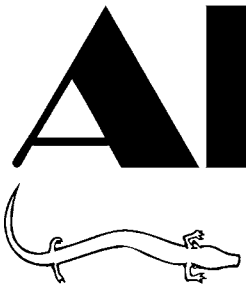

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Seasonal changes in the contents of nutrients in five macrophyte species from the lake Velenjsko jezero (Slovenia)

Sezonske spremembe vsebnosti hranil v petih vrstah makrofitov iz Velenjskega jezera (Slovenija)

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Abstract. The study was designed to depict the seasonal dynamic in relative abundance of macrophyte species, nutrient availability and their content in macrophytes to assess the capability of different species to store nutrients in nutrient rich lake Velenjsko jezero. The concentrations of total nitrogen and total phosphorus in the lake sediment, water and aboveground biomass of macrophytes (*Nuphar luteum*, *Najas marina*, *Najas minor*, *Potamogeton lucens* and *Potamogeton pectinatus*) were measured at three locations monthly from June to September 2004. Seasonal variability in the contents of total phosphorus in macrophyte tissues was high, but all examined species reached similar maximal concentration in the beginning of their growth. Later in the season, concentrations declined to a high degree. Seasonal variability in the contents of total nitrogen was smaller. Floating-leaved species *Nuphar luteum* was present in a low amounts and contained much more total nitrogen in its above-ground tissues than the submersed species. Because of different species life spans, differences in the content of nutrients among species were very high in particular month. *Nuphar luteum, Najas minor* and *Potamogeton lucens* were rarely present in the lake and they contributed less to storing nutrients in their biomass. *Potamogeton pectinatus* was commonly present in the early summer, but in August *Najas marina* prevailed by far and its growth had high effect on the concentration of nutrients in sediment and water. Therefore removing of 1 t dry weight of *Najas marina* biomass from the lake would contribute to removal of 2.7 kg of phosphorus and 28.2 kg of nitrogen from the lake.

Key words: lake, total nitrogen, total phosphorus, *Nuphar luteum* (L.) Sibth et Sm., *Najas marina* L., *Najas minor* All., *Potamogeton lucens* L. and *Potamogeton pectinatus* L.

Izvleček. V pričujoči raziskavi smo spremljali sezonske spremembe v abundanci makrofitskih vrst ter vsebnosti celotnega dušika in celotnega fosforja v sedimentu, vodi in v makrofitih (*Nuphar luteum*, *Najas marina*, *Najas minor*, *Potamogeton lucens* in *Potamogeton pectinatus*) Velenjskega jezera. Sezonska nihanja v vsebnosti TP v rastlinah so bila zelo velika, vendar so vse vrste v začetku svoje rasti dosegale podobne maksimalne vsebnosti TP, nato pa so koncentracije s časom večinoma pri vseh vrstah hitro upadale. Sezonska nihanja v vsebnosti TN v rastlinah so bila manjša. Največja maksimalna koncentracija celotnega dušika je bila izmerjena v nadzemnih tkivih v jezeru redko prisotne submerzne vrste s plavajočimi listi – *Nuphar luteum*. Maksimalne koncentracije v popolnoma potopljenih makrofitih (*Najas marina*, *Najas minor*, *Potamogeton pectinatus* in *Potamogeton lucens*) pa so bile zelo podobne. Zaradi različnega življenjskega cikla makrofitskih vrst, so bile sicer razlike med vrstami v določenem mesecu zelo velike. Prisotnost vrst *Nuphar luteum*, *Najas minor* in *Potamogeton lucens* je bila v jezeru redka, zato je bil njihov prispevek k zmanjšanju hranil v sedimentu in vodi jezera majhen. *Potamogeton pectinatus* je bila s svojo relativno zmerno prisotnostjo prevladujoča vrsta v jezeru spomladi, poleti pa je nad njo

močno prevladala vrsta *Najas marina*. Njena razrast je imela večji učinek na zmanjšano koncentracijo hranil v sedimentu in v vodi. Odstranitev 1 tone suhe biomase makrofita *Najas marina* v mesecu avgustu, bi prispevala k odstranitvi 2,7 kg fosforja in 28,2 kg dušika iz jezera.

Ključne besede: jezero, celotni dušik, celotni fosfor, *Nuphar luteum* (L.) Sibth et Sm., *Najas marina* L., *Najas minor* All., *Potamogeton lucens* L. in *Potamogeton pectinatus* L.

Abbreviations:

TP – total phosphorus, SRP – soluble reactive phosphate, TN – total nitrogen, Pot pec – *Potamogeton pectinatus* L., Pot luc – *Potamogeton lucens* L., Naj mar – *Najas marina* L., Naj min – *Najas minor* All., Nup lut – *Nuphar luteum* (L.) Sibth et Sm.

Introduction

Velenjsko jezero is more and more popular as a recreational resource, which increases the threat of its rapid eutrophication. The lake offers good conditions for the growth of macrophytes, which allowed for their rapid spread within only a few years. There is strong evidence that nutrient availability plays an important role in controlling the development and abundance of macrophytes (Carr & Chambers 1998). After 1997 the aquatic plant *Najas marina* L. prevailed in Velenjsko jezero, forming weed beds that covered larger areas in August and September. *Najas marina* is a summer-annual plant that is highly fertile and produces great quantities of seeds. Its presence in Velenjsko jezero disturbs swimmers, fisheries and other lake users.

Aquatic macrophytes are often suggested to be accumulators of contaminants and nutrients in surface waters (Wolverton & McDonald 1979). Nutrient absorption in submersed macrophytes occurs both from the water by foliage and from the sediments by root and rhizoid systems. The relative contribution of roots and shoots, at least to N and P uptake, depends on the sediment:water nutrient ratio (Carignan 1982). But Wetzel (2001) emphasized that under most circumstances, even in nutrient-rich waters, roots are the dominant sites of nutrient uptake and assimilation for aquatic plants, although some evidence is contradictory (e.g. Swanepoel & Vermaak 1977). Experimental analyses have demonstrated that most rooted submersed angiosperms obtain most of their phosphorus from the interstitial water of the sediments (e.g. Chambers & al. 1989; Carr & Chambers 1998), since the absorbable nutrient concentration is much higher in sediment pore water than in the water column (Carr & Chambers 1998). Because of that, the value of tissue analyses of element concentrations in aquatic macrophytes is suspect as an index of the fertility of the lake water.

Nutrient content of the water can be quite unrelated to plant growth of those species having ready access to the abundant nutrient in the sediment. The rooted plants can function as a "pump" of nutrients from the sediment; some of those nutrients can then be lost to the water during both active growth and decomposition (Wetzel 2001). Nutrient concentrations in macrophyte species differ greatly even among closely related species and within the same species from site to site (Kufel & Kufel 2002; Garbey & al. 2004). This can be explained by the fact that nutrient uptake and accumulation in macrophytes does not only depend on the physiological capacity of the species, but also on the nutrient concentration in water and sediment (Carignan 1982; Shardendu & Ambasht 1991; Fernández-Aláez & al. 1999) and on N and P reserves in the plant tissue (Taheruzzaman & Kushari 1989).

In the present study we attempted to depict the seasonal dynamics in relative abundance of macrophyte species, as well as nutrient availability and content in macrophytes, to assess the capability of different species to store nutrients in nutrient rich lake Velenjsko jezero. For this purpose we carried out a comparative analysis of nitrogen and phosphorus content of macrophyte species: the submersed annual species *Najas marina* and *Najas minor* All., the submersed perennial annual species *Potamogeton lucens* L. and *Potamogeton pectinatus* L. and the floating-leaved perennial species – *Nuphar luteum* (L.) Sibth et Sm.

Materials and Methods

Description of the site

Lake Velenjsko jezero is located in central Slovenia, in the Šalek Valley. It is situated at an altitude of 366 m, with a surface area of 135,000 m^2 and a maximal depth of 54 m. It is an artificial lake resulting from mining activity. The detailed description is available in Šterbenk (1999) and Mazej and Epšek (2005).

Presence and abundance of macrophytes

The distribution of macrophyte species over the entire littoral was assessed using a boat, depth meter, view box and sampling rake to choose sampling locations. After that, three sampling transects (L1, L2, L3) were chosen for comparative analyses on the south-eastern part of the lake, where macrophyte species richness was greatest. Each transect was 200±2 m in length. These transects were surveyed every month from June to September. Species abundance in each section was evaluated according to Kohler (1978) on a five level descriptor scale (1 – very rare, 2 – infrequent, 3 – common, 4 – frequent, 5 – abundant, predominant).

Content of nutrients in water, sediment and macrophytes

Samples of water, sediment and macrophytes were taken from the three locations every month from June to September 2004. The sediment samples were collected from the top 5 cm of bottom sediments using a grab sampler. Samples of water were collected by hand from a boat by submerging precleaned PE bottles approximately 50 cm beneath the water surface. Plants were collected from the boat with a rake. The contents of total nitrogen (TN), total phosphorus (TP) and soluble reactive phosphate (SRP) in sediment were analysed by the standard methods: ISO 11261:1995, ISO 11263:1995 and ÖNORM L 1088:2005, while TN and TP in the water were determined according to the standard methods ISO 10304-2:1995 and ISO 6878:2004.

Above ground tissues of plants were washed carefully in the laboratory to remove sediment and periphyton. The specimens of different macrophyte species were analysed for nutrient content. Plant material was oven-dried at 75 °C to constant weight and ground to a fine powder by milling. TN in plants was determined by the standard method ISO 11261:1995, while TP was determined by EPA Method 3050B Mod Block.

Statistical analysis

Statistical procedures were performed using the Statistica software package (Statistica for Windows, version 7.0). After verifying the normality and homoscedasticidy of the variables (K-S and Liliefors test for normality), standard one-way analysis of variance (ANOVA; LSD test) was used to check the existence of significant differences between the locations regarding the contents of nutrients in water, sediment and macrophytes. The Spearman correlation coefficients between the content of nutrients in macrophytes and the contents of nutrients in water and sediment were calculated.

Results

Table 1: Abundance of different macrophyte species in Lake Velenjsko jezero in the year 2004 at the three selected transects. Each transect was 200±2 m in length. Five level descriptor scale of abundance was used: 0 – absent, 1 – very rare, 2 – infrequent, 3 – common, 4 – frequent, 5 – abundant, predominant (KOHLER 1978).

Tabela 1: Prisotnost in pogostost makrofitov na treh izbranih transektih Velenjskega jezera od junija do septembra 2004. Dolžina transektov je merila 200±2 m. Abundanca po KOHLER (1978) temelji na petstopenjski skali: 0 – odsotna, 1 – posamična, 2 – redka, 3 – pogosta, 4 – množična, 5 – prevladujoča).

