the standard method for assessing the abundance of *Phengaris alcon* (Maes et al. 2004, Van Swaay et al. 2012). The white eggs are very conspicuous on the green flower buds of *G. pneumonanthe*; and most of the (empty) egg shells remain on the host plant until about two weeks after the flight season (Maes et al. 2004), because the larvae do not eat them after eclosion (Ebert & Rennwald 1993, as cited in Verovnik 2002).

Potential habitat patches for *P. alcon* and *G. pneumonanthe* were determined by existing data from Verovnik & Škvarč (2002), data in the database of the Centre for cartography of fauna and flora (15.7.2015; unpublished data of A. Škvarč, M. Govedič, B. Frajman, V. Zakšek and R. Verovnik) and data of locations of *G. pneumonanthe* mentioned by Ivanovič (1983) and Dražumerič (1992). All these locations were subsequently checked for *P. alcon* and *G. pneumonanthe*, including the wet meadows around Podturščica where data for the host plant exist but are spatially inaccurate. The locations were first examined for *G. pneumonanthe*, for which we inspected the meadows and the forest edge (Fig. 1). At locations with small populations of *G. pneumonanthe* we counted all the genets and the number of eggs on each genet. At locations with bigger populations we divided the meadow into different plots according to clear difference in density of genets or a human factor (one part of the meadow being mowed). Afterwards we delimited the habitat patches according to our field survey in ArcGIS (ver 10.2.2, ESRI 2014). In two of our study sites, where *G. pneumonanthe* was abundant (Lahinjske luge and Obrh), we estimated the population of genets and eggs using quadrat method. We randomly threw a stick in the meadow where we placed a 5×5 m^2 quadrat. Eggs and genets were then counted in ten randomly selected quadrats at Lahinjske luge and five at Obrh. Because the data of eggs and genets per quadrats were not normally distributed we log transformed the data to calculate the confidence intervals. The total number of genets on sites where their populations were small was probably underestimated, because some genets did not flower and therefore were very difficult to spot among the grass. However, we consider the number of such genets to be relatively small as we thoroughly inspected the meadows. To get an estimate of population size of imagos (EPS) we assumed that: (1) a female lays on average 80 eggs (Maes et al. 2004, Mouquet et al. 2005), (2) the sex ratio in the population is 1:1. The EPS was calculated by:

$$\text{EPS} = \frac{no.\ eggs * 2}{80}$$

In case of fewer than 40 eggs the number was rounded to 1 individual.

Maes et al. (2004) determined the maximum local movement distance based on mark-release-recapture as 500 m. They treated flight areas separated by more than 500 m as different populations. Maes et al. (2004) also determined the maximum observed colonization capacity as 2000 m, based on distances from newly colonized habitat patches to the previously existing ones. Thus we defined distinct populations as habitat patches > 500 m apart. Conversely we treated disjoint habitat patches of < 500 m as one population.

Results

In our field study we investigated a total of 35.7 ha potentially suitable habitat in Bela krajina (Fig. 1). Figure 1 shows the current distribution of *P. alcon* and *G. pneumonanthe* based on our study. We recorded 7 populations of *P. alcon* within the studied area. The discoveries of *P. alcon* at the locations Dragatuš and Podlog (habitat patches 6 and 2 in Fig. 1, respectively) are new for the area. *P. alcon* disappeared from the meadow »F« at the location of Nerajske luge (Fig. 3). The adult *P. alcon* on meadow »8« (Fig. 1) found in 2001 (Verovnik & Škvarč 2002) was probably a dispersing individual, as no *G. pneumonanthe* were recorded at the location (neither in 2001 or 2015).

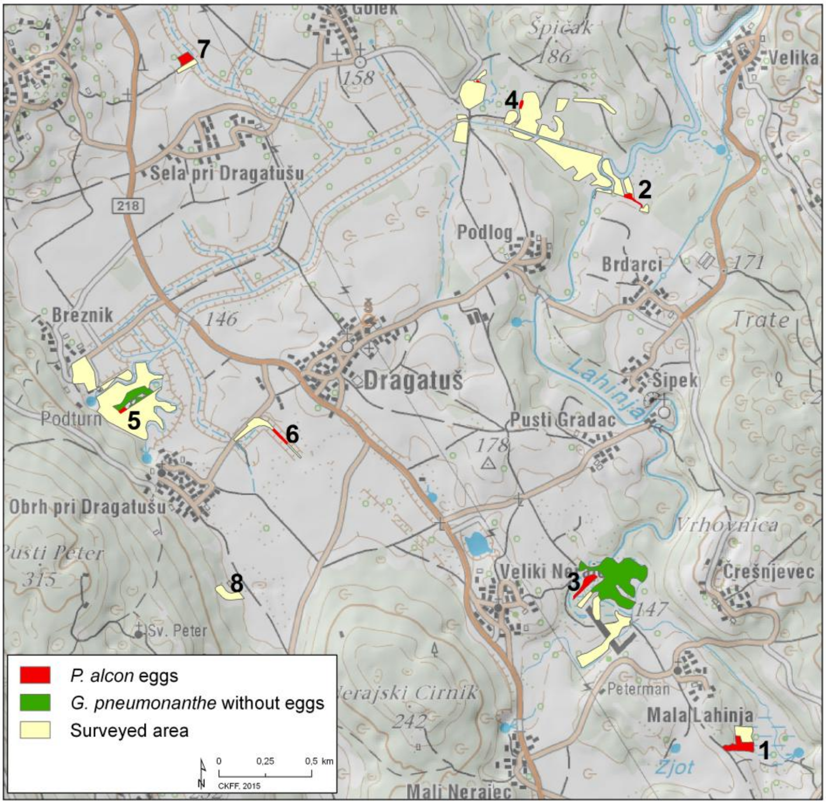

Figure 1. Current distribution of *P. alcon* and its larval host plant *G. pneumonanthe* in the study area in Bela krajina. Location numbers correspond to: 1 – Lahinjske luge, 2 – Podlog, 3 – Nerajske luge, 4 – Podturnščica, 5 – Obrh, 6 – Dragatuš, 7 – Sela and 8 – Pašnik pri Obrhu

Slika 1. Trenutna razširjenost sviščevega mravljiščarja (*P. alcon*) in njegove hranilne rastline močvirskega svišča (*G. pneumonanthe*) na študijskem območju Bele krajine. Številke lokacij predstavljajo: 1 – Lahinjske luge, 2 – Podlog, 3 – Nerajske luge, 4 – Podturnščica, 5 – Obrh, 6 – Dragatuš, 7 – Sela and 8 – Pašnik pri Obrhu

The lowest number of eggs was at Sela, which held 5 eggs (Tab. 1), and the highest at Lahinjske luge with an estimated number of 4,032 eggs (532–4593, p=0.05). The largest number of eggs present on one genet was 289 at the location Podlog.

Table 1. Estimation of the population size of *P. alcon* in Bela krajina. With information on the number of the habitat patch corresponding to Fig. 1 (No), area of habitat patch hosting *P. alcon* eggs (A), the number of *G. pneumonanthe* in habitat patch (#GP), number of eggs (#eggs), estimated population size of imagos based on number of eggs (EPS), density of *G. pneumonanthe* per hectare (GP/ha), presence of adult butterflies at the habitat patch during egg count (Adults, Y = present, N = not present), existence of old data for presence (G = *G. pneumonanthe*, A = *P. alcon*) and the year of the old data. The numbers which are represented with confidence intervals were obtained by quadrat sampling. Otherwise the census on the habitat patch was complete.

Tabela 1. Ocena velikosti populacije sviščevega mravljiščarja (*P. alcon*) v Beli krajini. S podatki o številki habitatne krpe (No), površini habitatne krpe s prisotnimi jajčeci sviščevega mravljiščarja (A), številu močvirskih sviščev (*G. pneumonanthe)* na habitatni krpi (#GP), številu jajčec (#eggs), ocenjeni velikosti populacije odraslih osebkov na podlagi štetja jajčec (EPS), gostoti močvirskega svišča na hektar (GP/ha), prisotnost odraslih osebkov na habitatni krpi med štetjem jajčec (Adults; Y = prisotni, N = odsotni), starejših obstoječih podatkih prisotnosti (G = *G. pneumonanthe*, A = *P. alcon*) s pripisom letnice najdbe. Številke zapisane z intervali zaupanja smo pridobili z metodo kvadratov. V ostalih primerih smo popisali celotno površino habitatne krpe.

No	Location	A (m^2)	#GP	#eggs	EPS	GP/ha	Adults	Old data	Year
1.	Lahinjske luge	5929	6735 (1112-7143)[a]	4032 (532-4593)[a]	101 (13-115)[a]	11360 (1877-12048)[a]	Y	G, A	2001
2.	Podlog	1037	405	2654	66	3909	Y	–	–
3.	Nerajske luge						Y		
	Patch »D«[b]	4143	322	514	13	777	Y	G, A	2008
	Patch »E«[b]	49533	18	0	–	4	N		
	Patch »F«[b]	20397	0	–	–	–	N	G, A	2001
4.	Podturnščica	627	29	172	5	462	N	–	–
5.	Obrh[c]	3396[c]	2853 (1887-3769)[ad]	120[d]	3	8402 (5556-11097)[a]	Y	G	2008
6.	Dragatuš	1155	109	32	1	944	N	G	2001
7.	Sela	3784	18	5	1	48	N	G, A	2001
8	Pašnik pri Obrhu	6140	0	–	–	–	N	A	2001

[a] p = 0,05
[b] position of patches shown in Fig. 3
[c] two different methods were used at this location, see Fig. 2
[d] the mown part of the meadow was not counted

The two locations where we used different methods to estimate the population of eggs and genets are shown in Figs. 2 and 3. The patch »E« (Fig. 3) had 18 genets, but without eggs. The patch »D« (Fig. 3), on the other hand, hosted a population of 322 genets with 514 eggs. The patch »F« (Fig. 3) hosted a population of genets with eggs in 2001 (Verovnik & Škvarč 2002) but is now locally extinct.

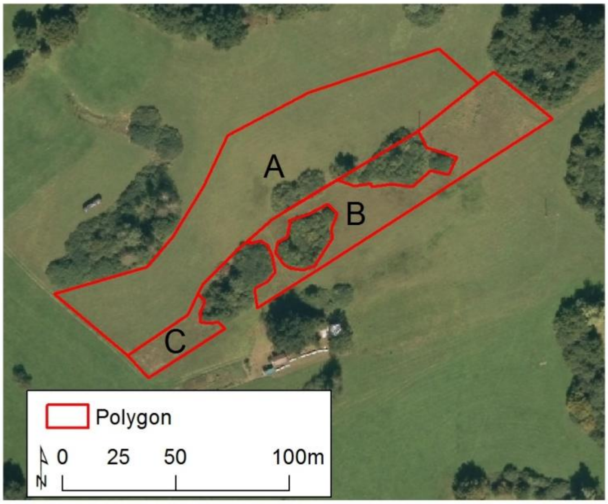

Figure 2. Orthophoto of the location Obrh with polygons delineating different parts of the meadow. Mowing status (on 29. 7. 2015) and method used, **A**: mown, *G. pneumonanthe* present but not counted; **B**: not mown, quadrat sampling; **C**: not mown, total census of eggs and host plant.

Slika 2. Ortofoto posnetek lokacije Obrh. Poligoni razmejujejo različne dele travnika. Pokošenost (dne 29. 7. 2015) in metoda, **A**: pokošeno, močvirski svišč (*G. pneumonanthe*) prisoten ampak brez štetja rastlin; **B**: ni pokošeno, metoda kvadratov; **C**: ni pokošeno, popolno štetje jajčec in rastlin.

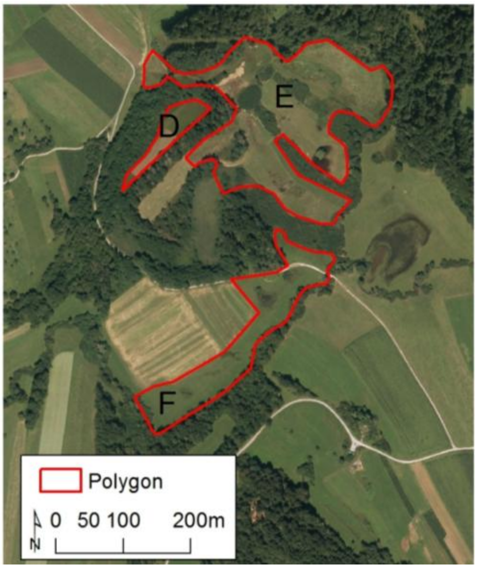

Figure 3. Orthophoto of the location Nerajske luge with polygons delineating different parts of the meadow. Mowing status (on 29.7. 2015) and method used; **D**: not mown, total census of eggs and host plant; **E**: mown, 18 genets with no eggs; **F** mown, no genets, locally extinct.

Slika 3. Ortofoto posnetek lokacije Nerajske luge. Poligoni razmejujejo različne dele travnika. Pokošenost (dne 29. 7. 2015) in metoda, **D**: ni pokošeno, popolno štetje jajčec in rastlin; **E**: pokošeno, 18 močvirskih sviščev (*G. pneumonanthe*); **F**: pokošeno, brez močvirskega svišča, lokalni izgin vrste.

Discussion

P. alcon often occurs in small and isolated, but stable habitat patches, supported by a few hectares of suitable land (Nowicki et al. 2005). This might also be the case in Bela krajina. If we strictly follow the 500 m parameter for determining different populations, we have seven separate populations of *P. alcon* in Bela krajina (Lahinjske luge, Nerajske luge, Obrh, Dragatuš, Podlog, Podturnščica and Sela). However, because the distances between adjacent populations are never more than the colonization capacity of 2000 m, we can expect occasional migrations between populations. This implies a classic metapopulation (sensu Hanski 1999) of *P. alcon* in Bela krajina. In its classic form (Hanski 1999), metapopulations experience frequent extinctions and colonisation events. In Bela krajina four populations (Obrh, Dragatuš, Podturnščica and Sela) have less than 5 EPS and relatively small *G. pneumonanthe* population (< 110, except Obrh). This makes them highly vulnerable to extinction but also to re-colonization from bigger adjacent populations. *P. alcon* densities predominantly depend on density of *G. pneumonanthe* (Nowicki 2007) and Obrh is the only location in our study that stands out in this view (Tab. 1). The population of *G. pneumonanthe* at Obrh is extensive with more than 2853 genets (without counting the mown half of the meadow, see Fig. 2) but the EPS is only 3. Although we have certainly missed some eggs using the quadrat method on the patch »B« (Fig. 3) we could expect a bigger population of *P. alcon*. The location was known for *G. pneumonanthe* but not *P. alcon* in 2008 (Zakšek, CKFF database), although eggs could had been overlooked. The meadows were certainly mown in the last years which might account for the low local population. Even during our study, half of the suitable habitat with *G. pneumonanthe* was mown (Fig. 2). Early mowing (June and July) is not detrimental to *G. pneumonanthe* but makes it impossible for the larvae of *P. alcon* to survive or eliminates stems suitable for egg laying. It will depend on the mowing regime in the years to come whether this population might increase or go extinct. Local extinction occurred in 2001 at location Nerajske luge habitat patch »F« (Fig. 3) where no *G. pneumonanthe* has been found in 2015. The cause might be excessive mowing and fertilization (Oostermeijer 1994). It is interesting that the small and isolated location Sela hosted 5 eggs and 18 genets (2015), which is very similar to 6 eggs and 20 genets in 2001 (Verovnik & Škvarč 2002). *G. pneumonanthe* can reach a high age of more than 30 years (Oostermeijer 1994), so it is possible these 18 plants are the same as in 2001. It is doubtful that this is a self-sustained population, and it probably depends on frequent recolonizations from the nearby bigger populations.

We can determine three main populations for *P. alcon* in Bela krajina: Lahinjske luge, Podlog and Nerajske luge with 101, 66 and 13 EPS, respectively. We estimate that Obrh is also an important local population based on arguments already stated and a high potential for increase of the population (which presumably depends on the mowing regime).

Implications for conservation

The current populations of *G. pneumonanthe* in Bela krajina are small and localized and thus prone to extinctions. All of them host eggs, implying that the host plant is the main limiting factor for *P. alcon*. The summed number of imagos in the whole metapopulation is 190. Nowicki (2007) surveyed a population of slightly more than 500 individuals of *P. alcon* in Poland by the method of egg count and the population remained stable over the course of two years. The metapopulation in Bela krajina might thus be stable even though the number of individuals seems low. Monitoring in the next years would show the trend for *P. alcon* in Bela krajina and discoveries of new habitat patches with *P. alcon* are also possible.

At the time of our field work some patches with *G. pneumonanthe* were already mown. The biggest patch already mown was at Obrh (Fig. 1). We assume that the frequent mowing at this patch might be the cause for the low number of individuals of *P. alcon*. Although mowing is used to promote grassland *G. pneumonanthe* populations (Oostermeijer et al. 1994), it must be done in late September when *P. alcon* larvae have already left *G. pneumonanthe* (Mouquet et al. 2005). Mowing in July and August should be avoided. Another unfavourable management practice is applying fertilizers, since this leads to eutrophication which deteriorates the living conditions for the *G. pneumonanthe* and should be avoided in meadows where the plant grows.

All the core populations except Lahinjske luge are part of valuable natural features (Ur. l. RS2004bc).

Povzetek

Močvirska forma sviščevega mravljiščarja (*Phengaris alcon* f. *alcon*) je ena najbolj ogroženih vrst metuljev v Sloveniji in je že izginila iz več območij prvotne razširjenosti (Verovnik 2012). Na Rdečem seznamu metuljev (Lepidoptera) je v kategoriji prizadeta vrsta (E). Življenjski prostor so mokrotni travniki, kjer uspeva močvirski svišč (*Gentiana pneumonanthe*). To so pri nas oligotrofni mokrotni travniki (Rebeušek 2006).

