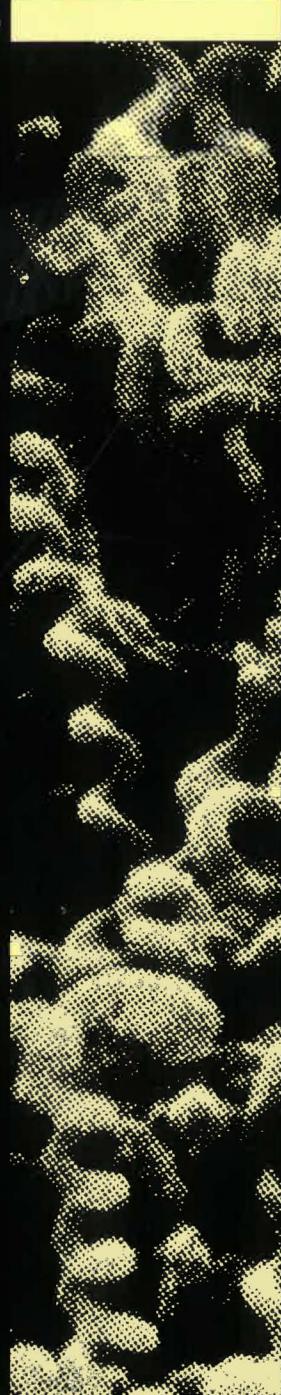


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Food choice experiments with cadmium nitrate dosed food in terrestrial isopod *Oniscus asellus* (Crustacea)

Poskusi izbire hrane z različno vsebnostjo kadmijevega nitrata na kopenskem enakonožcu *Oniscus asellus* (Crustacea)

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Abstract. The influence of different concentrations of cadmium nitrate on food choice behaviour was studied in the terrestrial isopod *Oniscus asellus*. In paired food choice tests, consumption rates were compared in relation to cadmium nitrate concentrations and duration of feeding. The consumption of contaminated food was reduced at even the lowest cadmium concentration already in the first week of feeding. In the third week the consumption rates for uncontaminated and contaminated food reached a ratio of 6:4 in all animal groups. Consumption of contaminated food during the experiment resulted in increased cadmium content in the animals. It is presumed that *O. asellus* cannot distinguish food according to cadmium concentration. The difference in consumption rates between uncontaminated and cadmium-contaminated food could be based on integration of feeding behaviour and the adverse metabolic effects of cadmium.

Keywords: Isopods, *Oniscus asellus*, food-choice, cadmium nitrate, contamination, consumption rate, accumulation

Izvleček. Proučevali smo vpliv različnih koncentracij hrani dodanega kadmijevega nitrata na izbiro hrane pri kopenskem enakonožcu *Oniscus asellus*. V poskusih izbire smo primerjali stopnjo hranjenja z neonesnaženo in onesnaženo hrano, v odvisnosti od koncentracije kadmijevega nitrata in trajanja hranjenja. Živali so zaužile manj onesnažene hrane že v prvem tednu poskusa izbire tudi pri najnižji koncentraciji kadmijevega nitrata dodanega hrani. V tretjem tednu poskusa je bilo pri vseh skupinah živali razmerje med stopnjo hranjenja z neonesnaženo in onesnaženo hrano 6:4. Pri živalih, ki so med poskusom jedle hrano z dodanim kadmijevim nitratom je bila telesna vsebnost kadmija povečana. Iz rezultatov sklepamo, da raki enakonožci verjetno ne razlikujejo med hrano z različno vsebnostjo kadmijevega nitrata. Razlike v stopnji

hranjenja z neonesnaženo in onesnaženo hrano so verjetno posledica prehranjevalnega vedenja živali povezanega s presnovnimi učinki kadmija.

Ključne besede: enakonožci, *Oniscus asellus*, izbira hrane, kadmijev nitrat, onesnaževanje, stopnja hranjenja, akumulacija

Introduction

Faced with an ever growing input of waste substances to the chemical cycles of the biosphere due to anthropogenic activities, the importance of assessing levels of pollution by biomonitoring programmes in various ecosystems has gained increasing recognition over the last decades (BAYNE 1979, HOPKIN 1989, GOLDBERG & BERTINE 2000). The success of the 'Mussel Watch Program' in marine ecosystems has led to considerable efforts to establish similar programmes for terrestrial ecosystems. Terrestrial isopods are amongst the most promising animals for global terrestrial biomonitoring, and the species *Porcellio scaber* has been proposed as the terrestrial biomonitoring equivalent of *Mytilus edulis* (CORTET & al. 1999, COUGHTREY & al. 1997, DROBNE 1997, HOPKIN & al. 1986, HOPKIN 1990, HOPKIN & al. 1993, PAOLETTI & HASSALL 1999). However, in recent years some data on possible pollutant-dependent food selection behaviour have been presented (VAN CAPELLEVEEN & al. 1986, DALLINGER 1977, ODENDAAL & REINECKE 1999, DROBNE & al. 1995). If terrestrial isopods are able to discriminate between differently contaminated food, their value as biomonitoring organisms would diminish.

Terrestrial isopods play an important role in decomposition of organic material and fulfill most of the criteria required of a good biomonitor (HOPKIN 1989, HOPKIN & al. 1993, COUGHTREY & al. 1997, PAOLETTI & HASSALL 1999). Many aspects of the ecology of *Porcellio scaber* are well known, and it is one of the most studied isopods (CORTET & al. 1999, DROBNE 1997, GUNNARSON 1987, ODENDAAL & REINECKE 1999, PAOLETTI & HASSALL 1999). Field studies and monitoring programmes have used the species successfully in assessing the bioavailability of pollutants (HAMES & HOPKIN 1989, HOPKIN 1990, RABITSCH 1995). Terrestrial isopods accumulate the highest tissue concentrations of cadmium, copper, lead, and zinc known for any invertebrate (HOPKIN 1989, HOPKIN & MARTIN 1984). The robustness against pollution with metals in isopods apparently stems from a compartmentalization mechanism dependent on metal-containing granules in small cells of the hepatopancreas and possibly a detoxification mechanism with binding metallothioneins, which yet remains to be verified (CROMENTUJIN & al. 1994, HAMES & HOPKIN 1991, HOPKIN 1989, PROSI & al. 1983). The shiny woodlouse *Oniscus asellus* has been proposed as a substitute for *P. scaber* in areas where the latter is scarce or missing from the fauna (DROBNE 1997). *O. asellus* shows higher accumulation rates for cadmium and other metals than *P. scaber*, and has likewise a wide distribution, thus constituting a suitable alternative to *P. scaber* (HAMES & HOPKIN 1991, HOPKIN 1990).

In recent years, much information has been gathered about the sensory equipment of terrestrial isopods. They are able to distinguish between and show preferences for different qualities of food, e.g. different levels of fungal permeation of their food (ZIMMER & al 1996, GUNNARSON 1987, SZLAVECZ & MAIORANA 1990). In addition, some results of food choice experiments have suggested that terrestrial isopods may also sense different levels of contaminants in their food, e.g. *P. laevis* has been shown to discriminate against cadmium sulfate added to leaves and *P. scaber* can apparently discriminate and avoid lead and copper in its food (VAN CAPELLEVEEN & AL. 1986, DALLINGER 1977, ODENDAAL & REINECKE 1999). If terrestrial isopods have evolved mechanisms to avoid uptake of biotoxic substances from their food, then the concentrations of these substances in their tissues do not represent levels of habitat pollution, but are the result of an integrated behavioural response to pollution (DROBNE & al 1995, DONKER & BOGERT 1991). However, one of the criteria for the use of an organism as a biomonitor

is that accumulation of a pollutant should reflect exposure. Results from biomonitoring experiments using terrestrial isopods could therefore be unreliable and should be treated with caution (DROBNE & al. 1995).

In the present work we investigated the possibility of an avoidance mechanism for the consumption of cadmium-contaminated food by the terrestrial isopod *O. asellus* in paired food choice experiments. We aimed to show that the results from our experiments, which document preference for uncontaminated food, in correlation with previous publications, can be explained without assuming the existence of a sensory detection mechanism for cadmium in the diet, thus rendering the species an accurate biomonitor of bioavailable cadmium in its habitat. We assumed that the observed preference for uncontaminated food could be explained by the combined influence of feeding behaviour and the adverse metabolic effects of cadmium in the isopods.

Materials and Methods

Sampling of animals from the field

About 250 specimens of *Oniscus asellus* were collected in October 2001 from the litter layer of a woodland area near a former smelter site in the vicinity of Nussloch near Heidelberg, Germany. For three weeks the animals were kept in a glass container on moist plaster of Paris and fed on partly decomposed unpolluted leaves of various tree species. The temperature in the glass container was kept steady at about 17°C, and exposure to direct light was prevented. To avoid desiccation, the leaves and the plaster of Paris were lightly sprayed with commercial bottled water every two days. After three weeks, ten animals were selected at random, lyophilised and weighed, and digested in a hot acid mixture ($\text{HNO}_3:\text{HClO}_4 = 7:1$; final temperature 185°C) until dryness. The residue was suspended in 1.5 ml HNO_3 (0.2%) and analysed for cadmium content by flame atomic absorption spectrophotometry (AAS), using a Perkin Elmer AAAnalyst 100 atomic absorption spectrophotometer. All further animal samples were digested and analysed according to this method.

Sampling of soil

From the same site, five soil samples of the top 10 cm of the soil horizon were collected, cleaned of all visible organic components, and dried for two hours at 110°C, then homogenised in a mortar and digested in hot acid mixture ($\text{HNO}_3:\text{HCl} = 1:3$) until dryness. As the samples were not digested completely by this procedure, it was repeated once. The samples were then diluted with weak nitric acid (0.2 %), and filtered. The filtrate was subsequently analysed for cadmium content by flame AAS analysis.

Experimental set-up

The animals were separated, sexed and weighed, and kept individually on moist filter paper in plastic petri dishes ($\varnothing = 9\text{ cm}$). Only males and non-gravid females were selected for the experiment. The petri dishes were kept in a climate chamber at a relative humidity of 100% and under a 16 hours light and 8 hours dark regime. Temperature was kept constant at 21°C ($\pm 1^\circ\text{C}$). Animals were checked every two days, and the filter paper was moistened with commercial bottled water if necessary. Any dead animals were removed immediately and their data excluded from the results. Moulting animals or animals that did not produce faecal pellets were marked and counted.

The animals were fed exclusively with complex food pellets designed especially for this experiment. For the production of food pellets, partly decomposed hazel leaves (*Corylus avellana*) were collected in an unpolluted woodland area in the vicinity of Cerknica near Ljubljana, Slovenia. After drying the leaves at room temperature for several days, leaf stems were removed and the leaves pulverised with a coffee mill and subsequently sieved through a 0.25 mm mesh net. The leaf powder was dried for 2

hours at 60°C. Other components of the complex food pellets were commercial Dr. Oetker's gelatine, which was dried at 40°C, and fish food for aquarium fish (JBL Novobel; JBL GmbH& Co. KG), which was homogenised by hand in a glass mortar and dried for two hours at 60°C.

Dry leaf powder, gelatine, and fish food were mixed in a 63:34:3 ratio and turned into a paste with demineralized water (15 ml per gram gelatine). For the preparation of contaminated food, four different amounts of cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$) solution (2712.5 mg Cd/l) were added to the food paste to give nominal concentrations of 0, 20, 45, 200 and 450 mg Cd kg⁻¹ dry weight of food. In order to exclude food choice due to nitrate content (Hopkin 1989), corresponding amounts of potassium nitrate (KNO₃) were added to the respective control food. Food pellets were formed out of the paste by depositing equal amounts in plastic blisters ($V = 0.3 \text{ ml}$). The food pellets were allowed to solidify for 24 h at 5°C, dried at room temperature for 24 h and then dried at 70°C for the next 48 h. The actual concentrations of cadmium in the food pellets were measured by AAS analysis and compared to the nominal cadmium contents (Tab. 1). The amount of water-soluble cadmium in the food pellets was measured by soaking food pellets in demineralized water for 24 h at increasing temperatures up to 40°C, centrifuging the solution and analysing the supernatant for Cd content by flame AAS (Tab. 1). The water soluble concentration of cadmium in food pellets was observed to be less than 10 % of the actual cadmium concentration.

Table 1: Concentration of cadmium in food pellets as measured by flame AAS (* = concentration below detection limit)

Preglednica 1: Koncentracije kadmija v hrani izmerjene s plamensko AAS (* = koncentracija pod zaznavno mejo)

Nominal Conc. (mg/kg)	Measured Conc. (mg/kg) n=20		Water Soluble Conc. (mg/kg) n=10	
	Mean	SE	Mean	SE
0	0.06	0.02	*	
20	20.55	0.30	1.14	0.13
45	45.34	0.51	3.78	0.07
200	194.55	2.69	14.05	0.20
450	445.26	1.58	36.58	0.40

Food pellets were offered in small plastic dishes ($\varnothing = 2 \text{ cm}$, height of rim ca. 3 mm). Prior to food choice experiments, the animals were acquainted with the form and taste of the food pellets by offering them uncontaminated food for one week. After seven days, the animals were starved for 24 h to empty their guts (HAMES & HOPKIN 1991). Ten animals were selected randomly and analysed for tissue cadmium content by flame AAS.

Food choice experiments

The isopods were assigned to five groups of 35 individuals each, giving a total number of 175 animals. Each animal was offered a choice between an uncontaminated and a Cd-contaminated food pellet; in the group "0-20" the nominal cadmium concentration in the contaminated pellet was 20 mg kg⁻¹ dry food weight, in the group "0-45" 45 mg kg⁻¹ dry food weight, in the group "0-200" 200 mg kg⁻¹ dry food weight, and in the group "0-450" 450 mg kg⁻¹ dry food weight. Animals in the control group "0-0" were offered a choice of two uncontaminated, but differently marked food pellets. The dry weight of food pellets was determined before and after exposure to animals using a micro-balance.

After seven days of the food choice experiment, the animals were starved for 24 h to empty their guts (HAMES & HOPKIN 1991). Eight animals of each group were randomly selected and analysed for tissue cadmium content by AAS analysis. The food pellets were collected, dried for two days at room

temperature, cleaned from faeces and dried at 70 °C for 48 h. Consumption of food was calculated according to the differences in weight before and after the seven day period of exposure to the animals.

The remaining animals were offered a new choice between new food pellets with the same Cd-concentrations as before. The whole cycle of feeding, removal of food, starvation for 24 h, and random selection of eight animals for analysis of cadmium content by AAS analysis was repeated twice, resulting in a total exposure period of twenty-one days for the last cohort of animals.

Analysis of data

To compare food consumption rates (CR), the absolute consumption of food per week was divided by the dry weight of animals. The data of the experiment were analysed using Microsoft Excel 97 computer software. Ratios of consumption for uncontaminated food and contaminated food were calculated from consumption rates for each individual. The percentage of uncontaminated food consumed was calculated and compared to a null-hypothesis of 50 % with a two-tailed Student's t-test. Standard errors (SE) and 95 % confidence intervals were calculated where appropriate. Weekly consumption rates within groups and weekly consumption rates between groups were analysed for significant differences with a one-way ANOVA test and a Tukey test, using SPSS for Windows statistics software.

Results

Concentration of cadmium in soil samples and animals

Analysis of soil samples by flame Atomic Absorption Spectrometry yielded a mean concentration of 11.42 (SE=1.63) mg Cd per kg dry weight of soil. Analysed animal samples from the field contained a mean of 64.54 (SE=5.26) mg Cd per kg dry weight of animals. Analysed animal samples from food choice experiment yielded increased levels of cadmium correlated to the duration of the experiment and the concentration of cadmium in the offered food (Fig. 1).

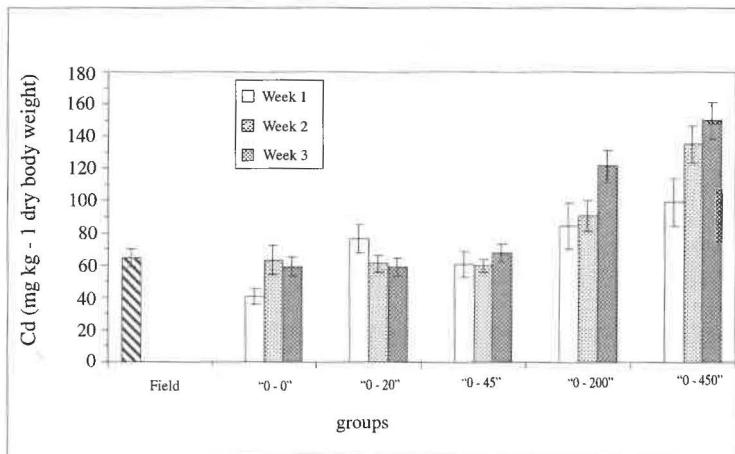


Figure 1: Concentration of cadmium in whole *Oniscus asellus* measured by flame AAS followed over three weeks of the food choice experiment. Concentration of cadmium in field animals is represented on the far left (mean \pm SE).

Slika 1: Koncentracije kadmija v telesu raka enakonožca *Oniscus asellus*, izmerjene s plamensko AAS v treh tednih poskusa izbire hrane. Povprečna koncentracija kadmija v živalih iz okolja je prikazana skrajno levo (povp. \pm stand. napaka).

Consumption rates

During the preliminary experiment, the mean food consumption rate was 0.52 (SE=0.03) mg dry food weight per kg dry weight of animals. In the third week of the food choice experiment, the mean food consumption rate increased to 1.24 (SE=0.05).

Combined consumption rates of contaminated and uncontaminated food increased significantly between the first and third week in group “0-0” (Tukey test: $p < 0.05$) (Fig. 2). In group “0-20”, combined consumption rates between the first and second week are significantly different (ANOVA: $p < 0.05$); however, with the Tukey test no statistically significant increase between weekly consumption rates could be shown. Combined consumption rates in group “0-45” were significantly higher in the second and third week (Tukey test: $p < 0.005$) than in the first week. In group “0-200”, combined consumption rates in the third week were significantly higher than in the first week (Tukey test: $p < 0.05$); between the first and second and the second and third week there was no statistically significant difference. In group “0-450”, no statistically significant increase in weekly consumption rates could be shown.

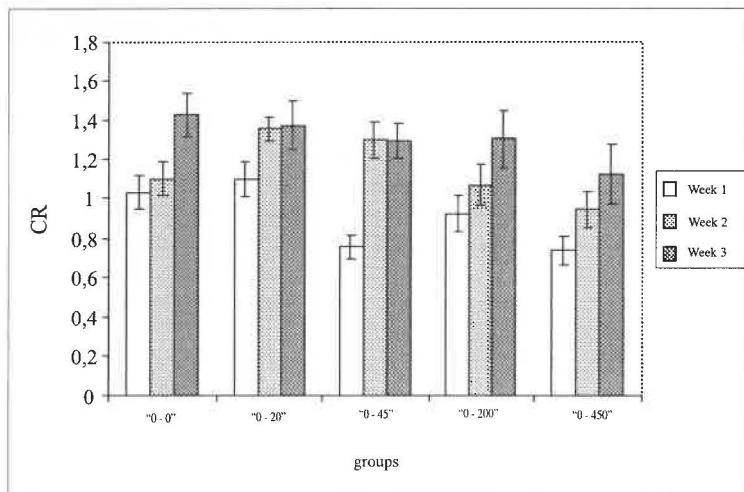


Figure 2: Combined consumption rates (CR) of uncontaminated and contaminated food in different groups of animals for each of the three weeks of the food choice experiment (mean \pm SE).

Slika 2: Stopnja hranjenja (CR) z neonesnaženo in onesnaženo hrano skupaj v posameznih tednih poskusa izbiре, pri različnih skupinah živali (povp. \pm stand. napaka).

Consumption rates of contaminated and uncontaminated food in different groups

Consumption rates show that animals within group “0-0” did not significantly discriminate between food pellets during the three weeks (Fig. 3, Tab. 2). Animals within group “0-20” and “0-45” significantly preferred uncontaminated food to contaminated food in the first and third week but not in the second week. Animals of the group “0-200” consumed significantly more uncontaminated food in all three weeks. In group “0-450”, there was no significant preference for uncontaminated over contaminated food in the first week; however, in the second and third week, animals consumed significantly less contaminated food.

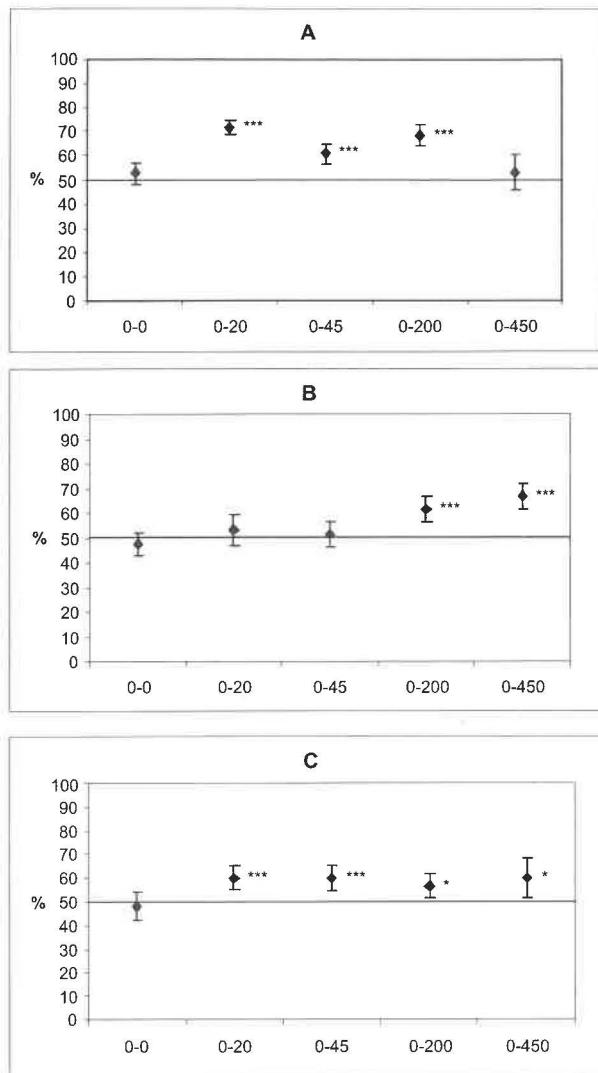


Figure 3: Consumption rate (CR) of uncontaminated food shown as percentage values (♦) of combined CR of uncontaminated and Cd-contaminated food (means and 95% conf. int.): A = 1st week, B = 2nd week, C = 3rd week of food choice experiments. Stars above bars represent significant differences between CR of uncontaminated food and 50 % (horizontal line) (t-test: * = p < 0.05; ** = p < 0.01; *** = p < 0.005).

Slika 3: Stopnja hranjenja (CR) z neonesnaženo hrano prikazana kot odstotek (♦) skupne stopnje hranjenja z neonesnaženo in s kadmijem onesnaženo hrano (povp. in 95% interval zaupanja): A = 1. teden, B = 2. teden, C = 3. teden poskusov izbire hrane. Statistična značilnost razlik med stopnjo hranjenja z neonesnaženo hrano in 50% (vodoravna črta) je prikazana z zvezdicami (t-test: * = p < 0.05; ** = p < 0.01; *** = p < 0.005).

Differences in combined consumption rates between the groups in the first week were mainly due to differences in consumption of uncontaminated food (Tab. 2). In the second week, the differences between combined CRs of the groups were mainly connected with consumption of contaminated food, whereas consumption levels of uncontaminated food were roughly similar in all groups (Tab. 2). During the third week, CRs between uncontaminated and contaminated food reached almost identical ratios of about 6:4 in all groups (Tab. 2, Fig. 3).

Table 2: Feeding and moulting behaviour of *Oniscus asellus* in different groups over three weeks of food choice experiment. (0 = uncontaminated food, Cd = food contaminated with cadmium; CR = Consumption Rate)

Preglednica 2: Prehranjevanje in levitev v treh tednih poskusa izbire pri *Oniscus asellus* iz različnih skupin. (0 = neonesnažena hrana, Cd = hrana onesnažena s kadmijem; CR = stopnja hranjenja)

		“0-0”		“0-20”		“0-45”		“0-200”		“0-450”	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Week 1	CR 0	0.53	0.05	0.79	0.07	0.47	0.05	0.64	0.07	0.41	0.05
	CR Cd	0.50	0.06	0.31	0.03	0.29	0.02	0.29	0.03	0.36	0.05
	% of moulting animals	33.33		21.43		42.86		24.14		38.46	
	Number of animals	26		27		27		28		24	
Week 2	CR 0	0.54	0.06	0.71	0.05	0.67	0.07	0.67	0.07	0.64	0.07
	CR Cd	0.56	0.04	0.65	0.06	0.63	0.05	0.40	0.05	0.30	0.04
	% of moulting animals	5.26		10.00		10.00		33.33		41.18	
	Number of animals	18		19		19		20		16	
Week 3	CR 0	0.66	0.04	0.81	0.06	0.78	0.06	0.75	0.10	0.70	0.10
	CR Cd	0.77	0.10	0.56	0.07	0.51	0.05	0.56	0.06	0.43	0.05
	% of moulting animals	18.18		8.33		0		0		11.11	
	Number of animals	11		12		12		12		9	

Discussion

Compared with data from HOPKIN (1989), the soil samples from Nussloch show intermediate to slight pollution levels with cadmium. Variability in body concentrations of cadmium in field animals was shown. It has been demonstrated that there may be variability of metal concentrations in sampled field populations, and that levels of metals in the hepatopancreas of individual woodlice may be more than twice the mean value for the whole population within a site (HOPKIN & MARTIN 1984, HOPKIN & al. 1986). Furthermore, the amount of pollutants in soil and litter are not always predictive of bioavailability and actual accumulation of pollutants, especially in environments that are polluted due to anthropogenic activity, such as former smelters, mines, and roadsides (HOPKIN & al. 1986). The variable cadmium concentrations in the sampled animals may be further explained by the variable age of the sample animals (reviewed in DROBNE 1997).