	L1				L2				L3			
	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
Nup lut	2	2	2	2	0	0	0	0	0	0	0	0
Pot pec	3	3	3	2	0	0	0	0	3	3	2	2
Pot luc	0	0	0	0	1	2	2	1	1	2	2	1
Naj mar	0	1	5	4	0	2	5	3	0	2	5	3
Naj min	0	0	0	0	0	0	2	2	0	1	2	2

The distribution pattern of the species was patchy, no species examined being found at all three chosen locations, with the exception of *Najas marina*, which in August overgrew almost the whole littoral (Tab. 1). *Potamogeton pectinatus*, *Najas minor* and *Potamogeton lucens* throve at two locations and *Nuphar luteum* at only one. We detected large seasonal changes in the presence and abundance of macrophyte species at the sampling locations due to their different life histories. The perennial spe-

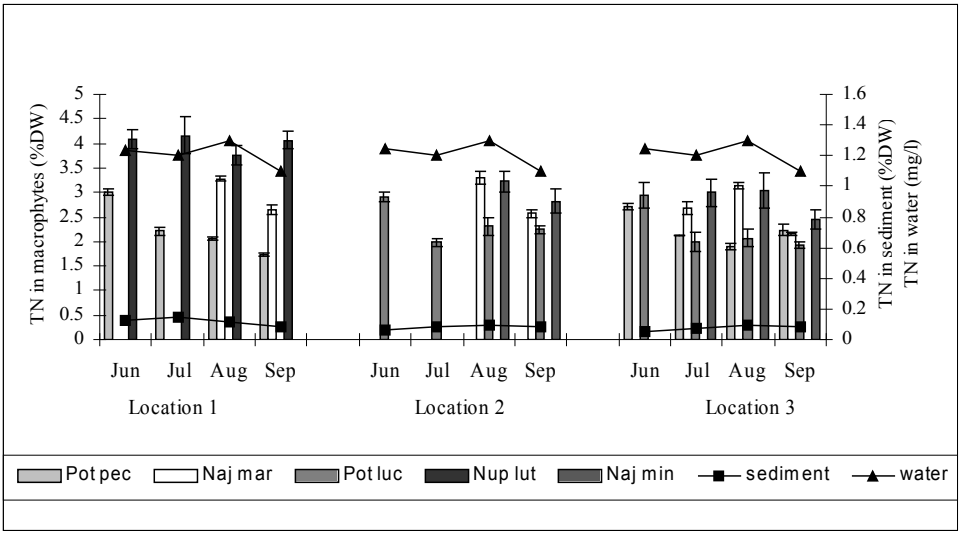

Figure 1: Total nitrogen (TN) content in above-ground tissue of five species of macrophytes (whole plant homogenate) – *Nuphar luteum*, *Potamogeton pectinatus*, *Potamogeton lucens*, *Najas minor* and *Najas marina* (in columns). Each value is the mean of three replicates. Lines represent the content of TN in sediment and TN in the water column during the summer growth season.

Slika 1: Vsebnost celotnega dušika (TN) v nadzemnih tkivih petih makrofitskih vrst – *Nuphar luteum*, *Potamogeton pectinatus*, *Potamogeton lucens*, *Najas minor* and *Najas marina* (v stolpcih). Vsaka vrednost je povprečje treh paralelk. Linije predstavljajo vsebnost TN v jezerskem sedimentu in v vodi.

cies *Nuphar luteum*, *Potamogeton lucens* and *Potamogeton pectinatus* started their vegetative growth earlier in the season. *Potamogeton pectinatus* prevailed in June and July, while in August *Najas marina* became the most abundant and overgrew almost all littoral. Annual species *Najas marina* and *Najas minor* had short life spans, appearing in the lake from July to September only.

The contents of TN, and especially of TP, in plants were higher at the beginning of their development (Figs. 1–2). Thus the comparison of different species regarding their content of nutrients was influenced by differences in phenological phase. Extreme values of TN stand out, the highest being 4.16% DW in *Nuphar luteum* in July (Fig. 1). This species was found only at the first location (L1). Concerning TP, the species *Nuphar luteum* (0.41% DW) and *Potamogeton pectinatus* (0.37% DW) from the first location (L1) and *Najas marina* (0.41% DW), *Najas minor* (0.42% DW) and *Potamogeton lucens* (0.38% DW) from the second location (L2) contained similar maximal concentrations at the beginning of their development (Fig. 2).

With respect to water and sediment, there were no significant differences in chemical characteristics in these two compartments between the three locations, except that the sediment of the first location contained a significantly higher amount of TP than the sediment from the third location ($p = 0.015$). Accordingly, *Potamogeton pectinatus* and *Najas marina* from the first location contained significantly higher average contents of TP than the same species from the third location – (*Potamogeton pectinatus* – $p = 0.034$, n=12 and *Najas marina* – $p = 0.002$, n=12). No significant differences were found

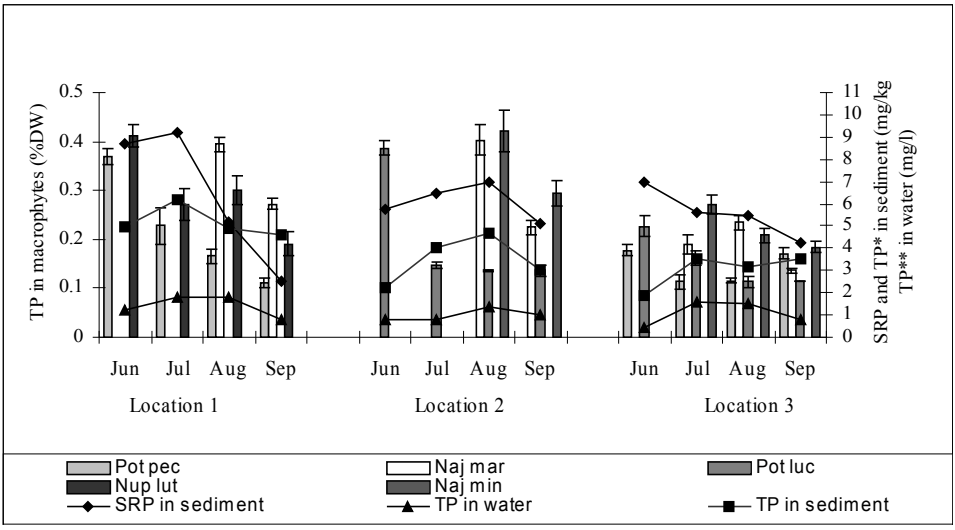

Figure 2: Total phosphorus (TP) content in above-ground tissue of five species of macrophyte (whole plant homogenate) – *Nuphar luteum*, *Potamogeton pectinatus*, *Potamogeton lucens*, *Najas minor* and *Najas marina* (in columns). Each value is the mean of three replicates. Lines represent the content of soluble reactive phosphorus (SRP) and TP in sediment and TP in the water during the summer growth season.
* Values of TP in sediment were in fact 100 times higher than is represented on the figure.
** Values of TP in water were in fact 100 times lower than is represented on the figure.

Slika 2: Vsebnost celotnega fosforja (TP) v nadzemnih tkivih petih makrofitskih vrst – *Nuphar luteum*, *Potamogeton pectinatus*, *Potamogeton lucens*, *Najas minor* and *Najas marina* (v stolpcih). Vsaka vrednost je povprečje treh paralelk. Linije predstavljajo vsebnost lahko dostopnega fosforja (SRP) in TP v jezerskem sedimentu in TP v vodi.
* Vsebnosti TP v sedimentu so bile 100 krat večje, kot je prikazano na sliki.
** Vsebnosti TP v vodi so bile 100 krat manjše kot je prikazano na sliki.

between different locations regarding concentrations of TN in macrophytes. Concentrations of SRP in the sediment, and TP and TN in water, drastically decreased in September, while content of TN in sediment remained constant.

Table 2: Correlation (Spearman coefficient) between the concentrations of nutrients in some macrophyte species and the concentrations in water and sediment.
Tabela 2: Korelacija (Spearmanov koeficient) med koncentracijo hranil v nekaterih makrofitskih vrstah in med koncentracijo hranil v vodi in sedimentu.

	Annual species				Perennial species					
	Naj mar (15)		Naj min (15)		Pot pec (24)		Nup lut (12)		Pot luc (24)	
	TN	TP	TN	TP	TN	TP	TN	TP	TN	TP
Water (TN, TP) (12)	0.60*	0.42*	0.56*	0.43*	–0.3	–0.38	–0.88**	–0.15	–0.3	0.23
Sediment (TN, SRP) (12)	0.05	0.41*	–0.4	0.67*	–0.02	0.57*	0.41	0.47	0.01	–0.33

Level of statistical significance: *** $p<0.001$, ** $p<0.01$, * $p<0.05$
Numbers of replicates are in brackets.

Relationship between the content of nutrients in the macrophytes and in water and sediment was determined (Tab. 2). The contents of total phosphorus in *Najas marina* and *Najas minor* correlated positively with the contents of SRP and TP in sediment, while their contents of TN correlated positively with that in the water. Negative significant correlation was observed between the content of TN in *Nuphar luteum* and its content in water.

Discussion

Lake Velenjsko jezero can be classified as eutrophic on the basis of the level of total phosphorus (0.1 mg/L) and total nitrogen (1.34 mg/L) in the water (OECD 1982). Macrophytes of Velenjsko jezero showed no evidence of TP and TN limitation. The TN and TP contents in macrophytes were generally higher than the critical concentrations of 0.13 % for TP and 1.3 % for TN. The critical concentration of a nutrient in a plant has been defined as that concentration in plant tissues which permits the maximum yield (Gerloff & Krombholz 1966).

The variation in macronutrient concentration in macrophyte tissues was found to correlate with the growth form of the plant (Jackson & Kalff 1993; Fernández-Aláez & al. 1999). The macroalgae showed the lowest nutrient content, while an impoverishment in N was observed in emergent species (Fernández-Aláez & al. 1999). The lower N in tissues of emergent plants as related to submersed and rooted floating-leaved macrophytes is explained by their greater biomass, which means a greater proportion of non-nitrogenou supporting tissue (Fernández-Aláez & al. 1999). The above-ground tissues of *Nuphar luteum* contained much more TN than those of submersed species, in spite of the fact that the structure of floating leaves is similar to that of leaves of emergent species. Floating-leaved plants have a morphological adaptation to increase inorganic carbon and mineral acquisition, which enables their higher productivity (Boston & al. 1989). The floating and submersed leaves of *Nuphar luteum* enable it to exploit CO_2 from air and from water. Like emergent macrophytes, *Nuphar luteum* possesses operationally active transpiration-mediated root-pressure systems, which enable nutrient absorption and translocation from the roots to the foliage (Wetzel 2001). Maximum concentration of TP in *Nuphar luteum* did not differed from those of submersed species.

Furtado (1998) registered the changes in concentrations of both nutrients (P and N) as a function of season, plant age and stage of vegetative development. Our results show great seasonal variability in the content of nutrients in macrophyte tissues in which fluctuations of the content of TP were most marked (Figs. 1–2). Seasonal variability in the contents of total phosphorus in macrophyte tissues

was high, but all examined species reached similar maximum concentration in the beginning of their growth. Later in the season, concentrations declined to a considerable degree. Seasonal variability in the contents of total nitrogen was smaller. The highest content of nutrients, especially phosphorus, at the beginning of the growth season is known as "luxury" uptake. Such a strategy may benefit the plant later, should nutrient concentrations diminish (Garbey & al. 2004), and allow the abundance of submerged plants to increase (Palma-Silva & al. 2002).