V članku so predstavljeni rezultati štetja jajčec sviščevega mravljiščarja (*Phengaris alcon*) v Beli krajini. Najprej smo poiskali stare podatke o pojavljanju sviščevega mravljiščarja in močvirskega svišča v Beli krajini. Terensko delo smo opravili 28. in 29. 7. 2015. Na terenu smo pregledali stare lokacije ter bližnjo okolico za morebitne nove najdbe. Na lokacijah z manjšimi populacijami močvirskega svišča smo prešteli vse rastline ter jajčeca. Kjer so bile populacije večje (Obrh in Lahinjske luge), smo uporabili metodo kvadrata velikosti 5×5 m. Sviščev mravljiščar leti v Sloveniji od začetka junija do začetka septembra, odvisno od lokalitete (Verovnik et al. 2012). Kljub temu, da smo na treh lokalitetah opazovali leteče samice (skupaj tri osebke), ki so še odlagale jajčeca, ocenjujemo, da se je sezona odlaganja jajčec zaključevala in zato končno število jajčec ne bi bilo bistveno višje, če bi šteli kasneje v sezoni.

Skupno smo pregledali 35,7 ha površin primernih habitatov (mokrotni travniki, gozdni rob, travniki v zaraščanju itd.) v Beli krajini. Zabeležili smo 7 populacij sviščevega mravljiščarja. Ker razdalje med sosednjimi populacijami metulja nikoli niso bile večje od 2000 m, so migracije med habitatnimi krpami možne. Sklepamo lahko, da gre za obstoj ene metapopulacije (sensu Hanski 1999). Največje lokalne

populacije vrste so bile v Lahinjskih lugah, Podlogu in Nerajskih lugah s 101, 66 in 13 ocenjenimi osebki (EPS), v tem vrstnem redu.

Večina trenutnih populacij močvirskega svišča v Beli krajini je majhnih in lokalnih ter zato izpostavljenih večji verjetnosti izumrtja. Vse gostijo populacije sviščevega mravljiščarja, zato menimo, da je prav omejeno pojavljanje rastline glavni omejujoči faktor za sviščevega mravljiščarja v Beli krajini. Pojavljanja mravelj iz rodu *Myrmica,* ki so nujne za življenjski cikel sviščevega mravljiščarja, v naši raziskavi nismo preverjali. Celotno metapopulacijo ocenjujemo na 190 osebkov. Sviščev mravljiščar je znan po tem, da preživi v majhnih populacijah na nekaj hektarih primernega habitata (Nowicki et al. 2005). Spremljanje stanja v naslednjih letih bo pokazalo, kakšna prihodnost čaka sviščevega mravljiščarja v Beli krajini.

Za obstoj sviščevega mravljiščarja je ključno ustrezno upravljanje. Košnja je primerna za ohranjanje travniških populacij močvirskega svišča (Oostermeijer et al. 1994). Le-ta mora biti opravljena pozno v septembru, ko gosenice sviščevega mravljiščarja že zapustijo svišč (Mouquet et al. 2005). Košnja v juliju in avgustu onemogoči odlaganje jajčec, oziroma vodi v propad gosenic. Gnojenje travnikov poslabša rastne razmere za močvirski svišč, zato bi morali na travnikih, kjer raste močvirski svišč, prenehati z gnojenjem. V prihodnje bo potrebna komunikacija z lastniki zemljišč, kjer raste močvirski svišč, in doseči mravljiščarju prijazen način upravljanja.

Vse največje populacije razen Lahinjskih lug so v območju naravnih vrednot (Ur. l. RS2004b).

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Prispevek k poznavanju ihtiofavne pritokov Ljubljanice: Podlipščica, Gradaščica in Horjulščica

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Izvleček. Leta 2013 smo v pritokih Ljubljanice Podlipščici, Gradaščici in Horjulščici z enkratnim izlovom raziskovali vrstno sestavo in maso rib in piškurjev. V Podlipščici smo na dveh lokacijah popisali 13 vrst, ugotovili povprečno maso 329,14 kg/ha in zabeležili 10450 osebkov/ha. V Gradaščici smo, prav tako na dveh lokacijah, popisali 16 vrst, ugotovili maso 180,82 kg/ha in zabeležili 2840 osebkov/ha. V Horjulščici smo popisali 18 vrst, ugotovili maso 756,94 kg/ha in zabeležili 9238 osebkov/ha.

Ključne besede: sladkovodne ribe, piškurji, vrstni sestav, porečje Ljubljanice, Slovenija

Abstract. Contribution to the knowledge of ichthyofauna of the Ljubljanica river tributaries: the Podlipščica, the Gradaščica and the Horjulščica – In 2013, fish and lamprey species were sampled in the tributaries of the Ljubljanica river, i.e. the Podlipščica, the Gradaščica and the Horjulščica, to determine the species composition and weight of fish. In the Podlipščica we recorded 13 species, the weight of fish was 329.14 kg/ha and 10,450 N/ha. In the Gradaščica we recorded 16 species, the weight of fish was 180.82 kg/ha and 2,840 N/ha, while in the Horjulščica we recorded 18 species, the weight of fish was 756.94 kg/ha and 9,238 N/ha.

Key words: freshwater fishes, lampreys, species composition, the Ljubljanica river Basin, Slovenia

Uvod

Celostnih ihtioloških raziskav z oceno velikosti ribjih in piškurjevih populacij na porečju Ljubljanice doslej ni bilo. Edini razpoložljivi podatki izvirajo iz ribiško gojitvenih načrtov ribiških družin Vrhnika, Barje in Dolomiti (RGN 1986-2006), arhivirani so tudi v bazi Ribkat (Zavod za ribištvo Slovenije). V porečju Ljubljanice živi skupaj s piškurjem 41 različnih vrst iz 13 družin. Med temi je 32 domorodnih (vključno s piškurjem) in devet tujerodnih vrst. Prevladujejo krapovske vrste rib (Povž 2008, Urbanc-Berčič et al. 2008).

Leta 2013 so potekale enkratne ihtiološke raziskave pritokov Ljubljanice, Gradaščice, Podlipščice in Horjulščice. Cilj raziskave je bil oceniti razširjenost in velikost populacij rib in piškurja.

Materiali in metode

Gradaščica je od izvira do razcepa v dva kraka dolga približno 14,5 km (ARSO 2007). V bližini gradu Bokalce se razcepi v Mestno Gradaščico, ki je v celoti regulirana kot betonski kanal, in v Mali graben, ki ima zemeljske brežine (ARSO 2007). Oba vodotoka se ločeno izlivata v Ljubljanico.

Horjulščica izvira kot Šujica. Dolga je okoli 16 km (ARSO 2007) in se pri Dobrovi izliva v Gradaščico.

Podlipščica je okoli 13 km dolg levi pritok Ljubljanice pri Sinji Gorici (ARSO 2007). Brežine Horjulščice in Podlipščice so na lokacijah vzorčenja zemeljske in porasle z obrežnim rastlinjem.

Začetek in konec lokacije izlovnih mest smo določili z geografskimi koordinatami (Tab. 1).

Tabela 1. Lokacije vzorčnih mest v pritokih Ljubljanice: Podlipščici, Gradaščici in Horjulščici z okrajšavami (O), datumi vzorčenja in opisi.
Table 1. Sample sites in tributaries of the Ljubljanica river: the Podlipščica, the Gradaščica and the Horjulščica. Including sampling dates and description of sites.

O	vodotok/lokacija	datum vzorčenja	Dolžina (d), širina (š), površina vzorčenja (P)	X	Y
P1	**Podlipščica 1** (Podlipa)	26.9.2013	d = 70 m; š = 2–4 m; **P = 210 m^2**	14° 16' 39.48"	45° 59' 22.27"
P2	**Podlipščica 2** (Velika Ligojna)	26.9.2013	d = 50 m; š = 2–3 m; **P = 125 m^2**	14° 15' 45.7"	45° 59' 23.7"
G1	**Gradaščica 1** Srednja vas	18.9.2013	d = 100 m; š = 12 m; **P = 1.200 m^2**	14° 19' 12.30"	46° 3' 27.44"
G2	**Gradaščica 2** izliv Ostrožnika	18.9.2013	d = 100 m, š = 8 m; **P = 800 m^2**	14° 26' 3.41"	46° 3' 9.46"
H	**Horjulščica** (Brezje pri Dobrovi)	18.9.2013	d = 80 m; š = 4–5 m; **P = 360 m^2**	14° 21' 57.85"	46° 2' 5.11"

Vzorčenje rib in piškurjev je potekalo po dveh različnih metodah, in sicer po metodi za majhne prebrodljive potoke in majhne prebrodljive reke, ker smo v okviru raziskave vzorčili v vodotokih različnih lastnosti (Anonymus 2009). Podlipščica in Horjulščica spadata po normativih Metodologije za vzorčenje in laboratorijsko obdelavo vzorcev za vrednotenje ekološkega stanja rek z ribami (Anonymus 2009) v kategorijo majhen prebrodljiv potok širine do 5 m, z najmanjšo predpisano dolžino izlovljenega odseka 100 m, Gradaščica pa v kategorijo majhna prebrodljiva reka, širine 5–15 m, z najmanjšo predpisano dolžino izlovljenega odseka 100–150 m, z več do veliko avtohtonimi vrstami rib (Anonymus 2009).

Med ogledom in izbiro terena za vzorčenje rib in piškurjev smo izbrali lokacije s čim več različnimi in reprezentativnimi habitati.

Pred začetkom vzorčenja smo vodotok zaprli z mrežo. Z vzorčenjem smo začeli nizvodno od razpete mreže in lovili po toku navzgor vse do zapore z mrežo. Ribe in piškurje smo lovili po standardizirani metodi vzorčenja rib z elektroribolovom (EN14011 2003, Anonymous 2009) z elektroagregatom Hans Grassel z dvema elektrodama (7,5 kW, 600 V, 12 A). Dolžine vzorčnih mest niso bile v predpisanih okvirih, ker konfiguracija struge vodotokov tega ni dopuščala.

Ujete ribje vrste in piškurje smo prešteli, jih določili do rodu oz. do vrste in stehtali na g natančno. Številčnost in maso rib smo ocenjevali relativno, s številom oziroma maso ujetih rib in piškurjev ob enkratnem izlovu na enoto površine (osebki/ha, kg/ha) (Anonymus 2009). Predstavnika rodu *Cottus* nismo določili do vrste, ker je determinacija zahtevna (Bravničar 2012).

Rezultati in diskusija

V pritokih Ljubljanice Podlipščici in Gradaščici in pritoku Gradaščice Horjulščici smo 18. in 26. 9. 2013 vzorčili ribe in piškurje. Ujeli smo 21 različnih vrst (Tab. 2).

V Podlipščici smo na dveh lokacijah ujeli 13 različnih vrst (Tab. 2). Na prvi lokaciji smo ujeli 12 različnih vrst s skupno maso 327,18 kg/ha oziroma 9692 osebkov/ha (Tab. 2). Na drugi lokaciji smo ujeli 11 različnih vrst, skupna masa je bila 331,1 kg/ha oziroma 11209 osebkov/ha (Tab. 2). Povprečna skupna masa ulovljenih rib v Podlipščici, ugotovljena z enkratnim izlovom na dveh lokacijah, je bila 329,14 kg/ha oziroma 10450 osebkov/ha.

Dne 18. 9. 2013 smo opravili ihtiološko raziskavo Gradaščice na dveh vzročnih mestih in Horjulščice na enem vzorčnem mestu.

V Gradaščici smo na prvi lokaciji ujeli devet različnih vrst (Tab. 2). Na prvi lokaciji je bila masa vrst 171 kg/ha zabeleženih je bilo 2115 osebkov/ha (Tab. 2). Na drugi lokaciji smo ujeli 15 različnih vrst (Tab. 2). Masa vrst je bila 190,64 kg, število osebkov pa je bilo 351,4/ha. Povprečna skupna masa ulovljenih rib je bila 180,82 kg/ha, zabeleženih je bilo 2840 osebkov/ha (Tab. 2).

V Horjulščici smo ujeli 18 različnih vrst (Tab. 2). Masa vseh rib je bila 756,44 kg/ha, zabeleženih je bilo 9238 osebkov/ha (Tab. 2).

Ob samo enkratnem izlovu rib in piškurjev smo v treh pritokih Ljubljanice registrirali 21 izključno avtohtonih vrst rib in piškurjev, kar je skoraj 50 % vseh v povodju Ljubljanice živečih vrst (Urbanc-Berčič et al. 2008). Zbrani podatki dokazujejo, da so obravnavani pritoki Ljubljanice z vidika ihtiološke pestrosti in naseljenosti vrst izjemno pomembno območje za celotno nizvodno porečje Ljubljanice do izliva v Savo.

Tabela 2. Seznam vrst, število osebkov na hektar (N/ha) in masa na hektar (kg/ha) rib in piškurjev, ki smo jih ujeli na posameznih vzorčnih mestih Podlipščice (P1 in P2; 26.9.2013), Gradaščice (G1 in G2; 18.9.2013) in Horjulščice (H; 18.9.2013). Spodaj so podana celokupna števila za posamično lokacijo in vodotok. Opis lokacij je podan v Tab. 1.
Table 2. The list of species, number of individuals per hectare (N/ha) and weight per hectare (kg/ha) of fish and lamprey, that were caught at each sampling site in Podlipščica (P1 and P2; 26.9.2013), Gradaščica (G1 and G2; 18.9.2013) and Horjulščica (H; 18.9.2013). In the lowest lines the overall numbers per location and per each river are given. For description of localities see Tab. 1.

	P1		**P2**		**G1**		**G2**		**H**	
Vrsta	N/ha	kg/ha	N/ha	kg/ha	N/ha	kg/ha	N/ha	kg/ha	N/ha	kg/ha
blistavec *Telestes souffia*	2185	18,76	1529	13,12	316	3,60	738	8,44	360	2,76
rečna babica *Barbatula barbatula*	95	0,57	880	3,92	25	0,27	25	0,21	28	0,28
potočna mrena *Barbus balcanicus*	95	1,00	720	12,88	83	0,60	50	0,43	111	9,89
rdečeperka *Scardinius erythrophthalmus*							*	*		
kapelj *Cottus sp.*	143	1,05	1.760	13,52	683	5,70	1.375	7,56	56	0,67
klen *Squalius cephalus*	1.140	163,14	1.440	256,48	8	0,37	50	47,90	420	149,83
pisanec *Phoxinus phoxinus*	95	1,05			17	0,06			336	1,39
podust *Chondrostoma nasus*							50	52,90	1.568	409,03
donavski piškur *Eudontomyzon vladykovi*	333	6,43	400	7,76			13	0,26	56	1,58
navadni globoček *Gobio obtusirostris*	95	1,52	320	4,56			13	0,31	644	5,78
beloplavuti globoček *Romanogobio vladykovi*			80	0,48					28	0,11
potočna postrv *Salmo trutta* m. *fario*	903	60,86	80	6,80	33	3,20		2,23	28	9,17
sulec *Hucho hucho*	95	49,00	*	*	50	82,00	25	28,58	56	77,78
lipan *Thymallus thymallus*					900	74,00	925	39,44		
pisanka *Alburnoides bipunctatus*	4.465	15,62	4.000	11,68			300	2,00	4.456	23,92
navadna nežica *Cobitis elongatoides*							*	*	*	*
mrena *Barbus barbus*									84	21,67
platnica *Rutilus virgo*									252	30,22
rdečeoka *Rutilus rutilus*								0,38	644	12,30
pezdirk *Rhodeus amarus*									111	0,56
ščuka *Esox lucius*	48	8,18								
št. vrst // št. osebkov/ha // masa (kg/ha) na lokacijo	12 // 9692 // 327,18		11 // 11209 // 331,10		9 // 2115 // 171,00		15 // 3564 // 190,64		18 // 9238 // 756,94	
št. vrst // masa (kg/ha) // št. osebkov/ha na vodotok	13 // 10450 // 329,14				16 // 2840 // 180,82				18 // 9238 // 756,94	

V Horjulščici in v Podlipščici je bil prvič ujet beloplavuti globoček. Ta vrsta je v Sloveniji razširjena v porečjih Save, Drave in Mure. V Savi je pogostejša v spodnji Savi, v zgornji in srednji Savi in pritokih so nahajališča redka (Povž et al. 2015), kar je posledica neraziskanosti ihtiofavne zgornje in srednje Save in pritokov. Domnevamo, da živi v številnejših vodotokih Ljubljanskega barja, saj smo ga samo v okviru obravnavane raziskave ujeli v dveh od treh pregledanih rek. Po podatkih o ribah, zbranih v ribiško gojitvenih načrtih ribiških družin Barje, Dolomiti, Vrhnika (1986–2006), ki upravljajo z ribjim življem vodotokov na Ljubljanskem barju, naj bi bila v pritokih Ljubljanice tudi velika nežica (*Cobitis elongata*), kar omenjajo tudi Povž (2008) in Urbanc-Berčič et al. (2008). Za zdaj obstaja en sam podatek iz l. 1998 (osebno opažanje) o njenem pojavljanju v Malem grabnu (Povž et al. 2015). Domnevamo, da je v vodotokih Ljubljanskega barja pogostejša, kot je evidentirano, in da je nepoznavanje razširjenosti posledica neraziskanosti Na Ljubljanskem barju je bil prvič ujet in opisan barjanski kapelj (*Cottus metae*). Vendar je determinacija te vrste zelo zahtevna, zato je navedena v okviru rodu *Cottus sp.* (Bravničar 2012). Tudi ta vrsta zahteva raziskavo razširjenosti tako v pritokih Ljubljanice kot v vseh vodotokih po Sloveniji.