Cadmium concentrations in experimental animals increased with increasing concentrations of cadmium in the food. This is in accordance with laboratory experiments on the accumulation of pollutants from food according to consumption and dry weight (DROBNE 1996, HOPKIN & MARTIN 1984).

Food consumption rates in all groups increased with the course of the experiment. This may be explained by the great numbers of moulting animals in the first week, and the fact that animals were not adapted to laboratory conditions. It is recommended to accustom isopods to laboratory conditions for some weeks before the start of an experiment, as moulting is stimulated by changed conditions and

thus interferes with normal behavioural responses to a stimulus (STEEL 1980; DROBNE 1996; ZIDAR & al. 1998). An interval of 28 days between two ecdyses has been reported for *O. asellus* (STEEL 1982), so the impact of moulting behaviour on food consumption rate cannot be excluded in long-term experiments. Almost 80% of animals moulted during the experiments which resulted in decreased food consumption rates. Another factor influencing the food consumption rate might be the phenomenon of hyperphagia which describes the observation that isopods in the laboratory tend to consume more food than a comparable group of field animals (BECK & BRESTOWSKY 1980, HOPKIN 1990).

The combined consumption rates for the whole duration of the food choice experiment show that low levels of cadmium (20 and 45 mg kg⁻¹ dry food weight) seem to have only little or no adverse effects on consumption rate. This is in accordance with data from the literature, which reports the low observed effective concentration (LOEC) for Cd in *P. scaber* at about 20 mg Cd kg⁻¹ (DONKER & BOGERT 1991). For intermediate and high levels of Cd-pollution in the food a decrease of food consumption was observed.

After three weeks of the food choice experiment, the amounts of uncontaminated food and contaminated food consumed reached an apparently stable ratio of 6:4 in all groups, thus suggesting some kind of avoidance reaction to cadmium independent of cadmium concentration. If *O. asellus* was indeed able to taste and discriminate against cadmium contaminated food, we would expect a reaction in relation to cadmium concentration.

However, some interesting patterns of discrimination against contaminated food related to the concentration of cadmium and the duration of exposure can be observed. In groups "0-20" and "0-45" we found a similar pattern of rejection over three weeks. In the first week, there was a strong preference for uncontaminated food, whereas in the second week, the avoidance reaction against cadmium contaminated food was barely observable. In the third week, animals again discriminated significantly against contaminated food. In group "0-200", preference for uncontaminated food dropped from a ratio of 7:3 in the first week to a final 6:4 in the third week. In group "0-450", discrimination against contaminated food in the first week was not very pronounced, whereas in the second week, animals strongly discriminated against contaminated food, reaching a consumption ratio of about 6:4 in the third week.

The differences and time-dependent pattern of the avoidance reactions to cadmium contaminated food observed in the food-choice experiment might be explained by the assumption of two combined physiological effects: Firstly, an animal that starts to feed on cadmium contaminated food will suffer adverse metabolic effects from cadmium which will cause a premature cessation of feeding. Secondly, the intake of cadmium initiates a detoxification mechanism.

As described in the literature, isopods show a regular cycle of feeding behaviour (HAMES & HOPKIN 1990). After a phase of resting, animals void their guts, search for food, and, upon finding suitable food, start feeding. It may be assumed that once an animal starts feeding on suitable food, it will continue to feed on the same food if not disturbed. If animals start to feed on contaminated food, they will ingest this food until the ingested cadmium produces the assumed adverse metabolic effect, which will result in cessation of feeding. The higher the concentrations of ingested cadmium, however, the more pronounced the toxic reaction will be, thus reducing uptake even of uncontaminated food. The effect observed in group "0-450", where consumption levels of contaminated and uncontaminated food are equally low, might therefore be explained by assuming that the ingested levels of Cd are already so toxic that it also reduces the consumption of uncontaminated food.

Comparing the course of food consumption in the respective groups over time supports the second assumption. If animals that are exposed to small concentrations of cadmium acclimatise to these levels of contaminants in their food, after some time they will not be subject to the negative metabolic effects of low doses of cadmium in their food. Consumption rates of uncontaminated and contaminated food will be more equal, as was observed in the second week for groups exposed to low cadmium concentrations (groups "0-20" and "0-45"), and to a lesser degree in the group exposed to intermediate

cadmium concentrations (group "0-200"). In the group exposed to high cadmium concentrations (group "0-450"), this acclimatisation process will take longer. In the third week, the acclimatisation to cadmium in the food reached a steady state, which allows consumption of contaminated food, but still favours consumption of uncontaminated food. This state is represented by similar consumption ratios for uncontaminated and contaminated food in all groups.

Conclusions

In paired food choice tests, the consumption of cadmium contaminated food by terrestrial isopods is reduced. This has formerly been interpreted as the ability of isopods to detect and discriminate against pollutants in their food, rendering results from biomonitoring experiments less significant. In our experiment, we could show that *O. asellus* does eat less contaminated food than uncontaminated if offered a choice. However, this effect is not related to the concentrations of the pollutant or the duration of exposure. This leads to the presumption that *O. asellus* (and perhaps other terrestrial isopods) cannot distinguish food according to cadmium concentration. The difference in consumption rates between uncontaminated and contaminated food is based on integration of feeding behaviour and the adverse metabolic effects of cadmium.

If this presumption is proven to be correct, results from experiments where *O. asellus* is employed as biomonitoring organism are accurate. The findings of this paper strongly support the generally held concept that the maximum amount of knowledge has to be accumulated about a species prior to its potential use as a biomonitor.

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Povzetek

Kopenski raki enakonožci v telesu kopijo kovine, kar jih uvršča med možne pokazatelje dostopnosti kovin v okolju. Nekatere raziskave so pokazale, da enakonožci izbirajo hrano z nižjo vsebnostjo kovin. Izberi hrane z manj kovin lahko pomeni, da vsebnost kovin v enakonožcih ne kaže količine dostopnih kovin v okolju. V tem primeru bi bila vprašljiva uporaba enakonožcev kot pokazateljev obremenjenosti okolja s kovinami.

V predstavljenem delu smo proučevali vpliv različnih koncentracij hrani dodanega kadmijevega nitrata na izbiro hrane pri kopenskem enakonožcu *Oniscus asellus*. Živalim smo hkrati ponudili hrano brez dodanega kadmija in hrano z dodanim kadmijevim nitratom (20, 45, 200 in 400 mg Cd kg⁻¹ suhe teže hrane). Hrano smo pripravili kot mešanico mletih listov leske, ribje hrane in želatine v razmerju 63:3:34. Količino nitratnih ionov v ponujeni hrani smo uravnovežili z dodajanjem raztopine kalijevega nitrata (KNO₃). Primerjali smo stopnjo hranjenja z neonesnaženo in onesnaženo hrano, v odvisnosti od koncentracije kadmijevega nitrata v hrani in trajanja hranjenja.

Živali so zaužile manj s kadmijem onesnažene hrane že v prvem tednu poskusa izbire tudi pri najnižji koncentraciji kadmijevega nitrata dodanega hrani. V tretjem tednu poskusa je bilo pri vseh skupinah živali razmerje med stopnjo hranjenja z neonesnaženo in onesnaženo hrano 6:4. Pri živalih,

ki so med poskusom jedle hrano z dodanim kadmijevim nitratom je telesna vsebnost kadmija naraščala s koncentracijo kadmija v hrani in časom hranjenja.

Rezultati poskusov izbere hrane so pokazali, da *O. asellus* zaužije manj s kadmijem onesnažene hrane, če ima na voljo neonesnaženo. Vendar razmerje med zaužitjem neonesnažene in onesnažene hrane ne narašča s koncentracijo kadmija v hrani kakor tudi ne s časom hranjenja. Tako sklepamo, da *O. asellus* verjetno ne razlikujejo med hrano z različno vsebnostjo kadmijevega nitrata. Razlike v stopnji hranjenja z neonesnaženo in onesnaženo hrano so verjetno le posledica prehranjevalnega vedenja živali povezanega s presnovnimi učinki kadmija. Če je naša domneva pravilna, uporaba *O. asellus* kot pokazatelja dostopnosti kovin v okolju ni vprašljiva.

Literature:

- BAYNE B.L. 1979: Assessing effects of marine pollution. *Nature* **280**: 14-15.
- BECK L. & E. BRESTOWSKY 1980: Auswahl und Verwertung verschiedener Fallaubarten durch *Oniscus asellus* (Isopoda). *Pedobiologia* **20**: 428-441.
- CORTET J., A. GOMOT-DE VAUFLERY, N. POINSOT-BALAGUER, L. GOMOT, C. TEXIER & D. CLUZEAU 1999: The use of invertebrate soil fauna in monitoring pollutant effects. *Eur. J. Soil. Biol.* **35** (3): 115-134.
- COUGHTREY P. J., M. H. MARTIN & E. W. YOUNG 1997: The woodlouse, *Oniscus asellus*, as a monitor of environmental cadmium levels. *Chemosphere* **12**: 827-832.
- CROMMENUIJ T., J. A. M. DOODEMAN & A. DOORNEKAMP 1994: Lethal body concentrations and accumulation patterns determine time-dependent toxicity of Cadmium in soil arthropods. *Environmental Toxicology and Chemistry* **13** (11): 1781-1789.
- DONKER M.H. & C.G. BOGERT 1991: Adaptation to cadmium in three populations of the isopod *Porcellio scaber*. *Comp. Biochem. Physiol.* **12**: 143-146.
- DALLINGER R. 1977: The flow of copper through a terrestrial food chain: III. Selection of an optimum copper diet by isopods. *Oecologia* **30**: 273-276.
- DROBNE D., P. ZIDAR & P. BJRREGAARD 1995: Could proposed bio-monitoring organisms select differently contaminated food? Abstracts, Sixth International Symposium, Metal Compounds in Environment and Life, Jülich, Germany, May 9-12, p. 35.
- DROBNE D. 1996: Zinc toxicity to terrestrial isopods (Isopoda, Crustacea). Dissertation Thesis. Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo, Ljubljana, Slovenia.
- DROBNE D. 1997: Terrestrial isopods – a good choice for toxicity testing of pollutants in the terrestrial environment. *Environmental Toxicology and Chemistry* **16**: 1159-1164.
- GOLDBERG E. D. & K. K. BERTINE 2000: Beyond the mussel watch – new directions for monitoring marine pollution. *The Science of the Total Environment* **247**: 165-174.
- GUNNARSSON T. 1987: Soil arthropods and their food: choice, use and consequences. Doctoral thesis. Department of Ecology, University of Lund, Sweden.
- HAMES C. A. C & S. P. HOPKIN 1989: The structure and function of the digestive system of terrestrial isopods. *J. Zool.* **217**: 599-627.
- HAMES C. A. C. & S. P. HOPKIN 1990: A daily cycle of apocrine secretion by the B cells in the hepatopancreas of terrestrial isopods. *Can. J. Zool.* **69**: 1931-1937.
- HAMES C. A. C. & S. P. HOPKIN 1991: Assimilation and loss of ¹⁰⁹Cd and ⁶⁵Zn by the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. *Bull. Environ. Contam. Toxicol.* **47**: 440-447.
- HOPKIN S. P. & M. H. MARTIN 1984: Heavy metals in woodlice. *Symp. Zool. Soc. (London)* **53**: 143-166.
- HOPKIN S. P, G. N. HARDISTY & M. H. MARTIN 1986: The woodlouse *Porcellio scaber* as a 'biological Indicator' of zinc, cadmium, lead and copper pollution. *Environmental Pollution* **11**: 271-290.

- HOPKIN S. P. 1989: Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Sciences, London and New York.
- HOPKIN S. P. 1990: Species-specific differences in the net assimilation of zinc, cadmium, lead, copper and iron by the terrestrial isopod *Oniscus asellus* and *Porcellio scaber*. Journal of Applied Ecology 27: 460-474.
- HOPKIN S. P., DT. JONES & D. DIETRICH 1993: The isopod *Porcellio scaber* as a monitor of the bioavailability of metals in terrestrial ecosystems: towards a global 'woodlouse watch' scheme. The Science of the Total Environment, Supplement 1993, Elsevier Science Publishers B.V., Amsterdam, 357-365.
- ODENDAAL J. P. & A . J. REINECKE 1999: The sublethal effects and accumulation of cadmium in the terrestrial isopod *Porcellio laevis* Latr. (Crustacea, Isopoda). Arch. Environ. Contam. Toxicol. 36: 64-69.
- PAOLETTI M. G. & M. HASSALL 1999: Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems and Environment 74: 157-165.
- PROSI F., V. STORCH & H. H. JANSEN (1983): Small cells in the midgut glands of terrestrial Isopoda: Sites of heavy metal accumulation. Zoomorphology 102: 53-64.
- RABITSCH W. B. 1995: Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. I. Environmental Pollution 90, No. 2: 221-237.
- STEEL C. G. H. 1980: Mechanisms of coordination between moulting and reproduction in terrestrial isopod (Crustacea). Biol. Bull. 159, 206-218.
- STEEL C. G. H. 1982: Stages of the intermoult cycle in the terrestrial isopod *Oniscus asellus* and their relation to biphasic cuticle secretion. Can. J. Zool. 60: 429-437.
- SZLAVECZ K. & V. C. MAIORANA 1990: Food selection by isopods in paired choice tests. In: Biology of Terrestrial Isopods. Third International Symposium. Poitiers, July 10-12, 1990.
- VAN CAPELLEVEEN H. E., N. M. VAN STRAALEN, M. VAN DEN BERG & E. VAN WACHEM 1986: Avoidance as a mechanism of tolerance for lead in terrestrial arthropods. In: Proc 3rd Eur. Congress. Entom. Part 2 (H.H.W. Veldhuis, Ed.), 251-255. N. Ev., Amsterdam.
- ZIDAR P., D. DROBNE & J. ŠTRUS 1998: Determination of moult stages of *Porcellio scaber* (Isopoda) for routine use. Crustaceana 71, 6: 646-654.
- ZIMMER M., G. KAUTZ & W. TOPP 1996: Olfaction in terrestrial isopods (Crustacea: Oniscidea): responses of *Porcellio scaber* to the odour of litter. Eur. J. Soil. Biol. 32 (3): 141-147.

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Adaptive mutation: shall we survive bacterial genetic skills?

Adaptivna mutacija: bomo preživelci genetske veščine bakterij?

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Abstract. The origin and dynamics of genetic variations is one of the key questions in the modern science that has still not come out with a final answer. Emerging concepts regarding genetic variation have always produced a great controversy because they hold a key to unlock a great mystery of evolution. With such a powerful motivation scientist working in the molecular biology, genetics and biochemistry gathered a vast amount of experimental data showing us that a genome is a dynamic, hierarchically organized and complex integrated system for storing and processing information. Dynamic balance between stability and mutability of DNA nucleotide sequences is essential for a proper functioning of the organism. Beside many DNA repairing proteins and DNA protective mechanisms organisms possess also biochemical systems capable of changing DNA information. One of the most controversial and at the same time the most informative one is a phenomenon called adaptive mutation. We shall review findings concerning the phenomenon of adaptive mutation in prokaryotes and point out an urgent need for the upgrade of the awkward neo-darwinistic view on the origin of the genetic variation.

Keywords: adaptive mutation, inducible mutagenesis, transposable elements, signal transduction network, neo-darwinism

Izvleček. Izvor in dinamika genetskih variacij je eden od ključnih vprašanj moderne znanosti, ki še vedno čaka na dokončen odgovor. Koncepti v zvezi z genetskimi variacijami povzročajo veliko polemik, saj nosijo ključ do skrivnosti evolucije. Molekularni biologi, genetiki in biokemiki, so zavoljo tako močnega motiva zbrali ogromno količino eksperimentalnih podatkov, ki kažejo, da je genom dinamičen, hiearhično organiziran in kompleksno integriran sistem za shranjevanje in obdelovanje informacij. Dinamično ravnotežje med stabilnostjo in spremenljivostjo DNK zapisa je nujno za pravilno funkcioniranje organizma. Poleg številnih DNK popravljalnih proteinov in DNK varovalnih mehanizmov, imajo organizmi tudi biokemične sisteme, sposobne spremnjanja DNK informacije. Eden najbolj polemičnih in hkrati najbolj poučnih je fenomen imenovan adaptivna mutacija. Nujen namen je pregledati doganjaj

o adaptivni mutaciji pri prokariontih in pokazati, da je nadgradnja togega neodarvinističnega pogleda na izvor genetskih variacij, nujno potrebna.

Ključne besede: adaptivna mutacija, inducibilna mutageneza, transponibilni elementi, signal prevajalna mreža, neodarvinizem

Introduction

In its essence genetic variation stirred up scientists as far back as the Darwin's era in the 19th century. Despite the fact that Darwin possessed no knowledge about genetics, he suspected that variations between individuals or mutations did not originate only from random events. He thought that there were also adaptive mutations induced by the environment. However, he commented that it was reasonable to treat them as random, as long as we do not know their origin (Darwin 1859).

At the start of the antibiotic era in 1940' the emergence of antibiotic resistant mutants stimulated Luria & Delbrück (1943) to perform a classical experiment, where they exposed bacterial population to a lethal selective pressure of a bacteriophage T1. Phage immediately killed non-resistant cells and only cells with a pre-existing specific mutation could survive the exposure to the phage. A thorough analysis of a number of surviving colonies and their distribution in independent cultures proved the existence of random mutations that arose during the growth with no relation to the selective pressure. Together with the persuasive results from Lederberg & Lederberg (1952) and Cavalli-Sforza & Lederberg (1956) researchers concluded that all mutations arose randomly, prior to or in the absence of the selective pressure. They considered mutations as only a consequence of a non-perfection DNA replication machinery. Genetic variations thus appeared to be totally independent from the needs of the organisms in the environment with natural selection as a final statistical filter to decide which organism will survive. This notion became the central idea of the neo-darwinistic evolutionary biology.

First results that contradicted this well established dogma came already in the 50' when Ryan demonstrated genomic changes without the DNA replication (Ryan & Wainwright 1954, Ryan 1955), but he failed to show that changes follow the applied selective pressure. First indication that the environment can influence the mutation process came much later when Shapiro (1984) used genetically engineered bacterial cells with a mutation that prevented them to use a specific carbon source. By using a non-lethal selective pressure, he observed accumulation of mutants on the plates during the selection.

The turning point in the research on the origin and dynamics of the genetic variation came four years later when Cairns and co-workers (1988) challenged the established biological dogma with an argument that mutations in the direction of phage or antibiotic resistance are not expressed until after the period of growth. Since lethal conditions used killed bacterial cells or at least completely inhibited their growth, classical experiments could not detect and did not exclude the existence of mutations that could arise *after* the selective pressure was applied. The temporal and numerical distribution of surviving mutant colonies clearly demonstrated that during non-lethal and non-mutagenic selective pressure non-growing or slowly growing bacterial cells experience a specific mutation, named *adaptive mutation* that relieves the selective pressure.

The notion that mutations arise in non-dividing stationary cells (Cairns & Foster 1991) was strongly reminiscent of Lamarck's ideas. This, of course, provoked a great upheaval in the scientific community (Lenski & al. 1989, Lenski & Mittler 1993), and it was mainly due to the fact that adaptive mutations arise only after the selective pressure was applied and in the presence of the selective agent. So it seemed that in some way the applied stress directs mutations in a useful way on appropriate sites (Cairns & al. 1988, Foster & Cairns 1992).

After the presentation of such challenging results and thinking, researchers from various countries and different backgrounds performed many new studies and a great deal of experimental data were collected. Improvements in experimental techniques enabled researchers to get a deeper understanding

of the complexity of molecular events taking place during the processes of mutation, which inevitably lead to a general agreement that an awkward classical neo-darwinistic doctrine, claiming that there is no relation between the needs of the organism and its mutability, must be upgraded.

Current findings in adaptive mutation research

Great efforts from many researchers in the last 14 years gave rise to numerous mutational systems, from which some of them became representative and often more explored and understandable. Our intention is to arrange a review of current findings in adaptive mutation research according to such systems and at the end present a discussion on the origin and dynamics of the bacterial genetic variation with pointing out some possible effects of such bacterial genetic variability on the species *Homo sapiens sapiens*.

Cairns system

Escherichia coli cells strain FC40 carry an a+1 frameshift mutation in an F'-located lacI-lacZ fusion gene (Cairns & Foster 1991, Foster 1999) and therefore cannot metabolise lactose. After plating on a minimal medium with lactose as the only carbon and energy source, they readily revert to lactose utilization. After many studies and many proposals it turned out that this most popular adaptive mutation assay system, also called Cairns system, is explainable by a standard Darwinian process (Andersson & al. 1998, Hendrickson & al. 2002). The explanation of this phenomenon, called the amplification-mutagenesis model, presupposes that a non-selective growth before plating enables few cells to acquire a simple duplication of the leaky lac mutant allele. After plating, such cells initiate slowly growing clones and with further amplification soon dominate the colony. Eventually a cell of the clone experiences an adaptive mutation that enables it to utilize lactose as a sole carbon and energy source. Selection then favours only stable mutants carrying only the revertant allele; these cells overgrow the clone and begin strongly to predominate in the mature revertant colony.

A recombination between repeated sequences of amplified alleles releases DNA fragments, which are degraded to single DNA strands that induce the SOS system. Induction of the SOS system was confirmed in the *E. coli* strain FC40 (McKenzie & al. 2000). The SOS response induces an error-prone DNA polymerase DinB (Wagner & al 1999, Wagner & Nohmi 2000, Tang & al. 2000, McKenzie & al. 2001) which leads to extra mismatches that exceed the capacity of the mismatch repair system and therefore a cell experiences a genome-wide mutagenesis known as hypermutation (Hall 1990, Foster 1997, Torkelson & al. 1997, Rosenberg & al. 1998, Rosche & al. 1999, Lombardo & al. 1999, Bull & al. 2000a,b, Cairns 2000, Godoy & Fox 2000a, Godoy & al. 2000b, Foster 2000, Rosenberg 2001, Bridges 2001). Temporary hypermutability is therefore a side effect of the lac operon amplification (Hendrickson & al. 2002).

The amplification-mutagenesis model is totally in tune with the neo-darwinistic view of the origin and dynamics of the genetic variation because here the environment does not direct mutation in specific regions, does not have direct effects on the general mutation rate and mutations do not occur in non-growing stationary cells but in growing subpopulations. But as it will be seen, this model cannot explain other cases of the adaptive mutation in cells under a specific selection pressure.

Adaptive mutation mediated by Mu prophage

Probably the clearest example showing us the incompleteness of the classical neo-darwinistic paradigm is a Mu-mediated adaptive mutation. Bacteriophage Mu is a very notorious mutator phage

that induces mutations in a host genome. Mu element is integrated into the bacterial genome and exists inside the cell in a latent prophage state, as long as the genes controlling its lytic pathway are not expressed. A phage-encoded repressor maintains the control of the expression. In the case of *E. coli* the repressor of the strain used by Shapiro (1984) was temperature sensitive and the Mu prophage was inserted into the araB gene. The prophage represents both a translational and a transcriptional block preventing the expression of downstream located genes lacZ and lacY. When the prophage excises, genes lacZY can be fused to an araB gene and the cell encodes a hybrid AraB-LacZ protein.

Shapiro (1984) reported that the Mu-mediated formation of araB-lacZ fusions occurred when cells were plated on the selective lactose minimal medium with the arabinose as an inducer. Mutants accumulate on the selective media more frequently than during the normal growth. Later it was demonstrated that fusions occurred only in the presence of the lactose (Cairns & al. 1988), but this was subsequently denied. Today it is known that an aerobic starvation could induce the formation of araB-lacZ fusions also in the absence of lactose (Mittler & Lenski 1990, Maenhaut-Michel & Shapiro 1994, Foster & Cairns 1994, Sniegowski 1995).

But it turned out that the most staggering point regarding Mu-mediated adaptive mutations was a demonstration that the structures of araB-lacZ fusions occurring during aerobic starvation on the glucose or on the lactose-arabinose medium differed from each other (Maenhaut-Michel & Shapiro 1994, Maenhaut-Michel & al. 1997). This means that selective conditions have some influence on the mechanism by which the fusions occur. Through the known complexity of the fusion formation (Shapiro & Leach 1990, Gomez-Gomez & al. 1997, Lamrani & al. 1999) it becomes clear that we are witnessing a multi-step process and not some stochastic individual mutational event. This process enables bacterial cells to control their genetic variation according to the environmental conditions.

Adaptive mutation mediated by insertion sequences

Another example of a mutational event affected by the selective environment represents adaptive mutations in the ebg operon mediated by insertion sequences (IS). IS are less than 2,5kb long segments of the DNA that code only elements responsible for their mobility (Majillion & al. 1998). They are known because of their ability to affect different parts of the genome (Naas 1994 & al., Fedoroff 1999, Shneider & al. 2000) and together with transposons and viruses like Mu constitute a storehouse of mobile DNA elements. IS not only inactivating genes but are also capable of activating the so-called silent or cryptical genes (Reynolds & al. 1981, Hall 1998a, Hall 1999a,b).

E. coli possesses at least four silent systems for the uptake and metabolism of the beta-glucoside sugars (Hall 1998a and references therein). One of them is called the *ebg operon* and it is organized in the same way as the *lac operon*. Ebg operon encodes a repressor EbgR and a beta-galactosidase EbgAC (Hall 1989). If a strain is deleted for the *lacZ*, the *ebgAC* represents the only beta-galactosidase in the cell. The expression of the *ebgAC* is under the control of the repressor *ebgR* and mutations in the *ebgR* gene allow cells to grow on the lactose or related sugars such as lactulose, as a sole source of energy and carbon.