The rarely present floating-leaved species *Nuphar luteum* contained much more total nitrogen in its above-ground tissues than submersed species. Because of different species life spans, differences in the content of nutrients among species were very large in particular month. *Nuphar luteum*, *Potamogeton lucens* and *Potamogeton pectinatus* overwinter, and then grow rapidly in the spring. They are able to recycle and withdraw nutrients from their senescing parts or storage organs for reuse (Vitousek 1982) and so are less dependent on the nutrient concentration in their environment at the beginning of their growth, other than annual plants. Their growth in the lake was not significantly influenced on nutrient concentration in sediment and water. In contrast annual plants like *Najas marina* and *Najas minor*, are more dependent on the concentration of nutrients in their environment and required high levels for new growth in the summer, resulting in intensive growth. In July the soft sediment of the littoral is still not colonized and the amounts of nutrients in the water and sediment do not decrease before September. Both *Najas* species propagate from seeds that enable quick colonization of new habitats (Agami & Weisel 1986). Favourable nutrient conditions in summer offered *Najas marina* to overgrow almost the whole lake littoral in one month, reflecting the nutrient status of its environment. Concentrations of SRP in the sediment and TP in water drastically decreased in September, after the rapid expansion of *N. marina* in August. The content of TN in water also decreased drastically in September, while its content in sediment remained constant. Removing of 1 t dry biomass of *Najas marina* from the lake would contribute to removal of 2.7 kg of phosphorus and 28.2 kg of nitrogen from the lake.

Conclusions

1. Floating-leaved species *Nuphar luteum* contained much more total nitrogen in its above-ground tissues than submersed species.
2. Macrophytes showed great seasonal variability of TP content in their tissues. The stage of plant vegetative development appears to be an important factor, influencing the content of nutrients in plants. A high content of TP, very similar at all species, was detected in the initial phase of plant development. Because of different species life spans, differences in the content of nutrients among species were high in particular months.
3. While the development of four other species did not significantly influenced the nutrient concentration in sediment and water, rapid development of annual *Najas marina* had a significant effect on the concentration of nutrients in these two compartments. Therefore removal of at least part of the enormous biomass of this species in August would contribute to a high export of nutrients from Velenjsko jezero.

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Competitive advantages of *Najas marina* L. in a process of littoral colonization in the lake Velenjsko jezero (Slovenija)

Tekmovalne prednosti vrste *Najas marina* L. pri kolonizaciji litorala Velenjskega jezera (Slovenia)

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Abstract. *Najas marina* is the dominant macrophyte species in Velenjsko jezero. It appeared in the lake in 1997 and soon prevailed over the species *Myriophyllum spicatum* L. and *Potamogeton crispus* L., which used to be the two most abundant species in the lake in the past. The physico-chemical and geomorphological characteristics of the lake are discussed in relation to the attributes of *Najas marina*, presenting competitive advantages in this environment. Conditions in the lake such as warm water and unstable sediment enabled successful growth and life strategy of *Najas marina*, which is a summer-annual plant with short life cycle, quick propagation from seeds and a very extensive root system.

Key words: artificial lake, physico-chemical and geomorphological characteristics of the lake, plant invasion, aquatic macrophytes, *Najas marina* L.

Izvleček. *Najas marina* je prevladujoča makrofitska vrsta v Velenjskem jezeru. V jezeru se je pojavila leta 1997 in kmalu prevladala nad vrstama *Myriophyllum spicatum* L. in *Potamogeton crispus* L., ki sta do takrat prevladovali v jezeru. V članku so izpostavljene fizikalno kemijske in geomorfološke značilnosti jezera, ki so ene izmed pomembnih dejavnikov, ki vplivajo na kompeticijske lastnosti določene vrste. Razmere v jezeru, kot so topla voda in nestabilen sediment, omogočajo uspešen razvoj in rast vrste *Najas marina*, ki je toploljubna enoletnica s kratkim življenjskim ciklom, hitrim načinom razmnoževanja iz semen in z zelo ekstenzivnim koreninskim sistemom.

Ključne besede: umetno jezero, fizikalno kemijske in geomorfološke značilnosti jezera, invazivnost rastlin, vodni makrofiti, *Najas marina* L.

Introduction

The ability of a plant species to invade a region depends not only on the attributes of the plant, but also on the physico-chemical characteristics of the lake habitat invaded (Ali & Soltan 2006). Besides penetration of radiation and temperature, the key factor determining the composition and vertical distribution of submersed macrophyte communities is substrate composition (average grain size, sorting level, silt fraction, organic matter). Sediments influence vegetation in two ways, i.e. by serving as an anchor for roots and rhizoids (Handley & Davy 2002) and as potential nutrient reservoir

(Peralta & al. 2003). Sculthorpe (1967) pointed out that the principal influence of the substrate on the distribution of rooted aquatic plants is due to its physical texture rather than chemical composition. The sediment texture is a very important characteristic, which has very great influence on the physical, chemical and, indirectly, on the biological properties of the sediment. The mechanical properties and instability of the substrate in aquatic habitats might prevent seedling establishment and increase the chances of dislodgement (Titus & Hoover 1991) in many species. In contrast seedlings of *Najas marina* L. can not colonize on firm sediments (Handley & Davy 2002). Authors who have investigated the interaction between vegetation and sediment (Lindner 1978, Selig & al. 2007) clearly distinguished between plant communities' characteristic for mineral sediments with low nutrient content and plant communities' characteristic for muddy sediments with high nutrient content.

Najas marina is a rare species in Slovenia, registered in only six locations (Vreš & Kaligarič 1999, Germ & al. 2008) beside Velenjsko jezero, where *Najas marina* is the dominant species. It appeared in the lake in 1997 (Mazej 1998) and outcompeted both *Myriophyllum spicatum* L. and *Potamogeton crispus* L., which used to be abundant in previous years. However, they had never occurred in the lake to such an extent as *Najas marina*. *Myriophyllum spicatum* and *Potamogeton crispus* are known as very competitive species, which establish large monospecific weed beds in many lakes (Nichols & Show 1986; Bolduan & al. 1994), including some Slovenian meso-eutrophic lakes (Mazej 1998; Mazej & Gaberščik 1999).

We hypothesize that physico-chemical and geomorphological characteristics of the lake are the main factors, which allow the broad expansion of *Najas marina*. Species composition was related to environmental characteristics in order to point out the reasons for the successful growth of *Najas marina*, which prevailed over other macrophytes. Some of the attributes of *Najas marina* were also stressed.

Materials and methods

Study Area

Velenjsko jezero is situated in the Šalek valley, in the Sub-alpine part of Slovenia near the Austrian border at an altitude of 366 m, with a surface area of 135000 m^2 and a maximum depth of 54 m. Huge lignite-coal reserves, which are dug in Velenje Colliery are the crucial factor of human caused changes and pollution of the Šalek valley. The most remarkable consequences of coal mining are three subsidence lakes, Škalsko, Velenjsko and Družmirsko jezero. Velenjsko jezero came into existence after the World War II. At the beginning of its existence it was used as a reservoir for ash transport water from the Šoštanj thermal plant. The pH of transport water is around 12. The pH of lake was the same so any sorts of organism could not survive in such an alkaline environment. Up to early eighties ash slurry had run into lake, but afterwards the building of the ash landfill was begun. Ash reminded on the landfill and only transport water ran to lake. The pH remained 12 because the only reason for high alkinity was transport water. The closed loop system for the ash was built in 1994 and this has an impact on the lake quality. In only three years the lake pH has almost been normalized and biota appeared in the lake again (Šterbenk, 1999). It was recolonized by phyto- and zooplankton, fish, macrophytes and other organisms. The pH-value of the lake is now around 8.

Sampling

Three sampling locations (L1, L2, L3) with highest species richness and similar morphometric characteristics of littoral on the south-eastern part of the lake, were chosen. Samples of water and sediment were taken adjacent to vegetation from three sampling locations monthly from June to September 2004. Samples of water were taken at 0.5 m depth in plastic bottles, while sediment was sampled by a

handled plastic scoop. Water transparency was measured in the middle of the lake with a Secchi disk. Temperature at 30 cm and pH at all three locations were measured with a MultiLine P4.

Presence and abundance of macrophytes

At the same time the distribution of macrophyte species at the three sampling locations was assessed using a boat, a depth meter, a viewing box and a sampling rake. Species abundance was evaluated according to Kohler (1978) on a five level descriptor scale (1 – very rare, 2 – infrequent, 3 – common, 4 – frequent, 5 – abundant, predominant).

Water and sediment analysis

The samples of water and sediment were brought to the laboratory and stored at 4 °C. The contents of total nitrogen (TN), total phosphorus (TP) and soluble reactive phosphate (SRP) in sediment were analysed by the standard methods: ISO 11261:1995, ISO 11263:1995 and ÖNORM L 1088:2005, while TN and TP in the water were determined according to the standard methods ISO 10304-2:1995 and ISO 6878:2004.

Soil texture, sorting level and the content of organic matter were also determined in the sediment. Soil texture was determined by mechanical analysis – the sedimentation stactometer method with American classification according to Hodnik (1988). The percentages of four fractions were determined: sand (grain size > 0.2 mm), coarse silt (grain size 0.05<x<0.2 mm), fine silt (grain size 0.02<x<0.05 mm) and loam (grain size < 0.02 mm). The sorting level was calculated according to Nausch & Schlungbaum (1984). The organic matter was determined according to the method described in international standard ISO 14235.

Results

The littoral of Velenjsko jezero is relatively steep (Table 1). The geomorphology of the chosen transects was quite homogenous, as the areas of the littoral at different depth zones were similar.

Table 1: Average distance and area of the littoral from the shore to a defined depth of the lake basin measured at three sections each 200±2 m in length.

Tabela 1: Povprečna razdalja od obale do določene globine jezera in površina enometerskih globinskih pasov v treh izbranih transektih (širina enega 200±2 m).

Depth of lake basin (m)	Distance from the shore (m)	Depth zone (m)	Area of the littoral of the three sections (m^2)		
			L1	L2	L3
1	6.1±2.30	0 – 1	710	1396	1572
2	11.6±2.09	1 – 2	644	1177	1473
3	16.9±1.40	2 – 3	784	1047	1357
4	22.2±1.43	3 – 4	808	948	1368
5	27.4±1.15	4 – 5	874	959	1307
6	32.7±1.00	5 – 6	971	933	1298
7	38.1±1.00	6 – 7	1013	921	1308

We detected large seasonal changes in the presence and abundance of macrophyte species in the three chosen locations of the lake (Table 2). *Potamogeton pectinatus* L. prevailed in June and July, while in August *Najas marina* became the most abundant and colonized almost all littoral. *Potamogeton crispus* was abundantly present mainly in June, while its abundance drastically decreased during

July. *Chara* sp., *Potamogeton lucens* L., *Najas minor* All. and *Myriophyllum spicatum* were present in low abundance over the whole season.