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SHORT COMMUNICATION

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First records of the American bullfrog *Lithobates catesbeianus* (Shaw, 1802) in Slovenia

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Abstract. The American bullfrog *Lithobates catesbeianus* is one of the 100 worst alien species in the world. It has been introduced to several European countries. During our field search activities in May 2014 and June 2015, a male bullfrog advertisement call was heard at Fiesa Lakes in Slovenia. On September 2015, a male specimen of the American bullfrog was caught in a funnel trap at the same location. These are the first records of the occurrence of this invasive species in Slovenia. Further studies should be conducted to evaluate if an actual population is present in the area. Eradication measures should commence immediately as chances for the establishment of naturalized bullfrog populations here are high.

Key words: invasive species, *Lithobates catesbeianus*, Slovenia, first records

Izvleček. Prvi podatki o pojavljanju volovske žabe *Lithobates catesbeianus* (Shaw, 1802) v Sloveniji – Volovska žaba *Lithobates catesbeianus* je ena izmed 100 najhujših invazivnih vrst na svetu. Naseljena je bila v številne evropske države. Med terenskim popisom maja 2014 in junija 2015 je bil pri jezerih v Fiesi v Sloveniji slišan paritveni klic samca volovske žabe. Septembra 2015 se je na isti lokaciji v vršo ujel samec volovske žabe. To so prvi podatki o pojavljanju te invazivne vrste v Sloveniji. Nadaljnje raziskave bi bile potrebne, da se oceni ali je na območju prisotna populacija te vrste. Ukrepi odstranitve te vrste iz okolja morajo steči nemudoma, saj je verjetnost naturalizacije vrste na tem območju visoka.

Ključne besede: invazivne vrste, *Lithobates catesbeianus*, Slovenija, prvi podatki

Introduction

The American bullfrog is one of the 100 worst invasive alien species in the world (Lowe et al. 2000, DAISIE 2008), playing a certain role in the decline of some native species in Europe (Veenvliet & Kus Veenvliet 2002, DAISIE 2008, Kraus 2009). Invasive bullfrogs have a negative impact on native species via competition, predation, and habitat displacement (Pearl et al. 2004). They are also known to transmit diseases like chytridiomycosis to local amphibian fauna (Garner et al. 2006). The American bullfrog is native to western North America but has been introduced to over 40 countries and four continents since the 19th century (Ficetola et al. 2007a). The cause of its introductions was mainly their use as edible livestock (Nava 2010). Before its import ban in 1997, they were also widely sold as animals for garden ponds in garden centres (Parrott & Roy 2009). Despite the fact that new introductions are prohibited by law, translocations by personal initiatives seem to be the primary cause of current introductions (Spitzen-van der Sluijs & Zollinger 2010). In Europe, introductions have been observed in Belgium, France, Germany, Greece, the Netherlands, Italy, Spain and the United Kingdom (Ficetola et al. 2007b, IUCN... 2015). Naturalized populations were recorded at least in Belgium, France, Germany, Greece, Italy and the United Kingdom (Ficetola et al. 2007b). American bullfrogs are known to occur in the neighbouring Italy, with some observations from the vicinity of Udine province (Villa Bruna, Carlino: Lapini et al. 1999, Lapini 2007), where the species had been recorded till 1962 and is now considered extinct (Lapini et al. 1999). Another introduction of the bullfrog was recently quoted by Lapini et al. (2014), but it was probably limited to the release of a single male in a garden fishpond in the village of Buttrio near Udine (L. Lapini, pers. comm. 2016).

Materials and methods

On 30. 5. 2014 and 9. 6. 2015, the area of the two lakes in Fiesa on the Slovenian coast (Gauss–Krüger coordinates Y: 389643, X: 43056 [lat.: 45.523007, long.: 13.582537]) was checked for amphibian activity. Afterward, a two-day field work on invasive species within the framework of activities carried out by Societas herpetologica slovenica was conducted on 27. and 28. 9. 2015. Because of the steep and overgrown water edges, most of the work was done from boats. Both lakes were checked for amphibian activity and sampled with dipnets where possible. 20 funnel traps were set on the shore of both lakes.

The two lakes are located between Piran and Strunjan in Fiesa Bay, at the seafront of the Adriatic Sea. The lakes are man-made and used to be part of a clay pit. The larger lake closer to the sea contains brackish water, while the smaller one is a freshwater lake. Although the lakes were declared a natural monument, they are known to harbour different invasive species, mostly fish (Veenvliet 2007) and turtles (Standfuss et al. 2016).

Results and discussion

On 30. 5. 2014, a male bullfrog advertisement call was heard and recorded in the smaller of the two Fiesa lakes. Subsequently, on 9. 6. 2015, the calling song was heard again at the same locality. On the 28. 9. 2015, one male bullfrog was caught in a funnel trap at the smaller lake (Fig. 1). These are the first known records of the American bullfrog in Slovenia. We also recorded seven other aquatic vertebrate species. Five of them were alien: red-eared slider (*Trachemys scripta elegans*), eastern mosquitofish (*Gambusia holbrooki*), pumpkinseed (*Lepomis gibbosus*), largemouth bass (*Micropterus salmoides*) and goldfish (*Carassius auratus*).

Figure 1. a) Male specimen of American bullfrog caught in the funnel trap in Fiesa, western Slovenia (photo: Katja Poboljšaj), and b) the location where the specimen was caught (red mark).
Slika 1. a) Samec volovske žabe, ujet v vršo v Fiesi, zahodna Slovenija (foto: Katja Poboljšaj), in b) lokacija, kjer smo osebek ujeli (rdeči kvadrat).

The caught specimen was in a poor physical condition and had a trematode infection in the right eye. It was a fully adult male, with snout-to-vent length of 147 mm and a weight of 224 grams. It was taken to the Ljubljana ZOO, where it was tested for chytridiomycosis and ranavirus infection. The results were negative. Although there is no direct evidence, this specimen was likely introduced to the location intentionally or maybe unintentionally with the introduction of fish. There are no known garden ponds or other freshwater standing waters within the dispersal distance.

In the ensuing years, further efforts should be made by the state institutions to check whether this was a single introduced individual, or an actual population is present at the lake and other potentially appropriate locations in the area. In case more individuals are found, the species should be eradicated to prevent further spread and the potential diseases it transmits. Italian populations of bullfrog are often infected by the chytrid fungus *Batrachochytrium dendrobatidis*, playing the role of healthy carriers (Ficetola & Scali 2010). Eradication measures should commence immediately as chances for the establishment of naturalized bullfrog populations here are high. According to the prediction model that is based on the maximum and minimum temperatures, precipitation and human footprint, coastal areas of the Northern Adriatic (including Slovenia) hold a high potential for successful establishment and invasion of this species (Ficetola et al. 2007b). Furthermore, Fiesa Lakes are even more susceptible to naturalization as this 'warm-adapted species' prefers permanent, large, densely vegetated deep waters, where tadpoles can overwinter (Spitzen-van der Sluijs & Zollinger 2010).

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New record of Bechstein's bat *Myotis bechsteinii* (Kuhl, 1817) in NE Slovenia

Nova najdba velikouhega netopirja *Myotis bechsteinii* (Kuhl, 1817) v SV Sloveniji

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In September 2015, I received, as a member of SDPVN - Slovenian Association for Bat Research and Conservation, a phone call from Barbara Makoter, informing me of a dead bat found glued to flypaper in a vineyard (Fig. 1) located in the village of Desnjak (lat. 46.4885 °N, long. 16.1282 °E, 300 m a. s. l), SW of Ljutomer in NE Slovenia. Desnjak is a small settlement of about 130 inhabitants in the wine growing hills of Slovenske gorice, where villages are comprised of scattered houses, interrupted by vineyards, cultivated fields and forest residues. Eight flypapers were set in the vineyard on 18. 9. 2015, and the bat was found there next day – it had stuck on one of the flypapers during the night from 18. to 19. 9. 2015.

I soon received the carcass, which turned out to be an adult or subadult male of Bechstein's bat *Myotis bechsteinii* (Kuhl, 1817). This species has already been found as a victim of flypaper trap in Slovenia, specifically in an orchard in N Slovenia in July 2008 (Lampič & Kodba 2008). In our case, it is the location that makes the finding interesting. Generally, the Bechstein's bat is strongly associated with old deciduous forest stands, which contain many potential roosts in tree holes or trunk crevices (Presetnik & Govedič 2006, Dietz et al. 2009) and present a foraging habitat that is, according to Kerth et al. (2001), less than 1 km from the roost. We can assume that patches of very fragmented forest stands around the finding site offer minimum conditions at least for the species' occasional occurrence in the area. Since the bat was found in autumn, when the time comes for mating and migration between summer and winter roosts (Dietz et al. 2009), only further finds (especially pregnant or lactating females) will provide proof of the species' permanent residency in the area.

This finding of Bechstein's bat is interesting also due to the scarcity of data on this species in Slovenia (Koselj 2009). Our record is the second observation of this species in NE Slovenia; the first concerns an individual from Grad Castle in the region of Goričko (36.5 km north of Desnjak), where a female was found hibernating in the cellars in 2005 (Presetnik et al. 2009). The closest observation of this species comes from the village of Hruščica near Varaždin in Croatia (22 km SE from Desnjak), where one individual was found in a cellar in September 1989 (Pavlinić et al. 2010). Our observation therefore fills in the gap in the knowledge on distribution of Bechstein's bat in NE Slovenia.

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Figure 1. Bechstein's bat (*Myotis bechsteinii*) stuck to flypaper set in vineyard in the village of Desnjak in NE Slovenia (photo: Jasmina Kotnik).

Slika 1. Velikouhi netopir (*Myotis bechsteinii*), prilepljen na muholovec pri vasi Desnjak v SV Sloveniji (foto: Jasmina Kotnik).

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»SOS PROTEUS« – EDITORIAL
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International workshop »SOS PROTEUS: Implementation of monitoring and practical actions for the conservation of *Proteus* in Slovenia« / Mednarodna delavnica »SOS PROTEUS: Zagon monitoringa in praktičnih ukrepov za varstvo človeške ribice v Sloveniji«

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Within the project »With *Proteus* we share dependence on groundwater«, the Tular Cave Laboratory (Society for Cave Biology) organised, together with its hosting partner, the Municipality of Dobrepolje, the international workshop »SOS PROTEUS: Implementation of monitoring and practical actions for the conservation of *Proteus* in Slovenia«. It was held on 12. 12. 2015 in Videm – Dobrepolje, Slovenia, in memory of the cave laboratory in Podpeška jama (1928–1931), and in celebration of 55 years of the Tular Cave Laboratory.

The International workshop gathered 40 researchers and experts on *Proteus*, speleobiology, karstology, water quality, herpetology, conservation and public outreach from Slovenia, Italy, Bosnia and Herzegovina, the Netherlands and Brazil (Fig. 1). The program of the workshop was divided into three sessions: 1. Present status and threats; 2. New methods and approaches; 3. Discussion, conclusions and next steps (Aljančič & Aljančič 2015). Most lectures from the 1st and 2nd sessions are resumed in this issue of Natura Sloveniae.

Figure 1. Participants of the international workshop »SOS PROTEUS: Implementation of monitoring and practical actions for the conservation of *Proteus* in Slovenia« (photo: Gregor Aljančič).
Slika 1. Udeleženci mednarodnega posveta »SOS PROTEUS: zagon monitoringa in praktičnih ukrepov za varstvo človeške ribice v Sloveniji« (foto: Gregor Aljančič).

The discussions in the 3rd session focused on three main topics composed of the critical issues presented below:

1. Implementation of monitoring schemes and conservation management of *Proteus*

- Systematic collection of reliable data on *Proteus* and groundwater status is urgently needed; standards of future monitoring scheme should be carefully defined.
- Protection of *Proteus* groundwater habitat has only recently been associated with the management of the groundwater dependent ecosystems, hence the ecosystemic approach should be integrated in conservation of *Proteus*; the current assessment of chemical parameters should be supplemented by ecological monitoring of groundwater.
- The implementation of monitoring alone cannot improve the status of *Proteus* if not followed by conservation actions.
- Decision-makers should promptly response to emerging threats, and financial support should be provided for research and conservation of *Proteus* and groundwater in order to meet national, EU and international environmental commitments.
- The extremely rare population of *Proteus anguinus parkelj* is particularly in need of urgent protection; a new threat is rising from overuse of biogas slurry in intensive agriculture, and conservation authorities fail to consider the high vulnerability of karst terrains to pollution caused by nitrates from agriculture.

2. Interdisciplinary partnerships, involvement of the international conservation community

- Participants expressed the necessity to strengthen the impact of the *Proteus* and karst groundwater research and conservation community by fostering partnerships and interdisciplinary approaches.
- Sharing knowledge and experience on the preservation of *Proteus*, best practices, particularly on the implementation of the Natura 2000 network is needed among all countries of the Dinaric Karst, particularly in Bosnia and Herzegovina where only around 100 ha are presently protected, with no clear conservation measures defined.
- The present taxonomic status of *Proteus* as a single species does not reflect its true evolutionary diversity and hence hampers appropriate conservation attention to the most endangered evolutionary units.
- Pressure on decision-makers, including petitions and support from environmental lawyers should be applied in order to fully implement obligations from national legislation and EU Directives.
- Major international conservation organizations (herpetological in particular) and EU institutions should be promptly informed on current *Proteus* and groundwater status in the Dinaric Karst in order to raise their attention in urgent search for solutions; a report on the workshop should be sent to the Societas Europaea Herpetologica and IUCN.
- Reassessment of the present status of *Proteus* in the IUCN Red List should be considered.

3. Role of public promotion at local and international levels

- All available communication channels (local, national and international) should be used to raise public opinion, explaining the status of *Proteus* and groundwater, emphasizing importance of groundwater ecosystem services such as drinking water supply.

The discussion was closed by a report of Brazilian colleagues on the karst of Minas Gerais, where a rich subterranean biodiversity is threatened by large scale habitat degradation (Travassos 2015). To conclude, the workshop showed that *Proteus* as a flagship species of the endangered subterranean biodiversity could symbolise the joint aspiration of conservationists around the world to solve environmental problems in karst landscapes.

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Groundwater dependent ecosystems – groundwater status indicators

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Abstract. Within the framework of »Water Management Plan for the Danube River Basin and the Adriatic Sea 2015-2021 (NUV II)«, a study on the groundwater status of karst aquifers which are habitats of groundwater dependent ecosystems (GDEs) was carried out. Among them are the habitats of olms (*Proteus anguinus*), the endemic species of Dinaric karst. These habitats have been included in the Slovenian part of the Natura 2000 network, which aims at protecting and preserving rare, endangered and endemic animal and plant species and habitat types. However, the conservation status of some olm habitats is unfavourable given that the number of specimens is declining. For this purpose we prepared hydrogeological conceptual models, where recharge areas were delineated and quantitative as well as qualitative status of surface and ground-waters were studied. Knowing the basic characteristics of olms, their habitats and critical parameters that adversely affect their conservation status, we identified locations where critical parameters could exceed the threshold values in groundwater. We also identified the areas where and what are the trends of the observed parameters that exceed the natural background (As, Zn) or are undoubtedly of anthropogenic origin (PCB).

Key words: groundwater, groundwater dependent ecosystems, olm, Water Framework Directive

Izvleček. Ekosistemi, odvisni od podzemne vode – indikatorji stanja podzemne vode – V okviru priprave »Načrta upravljanja voda za vodni območji Donave in Jadranskega morja 2015-2021 (NUV II)« smo preučevali tudi kemijsko in količinsko stanje podzemnih voda kraških vodonosnikov, ki so habitat ekosistemov, odvisnih od podzemne vode (EOPV). Med temi habitati je tudi habitat človeške ribice (*Proteus anguinus*), ki velja za endemit dinarskega krasa. Habitati človeških ribic so uvrščeni v slovenski del omrežja Nature 2000, katerega cilj je varovati in ohraniti redke, ogrožene ali endemične vrste živali in rastlin ter habitatnih tipov. Kljub temu je stanje ohranjenosti nekaterih habitatov človeških ribic neugodno, saj število osebkov upada. V ta namen smo na podlagi razpoložljivih podatkov izdelali hidrogeološke konceptualne modele, kjer smo določili prispevna območja virov podzemne vode, od katerih so odvisni ekosistemi, in preučili kemijsko in količinsko stanje podzemne ter površinske vode na podlagi rezultatov različnih monitoringov. S poznavanjem osnovnih značilnosti človeških ribic in njihovega habitata ter z naborom poznanih kritičnih parametrov, ki neugodno vplivajo na njihovo stanje ohranjenosti, smo ugotavljali, na katerih mestih so mejne vrednosti kritičnih parametrov v podzemni vodi presežene ter kje in kakšni so trendi koncentracij onesnaževal v podzemni vodi, ki presegajo naravna ozadja (As, Zn), ali pa so zagotovo antropogenega izvora (PCB).

Ključne besede: podzemna voda, ekosistemi, odvisni od podzemne vode, človeška ribica, Vodna direktiva

Introduction

Groundwater presents the main source of drinking water in Slovenia since it supplies more than 97% of its population (Krajnc et al. 2008). It is also very important for agricultural and industrial use. Actually, more attention is given to the groundwater dependent ecosystems (GDEs) which also play an important role in characterization of the groundwater body status (ARSO 2009). These GDEs require permanent or intermittent access to groundwater to meet all or some of their water requirements, so as to maintain their communities of plants and animals, ecological processes and ecosystem services (Richardson et al. 2011). GDEs include groundwater ecosystems (GWE), groundwater dependent terrestrial ecosystems (GWDTE) and groundwater associated aquatic ecosystems (GWAAE).