It was shown that during a prolonged starvation on the lactulose 61% of the growth dependent and 80% of adaptive mutations in the *ebgR* gene are mediated by IS (Hall 1999a). 6% of the growth dependent and 39% of adaptive mutations were due to insertions of IS30 in the *ebgR*, so it appears that IS30 transposition may be at least partially directed by some adequate environmental condition (Foster 1999). It was also demonstrated that only adaptive mutations in the *ebgR* gene and not growth dependent mutations are positively regulated by a two-component regulatory system PhoPQ (Hall 1998b).

PhoPQ is a typical two component regulatory system with a regulatory protein in the cytoplasm and a sensory kinase located in the membrane (Stock & al. 2000). Primary signal for the PhoQ sensory

kinase is an extracellular Mg²⁺ (Sonicini & al. 1996, Vescovi & al. 1997). After an extracellular signal is sensed by the sensory kinase, an autophosphorylation takes place at the histidine residue of the sensory kinase, thus creating a high-energy phosphoryl group. This group is then transferred to an aspartate residue in the regulatory protein, which induces a conformational change in the regulatory domain. The result is an activation of at least 50 genes in the *E. coli* (Kasahara & al. 1992, Groisman 2001). PhoPQ regulated proteins are therefore directly or indirectly responsible for the adaptive mutation at the ebgR repressor gene.

Promoter-creating mutations

An incredible adaptation to a starving condition still unexplainable by the classical neo-darwinistic approach was shown with *Pseudomonas putida* cells carrying a plasmid with a promotorless pheAB operon (Kasak & al. 1997) that encodes for the enzyme that decompose phenol. Phenol utilising mutants that accumulated in a starving culture experience base substitutions, deletions and insertions of Tn4652 that created active promoters permitting starving *P.putida* cells to use phenol as a sole source of carbon. Mutants accumulate only in the presence of the phenol and plating stationary phase cells showed higher rate of mutants' accumulation than plating exponential cells.

Another exiting and still unexplainable case of the adaptive mutation is the accumulation of resistant mutants during a non-lethal selective pressure from the antibiotic chloramfenicol (Lioy & al. 2001). During stressful selective conditions sensitive *E. coli* cells carrying a plasmid with a cam promotorless gene, encoding for chloramfenicol acetyl transferase enzyme, experience the insertion of the IS10R mobile element into the upstream of the cam gene. Mobile element is transposed from the bacterial genome and allowed the bacterial RNA polymerase to efficiently transcript cam gene located on the plasmid, which in turn enable cell to survive a non-lethal selective pressure from the antibiotic.

Discussion

Ever since the discovery of the transposable elements in 1950 (McClintock 1984) genome has been viewed as a complex, dynamical and highly organised system for storing and processing information (Berg & Howe 1989, Shapiro 1991, Shapiro 1992, Shapiro 1999a,b, Fedoroff 1999). This means that a bacterial genome is not only a conservative library of triplet codes for cell's constituent elements but also represents a dynamical storage system subject to constant and at least partially regulated changes. After this short but thorough review of the current status in the adaptive mutation research it is obvious that bacterial genetic change can originate from growth dependent random events independently from the environment or from some processes that show sensitivity to specific environmental conditions. The big question is which mode of the mutation generation is more biologically relevant.

To answer that we have to bear in mind the fact that within bacterial genomes coexist highly mutable 'contingency' genes and so called 'housekeeping' genes with low mutation rates (Moxon & al. 1994). This means that not all parts of the bacterial genome are equally mutable and bacterial cells obviously have capacities to control and maintain a balance between stability and mutability of DNA nucleotide sequences. With this in mind it is not surprising that bacterial cells are known to possess many DNA repairing proteins and DNA protecting mechanisms which preserve nucleotide sequences (Radman & al. 1999) and protect organism's needing triplet codes from unpredictable random mutations. However, on the other hand, many times bacterial cells exit stressful conditions only if they acquire specific genetic information. Cells that gain such information sometimes in the past through a random mutational event are not sensing any stress at all. But other sensitive cells that are threatened can

accomplish such a task with *de novo* mutation or with a reorganization of the already existing genetic information that may include a horizontal gene transfer or insertions of transposable elements.

Knowledge gained from the adaptive mutation research demonstrates that during non-lethal stress bacterial cells can experience controlled DNA changes influenced by selective environmental conditions. Bacterial cells must therefore possess some biochemical systems, active only under stress, capable of inducing changes or the reorganisation of genetic information (Shapiro 1991, Shapiro 1992, Shapiro 1997, Radman & al. 1999, Shapiro 1999a,b, Capy 2000). Biochemical systems such as ROSE mutagenesis (Taddei & al. 1995, Taddei & al. 1997), SOS mutagenesis (Fijalkowska & al. 1997) and the adaptive mutation process constitute strategic mechanisms for the genetic variation available to cells during stressful situations. Which cell's response or which inducible biochemical system will predominate depends on many external and internal signals sensed by the cellular signal transduction network. Or in another words, these inducible biochemical systems are regulated by specific controlling mechanisms and show sensitivity to environmental conditions sensed through a complex bacterial signal transduction network (Shapiro 1999a, Massey & al. 1999, Foster 1999).

Conclusions

It may be concluded that both random mutations and controlled genetic changes are present as a constituent elements of the bacterial life cycle. Growth dependent random mutations are unpredictable and bacterial cells are trying to suppress them by numerous repairing strategies. On the other hand, controlled inducible DNA changes are needed during stressful conditions and help many bacterial cells to conquer stress and stay alive. Therefore, we must acknowledge that bacterial cells possess genetic skills that enable a bacterial cell to gain the needed DNA information to exit stress state. We can say that the bacterial genome is evolved to cope with the predictable and also unpredictable challenges that come out from the environment (Caporale 1999).

Last but not least, let us discuss some of the consequences of such bacterial genetic skills on our human lives. Adaptive mutations were demonstrated to be important in a development of antibiotic resistance mutations (Riesenfeld & al 1997, Alonso & al. 1999, Martinez & Baquero 2000, Karunakaran & Davies 2000) and may also provide models for human cancer (Strauss 1992, Hall 1995 Cairns 1998). Needless to say, these two phenomena represent a gigantic problem for modern human society. But at the same time they present a high motivation for researchers to try to understand more deeply and thoroughly the nature of bacterial genetic variation. Therefore it rests on us, researchers, to stay open minded and admit that bacterial cells possess genetic skills that go beyond the ideas of the ordinary neo-darwinian evolutionary doctrine and should not be underestimated.

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References

- ALONSO A., E. CAMPANARIO & J.L. MARTINEZ 1999: Emergence of multidrug-resistant mutants is increased under antibiotic selective pressure in *Pseudomonas aeruginosa*. *Microbiology* (UK) **145**: 2875-2862.
- ANDERSSON D. I., E. S. SLECHTA & J. R. ROTH 1998: Evidence that gene amplification underlies adaptive mutability of the bacterial operon. *Science* **282**: 1133-1135.
- BERG D. E. & M. M. HOWE (ed.) 1989: Mobile DNA. ASM Press, Washington D.C.
- BRIDGES B. A. 2001: Hypermutation in bacteria and other cellular systems. *Philos. Trans. R. Soc. Lond. B* **356**: 29-39.
- BULL H. J., G. J. MCKENZIE, P. J. HASTINGS & S. M. ROSENBERG 2000a: Response to John Cairns: Contribution of transiently hypermutable cells to mutation in stationary phase. *Genetics* **156**: 925-926.
- BULL H. J., G. J. MCKENZIE, P. J. HASTINGS & S. M. ROSENBERG 2000b: Evidence that stationary-phase hypermutation in the *E.coli* chromosome is promoted by recombination. *Genetics* **154**: 1427-1437.
- CAIRNS J. 2000: The Contribution of bacterial hypermutators to mutation in stationary phase. *Genetics* **156**: 923.
- CAIRNS J. & P.L. FOSTER 1991: Adaptive reversions of a frameshift mutation in *Escherichia coli*. *Genetics* **128**: 695-701.
- CAIRNS J. 1998: Mutation and cancer: The antecedents to our studies of adaptive mutation. *Genetics* **148**: 1433-1440.
- CAIRNS J., J. OVERBAUGH & S. MILLER 1988: The origin of mutants. *Nature* **335**: 142-145.
- CAPORALE L.H. 1999: Chance favors the prepared genome. *Ann. N.Y. Acad. Sci.* **870**: 1-21.
- CAPY P., G. GASPERI, C. BIEMONT & C. BAZIN 2000: Stress and transposable elements: co-evolution or useful parasites? *Heredity* **85**(2): 101-106.
- CAVALLI-SFORZA L. L. & J. LEDERBERG 1956: Isolation of pre-adaptive mutants in bacteria by sib selection. *Genetics* **41**: 367-381.
- DARWIN C. 1859: The origin of species., J. Murray, Albemarle Street, London
- FEDOROFF N. V. 1999: Transposable elements as a molecular evolutionary force. *Ann. N.Y. Acad. Sci.* **870**: 251-264.
- FJALKOWSKA I. J., R. L. DUNN & R.M. SCHAAPEL 1997: Genetic requirements and mutational specificity of the *E.coli* SOS mutator activity. *J. Bacteriol.* **179**: 7435-7445.
- FOSTER P. L. & J. CAIRNS 1992: Mechanisms of directed mutation. *Genetics* **131**: 783-789.
- FOSTER P. L. & J. CAIRNS 1994: The occurrence of heritable Mu excisions in starving cells of *E.coli*. *EMBO J.* **13**: 5240-44.
- FOSTER P. L. 1997: Non-adaptive mutations occur on the F' episome during adaptive mutation conditions in *Escherichia coli*. *J. Bacteriol.* **179**: 1550-1554.
- FOSTER P. L. 1999: Mechanisms of stationary phase mutation: A decade of adaptive mutation. *Annu. Rev. Genet.* **33**: 57-88.
- FOSTER P. L. 2000: Adaptive mutation: implications for evolution. *BioEssays* **22**: 1067-1074.
- GALITSKI T. & J. R. ROTH 1996: A search for a general phenomenon of adaptive mutability. *Genetics* **143**: 645-659.
- GODOY V. G. & M. S. FOX 2000a: Transposon stability and a role for conjugational transfer in adaptive mutability. *Proc. Natl. Acad. Sci. U. S. A.* **97**: 7393-7398.
- GODOY V. G., F. S. GIZATULLIN & M.S. FOX 2000b: Some features of the mutability of bacteria during nonlethal selection. *Genetics* **154**: 49-59.
- GOMEZ-GOMEZ J. M., J. BLAZQUEZ, F. BAQUERO & J.L. MARTINEZ 1997: H-NS and RpoS regulate emergence of LacAra+ mutants of *E.coli* MCS2. *J. Bacteriol.* **179**: 4620-4622.

- GROISMAN E. A. 2001: The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* **183**: 1835-1842.
- HALL B. G. 1988: Adaptive evolution that requires multiple spontaneous mutations. I. Mutations involving an insertion sequence. *Genetics* **120**: 887-897.
- HALL B. G. 1990: Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* **126**: 5-16.
- HALL B. G. 1995: Adaptive mutations in *E.coli* as a model for the multiple mutational origins of tumors. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 5669-5673.
- HALL B. G. 1998a: Activation of the bgl operon by adaptive mutation. *Mol. Biol. Evol.* **15**(1): 1-5.
- HALL B. G. 1998b: Adaptive mutagenesis at ebgR is regulated by PhoPQ. *J. Bacteriol.* **180**: 2862-2865.
- HALL B. G. 1998c: Adaptive mutagenesis: a process that generates almost exclusively beneficial mutations. *Genetica* **102/103**: 109-125.
- HALL B. G. 1999a: Spectra of spontaneous growth-dependent and adaptive mutations at ebgR. *J. Bacteriol.* **181**: 1149-1155.
- HALL B. G. 1999b: Transposable elements as activators of cryptic genes in *E.coli*. *Genetica* **107**: 181-187.
- HALL B. G., P.W. BETTS & J.C WOOTTON 1989: DNA sequence analysis of artificially evolved ebg enzyme and ebg repressor genes. *Genetics* **123**: 635-648.
- HARRIS S. B., G. FENG, K. J. ROSS, R. SIDHU, C. THULIN, S. LONGERICH, S. K. SZIGETY, M. E. WINKLER & S.M. ROSENBERG 1997: Mismatch repair protein MutL becomes limiting during stationary-phase mutation. *Genes Dev.* **11**: 2426-2437.
- HENDRICKSON H., E. S. SLECHTA, U. BERGTHORSSON, D. I. ANDERSSON & J. R. ROTH 2002: Amplification-mutagenesis: Evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. *Proc. Natl. Acad. Sci. U. S. A.* **99**: 2164-2169.
- JAYARAMAN R. 2000: Modulation of allele leakiness and adaptive mutability in *E.coli*. *J. Genetics* **79**(2): 55-60.
- JAYARAMAN R. 1995: Leakiness of genetic markers and susceptibility to post-plating mutagenesis in *E.coli*. *J. Genetics* **74**(3): 85-79.
- KARUNAKARAN P. & J. DAVIES 2000: Genetic antagonism and hypermutability in *Mycobacterium smegmatis*. *J. Bacteriol.* **182**: 3331-3335.
- KASAHARA M., A. NAKATA & H. SHINAGAWA 1992: Molecular analysis of the *Escherichia coli* phoP-phoQ operon. *J. Bacteriol.* **174**: 492-498.
- KASAK L., R. HORAK & M. KIVISAAR 1997: Promoter-creating mutations in *Pseudomonas putida*: A model system for the study of mutation in starving bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 3134-3139.
- LAMRANI S., C. RANQUET, M-J. GAMA, H. NAKAI, J. A. SHAPIRO, A. TOUSSAINT & G. MAENHAUT-MICHEL 1999: Starvation-induced Mucts62-mediated coding sequence fusion: a role for ClpXP, Lon, RpoS and Crp. *Mol. Microbiol.* **32**: 327-343.
- LEDERBERG J. & E. M. LEDERBERG 1952: Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63**: 399-406.
- LENSKI R. E. & J. E. MITTLER 1993: The directed mutation controversy and neo-darwinism. *Science* **259**: 188-194.
- LENSKI R. E., M. SLATKIN & F. J. AYALA 1989: Mutation and selection in bacterial populations: Alternatives to the hypothesis of directed mutation. *Proc. Natl. Acad. Sci. U. S. A.* **86**: 2775-2778.
- LIOY M., S. DABIZZI, S. AMMANATO, A. CACIOTTI, L. CIONI & R. FANI 2001: Activation of cam promoterless gene by ISR10 transposition in an *Escherichia coli* population under stress conditions. *Ann. Microbiol.* **51**: 225-233.

- LOMBARDO M. J., J. TORKELSON, H. J. BULL, G. J. MCKENZIE & S. M. ROSENBERG 1999: Mechanisms of genome-wide hypermutation in stationary phase. Ann. N.Y. Acad. Sci. **870**: 275-289.
- LURIA S. E. & M. DELBRÜCK 1943: Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**: 491-511.
- MADIGAN M. T., J. M. MARTINKO & J. PARKER (ed.) 2000: Brock Biology of microorganisms. Ninth edition. Prentice Hall, NY.
- MAENHAUT-MICHEL G. & J. A. SHAPIRO 1994: The roles of starvation and selective substrates in the emergence of araB-lacZ fusion clones. EMBO J. **13**: 5229-5239.
- MAENHAUT-MICHEL G., C. E. BLAKE, D. R. F. LEACH & J. A. SHAPIRO 1997: Different structures of selected and unselected araB-lacZ fusions. Mol. Microbiol. **23**: 1133-1145.
- MAHILLON J. & M. CHANDLER 1998: Insertion sequences. Microbiol. Mol. Biol. Rev. **62**(3): 725-774.
- MARTINEZ J. L. & F. BAQUERO 2000: Mutation frequencies and antibiotic resistance. Antimicrob. Agents. Chemother. **44**: 1771-1777.
- MASSEY R. C., P. B. RAINES, B. J. SHEEHAN, KEANE O. M. & C. J. DORMAN 1999: Environmentally constrained mutation and adaptive evolution in *Salmonella*. Curr. Biol. **9**: 1477-1480.
- MCCLINTOCK B. 1984: The significance of responses of the genome to challenge. Science **226**: 792-801.
- MCKENZIE G. J., P. L. LEE, M. J. LOMBARDO, P. J. HASTINGS & S. M. ROSENBERG 2001: SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Mol. Cell. **7**: 571-579.
- MCKENZIE G. J., R. S. HARRIS, P. L. LEE & S. M. ROSENBERG 2000: The SOS response regulates adaptive mutation. Proc. Natl. Acad. Sci. U. S. A. **97**: 6646-6651.
- MITTLER J. E. & R. E. LENSKI 1990: New data on excisions of Mu from *E.coli* MCS2 cast doubt on directed mutation hypotheses. Nature **344**: 173-175.
- MOXON E. R., P. B. RAINES, A. M. NOWAK & R. E. LENSKI 1994: Adaptive evolution of highly mutable loci in pathogenic bacteria. Curr. Biol. **4**: 24-33.
- NAAS T., M. BLOT, W. M. FITCH & W. ARBER 1994: Insertion sequence-related genetic variation in resting *E.coli* K-12. Genetics **136**: 721-730.
- RADMAN M., I. MATIC & F. TADDEI 1999: Evolution of evolvability. Ann. N.Y. Acad. Sci. **870**: 146-155.
- REYNOLDS A. E., J. FELTON & A. WRIGHT 1981: Insertion of DNA activates the cryptic bgl operon in *E.coli* K12. Nature **293**: 625-629.
- RIESENFELD C., M. EVERETT, L.J.V. PIDDOCK & B.G. HALL 1997: Adaptive mutations produce resistance to ciprofloxacin. Antimicrob. Agents Chemoter. **41**: 2059-2060.
- ROSCHE W. A. & P. L. FOSTER 1999: The role of transient hypermutators in adaptive mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **96**: 6862-6867.
- ROSENBERG S. M. 2001: Evolving responsively: adaptive mutation. Nat. Rev. Genet. **2**: 504-515.
- ROSENBERG S. M., C. THULIN & R. S. HARRIS 1998: Transient and heritable mutators in adaptive evolution in the lab and in nature. Genetics **148**: 1559-1566.
- RYAN F. J. & L. K. WAINWRIGHT 1954: Nuclear segregation and the growth of the clones of spontaneous mutants of bacteria. J. Gen. Microbiol. **11**: 364-379.
- RYAN F. J. 1955: Spontaneous mutations in non-dividing bacteria. Genetics **40**: 726-738.
- SCHNEIDER D., E. DUPERCHEY, E. COURSANGE, R. E. LENSKI & M. BLOT 2000: Long-term experimental evolution in *Escherichia coli*. IX. Characterization of insertion sequence-mediated mutations and rearrangements. Genetics **156**: 477-488.
- SHAPIRO J. A. & D. LEACH 1990: Action of a transposable element in coding sequence fusions. Genetics **126**: 293-299.
- SHAPIRO J. A. 1984: Observations on the formation of clones containing araB-lacZ cistron fusions. Mol. Gen. Genet. **194**: 79-90.
- SHAPIRO J. A. 1991: Genomes as smart systems. Genetica **84**: 3-4.
- SHAPIRO J. A. 1992: Natural genetic engineering in evolution. Genetica **86**: 99-111.

- SHAPIRO J. A. 1995: Adaptive mutation: Who's really in the garden? *Science* **268**: 373-374.
- SHAPIRO J. A. 1997: Genome organization, natural genetic engineering and adaptive mutation. *Trend Genet.* **13**(3): 98-104.
- SHAPIRO J. A. 1999a: Genome system architecture and natural genetic engineering in evolution. *Ann. N.Y.Acad. Sci.* **870**: 23-35.
- SHAPIRO J. A. 1999b: Transposable elements as the key to a 21st century view of evolution. *Genetica* **107**: 171-179.
- SNIEGOWSKI P. D. 1995: A test of the directed mutation hypothesis in *E.coli* MCS2 using replica plating. *J. Bacteriol.* **177**: 1119-1120.
- SONCINI F. C. & E. A. GROISMAN 1996: Two component regulatory systems can interact to process multiple environmental signals. *J. Bacteriol.* **178**: 6796-6801.
- STOCK A. M., V. L. ROBINSON, P. N. GOUDREAU 2000: Two-component signal transduction. *Annu. Rev. Biochem.* **69**: 183-215.
- STRAUSS B. S. 1992: The origin of point mutations in human tumor cells. *Cancer Res.* **52**: 249-253.
- TADDEI F., I. MATIC & M. RADMAN 1995: cAMP-dependent SOS induction and mutagenesis in resting bacterial populations. *Proc. Natl. Acad. Sci. U. S. A.* **92**: 11736-11740.
- TADDEI F., J.A. HALLIDAY, I. MATIC & M. RADMAN 1997: Genetic analysis of mutagenesis in aging *E.coli* colonies. *Mol. Gen. Genet.* **256**: 277-281.
- TANG M., P. PHAM, X. SHEN, J.S. TAYLOR, M. O'DONNEL, R. WOODGATE & M.F. GOODMAN 2000: Roles of *E.coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis. *Nature* **404**: 1014-1018.
- TORKELSON J., R. S. HARRIS, M.J. LOMBARDO, J. NAGENDRAN, C. THULIN & S.M. ROSENBERG 1997: Genome-wide hypermutation in a sub-population of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* **16**: 3303-3311.
- VESCOVI E. G., Y. M. AYALA, E. DI CERA & E.A. GROISMAN 1997: Characterization of the bacterial sensor protein PhoQ. Evidence for distinct binding sites for Mg²⁺ and Ca²⁺. *J. Biol. Chem.* **272**: 1440-1443.
- WAGNER J. & T. NOHMI 2000: *Escherichia coli* DNA polymerase IV mutator activity: genetic requirements and mutational specificity. *J. Bacteriol.* **182**: 4587-4595.
- WAGNER J., P. GRUZ, S. R. KIM, M. YAMADA, K. MATSUI, R.P. FUCHS. & T. NOHMI 1999: The dinB gene encodes a novel *E.coli* DNA polymerase, DNA pol IV, involved in mutagenesis. *Mol. Cell.* **4**: 281-286.

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Aquatic macrophytes of the mountain lake Krnsko jezero, Slovenia

Vodni makrofiti Krnskega jezera, Slovenija

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Abstract. The macrophyte vegetation in the mountain lake Krnsko jezero has been monitored since 1991. Five species of aquatic macrophytes, occupying different depths, were present in the lake. Changes in depth distribution were detected due to accelerated eutrophication caused by inputs of nutrients from the watershed. In the period between 1997 and 1998 repeated earthquakes additionally influenced the processes in the lake Krnsko jezero, by increased input of matter. The occasional phytoplankton blooms reduced water transparency and consequently disturbance to growth, development and distribution of anchored macrophytes occurred. In 1998 macrophytes spread down to 7.0 m, reaching the maximum at 5.8 kg DW m⁻² at 2 m depth. The total primary production in the lake was estimated at 4991 kg organic matter per year out of which *Chara* and *Potamogeton* species presented 95.5 % and 0.6 %, respectively.

Key words: aquatic macrophytes, chlorophyll *a*, lake Krnsko jezero, mountain lake, nutrients, organic matter, primary production

Izvleček. Makrofite v Krnskem jezeru spremljamo od 1991 leta. V jezeru je prisotnih pet vrst podvodnih rastlin, ki uspevajo na različnih globinah. Zaradi pospešene evtrofikacije, ki je odraz povečanega vnosa nutrientov iz zaledja, se globina uspevanja spreminja. Med letoma 1997 in 1998 je bila serija potresov, zaradi česar se je vnos snovi iz zaledja povečal in dodatno vplival na procese v jezeru. Občasno cvetenje fitoplanktona je zmanjšalo prosojnost vode, kar se je odrazilo na rasti, razvoju in razporeditvi submerznih makrofitov. Tako so v letu 1998 makrofiti segali do globine 7 m, njihova največja biomasa je bila 5,8 kg suhe teže m⁻² na globini 2 m. Celotna primarna produkcija organske snovi v jezeru je bila ocenjena na 4991 kg leto⁻¹, pri čemer je bil delež parožnic 95,5 %, delež dristavcev pa le 0,6 %.

Ključne besede: vodni makrofiti, klorofil *a*, Krnsko jezero, gorsko jezero, nutrienti, organska snov, primarna produkcija

Introduction

Mountain lakes, usually located in remote natural environment, are becoming interesting sites of ecological research for two reasons *inter alia*: they are relatively small and they are sensitive to different influences from the environment. The alpine lake Krnsko jezero is an eutrophic lake (GABERŠČIK & URBANC-BERČIČ 1996). In the last century some events had put an evident mark on this ecosystem. During the First World War the wider area was the centre of severe battles that had resulted in increased input of matter from the watershed. Latter on two species of fish were introduced in the lake; in late twenties *Salvelinus alpinus* (Linnaeus, 1758) and two decades latter *Phoxinus phoxinus* (Linnaeus, 1758) (BRANCELJ & al. 1997). Other human activities, pasturing and mountaineering at most, presented an influential activity, the latter being intensified in the last decade. The influence of these activities had been closely studied in the nearby lake (BRANCELJ & al. 2000).

The first investigation of basic geographic and hydrobiologic characteristics was carried out in the late fifties (GAMS 1962), but no record on macrophytes was made until 1988 (BLAŽENČIČ & al. 1990). In 1996 the systematic research of 14 alpine lakes started as an addition to the previous 5-year monitoring. The study had revealed that the most abundant aquatic vegetation was in the lake Krnsko jezero (GABERŠČIK & URBANC-BERČIČ, 1996). Comparable studies to ours on primary production in mountain lakes was not found but in our case it was evident that macrophytes contributed a great deal to the production of the lake. Researches on lowland lakes, rich with aquatic vegetation showed, that macrophytes present an important factor maintaining the stability of the ecosystem (OZIMEK & al. 1990, RØRSLETT 1991, RASPOPOV & al. 1996).