Table 2: Average relative abundance of different macrophyte species in Velenjsko jezero in the year 2004 at the three chosen transects 200±2 m in length. Abundance: a five level descriptor scale (0 – absent, 1 – very rare, 2 – infrequent, 3 – common, 4 frequent, 5 – abundant, predominant) (Kohler 1978).

Tabela 2: Prisotnost in pogostost makrofitov v Velenjskem jezeru v treh izbranih transektih od junija do septembra 2004. Abundanca temelji na petstopenjski skali: 0 – odsotna, 1 – zelo redka, 2 – posamična, 3 – pogosta, 4 – množična, 5 – prevladujoča) (Kohler 1978).

	L1				L2				L3			
	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
Chara sp.	1	1	0	0	1	0	0	0	0	0	0	0
Myriophyllum spicatum	0	1	1	2	1	1	0	0	1	1	0	0
Najas marina	0	1	5	4	0	2	5	3	0	2	5	3
Potamogeton crispus	0	2	0	0	2	1	0	0	2	1	0	0
Potamogeton pectinatus	3	3	3	2	0	0	0	0	3	3	2	2
Potamogeton lucens	0	0	0	0	1	2	2	1	1	2	2	1
Najas minor	0	0	0	0	0	0	2	2	0	1	2	2

The mean values of physical characteristics of water observed in Velenjsko jezero throughout the season 2004 are shown in Table 3. The temperature of the water was the highest in August (23.9 °C), when the transparency of the lake was the lowest (3.1 m). In September lake water was still relatively warm (22.5 °C), but the transparency had increased (8 m). pH value was stable and the water was well aerated throughout the whole season.

Table 3: Physical characteristics of water over the period from June to September 2004 at three chosen locations (average±SD; n=3).

Tabela 3: Rezultati fizikalnih meritev in kemijskih analiz vode opravljenih enkrat mesečno od junija do septembra 2004 na izbranih transektih (povprečna vrednost ±SD; n=3).

Date 2004	Temperature (°C)	Oxygen (mg/L)	Oxygen saturation (%)	pH	Transparency (m)
08.06.	19.0±0.5	10.0±0.3	111.0±1.1	8.2±0.1	4.5
14.07.	21.0±0.1	11.7±0.5	127.4±0.9	8.1±0.1	3.7
18.08.	23.9±0.2	7.8±0.2	100.0±1.2	8.1±0.1	3.1
08.09.	22.5±0.2	7.5±0.3	93.9±0.9	8.2±0.1	8.0

The contents of TN and TP in water and sediment and SRP in sediment varied throughout the season (Table 4), at a rather high level.

Sediment parameters were variable, when comparing different sampling sites, but on average sand (83.6%) was the prevailing fraction. The sorting level (S=1.6) reflects the fact that one-grain size fractions clearly dominated (more than 50%), which is often the case in exposed areas. The level of organic matter varied from 2.5 to 13 % (Table 5).

Table 4: The contents of nutrients in sediment and water measured every month from June to September at three chosen locations (average±SD; n=3).
Table 4: Povprečna vsebnost hranil v sedimentu in vodi, merjena enkrat mesečno od junija do septembra (povprečna vrednost ±SD; n=3).

	SEDIMENT			WATER	
Date	Total N (%DW)	Total P (mg/kg)	SRP (mg/kg)	Total N (mg/l)	Total P (mg/l)
08.06.	0.067±0.05	302±169	7.14±1.51	1.23±0.01	0.06±0.02
14.07.	0.102±0.04	456±140	6.40±2.32	1.20±0.00	0.14±0.05
18.08.	0.098±0.01	456±93.2	6.75±1.89	1.83±0.58	0.17±0.02
08.09.	0.098±0.008	505±54.6	6.51±1.31	1.10±0.00	0.08±0.01

Table 5: Results of the analysis of sediment texture, sorting level and organic matter content at three chosen locations (povprečna vrednost ±SD; n=3).
Tabela 5: Tekstura in sortirna stopnja sedimenta ter vsebnost organskih snovi v sedimentu iz treh izbranih transektov (average±SD; n=3).

loam (%)	fine silt (%)	coarse silt (%)	sand (%)	sorting level	organic matter (%)
5.42±5.80	7.06±5.42	3.88±1.58	83.6±11.5	1.62±0.29	5.8±4.7

Discussion

Many authors reported *Myriophyllum spicatum* as outcompeting *Najas marina* in different lake ecosystems (Agami & Waisel 1986, Simons & al. 1994, Bootsma & al. 1999, Ali & Soltan 2006). In Velenjsko jezero the situation is the opposite (Tab. 2). Prior to 1997, when *Najas marina* appeared in the lake, *Myriophyllum spicatum* and *Potamogeton crispus* were the most abundant species (Mazej 1998, Mazej & Gaberščik 1999, Mazej & Epšek 2005). After 1997 *Najas marina* had spread rapidly within a few years, forming weed beds that covered larger areas. Abundance of *Myriophyllum spicatum* and *Potamogeton crispus* declined even in spring (Mazej & Epšek 2005), when *Najas marina* is not competitive, since it is a summer-annual plant, starting its life cycle in July. *Potamogeton crispus* and *Myriophyllum spicatum* show different life cycles in comparison to *Najas marina*. They overwinter, and then grow rapidly in the spring. By early summer the plants undergo senescence processes and then remain dormant until autumn (Bolduan & al. 1994). The biomass production of *Potamogeton crispus* often reaches its maximum in early summer, avoiding competition with other species (e.g. with *Najas marina*) in the same habitat, which begins their growth later in the season (Tobiessen & Snow 1984). In contrast to the other species in the lake, *Najas marina* propagates from seeds (Agami & Weisel 1986) that enable quick colonization of new habitats.

Optimum germination temperatures of *Najas marina* are between 20 and 25 °C (Proctor 1967). That might be a reason why the species is rare throughout Europe (Handley & al. 2002). It begins its growth in July, when nutrients in lakes are usually impoverished and the littoral is fully overgrown with other macrophytes. This is not the case in Velenjsko jezero. In July soft sediment littoral is still not colonized and the amounts of nutrients in the water and sediment do not decrease before September (Tabs. 2, 4). Velenjsko jezero could be classified as eutrophic according to the level of total phosphorus (0.11 mg/L) and total nitrogen (1.34 mg/L) in the water (OECD 1982). *Najas marina* is reported to thrive in oligotrophic and mesotrophic shallow freshwater habitats (TP in sediment <50 $\mu g\ g^{-1}$ dw) and was considered to be nearly extinct due to hypereutrophication (Selig & al. 2007). For

example, Simons & al. (1994) beside the decline of charophytes, described the decline of *Najas marina* stands in a shallow peat lake (Botshol, The Netherlands) due to nutrient input. The same effect was observed in the research of Bootsma & al. (1999) in the shallow lakes of Naardemeer nature reserve (The Netherlands). *Najas marina* disappeared from the lakes when the concentration of total P in the water was 0.1 mg/l and it reappeared when the concentration of total phosphorus had dropped to 0.05 mg/l. Our results cannot confirm these findings as the sediment (mean TP in sediment is 329 $\mu g\ g^{-1}$ dw) and water (mean TP in water is 0.11 mg/l) of Velenjsko jezero are very rich in phosphorus. However, Velenjsko jezero, in contrast to Botshol and the lakes in Naardemeer, is a deep lake (max depth is 54.6 m) with steep slopes along greater parts of the lakeshore (Tab. 1). Its transparency (mean Secchi depth was 4.8 m) is much better than the transparency in the lakes of Naardmeer (from 0.3 to 1.05 m). Stelzer & al. (2005) claim, that *Najas marina* is a taxon with a high preference for good ecological status in a case of German lakes. Selig & al. (2007) found *Najas marina* stands in eutrophic waters, even though TP was comparatively low.

The littoral of Velenjsko jezero is decisive for low sediment stability. Therefore species, which develop extensive root systems, were expected to be more successful. Unstable sediment that prevails in Velenjsko jezero prevented spreading of *Myriophyllum spicatum* and *Potamogeton crispus*. *Myriophyllum spicatum* is reported to have difficulty in becoming rooted in a soft and moving bottom (Nichols & Shaw 1986). *Najas marina* with a branching growth form (Handley & Davy 2000) forms dense beds and develops very extensive root systems (Handley & Davy 2002). Roots can account for more than 30% of plant biomass compared to the values of 10% or less for the majority of submersed aquatic plants (Agami &Weisel 1986). The sorting level and sediment fraction type reflected other abiotic parameters such as wave exposure and water current. Selig & al. (2007) found that *Najas marina* stands prefer sediment with a higher sorting level in the range similar to the sediment of Velenjsko jezero (S =1.6) (Tab. 5). This level describes open sandy areas with little sedimentation. *Myriophyllum spicatum* and *Potamogeton pectinatus* can be found in sediments with a wide range of sorting level but with the lower median (1.3) (Selig & al. 2007). It is known from literature that *Myriophyllum spicatum* (and also *Potamogeton crispus*) grow best on fine sediment with a relatively high organic matter concentration, e.g. 10-25% (Nichols & Shaw 1986) and 9-13% (Van Wijck & al. 1994), which is higher than the concentration in the sediment of Velenjsko jezero.

Conclusion

This investigation revealed that species composition in a particular lake depends on plant strategy and on the physico-chemical and geomorphological characteristics of the lake. The same species cannot be equally successful in habitats with differing physico-chemical and geomorphological characteristics. Favourable circumstances in the lake Velenjsko jezero like warm water, unstable sediment and life strategy of *Najas marina* as a summer-annual plant with short life cycle, rapid propagation by seed and a very extensive root system, enable this species to grow successfully and dominate over other macrophytes in the lake.

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Prevalence, distribution and genetic association of adhesin gene sequences of *Escherichia coli* isolates from urinary tract infections in Slovenia

Prevalenca, porazdelitev in genetska asociacija zapisov za adhezine v izolatih bakterije *Escherichia coli* iz okužb sečil v Sloveniji

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Abstract. 110 uropathogenic *Escherichia coli* (UPEC) strains obtained from the Institute of Microbiology and Immunology of the Medical Faculty in Ljubljana, Slovenia were screened with molecular biology methods for the well characterized adhesin gene sequences: *fimH* (Type 1 fimbriae), *papC*, *papGII* and *papGIII* (P-fimbriae), *sfa* (S-fimbriae) and *afa/dra* (Afa/Dr adhesins). The *fimH* gene nucleotide sequences were detected in 97% of the isolates, *papC* in 49%, *papGII* in 34%, *papGIII* in 13%, *sfa/foc* in 24% and *afa/dra* sequences were harbored by 2% of the tested isolates. *FimH* sequences were found with similar prevalence in *E. coli* strains of all four phylogenetic groups A, B1, B2 and D. *papC* sequences were also found in all phylogenetic groups, but they were the most prevalent (64%) in the B2 group. The *papGII* showed the highest prevalence in the D group (48%), but *papGIII* adhesin sequences were exclusively found in the B2 group. A very high prevalence of S-fimbriae in the B2 group was detected. The analysis of co-associations of adhesin gene sequences and some other traits revealed that *papC* gene sequences were co-associated with P-fimbriae adhesin gene sequences *papGII* and *papGIII* and with S-fimbriae *sfa/foc* sequences. A negative association was found between *papGIII* and *traT* and between *papGIII* and RepFIB sequence. Interestingly, a negative association was also visible between integrons and P- and S-fimbriae, albeit the association was not statistically significant.