Habitats of olms, GDEs studied in this paper, are part of the Natura 2000 protected areas (the EU's ecological network). The aim of Natura 2000 Management Programme for Slovenia for the 2015–2020 period is to ensure the favourable conservation status of species and habitat types (Vlada RS 2015). It includes various GDEs, such as forests, wetlands, caves, aquatic animals, petrifying springs with tufa formation (*Cratoneurion*), etc. Subject of our research were endemic species of olms (*Proteus anguinus*), karst GDEs that have unfavourable conservation status and require restoration and/or preservation. Amphibians are found in groundwater of karst channels and caves in the SW, S and SE parts of Slovenia. Chemical status of groundwater in vulnerable karst aquifers in Slovenia is good on the regional scale but experts claim that olm population, which undoubtedly depends on groundwater status, is declining in some areas (Sket 1997, Bulog 2012). For this reason we studied, within the framework of »Water Management Plan for the Danube River Basin and the Adriatic Sea 2015-2021 (NUV II)«, the relationship between the groundwater quality and quantity and ecosystem’s conservation status.

Materials and methods

Olms are also known as proteus, human fish or salamander (*Proteus anguinus*). This cave-dweller is considered an endemic species of Dinaric karst (ZRSVN 2008). It lives only in groundwater along the Adriatic Sea (Sket 1997). Olms can be found in more than 70 caves in Slovenia (ZRSVN 2008). Beside the white olm (*Proteus anguinus anguinus*), there is also a black pigmented subspecies of olm (*Proteus anguinus parkelj*), which is located around Črnomelj and is known as endemic species of Bela krajina in SE Slovenia (Sket et al. 2003). Olms live in the karst groundwater cave systems and prefer calm and well oxygenated water with stable temperature (Honegger 1981, Sket 1997). They live in phreatic zones that are occasionally flooded into epiphreatic zones during peak discharges, so they depend exclusively on fresh groundwater and do not survive on the dry land (Aljančič et al. 2014). Ecosystems of olms (Tab. 1, Fig. 1) are found in the karst areas where surface water mostly or completely sinks into the ground. Conservation status of olms is classified into 3 priority classes (ZRSVN 2014). 9 GDEs have unfavourable conservation status (the 1^{st} and the 2^{nd} priority), whereas 16 ecosystems achieve favourable conservation status. In our study we focused on olms in unfavourable conservation status, where their population is believed to be declining. Experts estimate that the critical oxygen concentration in groundwater for olms is 2.9 mg O_2/L (at 10° C), but they can also survive in groundwater with less than 1% O_2/L (Bulog 2007). Experts also estimate that groundwater nitrate concentrations (NO_3^-) above 10 mg/L can harm the adult olms as well as the larval stages (Hudoklin 2011).

For the identification and understanding the dependence of GWE on groundwater it was necessary to collect available information on hydrogeological and hydrodynamic characteristics of aquifers in the areas of GDEs. Based on results of past tracer tests and studies for water protection areas, groundwater recharge areas of groundwater resources on which GWEs depend on were delineated and conceptual models were elaborated. Conceptual models describe in the first place the aquifer type, groundwater flow directions and groundwater - surface water interaction. Groundwater chemical and quantity status was studied based on on-site or nearest monitoring sites to GWEs and, moreover, also using surface water monitoring sites where aquifer is recharged by surface water. Monitoring programme is implemented and performed by the Slovenian Environment Agency (ARSO) for ecological, chemical and quantity status, and by the Biotechnical Faculty (University of Ljubljana) for water quality. Additionally, we also examined whether the GDEs are located in the water protected areas, where the abstractions could have a certain impact. Further, we reviewed the published literature and available data from other groundwater monitorings (sources cited in Mezga et al. 2015), land use and possible sources of pollution in the GDEs' recharge areas such as settlements, agricultural land use, industrial facilities, landfills and emissions from facilities. Based on the known environmental supporting conditions as well as critical parameters and threshold values for the olm population, we identified locations with exceeded threshold values for the critical parameters and prepared a list of possible sources for ecosystem deterioration (Tab. 1).

Table 1. List of habitats of olms in the Natura 2000 area in unfavourable conservation status (ZRSVN 2014), possible sources of ecosystem deterioration and measured concentrations of some pollutants in surface- and groundwater.
Tabela 1. Seznam habitatov človeških ribic na območju Nature 2000 v neugodnem stanju ohranjenosti (ZRSVN 2014), možni viri obremenitev ekosistemov in merjene koncentracije nekaterih onesnaževal v površinski in podzemni vodi.

GB ID*	Ecosystem (Natura 2000)	Priority	Possible sources for ecosystem deterioration*	Measured concentrations of some pollutants in surface- and groundwater
1010	Notranjski trikotnik	2	fertilizing Planinsko polje with chicken manure, intensive farming, uncontrolled discharges of municipal wastewater treatment plant, direct runoff from the sewage system, landfills, illegal dumpsites; occurrence of metals in water, poor ecological status of rivers Pivka, Cerkniščica, Reka	Pivka River at Planinsko polje (Bulog 2007, 2012): <5.0 µg Zn/L, <0.5–3.6 µg Cu/L, 3.17 µg Hg/L, 0.25–0.76 µg As/L
1011	Vir pri Stični	1	intensive agriculture, illegal discharges from the sewer system into the underground, settlement with unregulated sewage system, illegal dumpsites, caves and abysses filled with garbage, cultivation of birds and rabbits close to spring	Vir (2003–2006; Seibert 2008): 6.2–25.5 mg NO_3^-/L, 0.06–2.38 mg PO_4^{3-}/L

GB ID*	Ecosystem (Natura 2000)	Priority	Possible sources for ecosystem deterioration*	Measured concentrations of some pollutants in surface- and groundwater
1011	Gradac	2	old sources of pollution (landfills, installations, industrial sites, emission sites, etc.), settlement, agriculture, caves filled with garbage	Krupa (ARSO 2015): presence of PCB
	Dobličica (Jelševnik)	2	old unremediated pollution sources, uncontrolled discharges of urban waste, illegal dumping of waste, renovation of the road above the spring, manure and slurry spills; occurrence of metals in water	Jelševnik (2001–2015; Bulog 2007, 2012; ARSO 2015): 0.9–16 mg NO_3^-/L; 0.01–2.5 mg PO_4^{3-}/L; <5.0–14.6 µg Zn/L; <0.5–9.8 µg Cu/L; 0.29–5.05 ng Hg/L; <0.01–1.20 µg As/L
	Kotarjeva prepadna	2	agricultural and urban areas, illegal dumping of waste, caves filled with garbage	n.d.*
	Stobe - Breg	2	manure and slurry spills, caves filled with garbage, illegal dumping of waste, agriculture and irrational use of fertilizers and pesticides, uncontrolled discharges of municipal wastewater treatment plant, old impacts (insecticide)	Pački potok (2000-2009; Bulog 2012): 2.7 mg PO_4^{3-}/L; Pački breg (2014; ARSO 2015): 4.5–16.5 mg NO_3^-/L, 0.04–0.52 mg PO_4^{3-}/L; Otovški breg (2014; ARSO 2015): 6.5–20.5 mg NO_3^-/L, 0.02–0.32 mg PO_4^{3-}/L
	Petanjska jama	2	agricultural and urban areas, illegal dumping of waste, caves filled with garbage	n.d.*
	Kočevsko	2	manure from pig farm, cattle farming, unremediated old deposits in pits, effluent of waste water into a pit, discharges from municipal waste water treatment plant, municipal landfill, industrial facilities, cave Jama pod Starim Bregom used for industrial landfill for chipboard, resin and melamine panels; poor ecological status of Rinža River	Listed locations (2015; Prelovšek 2015): Vodna jama (pri Klinji vasi) 12.64–16.97 mg NO_3^-/L; Vodna jama pri Cvišlerjih 11.66–22.33 mg NO_3^-/L; Velika Stankova jama 12.30–15.56 mg NO_3^-/L; Remihov mlin 172.36 mg SO_4^{2-}/L; Vodna jama (pri Klinji vasi) 111.15–242.18 mg SO_4^{2-}/L; Željnske jame 12.66–216.61 mg SO_4^{2-}/L; Jama v Šahnu 0.13–2.14 mg PO_4^{3-}/L; Vodna jama pri Cvišlerjih 0.10–2.94 mg PO_4^{3-}/L
5019	Kras	2	landfills, direct discharges of waste water from industrial and municipal waste water treatment plants in the cave, use of fertilizers and pesticides (golf course, horse breeding), winegrowing, unregulated dung and manure storage pits	Jama 1 v Kanjaducah (2004; Mihevc & Rijavec 2006): 207 mg NO_3^-/L, 63 mg SO_4^{2-}/L, 5 mg PO_4^{3-}/L, 204 mg Cl^-/L

* All sources are listed in Mezga et al. (2015); GB – Groundwater Body; n.d. – no data

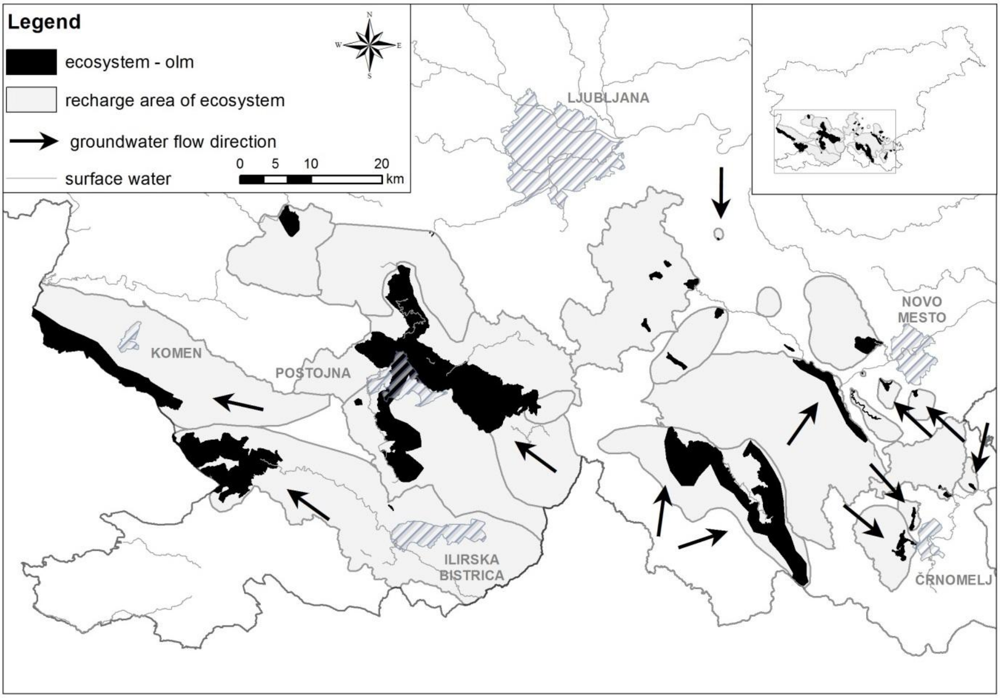

Figure 1. Habitats of olms in the Natura 2000 (ZRSVN 2014) and their recharge areas (Mezga et al. 2015).
Slika 1. Habitati človeških ribic na območju Nature 2000 (ZRSVN 2014) in njihova prispevna območja (Mezga et al. 2015).

Results and discussion

The main pressures on olms in unfavourable conservation status could be linked to poor groundwater quality arising from unremediated old impacts of pollution, illegal dumping of waste, intensive agriculture, unregulated disposal of manure, slurry and pesticides, waste water treatment plants malfunctions, unregulated drainage of industrial and municipal waste disposal sites (especially into caves), which make these GWEs highly vulnerable.

Detailed analysis of the results of national monitoring show that groundwater status at three monitoring sites (Krupa, Otovški breg and Pački breg) can have unfavourable impact on the conservation status of ecosystems due to the presence of polychlorinated biphenyls (PCB) and increased nitrate concentrations (NO_3^-) in groundwater (ARSO 2015). Additionally, the results of other monitorings (Mihevc & Rijavec 2006, Bulog 2007, Seibert 2008, Bulog 2012, Prelovšek 2015) showed that groundwater chemical status is locally deteriorated by increased concentrations of nitrate (NO_3^-), phosphates (PO_4^{3-}), chlorides (Cl^-), sulphates (SO_4^{2-}) (Jama 1 v Kanjaducah, Vodna jama (pri Klinji vasi), Vodna jama pri Cvišlerjih, Željnske jame, Jama v Šahnu, etc.) and metals (As and Zn) (Jelševnik). In the areas of the ecosystems, where no monitoring of groundwater has been carried out, we can assess the status of groundwater based on analysis of the pressures and impacts.

In order to achieve the environmental objectives according to the Water Framework Directive (EU WFD 2000, Article 4) and Decree on groundwater status (Ur. l. RS 2009, 2012), as part of the Water Management Plan for the Danube River Basin and the Adriatic Sea, supplementary measures need to be introduced to prevent any groundwater deterioration that has unfavourable impacts on GDEs. Thus, continuous upgrade of knowledge on GDEs is needed in the following years, including hydrogeological and ecological conceptual models of ecosystems as well as determination of critical parameters and their threshold values. In order to prepare quality basis for the preparation of the necessary supplementary measures, a close collaboration between experts of both mentioned interdisciplinary fields is required.

Groundwater monitorings in the recharge areas of groundwater resources, on which GWEs depend on, have pointed out an important role of GWEs in identifying local groundwater status. Specifically, the conservation status of ecosystems (favourable or unfavourable) can provide additional information on the groundwater status at the local level and can serve as an indicator of local groundwater status. Additional definition of critical parameters and their threshold values for olms would allow narrowing the list of sources of pollution which are affecting groundwater status and consequently ecosystems. The fact that olms are extremely sensitive and vulnerable to changes in the environment (Aljančič et al. 2014) and that their threshold critical values are much lower in comparison with the quality standards or threshold values according to the Decree on groundwater status (Ur. l. RS 2009, 2012), can help us to identify reliable local groundwater status.

In order to restore and preserve damaged GDEs, including endemic species, a favourable conservations status needs to be achieved. This could only be reached when there is sufficient quantity of groundwater of good quality, which also requires knowledge on critical threshold values of critical parameters for individual species and habitats conservation in groundwater.

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Stanje človeške ribice v omrežju NATURA 2000 v Sloveniji

The status of *Proteus* in the NATURA 2000 network in Slovenia

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Človeška ribica z obema podvrstama je ena izmed najbolj pozornost zbujajočih vrst slovenske favnistične zakladnice. Zavarovana je z Uredbo o prosto živečih živalskih vrstah (Ur. l. RS2004), na rdečem seznamu Svetovne zveza za varstvo narave (IUCN) pa opredeljena kot ranljiva. Na ravni Evropske unije je uvrščena v Dodatek II in Dodatek IV Habitatne direktive (92/43/EEC), zato smo v evropsko ekološko omrežje Natura 2000 v Sloveniji vključili 92 najpomembnejših lokalitet človeške ribice v okviru 24 območij, kjer ciljno skušamo vrsto in njen habitat obdržati v ugodnem ohranitvenem stanju.

Države članice EU morajo po 17. členu Habitatne direktive vsakih šest let poročati o uresničevanju ukrepov po tej direktivi (2006 in 2013). Ključni del poročila obsega ocene kazalcev stanja ohranjenosti vrst in habitatnih tipov z vseh prilog. Končno oceno stanja sestavljajo kazalci razširjenosti, ohranjenosti populacije in habitata ter obetov za prihodnost. Največjo zadrego pri oceni stanja je povzročala ocena ohranjenosti habitata in populacije, saj podzemski habitat praviloma ni dostopen. Pri oceni stanja je bila možna le uporaba posrednih indikatorjev, v prvi vrsti kakovosti podzemske vode ter drugih podatkov o stanju habitata in delno populacije. Na državnem nivoju seveda ni vzpostavljen monitoring kakovosti podzemne vode lokacij človeške ribice, populacijski monitoring pa zaradi zahtevnosti ni izvedljiv.

Pri ocenjevanju stanja leta 2013 smo uporabili nekatere rezultate državnega monitoringa kakovosti površinskih in podzemskih voda, ki ga v skladu z zahtevami Vodne direktive (2000/60/EC od leta 2007 opravlja Agencija Republike Slovenije za okolje. V naboru 136 izvirov je le 11 takšnih, ki so habitat človeške ribice. Iz monitoringa stanja površinskih voda so uporabni podatki, ki opredeljujejo ekološko in kemijsko stanje nekaterih ponornic (Pivka, Unica, Ljubljanica, Rinža, Reka). Uporabili smo tudi druge objavljene podatke (npr. Oddelka za biologijo Biotehniške fakultete) ter druge vire (Hudoklin 2011, Kataster jam Slovenije Jamarske zveze Slovenije, Zavoda RS za varstvo narave, lokalni poznavalci).

Pri postavljanju kriterijev za oceno stanja smo izhajali iz predpostavke, da so standardi kakovosti oziroma vrednostni pragi, določeni z Uredbo o standardih kakovosti podzemne vode (Ur. l. RS 2005), za večino parametrov ustrezni tudi za človeško ribico. Izjema so nitrati, pri katerih je vrednosti prag 50 mg/l (normativ za podzemsko in pitno vodo) težko sprejemljiv za človeško ribico. Raziskave namreč kažejo (Blaustein & Wake 1998, Rouse et al. 1999), da dušikova umetna gnojila (amonijev nitrat, kalijev nitrat in natrijev nitrat) skupaj s pesticidi odločilno prispevajo k upadanju dvoživk. Nitrati v obliki natrijevega nitrata imajo zelo škodljiv vpliv predvsem na larvalne stadije in neotenične oblike, kot je človeška ribica, ki so permanentno v vodnem okolju. Vode, v katerih je presežen nivo 10 mg NO_3/L, so za populacijo človeške ribice ocenjene kot zelo neugodne.

Lokacije, za katere smo pridobili podatke o ekološkem ali kemijskem stanju podzemnih ali ponornih voda ali druge objavljene podatke, smo ocenili kot ugodne oziroma neugodne, če je kvaliteta podzemne vode ustrezala oziroma ni ustrezala merilom Uredbe o stanju podzemne vode (Ur. l. RS 2009). Izjema so bili nitrati, ki niso smeli presegati praga 10 mg NO_3/l. Lokacije, za katere ni bilo dovolj ustreznih podatkov, so bile ocenjene na osnovi ocene stanja v zaledju izvirov, ali pa so ostale neopredeljene.