The present study was carried out in order to evaluate the abundance and production of anchored macrophytes, related to the main trophic parameters and their contribution to the primary production of the lake.

Material and methods

Site description

The lake Krnsko jezero is located well bellow the timberline at the altitude of 1383 m. It is of glacial origin. Lake is the largest and the deepest in the Triglav National Park, covering the area of 49600 m². The lake is 17.6 m deep (Fig. 1). At the highest water level the total volume of 446700 m³ of water accumulates in the lake. At heavy rains fluctuations of water level could be as high as 4 m, reaching the normal level within two days. The lake is ice covered usually from November to May. During the summer it is stratified, with late spring (after ice-cover melting) and late autumn homothermy. The nearby watershed is diverse, formed of stones, gravel, rocks, meadows and pastures. The wider area is geologically unstable and some severe earthquakes with soil avalanches have caused strong disturbances in watershed and increased the input of matter.

Water chemistry

The sampling of water for chemical analyses and chlorophyll *a* was carried out with Van Dorn sampler. Samples were collected on the vertical profile every 2.5 m (0 m, 2.5 m, 5 m, 7.5 m, 10.0 m, 12.5 m and above bottom) in monthly intervals in the ice-free period from May 1996 to the end of 1999. Electric conductivity was measured in laboratory at 25 °C with conductivity meter (MA 5950, Iskra, Slovenia) and concentration of oxygen was measured *in situ* with an oxygenmeter (WTW Oxi 320, Germany). To evaluate chemical variables analytical methods according to APHA (1992) were applied. All spectrophotometric measurements were performed with spectrophotometer Perkin Elmer (UV/VIS, Lambda 12). The temperature was measured with a thermistor (Iskra, Slovenia). Chlorophyll

a concentration was determined by photometry (GOLTERMAN & al. 1978, WETZEL & LIKENS 1995). Biomass of phytoplankton was calculated on the basis of chlorophyll *a* concentrations (TALLING 1975). Transparency was estimated by means of Secchi disc. The physical and chemical data are presented as median, minimum and maximum of total measured values.

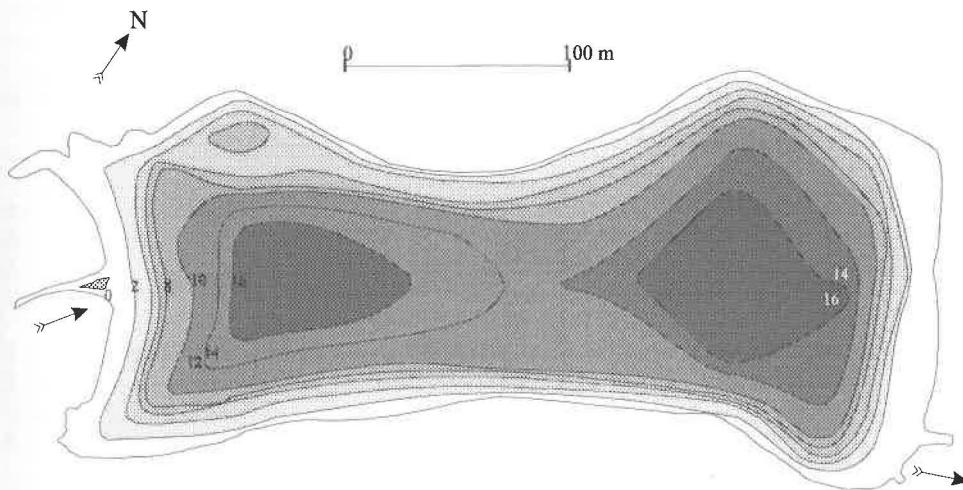


Figure 1: Bathymetric map of the lake Krnsko jezero

Slika 1: Batimetrična karta Krnskega jezera

Macrophyte survey

The survey of submersed vegetation was carried out from 1991 to 1999 along 20 permanent transects. The shore line was divided into the sections of different lengths, based on the uniformity of morphological characteristics (bathymetric map), water depth and substrate types. For graphic presentation the homogenous sections were additionally divided into the final 31 sections, with the length of 35 ± 2 m (Fig. 2). Abundance of the observed macrophytes was estimated semi-quantitatively using a 5-level scale (1 - present, 2 - rare, 3 - common, 4 - abundant, 5 - predominant) (MELZER 1992, PALL & JANAUER 1995). The survey was made from the boat using depthmeter, view box and sampling rake. In September 1998, macrophyte community was surveyed by scuba-diving on six representative transects. The total biomass of macrophytes was collected in the area of 0.1 m^2 on the depths of 1, 2, 3, 4 and 7 m, washed thoroughly to remove periphyton, oven dried at 105°C and weighted. The ratio of organic matter was determined by ignition at 550°C following the method from APHA (1992).

Results and Discussion

As the majority of lakes world-wide the lake Krnsko jezero is subjected to accelerated eutrophication due to diverse reasons. The disturbances to the lake were caused by the First World War, introduction of fish, traditional pasturing, earthquakes and mountaineering (GABERŠČIK & al. 1997). In the period between 1997 and 1998 after repeated earthquakes the sedimentation rate in the lake increased. The analyses of the material in the sediment traps revealed that the annual amount of gathered matter was increasing from 0.84 g DW m^{-2} in 1997, 1.01 g DW m^{-2} in 1998 to 3.68 g DW m^{-2} in 1999 (MURI & al. 2002).

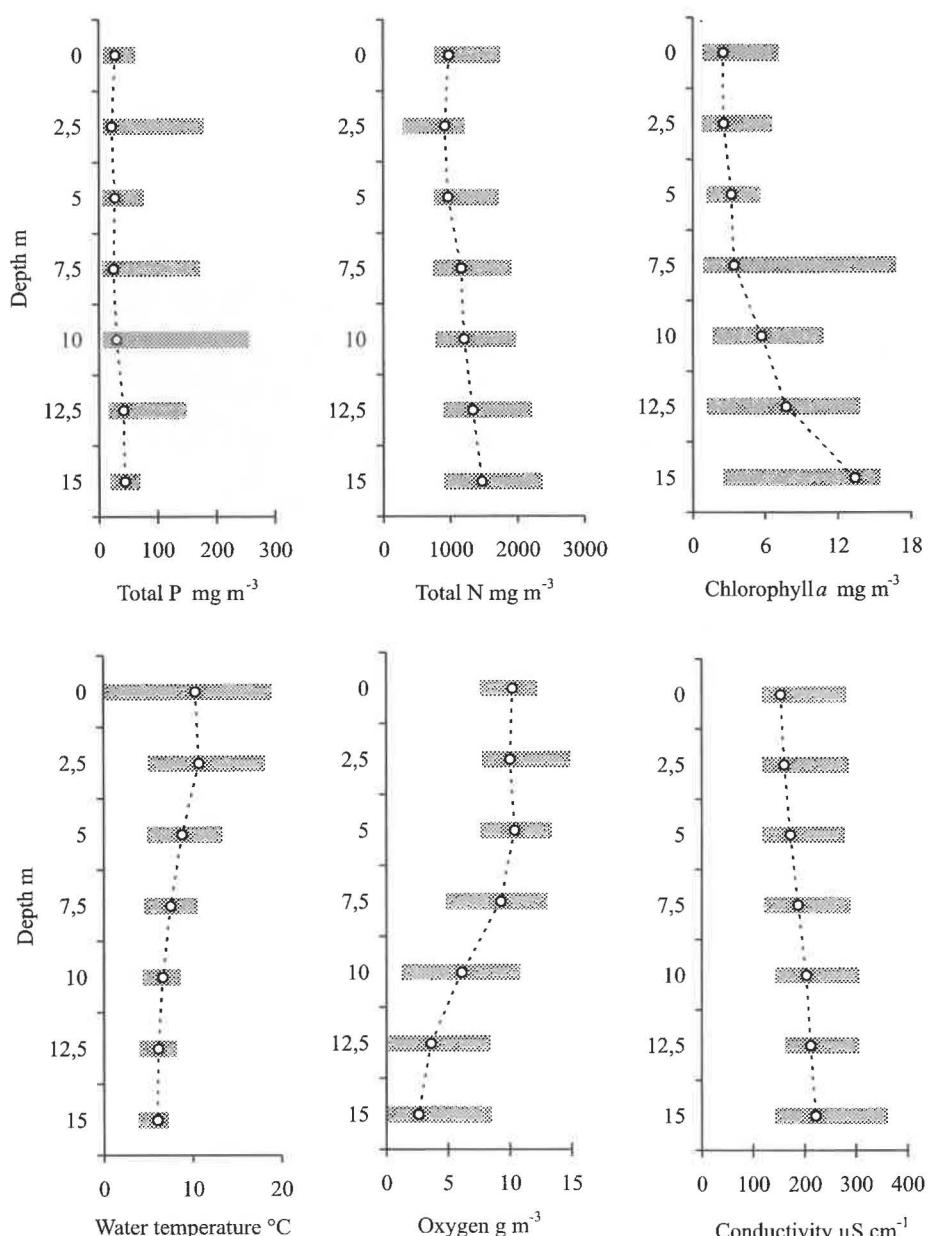


Figure 2: Main physical and chemical parameters measured in the depth profile of the lake Krnsko jezero in the period from 1996 to 1999. Median values, minima and maxima are presented.

Slika 2: Glavni fizikalni in kemični parametri merjeni na globinskem profilu v Krnskem jezeru, v obdobju od 1996 do 1999 leta. Pedstavljeni so mediane, minimalne in maximalne vrednosti.

Concentrations of nutrients in water column measured in a period from 1996 to 1999 varied to a great extent (Fig. 2). The median values of nutrients increased with depth, the total phosphorus ranging from 21 to 44 mg m⁻³ and the total nitrogen from 915 to 1463 mg m⁻³. The concentrations of total phosphorus in the water column differed a lot, exhibiting the lowest variability in the bottom samples, due to releasing of phosphorus from the sediment under anaerobic conditions. The concentrations of total nitrogen and dissolved solids measured as electric conductivity showed less variability than total phosphorus.

The introduction of fish influenced the food web in the lake, and consequently only one zooplankton species, *Cyclops vicinus* (Uljanin 1875) was present. In the nearby lakes zooplankton was still diverse and up to 6 species could be found (BRANCELJ & al. 1997). The reduction of zooplankton diversity resulted further in the increase of chlorophyll *a* concentration and in lower water transparency (Tab. 1). During the monitoring Secchi depth varied in the range from 2.5 m to 11 m, median value being 4 m. The concentration of chlorophyll *a* as a measure of phytoplankton production was rather constant in the upper layers, reaching median value about 3 mg m⁻³. The highest concentration was measured at the bottom layer (median value 13 mg m⁻³), likely due to algal sedimentation, while the variability from 1 to 17 mg m⁻³ was determined at the depth of 7.5 m, where thermocline usually occurred.

Table 1: Data on minimal water tranparencies and maximal depth distribution of *Characeae* in the lake Krnsko jezero from 1994 to 1999; n = 3 - 6, * BLAŽENČIČ & al. (1990).

Tabela 1: Najnižje vrednosti prosojnosti vode in največja globina uspevanja parožnic (*Characeae*) v Krnskem jezeru v obdobju od 1994 do 1999 (n=3-6). Podatek * BLAŽENČIČ & al. (1990).

Year	1988	1991	1992	1993	1994	1995	1996	1997	1998	1999
Secchi depth (m)	-	-	-	-	-	-	4.2	3.5	3.0	2.5
Maximal depth distribution (m)	10.0*	8.0	8.0	8.0	8.0	7.0	7.0	7.5	7.0	7.5

By preventing the penetration of light, planktonic algae posed depth limits for anchored macrophytes. In spite of this phenomenon the littoral area of the lake Krnsko jezero is large. We defined it by the extension of macrophyte community (WETZEL 1990) on the basis of bathimetric map. In 1998 it presented about 19600 m², that is nearly 40 % of the projected lake area. Five species of submersed macrophytes i.e. *Chara delicatula* Ag., *Chara contraria* f. *capillacea* Mig., *Potamogeton alpinus* Balbis, *Potamogeton pusillus* L. and *Batrachium trichophyllum* Chaix. colonising different depths, were determined in the lake (Fig. 3). The community is considered as floral rich in comparison to other 13 Slovene alpine lakes (GABERŠČIK & URBANC-BERČIČ 1996). This coincides with the data of northern European lakes elaborated by RØRSLETT (1991) which revealed that upland lakes exhibited proportionally fewer species than lowland sites.

During the monitoring the maximal depths colonised by macrophytes oscillated (Tab. 1) showing trend of gradual reduction. In 1988 *Chara delicatula* spread down to 10 m. The maximum depth of this species was 8 m from 1991 to 1994 and 7 - 7.5 m since then (BLAŽENČIČ & al. 1990, GABERŠČIK & URBANC-BERČIČ 1996). The competition for light and nutrients with filamentous and planktonic algae was estimated to be the main reason for this feature. BLINDOW (1992a,b) found out that charophytes were sensitive to the reduction of light more than to the increase of total phosphorus, as it was indicated in the previous studies (FORSBERG 1964). LEHMANN & LACHAVANNE (1999) compared the relevance of two indices to refine changes in macrophyte community in Lake Geneva. They demonstrated that saprobic index based on organic matter inputs, was more closely related to subtle vegetation changes than index, based on nutrients load. Our survey indicated that increased inputs detected in sediment traps, originating from the disturbed watershed supposed to be a trigger for changing of submersed vegetation. Survey of 116 high mountain Pyrenean lakes, led by GACIA & al. (1994) pointed out two environmental variables, vegetation coverage of the catchment and altitude as the main factors that influence the distribution and composition

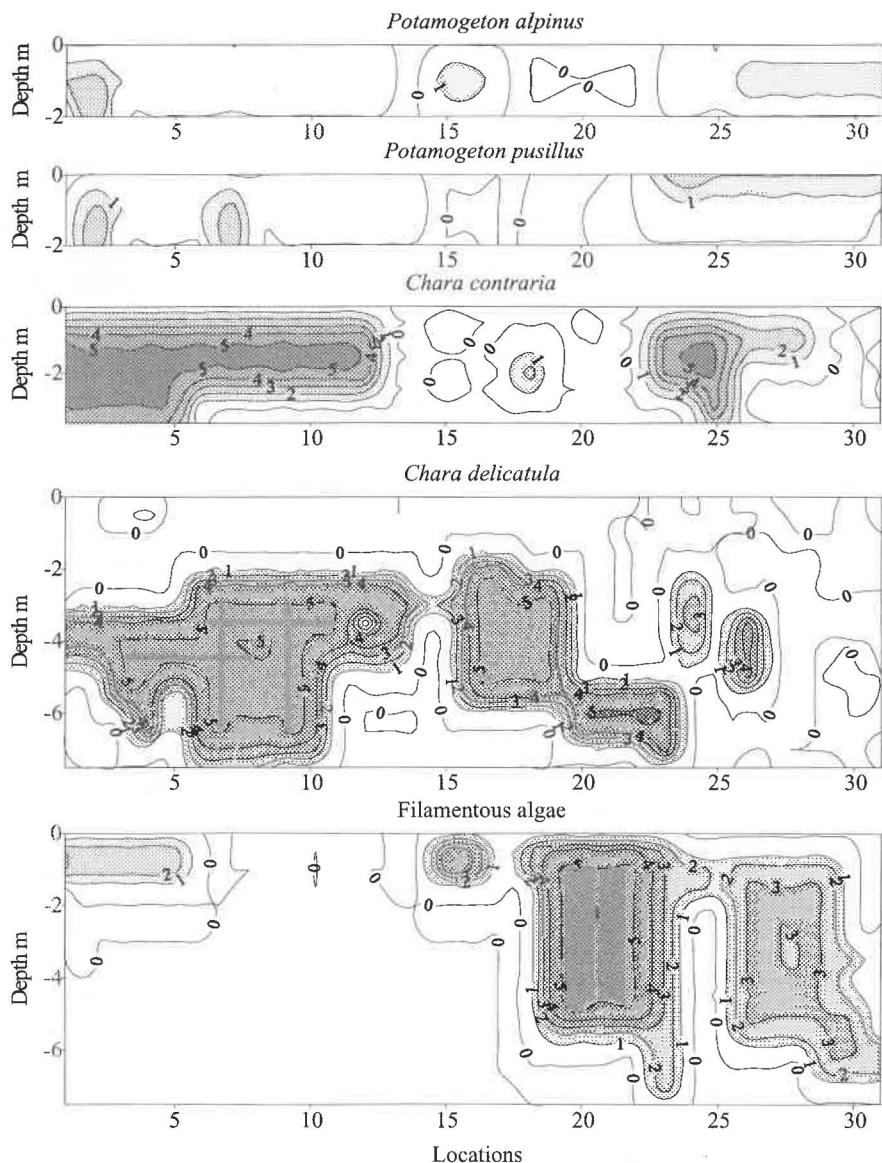


Figure 3: Depth distribution and abundance (rated from 1 to 5) of macrophytes in 31 sections (length 35 ± 2 m) in the littoral of the lake Krnsko jezero in September 1998. Section 1 is to the right of the lake outflow (counter clockwise).

Slika 3: Globinska razporeditev in številčnost makrofitov, ocenjena od 1 do 5, na 31 odsekih litorala v Krnskem jezeru, september 1998. Odseki (1 je desno od iztoka) potekajo v obratni smeri urinega kazalca.

of macrophytes. These two determinants reflect potential inputs in whole while a nutrient availability in the water as a relevant variable for the primary producers, depends on the processes in lake, as well.

In 1998, when a detailed survey was made by scuba-divers, the maximum biomass of macrophytes was determined between the depths of 2 and 3 m (the estimation of abundance was 5) even though the continuous stands of stoneworts spread to the depth of 7.5 m (Fig. 3). Plants were extremely elongated reaching the length up to 1 m at the depth of 3 m. The prevailing species in the lake were *Chara delicatula* and *Chara contraria f. capillacea*. In 1998 their share per lake was 2700 and 2068 kg of organic matter, respectively. The former reached up to 3 kg DW m⁻² on the depth of 3 m. The highest dry weight 5.8 kg m⁻² of *Chara contraria f. capillacea* was determined on the depth of 2 m (Fig. 4).

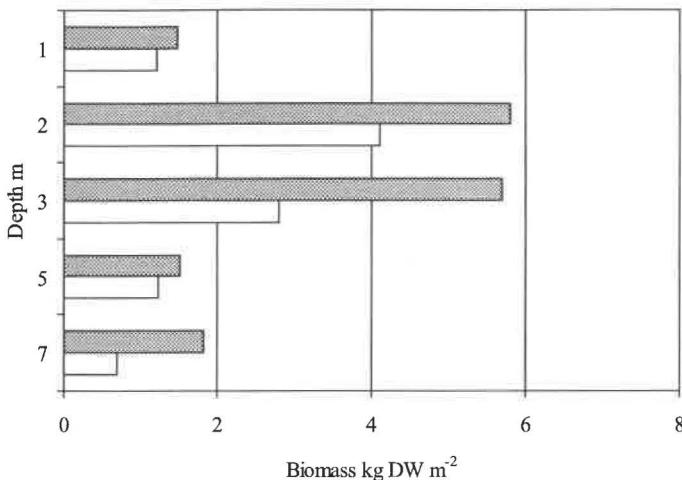


Figure 4: Depth distribution of macrophytes biomass in September 1998 (white column – average, grey column – maximum value). Figures present the values calculated from the macrophytes' samples obtained by scuba diving on 6 transects.

Slika 4: Globinska razporeditev biomase makrofitov v septembru 1998 (bel stolpec so povprečne, siv stolpec maximalne vrednosti). Številke predstavljajo biomaso, preračunano iz vzorcev makrofitov, zbranih s pomočjo potapljačev na 6 transektilih.

Both species contributed a lot to the total dry weight which was determined at 17537 kg. The value corresponds to 4991 kg of organic matter. Converted to energy content it means that about 10⁸ KJ were integrated in the ecosystem from solar energy. With such a production the lake reaches the level of fertile ecosystems (OZIMEK & al. 1990). The predominance of stonewort species which function as a stabiliser of the sediment is beneficial for the system resilience concerning inputs from the watershed i.e. nutrients, as well as particulate matter. The other two submersed macrophytes *Potamogeton alpinus* and *Potamogeton pusillus* were not abundant and their biomass presented a negligible share of 21 and 33 kg DW per lake in 1998 (Fig.5) but having the highest organic content (Fig. 6). In spite of low production pondweeds present a factor stabilising the shallow, sensitive parts of the littoral and promoting sedimentation of fine particles. The macrophytic biomass distribution in the lake Krnsko jezero reflects the characteristics of the lake, i.e. basin configuration, high availability of nutrients, water level fluctuations and modest light conditions. Stoneworts that have lower light demands for photosynthesis (BLINDOW 1992a,b) express a competitive advantage over higher submersed plants. Occasionally, when the runoff of nutrients from the watershed was increased, the biomass of filamentous and planktonic algae increased. Even though planktonic algae presented a minor share in the primary production of the lake, they affected macrophytes (LACHAVANNE 1985, HOUGH & al. 1989). Calculated

to the whole lake volume their average concentration would be 4 mg m^{-3} , which is equivalent to the production of 92 kg organic matter per lake, presenting 1.8 % of the lake primary production (Fig. 5). In 1998, nearly the same share of primary production was contributed from filamentous algae. They

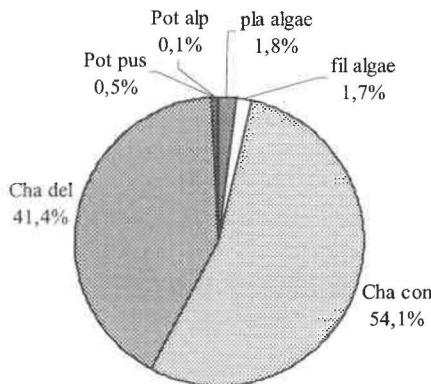


Figure 5. Partition of total organic matter among different primary producers in the lake Krnsko jezero in September 1998 when the total primary production was estimated to 4991 kg DW of organic matter. Periphyton biomass is excluded.

Slika 5: Delež organske snovi med različnimi primarnimi producenti v Krnskem jezeru, september 1998. Celotna primarna produkcija je bila ocenjena na 4991 kg suhe organske snovi brez biomase perifitona.

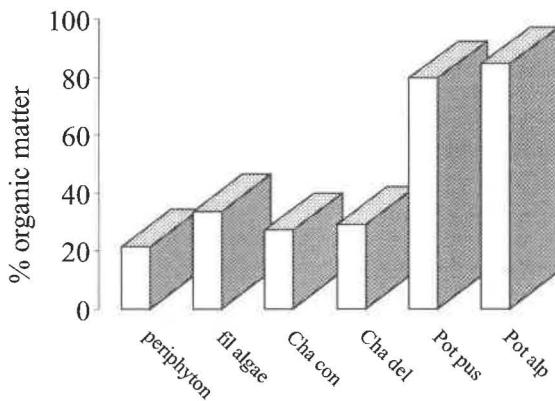


Figure 6: The ratio of organic matter in the biomass of different primary producers from the lake Krnsko jezero.

Slika 6: Razmerje organske snovi v biomasi različnih primarnih producentov v Krnskem jezeru.

appeared mainly in the shallow water competing with pondweeds. On the sites with steep slopes where the conditions for anchoring were not favourable, higher plants and stoneworts were scarce. On these locations filamentous algae were not in a position of direct competitors (locations from 18 to 30 on Fig. 3).

Conclusions

In the lake Krnsko jezero accelerated eutrophication resulted in high primary production that presented very high absorbance of solar energy into the lake. In 1998, littoral presented 40 % of a

projected lake area and was densely colonised with macrophytes. The major share of biomass belonged to charophyte species (95.5%) while planktonic and filamentous algae contributed 3.5%, only. In spite of this minority they presented an influential competitor to stonewort for light. Reduction of light penetration was a feature which have been measured in the last years. Successful competition of filamentous algae for nutrients was evident in the shallowest parts, with exception of the locations with steep slopes and unsuitable substrates for stoneworts and higher plants to anchor. The colonisation of littoral with anchored macrophytes increases the ecosystem resilience, by stabilising the erosion zone which overtakes function of a sedimentation zone.

In spite of different influences from the watershed, the direct impact of a single disturbance was difficult to define on a temporal scale, but the consequences of integral impact resulted in reduction of water transparency, enhanced appearance of filamentous algae and shrinking of littoral zone. Those features indicated the eutrophication in lake Krnsko jezero and showed trend of accelerated succession in a remote mountain lake ecosystem.

Povzetek

Pospošena evtrofikacija Krnskega jezera se odraža v visoki primarni produkciji, kar pomeni zelo visoko vezavo sončne energije v jezeru. Litoral je v letu 1998 zavzemal kar 40 % jezerske površine, ki je bila gosto poseljena z makrofiti. Večinski delež biomase je pripadel parožnicam (95,5%), medtem ko je bil delež planktonskih in nitastih alg samo 3,5%. Kljub podrejenemu deležu so bile alge vpliven kompetitor za svetlobo, katere intenziteta se je v jezeru zmanjševala. Uspešnost nitastih alg v tekmovanju za hranila je bila očitna predvsem v plitvejših predelih, z izjemo odsekov na strmih brežinah, kjer je bil substrat neprimeren za zakoreninjenje parožnic in višjih rastlin. Poselitev litorala z makrofitskimi vrstami, ki se zakoreninijo, povečuje elastičnost ekosistema, saj te vrste s koreninskim sistemom stabilizirajo potencialne erozijske cone, ki tako postanejo sedimentacijske cone.