Key words. uropathogenic *Escherichia coli*, UPEC, adhesin, fimbriae

Izvleček. 110 uropatogenih sevov *Escherichia coli*, ki so jih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani osamili iz diagnostičnih vzorcev urina, smo z molekularnobiološkimi metodami analizirali z namenom določiti gene, ki imajo zapise za adhezine: *fimH* (fimbrije tipa 1), *papC*, *papGII* in *papGIII* (fimbrije P), *sfa* (fimbrije S) in *afa/dra* (adhezini Afa/Dr). Nukleotidno zaporedje gena *fimH* smo odkrili v 97 % izolatov, *papC* v 49 %, *papGII* v 34 %, *papGIII* v 13 %, *sfa/foc* v 24% in zaporedja *afa/dra* smo našli v 2 % vseh preučevanih izolatih. Prevalenca zaporedja *fimH* po posameznih filogenetskih skupinah A, B1, B2 in D je primerljiva. V vseh štirih filogenetskih skupinah smo našli tudi zaporedja *papC*, največja prevalenca je bila v skupini B2, kjer je kar 64 % izolatov vsebovalo to zaporedje. Zaporedje *papGII* je imelo največjo prevalenco v skupini D. Zaporedje *papGIII* smo našli izključno v skupini B2. V skupini B2 smo odkrili tudi veliko prevalenco fimbrij S (45 %). Analiza asociacij zapisov za adhezine z drugimi zapisi je pokazala, da je zaporedje *papC* asociirano z zaporedji *papGII* in *papGIII* ter zaporedji *sfa/foc*. Negativno povezavo smo našli med *papGIII* in *traT* ter med *papGIII* in zaporedji RepFIB. Med integroni in fimbrijami P in S smo tudi našli negativno povezavo, a le ta ni bila statistično značilna.

Ključne besede. uropatogena *Escherichia coli*, UPEC, adhezin, fimbrija

Introduction

Escherichia coli (*E. coli*) is a very diverse bacterial species found naturally in the intestinal tract of all humans and many other animal species. Even though *E. coli* is known to be part of the normal gut flora, some strains – that are pathogenic – cause a wide variety of different intestinal and extraintestinal diseases (Marrs & al. 2005). Typical extraintestinal infections due to *E. coli* include urinary tract infections (UTI) (Russo & Johnson 2006).

Any component of a microbe that is required for, or potentiates its ability to cause disease is called a virulence factor. The best known virulence factors are adhesins, toxins, polysaccharide coatings, invasins and iron uptake systems. Among the first virulence factors that come into play during establishment of an infection are adhesins. Besides their primary role as adhesin molecules, they can also function as invasins, promoters of biofilm formation and transmitors of signals to epithelial cells resulting in inflammation.

Type 1 fimbriae are the most common adhesive organelles of *E. coli* strains. They are encoded by the vast majority of uropathogenic *E. coli* (UPEC) isolates and many other pathogenic and commensal isolates (Bower & al. 2005). They are also found in other bacteria, such as *Salmonella typhimurium*, *Pseudomonas putida* and *Klebsiella pneumoniae* (Capitani & al. 2006). Receptors for type 1 fimbriae are present on erythrocytes, buccal epithelial cells, intestinal cells, vaginal cells and uroepithelial cells (Johnson 1991). The *fimH* gene encodes the minor subunit protein FimH that mediates binding to the receptor. FimH has several variants: UPEC strains have a FimH that binds both monomannose and trimannose containing gycoprotein receptors, while commensal *E. coli* isolates typically show high affinity binding to only trimannose residues (Bower & al. 2005). Type 1 fimbriae function not just as adhesins, but also as invasins for bladder epithelial cells (Martinez & al. 2000).

P fimbriae are among the best studied fimbrial adhesive fibres of UPEC strains. The P fimbrial adhesin molecule (PapG) recognizes globoseries of glycolipids as receptors (Zhang & Foxman 2003). The *papC* gene encodes the outer membrane usher protein that is required for ordered P fimbriae assembly (Thanassi & al. 1998). Many studies showed that P fimbriae occur more frequently among UPEC than fecal isolates. It was estimated, that about 80% of *E. coli* isolates from patients with pyelonephritis possess P-fimbriae. Based on binding specificities, P fimbriae are grouped into three major classes (I, II and III); class II (*papGII*) is more often found in pyelonephritic strains and class III (*papGIII*) in cystitis strains (Zhang & Foxman 2003).

S fimbriae bind to sialyl galactosides. Studies showed that *E. coli* UTI isolates were at least two times more likely to carry S fimbriae genes (*sfa* operon) than fecal strains (Zhang & Foxman 2003).

The 13 known adhesins of the Afa/Dr family all bind to the Dr^a blood group antigen present on the complement regulatory molecule CD55, also known as decay-accelerating factor (DAF) (Bower & al. 2005). The *E. coli* strains harboring these adhesins have been found to be associated with UTIs and also with various enteric infections (Servin 2005).

The subunit proteins of adhesins are seriously considered as possible vaccines against *E. coli* infections (Oelschlaeger & al. 2002). Since UTIs and other extraintestinal infections due to *E. coli* cause considerable costs to the health system vaccines against *E. coli* are searched for (Russo & Johnson 2006). To evaluate the potential of different adhesins as vaccines it is necessary to investigate the presence of individual adhesins among pathogenic strains. To our knowledge, no such data are available for Slovenian uropathogenic *E. coli* (UPEC) strains. We therefore, analyzed a collection of 110 UPEC strains, that were previously screened for antibiotic resistance and horizontal gene transfer elements (Rijavec & al. 2006), for the presence of the following adhesin gene sequences: *fimH* (Type 1 fimbriae), *papC*, *papGII* and *papGIII* (P fimbriae), *sfa* (S-fimbriae) and *afa/dra* (afimbrial adhesin).

Table 1: Oligonucleotide primers and PCR conditions to detect adhesin genes
Tabela 1: Oligonukleotidni začetniki in pogoji PCR za ugotavljanje genskih zapisov za adehezine

Gene	Oligonucleotide sequence (5' to 3')	Size of product (bp)	PCR conditions			Reference
fimH	tgcagaacggataagccgtgg gcagtcacctgccctccggta	508	95°C 94°C 60°C 72°C 72°C	2,5 min 0,5 min 0,5 min 1 min 10 min	1× 30× 1×	Johnson & Stell, 2000
papC	gacggctgtactgcagggtgtggcg atatccttttctgcagggatgcaata	328	94°C 94°C 65°C 72°C 72°C	3 min 2 min 1 min 2 min 10 min	1× 25× 1×	Le Bouguenec & al., 1992
papGII	gggatgagcgggcctttgat cgggccccaagtaactcg	190	95°C 94°C 55°C 72°C 72°C	2,5 min 0,5 min 1 min 0,5 min 7 min	1× 25× 1×	Johnson & Brown, 1996
papGIII	ggcctgcaatggatttacctgg ccaccaaatgaccatgccagac	258	94°C 94°C 63°C 72°C 72°C	2,5 min 0,5 min 0,5 min 3 min 10 min	1× 25× 1×	Johnson & Brown, 1996
sfa/foc	ctccggagaactgggtgcatcttac cggaggagtaattacaaacctggca	410	94°C 94°C 65°C 72°C 72°C	3 min 2 min 1 min 2 min 10 min	1× 25× 1×	Le Bouguenec & al., 1992
afa/dra	gctgggcagcaaactgataactctc catcaagctgtttgttcgtccgccg	750	94°C 94°C 65°C 72°C 72°C	3 min 2 min 1 min 2 min 10 min	1× 25× 1×	Le Bouguenec & al., 1992

Material and methods

Bacterial strains and media

A total of 110 *E. coli* isolates (DL strains) from humans with urinary tract infections collected in 2002, at the Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia were studied. Only one isolate from each patient was analyzed. Ninety-four (86%) of the patients were women. The strains had already been examined for prevalence of antibiotic resistances further, the serotype and phylogenetic groups were assigned and traits typical of horizontal gene transfer (*traT*, integrons, *rep*) were searched for (Rijavec & al. 2006). For cultivation of strains Luria Bertani medium or agar were used.

Detection of adhesin genes

The primers and PCR conditions used to amplify adhesin genes with polymerase chain reaction (PCR) are listed in Table 1. DNA to be amplified was released from whole organisms by boiling according to Le Bouguenec et al. (Le Bouguenec & al. 1992). Amplification was performed in an automated thermal cycler (UNOII, Biometra, Göttingen, Germany) in a 50 µl reaction mixture containing template DNA (10 µl of boiled lysate), 20 pmol of forward and reverse primer, 0,2 mM of dNTP mixture, 1,25 U *Taq* DNA polymerase and 2,5 mM $MgCl_2$ in 1× PCR buffer (Fermentas, Vilnius, Lithuania).

Dot blot hybridization experiments using the DIG DNA labelling and detection kit (Roche, Mannheim, Germany) were performed to validate the PCR assays. Probes were prepared using the same primers as for the PCR experiments and labelled with digoxigenin. The template DNA samples were the same as in the PCR experiments.

Statistical analysis

The significance of the results was established using the Fisher's exact test (2-tailed) available on-line on the web site http://www.matforsk.no/ola/fisher.htm and the level of significance was set at a P value < 0.05.

Results

Prevalence of adhesin genes

The presence of adhesin genes in the genomes of DL strains was screened by PCR and validated in the hybridization experiments. Figure 1 gives an example for *papGIII* detection. Among the tested adhesive organelles, the type 1 fimbriae were the most prevalent – the *fimH* gene nucleotide sequences were detected in 107 strains (97%). The P-fimbriae were also abundant, in 54 strains (49%) *papC* encoding gene sequence was found, 37 strains (34%) harbored the class II *papG* adhesin sequence and 14 strains (13%) harbored the class III *papG* adhesin. 26 (24%) possessed the S fimbriae typical gene sequence *sfa/foc*. Only 2 strains (2%) harbored *afa/dra* sequences (Figure 2).