Tako smo ovrednotili 98 lokalitet človeške ribice, nekatere tudi v vplivnem območju Natura 2000, in ugotovili, da je v neugodnem stanju kar 23 (23 %) lokalitet, v ugodnem stanju 56 (57 %), neopredeljenih pa je ostalo 19 (20 %).

Rezultat je zaskrbljujoč. Za vrsto so najbolj obremenjujoča nekatera bremena starih nelegalnih industrijskih deponij. Največ pozornosti zbujata izvir Krupe, onesnažen s kancerogenimi PCB-ji (poliklorirani bifenili), ki so med najbolj toksičnimi in kancerogenimi snovmi (Pezdirc et al. 2011), ter izvir Jelševnik, ki je obremenjen z aromatskimi ogljikovodiki in kovinami. V obeh primerih so bila onesnaževala v visokih koncentracijah zaznana tudi v tkivih živali (Bulog et al. 2002, Bulog 2007).

Zaskrbljujoči so nekateri podatki občasnih preseženih vrednosti pesticidov v podzemskem zaledju izvira Krke in Temenice v Luknji. Vse bolj problematično je obremenjevanje podzemske vode z nitrati iz kmetijskih virov na območju plitvih kraških ravnikov v Beli krajini, na Kočevskem polju ter v okolici Stične. Nekatere podzemske habitate ogroža slabo stanje ponornic Cerkniščice, Pivke med Prestrankom in Postojnsko jamo ter Rinže. V primeru podzemske Reke so bili poudarjeni direktni iztoki neprečiščene vode iz divaške čistilne naprave v Kačno jamo, iz sežanske pa v jamo Bjekovnik in dalje v Jamo 1 v Kanjeducah. Unico bremeni intenzivno gnojenje Planinskega polja s problematičnim kurjim gnojem.

Skupna ocena stanja ob poročanju leta 2013 je bila zaradi ugotovljenega slabega stanja ohranjenosti habitata ocenjena kot neugodna (U1). Enaka ocena za vrsto je bila tudi ob prvem poročanju leta 2006, vendar je bila podana le na osnovi slabih obetov za prihodnost. Ugotovljeno stanje zahteva ukrepanje. Izhodišče zanj podaja Program upravljanja območij Natura za obdobje 2015–2020. Med cilji in ukrepi (priloga 6.1), ki jih je potrdila Vlada RS 28. 5. 2015, so za vrsto podani naslednji:

- v sodelovanju s strokovnjaki razviti sistem monitoringa (vrste/habitata),
- razviti sistem monitoringa intoksiciranih populacij (Krupa, Jelševnik),
- v državni monitoring površinske in podzemske vode vključiti ogrožene lokalitete,
- v izvirih zagotavljati nivo nitratov do 10 mg/l, nivo pesticidov kot v pitni vodi (sprememba zakonodaje),
- sistemsko urediti mejne vrednosti za izpuste iz čistilnih naprav, ki vplivajo na kraški vodonosnik (sprememba zakonodaje),
- sistemsko urediti mejne vrednosti za gnojilne načrte in povečati kontrolo,
- spodbuditi sanacijo lokalitet, kjer je bilo ugotovljeno neugodno stanje,
- povečati ozaveščenost javnosti na problematičnih območjih.

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Functional morphology and environmental studies on *Proteus*

Funkcionalno-morfološke in okoljske raziskave na proteju

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The purpose of this paper is to summarize some recent studies of the research group, Functional Morphological Studies of Vertebrates, of Prof. Dr. Boris Bulog (recently retired). We focused our investigations on the biological adaptations of the blind cave salamander (*Proteus anguinus*) to its habitat in the context of vulnerability to toxic chemical and organic pollution. Our long-term goal was to understand factors that can affect its survival in its natural environment. The following sections highlight some of the main areas of our recent studies.

Reproductive biology. Information on reproductive biology is important for the long-term goal of establishing a successful captive breeding program of this endangered cave amphibian. We have focused on the development of non-destructive methods for identifying sex, since *Proteus* males and females are indistinguishable by external morphological criteria. We have also examined variation in gonad morphology and the dynamics of gametogenesis. Our initial prediction was that we could use chromosomes from cultured blood to identify sex since the closest relative of *Proteus*, *Necturus,* has heteromorphic sex chromosomes. However, our results showed that the sex chromosomes of *Proteus* are homomorphic and therefore not useful for sex identification. On the other hand, we found interesting banding patterns indicating an X-Y translocation (Sessions et al. 2016). The significance of this finding is the basis for future research. The optimization of the non-destructive in vitro method using the cultivation of blood cells has been successfully achieved. We anticipate that we can utilize the culturing methods we have developed to answer several important questions about the cell biology and possibly even developmental genetics of *Proteus*. We are also currently trying to develop molecular markers for identifying sex.

The morphology and histology of gonads, as well as the ultrastructural characteristics of maturation stages of gametes, have been described in detail (Bizjak Mali & Bulog 2010, Kovačič 2013). Our research on gonads shows that reproductive characteristics reflect the fact that *Proteus* lives in a relatively stable habitat with environmental conditions that are conducive to non-seasonal reproductive cycles, however, some peculiarities have been revealed. We found that 1) there is no correlation between the size of the adult animal and condition of the gonads, 2) testis morphology is highly variable and at least three testis morphologies were found, 3) gametogenesis is mainly non-seasonal but with a tendency for maturation of gametes during the autumn for ♂ and winter for ♀, and 4) spermatogenesis and oogenesis may not be synchronous; courtship and insemination via spermatophore can probably occur weeks or months before oviposition as is common for some other salamanders. Surprisingly, numerous atretic follicles were revealed in the ovaries and were more frequent in ovaries of food-deprived specimens (Bizjak et al. 2011, 2013). The affected oocytes were mainly in vitellogenic stages, underlining the fact that yolk resorption by phagocytic cells can be an important energy-conservation process essential for normal development of the remaining oocytes, ensuring the reproductive potential of the species. The most remarkable discovery is that we found testis-ova in testes regardless of the morphology or meiotic condition of the testes (Bizjak et al. 2015, Bizjak & Bulog 2015). Testis-ova in other species of amphibians are usually associated with hormonal dysfunction or possible exposure to endocrine disruptors. In the case of *Proteus* we think they might be related to current evidence that *Proteus* has undergone a sex-chromosome turnover involving X-Y translocation (Sessions et al. 2016).

Conservation biology and monitoring pollution. From the early 1990's to the present, we have conducted environmental investigations of contamination of the cave habitats of *Proteus* and the accumulation of toxic compounds in its tissues. We paid special attention to the underground waters in the SE region of Slovenia, the locality of the black subspecies *P. a. parkelj*, which is an area smaller than 15–20 square kilometres. This is potentially one of the most threatened populations of *Proteus*. The water resources in karst underground are extremely vulnerable to all kinds of pollution and contamination with hazardous organic and inorganic compounds originating from agricultural and industrial development, and require long-term monitoring. Among the most serious chemical pollutants are pesticides, polychlorinated biphenyls (PCBs), and heavy metals which persist in the environment, being slowly, if at all, degraded by natural processes. Our studies revealed the accumulation of zinc and arsenic as well as PCBs in both tissues and habitat of *Proteus*, and we also found a very high level of nitrates in the underground water (Bulog et al. 2002). The elevated concentrations of zinc in the tissues of *P. a. parkelj* were connected with the Zn-rich sludge deposit from an adjacent factory. After our intervention, the deposits were removed and the situation was improved. In the nearby vicinity of Jelševnik there are many vineyards and fields that use arsenic-containing pesticides in agricultural processes that can lead to arsenic accumulation in sediments and consequently to its bio-accumulation in living organisms. The concentration of arsenic found in the tissues of the black *Proteus* was more than 42 times higher than in the underground water of its habitat and about 65 times higher than in the tissues of white species of *Proteus* from unpolluted areas (Bulog et al. 2002). A recent study also revealed the accumulation of high levels of PCBs in the tissues of *Proteus* from the Krupa River, which is at least 28 times higher than those from unpolluted sites (Pezdirc et al. 2011). The results also showed that the Krupa River and its hinterland are still burdened with PCBs. The ability of *Proteus* to survive such a high PCB loading in its tissues is remarkable and deserves further study. The results of the monitoring of physical-chemical parameters in the period from 2000 to 2009 at the localities of the black subspecies of *Proteus* showed high levels of nitrates ranging between 1–9.7 mg/L (in unpolluted waters it is usually not higher than 1 mg/L), and the level increased in 2010 and 2011 when a biogas plant at Lokve in Črnomelj began to operate (Bulog 2012). The values were significantly increased in the spring and reached values higher than 15 mg/L, probably as a consequence of intensive manuring and pouring of slurry onto agricultural surfaces, and the consequent leaching of nitrates into underground water. Therefore, further long-term regular monitoring of potentially harmful pollutants in the underground water, in conjunction with continued studies of the reproductive biology of *Proteus*, is crucial.

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Proteus survival close to an industrial, agricultural and urbanized basin – the case of Kočevsko polje

Preživetje človeške ribice na meji močno industrializiranega, kmetijskega in urbaniziranega prostora – primer s Kočevskega polja

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Together with interconnected Ribniško polje, Kočevsko polje represents the biggest karst polje in Slovenia. Varied geological and geomorphological settings make it heterogeneous and hydrologically complex. Outflow from the middle part of Kočevsko polje is radial due to impermeable strata with thin layers of mined Pliocene brown coal. Water either flows underground directly toward the Radeščica (catchment of the Krka River) or to the Bilpa (catchment of the Kolpa River) via the Rinža River (Fig. 1). The latter forms a parallel hydrological system, draining the waters of the Stojna mountain ridge into sinks between Kočevje and Črni Potok. The presence of sulfide and its oxidation to sulfuric acid at the impervious Pliocene sediments generates highly undersaturated waters (aggressive with respect to calcite) that flow to the contact between non-carbonate and carbonate bedrock. As a result, several cave systems were formed near the settlements Željne, Mahovnik, and Klinja vas. More detailed information on hydro(geo)logy and speleology of Kočevsko polje is provided by Kranjc (1972), Kranjc & Lovrenčak (1981) and Novak (1974, 1987), while Kogovšek & Petrič (2010) provide an overview of all reliable underground water tracing tests in the region.

Anthropogenic pressure at Kočevsko polje is high due to the presence of Kočevje as a regional urban centre surrounded by a suburban belt (wastewater, solid wastes), coal mining (mine wastes; abandoned in 1978) and extensive agricultural production accompanied by intensive animal farming (fertilization, plant protection products, overuse of manure). Especially in the late 1960s, 1970s and 1980s, all of these activities devastated underground habitats which led to the elimination of extremely rich *Proteus anguinus* populations known from two caves (Jama v Šahnu and Vodna jama 1 pri Klinji vasi; Mramor 1968, Belšak 1973, Kranjc 1981). To support and direct concrete conservation actions within the LIFE project Kočevsko, the following research needs were identified:

- definition of current underground water quality and identification of the main polluters responsible for bad water quality,
- the degree of proteus' tolerance to pollution, and
- the use of a biospeleological inventory to estimate the ecological status of underground waters.

To address the first two topics, water was analyzed once per season during 2015/2016 in the stream caves of Kočevsko polje (Vodna jama pri Klinji vasi 1 and 3, Željnske jame, Velika and Mala Stankova jama, Vodna jama pri Cvišlerjih, and Jama v Šahnu), regional springs (Radeščica and Bilpa), and sinking streams (Rinža River, Remihov mlin; Fig. 1; Prelovšek 2016). Water temperature, electrical conductivity, and the saturation as well as the concentration of oxygen were measured in the field. Water samples were taken to the lab to determine alkalinity, as well as concentrations of Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^-, PO_4^{3-}, and Cl^-. Some parameters were calculated (calcite saturation index (SI_{Cal}), equilibrium CO_2 pressure (pCO_2) before and after CO_2 outgassing. Parameters were chosen to describe basic water quality, to roughly delineate catchment area and to identify possible present-day pollutants. At two sites formerly holding rich proteus populations (Jama v Šahnu and Vodna jama 1 pri Klinji vasi), continuous monitoring with an Eijkelkamp CTD Diver with 15-minute sampling interval was performed to get more details on pollution characteristics and sources.

In addition to known localities of proteus at Kočevsko polje (Trata quarter, springs at the foot of the Stojna mountain ridge), two new ones were identified: Velika Stankova jama and a sink near Remihov mlin. The latter, together with much better known Željnske jame, was severely degraded by several metres high deposits of coal washing material (Novak 1974) – during pollution these sites were not likely to be suitable for proteus and represent site where proteus were naturally recolonized after mine closure. In the first lake of Velika Stankova jama, 2–5 animals per visit were usually observed despite poor water quality that may also be a result of an illegal deposit of 30–40 m^3 waste in Mala Stankova jama, located upstream. Observations in Jama v Šahnu and Vodna jama 1 pri Klinji vasi confirmed the absence of any proteus. Natural recolonization of proteus into the cave Jama v Šahnu (if some fraction of the population survived) is not likely due to:

- continued poor water quality as a result of organic pollution that reduced saturation with oxygen below 20% (1.83 mg/L),
- relatively high concentrations of Cl^- and PO_4^{3-} (up to 21.40 mg/L and 2.14 mg/L, respectively) indicating city and agricultural sources, and
- the occasional presence of fish specimens in water.

The source of pollution might be the settlement Spodnji Cvišlerji, which currently has no sewage system and probably drains through the cave (we detected occasionally very high pCO_2 characteristic of percolation water), agricultural production and the Rinža River. Underground flow of the Rinža River was identified by regular daily oscillations of water temperature for more than 1 °C during summer using the CTD Diver in the cave (daily temperature oscillation of percolation water should be in much narrower range) and underground stream discharge (several hundreds of litres per second) that can be supplied only by the Rinža River in Kočevje region. During low water level, underground connection of Jama v Šahnu and Rinža River was successfully proved by tracing test (Novak 1974). Proteus were never observed in Vodna jama 1 pri Klinji vasi despite much better quality of water in comparison with Jama v Šahnu and past conditions. Other caves do not seem to be favourable habitat for proteus due to small upstream water bodies characteristic of the shallow vadose zone and, in the case of Vodna jama pri Cvišlerjih, also severe ongoing pollution.

Despite significantly improved quality of the Rinža River water in the last decade, organic pollution levels are still too high for potential natural recolonization of proteus in the area of Jama v Šahnu. The problem of another source of pollution (untreated sewage water from Spodnji Cvišlerji) should also be resolved to reach adequate water quality during high water levels. The impact of agricultural production above passages of Vodna jama 1 pri Klinji vasi can be observed with increased concentrations of NO_3^- and PO_4^{3-}, but these do not seem to be limiting factors for the recolonization by proteus since water quality in Velika Stankova jama is even worse with respect to NO_3^-, PO_4^{3-} and Cl^-. A more probable reason for the absence of natural recolonization seems to be a complete elimination of the proteus population in the past. Recolonization in areas where sources of pollution were abolished (e.g. Kočevje coal mine closure, sewage system built in the catchment area of Željnske jame) is possible only if some fractions of proteus populations survived in less affected parts of the aquifer – population of 4–14 small animals (up to 15 cm long) in once devastated Remihov mlin seems to indicate proteus recolonization in the last decades. Further downstream toward the regional spring of Bilpa, dilution of polluted waters from Jama v Šahnu with clean percolation water is strong enough to sustain proteus populations even during low discharge when water quality is usually worse. This strongly differentiates the vulnerability of local upstream aquifers in comparison with the downstream regional ones.

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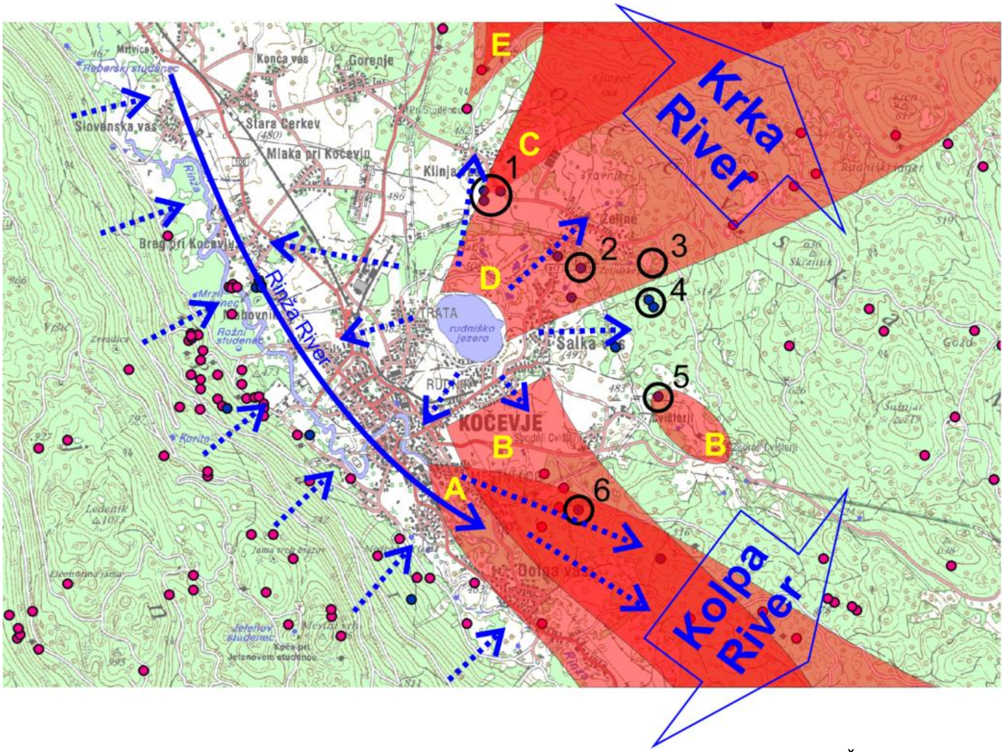

Figure 1. Map of the survey area with sampling locations (1 – Vodna jama 1 and 3 pri Klinji vasi, 2 – Željnske jame, 3 – Remihov mlin, 4 – Velika and Mala Stanova jama, 5 – Vodna jama pri Cvišlerjih, 6 – Jama v Šahnu), general outflow from Kočevsko polje (blue arrows) and major known sources of pollution (A – city of Kočevje/Rinža River (problem partly solved), B – intensive agricultural production, C – untreated manure from pig farm (problem solved), D – coal mining activity (problem partly solved), E – caves with 50+ m^3 of illegally disposed waste). Source of topographic map: Geodetska uprava Republike Slovenije.