Kljub očitnim vplivom iz zaledja je neposreden vpliv posamezne motnje tako časovno kot prostorsko težko razločiti. Vendar pa so posledice vseh motenj določljive in prepoznane kot znižanje prosojnosti vode, kot povečano pojavljanje nitastih alg in kot krčenje littoralne cone. Vsi ti pojavi, ki so prepoznani kot odraz pospešene evtrofikacije, so v Krnskem jezeru časovno zgoščeni in kot taki dober prikaz sukcesijskih procesov v oddaljenem gorskem jezerskem ekosistemu.

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References

- APHA 1992: Standard Methods for the examination of Water a Wastewater, 18th edition. Washington DC, USA.
- BLAŽENČIČ, J., O. URBANC- BERČIČ & D. VRHOVŠEK 1990: Makrofiti v jezerih Triglavskega narodnega parka. Biološki vestnik **38:** 1-14.
- BLINDOW, I., 1992a: Decline of charophytes during eutrophication; a comparison to angiosperms. Freshwater Biology, **28:** 9 -14.

- BLINDOW, I. 1992b: Long and short term dynamics of submerged macrophytes in two shallow eutrophic lakes. *Freshwater Biology*, **28**: 15-27.
- BRANCELJ, A., G. KOSI & M. ŠIŠKO 1997: Distribution of algae and crustacea in mountain lakes in Slovenia with different trophic levels. *Periodicum Biologorum* **99**: 87-96.
- BRANCELJ, A., M. ŠIŠKO, I. REJEC BRANCELJ, Z. JERAN & R. JAČIMOVIĆ 2000: Effects of land use and fish stocking on a mountain lake - evidence from the sediment. *Periodicum Biologorum* **102**: 259-268.
- FORSBERG, C. 1964: Phosphorus, a maximum factor in the growth of Characeae, *Nature*, **201**: 517 - 518.
- GABERŠČIK, A. & O. URBANC-BERČIČ 1996: Lakes of the Triglav National park: water chemistry and macrophytes. In: GABERŠČIK A., O. URBANC-BERČIČ, G.A. JANAUER (eds.): Proceedings of the International Workshop and 8th Macrophyte Group Meeting LAD-SIL, Bohinj 1996, pp. 23-29.
- GABERŠČIK, A., O. URBANC-BERČIČ, A. BRANCELJ & M. ŠIŠKO 1997: Mountain lakes - remote but endangered. In: ROŠ, M. (ed.): Proceedings 1st International Conference on environmental restoration, IAWQ, SDZV, Ljubljana 1997, pp. 452-457.
- GACIA, E., E. BALLESTEROS, L. CAMARERO, O. DELGADO, A. PALAU, J.L. RIERA & J. CATALAN 1994: Macrophytes from lakes in the eastern Pyrenees: community composition and ordination in relation to environmental factors. *Freshwater Biology*, **32**: 73 - 81.
- GAMS, I. 1962: Visokogorska jezera. Poročilo, pp. 197-261.
- GOLTERMAN, H. L., R.S. CLLYMO, & M. A. M OHNSTAD 1978: Methods for Physical and Chemical Analysis of Freshwaters. Billing and Sons Limited, pp. 162.
- HOUGH, R. A., M. D FORNWALL, B. J. NEGELE, R. L. THOMPSON & D. A. PUTT 1989: Plant community dynamics in a chain of lakes: principal factors in the decline of rooted macrophytes with eutrophication. *Hydrobiologia*, **173**: 199 - 217.
- LACHAVANNE, J. - B. 1985: The influence of accelerated eutrophication on the macrophytes of Swiss lakes: Abundance and distribution. *Verh. Int. Ver. Limnol.* **22**: 2950 -2955.
- LEHMANN, A. & J. - B. LACHAVANNE 1999: Changes in the water quality of Lake Geneva indicated by submersed macrophytes. *Freshwater Biology*, **42**: 457-466.
- MELZER A.1992: Submersed macrophytes. In: Scharf, B.W. & S. Bjorks (eds.): Limnology of Eifel maar lakes, *Ergebnisse der Limnologie* **38**: pp. 223-237.
- MURI, G., Z. JERAN, R. JAČIMOVIĆ & O. URBANC-BERČIČ 2002: Fizikalne lastnosti sedimentov in onesnaževalci v sedimentih. V: (ur. A. Brancelj)Visokogorska jezera vzhodnem delu Julijskih Alp. Založba ZRC, ZRC - SAZU.159 - 177.
- OZIMEK, T., R.D. GULATI & E. VAN DONK 1990: Can macrophytes be useful in biomanipulation of lakes? The Lake Zwemlust example. *Hydrobiologia* **200/201**: 39-407.
- PALL, K. & G.A. JANAUER 1995: Die Makrophytenvegetation von Flussstauen am Beispiel der Donau zwischen Fluss-km 2552,0 und 2511,8 in der Bundesrepublik Deutschland. *Arch. Hydrobiol. Suppl. 101, Large Rivers* **9, 2**: 91-109.
- RASPOPOV I. M., I. N. ANDRONIKOVA, O.N. DOTSENKO, G.I. KURASHOV, G. I LETANSKAYA,, V.E. PANOV, M.A. RYCHKova, I.V. TELESH, O.A. TCHERNYKH & F.F. VORONTSOV 1996: Littoral zone of Lake Ladoga: ecological state evaluation, *Hydrobiologia* **322**: 39 - 47.
- RØRSLETT, B. 1991: Principal determinants of aquatic macrophytes richness in Northern European lakes. *Aquatic Botany* **39**: 173 - 193.
- TALLING, J. F. 1975: Primary production of aquatic plants - conclusions. In: Cooper J. P. (ed.): *Photosynthesis and productivity in different environments*. Cambridge Univ. Press, Cambridge, pp 281-294.
- WETZEL, G. R. & G. E. LIKENS 1995: Limnological analyses. Second Edition. Springer –Verlag, pp. 156-161.
- WETZEL, G. R. 1990: Land – water interfaces: Metabolic and limnological regulators. In Edgardo Baldi Memorial Lecture. *Verh. Int. Verein. Limnol.* **24**: 6 -24.

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Goli polži (Gastropoda: Pulmonata: Milacidae, Limacidae, Boettgerillidae, Agriolimacidae, Arionidae) Slovenije

Slugs (Gastropoda: Pulmonata: Milacidae, Limacidae, Boettgerillidae, Agriolimacidae, Arionidae) of Slovenia

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Izvleček. Podan je prvi obsežnejši pregled favne golih polžev Slovenije. Z obdelavo približno 600 vzorcev z golimi polži (Milacidae, Limacidae, Boettgerillidae, Agriolimacidae in Arionidae) iz treh zbirk in z analizo podatkov iz literature je bilo na območju Slovenije ugotovljenih 27 vrst. Med njimi je šest vrst, za katere so to prvi podatki o prisotnosti na območju Slovenije. Največ vrst golih polžev v Sloveniji je iz družine Milacidae (8). Seznam vrst je dopolnjen s kartami njihove razširjenosti po kvadratih UTM (10 x 10 km).

Ključne besede: polži (Gastropoda), goli polži, favna, razširjenost, Slovenija.

Abstract. Slugs are among the less investigated gastropods in Slovenia. The study of approximately 600 samples of slugs (Milacidae, Limacidae, Boettgerillidae, Agriolimacidae and Arionidae) from three collections and the analysis of data from the literature gave a total of 27 species for the territory of Slovenia. For six species this is the first record for Slovenia. Most slugs in Slovenia belong to the family Milacidae (8). A list of species with distribution maps in UTM grids (10 x 10 km) is given.

Key words: snails (Gastropoda), slugs, fauna, distribution, Slovenia.

Uvod

Na območju Slovenije živijo predstavniki petih družin golih polžev: Milacidae - grebenarji, Limacidae - slinarji, Boettgerillidae - črvarji, Agriolimacidae - poljski slinarji, Arionidae - lazarji. Po ocenah WIKTORJA (1997) obsegajo te družine vsega približno 300 vrst. V Sloveniji so goli polži med slabše raziskanimi skupinami polžev.

V starejši literaturi že zasledimo nekaj podatkov o golih polžih iz Slovenije ali njene bližnje sosedstvo. SCHMIDT (1847) v prvem favničnem popisu mehkužcev na slovenskih tleh tedanje

avstro-ogrške dežele Kranjske še ne navaja golih polžev. Šele ERJAVEC (1877) omenja deset vrst iz Posočja. SIMROTH je opisal nove vrste golih polžev tudi iz Slovenije, leta 1885 vrsto *Amalia robici* (danes jo imenujemo *Tandonia robici*) iz Suhega dola nad Zgornjo Kokro v Savinjskih Alpah in nato leta 1910 vrsto *Amalia kobelti* z Mangarta (danes je to *Tandonia simrothi*). BOLE (1969) navaja štirinajst vrst v določevalnem ključu za mehkužce Slovenije. Tudi v nekaterih drugih delih navaja podatke o golih polžih Slovenije (BOLE 1962, 1966, 1976a, 1976b, 1977, 1979a, 1979b, 1981, 1992a, 1992b, 1994). V delu o golih polžih nekdanje Jugoslavije so bili prvič (WIKTOR 1996) zbrani natančnejši podatki o vrstah iz Slovenije. VAUPOTIČ & VELKOVRH (1997) sta z intenzivnim vzorčenjem favne mehkužcev v Pomurju ugotovila 11 vrst golih polžev, med njimi je bila prvič odkrita *Boettgerilla pallens*. Gole polže z ozemlja Slovenije navajajo v svojih delih še STOSSICH (1899), SAJOVIC (1908), WAGNER (1931), KOS (1933), JAECKEL et al. (1958), ALTENA (1973, 1977), REISCHÜTZ (1978), WIKTOR (1982), WOLF & RÄHLE (1987), SLAPNIK (1998).

Predloženo delo je prikaz trenutnega poznavanja favne golih polžev Slovenije (Sl. 1) in temelji na analizi podatkov iz literature ter taksonomski obdelavi skoraj 700 vzorcev, ki jih je prvi avtor članka pregledal v okviru svojega magistrskega dela. Vsi podatki so iz 112 kvadratov UTM (10 x 10 km).

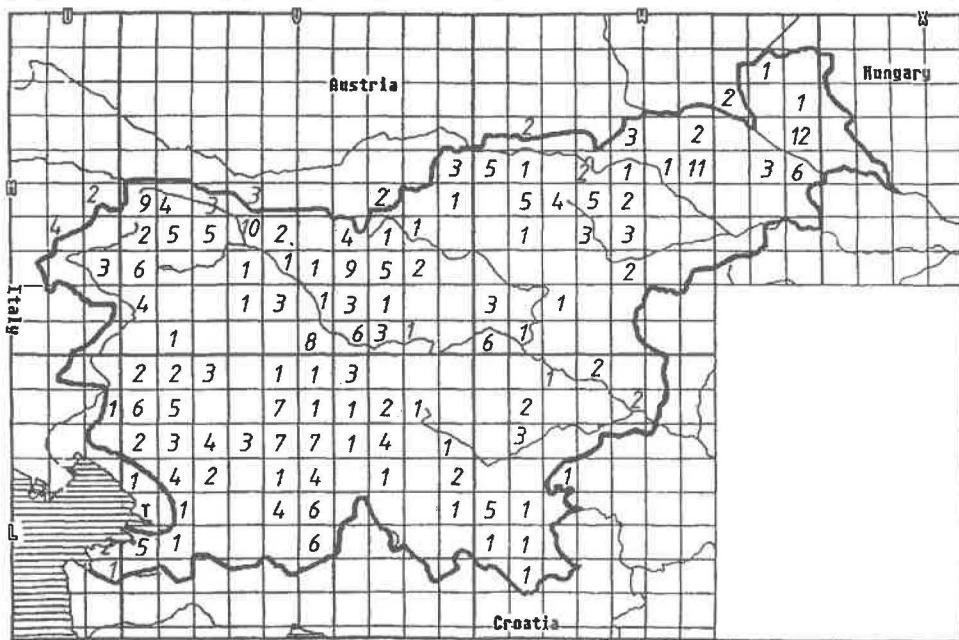


Figure 1: Number of slug species known in individual UTM squares in Slovenia.

Slika 1: Število ugotovljenih vrst golih polžev v posameznih kvadratih UTM v Sloveniji.

Material in metode dela

Pregledala sva vzorce iz zbirke mehkužcev J. Boleta na Biološkem inštitutu ZRC SAZU (BI), in lastnih zbirk (FV in MV). Vseh vzorcev v zbirkah je bilo 686, od tega največ v zbirki FV (562 vzorcev), manj v zbirki MV (117 vzorcev) in najmanj v zbirki Biološkega inštituta (7 vzorcev). V 38 vzorcih so bili le rudimenti hišic golih polžev, ki zaenkrat niso uporabni za določitev vrste (SOUTH 1992 cit. REUSE 1983). Pregledani material je bil nabran v obdobju od leta 1957 do 1998. Živali so shranjene v 70 % etanolu. Vseh živali v vzorcih je bilo približno 4000. Nabirali smo na dva različna

načina: naključno in intenzivno. Pogosteje je bil naključni način vzorčenja, ko število primerkov v vzorcih ni presegalo štivila 10. V nekaterih vzorcih, ki so rezultat intenzivnega vzorčenja, je bilo tudi več sto živali, zaradi zelo uspešne metode nabiranja, ki jo je drugi avtor članka izvajal le na eni lokaliteti (Lenart v Slovenskih Goricah, WM65). Zvečer je na namočena tla nastavljal vlažne, 4-5 cm debele deske. Čez noč so se pod te deske zatekli mnogi polži, ki jih je naslednji dan pobral, še preden je dopoldansko sonce segrelo deske.

Taksonomsko pomembni znaki za določitev vrste so na spolnem aparatu, zato je bilo večinoma treba opraviti sekcijsko živali pod stereo-mikroskopom. Za določanje vrst so bila uporabljena dela več avtorjev (KERNEY et al. 1983; LIKHAREV & WIKTOR 1980; QUICK 1960; WIKTOR 1973, 1982, 1983, 1987, 1989, 1996; BACKELJAU & VAN BEECK 1986; BACKELJAU & DE BRUYN 1990; GARRIDO et al. 1995; LUPU 1977; VON PROSCHWITZ 1989; DE WINTER 1984, 1989; WIKTOR & MILANI 1995).

Ves pregledani material je shranjen v zbirkri Franceta Velkovrha, Lenart v Slovenskih goricah, Lackova ulica 52.

Rezultati

Ugotovljene vrste so naštete v seznamu. Pri vsaki vrsti je navedena njena razširjenost v Sloveniji. Prvi podatki so iz literature, večinoma po WIKTORJU (1996), ki navaja tako rezultate lastnih raziskav favne tedanje Jugoslavije, kot tudi podatke drugih avtorjev (ALTENA 1973, 1977; BOLE 1962, 1966, 1976a, 1976b, 1977, 1979a; GROSSU 1972; JAECKEL & MEISE 1956; SIMROTH 1885, 1910; WAGNER 1931; WOLF & RÄHLE 1987). Nekatera dela pa v prej navedeni monografiji niso upoštevana (BOLE 1969, 1981, 1992b, 1994; REISCHÜTZ 1978; SLAPNIK 1998; VAUPOTIČ & VELKOVRH 1997). Sledijo lastni podatki po zgoraj omenjenih zbirkah. Štirideset vzorcev iz zbirke MV je bilo že uporabljenih in objavljenih (VAUPOTIČ & VELKOVRH 1997). V karte razširjenosti so iz literature vneseni podatki, ki so geografsko dovolj natančni, vrste pa identificirane po anatomskeh znakih. Upoštevane so bile tudi navedbe o vrstah, ki se jih da zanesljivo določiti že po zunanjih znakih (*Limax cinereoniger*). V literaturi navedene vrste, ki jih nisva odkrila v vzorcih iz zbirk, so v pregledu vrst označene s črtico (-).

Najdišča vrst so prikazana v mreži UTM. Pri navajanju podatkov iz literature je za imenom avtorja navedena samo koda UTM, medtem ko je pri podatkih iz sedaj obdelanih zbirk za kodo kvadrata UTM zapisana lokaliteta, datum nabiranja in kratica zbirke s kataloško številko. Število osebkov v vzorcu je navedeno le v primeru, ko gre za eno samo lokaliteto oziroma lokalitete iz enega samega kvadrata UTM. Če je bilo več vzorcev z istega nahajališča, je pri ponovitvah naveden samo datum vzorčenja na najdišču in kataloška številka vzorca; če pa je bil drugi vzorec nabran tudi isti dan, je navedena le kataloška številka.

Legenda: L - podatki iz literatur

ZT - podatki, dobljeni z obdelavo vzorcev treh zbirk

FV - zbirka Franceta Velkovrha

BI - zbirka Biološkega inštituta SAZU

MV - zbirka Marjana Vaupotiča

? - podatki, ki niso vneseni v zemljevide, ker geografsko niso dovolj natančni ali pa je taksonomska določitev nezanesljiva

Table 1: Sistematic check-list of the slugs of Slovenia.

Tabela 1: Sistematski pregled vrst golih polžev Slovenije.

DRUŽINA	VRSTA
Milacidae	1. <i>Milax nigricans</i> 2. <i>Tandonia budapestensis</i> 3. <i>Tandonia rara</i> 4. <i>Tandonia reuleauxi</i> 5. <i>Tandonia robici</i> 6. <i>Tandonia rustica</i> 7. <i>Tandonia simrothi</i> 8. <i>Tandonia sowerbyi</i>
Limacidae	9. <i>Malacolimax mrazekii</i> 10. <i>Lehmannia marginata</i> 11. <i>Limax cinereoniger</i> 12. <i>Limax maximus</i> 13. <i>Limax flavus</i>
Boettgerillidae	14. <i>Boettgerilla pallens</i>
Agriolimacidae	15. <i>Deroceras laeve</i> 16. <i>Deroceras lothari</i> 17. <i>Deroceras reticulatum</i> 18. <i>Deroceras rodnae</i> 19. <i>Deroceras sturanyi</i> 20. <i>Deroceras turanicum</i>
Arionidae	21. <i>Arion lusitanicus</i> 22. <i>Arion subfuscus</i> 23. <i>Arion circumscriptus</i> 24. <i>Arion fasciatus</i> 25. <i>Arion silvaticus</i> 26. <i>Arion alpinus</i> 27. <i>Arion distinctus</i>

DRUŽINA: Milacidae – grebenarji (ali vrtni lazari)– *Milax gagates* (Draparnaud, 1801) – temni grebenar

L: ? BOLE (1969), ? BOLE (1981).

1. *Milax nigricans* (Schulz, 1836) – črni grebenar (Sl. 2 A)Z: **VL04:** Pridvor = Sv. Anton, Dekani, Koper, 8.1995, FV48653. V vzorcu je 5 osebkov.2. *Tandonia budapestensis* (Hazay, 1881) – madžarska grebenarka (Sl. 2 A)Z: **VL08:** Dornberk, 11.10.1998, MV697; **VL18:** Vipavski Križ, 11.10.1998, MV705; **WM65:** Lenart v Slovenskih Goricah, 8.1986, FV45623; 1995, FV49989; 7.-10.1996, FV50877; 7.-10.1996, FV51065; 7.-10.1997, FV51442; 5. - 7.1994, MV607; **WM96:** Generala Maistra 5, Murska Sobota, 29.9.1996, MV616; **WM96:** žive meje, vrtovi, Murska Sobota, 8.-9.1993, MV674.– *Tandonia fejervaryi* (Wagner, 1929) – Fejervaryjeva grebenarka*Milax (Milax) Adensameri* Wagner 1931.L: ALTENA (1977)/ **VM63**.3. *Tandonia rara* Wiktor, 1996 – redka grebenarka (Sl. 2 A)*Tandonia schleschi* partim in Wiktor 1982Z: **VM62:** Planina Koren, Kravavec, 6.1974, FV26345. V vzorcu so 3 osebki.4. *Tandonia reuleauxi* (Clessin, 1887) – Reuleauxjeva grebenarka (Sl. 2 A)*Aspidoporus limax* Fitzinger 1883L: WAGNER (1931) – ? Krain (as *Aspidoporus limax*) (Kranjska (kot *Aspidoporus limax*)).Z: **VL06:** Jama v Doktorjevi ogradi, Pliskovica, Tomaj, 1.6.1996, MV654. V vzorcu je en sam osebek.5. *Tandonia robici* (Simroth, 1885) – Robičeva grebenarka (Sl. 2 B)*Amalia Robici* Simroth 1885, *Milax robici* (Simroth 1885)

L: SIMROTH (1885)/ **VM62:** WAGNER (1931)/ **UM92:** ? JAECKEL ET AL. (1958); ? BOLE (1969); WIKTOR (1982)/ **VM23:** WOLF & RÄHLE (1987)/ **VM04:** WIKTOR (1996)/ **VL27:** **VL54.**

Z: **VL45:** melišče, pod steno Pešiče, Koritnice, 10.11.1994, MV656; **VL47:** Rakov Škocjan, 10.1969, FV10123; pred vhodom: Planinska jama, Planina, 6.1976, FV32608; pred jamo: Mačkovica, Planina, 4.1977, FV36015; Pod stenami, Planinsko polje, 5.1982, FV44616; Laška kukava, Logaška planota, Laze, Planina, 6.1995, FV48795; vhodna udornica, Vranja jama, Planinsko polje, N rob, 6.1995, FV48998; Pod stenami, Planinsko polje, Planina, 6.1982, FV51328; 7.1982, FV50876; **VL48:** N Ljubljanski vrh, Vrhnika, 6.1977, FV36046; Planinski polje, N rob, Planina, 6.1985, FV50865; **VL57:** Slivnica, Cerknica, 6.1971, FV50870; 6.1971, FV51307; FV51323; **VL95:** Stojna, Dolga vas, Kočevje, 9.1996, FV51451; **VM04:** Prisojnik, W pobočje, Vršič, 6.1981, FV46026; **VM43:** vas Dolina, Tržič, 5.1974, FV25433; **VM50:** Turnc, Grmada, Tacen, Ljubljana, 6.1997, FV50615; Podutik, nad kamnolomom, Ljubljana, 6.-7.1997, FV50982; **VM61:** stena nad vasjo Rašica, Ljubljana, 7.1994, FV47407, FV47408; Rašica SW pobočje, Ljubljana, 6.1995, FV48883; **VM62:** Planina Koren, Krvavec, 6.1974, FV26348; Dolge njive, Krvavec, 6.1978, FV38691; Dolina Korošice, Krvavec, 6.1978, FV38775; desno pobočje: Dolina Korošice, Stahovica, 6.1998, FV51058; Korošica- Krvavec, 4.1970, FV51319; Davovec, Cerklje, 6.1998, FV51622; **VM95:** pred Jamo v Votli peči, Ravne DRJS-3263, 5.1975, FV50875; **WL05:** Podstene, Kočevski Rog, 6.1980, FV45783; 6.1986, FV45662; **WL15:** Semič, Bela Krajina, 14.4.1990, FV47476; **WL17:** gozd, Otočec, 26.9.1998, MV701.

6. *Tandonia rustica* (Millet, 1843) – podeželska grebenarka (Sl. 2 B)

Milax rusticus (Millet 1843)

L: ? BOLE (1969); ? BOLE (1979a); WOLF & RÄHLE (1987)/ **UM83.**

Z: **VL08:** Dornberk, 11.10.1998, MV696; **VL16:** W Dane, Sežana, 9.1997, FV50636; **VL18:** Vipavski Križ, 11.10.1998, MV698.

7. *Tandonia simrothi* (Hesse, 1923) – Simrothova grebenarka (Sl. 2 A)

Amalia Kobelti Simroth 1910, *Milax Simrothi* Hesse 1923, *Milax (Milax) Schleschi* Wagner 1930, *Tandonia schleschi* (Wagner 1930)

L: SIMROTH(1910)/ **UM94:** BOLE (1969)/ **UM94:** WOLF & RÄHLE (1987)/ **VM04,** **UM92:** WIKTOR (1996)/ **VM63.**

Z: **UM92:** Planina Duplje, Julijске Alpe, 7.1981, FV41412; pri Dupeljskem jezeru, Planina Duplje, Julijске Alpe, 7.1982, FV43645; **UM94:** Mangart, pod Rdečo skalo, 9.1970, FV12602; **VM02:** Komna, 8.1958, FV11387; 8.1958, FV11388; Planina Govnač, Komna, 5.1972, FV20525; Planina Govnač, Komna, 7.1982, FV43614; Komna - Bogatinsko sedlo, 7.1985, FV45008; 7.1985, FV45891/51311; **VM04:** Erika (koča), Kranjska Gora - Vršič, 6.1975, FV32163; Vršič - Trenta, 6.1975, FV32178; 6.1975, FV32179; Vršič, 7.1975, FV35407; Prisojnik, S pobočje: pod oknom, Kranjska Gora, 6.1977, FV36616; Mihov dom, Vršič - Kranjska Gora, 7.1981, FV42013; Prisojnik, W pobočje, Vršič, 7.1980, FV45416; 7.1984, FV45773; 6.1984, FV45826; 7.1984, FV45867; Kranjska Gora - Vršič, 6.1981, FV45944; **VM73,** Kamniško sedlo, Kamniška Bistrica (S1640a), 21.8.1994, BI003; Kamniško sedlo, 8.9.1987, BI004.

8. *Tandonia sowerbyi* (Férussac, 1823) – Sowerbyjeva grebenarka (Sl. 2 C)

Z: **UL94:** Portorož - Beli Križ - Fiesa, 24.10.1998, MV703; Piran, 24.10.1998, MV704; **VL04:** Pridvor = Sv. Anton, Dekani, Koper, 8.1995, FV50860; **VL18:** Vipavski Križ, 11.10.1998, MV699; **VM60:** Ljubljana, 10.1976, FV33182; Ljubljana, Kolodvor, 10.1976, FV34231.

DRUŽINA: **Limacidae** - slinarji

9. *Malacomimax mrazekii* (Simroth, 1904) – Mrazekov mali slinar (Sl. 2 D)

Malacolimax kostali Wiktor 1982, nec Babor 1900

L: WIKTOR (1982)/ **VL55**; WIKTOR (1996)/ **VM14, VL54**.