Distribution of adhesin genes among phylogenetic groups

E. coli isolates can be divided into four main phylogenetic groups A, B1, B2 and D (Herzer & al. 1990). Analysis of the distribution of adhesin gene sequences among the previously determined phylogenetic groups of DL strains (Rijavec & al. 2006) revealed that different adhesin gene sequences were differently distributed (Table 2). *FimH* sequences were found with similar prevalence in strains of all four phylogenetic groups, *papC* sequences were found in all phylogenetic groups, but they were most prevalent (64%) among B2 group strains. The association of *papC* with the B2 group was statistically significant. The distribution of the P-fimbriae adhesins *papGII* and *papGIII*, however, differed. *papGII* sequences showed the highest prevalence in the D group (48%), albeit the association was not statistically significant. In contrast, *papGIII* adhesin sequences were exclusively found among strains of the B2 group. Further, a very high, statistically significant, prevalence of S-fimbriae in the B2 group was detected.

Co-associations of adhesin genes

The analysis of co-associations of adhesin gene sequences and some other traits revealed (Table 3) that the P-fimbriae usher *papC* gene sequences were 100% co-associated with P-fimbriae adhesin

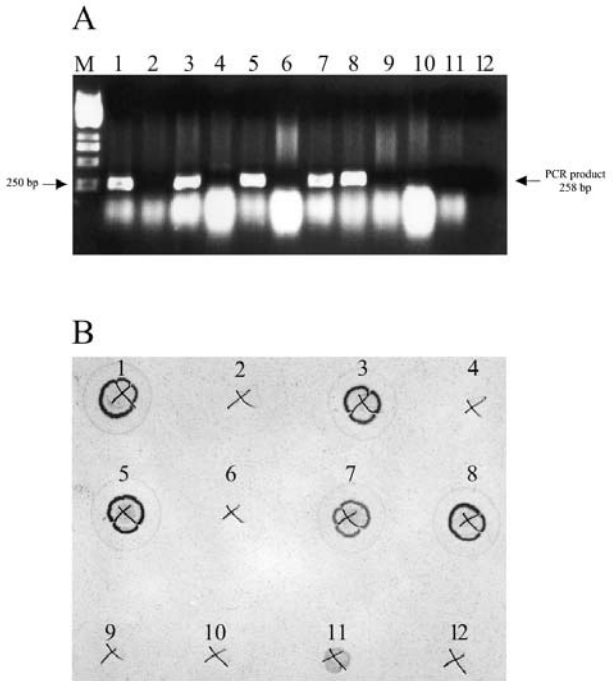

Figure 1: An example of detection of adhesin genes – detection of the *papGIII* gene

(A) Visualization of PCR products obtained in PCR reactions on lysates of DL strains with primers specific for the *papGIII* gene (1% agarose gel, stained with ethidium bromide).

M: marker – 1 kb DNA ladder (Fermentas, Vilnius, Lithuania); 1: strain DL1 (*papGIII*+); 2: strain DL9 (*papGIII*-); 3: strain DL12 (*papGIII*+); 4: strain DL23 (*papGIII*-); 5: strain DL30 (*papGIII*+); 6: strain DL49 (*papGIII*-); 7: strain DL59 (*papGIII*+); 8: strain DL62 (*papGIII*+); 9: strain DL89 (*papGIII*-); 10: strain DL90 (*papGIII*-); 11: laboratory strain DH5α (*papGIII*-) and 12: negative control – a PCR reaction with sterile water instead of a lysate.

(B) Validation of the PCR assay with DIG hybridization of a *papGIII* specific probe on *papGIII* PCR products (10 µl) bound to a nylone membrane.

1: strain DL1 (*papGIII*+); 2: strain DL9 (*papGIII*-); 3: strain DL12 (*papGIII*+); 4: strain DL23 (*papGIII*-); 5: strain DL30 (*papGIII*+); 6: strain DL 49 (*papGIII*-); 7: strain DL59 (*papGIII*+); 8: strain DL62 (*papGIII*+); 9: strain DL89 (*papGIII*-); 10: strain DL90 (*papGIII*-); 11: laboratory strain DH5α (*papGIII*-) and 12: negative control – a PCR reaction with sterile water instead of a lysate.

Slika 1: Primer detekcije genskega zapisa za adhezin – detekcija gena *papGIII*

(A) Vizualizacija produktov PCR dobljenih v reakcijah PCR na lizatih sevov DL z začetnimi oligonukleotidi specifičnimi za gen *papGIII* (1 % agarozni gel, obarvan z etidijevim bromidom).

M: standard – 1 kb DNA-lestvica (Fermentas, Vilnius, Litva); 1: sev DL1 (*papGIII*+); 2: sev DL9 (*papGIII*-); 3: sev DL12 (*papGIII*+); 4: sev DL23 (*papGIII*-); 5: sev DL30 (*papGIII*+); 6: sev DL49 (*papGIII*-); 7: sev DL59 (*papGIII*+); 8: sev DL62 (*papGIII*+); 9: sev DL89 (*papGIII*-); 10: sev DL90 (*papGIII*-); 11: laboratorijski sev DH5α (*papGIII*-) in 12: negativna kontrola – reakcija PCR s sterilno vodo namesto lizata.

(B) Preverjanje PCR z DIG-hibridizacijo z vezavo sonde specifične za *papGIII* na produkte *papGIII* iz PCR (10 µl) vezane na nylonski membrani.

1: sev DL1 (*papGIII*+); 2: sev DL9 (*papGIII*-); 3: sev DL12 (*papGIII*+); 4: sev DL23 (*papGIII*-); 5: sev DL30 (*papGIII*+); 6: sev DL 49 (*papGIII*-); 7: sev DL59 (*papGIII*+); 8: sev DL62 (*papGIII*+); 9: sev DL89 (*papGIII*-); 10: sev DL90 (*papGIII*-); 11: laboratorijski sev DH5α (*papGIII*-) in 12: negativna kontrola – reakcija PCR s sterilno vodo namesto lizata.

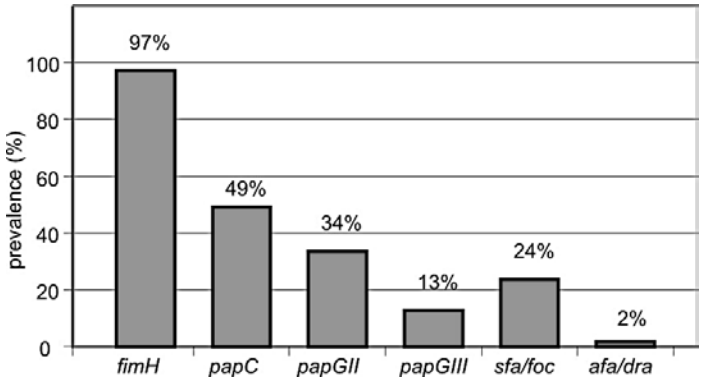

Figure 2: Prevalence (in % of the total 110 DL strains) of adhesin gene sequences
Slika 2: Prevalenca (v % od 110 preučevanih sevov E. coli) genskih zapisov za adhezine

gene sequences *papGII* and *papGIII*. Further, *papC* gene sequences were also statistically significantly co-associated with S-fimbriae *sfa/foc* sequence. The P-fimbriae adhesin gene sequences *papGIII*, but not *papGII*, were statistically significantly co-associated with S-fimbriae *sfa/foc* sequence. A statistically significant negative association was found between *papGIII* and *traT* and between *papGIII* and RepFIB sequences. Interestingly, a negative association was also visible between integrons and P- and S-fimbriae, albeit the association was not statistically significant.

Co-associations of adhesin genes (Table 3) *fimH* and *afa/dra* were not analyzed, due to either very high or low prevalence, respectively.

Discussion

In the presented study 110 UTI *E. coli* strains isolated in Ljubljana, Slovenia, were characterized using PCR with primers specific for adhesin genes: *fimH*, *papC*, *papGII*, *papGIII*, *sfa/foc* and *afa/dra*.

Among the tested adhesin gene sequences the prevalence of *fimH* gene sequences was the highest, almost 100%. The high prevalence of *fimH* sequences found in our study assures a good possibility

Table 2: Distribution of adhesin gene sequences among *E. coli* phylogenetic groups
Tabela 2: Razporeditev genskih zapisov za adhezine po filogenetskih skupinah *E. coli*

	Prevalence of trait (no. [%] of isolates) within phylogenetic group[a]			
Toxin gene	A (n = 28)	B1 (n = 6)	B2 (n = 55)	D (n = 21)
fimH	28 (100)	5 (83)	53 (96)	21 (100)
papC	8 (29)$^{(*)}$	1 (17)	35 (64)**	10 (48)
papGII	5 (18)	1 (17)	21 (38)	10 (48)
papGIII	0 (0)$^{(*)}$	0 (0)	14 (25)***	0 (0)
sfa/foc	1 (4)$^{(**)}$	0 (0)	25 (45)***	0 (0)$^{(**)}$
afa/dra	1 (4)	0 (0)	1 (2)	0 (0)

[a]*P* values (Fisher's exact test) are indicated by asterisks where *P* is <0,05. Symbols: *, *P*<0,05; **, *P*≤0,01; ***, *P*≤0,001. Parentheses indicate negative associations.

[a] vrednost *P* (Fisherjev eksaktni test) <0,05 je nakazana z zvezdicami: simboli *, *P*<0,05; **, *P*≤0,01; ***, *P*≤0,001. Negativne povezave so označene z oklepajem.

Table 3: Co-association of tested adhesin genes and some other traits
Tabela 3: Vezanost genskih zapisov za adhezine in nekaterih drugih lastnosti

	Prevalence of trait (no. [%] of isolates)[a]																			
	C+		*C-*		*P*	*GII+*		*GII-*		*P*	*GIII+*		*GIII-*		*P*	*sfa/foc+*		*sfa/foc-*		*P*
Trait	n = 54		n = 56			n = 37		n = 73			n = 14		n = 96			n = 26		n = 84		
PapC																				
papGIII	37	(69)	0	(0)	<0,001															
papGIII	14	(26)	0	(0)	<0,001	3	(8)	11	(15)	NS[b]										
sfa/foc	23	(43)	3	(5)	<0,001	12	(32)	14	(19)	NS	11	(79)	15	(16)	<0,001					
TraT	26	(48)	37	(66)	0,082-NS	20	(54)	43	(59)	NS	4	(29)	59	(61)	0,040	11	(42)	52	(62)	NS
integron	14	(26)	20	(36)	NS	7	(19)	27	(37)	0,080-NS	2	(14)	32	(33)	NS	4	(15)	30	(36)	0,060-NS
RepFIA	12	(22)	8	(14)	NS	7	(19)	13	(18)	NS	2	(14)	18	(19)	NS	6	(23)	14	(17)	NS
RepFIB	27	(50)	30	(54)	NS	21	(57)	36	(49)	NS	3	(21)	54	(56)	0,020	10	(38)	47	(56)	NS
RepFIIA	13	(24)	11	(20)	NS	9	(24)	15	(21)	NS	1	(7)	23	(24)	NS	4	(15)	20	(24)	NS