Slika 1. Karta raziskovalnega območja z mesti vzorčevanja (1 – Vodna jama 1 in 3 pri Klinji vasi, 2 – Željnske jame, 3 – Remihov mlin, 4 – Velika in Mala Stanova jama, 5 – Vodna jama pri Cvišlerjih, 6 – Jama v Šahnu), generalnim vodnim tokom na Kočevskem polju (modre puščice) in glavnimi identificiranimi viri onesnaževanja (A – mesto Kočevje/reka Rinža (problem deloma odpravljen), B – intenzivno kmetijstvo, C – iztok neobdelane gnojevke iz prašičje farme (problem odpravljen), D – premogovništvo (problem deloma odpravljen), E – jame s preko 50 m^3 ilegalno odloženih odpadkov). Vir topografske podlage: Geodetska uprava Republike Slovenije.

Ogroženost Virskega izvira – klasičnega najdišča človeške ribice

Threats to the karst spring Vir pri Stični – the classical locality of *Proteus*

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Na območju Stične prihajajo človeške ribice na plano v treh izvirih. Virski izvir ali po domače Studenec je izvir Virskega potoka. Leži ob cesti Stična–Šentvid pri Stični pod vasjo Vir pri Stični. Estavela Rupca leži v neposredni bližini glavnega izvira in tudi dodaja ali jemlje vodo Virskemu potoku. Opazovanja kažejo, da se človeške ribice v njej pojavljajo v večjem številu kot v glavnem izviru. V obeh pa se pojavljajo ne glede na to, ali dežuje ali ne. Tretji pa je izvir potoka Rupnica na drugi strani stiškega polja v zaselku Rupe. Potok Rupnica se pojavlja samo ob dežju. Takrat se celotno polje napolni z vodo, a ga gotovo polnijo tudi številni bruhalniki, saj je Rupnica kratek potok, ki ne seže tako daleč, kot je poplavljeno polje. Tu človeške ribice prihajajo na plano le ob dežju, ko jih iz podzemlja potisne bruhajoča voda. O samem pojavu je malo znanega in o človeških ribicah iz Rupnice tudi.

Gotovo je najbolj zanimiv Virski izvir ali Studenec, ker ima bogato zgodovinsko in aktualno zgodbo. Je klasično najdišče človeške ribice. Tu jo je Scopoli odkril leta 1762 (Aljančič 1997, Soban 2004). Iz leta 1825 se je ohranil zapisnik, ki ga poznamo po imenu Stratilov protokol, poroča pa o opazovanju človeške ribice iz tega izvira, ko je »porajala mladiče« (Stratil 1831). Poleg tega, da je nahajališče še vedno aktualno in v njem človeške ribice videvamo dokaj pogosto, je to tudi najdišče prvih dveh v naravi najdenih jajčec. V jajčecih sta bila že kar precej razvita zarodka (Sket & Velkovrh 1978). V zadnjem času pa stroka ugotavlja, da se človeške ribice iz stiških izvirov razlikujejo od vseh drugih populacij te živali (Gorički Š., osebno).

Virski izvir je ogrožen v kar nekaj pogledih. Njegova voda je onesnažena, zlasti visoke so vrednosti nitratov (analize opravil prof. dr. Boris Bulog), k sreči pa v njem niso našli težkih kovin, kljub temu da so v nasutjih okoli njega našli več avtomobilskih akumulatorjev. Vzrok za onesnaženje so vsi videli predvsem v tem, da vasica Vir ni imela kanalizacije. Hiši nad izvirom in čez cesto sta svoje odplake pošiljali kar direktno v potok tik za izvirom. A izvor onesnaženja so lahko tudi fekalije številnih eksotičnih ptic in domačih kuncev, ki jih gojijo pri hiši nad izvirom. Ker je Vir letos dobil kanalizacijo, bi bilo smiselno naslednje leto spet pregledati vodo. Tako bi ugotovili, ali je bil edini vzrok za onesnaženje v tem, da takrat še ni bilo kanalizacije. Tudi če je tako, ostaja še vedno nevarnost okužbe vode in človeških ribic z različnimi paraziti gojenih živalih nad izvirom.

Iz izvira po ceveh črpajo vodo k hiši nad njim. Potrebujejo jo za izpiranje ptičjih in kunčjih kletk in napajanje živali. V preteklosti so celo s svojega dvorišča vrtali v zaledje izvira, da bi kar tam črpali vodo (po izjavi same lastnice hiše nad izvirom). Po pripovedovanju domačinov potekajo vrtanja tako, da v dokaj široko vrtino zlivajo večje količine betona, da bi pod zemljo pregradili vodni tok, za pregrado pa napeljali cev in po njej vodo k sebi. Tukaj jim to ni uspelo, je pa prav v tistem času usahnil tok vode v eni izmed odprtin izvira, čez čas pa je voda našla izhod drugje in zdaj spet teče skozi dve odprtini (po izjavi domačega sina iz hiše nad izvirom).

Ker so prej pri hiši nad izvirom redili konje, so okoli izvira nasuli zemljo, da si konji med skalami ne bi polomili nog (po izjavi same lastnice hiše nad izvirom). Ta grobi poseg so opravili kljub temu, da je izvir na občinski in ne na njihovi parceli. Obstaja fotografija, ki je žal slabe kakovosti, a na njej lahko vidimo, kako so učenci leta 1958 sedeli na eni od skal in z nogami bingljali nad vodo. Zdaj je ta skala kar nekaj metrov oddaljena od vode. To priča, da se je videz izvira z nasipanjem zelo spremenil. Gospodar hiše nad izvirom je tudi vabil vaščane, naj odpadni material nasipajo med cesto in izvirom. Motiv za to nasipanje ni znan. So pa bili do čistilne akcije leta 2004 v tistem nasutju vsakovrstni gradbeni in drugačni odpadki.

Obstaja sum, da iz izvira odtujujejo človeške ribice, ki priplavajo na plano. Sum se je pojavil že, ko je domači sin, ne da bi ga kdo o tem kaj spraševal, hitel razlagati, kako ga neki ljudje napeljujejo h kraji človeških ribic. Povečal se je, ko so fantje iz vasice Vir, ki jih mladi stiški gostilničar ni hotel imenovati, temu gostilničarju ponujali, da mu nalovijo človeške ribice za gojenje v atraktivnem podzemnem gostinskem prostoru za mladino. Sum podpirajo tudi vaške govorice o kraji človeških ribic.

Izvir je ogrožen tudi zato, ker gospodar hiše nad izvirom skuša preprečevati obiske izvira drugim ljudem, med njimi biologom – raziskovalcem človeške ribice. Taki obiski se občasno končajo z grožnjami, da bo na obiskovalce spustil pse, obiskovalci pa so morali tudi že klicati na pomoč policijo.

Leta 2003 se je za izvir začela zanimati Občinska turistična zveza z namenom, da ga kot zanimiv zgodovinski spomenik uredi v turistično točko. Idejo za to sta zvezi dala biologa zakonca Stane in Marjana Peterlin iz Grosupljega. Turistična zveza je k sodelovanju pritegnila mene, priseljenko biologinjo Tatjano Kordiš. Vsi skupaj smo šele ob tej priložnosti ugotovili, da v izviru človeške ribice še vedno ob večerih prihajajo na plano, in se tako zavedli, da bi bil izvir potreben predvsem zavarovanja in temu primerne ureditve.

Od leta 2003 do leta 2015 se je nabralo že precej izkušenj v prizadevanjih za ureditev in zavarovanje izvira. Mogoče jih je razvrstiti v tri skupine: v prizadevanja, ki so rodila nekaj uspehov, v tista, ki so rodila le navidezne kratkotrajne uspehe, in neuspešna prizadevanja.

Od vseh prizadevanj je dolgotrajen, morda celo trajen uspeh rodilo samo informiranje in ozaveščanje domačinov o pomenu izvira. S tem je skupina, ki se je ukvarjala z izvirom, začela na pobudo Marka Aljančiča, in sicer takoj ko se je seznanila s stanjem. Danes o izviru in človeških ribicah v njem vedo že otroci v vrtcu, kandidati na vsakih županskih volitvah pa dajejo ureditev izvira v svoj predvolilni program (ki pa ga potem ne uresničijo). Morda je to splošno vèdenje o pomenu izvira dovolj trden temelj, na katerem vendarle tli upanje za njegovo ureditev.

V omenjenem obdobju je bilo na Viru ustanovljeno Društvo Vir za ohranjanje naravne in kulturne dediščine. Ukvarjalo naj bi se predvsem z izvirom in bližnjo halštatsko naselbino. Društvo je doseglo, da so izvir vendarle uvrstili na seznam naravnih vrednot državnega pomena, Virski potok z okolico pa na seznam naravnih vrednot lokalnega pomena. Organiziralo je dve odmevni akciji – čistilno in akcijo SOS za človeško ribico, a delovanje društva je kasneje zamrlo.

Poskusi sodelovanja z občino, Zavodom RS za varstvo narave in Ministrstvom za okolje praktično niso rodili nobenega uspeha. Občina je ostala nema in je šele z menjavo župana prenehala z arogantnimi odzivi ter se je vsaj na videz začela zanimati za izvir. V praksi ni storila še nič. Zavod RS za varstvo narave je poskrbel, da je izvir uvrščen na seznam naravnih vrednot, a njihov ukrep ne seže od papirnate uredbe v življenje samo. Ministrstvo je po dveh sestankih z županom občini dodelilo sredstva za dokumentacijo kanalizacije na Viru z obljubo, da bo po ureditvi dokumentacije nadaljevalo tudi z dotiranjem same graditve kanalizacije. Kljub opozorilu Društva Vir od občine niso zahtevali nikakršnih povratnih informacij o porabljenem denarju. Občina je zato denar, namenjen virski kanalizaciji, porabila za kanalizacijo v Šentvidu pri Stični, Vir pa je dobil kanalizacijo šele po 12 letih ob pomoči evropskih sredstev. Sodelovanje s temi inštitucijami je bilo za člane društva izčrpavajoče pehanje v brezup.

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Genetic monitoring of *Proteus* populations

Genetski monitoring populacij človeških ribic

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The European cave salamander, or proteus, is a Slovenian national symbol. Endemic to the Dinaric Karst, it is the largest cave animal on our planet and the only European cave vertebrate (Parzefall et al. 1999). As a long-living top predator and charismatic species of karst underground waters it is of outstanding ecological and conservational importance. It is protected by several national and international regulations. Despite all this, proteus populations have not been monitored or surveyed in a way that is standard for most European amphibian species and other species protected under the EU Habitats Directive. From various scattered sources and anecdotic reports we can conclude that, over the last three decades or so, there has been a moderate to substantial decline at a number of sites with regular occurrence, and a decline in number of animals at some sites (Hudoklin 2011). These reports are mostly from the regions of Dolenjska and Bela krajina (southeastern Slovenia) as well as Istria (Croatia), and to a much smaller extent from other parts of the proteus range.

Nevertheless, at present we can hardly distinguish between true population decline on one side and periodic fluctuations, minor spatial shifts of habitat, or differences in observation effort on the other. There are three major reasons why reliable information on proteus population size, population density and frequency of occurrence is hard to obtain. The first and most important is the inaccessibility of its habitat. Karst groundwater and aquifers are accessible only through small »windows« in the form of caves or, sometimes, by cave diving. The most basic faunistic surveys are technically extremely demanding and expensive. At the same time, we do not know enough about the position, volume and extent of karst aquifers to infer indirect estimates of the populations' status and size. The second impediment is posed by several peculiarities in the life history and ecology of proteus that make most standard zoological research approaches inapplicable, even when the animals can be accessed in their natural environment. Common surface-dwelling amphibian species can be surveyed during seasonal migrations and mating, at different developmental stages, etc. None of these techniques can be applied to survey proteus populations. In theory, it is possible to count animals in cave lakes or along the few accessible stretches of subterranean rivers, but any interpretation of numbers would be completely arbitrary. It would be unclear whether fluctuations in numbers of counted individuals are caused by seasonal habitat shifts, random movements, disturbance avoidance, or by actual changes in population size. The third factor hindering effective monitoring and conservation management is the unresolved true taxonomic structure of the taxon. Unclear population and taxonomic boundaries hamper conservation decisions like setting of conservation priorities and management units.

The applied research project under the title »Toward the conservation of the European cave salamander (*Proteus anguinus*): monitoring guidelines, current status estimation and identification of evolutionarily significant units«, funded by the Slovenian Research Agency, the Ministry of the Environment and Spatial Planning and Centre for Cartography of Fauna and Flora, is aimed at overcoming these obstacles. Within the framework of this project we are developing methods and tools that will enable us to asses and monitor population sizes as well as establish their geographic and taxonomic structure. We have developed an efficient method for catching live proteus in open cave waters with the use of diving equipment and hand nets. This method allows capture rates of up to 80% of all observed individuals, which is much higher than any other means used so far. With it, we managed to catch about 800 animals from several Slovenian caves. The highest numbers were caught in the Postojna-Planina Cave System, followed by the subterranean Reka River in the South West and the Šica System in the South East. DNA is obtained non-invasively, by skin swabs (Prunier et al. 2012), and the animals are released immediately at the spot after their weighing and measuring. For genetic analysis we have developed a wide array of polymorphic tetranucleotide microsatellite markers, which will be used both for individual genotyping within populations as well as for inferring the global population structure. A subset of 23 highly polymorphic loci is optimized for the Postojna-Planina Cave System, where we already recaptured some of the genetically »marked« individuals. Eventually, a full recapture is planned for the coming season in order to estimate the population by means of mark-recapture modelling (Amstrup et al. 2005). The developed loci have passed the tests of Hardy-Weinberg, linkage disequilibrium, probability of identity, and reliability of genotyping. Comparisons within and between hydrological systems revealed strong gene flow between the adjacent Postojna and Planina Caves (Fst = 0.006) and a strong differentiation between the Ljubljanica and other, more remote drainages, where populations had only few alleles in common. The strong differentiation at nuclear DNA level supports the previously established mitochondrial DNA lineages (Trontelj et al. 2009). In the following phases of the project, we plan to spatially delineate those lineages, to formalize their taxonomic status, and to provide for them first estimates of population size and conservation status.

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Application of environmental DNA for detection of *Proteus*

Uporaba okoljske DNA pri iskanju človeške ribice

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The rare and highly endangered olm (*Proteus anguinus*) is the only obligate subterranean vertebrate of Europe. It inhabits subterranean waters of the Dinaric Karst in the north-western Balkan Peninsula (Sket 1997). As only small fragments of its subterranean habitat are accessible, the basic but important question of its exact distribution has been difficult to address. Its presence can only rarely be confirmed by classical survey methods such as trapping and visual encounters. For this reason, alternative methods are required to test for its presence in new potential localities. Detection of species-specific DNA released into the aquatic environment (environmental DNA or eDNA) has already been shown as an appropriate approach to monitoring the distribution of vertebrates from surface waters. In aquatic environments rapid diffusion of eDNA from its source means that the presence of a specific animal could be detected anywhere within such water body and not just at its point of origin, thus making this approach particularly useful for those species that are difficult to detect using conventional methods or are very rare (Rees et al. 2014). We developed an adjusted eDNA approach for filtering water samples from karst springs, wells and caves and provide two specific primer sets that can be used to amplify short conserved fragments of *P. anguinus* mitochondrial DNA (Gorički 2006, Gorički & Trontelj 2006) by real-time PCR based on SYBR chemistry. The specificity of the assay was first tested on trout, crested newt and human DNA. In controlled conditions at the Tular Cave Laboratory the minimum density at which its DNA could still be detected corresponded to one animal per 256 m^3 of standing water, when sampling 20 L of water. The method, tested at three Slovenian field test sites occupied by different lineages of *P. anguinus*, was 100% effective. Subsequently, a pilot survey of its distribution was conducted along the southern limit of its known range in Herzegovina and Montenegro. Using DNA-based identification, we unequivocally established the presence of *P. anguinus* at four sites, and found its likely traces at additional eight sites – most of them new localities for this species. Even though the SYBR chemistry-coupled real-time PCR approach was shown to be very successful and time-efficient method for detection and monitoring of *P. anguinus* that can be applied with fidelity anywhere within its known range of occurrence, detectability can still be increased using TaqMan probes (Gorički et al. 2016).

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Searching for the black *Proteus* with the help of eDNA

Iskanje črnega močerila s pomočjo okoljske DNA

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Proteus is a specialized, blind and depigmented subterranean salamander inhabiting groundwaters of the Dinaric Karst. In Bela krajina, Slovenia, a unique black population is known from only four springs in the proximity of a closely related white, troglomorphic population (Aljančič et al. 1986, Sket 1997, Ivanovič 2012, Škedelj Petrič et al. 2014). In the face of the scarcity of the distributional information, potentially very limited distribution and impending threat due to heavy fertilizer load, establishing the detailed distribution of these populations was the focus of our work. Because its subterranean habitat is inaccessible, several techniques were employed at springs, including traps and visual observation in the dark using night goggles, but the survey predominantly aimed to introduce a novel approach – forensic analysis of traces of *Proteus* DNA released in water (environmental DNA or eDNA).