Z: **VL47:** gozd, pasti, Laze, Planina, 3.1995, FV49911; **VM04:** pod slapom, Gozd Martuljek, 6.1974, FV26395; Erjavčeva koča, Vršič, 7.1981, FV41422; Prisojnik, W pobočje, Vršič, 6.1981, FV51441; **VM72:** Velika planina, Savinjske Alpe, 6.1976, FV38659; **WL13:** hrib Sebetih, Sinji vrh, Črnatelj, 8.1974, FV28582; **WL17:** Otočec, gozd, 26.9.1998, MV694; **WM01:** Marija Reka, Prebold, 9.1996, MV659; **WM05:** Jesenkov vrh, Slovenj Gradec, 9.1996, FV51303; 9.1996, MV661; **WM33:** Oplotnica, Pohorje, 8.1967, FV11474; **WM34:** Šumnik, Pohorje, 8.1967, FV11486; Ruško Pohorje, 8.1995, FV48915; **WM35:** kmetija Strnad, Smolnik, Ruše, 9.1996, MV668; **WM65:** Lenart v Slovenskih Goricah, 8.1986, FV45726; **WM66:** Zgornja Ščavnica, Slovenske Gorice, 10.1987, FV46544; **WM97:** Sebeborci, gozd, Goričko, Prekmurje, 9.9.1995, MV617.

- *Malacolimax tenellus* (O. F. Müller, 1774) – gobji mali slinar

Limax tenellus Nillson 1822

L: ? BOLE (1969) – samo v Karavankah, ? BOLE (1979a) – v gozdovih na Javornikih, ? WOLF & RÄHLE (1987) – N of Vršič pass (severno od prelaza Vršič/**VM04**).

10. *Lehmannia marginata* (O. F. Müller, 1774) – obrobljena slinarka (Sl. 2 E)

Limax marginatus O.F. Müller 1774

L: BOLE (1962)/ **VM03**; BOLE (1969)/ **VL19, VL54**; BOLE (1977)/ **VM50**; WOLF & RÄHLE (1987)/ **VM04, UM83**; WIKTOR (1996)/ **VM13, VM33, VL27, WM00**; VAUPOTIČ & VELKOVRIH (1997)/ **WM95, WM96**; SLAPNIK (1998)/ **WM00**.

Z: **VL09:** Trnovski gozd, 1969, FV09352; **VL47:** Pod stenami, Planina, 6.1982, FV43483; 7.1982, FV43659; Vranja jama, vhodna udornica, Planinsko polje, N rob, 6.1995, FV51315; Rakov Škocjan, 10.1969, FV51429; **VL48:** S Ljubljanski vrh, Vrhnik, 6.1977, FV36047; Planinsko polje, N rob, Planina, 6.1985, FV46530; **VL48:** Bistra, izvir v Bistri, Vrhnik, 25.6.1990, FV47623; **VL55:** grad Snežnik, Cerknica, 9.1977, FV36518; FV36520; **VL56:** pri Mrzli jami, Bločice, 29.7.1994, MV655; **VL57:** Slivnica, 6.1971, FV15846; FV19776; **VL78:** Vodice na Dolenjskem, Dobropolje, 6.1958, FV11374; FV11375; **VM01:** dolina pri: Doblar, Doblar, 6.1998, FV51632; **VM02:** Savica - Komna, Bohinj, 7.1985, FV45249; 7.1986, FV45794; FV45997; 7.1982, FV46439; 7.1981, FV47719; 6.1981, FV51542; **VM04:** Vršič, 7.1975, FV35401; Prisojnik, W pobočje, Vršič, 7.1980, FV45412; 6.1981, FV51320; 6.1984, FV45817; 7.1984, FV45866; Mihov dom, Kranjska Gora - Vršič, 6.1980, FV45805; 6.1981, FV50866; 7.1981, FV50873; FV51324; Kranjska gora - Vršič, 6.1981, FV45935; FV45937; Vršič - Trenta, 6.1975, FV51312; Vršič, 7.1975, FV51325; **VM24:** Mežakla, 6.1969, FV11144; FV50867; FV51435; 6.1971, FV18795; Srednja Radovna, 6.1970, FV14657; **VM33:** gozd, Mošnje, Dobruša, levi breg, Brezje, 6.1995, FV48710; FV48711; FV48712; **VM41:** Moškrin, Škofja Loka, 6.1997, FV50750; Grenc, Škofja Loka, 6.1997, FV50850; **VM50:** Turnc, Grmada, Tacen, Ljubljana, 6.1997, FV50612; **VM62:** Dolina Korošice, Kamniška Bistrica, 6.1974, FV26313; Dolge njive, Krvavec, 6.1978, FV38692; Planina Koren, Krvavec, 6.1978, FV38751; **VM63:** Rokovnjaške luknje, Kamniška Bistrica, 7.1979, FV41243; **VM72:** Kamniška Bistrica, 7.1979, FV41699; **VM82:** Javoršek, Tuhinj, 5.1970, FV11780; FV11785; **VM82:** Mačkin kot, Gornji grad, 5.1976, FV25529; **VM95:** pred Jamo pri Votli peči, Ravne DRJS-3263, 5.1975, FV32534; **VM95:** Jama v Votli peči, Ravne, Koroška, 3.1978, FV42066; 1.1978, FV45394; S pobočje, Stražiščec (karbonati), NE Prevalje, 9.1994, FV47342; **WL05:** Podstene, Kočevski Rog, 7.1980, FV42778; FV51431; 6.1980, FV45784; FV51423; **WL26:** Gospodična, Gorganci, 5.1974, FV26109; **WL48:** Vitovec, E Malence, Brežice, 7.1994, FV47326; **WM05:** Jesenkov vrh, Slovenj Gradec, 9.1996, MV660; **WM16:** Kremenice, Šmartno Pohorje, 7.1986, FV46080; **WM24:** Pohorje, 8.1967, FV50880; **WM33:** Oplotnica, Pohorje, 8.1967, FV50883; **WM34:** Pragozd, Lobnica,

Pohorje, 6.1975, FV30199; FV30200; Šumnik, Pohorje, 8.1967, FV50886; **WM42:** pragozd, Boč, Poljčane, Štajerska, 8.1997, FV50555; **WM43:** Bistriški vindgar, N Slovenska Bistrica, 6.1997, FV50564; **WM44:** Areh, Pohorje, 8.1967, FV46172; **WM46:** Bresterica, 9.1996, MV665; **WM65:** Lenart v Slovenskih Goricah, 8.1985, FV44326; 9.1986, FV45705; **WM85:** gozd, Desnjak, SW Žerovinci, Ljutomer, Prlekija, 7.8.1994, MV646; **WM95:** vrt, Veržej, Prlekija, 6.11.1993, MV641; **WM96:** vrtovi, žive meje, Murska Sobota, Prekmurje, 8.+9.1993, MV626; vrtovi, žive meje, Murska Sobota, Prekmurje, 9.9.1993, MV627; Murski logi, SW Dokležovje, Prekmurje, 11.8.1993, MV630; dvorišča, vrtovi, Dokležovje, Prekmurje, 9.9.1993, MV638.

11. *Limax cinereoniger* Wolf, 1803 – pepelnočrni slinar (Sl. 2 F)

Limax cinereo-niger Wolf 1803, *Limax maximus* Linnaeus 1758 (partim), *Limax lineolatus* Dumont et Mortillet 1852.

L: BOLE (1962)/ **VL47:** BOLE (1969); BOLE (1976a)/ **VL19, VL08, VL47 (3), VL49, VL47, VL54, VL95:** BOLE (1976b)/ **VL54:** BOLE (1977)/ **VM50:** BOLE (1978)/ **VL46:** WIKTOR (1982)/ **VL55:** WOLF & RÄHLE (1987)/ **VM04, UM83 (3):** WIKTOR (1996)/ **WM10, VL37, VM14, VM33, VM13, VL27:** VAUPOTIČ & VELKOVRH (1997)/ **WM96.**

Z: **UM94:** Mangart, pod: Rdeča skala, 9.1970, FV12603; **VL16:** Laze, Novo mesto, 6.1997, FV50350; FV50351; **VL17:** Mlake pri Vipavi, 11.10.1998, MV682; **VL18:** Vipavski Križ, 11.10.1998, MV683; **VL29:** Godovič, Idrija, 6.1997, FV50551; **VL45:** Koritnice, Boršt - hrastov gozd, Knežak, 10.10.1988, MV708; MV709; Jurišče, Gradec, Pivka, 10.10.1988, MV710; Koritnice, pod lesom, Knežak, 15.10.1998, MV717; **VL47:** Rakov Škocjan, 10.1969, FV10122; FV51317; 1968, FV11550; pred vhodom: Planinska jama, Planina, 6.1976, FV32605; Pod stenami, Planinsko polje, Planina, 6.1982, FV43481; 6.1992, FV47890; Vranja jama, vhodna udornica, Planinsko polje, N rob, 6.1995, FV48987; **VL48:** Bistra, Vrhnika, 4.1971, FV14913; 5.1977, FV36762; 6.1980, FV42056; S Ljubljanski vrh, Vrhnika, 6.1977, FV51422; FV36022; FV36032; Laška kukava, Logaška planota, Laze, Planina, 6.1995, FV48790; **VL54:** Sviščaki, Snežnik, 6.1978, FV38469; FV38471; **VL55:** grad Snežnik, Cerknica, 9.1977, FV36517; Leskova dolina, Snežnik, 6.1978, FV38717; Mašun, Vidovi lazi, 10.10.1988, MV713; **VL56:** Gorica, Cerkniško jezero, Cerknica, 6.1980, FV42053; **VL57:** Slivnica, 6.1971, FV15843; Slivnica, gozd pod vrhom, 6.1971, FV19773; FV19774; FV51322; FV50869; **VL58:** Pekel, Borovnica, 6.1976, FV32653; **VL67:** Turjaški grad, Turjak, Velike Lašče, 6.1997, FV50786; **VL68:** Draga, Ig, 5.1967, FV19778; **VL78:** Vodice, Dolenjsko, 8.6.1958, FV11373; Limberk, Predole, 6.1998, FV51727; **VL88:** Vir pri Stični, 6.1976, FV32652; **VL95:** Stojna, Dolga vas, Kočevje, 9.96, 6.97, FV50357; FV50358; FV50359; **VL97:** Dvor pri Žužemberku, 6.1970, FV18796; FV18797; **VM01:** Tolmin, ob Soči, 4.1977, FV36804; **VM02:** Komna, 8.1958, FV11390; Savica - Komna, Julijiske Alpe, 7.1982, FV46435; 7.1983, FV43502; 7.1982, FV43630; 7.1985, FV45250; 6.1986, FV45584; Komna - Bogatinsko sedlo, Julijiske Alpe, 7.1985, FV45891?; **VM04:** Gozd Martuljek pod slapom, 6.1974, FV26416; Mihov dom, Kranjska Gora - Vršič, 6.1980, FV50871; FV45798; 6.1981, FV45966; 7.1981, FV42009; FV51309; Kranjska Gora - Vršič, 6.1981, FV45931; FV50872; Prisojnik, W pobočje, Vršič, 6.1981, FV51440; **VM23:** Kranjska dolina, Pokljuka, 9.1967, FV11553; FV24762; **VM24:** Mežakla, Ravne, 6.1969, FV09901(4); FV50868; 6.1971, FV18794; Srednja Radovna, 6.1970, FV14658; FV17213; Črni vrh, Jesenice, 7.1957, FV24777; **VM33:** Mošnje, gozd, Dobruša: levi breg, Brezje, 6.1995, FV48725; **VM34:** Završnica, Jesenice, 6.1974, FV26136; **VM41:** Grenc, Škofja Loka, 6.1997, FV50851; **VM50:** Turnc, Grmada, Tacen, Ljubljana, 6.1997, FV50622; Podutik, nad kamnolomom, 6.-7.1997, FV50779; FV50972; **VM61:** Rašica SW pobočje, Ljubljana, 6.1995, FV48882; **VM62:** Korošica - Krvavec, travniki, 9.1970, FV12787; Dolina Korošice, S Kamniška Bistrica, 6.1974, FV26318; Planina Koren, Krvavec, 6.1974, FV26339; 6.1978, FV38750; FV38753; Kopišče, Kamniška

Bistrica, 6.1977, FV36733; 6.1976, FV36737; desno pobočje, Dolina Korošice pod Krvavcem, 6.1988, FV51052; FV51053; Davovec, Cerkle, 6.1998, FV51623; **VM71:** Boštanova jama, Krtina, Moravče, 1.1980, FV40366; **VM72:** Velika planina, Savinjske Alpe, 6.1976, FV38657; **VM74:** Olševa, gora, 7.1974, FV26591; **VM80:** Kresnice, Litija, 6.1974, FV26192; **VM82:** Javoršek, Tuhinj, 5.1970, FV11775; FV11776; FV11782(3?), FV51438; Mačkin kot, Gornji grad, 5.1976, FV51447; **VM94:** Skobirjeva votlica, Zg. Razbor, Slovenj Gradec, 10.1978, FV38154; **VM95:** Ošven, Podgora, Ravne na Koroškem, 6.1988, FV51670; **WL05:** Podstene, Kočevski Rog, 7.1980, FV41124; **WL17:** gozdovi, Otočec, 1998, MV689; **WL29:** Studenec, Sevnica, 8.1972, FV20516; **WL39:** Bučerca, Krško, 6.1970, FV24902; Anže, Brestanica, 5.1976, FV32568; **WM00:** Škofja Riba, 5.6.1991, BI001; Kum, zgornji del ob pretvorniku, 6.5.1988, BI005; **WM01:** Marija Reka, Prebold, 9.1996, MV658; **WM05:** Gradišče (silikati), N Slovenj Gradec, Pohorje, 9.1994, FV47243; FV47244; Jesenkov vrh, Slovenj Gradec, 9.1996, MV657; **WM13:** Huda luknja, okolina, Velenje, 6.1994, FV50859; FV50895; **WM14:** Špehovka, jama, vhod, Mislinja, 9.1970, FV13652; **WM16:** Suh Vrh nad: Radlje ob Dravi, 7.1986, FV45629; **WM24:** Pohorje, 8.1967, FV11547; **WM34:** Pragozd, Lobnica, Pohorje, 6.1975, FV30197; **WM35:** Ruško Pohorje, 8.1995, FV48910; kmetija Strnad, Smolnik, Ruše, 9.1996, MV667; **WM42:** pragozd, Boč, Poljčane, Štajerska, 8.1997, FV50554; 8.1997, FV50556; okolina vasi, Hrastje, Poljčane, 7.1997, FV50572; FV50576; **WM43:** Slovenska Bistrica, 6.1988, FV51798; FV51802; **WM45:** dolina: Razvanjski potok, Razvanje, Pohorje, Maribor, 6.1997, FV50756; **WM46:** Bresterica, 9.1996, MV663; **WM65:** Lenart v Slovenskih Goricah, 1995, FV49990; 7.-10.1997, FV51445; **WM66:** Zgornja Ščavnica, Lenart v Slovenskih Goricah, 6.1985, FV44657; FV44658; Krivi Vrh, Sv. Ana, Lenart v Slovenskih Goricah, 8.1986, FV45622; **WM96:** Murski logi, W Stara Mura, S Dokležovje, Prekmurje, 12.8.1993, MV622.

12. *Limax maximus* Linnaeus, 1758 – veliki slinar (Sl. 2 G)

Limax cinereus Lister 1678.

L: ? BOLE (1969); VAUPOTIČ & VELKOVRH (1997)/ **WM95, WM96.**

Z: rastlinjak: B. Sket, Aškerčeva 12, Ljubljana, FV42237; **UL93:** Sečovlje, 9.1971, FV19646; **VL04:** Šalara, Koper, 6.1974, FV26337; Pridvor = Sv. Anton, Dekani, Koper, 8.1995, FV48649; **VL48:** Bistra, Vrhnika, 6.1989, FV46953; **VL55:** Mašun, Vidovi, Lazi, 10.10.1988, MV707; **VL59:** Vrhovci, Ljubljana, 1958, FV11348; **WM14:** Pečenikov vrh, Mislinja, 6.1998, FV51764; **WM65:** Lenart v Slovenskih Goricah, 8.1987, FV46433; 5. - 7.1994, MV608; 1995, FV49992; 7.-10.1996, FV51064; 7.-10.1997, FV50862; **WM95:** vrt, Verzej, Prlekija, 6.11.1993, MV643; MV612; **WM95:** Noršinci, Ljutomer, Prlekija, 3.5.1995, MV670; Noršinci, Ljutomer, 3.5.1995, MV618; **WM96:** dvorišča, žive meje, Dokležovje, 9.9.1993, MV610; vrtovi, žive meje, Beltinci, Prekmurje, 9.9.1993, MV611; vrtovi, žive meje, Murska Sobota, Prekmurje, 8. + 9.1993, MV621; Borovnjakova ulica, Murska Sobota, Prekmurje, 11.6.1994, MV651.

13. *Limax flavus* Linnaeus, 1758 – rumenkasti slinar (Sl. 2 H)

Limax variegatus Draparnaud 1801.

L: ? BOLE (1969); WOLF & RÄHLE (1987)/ **UM83.**

Z: **VL04:** Pridvor = Sv. Anton, Dekani, Koper, 8.1995, FV50861; **VL45:** Koritnice, pod lesom, 15.10.1998, MV711.

DRUŽINA: *Boettgrillidae* – črvaji

14. *Boettgerilla pallens* Simroth, 1910 – Boettgerjev črvvar (Sl. 2 H)

Boettgerilla vermiformis Wiktor 1959

L: VAUPOTIČ & VELKOVRH (1997)/ **WM96.**

Z: **WM96:** Generala Maistra 5, Murska Sobota, 29.9.1996, MV673; dvorišča, vrtovi,

Dokležovje, Prekmurje, 9.9.1993, MV637; vrtovi, žive meje, Murska Sobota, Prekmurje, 8.+9.1993, MV623.

DRUŽINA: Agriolimacidae – poljski slinarji

– *Deroceras agreste* (Linnaeus 1758) – enobarvni poljski slinar

Limax agrestis Linnaeus 1774, *Agriolimax agrestis* var. *Fedschenkoi* Koch et Heynemann 1874.

L: ? BOLE (1969); ? BOLE (1962); ? BOLE (1976b); ? BOLE (1977); ? BOLE (1992).

15. *Deroceras laeve* (O. F. Müller, 1774) – obvodni poljski slinar (Sl. 2 H)

Limax laevis O.F. Müller 1774.

L: ? BOLE (1981); ? BOLE (1994); WIKTOR (1996)/ **VM33**.

Z: rastlinjak v Biološkem središču, Ljubljana, 7.1994, MV620; **VL14**: Osp, Koper, 11.1984, FV46301.V vzorcu je en osebek.

16. *Deroceras lothari* Giusti, 1971 – Lotharjev poljski slinar (Sl. 3 I)

Deroceras klemmi Grossu 1972.

L: REISCHÜTZ (1978)/ **VL15**, **VM52**, **WM21**; WOLF & RÄHLE (1987)/**VM04**; WIKTOR (1996)/ **VM60**, **VM52**, **VM33**, **VM14**, **VM13**, **WM00**; ALTENA (1973)/ **VM01**, **VM62** (2); VAUPOTIČ & VELKOVRH (1997)/ **WM95**, **WM96**.

Z: **UL94**: Portorož- Fiesa, 24.10.1998, MV687; Piran, 24.10.1998, MV688; **VL04**: Pridvor = Sv. Anton, Dekani, Koper, 8.1995, FV48655; **VL08**: Dornberk, 11.10.1998, MV692; **VL29**: Idrija, 13.9.1998, MV706; **VL45**: Koritnice, na cesti, v dežju, 1.11.1998, MV714; Koritnice, v jami nad Koritnicami, 2.4.1995, MV715; pod lesom, Koritnice, 15.10.1998, MV716; **VL54**: gozd Sviščaki, Ilirska Bistrica, 8.-10.9.1991, FV51437; **VL69**: Ljubljansko barje, ob Izjanski cesti, 10.1976, FV33410; **VL77**: Velike Lašče, 9.1984, FV43827; 11.1984, FV43950; FV43951; FV51302; **VM02**: Komna, 8.1958, FV11389; **VM04**: Mihov dom, Kranjska Gora - Vršič, 7.1984, FV42022; **VM23**: Kranjska dolina, Pokljuka, 9.1967, FV24763; **VM50**: Rožnik, Ljubljana, 6.1972, FV22805; Koseze, Ljubljana, 12.10.1994, MV614; **WM24**: Pohorje, 8.1967, FV11548; **WM43**: Kajuhova cesta, Slovenska Bistrica, 9.1996, MV662; **WM65**: Lenart v Slovenskih Goricah, 11.1984, FV43939; 1995, FV49985; FV49986; FV50879; 7.-10.1996, FV51068; FV51436; 7.-10.1997, FV51443; **WM95**: vrt, Veržej, Prlekija, 6.11.1993, MV644; Noršinci, Ljutomer, 10.9.1994, MV672; **WM96**: vrtovi, žive meje, Beltinci, Prekmurje, 9.9.1993, MV628; poplavni travnik, S Dokležovje, Prekmurje, 12.8.1993, MV635.

17. *Deroceras reticulatum* (O. F. Müller, 1774) – mrežasti poljski slinar (Sl. 3 J)

L: WIKTOR (1996)/ **VM33**), ? BOLE (1994); VAUPOTIČ & VELKOVRH (1997)/ **WM95**, **WM96**; SLAPNIK (1998)/ **WM00**.

Z: **VL07**: Komen, 11.10.1998, MV702; **VL08**: Dornberk, 11.10.1998, MV691; **VL16**: Orlek, Sežana, 1.1977, FV35309; **VL17**: Mlake pri Vipavi, 11.10.1998, MV681; **VL18**: Vipavski Križ, 11.10.1998, MV700; **VL29**: Idrija, 13.9.1998, MV690; **VL57**: Slivnica, Cerknica, 6.1971, FV51304; **VL77**: Velike Lašče, Ljubljana, 4.1984, FV43472; 9.1984, FV43826; 11.1984, FV43949; FV43952; FV50887; **VM60**: Ljubljana, kolodvor, 10.1976, FV34232; stena nad vasjo, Rašica, Ljubljana, 7.1994, FV47405; Ljubljana, 10.1976, FV51450; **VM62**: Dolge njive, Kalški greben, Savinjske Alpe, 6.1967, FV11508; Planina Koren, Krvavec, 6.1974, FV26347; **WL05**: Podstene, Kočevski Rog, 6.1986, FV45661; **WL18**: Mokronog, 9.1998, MV693; 10.1998, MV712; **WM46**: Bresterica, 9.1996, MV664; **WM65**: Lenart v Slovenskih Goricah, 1995, FV49987; FV49988; 7.-10.1996, FV51066; 7.-10.1997, FV51078; 5.- 7.1994, MV606; **WM95**: Noršinci, Ljutomer, 10.9.1994, MV613; vrt, Veržej, Prlekija, 11.6.1993, MV645A-D; vrtovi, žive meje, Murska Sobota, Prekmurje, 8.+9.1993, MV625; **WM96**: vrtovi, žive meje, Beltinci, Prekmurje, 9.9.1993, MV629; dvorišča, vrtovi, Dokležovje, Prekmurje, 9.9.1993, MV639; Bratonce, Prekmurje, 7.1994, MV648; Borovnjakova ulica, Murska Sobota, Prekmurje, 11.6.1994, MV649; MV675.

18. *Deroceras rodnae* Grossu et Lupu, 1965 – svetli poljski slinar (Sl. 3 K)

Z: **VL57:** Slivnica, Cerknica, 6.1971, FV51305; FV51306; Slivnica, gozd pod vrhom, Cerknica, 6.1971, FV51449; **VM62:** Korošica - Krvavec, 9.1970, FV12789; Dolina Korošice, Kamniška Bistrica, 6.1974, FV26304.

19. *Deroceras sturanyi* (Simroth, 1894) – Sturanyjev poljski slinar (Sl. 3 K)

L: WIKTOR (1996)/ **VM33:**, VAUPOTIČ & VELKOVRH (1997)/ **WM95, WM96.**

Z: **VM50:** Koseze, okolica ribnika, Ljubljana, 6.1997, FV51119; **VM60:** Ljubljana, kolodvor, 10.1976, FV51308; **WL18:** Mokronog, 1998, MV680; **WM65:** Lenart v Slovenskih Goricah, 7.-10.1996, FV50878; FV51069; **WM95:** vrt, Veržej, Prlekija, 6.11.1993, MV671; **WM96:** vrtovi, žive meje, Murska Sobota, Prekmurje, 8.+9.1993, MV624; dvorišča, vrtovi, Dokležovje, Prekmurje, 9.9.1993, MV640.

20. *Deroceras turicum* (Simroth, 1894) – turški poljski slinar (Sl. 3 K)

?*Deroceras forcarti* Grossu et Lupu 1961.

L: ALTENA (1973)/ **VM72, VL56;** WIKTOR (1996)/ **VL26.**

Z: **VM01:** Tolmin, ob Soči, 4.1977, FV36805. V vzorcu je en sam osebek.