[a] In the table are not included *fimH* and *afa/dra* due to their too high or too low prevalence, 97% and 2%, respectively. Included are data for *traT*, integrons and replication regions RepFIA, RepFIB, RepFIIA (Rijavec & al. 2006).
[b] NS – not statistically significant

[a] V tabelo niso vključeni *fimH* in *afa/dra*, ker imata previsoko oz. prenizko prevalence (*fimH* = 97% in *afa/dra* = 2%). Vključeni so podatki za *traT*, integrone in replikacijske regije RepFIA, RepFIB, RepFIIA (Rijavec & al. 2006).
[b] NS – ni statistično značilno

Table 4: Comparison of results from different studies of UTI adhesin genes
Tabela 4: Primerjava rezultatov različnih raziskav genskih zapisov za adhezine

	Adhesin gene prevalence (%)						
Study	*fimH*	*papC/A*	*papGII*	*papGIII*	*sfa/foc*	*afa/dra*	Ref.
76 pyelonephritis strains (Japan)	na	78	na	na	42	12	Kanamaru & al. 2003, Yamamoto & al. 2001
74 cystitis strains (USA)	na	35	5	31	36	4	Johnson & al. 2001
170 pyelonephritis strains (USA)	99	68	60	9	na	17	Johnson & al. 2005b
194 cystitis strains (Japan)	na	64	na	na	37	9	Kanamaru & al. 2003, Yamamoto & al. 2001
100 cystitis strains (Israel)	na	46	31	17	37	14	Johnson & al. 2005a
78 UTI strains (Romunia)	86	36	na	na	23	14	Usein & al. 2001
110 UTI strains (Slovenia)	97	49	34	13	24	2	this study

for prevention of infection with the vaccine against the type 1 fimbriae that is already in phase II/III trial in theUS (Russo & Johnson 2006).

The P-fimbriae *papC* sequences were found in 49% of the tested DL strains. Comparison of our data with data on prevalences of *papC* or *papA* (encoding major fimbrial subunit PapA) from other studies (Table 4) showed a similar prevalence among UTI strains from Romania and cystitis strains from Israel and USA, but higher prevalence of *papC/A* among the pyelonephritis strains from USA and cystitis and pyelonephritis strains from Japan, compared to the prevalence in DL strains investigated in this study.

The *papC* sequences were 100% co-associated with the adhesin genes *papGII* and *papGIII*. This was expected, since *papGII* and *papGIII* are alleles of P-fimbriae adhesins and each strain harboring either *papGII* or *papGIII* sequences also harbors *papC* sequences.

Interestingly, in our study, as well as, in the study of Johnson *et al*. (Johnson & al. 2005b), *papGII* exhibited the highest prevalence among strains of the D group. This is in contrast to the results of *papC* and *papGIII* for which the highest prevalence was found among strains belonging to the B2 group, which is known to exhibit the highest prevalence of virulence traits.

Further, *papC* sequences were also strongly co-associated with *sfa/foc* sequences. This is surprising, as to our knowledge no previous study reported such a correlation. Co-association of virulence factors are expected, when they are physically joined, and it is well known that uropathogenic strains carry large chromosomal regions, termed pathogenicity islands (PAI) that encode several virulence factors. A number of PAIs have been identified in uropathogenic strains (Oelschlaeger & al. 2002), however to our knowledge no PAI harboring S-fimbriae and P-fimbriae has ever been described.

A negative association of P and S fimbriae with integrons is, even though not statistically significant, evident. Integrons are known to carry resistance genes for different/multiple antibiotics (Mazel 2006). Further, it is well known, that antibiotic-sensitive isolates possess more virulence factors than antibiotic-resistant isolates (Johnson & al. 2003, Starčič Erjavec & al. 2007). Therefore, it is reasonable that in strains with virulence associated adhesins, the prevalence of integrons is smaller.

It is worth to be noted, that not all studies (Table 4) support the assumption, that the *papGIII* allele is associated with cystitis isolates and *papGII* with pyelonephritis (Zhang & Foxman 2003). For example the study on Israelian cystitis isolates showed, that cystitis isolates have a higher prevalence of the *papGII* allele than the *papGIII* allele.

Further studies on different cystitis/pyelonephritis isolates are needed to clarify the importance of different *papG* alleles and to clarify the basis of the correlation between P and S fimbriae.

Conclusions

To summarise and conclude:

1. 110 uropathogenic *Escherichia coli* (UPEC) strains were screened with molecular biology methods for the well characterized adhesin gene sequences: *fimH* (Type 1 fimbriae), *papC*, *papGII* and *papGIII* (P-fimbriae), *sfa* (S-fimbriae) and *afa/dra* (Afa/Dr adhesins);
2. the prevalence, the distribution and the genetic associations of the tested adhesin gene sequences were determined;
3. the *fimH* gene nucleotide sequences were detected in 97% of the isolates, *papC* in 49%, *papGII* in 34%, *papGIII* in 13%, *sfa/foc* in 24% and *afa/dra* sequences were harbored by 2% of the tested isolates;
4. *fimH*, *papC*, *papGII* were found in all four *E. coli* phylogenetic groups, *sfa/foc* and *afa/dra* in A and B2 group and the *papGIII* was found only in the B2 group;
5. *papC* gene sequences were co-associated with P-fimbriae adhesin gene sequences *papGII* and *papGIII* and with S-fimbriae *sfa/foc* sequence.

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Povzetek

Bakterija *Escherichia coli* (*E. coli*) je del normalne flore prebavila človeka in toplokrvnih živali. A obstajajo sevi *E. coli*, ki imajo virulentne dejavnike (toksine, adhezine, kapsule, ...) in lahko povzročijo okužbe (driska, vnetje sečil, pljučnica, vnetje možganskih ovojnic, okužbe ran, ...). Okužba sečil je ena izmed najpogostejših bakterijskih infekcij in *E. coli* povzroča veliko večino teh okužb. Zaradi pogostnosti pojavljanja teh okužb so virulentni dejavniki sevov *E. coli*, ki povzročajo te okužbe (UPEC – uropatogena *E. coli*) za preučevanje zelo zanimivi. Kar nekaj adhezinov in fimbrij (fimbrije tipa 1, P-fimbrije, S-fimbrije, Afa/Dr-adheizini) povezujejo s patogenimi sevi UPEC. 110 uropatogenih sevov *Escherichia coli*, ki so jih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani osamili iz diagnostičnih vzorcev urina, smo z molekularnobiološkimi metodami analizirali z namenom določiti gene, ki imajo zapise za adhezine: *fimH* (fimbrije tipa 1), *papC*, *papGII* in *papGIII* (fimbrije P), *sfa* (fimbrije S) in *afa/dra* (adhezini Afa/Dr). Nukleotidno zaporedje gena *fimH* smo odkrili v 97 % izolatov, *papC* v 49 %, *papGII* v 34 %, *papGIII* v 13%, *sfa/foc* v 24% in zaporedja *afa/dra* smo našli v 2 % vseh preučevanih izolatih. Prevalenca zaporedja *fimH* po posameznih filogenetskih skupinah A, B1, B2 in D je primerljiva – je več kot 80 %. V vseh štirih filogenetskih skupinah smo našli tudi zaporedja *papC*, največ v skupini B2 (64 % izolatov). Zaporedje *papGII* je imelo največjo prevalenco v skupini D (48 %). Zaporedje *papGIII* smo našli izključno v skupini B2 (25 %). V skupini B2 smo odkrili tudi veliko prevalenco fimbrij S (45 %). Analiza asociacij zapisov za adhezine z drugimi zapisi je pokazala, da je zaporedje *papC* asociirano z zaporedji *papGII* in *papGIII* ter zaporedji *sfa/foc*. Negativno povezavo smo našli med *papGIII* in *traT* ter med *papGIII* in zaporedji RepFIB. Med integroni in fimbrijami P in S smo tudi našli negativno povezavo, a le ta ni bila statistično značilna. Zbrane informacije o pogostnosti zapisov za adhezine bi lahko bile osnova za načrtovanje cepiv proti patogenim sevom *E. coli*.

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How are Age and Gender Related to Attitude Toward Plants and Animals?

Kako sta starost in spol povezana z odnosom do rastlin in živali?

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Abstract. The article examines attitude towards plants and animals in a population of 210 pupils aged 9 to 18. The results support the proposition that animals are generally more interesting to pupils than plants. Their interest was strongly influenced by age, while gender-based difference was only one.

Key words: plants, animals, age, gender, attitudes, plant blindenss

Izvleček. Prispevek obravnava odnos 210 učencev, starih 9 do 18 let, do rastlin in živali. Rezultati podpirajo predpostavko, da se zdijo živali učencem v splošnem zanimivejše od rastlin. Na zanimanje učencev je močno vplivala njihova starost, medtem ko smo razliko med spoloma našli eno samo.

Ključne besede: rastline, živali, starost, spol, odnos, slepota za rastline

Introduction

We live in an "anthropocentric culture which assigns utilitarian value to other life forms" (Schneecloth 1989). Nevertheless, organisms provide a good starting point for grounding a student's interests in biology. At the same time direct contact with various living beings provides information and experiences that are not obtainable by reading, viewing static or moving pictures, or examining a model. Work with living, authentic materials is therefore a necessity in biology education.

People's attitude towards other living beings was always a matter of interest; many researches have studied it and written about it. In this context they also examined attitudes towards animals versus plants. Namely, research showed that in general people find animals more interesting than plants (Wandersee 1986, Kinchin 1999). In 1998 Wandersee and Schussler even introduced the term plant blindness (Wandersee & Schussler 1999), defined as the inability to see or notice the plants in one's environment, the inability to recognize the importance of plants, the inability to appreciate their aesthetic and biological features, and the anthropocentric ranking of plants as inferior to animals.

Earlier attempts to explain this attitude toward plants in comparison to animals included different aspects. Hershey (1992) quoted a number of articles showing that plants were rarely used in biology education. The authors gave five reasons for this situation: (a) the majority of people feel that animals are superior to plants; (b) teachers find plants less interesting than animals; (c) teachers are better prepared to teach about animals than plants; (d) textbooks contain less information on plants than animals; and (e) pupils prefer animals to plants. According to Hershey (1993) this was a case of a chain reaction: since biology teachers received little botany education, they themselves excluded plants from lessons to a great extent.

Various authors (Tunnicliffe & Reiss 2000, Wandersee & Schussler 2001) found that children in many countries seemed to be primarily animal-socialized because the majority of children's cartoon characters, books, shaped confectionary, stuffed toys, team mascots, songs, or games pay homage to animals and not to plants or other life forms.

Based on the literature and their own research Wandersee & Schussler (2001) came to the conclusion that all the previously stated reasons for neglecting plants in comparison to animals might be only secondary, a consequence of "the way that humans perceive plants-due to the inherent constraints of their visual information processing systems". They found it better to rely on the principles of human visual perception and visual cognition when looking for reasons for plant blindness. Schneecloth (1989) came to a similar conclusion. According to her it was the pervasiveness of the experience of vegetation that structured its position in human perception as a background. For that reason we do not notice plants but what differs from them, for instance the clearing in the forest or the edge of the woods.