To detect *Proteus* eDNA in samples of spring water and to discriminate between the black and white populations, we developed specific TaqMan probes and PCR primers, homologous to variable regions of *Proteus* mtDNA (Gorički 2006, Gorički & Trontelj 2006). Of the 19 spring water samples collected and filtered, six were positive for *Proteus* DNA, five of which were also positive for black *Proteus*. All five are new localities, where *Proteus* has neither previously nor during the survey been sighted or otherwise detected. Along with detection of *Proteus* eDNA in spring and cave water in southern Herzegovina and Montenegro (Aljančič et al. 2014, Stanković et al. 2016), this survey represents the first successful application of the eDNA approach in detection of a subterranean organism. The methodology developed and tested herein is the only quick and accurate method available for determining its presence and is fully appropriate for monitoring the distribution of this rare and endangered stygobiont in Bela krajina.

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Terenski pregled jam v hidrogeološkem zaledju izvira Krke

Field survey of caves in the hydrogeological catchment of the Krka River spring

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Hidrološko zaledje izvira Krke se razteza na približno 330 km^2. Obsega Grosupeljsko kotlino z zaledjem in precejšen del okolice Velikih Lašč, tja do roba Blok. Na vzhodnem robu Radenskega polja ponikajo trije vodotoki. Severni je Dobravka, ki odmaka večji del Grosupeljske kotline, ob visokih vodah teče v ponorno jamo Požiralnik v Ključu. Sredi Radenskega polja teče Zelenka, ki ponika v jami Pekel pri Kopanju, najjužneje pa teče Šica, ki ponika v jamskem sistemu s tremi vhodi – Viršnica, Lazarjeva jama in Zatočna jam. Podzemni režim pretakanja voda iz omenjenih jam je le v grobem poznan. Voda teče večinoma neposredno v Krško jamo, ob visokih vodah pa deloma tudi v Lučko jamo. Slednja je občasni izvir Radenščice, potoka, ki teče vzdolž kraškega polja Lučki dol in ponika v požiralnikih na jugu polja.

Kraški vodonosniki dinarskega krasa, med njimi tudi porečje Krke, so življenjski prostor človeške ribice. Vse večja onesnaženost vodonosnikov pa ogroža tudi človeško ribico, saj so zaradi dolgoživosti zanjo lahko usodne že nižje, a dolgotrajne koncentracije strupov, ki se kopičijo v njenem telesu in okolju.

Večletni rezultati monitoringa na izviru Krka, ki ga opravlja Agencija Republike Slovenije za okolje (ARSO), kažejo na širšo problematiko onesnaženja ozemlja Dolenjskega krasa. Na izviru Krke že od leta 2007 beležijo presežene koncentracije različnih pesticidov (ARSO, 2010). Stanje je bilo najbolj alarmantno v letu 2009, ko so bile ugotovljene izredno visoke vrednosti atrazina, metolaklora, simazina, prometrina, terbultilazina, terbutrina, metamitrona, izoproturona, metazaklora ter vsote pesticidov. V posamičnih vzorcih so bile vsebnosti terbutilazina šestkrat višje, vsebnosti metamitrona pa skoraj devetkrat višje od standarda (ARSO, 2009).

Glede na ekstremne vrednosti onesnaževal in obširno hidrogeološko zaledje izvira Krke so na ARSU sprva sklepali, da je vir onesnaženja v neposredni bližini vzorčnega mesta. Na širšem hidrogeološkem zaledju izvira, kjer vodovarstvena območja niso pogosta, je vsekakor možnih več virov onesnaženja, kot najbolj verjetna pa omenjajo dva vira:

- Kraške jame, saj so le-te nemalokrat črna, nelegalna odlagališča nevarnih odpadkov, ki so skrita v podzemlju.
- S kmetijstvom obremenjeni površinski del zaledja, ki se v ponorih Dobravke in Šice odvaja v vodonosnik in vpliva na režim ter kvaliteto izvira Krke (ARSO 2009).

Zaradi potencialnega onesnaženja z materialom, ki je morda odložen v kateri izmed jam v ožjem zaledju izvirov Krke, je ARSO naročil terenski pregled tamkajšnjih jam. Projekt je koordinirala Jamarska zveza Slovenije, na terenu pa sta ga uresničevali dve jamarski društvi: Jamarski klub Krka in Jamarski klub Železničar. V dobrih 30 akcijah, ki so bile opravljene konec leta 2010 in v letu 2011, je skupaj sodelovalo 34 jamarjev. Neposredno kraško zaledje izvira Krke, ki je bil predmet naših raziskav, je bilo na zahodu zamejeno z Radenskim poljem, ki je jugovzhodni podaljšek Grosupeljskega polja, in na severu z mejo kraškega sveta nekoliko južno od avtoceste Ljubljana–Novo mesto. Na jugu je bilo mejo preučevanega območja nekoliko težje določiti, približno pa gre ob lokalni cesti Krka–Dobrepolje. Obravnavano območje je imelo površino približno 37 km^2, na njem pa je bilo registriranih 58 jam. Razen prej naštetih aktivnih vodnih jam so vse druge jame kratke ali brezna, nobena od njih ne presega 70 m dolžine.

Osnovna naloga jamarjev je bila poiskati vse jame na obravnavanem območju, pri vsaki preveriti koordinate vhoda in jih po potrebi popraviti, pregledati vhodni del jame s posebnim poudarkom na morebitnih odpadkih, vzorčiti sediment in vodo (če sta v jami) ter izpolniti formular, pripravljen za ta projekt.

Izkazalo se je, da je na obravnavanem območju dejansko 57 jam, od tega smo jih 53 našli, štirih pa ne. Vzorce nam je uspelo odvzeti v 36 jamah, od tega v eni dvakrat.

Glede na onesnaženost, ki smo jo zaznali na vhodnih delih preučevanih jam, le-te lahko razdelimo v naslednje skupine (Sl. 1):

1) **Čiste jame.** Predvsem gre za jame, katerih vhod je dovolj oddaljen od cest. Pretežno čiste so tudi vse izvirne jame, kjer morebitne odpadke voda sama odplavi ob visokih vodah.
2) **Malo onesnažene jame.** Gre za jame, v katerih sicer najdemo smeti, vendar imamo opraviti z relativno majhno količino (pod 1 m^3), tako da jama še ni nepopravljivo degradirana. Takšno jamo je navadno razmeroma enostavno očistiti.
3) **Aktivne ponorne jame.** V teh jamah sicer večinoma ni neposrednega odlaganja odpadkov, pač pa se v njej kopičijo tisti odpadki, ki jih prinese voda (plovni odpadki, npr. embalaža). Poseben problem pa je odtekanje vseh vrst fekalij, ostankov pesticidov ipd. s površja v jame, kar redko opazimo s prostim očesom, so pa bili pobrani vzorci vode, v katerih je bilo mogoče določiti obstoj teh polutantov. Večina aktivnih ponornih jam sodi v skupino »malo onesnaženih jam«.
4) **Jame – smetišča.** Vhodi v tovrstne jame ležijo v neposredni bližini cest, vhodni deli pa so običajno vertikalni. V teh jamah najdemo lahko tudi več kubičnih metrov raznovrstnih odpadkov: komunalne odpadke, klavne odpadke, gradbene odpadke, gospodinjske aparate, sode z neznano vsebino; industrijskega odlaganja nismo zasledili.
5) **Zasute jame.** Kar osem jam na obravnavanem območju je zasutih, torej teh jam praktično ni več. Če je jama dovolj oddaljena od ceste, onesnaženja ni pričakovati – bodisi se je vhod zasul po naravni poti bodisi je npr. lastnik zemljišča nanj zvalil skalo, da ne bi kdo padel vanjo. Najdemo pa tudi primere, ko je jama do vrha zasuta s smetmi. Tedaj lahko na podlagi načrta (če je na voljo) zgolj grobo ocenimo količino odpadkov, ne pa tudi njihove sestave.

Primer zase, ki sicer spada v to zadnjo skupino, pa je **Mirniče jama** (kat. št. 2103). Vanjo so začeli odlagati odpadke že v prvi svetovni vojni (Brenčič 2001). Poleg tega, da je povsem zasuta s smetmi, pa je danes zasuta tudi cela vrtača, ob kateri je bila nekoč jama. Tam je zdaj nasut in zravnan plato, na katerega odlagajo nove odpadke. Primerjalna analiza topografije po topografskem načrtu (izdelan v 70. letih prejšnjega stoletja) in digitalnim modelom reliefa izpred nekaj let je pokazala, da je nasutega materiala za kar okoli 7.000 m^3.

Rezultati analiz vzorcev, pobranih v jamah, niso pokazali sledov pesticidov (viri onesnaževanja naj bi bili locirani na kmetijsko obremenjenem površinskem delu zaledja izvira Krke), kljub temu pa so naše raziskave osvetlile problematiko odlaganja odpadkov v hidrološkem zaledju izvira Krke. Verjetno je divjih odlagališč odpadkov na obravnavanem območju še veliko več, saj smo se v tem projektu omejili le na jame. Vse bolj je v navadi zapolnjevanje vrtač s smetmi, tudi v industrijskih razsežnostih. Čez odpadke se kasneje nasuje pesek in/ali zemlja, območje se zaraste, o odpadkih pa na površju ni več sledi.

Za jame – smetišča in manj onesnažene jame je edina rešitev čiščenje jam z organizacijo čistilnih akcij, po končanih akcijah pa bi bilo jame treba zaščititi pred nadaljnjim onesnaževanjem (npr. postavitev ograje). V primeru Mirniče jame bi bilo verjetno najprej treba preiskati, kaj vse tam utegne biti zakopano, najprej po dostopnih ustnih in pisnih virih, nato s sondiranjem. Končna rešitev bi seveda bila sanacija tega divjega odlagališča, kar pa bi bil ogromen finančni zalogaj.

Onesnaženost krasa in jam v Sloveniji je nasploh širši problem. Na podlagi projektov popisa onesnaženih jam, ki so bili opravljeni v zadnjem času, lahko ocenimo, da je v Sloveniji v manjši meri onesnaženih vsaj dva tisoč jam. Smetišč v jamah, kjer se kopičijo večje količine odpadkov, ki nenadzorovano odtekajo v kras in s tem v vodonosnik, pa je okoli 750. Potencialno največji viri onesnaževanja, če njihovo delovanje ni v okviru normativov, so tudi velike komunalne deponije in čistilne naprave. Prav iztoki čistilnih naprav direktno v kraško okolje pa so problem, na katerega so veliko opozarjali že v preteklosti.

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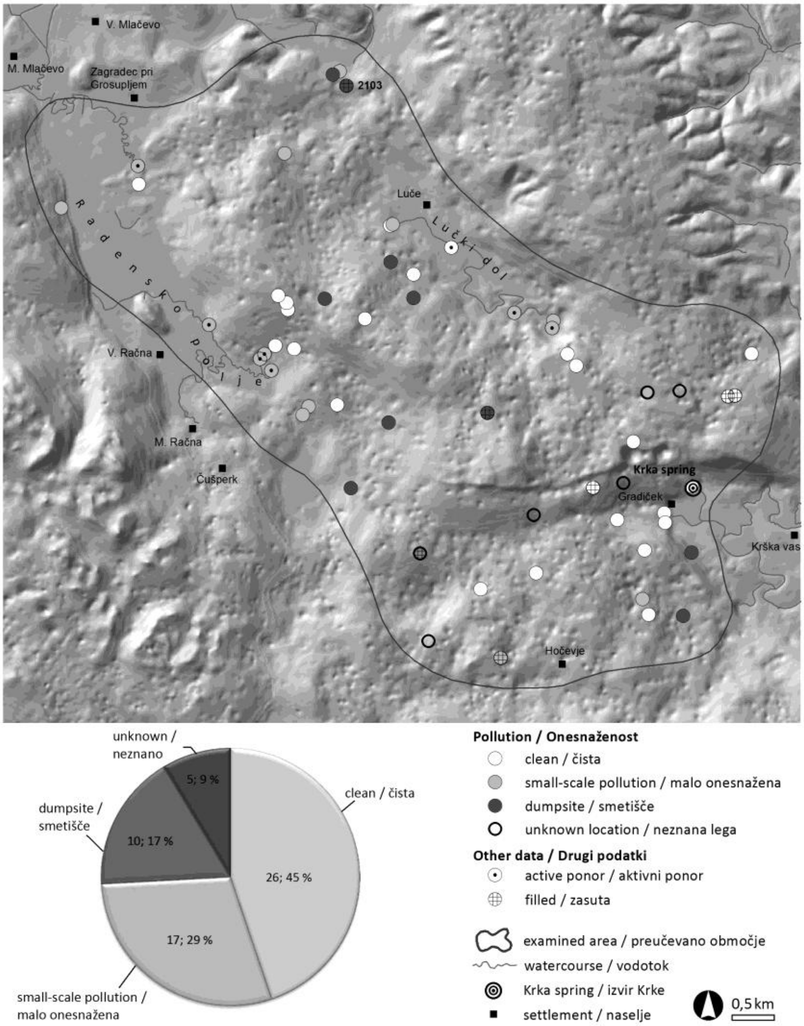

Slika 1. Pregledna karta obravnavanega območja z označenimi legami jam in njihovo onesnaženostjo.
Figure 1. An overview map of the examined area with cave entrance locations, shown according to their state of pollution.

Proteus and education at the Speleovivarium »Erwin Pichl« in Trieste (Italy)

Človeška ribica in izobraževanje v Speleovivariju »Erwin Pichl« v Trstu (Italija)

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The Speleovivarium »Erwin Pichl« is a museum and laboratory dedicated to cave biology and karst. It was started by Erwin Pichl and is currently run by Adriatic Caving Society in collaboration with the Civic Museum of Natural History of Trieste. It is located in the centre of the city of Trieste (Italy) in an artificial gallery that was an air-raid shelter excavated during the Second World War (Fig. 1). Physical characteristics of the gallery are very similar to those of karst caves, so it was easily converted into a laboratory for the study of *Proteus anguinus* (Fig. 2). It has been opened to the public as Speleovivarium in the 1990s. Our research on *Proteus* is now oriented towards ecological studies of feeding habits and distribution, with some direct ethological observation, and with the study of the skull, e.g. by X-ray computer microtomography using the TomoLab station at ELETTRA, Trieste. Special attention is paid to education, working with schools and tourists, in order to stimulate concrete conservation action and groundwater-friendly practice in karst areas (around 4,000 people visited the museum in 2014). Students (from schools and universities) have also been included in our scientific research with training courses and practical field activities. The next project will start in March 2016 with students of Scientific High School Galileo Galilei of Trieste. We will investigate caves and springs in the area of Doberdò (Gorizia, Italy) to monitor *Proteus* distribution and to better understand the night-time occurrence of *Proteus* in surface waters. The research will improve our knowledge on conservation status of Proteus and its habitat in Italy, with international cooperation, especially with scientists and cavers from Slovenia.

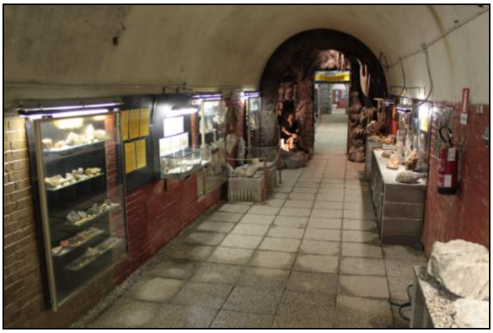

Figure 1. Interior of the Speleovivarium »Erwin Pichl«.
Slika 1. Notranjost Speleovivarija »Erwin Pichl«.

Figure 2. *Proteus* in the Speleovivarium »Erwin Pichl«.
Slika 2. Človeška ribica v Speleovivariju »Erwin Pichl«.

Salvaging the washed-out *Proteus*

Reševanje izplavljenih človeških ribic

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As a stygobiont, the olm, *Proteus anguinus*, is restricted to its subterranean aquatic habitat. Occasionally, during the night, it may appear in springs close to cave entrances (Bressi et al. 1999). However, during seasonal flooding, some individuals get washed-out onto the surface, far from their subterranean environment. The earliest description of this phenomenon was presented already by Janez V. Valvasor in the 17th century (Valvasor 1689). In one of the classic books on Slovene karst written by Franc A. Steinberg, another such case was attributed to fisherman Primož Ziherle, who caught five white animals from the flooded Planinsko polje in 1751 (Steinberg 1758); these animals were subsequently recognised as *Proteus*. Until the early 19th century, collecting washed-out *Proteus* was the only way to obtain fresh specimens for studies, and has later revealed many new localities (Aljančič & Năpăruş 2012).

While being flushed downstream during floods may in fact be part of natural history of *Proteus* as a rare chance to disperse into new habitats (Franci Kljun, pers. comm.), the fate of stranded individuals is quite predictable as the odds to re-enter the underground and thus to survive are minimal. The washed-out animals are often deposited in temporarily flooded fields: when exposed to sunlight, being without pigment, their skin suffers severe sunburns and desiccation. In winter, they may be exposed to low temperatures, hence serious chilblains were documented. In extreme cases they may survive for up to several months, as long as high waters persist; some *Proteus* already developed dark brown skin pigment (Freyer 1846). However, after the high groundwater retreats, many *Proteus* fail to find their way back into the karst underground and eventually die. Others may be carried further into surface streams where they are effectively preyed upon by fish, birds or other predators (Aljančič et al. 2014).

Figure 1. Washed-out *Proteus* exposed to freezing air (Kljunov ribnik, Pivka, Slovenia, 28. 12. 2008; photo: Gregor Aljančič).
Slika 1. Izplavljena človeška ribica izpostavljena ledeno hladnemu zraku (Kljunov ribnik pri Pivki, Slovenija, 28. 12. 2008; foto: Gregor Aljančič).