DRUŽINA: Arionidae - lazari

21. *Arion lusitanicus* J. Mabille, 1868 – španski lazar (Sl. 3 L)

L: WIKTOR (1996)/ **VL37;** VAUPOTIČ & VELKOVRH (1997)/ **WM96.**

Z: **UL98:** ob potoku Lijak, Ajševica, 27.7.1998, MV679; **VL07:** Komen, 11.10.1998, MV684; **VL08:** Dornberk, 11.10.1998, MV686; **VL17:** Mlake pri Vipavi, 11.10.1998, MV685; **VM33:** gozd, Mošnje, levi breg Dobruše, 6.1995, FV48707; **VM50:** Biološko središče, Ljubljana, 7.7.1994, MV602; **VM60:** Tomačevo, Ljubljana, 6.1973, FV25637; **VM70:** bregovi, reka Sava, Dolsko, 6.1970, FV12083; **WL48:** Brežice, 15.9.1991, FV47255; **WM01:** Marija Reka, Prebold, 9.1996, MV666; **WM65:** Lenart v Slovenskih Goricah, 9.1985; 5.-7.1994, MV605; FV44603; 1995, FV49982; 7.-10.1996, FV51063; **WM85:** vrt, Ključarovci, Ljutomer, Prlekija, 7.8.1994, MV601; **WM96:** Murski logi, 0,5km W Babičov mlin, Veržej, Prlekija, 8.7.1995, MV603; Borovnjakova ulica, Murska Sobota, Prekmurje, 11.6.1994, MV609.

– ***Arion rufus* (Linnaeus 1758) – veliki lazar**

Arion ater Linnaeus 1758 (partim, *Arion ater rufus* Linnaeus 1758), *Arion empiricorum* Ferussac 1819 (partim), *Arion ater rufus* (Linnaeus 1758).

L: ? BOLE (1969), ? BOLE (1976b), ? BOLE (1977), ? BOLE (1978), ? BOLE (1994).

22. *Arion subfuscus* (Draparnaud, 1805) – rjavkasti lazar (Sl. 3 M)

L: ? BOLE (1962), ? BOLE (1966), ? BOLE (1969), ? BOLE (1976a), ? BOLE (1976b), ? BOLE (1979), ? BOLE (1994); WIKTOR (1982)/ **VM23, VL55;** WIKTOR (1996)/ **VM23, WM00, VM03, VM13, VM33, VL27, VL26;** VAUPOTIČ & VELKOVRH (1997)/ **WM96;** SLAPNIK (1998)/ **WM00.**

Z: **UM92:** pri Dupeljskem jezeru, Planina Duplje, Julisce Alpe, 7.1982, FV43464; 7.1982, FV43616; **VL09:** Trnovski gozd, 1969, FV50882; **VL47:** Rakov Škocjan, 4.1968, FV11301; 10.1969, FV51316; Pod stenami, Laze, Planina, 6.1982, FV43485; 6.1980, FV51427; Laze, gozd, pasti, Planina, 9.1994, FV49947; Vranja jama, vhodna udornica, Planinsko polje, N rob, 6.1995, FV51434; **VL48:** Bistra, Vrhnika, 4.1971, FV14912; 5.1977, FV36764; FV36765; 12.6.1980, FV51329; S Ljubljanski vrh, Vrhnika, 6.1977, FV36030; **VL54:** Sviščaki, Snežnik, 6.1978, FV38470; **VL55:** grad Snežnik, Cerknica, 9.1977, FV36519; **VL55:** Leskova dolina, Snežnik, 6.1978, FV38718; **VL56:** Gorica, Cerkniško jezero, 6.1980, FV42052; **VL57:** Slivnica, 6.1971, FV15844; Slivnica, gozd pod vrhom, 6.1971, FV19775; **VL69:** Mestni log, Ljubljana, 1967, FV50888; **VM02:** Komna, 8.1958, FV11386; 7.1971, FV16806; Planina Govnač, Komna, 5.1972, FV20524; Bogatinsko sedlo, 5.1972, FV20527; Komna - Sedmera jezera, 7.1973, FV25615; Savica - Komna, Bohinj, 7.1983, FV43503; 7.1985, FV51425; Planina Govnač, Komna, 7.1982, FV43613; Komna - Bogatinsko sedlo, Julijske Alpe, 7.1985,

FV45007; 7.1985, FV45892; Savica - Komna, Julijske Alpe, 7.1986, FV45949; Planina Na kraju, Komna, Julijske Alpe, 7.1985, FV46430; **VM03:** Ute, Dolina Triglavskih jezer, 9.1958, FV11370; **VM04:** Vršič, 6.1975, FV32177; 7.1975, FV35402; Prisojnik, W pobočje, Vršič, 7.1980, FV45413; FV50863; 6.1981, FV46025; 6.1984, FV45818; 7.1984, FV45766; FV45869; Prisojnik, S pobočje, 6.1977, FV46180; Kranjska Gora - Vršič, 6.1981, FV45936; Vršič - Trenta, 6.1975, FV51310; FV51313; Mihov dom, Vršič - Kranjska Gora, 6.1980, FV51318; 7.1981, FV51448; Erika (koča), Kranjska Gora - Vršič, 6.1975, FV51426; **VM23:** Kranjska dolina, Pokljuka, 9.1967, FV11554; **VM24:** Mežakla, Ravne, 6.1969, FV09902; FV09903; FV11145; 6.1971, FV51327; Srednja Radovna, 6.1970, FV17214; **VM31:** nad vasjo Prtovč, Ratitovec, 7.1960, FV11480; **VM34:** Završnica, Jesenice, 6.1974, FV26144; **VM50:** Turnc, Šmarca gora, 6.1971, FV19245; potok: Trnovec, dolina Ločnice, Sora, 6.1973, FV23526; Biološko središče, Rožnik, Ljubljana, 18.7.1994, FV49952; **VM61:** Rašica, SW pobočje, Ljubljana, 6.1995, FV48884; **VM62:** Dolge njive - Kalški greben, Savinjske planine, 6.1967, FV11473; Korošica - Krvavec, 4.1970, FV12790; Dolina Korošice, Kamniška Bistrica, 6.1974, FV26306; FV26314; Planina Koren, Krvavec, 6.1974, FV26344; 6.1978, FV38763; Dolge njive, Krvavec, 6.1978, FV51430; **VM63:** Kokrško sedlo, Savinjske planine, 1957, FV11364; **VM70:** breg, reka Sava, Dolsko, 6.1970, FV12089; FV51418; **VM72:** Velika planina, Savinjske Alpe, 6.1976, FV38658; **VM74:** Olševo, gora, 7.1974, FV26590; **WL04:** Medvedak, plato, 25.9.1998, MV695; **WL05:** Podstene, Kočevski Rog, 6.1980, FV45780; **WM00:** Kum, 6.5.1988, BI006; **WM05:** Jesenkova vrh, Slovenj Gradec, 9.1996, MV677; **WM14:** Pečenikov vrh, Mislinja, 6.1998, FV511765; **WM24:** Pohorje, 8.1967, FV50881; **WM33:** Pesek - Oplotnica, Pohorje, 8.1975, FV34481; Oplotnica, Pohorje, 8.1967, FV50884; **WM34:** Pragozd, Lobnica, Pohorje, 6.1975, FV30198; Ruško Pohorje, 8.1995, FV48911; Šumnik, Pohorje, 8.1967, FV50885; **WM44:** Areh, Pohorje, 7.1986, FV46087; Areh, Pohorje, 8.1967, FV46171; **WM55:** Zgornji Duplek, Maribor, 6.1991, FV47473; **WM77:** Cankova, Prekmurje, 1994, MV678; **WM85:** Stara Cesta, Ljutomer, Prlekija, 11.6.1994, MV604; **WM96:** Murski logi, W Stara Mura, S Dokležovje, Prekmurje, 12.8.1993, MV631.

23. *Arion circumscriptus* Johnston, 1828 – očrtani lazар (Sl. 3 O)

L: WIKTOR (1996)/ **VM33.**

Z: **WM77:** Cankova, Prekmurje, 1994, MV653. V vzorcu je 14 osebkov.

24. *Arion fasciatus* (Nilsson, 1823) – vrtičkarski lazар (Sl. 3 N)

L: VAUPOTIČ & VELKOVRH (1997)/ **WM95, WM96.**

Z: **WM14:** Pečenikov vrh, Mislinja, 6.1998, FV51766; **WM65:** Lenart v Slovenskih Goricah, 1995, FV49991; 7.-10.1996, FV51321; **WM95:** vrt, Veržej, Prlekija, 6.11.1993, MV642.

WM96: vrtovi, žive meje, Beltinci, Prekmurje, 9.9.1993, MV632; Murski logi, SW Dokležovje, Prekmurje, 11.8.1993, MV633; vrtovi, žive meje, Murska Sobota, Prekmurje, 8.+9.1993, MV634; poplavní travník, S Dokležovje, Prekmurje, 12.8.1993, MV636; Bratonce, Prekmurje, 7.1994, MV647; Borovnjakova ulica, Murska Sobota, Prekmurje, 11.6.1994, MV650; MV676.

25. *Arion silvaticus* Lohmander, 1937 – gozdní lazар (Sl. 3 O)

L: WIKTOR (1996)/ **VL37, VM13;** VAUPOTIČ & VELKOVRH (1997)/ **WM96.**

Z: **VL48:** Bistra, Vrhniška, 12.6.1980, FV47282; **VL57:** Slivnica, Cerknica, 6.1971, FV15845;

VL77: Velike Lašče, 4.1984, FV43471; **VM70:** bregovi, reka Sava, Dolsko, 6.1970, FV12090;

WL14: pred jamo Gadina, Črnatelj, 6.1975, FV34212; **WL39:** Anže, v vasi Brestanica, 5.1976, FV32551; **WM34:** Pragozd, Lobnica, Pohorje, 6.1975, FV51330; **WM96:** gozd N Ižakovci, Prekmurje, 6.11.1993, MV619.

26. *Arion alpinus* Pollonera, 1887 – gorski lazар (Sl. 3 P)

L: WIKTOR (1996)/ **VM33, VM14.**

Z: **VL47:** Pod stenami, Laze, Planina, 6.1980, FV43485; 7.1982, FV43658; Vranja jama, vhodna udornica, Planinsko polje, N rob, 6.1995, FV48990; **VL48:** S Ljubljanski vrh, Vrhniška,

6.1977, FV36031; FV51433; Planinsko polje, N breg, Planina, 6.1985, FV46537; **VL69:** Rudnik pri Ljubljani, 4.1969, FV09906; **VL76:** Travna gora, 6.1972, FV23744; **VL77:** Podgora, Dobrepolje, 1.1976, FV32038; **VM02:** Planina Govnač, Komna, 5.1972, FV20526; Savica - Komna, Julijske Alpe, 7.1982, FV43629; 7.1983, FV51420; 7.1985, FV45253; 7.1986, FV45787; FV46002; FV51424; **VM04:** Erika (koča), Kranjska Gora - Vršič, 6.1975, FV32157; Mojstrovka, E pobočje, 6.1974, FV34234; Mihov dom, Kranjska Gora - Vršič, 6.1980, FV45804; 7.1981, FV42021; Vršič, 7.1975, FV51326; **VM23:** Bohinjska Bela, Bled, 6.1995, FV48951; **VM32:** Brezovica, Kropa, 6.1998, FV51722; **VM33:** Mošnje, gozd, Dobruša, levi breg, Brezje, 6.1995, FV51428; **VM34:** ? levi breg od mosta do jezu, Breg, Žirovnica (S1893a), 23.5.1996, BI007; **VM41:** Moškrin, Škofja Loka, 6.1998, FV50746; **VM42:** Arnševa jama, okolica (Udin boršt), Sp. Duplje, Kranj, 6.1995, FV48682; 8.1995, FV48673; **VM43:** Jelendol, Tržič, 6.1974, FV28238; **VM50:** Turnc, Grmada, Tacen, Ljubljana, 6.1997, FV50614; Podutik, nad kamnolomom, Ljubljana, 6.-7.1997, FV50984; **VM51:** pobočje: reka Sava, Mavčiče, Smlednik, 6.1998, FV51781; **VM60:** stena nad vasjo, Rašica, Ljubljana, 7.1994, FV47404; **VM62:** Dolge njive, Krvavec, 6.1978, FV38690; desno pobočje: Dolina Korošice, Stahovica, 6.1998, FV51056; Stari grad, Kamnik, 6.1998, FV51478; Davovec, Cerkle, 6.1998, FV51619; **VM63:** Rokovnjaške luknje, Kamniška Bistrica, 7.1979, FV50864; **VM83:** Zamernikova jama, Konjski vrh, Luče v Savinjski dolini, 4.1978, FV45373; **WL48:** Vitovec, E Malence, Brežice, 7.1994, FV47332; **WM00:** Škofja Riba, Dobovec, Kum, 5.6.1991, BI002; **WM05?15:** Jesenkov vrh, Slovenj Gradec, 7.1994, FV47366; 9.1996, MV669; **WM14:** Pečenikov vrh, Mislinja, 6.1998, FV51452.

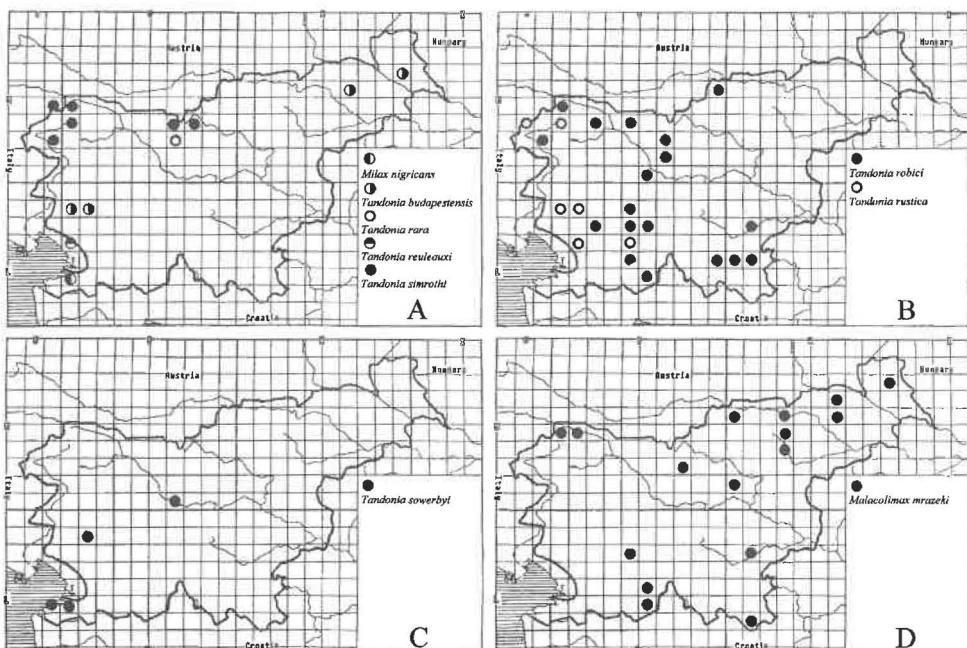
27. Arion distinctus J. Mabille, 1868 – navadni vrtni lazar (Sl. 3 O)

Z: **WM65:** Lenart v Slovenskih Goricah, 1995, FV49993; 7.-10.1996, FV51067; FV51079.

Skupaj je v vseh treh vzorcih 43 osebkov.

– **Arion hortensis Férrussac 1819** – vrtni lazar

L: ? BOLE (1962), ? BOLE (1969), ? BOLE (1979a).



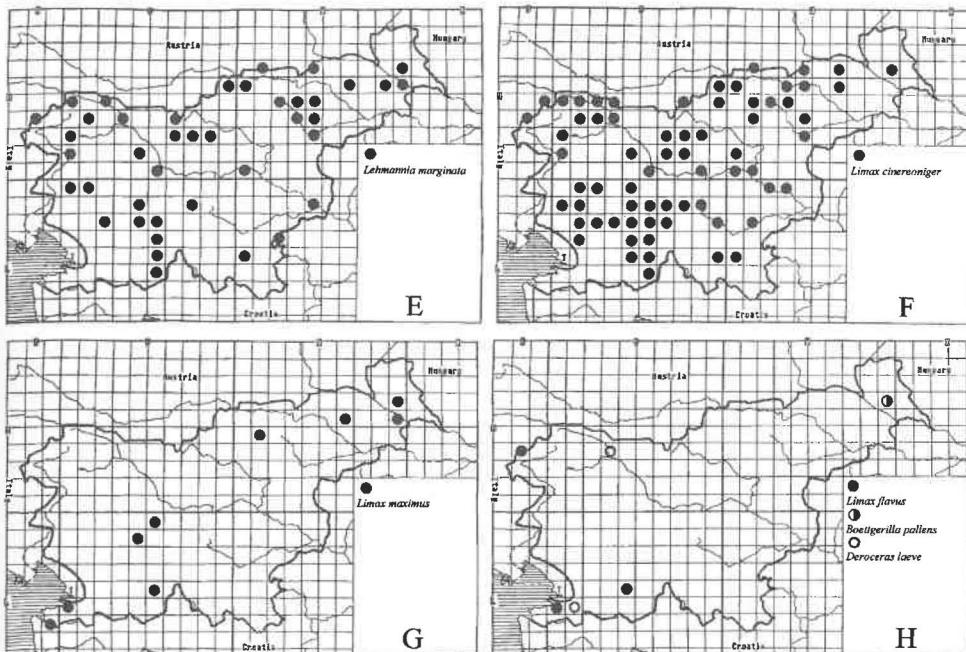
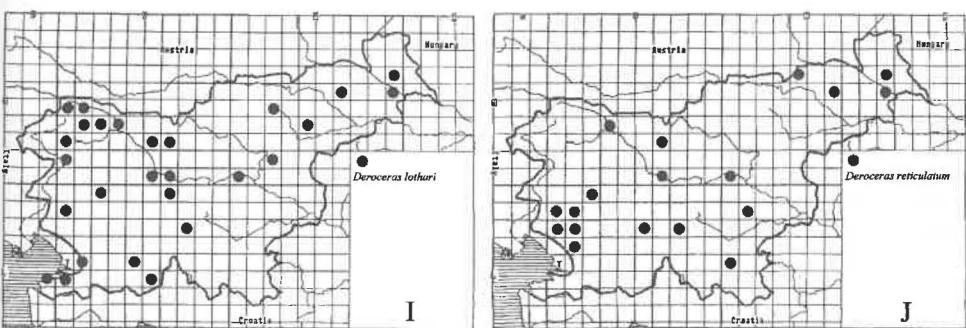


Figure 2: Distribution of slugs species in Slovenia; A - *Milax nigricans*, *Tandonia budapestensis*, *Tandonia rara*, *Tandonia reuleauxi*, *Tandonia simrothi*, B - *Tandonia robici*, *Tandonia rustica*, C - *Tandonia sowerbyi*, D - *Malacolimax mrazekii*, E - *Lehmannia marginata*, F - *Limax cinereoniger*, G - *Limax maximus*, H - *Limax flavus*, *Boettgerilla pallens*, *Deroceras laeve*.

Slika 2: Razširjenost vrst v Sloveniji; A - *Milax nigricans*, *Tandonia budapestensis*, *Tandonia rara*, *Tandonia reuleauxi*, *Tandonia simrothi*, B - *Tandonia robici*, *Tandonia rustica*, C - *Tandonia sowerbyi*, D - *Malacolimax mrazekii*, E - *Lehmannia marginata*, F - *Limax cinereoniger*, G - *Limax maximus*, H - *Limax flavus*, *Boettgerilla pallens*, *Deroceras laeve*.



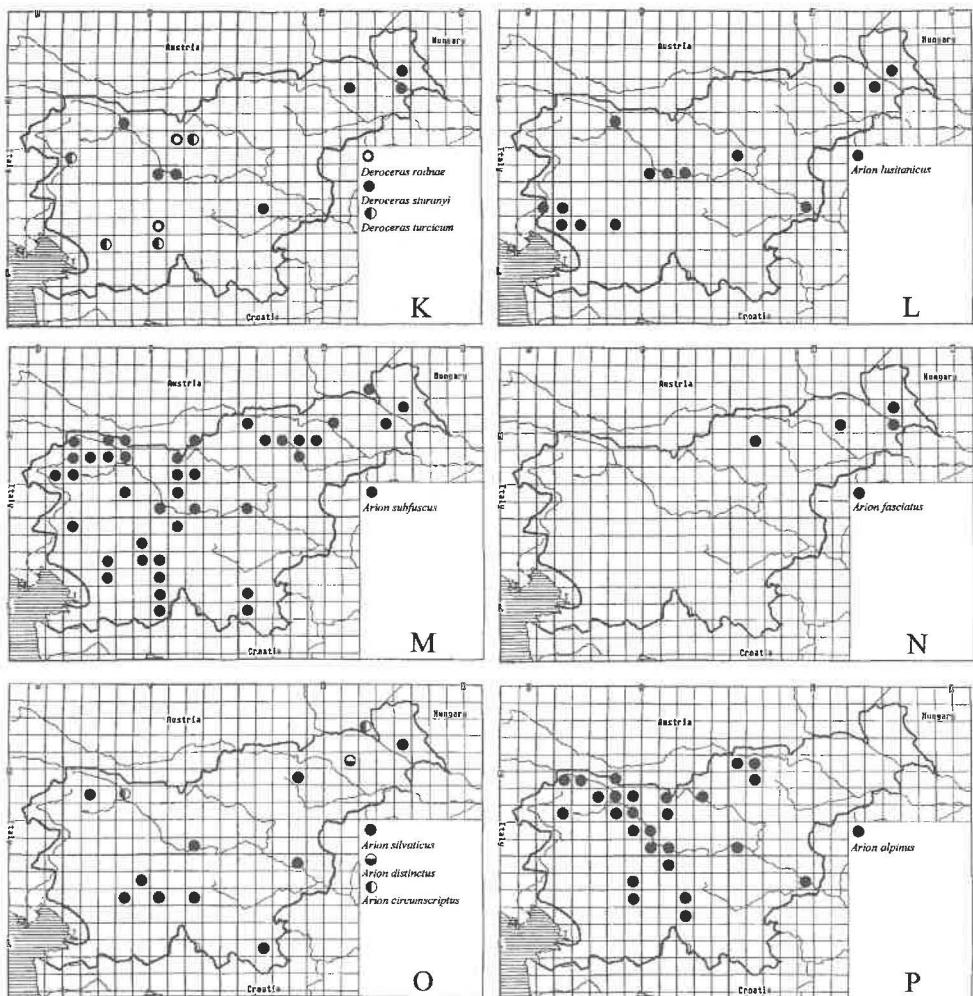


Figure 3: Distribution of slugs species in Slovenia; **I** - *Deroceras lothari*, **J** - *Deroceras reticulatum*, **K** - *Deroceras rodnae*, *Deroceras sturanyi*, *Deroceras turicum*, **L** - *Arion lusitanicus*, **M** - *Arion subfuscus*, **N** - *Arion fasciatus*, **O** - *Arion silvaticus*, *Arion distinctus*, *Arion circumspectus*, **P** - *Arion alpinus*.

Slika 3: Razširjenost vrst v Sloveniji; **I** - *Deroceras lothari*, **J** - *Deroceras reticulatum*, **K** - *Deroceras rodnae*, *Deroceras sturanyi*, *Deroceras turicum*, **L** - *Arion lusitanicus*, **M** - *Arion subfuscus*, **N** - *Arion fasciatus*, **O** - *Arion silvaticus*, *Arion distinctus*, *Arion circumspectus*, **P** - *Arion alpinus*.

Razprava

Po dosedanjih izsledkih živi na območju Slovenije sedemindvajset vrst golih polžev. Za enaindvajset od teh vrst lahko zasledimo podatke v literaturi, za šest vrst (*Milax nigricans*, *Tandonia budapestensis*, *Tandonia rara*, *Tandonia sowerbyi*, *Deroceras rodnae*, *Arion distinctus*) pa so to prvi podatki za Slovenijo. Od teh imajo tri vrste (*Milax nigricans*, *Tandonia rara*, *Arion distinctus*) samo po eno najdišče v Sloveniji. *Milax nigricans* in *Arion distinctus* sta zunaj Slovenije precej razširjeni, zato lahko pričakujemo, da sta tudi v Sloveniji bolj razširjeni. WIKTOR (1996) piše za vrsto *Tandonia rara*, da je razširjena na Velebitu in v Dalmaciji. Izgleda, da poteka severna meja njenega areala v Sloveniji. Podobno velja najverjetneje tudi za vrsto *Tandonia reuleauxi*. V Sloveniji je znana samo iz ene lokalitete. Vrsta je sicer razširjena v južnih delih nekdanje Jugoslavije (WIKTOR 1996), pod imenom *Aspidoporus limax* jo je z območja Kranjske navedel že WAGNER (1931). WIKTOR (1996) opozarja, da je starejše podatke v zvezi s to vrsto treba revidirati. Vseh vrst, za katere so znane lokalitete le iz enega ali dveh kvadratov UTM, je sedem (*Milax nigricans*, *Tandonia rara*, *Tandonia reuleauxi*, *Boettgerilla pallens*, *Deroceras laeve*, *Arion distinctus*, *Arion circumscriptus*).

WIKTOR (1996) navaja tudi šest vrst (*Milax gagates*, *Tandonia fejervaryi*, *Malacolimax tenellus*, *Deroceras agreste*, *Arion rufus*, *Arion hortensis*), za katere je našel podatke v literaturi, vendar jih sam na območju Slovenije ni ugotovil. Tudi pri obdelavi vzorcev iz naših zbirk nisva ugotovila nobene od navedenih vrst. Sinantropna vrsta *Milax gagates* je znana iz neke Jame blizu Trsta. Za vrste *Malacolimax tenellus*, *Deroceras agreste* in *Arion rufus* nimamo zanesljivih podatkov; so pa znana najdišča v Avstriji (REISCHÜTZ 1986), ki so blizu avstrijsko-slovenske meje in zato je mogoče, da te vrste živijo tudi v Sloveniji. WIKTOR (1996) piše, da bi bilo treba razširjenost vrste *Malacolimax tenellus* južno od Alp podrobnejše raziskati, zaradi možne zamenjave z vrsto *Malacolimax mrazekii*. Sodeč po lokalitetah blizu slovensko-avstrijske meje in po podatku, ki ga navajata WOLF & RÄHLE (1987), najverjetneje poteka južna meja areala vrste *Malacolimax tenellus* v Sloveniji. Vrsto *Arion rufus* je marsikje v Evropi izpodrinila vrsta *Arion lusitanicus* (FECHTER & FALKNER 1990). To najverjetneje velja tudi za Slovenijo, kjer so vrsto *Arion lusitanicus* ugotovili WIKTOR (1996) ter VAUPOTIČ & VELKOVRH (1997). Najstarejši vzorec s to vrsto v naših zbirkah je iz leta 1970, medtem ko vrste *Arion rufus* sploh nismo zanesljivo ugotovili.