Seasonal flooding has probably been an important selective force in the evolution of *Proteus* behaviour. We presume that *Proteus* has evolved several responses to reduce the danger of being washed out of its subterranean habitat as well as adapted its feeding and reproduction strategies (Aljančič & Prelovšek 2010). Due to the extreme lifespan of *Proteus* (estimated to around 100 years in captivity) on the one hand and long reproduction cycles (approx. every 7 years in captivity; Aljančič, pers. comm.) on the other, loss of every individual may considerably reduce the size of its population. A concern is raised on how this species might respond to unpredictable effects of climate change, which may include changes in timing, frequency and magnitude of flood events (Aljančič & Năpăruş 2011).

From 1964 onwards, the Tular Cave Laboratory has served as occasional sanctuary for the washed-out or injured *Proteus,* and until 1993 all such animals were permanently kept in asylum. Researchers at the Tular Cave Laboratory have been closely studying this phenomenon since 2008, and documented nearly thirty cases in Slovenia and Bosnia and Herzegovina. All animals were found by chance after reported by local people. Through this research we unexpectedly became involved in a rescue mission: seventeen of these animals were salvaged and returned to their source population. Veterinary inspection and animal care is conducted in partnership with the sanctuary for protected wildlife animals Golob d.o.o. Since 2013, Tular Cave Laboratory has again served as a sanctuary for injured *Proteus* and is now involved in the national network of sanctuaries for protected wildlife animals in Slovenia, under auspices of the Slovenian Environment Agency.

It is important for a sanctuary to have a precise action plan for rescuing the animals, providing first aid and treatment in controlled semi-natural conditions of the Tular Cave Laboratory in which animals are subjected to minimum stress and provided with optimal care. In case of the most rare and threatened black *Proteus* (*Proteus anguinus parkelj*), such service is particularly needed.

After being informed about a washed-out *Proteus*, we carefully examine and document (e.g., photograph and measure, skin-swab, etc.) the animal and then prepare it for transport in a container. The circumstances of the find and its location are also documented. The animal is kept in a quarantine tank at Tular Cave Laboratory, where it is clinically examined and treated if necessary. Quarantine is an essential part of the procedure, preventing uncontrolled transmission of disease to healthy populations. Handling of each specimen is documented; three months is the maximum period allowed by the Slovenian Environmental Agency to treat an animal in the sanctuary. After successful rehabilitation the animal is returned into the wild.

When a washed-out individual is ready to be returned to nature, its source population must be accurately identified. Screening for DNA markers powerful enough to detect ongoing gene flow, such as micro-satellites and single-nucleotide polymorphisms (SNPs), should minimize the danger of genetic mixing (Aljančič et al. 2014; compare Trontelj & Zakšek 2016). An accurate GIS distribution model (integrated georeferenced information on known *Proteus* localities, directions of groundwater flow, patterns of genetic variability of *Proteus* within the complex karst landscape, etc.) should guide the return of washed-out individuals to their source population. Researchers at the Tular Cave Laboratory have developed a method of detection of traces of *Proteus* environmental DNA in groundwater (see Stanković et al. 2016, Gorički et al. 2016) in order to efficiently survey *Proteus* distribution. However, this method can also be applied to identify which *Proteus* population is harbouring a potential release site (water cave or karst spring in the area where the washed-out *Proteus* was found).

If the washed-out individuals cannot be returned directly to their source population due to local inaccessibility of its subterranean habitat (e.g., no caves accessible to man, dry intermittent karst springs, etc.), these animals should be kept permanently for research, education or *ex situ* breeding program.

Researchers of the Tular Cave Laboratory put considerable effort into education and constant public promotion of *Proteus*, emphasizing its vulnerability and karst groundwater conservation issues. Besides addressing the general public, the Laboratory particularly focuses on nature conservation education in schools and local communities where *Proteus* is present. Through a program of regular lectures and education campaigns, designing local natural heritage information facilities, publishing leaflets and documentary films (Aljančič et al. 2015), the phenomenon of washed-out *Proteus*, instructions if finding washed-out *Proteus*, and the mission of the sanctuary are explained.

A case of good practice is the protected Texas blind salamander (*Eurycea rathbuni*), a species found in a few springs or artesian wells in San Marcos, Texas. There, washed-out individuals are collected for the purpose of a successful captive breeding program at the San Marcos National Fish Hatchery and Technology Center. To date, however, no individuals have been returned back to the wild (Andy Gluesenkamp, pers. comm.).

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Vodni krog – kratek film o soodvisnosti človeške ribice in človeka od čiste podzemne vode

Water Circle – a short movie on the mutual dependence of *Proteus* and man on clean groundwater

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Podzemski svet na območju Dinarskega krasa, od Slovenije do Črne gore, je po raznovrstnosti jamskih živali najbogatejši na svetu. Še več, ožje območje Slovenije je najbogatejše znotraj tega območja. Na prvi pogled bi morda rekli, da je na srečo ta krhki živi svet na varnem, saj je globoko pod zemljo dobro skrit pred vsemi nevarnostmi, vendar žal ni tako. Skozi vse vodne jame teče voda, pogosto zelo onesnažena, in nekatere jame so postale sčasoma pravi zbiralniki odpadkov. S tem so postale jamske živali zelo ogrožene in že eno samo večje razlitje strupenih snovi lahko povzroči izumrtje cele vrste endemičnih živali na ožjem območju. To velja tudi za človeško ribico, ki je endemit Dinarskega krasa, še posebej pa za črno podvrsto, ki je razširjena le v Beli krajini na nekaj kvadratnih kilometrih. Pri tem se moramo zavedati, da s tem ko z onesnaževanjem vode in kraškega podzemlja zastrupljamo jamsko živalstvo, obenem ogrožamo tudi svoje zdravje in obstoj, saj za pitje uporabljamo isto vodo, iz istega podzemlja.

V igrano-dokumentarnem filmu *Vodni krog* zanemarjen moški na stranišču simbolizira naš slabi odnos do okolja in čiste vode. Moški bere časopis z naslovom *Pitna voda naše bogastvo* in *S človeško ribico si delimo isto vodo*. Pod naslovom je fotografija človeške ribice. Moški zloži časopis in potegne vodo. Fekalije potujejo po ceveh, vse do kraškega podzemlja, kjer pljusknejo v vodo. Oblak potuje z vodnim tokom naprej, mimo človeške ribice, simbola jamskih živali, vse do sesalne cevi črpališča za vodo, ki posesa oblak. Zajete fekalije se dvigajo po cevi mimo črpalke in ventilov, po vodovodni napeljavi, do vodovodne pipe. Krog, ki ga naredi voda iz stranišča, se sklene in umazanija, ki smo ji sledili, se znajde v kozarcu našega junaka. Ta jo zadovoljen popije.

Film *Vodni krog* je zamišljen kot prispevek k skrbi za čisto pitno vodo, ki jo v veliki meri črpamo iz podzemlja in posredno za ohranjanje biotske raznovrstnosti v kraških jamah. Pretresljiv in provokativen, hkrati nekoliko humoren, vendar aktualen in vsakomur razumljiv nagovarja gledalce vseh starosti, predvsem pa jim za vedno ostane v spominu. Na mednarodnih filmskih festivalih je bil film *Vodni krog* razmeroma dobro sprejet. Poleg številnih uvrstitev v uradno selekcijo festivalov je na festivalu Vasteras na Švedskem leta 2015 prejel prvo nagrado. Seveda je od festivalskih uspehov pomembnejše ozaveščanje javnosti o omenjeni problematiki, a prav prek filmskih festivalov je film obkrožil svet in dosegel širšo, zelo različno publiko. Ta pa ima, simbolno gledano, glede problematike pitne vode povsod zelo podobne probleme.

NAVODILA AVTORJEM

NATURA SLOVENIAE objavlja izvirne prispevke, ki imajo za ozadje terensko delo s področja biologije in/ali prispevajo k poznavanju favne in flore osrednje in jugovzhodne Evrope. Prispevki so lahko v obliki znanstvenih člankov, kratkih vesti ali terenskih notic.

Znanstveni članek je celovit opis izvirne raziskave in vključuje teoretično ozadje tematike, območje raziskav in metode uporabljene pri delu, podrobno predstavljene rezultate in diskusijo, sklepe ter pregled literature. Dolžina naj ne presega 20 strani.
Kratka znanstvena vest je izvirni prispevek, ki ne vsebuje podrobnega teoretičnega pregleda. Njen namen je seznaniti bralca z delnimi ali preliminarnimi rezultati raziskave. Dolžina naj ne presega petih strani.
Terenska notica je krajši prispevek o zanimivih favnističnih ali florističnih opažanjih in najdbah na področju Slovenije. Dolžina naj ne presega treh strani.

Vsi prispevki bodo recenzirani. Avtorji lahko v spremnem dopisu sami predlagajo recenzente, kljub temu pa urednik lahko izbere tudi kakšnega drugega recenzenta. Recenziran članek popravi avtor oz. avtorji sami. V primeru zavrnitve se originalne materiale skupaj z obrazložitvijo glavnega urednika vrne odgovornemu avtorju.

Prispevki, objavljeni v reviji Natura Sloveniae, ne smejo biti predhodno objavljeni ali sočasno predloženi in objavljeni v drugih revijah ali kongresnih publikacijah. Avtorji se s predložitvijo prispevkov strinjajo, da ob njihovi potrditvi, ti postanejo last revije.

Prispevke lahko oddate na naslov Natura Sloveniae, Večna pot 111, SI-1111 Ljubljana, Slovenija (telefon: (01) 423 33 70, fax: 273 390, E-mail: maja.zagmajster@bf.uni-lj.si).

FORMAT IN OBLIKA PRISPEVKA
Prispevki naj bodo napisani v programu Word for Windows, v pisavi "Times New Roman CE 12", z levo poravnavo in 3 cm robovi na A4 formatu. Med vrsticami naj bo dvojni razmak, med odstavki pa prazna vrstica. Naslov prispevka in naslovi posameznih poglavij naj bodo natisnjeni krepko v velikosti pisave 14. Latinska imena rodov in vrst morajo biti pisana ležeče. Uredniku je potrebno prispevek oddati v primerni elektronski obliki (disketa, CD, elektronska pošta) v Rich text (.rtf) ali Word document (.doc) formatu.

Naslov prispevka (v slovenskem in angleškem jeziku) mora biti informativen, jasen in kratek. Naslovu naj sledijo celotna imena avtorjev in njihovi naslovi (vključno z naslovi elektronske pošte).

Izvleček v slovenskem jeziku mora na kratko predstaviti namen, metode, rezultate in zaključke. Dolžina izvlečka naj ne presega 200 besed za znanstveni članek oziroma 100 besed za kratko znanstveno vest. Pod izvlečkom naj bodo ključne besede, ki predstavljajo področje raziskave. Njihovo število naj ne bo večje od 10. Sledi abstract in key words v angleškem jeziku, za katere velja enako kot za izvleček in ključne besede.

Glavnina prispevka znanstvenega članka in kratke znanstvene vesti je lahko pisana v slovenskem jeziku čeprav je bolj zaželjen angleški jezik. Prispevek, ki je pisan v slovenskem jeziku mora vsebovati obširnejši angleški povzetek - summary, prispevek pisan v angleškem jeziku pa obširnejši slovenski povzetek (200-500 besed). Terenska notica je v celoti napisana v angleškem jeziku, brez izvlečka, ključnih besed in povzetka. Pri oblikovanju besedil naj se avtorji zgledujejo po zadnjih številkah revije.

SLIKE IN TABELE
Skupno število slik in tabel v prispevku naj ne bo večje od 10, njihovo mesto naj bo v članku nedvoumno označeno. Posamezne tabele z legendami naj bodo na ločenih listih. Naslovi tabel naj bodo nad njimi, naslovi slik in fotografij pa pod njimi. Naslovi in legenda slik in tabel naj bodo v slovenskem in angleškem jeziku. Pri navajanju slik in tabel v tekstu uporabljajte okrajšave (npr. angl: Tab. 1 ali Tabs. 1-2, Fig. 1 ali Figs. 1-2 in slo.: Tab. 1 in Sl. 1).

NAVAJANJE LITERATURE
Navajanje literature v besedilu mora biti na ustreznem mestu. Kadar citiramo enega avtorja, pišemo Schultz (1987) ali (Schultz 1987), če sta avtorja dva (Parry & Brown 1959) in če je avtorjev več (Lubin et al. 1978). Kadar navajamo citat večih del hkrati, pišemo (Ward 1991, Pace 1992, Amman 1998). V primeru, ko citiramo več del istega avtorja objavljenih v istem letu, posamezno delo označimo s črkami (Lucas 1988a, b). Literatura naj bo urejena po abecednem redu.

Primeri:

- članke iz revij citiramo:

Schultz J.W. (1987): The origin of the spinning aparatures in spiders. Biol. Rev. 62: 123-134.
Parry D.A., Brown R.H.J. (1959): The hydraulic mechanism of the spider leg. J. Exp. Biol. 36: 654-657.
Lubin Y.D., Eberhard W.G., Montgomery G.G. (1978): Webs of Miagrammopes (Araneae: Araneaidae) in the neotropics. Psyche 85: 1-13.
Lucas S. (1988a): Spiders in Brasil. Toxicon 26: 759-766.
Lucas S. (1988b): Spiders and their silks. Discovery 25: 1-4.

- knjige, poglavja iz knjig, poročila, kongresne povzetke citiramo:

Foelix R.F. (1996): Biology of spiders, 2. edition. Harvard University Press, London, pp. 155-162.
Nentwig W., Heimer S. (1987): Ecological aspects of spider webs. In: Nentwig W. (Ed.), Ecophysiology of Spiders. Springer Verlag, Berlin, 211 pp.
Edmonds D.T. (1997): The contribution of atmospheric water vapour to the formation of a spider's capture web. In: Heimer S. (Ed.), Proceedings of the 17th European Colloquium of Arachnology. Oxford Press, London, pp. 35-46.

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NATURA SLOVENIAE publishes original papers in Slovene and English which contribute to the understanding of the natural history of Central and Southeast Europe. Papers may be submitted as Scientific Papers, Short Communications or Field Notes.

Scientific Paper is a complete description of the original research including theoretical review, research area, methods, detailed presentation of the results obtained and discussion, conclusions and references. The length of the Scientific Paper may not exceed twenty pages.
Short Communication is an original paper without detailed theoretical review. Its purpose is to introduce partial or preliminary results of the research. The length of the Short Communication may not exceed five pages.
Field Note is a short report on interesting faunistical or botanical findings or observations in Slovenia. The lehgth of the Field Note may not exceed three pages.

All papers will be subject to peer review by one referee. Authors are invited to suggest the names of referees, although the editor reserves the right to elect an alternative referee to those suggested. The reviewed paper should be corrected by author or authors themselves. In the case of the rejection, the original materials will be sent back to the corresponding author with the editors explanation.

The submitted papers should not have been previously published and should not be simulatenously submiteed or published elsewhere (in other journals, bulletins or congress publications). By submitting a paper, the authors agree that the copyright for their article is transferred to the publisher if and when the article is accepted for publication.

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FORMAT AND FORM OF ARTICLES
Papers should be written with Word for Windows using "Times New Roman CE" size 12 font, align left and margins of 3 cm on A4 pages. Double spacing should be used between lines and paragraphs should be separated with a single empty line. The title and chapters should be written bold in font size 14. The latin names of all genera and species must be written italic. All submissions should be sent to the editor in the appropriate electronic version on diskette, CD or via e-mail in Rich text format (.rtf) or Word document (.doc) format.

Title of paper should be informative, understandable, and concise. The title should be followed by the name(s) and full adress(es) of the author(s), including E-mail adresse(s).

Abstract must give concize information about the objectives, methods used, results and the conclusions. The abstract length should not exceed 200 words for »Scientific Papers« and 100 words for »Short Communications«. There should be no more than ten keywords which must accurately reflect the field of research covered in the paper. Field notice does not include abstract and keywords. Author(s) should check the last issue of Natura Sloveniae when preparing the manuscript.

ILLUSTRATIONS AND TABLES
Papers should not exceed a total of ten illustrations and/or tables, with their positon amongst the text clearly indicated by the author(s). Tables with their legends should be submitted on separate pages. Titles of tables should appear above them, and titles of illustrations and photographs below. Illustrations and tables should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2).

LITERATURE
References should be cited in the text as follows: a single author is cited, as Schultz (1987) or (Schultz 1987); two authors would be (Parry & Brown 1959); if a work of three or more authors is cited, (Lubin et al. 1978); and if the reference appears in several works, (Ward 1991, Pace 1992, Amman 1998). 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. (Lucas 1988a, b). The literature should be arranged in alphabetical order.

Examples (use the the following forms):

- articles from journals:

Schultz J.W. (1987): The origin of the spinning aparatures in spiders. Biol. Rev. 62: 123-134.

Parry D.A., Brown R.H.J. (1959): The hydraulic mechanism of the spider leg. J. Exp. Biol. 36: 654-657.

Lubin Y.D., Eberhard W.G., Montgomery G.G. (1978): Webs of Miagrammopes (Araneae: Araneaidae) in the neotropics. Psyche 85: 1-13.

Lucas S. (1988a): Spiders in Brasil. Toxicon 26: 759-766.

Lucas S. (1988b): Spiders and their silks. Discovery 25: 1-4.

- for books, chapters from books, reports, and congress anthologies:

Foelix R.F. (1996): Biology of spiders, 2. edition. Harvard University Press, London, pp. 155-162.

Nentwig W., Heimer S. (1987): Ecological aspects of spider webs. In: Nentwig W. (Ed.), Ecophysiology of Spiders. Springer Verlag, Berlin, 211 pp.

Edmonds D.T. (1997): The contribution of atmospheric water vapour to the formation of a spider's capture web. In: Heimer S. (Ed.), Proceedings of the 17th European Colloquium of Arachnology. Oxford Press, London, pp. 35-46.