Zaradi medsebojne zunanje podobnosti vrst rodu *Lehmannia* bo v bodoče treba pregledati prav vse primerke v razpoložljivih vzorcih, saj bi bilo mogoče, da je tudi v Sloveniji razširjena katera izmed drugih vrst tega rodu. Vrsta *Lehmannia nyctelia* je na primer znana iz Madžarske (PINTÉR et al. 1979, WIKTOR & SZIGETHY 1983) in Avstrije (REISCHÜTZ 1986), vrsta *Lehmannia brunneri* pa iz Hrvaške (WIKTOR 1996).

V Avstriji (REISCHÜTZ 1986) in na Hrvaškem (WIKTOR 1996) je po 33 vrst golih polžev. Od sosednjih držav je manj vrst kot v Sloveniji le na Madžarskem, kjer je znanih 22 (WIKTOR & SZIGETHY 1983). Slovenija je na stičišču štirih naravnogeografskih makroregij (Alpe, Dinarsko gorstvo, Sredozemlje, Panonska kotlina) in s tem pod vplivom različnih dejavnikov, ki vplivajo na razširjenost vrst, zato bi tukaj pričakovali več kot 30 vrst golih polžev.

Pregled nahajališč golih polžev v Sloveniji (Sl. 1) kaže, da podatki prihajajo iz približno polovice kvadratov UTM. Nekatere podatke iz literature bi bilo treba preveriti s ponovnim pregledom navedenih lokalitet. Število ugotovljenih vrst v posameznem kvadratu UTM je največje tam, kjer je potekalo intenzivno nabiranje živali. Takšna sta kvadrata WM65 in WM96 z enajstimi oziroma dvanajstimi ugotovljenimi vrstami, kar predstavlja skoraj polovico vseh vrst, znanih iz Slovenije. Med ugotovljenimi vrstami iz teh kvadratov sta tudi dve v Sloveniji redki vrsti (*Arion distinctus* poznana iz kvadrata WM65 ter *Boettgerilla pallens* iz kvadrata WM96), kar poleg velikega števila ugotovljenih vrst kaže na uspešnost intenzivne metode vzorčenja. Slike razširjenosti vrst kažejo, da je poznavanje arealov mnogih vrst v Sloveniji še zelo nepopolno, iz nekaterih območij nimamo sploh nobenih podatkov in zato bi v bodoče bilo smiselno intenzivno vzorčiti predvsem na teh območjih.

Zahvala

Za vse nasvete se zahvaljujeva prof. dr. Borisu Sketu. Prav tako se zahvaljujeva prof. dr. Andrzeju Wiktorju za prijazno sodelovanje pri determinaciji nekaterih vzorcev.

Summary

Slugs are among the least investigated snails in Slovenia. SIMROTH described two new slug species from this area (1885, 1910). BOLE mentioned 14 species in his identification key (1969). WIKTOR (1996) presented the first accurate data about slugs in Slovenia in his work on slugs from the former Yugoslavia. VAUPOTIČ & VELKOVRH (1997) found out 11 species in the north-eastern Slovenia. That was the first recording of *Boettgerilla pallens* in Slovenia.

With the analysis of data from the literature and the study of 686 samples of slugs from three collections (J. Bole collection of Biological Institute ZRC SAZU, our own collections), collected in years from 1957 to 1998, a list of slug species and their distribution in Slovenia is given. Data of species distribution are presented in the UTM squares (10 x 10 km).

The number of identified slug species in Slovenia is 27. For six species (*Milax nigricans*, *Tandonia budapestensis*, *Tandonia rara*, *Tandonia sowerbyi*, *Deroceras rodnae*, *Arion distinctus*) this is the first data for Slovenia. In Slovenia is the northern border of the distribution area for the species *Tandonia rara* and *Tandonia reuleauxi*. We suppose that the real number of the species is over thirty; we expect some species known from neighbouring countries (for example *Milax gagates*, *Lehmannia nyctelia*, *Lehmannia brunneri*, *Deroceras agreste*, *Arion rufus*) to be present in Slovenia. The species *Malacolimax tenellus* is probably present in Slovenia due to the localities near the Slovene-Austrian border and according to the data by WOLF and RÄHLE (1987). WIKTOR (1996) writes that its wide range south of the Alps should be investigated in detail, because of the possibility to confound it with *Malacolimax mrazekii*.

The data on slugs localities in Slovenia is known from only one half of the UTM squares. The largest number of species was found in the UTM squares, where the sampling was very intensive; e.g., in squares WM65 with 11 species and in WM96 with 12 species. In square WM65, a very successful sampling was done in one locality, where 5 cm thick wet boards were placed during the night out on the ground, and under those boards the slugs were collected in the morning.

In the future, more intensive research will be needed to get a more accurate number of the slug species and their distribution in Slovenia. Our data enable us to direct future investigations. Some locations, which are mentioned in the literature, will have to be checked up and also the areas, for which there are no data for slugs, will have to be researched.

Literatura

- ALTENA C. O. VAN REGTEREN 1973: On two species of *Deroceras* from Jugoslavia and Austria. *Basteria* 37: 85-92.
- ALTENA C. O. VAN REGTEREN 1977: Notes on land slugs. 25. On little known species of the genus *Milax*. *Basteria* 41: 65-70.
- BACKELJAU T & M. VAN BEECK 1986: Epiphallus anatomy in the *Arion hortensis* species aggregate (Mollusca, Pulmonata). *Zool. scr.* 15(1): 61-68.
- BACKELJAU T & L. DE BRUYN 1990: On the infrageneric systematics of the genus *Arion* Ferussac, 1819 (Mollusca, Pulmonata). *Bull. – Inst. r. sci. nat. Belg.* 60: 35-68.

- BOLE J. 1962: Mehkužci Triglavskega narodnega parka in okolice (Mollusca. Gastropoda, Bivalvia). Varst. narave **1**: 57-85.
- BOLE J. 1966: Mehkužci in zoogeografski položaj Rakovega Škocjana. Varst. narave **5**: 129-137.
- BOLE J. 1969: Ključi za določevanje živali – Mehkužci. Mollusca. Inštitut za biologijo Univerze v Ljubljani in Društvo biologov Slovenije, Ljubljana, 115 pp.
- BOLE J. 1976a: Mehkužci Notranjskega Snežnika in okolice. Varst. narave **9**: 55-63.
- BOLE J. 1976b: Malakološke razmere v mraziščih. Razprave IV razreda SAZU **19**(5): 151-183.
- BOLE J. 1977: Mehkužci Šmarne gore. Varst. narave **10**: 57-62.
- BOLE J. 1979a: Mehkužci Cerkniškega jezera in okolice. Acta carsologica **8**(3): 204-236.
- BOLE J. 1979b: Malakološke raziskave v nekaterih fitocenozah Slovenije. Savez društava ekologa Jugoslavije (II. Kongres ekologa Jugoslavije), Zagreb: 183-190.
- BOLE J. 1981: Zoogeographische Analyse der Landschnecken des dinarischen Gebietes Sloweniens. Razprave IV razreda SAZU **23**(4): 125-146.
- BOLE J. 1992a: Rdeči seznam ogroženih kopenskih in sladkovodnih mehkužcev (Mollusca) v Sloveniji. Varst. narave **17**: 183-189.
- BOLE J. 1992b: Mehkužci Kraškega roba. Proteus **54**(6-7): 234-235.
- BOLE J. 1994: Polži tal. Prirodoslovno društvo, Ljubljana, 38 pp.
- DE WINTER A.J. 1984: The *Arion hortensis* complex (Pulmonata, Arionidae): designation of types, descriptions, and distributional patterns, with special reference to the Netherlands. Zool. Med. Leiden **59**: 1-17.
- DE WINTER A.J. 1989: *Arion lusitanicus* Mabille in Nederland (Gastropoda, Pulmonata, Arionidae). Basteria **53**: 49-51.
- ERJAVEC F. 1877: Die malakologischen Verhältnisse GEFÜRSTETEN GRAFSCHAFTGÖRZ im österreichischen Küstenlande, Görz, Mailing, 82 pp.
- FECHTER R. & G. FALKNER 1990: Steinbachs Naturführer: Weichtiere. Mosaikverlag, München, 287 pp.
- GARRIDO C., J. CASTILLEJO & J. IGLESIAS 1995: The *Arion subfuscus* complex in the eastern part of the Iberian Peninsula, with redescription of *Arion subfuscus* (Draparnaud 1805). Arch. Moll., Frankfurt a. M. **124** (1/2): 103-118.
- GROSSUA.V. 1972: Fünf neue Arten der Gattung *Deroceras* von der Balkanhalbinsel in der Sammlung des Naturhistorischen Museums in Wien (Gastropoda, Limacidae). Ann. Naturhist. Mus. Wien **76**: 639-648.
- JAECKEL S. sen. & W. MEISE 1956: Über Land- und Süßwasserschnecken Jugoslawiens und Albaniens. Mitt. Hamburg. Zool. Mus Inst. **54**: 21-32.
- JAECKEL S.G., W. KLEMM & W. MEISE 1958: Die Land- und Süßwasser-Mollusken der nördlichen Balkanhalbinsel. Dresden, Abh. Ber. Mus. Tierk. **23** (2): 141-205.
- KERNY M.P., R.A.D. CAMERON & J.H. JUNGBLUTH 1983: Die Landschnecken Nord- und Mitteleuropas. Verlag Paul Parey, Hamburg und Berlin, 384 pp.
- KOS F. 1933: Vodnik po zbirkah Narodnega muzeja v Ljubljani, Prirodopisni del. Narodni muzej v Ljubljani, Ljubljana, 219 pp.
- LIKHAREV I.M. & A. WIKTOR 1980: The fauna of slugs of the USSR and adjacent countries (Gastropoda terrestria nuda). Fauna SSSR (NS 122) Molluski III, 3 (5). Leningrad, 434 pp.
- LUPU D. 1977: Le polymorphisme chez quelques espèces appartenant aux familles *Limacidae* et *Arionidae* de Roumanie. Malacologia **16**(1): 21-33.
- PINTÉR L., A. RINCHOVSKY & A.S. SZIGETHY 1979: A magyarországi recens puhatestűek elterjedése. Budapest, A tudományos ismeretterjesztő tarsulat, Bacsikusk megyei szervezete, Biológiai szakosztályának, Időszakos malakológiai közleményei, Soosiana, 351 pp.
- QUICK H. E. 1960: British slugs (Pulmonata: Testacellidae, Arionidae, Limacidae), Bulletin of the British Museum (Natural History) Zoology **6**: 103-226.

- REISCHÜTZ P. L. 1978: Bemerkungen zu *Deroberas klemmi* Grossu, 1972. Mitt. Abt.Zool. Landesmus. Joanneum, Graz **7**(1): 39-44.
- REISCHÜTZ P. L. 1986: Die Verbreitung der Nacktschnecken Österreichs (Arionidae, Milacidae, Limacidae, Agriolimacidae, Boettgerillidae). Supplement 2 des Catalogus Faunae Austriae, Österreichische Akademie der Wissenschaften, Wien, 190 pp.
- SAJOVIC G. 1908: Kranjski mehkučci (Mollusca carniolica). Izvestja Muz. društva za Kranjsko, 21 pp.
- SCHMIDT F. J. 1847: Systematisches Verzeichniss der in der Provinz Krain vorkommenden Land- und Suesswasser-Conchylien (mit Angabe der Fund-Orte). Blasnik, Ljubljana, 27 pp.
- SIMROTH H. 1885: Versuch einer Naturgeschichte der deutschen Nacktschnecken und ihrer europäischen Verwandten. Zeitschrift für Wissenschaftliche Zoologie **42**: 203-366.
- SIMROTH H. 1910: Nacktschneckenstudien in den Südalpen. Abh. Senckenb. Natforsch. Ges. **32**: 275-348.
- SLAPNIK R. 1998: Polži Kuma (Gastropoda: Prosobranchia, Pulmonata), Posavsko hribovje (Slovenija). Acta biol. slov. **42**(1): 57-67.
- SOUTH A. 1992: Terrestrial slugs. Chapman & Hall, London, 428 pp.
- STOSSICH A. 1899: Contribuzione alla Fauna malacologica terrestre e Fluviale del territorio di Trieste. 35 pp.
- VAUPOTIČ M. & F. VELKOVRH 1997: Prispevek k poznovanju favne mehkužcev (Mollusca) v severovzhodni Sloveniji. Acta biol. slov. **41** (1): 37-45.
- VAUPOTIČ M. 2002: Taksonomska in biogeografska analiza kopenskih golih polžev (Gastropoda: Pulmonata p.p.) v Sloveniji. Magistrska naloga. Biotehniška fakulteta, Oddelek za biologijo, Ljubljana, 107 pp.
- VON PROSCHWITZ T. 1989: *Arion lusitanicus* Mabille – en för Sverige ny snigelart. Göteborgs Naturhistoriska museum: 43-53
- WAGNER H. 1931: Diagnosen neuer Limaciden aus dem Naturhistorischen Museum in Wien. Zoologischer Anzeiger 95: 194-202.
- WIKTOR A. 1973: Die Nacktschnecken Polens. Monografie fauny polski 1, Warszawa, Krakow, 279 pp.
- WIKTOR A. 1982: Contribution to the knowledge of the slugs of Yugoslavia (Arionidae, Milacidae, Limacidae, Agriolimacidae – Gastropoda, Pulmonata). Ann. Zool., **36**(24): 465-489.
- WIKTOR A. 1983: The Slugs of Bulgaria (Arionidae, Milacidae, Limacidae, Agriolimacidae – Gastropoda, Stylommatophora). Ann. Zool. **37**(3): 71-206.
- WIKTOR A. 1987: Milacidae (Gastropoda, Pulmonata) – systematic monograph. Ann. Zool. **41**(3): 154-313.
- WIKTOR A. 1989: Limacoidea et Zonitoidea nuda. Ślimaki pomrowiokształtne (Gastropoda, Stylommatophora), Fauna Poloniae 12, Warszawa, 208 pp.
- WIKTOR A. 1996: The Slugs of the Former Yugoslavia (Gastropoda terrestria nuda – Arionidae, Milacidae, Limacidae, Agriolimacidae). Ann. Zool. **46**(1-2): 1-110.
- WIKTOR A. 1997: Endemism of slugs within the Balkan Peninsula and adjacent islands (Gastropoda: Pulmonata: Arionidae, Milacidae, Limacidae, Agriolimacidae). Genus **8**(1): 205-221.
- WIKTOR A. & N. MILANI 1995: Contribution to the knowledge of two scarcely known Alpine slugs, *Tandonia simrothi* (HESSE, 1923) and *Deroberas planariooides* (SIMROTH, 1910) (Gastropoda: Pulmonata: Milacidae et Agriolimacidae). Malakol. Abh. **17**(12): 151-160.
- WIKTOR A. & A.S. SZIGETHY 1983: The distribution of slugs in Hungary (Gastropoda: Pulmonata), Soosiana **10**(11): 87-111.
- WOLF M., RÄHLE W. 1987: Ergebnisse einer Molluskenexcursion in die westlichen Julischen Alpen. Mitt. Dtsch. Malakozool. Ges. **41**: 31- 41.

NAVODILA AVTORJEM

1. Vrste prispevkov

a) ZNANSTVENI ČLANEK je celovit opis originalne raziskave in vključuje teoretični pregled tematike, podrobno predstavljeni rezultati z diskusijo in sklepi ter literaturni pregled: shema IMRAD (Introduction, Methods, Results And Discussion). Dolžina članka, vključno s tabelami, grafi in slikami, ne sme presegati 15 strani; razmak med vrsticami je dvojen. Recenzirata ga dva recenzenta.

b) PREGLEDNI ČLANEK objavi revija po posvetu uredniškega odbora z avtorjem. Število strani je lahko večje od 15.

c) KRATKA NOTICA je originalni prispevek z različnih bioloških področij (sistematike, biokemijske, genetike, mikrobiologije, ekologije itd.), ki ne vsebuje podrobnega teoretičnega pregleda. Njen namen je seznaniti bralca s preliminarnimi ali delnimi rezultati raziskave. Dolžina na srečo ne sme presegati 5 strani. Recenzira ga en recenzent.

d) KONGRESNA VEST seznanja bralce z vsebinami in sklepi pomembnih kongresov in posvetovanj doma in v tujini.

e) DRUŠTVENA VEST poroča o delovanju slovenskih bioloških društev.

2. Originalnost prispevka

Članek, objavljen v reviji Acta Biologica Slovenica, ne sme biti predhodno objavljen v drugih revijah ali kongresnih knjigah.

3. Jezik

Teksti naj bodo pisani v angleškem jeziku, izjemoma v slovenskem, če je tematika zelo lokalna. Kongresne in društvene vesti so praviloma v slovenskem jeziku.

4. Naslov prispevka

Naslov (v slovenskem in angleškem jeziku) mora biti kratek, informativen in razumljiv. Za naslovom sledijo imena avtorjev in njihovi polni naslovi (če je mogoče, tudi štev. faxa in e-mail).

5. Izvleček - Abstract

Podati mora jedrnato informacijo o namenu, uporabljenih metodah, dobljenih rezultatih in zaključkih. Primerna dolžina za znanstveni članek naj bo približno 250 besed, za kratko notico pa 100 besed.

6. Ključne besede - Keywords

Število naj ne presega 10 besed; predstavljati morajo področje raziskave, obravnavane v članku. Člankom v slovenskem jeziku morajo avtorji dodati ključne besede v angleškem jeziku.

7. Uvod

Nanašati se mora le na tematiko, ki je predstavljena v članku ali kratki notici.

8. Slike in tabele

Tabele in slike (grafi, dendrogrami, risbe, fotografije idr.) naj v članku ne presegajo števila 10, v članku naj bo njihovo mesto nedvoumno označeno. Ves slikovni material naj bo oddan kot fizični original (fotografija ali slika). Tabele in legende naj bodo tipkane na posebnih listih (v tabelah naj bodo le vodoravne črte). Naslove tabel pišemo nad njimi, naslove slik in fotografij pod njimi. Naslovi tabel in slik ter legenda so v slovenskem in angleškem jeziku. Pri citiranju tabel in slik v besedilu uporabljamo okrajšave (npr. Tab. 1 ali Tabs. 1-2, Fig. 1 ali Figs. 1-2; Tab. 1 in Sl. 1).

9. Zaključki

Članek končamo s povzetkom glavnih ugotovitev, ki jih lahko zapišemo tudi po točkah.

10. Povzetek - Summary

Članek, ki je pisan v slovenskem jeziku, mora vsebovati še obširnejši angleški povzetek. Velja tudi obratno.

11. Literatura

Uporabljene literaturne vire citiramo med tekstom. Če citiramo enega avtorja, pišemo ALLAN (1995) ali (ALLAN 1995), če sta dva avtorja (TRINAJSTIĆ & FRANJIĆ 1994), če je več avtorjev (PULLIN & al. 1995). Kadar navajamo citat iz večih del hkrati, pišemo (HONSIG-ERLENBURG & al. 1992, WARD 1994a, ALLAN 1995, PULLIN & al. 1995). V primeru, če citiramo več del istega avtorja, objavljenih v enem letu, posamezno delo označimo s črkami a, b, c itd. (WARD 1994a,b). Če navajamo dobesedni citat, označimo dodatno še strani: TOMAN (1992: 5) ali (TOMAN 1992: 5-6). Literaturo uredimo po abecednem redu, začnemo s priimkom prvega avtorja, sledi leto izdaje in naslov članka, mednarodna kratica za revijo (časopis), volumen poudarjeno, številka v oklepaju in strani. Npr.:

HONSIG-ERLENBURG W., K. KRAINER, P. MILDNER & C. WIESERR 1992: Zur Flora und Fauna des Webersees. *Carinthia II* **182/102** (1): 159-173.

TRINAJSTIĆ I. & J. FRANJIĆ 1994: Ass. *Salicetum elaeagno-daphnoides* (BR.-BL. et VOLK, 1940) M. MOOR 1958 (*Salicion elaeagni*) in the Vegetation in Croatia. *Nat. Croat.* **3** (2): 253-256.

WARD J. V. 1994a: Ecology of Alpine Streams. *Freshwater Biology* **32** (1): 10-15.

WARD J. V. 1994b: Ecology of Prealpine Streams. *Freshwater Biology* **32** (2): 10-15.

Knjige, poglavja iz knjig, poročila, kongresne povzetke citiramo sledeče:

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

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

TOMAN M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 17.

12. Format in oblika članka

Članek naj bo napisan v programu *Word for Windows* ali *WordPerfect*, v pisavi "Times New Roman CE 12" z dvojnim medvrstnim razmakom in levo poravnavo ter s 3 cm robovi na A4 formatu. Odstavki naj bodo med seboj ločeni s prazno vrstico. Naslov članka in poglavij naj bodo pisani krepko in v velikosti pisave 14. Vsa latinska imena morajo biti napisana ležeče. V besedilu navedemo uporabljene nomenklатурne vire. Tabele in slike so posebej priložene tekstu. Glavnemu uredniku je potrebno oddati original, dve kopiji in disketni zapis na disketi 3,5" (odda avtor po opravljenih strokovnih in jezikovnih popravkih).

13. Recenzije

Vsek znanstveni članek bosta recenzirala dva recenzenta (en domači in en tuji), kratko notico pa domači recenzent. Avtor lahko v spremnem dopisu predlaga tuje recenzente. Recenziran članek, ki bo sprejet v objavo, popravi avtor. Po objavi prejme 50 brezplačnih izvodov. V primeru zavrnitve se originalne materiale vrne avtorju skupaj z negativno odločitvijo glavnega urednika.

INSTRUCTIONS FOR AUTHORS

1. Types of Articles

a) SCIENTIFIC ARTICLES are comprehensive descriptions of original research and include a theoretical survey of the topic, a detailed presentation of results with discussion and conclusion, and a bibliography according to the IMRAD outline (Introduction, Methods, Results, and Discussion). The length of an article including tables, graphs, and illustrations may not exceed fifteen (15) pages; lines must be double-spaced. Scientific articles shall be subject to peer review by two experts in the field.

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

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

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

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

2. Originality of Articles

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

3. Language

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

4. Titles of Articles

Titles (in Slovene and English) must be short, informative, and understandable. The title should be followed by the name and full address of the author (and if possible, fax number and e-mail address).

5. Abstract

The abstract must give concise information about the objective, the methods used, the results obtained, and the conclusions. The suitable length for scientific articles is approximately 250 words, and for brief note articles, 100 words.

6. Keywords

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

7. Introduction

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

8. Illustrations and Tables

Articles should not contain more than ten (10) illustrations (graphs, dendograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided as physical originals (photographs or illustrations). Tables with their legends should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables should appear above the tables, and titles of photographs and illustrations below. Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2; Tab. 1 and Sl. 1).

9. Conclusions

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

10. Summary

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

11. Literature

References shall be cited in the text. If a reference work by one author is cited, we write ALLAN (1995) or (ALLAN 1995); if a work by two authors is cited, (TRINAJSTIĆ & FRANJIĆ 1994); if a work by three or more authors is cited, (PULLIN & al. 1995); and if the reference appears in several works, (HONSIG-ERLENBURG & al. 1992, WARD 1994a, ALLAN 1995, PULLIN & al. 1995). If several works by the same author published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc.: (WARD 1994a,b). If direct quotations are used, the page numbers should be included: TOMAN (1992: 5) or (TOMAN 1992: 5-6).

The bibliography shall be arranged in alphabetical order beginning with the surname of the first author followed by the year of publication, the title of the article, the international abbreviation for the journal (periodical), the volume (in bold print), the number in parenthesis, and the pages. Examples:

HONSIG-ERLENBURG W., K. KRAINER, P. MILDNER & C. WIESER 1992: Zur Flora und Fauna des Weberssees. *Carinthia II* **182/102** (1): 159-173.

TRINAJSTIĆ I. & J. FRANJIĆ 1994: Ass. *Salicetum elaeagno-daphnoides* (BR.-BL. et VOLK, 1940) M. MOOR 1958 (*Salicion elaeagni*) in the Vegetation in Croatia. *Nat. Croat.* **3** (2): 253-256.

WARD J. V. 1994a: Ecology of Alpine Streams. *Freshwater Biology* **32** (1): 10-15.

WARD J. V. 1994b: Ecology of Prealpine Streams. *Freshwater Biology* **32** (2): 10-15.

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

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

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

TOMAN M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. *Zbornik referatov s posvetovanja DZVS*, Gozd Martuljek, pp. 17.

12. Format and Form of Articles

Articles should be written with *Word for Windows* or *WordPerfect* using "Times New Roman CE 12" font with double spacing, align left and margins of 3 cm on A4 pages. Paragraphs should be separated with an empty line. The title and chapters should be written bold in font size 14. All scientific names must be properly italicized. Used nomenclature source should be cited. Tables and illustrations shall accompany the texts separately. The original manuscript, two copies, and a copy on a 3.5" computer diskette must be given to the editor-in-chief. All articles must be proofread for professional and language errors before submission.

13. Peer Review

All Scientific Articles shall be subject to peer review by two experts in the field (one Slovene and one foreign) and Brief Note articles by one Slovene expert in the field. Authors may nominate a foreign reviewer in an accompanying letter. Reviewed articles accepted for publication shall be corrected by the author. Authors shall receive fifty (50) free copies of the journal upon publication. In the event an article is rejected, the original material shall be returned to the author together with the negative determination of

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