Research established the existence of gender- and age- related differences concerning an interest in learning about animals and plants (Baird & al. 1984, Wandersee & Schussler 1986).

The literature therefore suggests that people hold different attitudes towards animals versus plants and that gender and age affect interest in individual objects. We therefore proposed to test the following three hypotheses:

- Pupils are more interested in animals than in plants.
- There are no differences between the genders regarding interest in animals and plants.
- There are no differences between age groups regarding interest in animals and plants.
- The above mentioned studies investigated gender- and age- related differences concerning interest in learning about animals or plants, while we proposed to test the attitude toward specific living beings.

Material and methods

In the experiment 210 pupils of three age groups took part:

- 67 pupils aged from 9 to 10 (4^{th} grade of elementary school),
- 86 pupils aged from 13 to 14 (8^{th} grade of elementary school),
- 57 pupils aged from 17 to 18 (grammar/high school).

All the schools in the project were urban schools. The students attending them came from different social backgrounds but the chi-square test among groups of the same age did not show statistically significant differences ($p < .05$). In the sample there were slightly more girls (54.8%) than boys (45.2%). Elementary school and grammar school were chosen because they are attended by the most general population which is least interest oriented. It is true that pupils in grammar school finished elementary school with better grades, but in our opinion this fact did not impact the results of our research which only focused on the attitude toward living beings, not on knowledge about them.

Twenty objects (plants and animals; Tab. 1) were used in the test, selected according to the following basic criteria: the objects should not be dangerous to people taking part in the test; they are clearly visible to the naked eye; are convenient for handling; and, since the test was conducted in schools, suitable for transportation. An additional selection criterion was the supposed attractiveness of the objects, based on our experience with pupils and previous research. These showed animals to be more attractive than plants, so we selected only 5, in our opinion not very attractive, animals. The remaining 15 objects were plants, 9 of them supposedly attractive and 6 unattractive.

One specimen (or its part) was used for each of the 20 animal and plant species, with the exception of the yellow meal worm beetle where around 100 larvae were used.

The 20 test objects were distributed randomly in the class, and set up in such a way the pupils were able to see each of them clearly and to touch or handle them. All the animals and cabomba that required containment were displayed in transparent glass containers without lids. The pupils were able

Table 1: List of objects
Tabela 1:

	Common Name	Scientific Name	Description
1.	Mongolian gerbil	*Meriones unguiculatus*	
2.	Fire salamander	*Salamandra salamandra*	
3.	Gambusia	*Gambusia* sp.	
4.	Indian stick-insect	*Carausius morosus*	
5.	Yellow mealworm beetle	*Tenebrio molitor*	Larva
6.	Pitcher plant	*Nepenthes* 'Coccinea'	
7.	Sundew	*Drosera aliciae*	
8.	Cactus	*Haageocereus versicolor*	Cylindrical, bearing artificial blooms
9.	Cactus	*Haageocereus versicolor*	Cylindrical, without blooms
10.	Cactus	*Mammillaria* sp.	Round, soft haired
11.	Formosa azalea	*Rhododendron simsii*	In bloom
12.	Formosa azalea	*Rhododendron simsii*	Without blooms
13.	Osage orange	*Maclura pommifera*	Fruit
14.	Artificial squash		Fruit
15.	Himalayan blue pine	*Pinus wallichiana*	25 cm long cone similar to *Pinus strobus* cone
16.	Eastern white pine	*Pinus strobus*	10 cm long cone
17.	Cutleaf teasel	*Dipsacus laciniatus*	Dry inflorescence on a stem
18.	Banana plant	*Musa* sp.	
19.		*Rhoicissus digitata*	
20.	Cabomba	*Cabomba caroliniana*	

to move among them freely for 10 minutes. The only explicit oral instruction was that they should observe the objects, get to know them and not damage them. After 10 minutes the pupils had to answer the following question: How interesting do you find each of these objects? Pupils evaluated all 20 objects, rating them on a 5-point scale where 1 was "worst" and 5 was "best".

The Mann-Whitney U test was performed to test the gender and age differences in ratings.

Results and discussion

Our results supported the first hypothesis, that pupils find animals more interesting than plants, since they rated animals with an average rating of 4.0, and placed plants a bit lower, at an average of 3.2. The average value of plants was lowered by 9 objects that were given a rating below 3.3, which means they were less interesting than the least interesting animal in this experiment – the gambusia fish (rating 3.4). As far as interest is concerned, 4 plant objects were able to compete with gambusia fish: cylindrical cactus with artificial blooms, artificial squash, banana plant and cone of Himalayan blue pine. Two animals that rated somewhere in the middle (ratings 3.6 and 3.9) were larvae of yellow meal worm beetle and stick insect, and were on the same interest level as the most interesting plants: fruit of osage orange and pitcher plant. This means the most interesting plants were rated about a grade lower than the most interesting animals. The most interesting objects for both genders and all age groups were the Mongolian gerbil (rating 4.7) and the fire salamander (rating 4.5).

The second hypothesis that gender does not affect interest in individual objects was accepted. Only one statistically significant gender-related difference in rating the interest factor of animals and plants was found in the 4th grade (Man-Whitney U test; $z = 2.3884$, $P < 0.05$), but there were none in

8th grade and in grammar school pupils. It may be concluded that gender does not affect the attitude towards living beings.

There are suggestions in the literature however that girls are more likely than boys to express an interest in learning about plants (Baird & al. 1984, Wandersee & Schussler 1986).

The third hypothesis that the age of pupils does not affect interest in individual objects was rejected. The rating of the test objects was strongly influenced by age; statistically significant differences were found with most of the test animals and plants (Tab. 2).

Table 2: Statistical significance of the differences between answers of each age group, obtained by the Mann-Whitney U test ($p < .05$)
Tabela 2:

	Female			Male		
Object	**ES4/ES8**	**ES4/GS**	**ES8/GS**	**ES4/ES8**	**ES4/GS**	**ES8/GS**
1 Animal	2.2108			2.7907		
2 Animal					2.2161	
3 Animal	3.6378			2.1726	3.0792	
4 Animal				3.1289	4.1055	
5 Animal			2.1239			
6 Plant	2.7199	2.1372		3.6818	3.0947	
7 Plant	2.3815	2.9367		2.7304	3.4990	
8 Plant	3.6789	2.7863		4.0460	3.7556	
9 Plant	2.2079	3.1711		4.1393	4.8526	
10 Plant		2.7666		2.4883	3.4213	
11 Plant	2.9657	2.7309			2.4027	
12 Plant	2.1524			2.1995	2.8459	
13 Plant						
14 Plant						
15 Plant	2.0597					
16 Plant						
17 Plant	3.7475	2.8465			2.4023	
18 Plant	2.4507	2.8394		2.3496	2.8039	
19 Plant	3.8823	4.3219		3.4268	4.0356	
20 Plant	5.1609	3.2058		2.7028	3.3435	

Note: ES4 = 4th grade elementary school pupils; ES8 = 8th grade elementary school pupils; GS = grammar school pupils.

The 4th grade elementary school pupils rated 60 – 65% of the objects higher than 8-graders and 50 – 70% of the objects higher than grammar school pupils. The 8-graders gave slightly higher ratings than grammar school pupils. There was only one significant difference between the ratings of 8-graders and grammar school students. The conclusion is that living objects were most attractive to younger pupils, and that their interest then decreases with age. Our results are in line with the findings by Baird & al. (1984), who established that preference for learning about animals decreases as grade level increases. Baird & al. also noted that plant study preferences rose slightly as grade level increased. We did not find this connection in our research, in which the ratings for both plants and animals decreased with age.

The 4-graders also gave high ratings to many of the objects, which means that they showed less fluctuations in the object rating than the other two age groups. The interest area of younger pupils is

therefore less specialized and their attention is governed by more diverse factors. In contrast, older students responded more to objects that were exceptional and surprising, which aroused cognitive conflict (fire salamander, pitcher plant, fruit of osage orange, artificial squash and cone of Himalayan blue pine). As Kellert established (as cited in Thompson & Mintzes 2002), pupils' attitude changes over the course of their school years, ranging from focusing primarily on an emotional relationship towards animals (ages 6 to 9 years) to focusing on cognitive or factual understanding (ages 10 to 13 years), and finally to a view that embraces an ethical concern and ecological awareness of the role of animals in their natural habitats. It seems that pupils' attitude toward plants might also have a similar age-dependent pattern.

Conclusions

Plants do engage pupil interest, but a great deal less than animals. How do we apply this finding to the educational process, since it is a school's task to present plants and animals on equal terms? Our starting point should be the education provided for teachers – if the teachers truly appreciated the difference in attitude towards various living beings, they would adjust their own views and take them into account when planning lessons. In this way lessons would not be based so much on animals; a competent teacher will also find enough interesting plants or ways to make plants attractive. For instance, on field trips pupils are more easily motivated when introduced to a plant through its connection with a certain animal.

Only one statistically significant gender-related difference in rating the interest factor of animals and plants was found.

The age of pupils strongly influenced their interest in living objects. Both animals and plants were most attractive to younger pupils, their interest then decreasing with age. Younger pupils also seemed to be attracted by various factors, while older ones responded more to features related to cognition. We can therefore implement our notion that biology for the youngest pupils should be based on pleasant direct experience with a great variety of living beings. In this way the pupils can gain a positive attitude towards the living world through emotions, and form a strong foundation on which they can build more abstract, higher level knowledge that will help them understand nature.

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The influence of direct experience on students' attitudes to, and knowledge about amphibians

Vpliv neposredne izkušnje na odnos in znanje učencev o dvoživkah

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Abstract. In the present study, we investigated how direct experience of certain amphibian species may affect a change in 7th grade science students' attitude to, and knowledge about them. For this purpose, we devised a 45-minute lesson in which we used live amphibian species, and one lesson in which lifeless animals were used. The results show that students with prior direct experiences of amphibians generally report a more positive attitude toward them and, on average, achieve higher pre-test scores. Using live animals in the classroom had a major effect on the students' attitudes to individual species, regardless of whether they had any previous experience with them. Students who have done both practical work with live animals in the classroom and had previous direct experience with them showed the highest level of knowledge and knowledge retention. Implications of the findings are discussed.

Key words: amphibians, direct experience, attitude change, knowledge

Introduction

Slovenian curricula state that, during the course of their formal education, students should acquire as much direct experience as possible of various organisms and their living environments. Only by working with live organisms as the primary teaching objects can students acquire the most vivid experiences and develop strong emotions about given objects. Moreover, students can truly understand living things when they personally make direct contact with them (Lock 1994, Lock & Alderman 1996).

The meaning of direct experience in this case involves the actual interaction or manipulation of the object in question. In contrast to direct experience, indirect experience involves only reading or being told about an object. It is known that attitudes based on direct experience are more persistent, stronger, held with greater certainty, more stable over time and more resistant to counter-influence (Fazio & Zanna 1981). Through direct contact or experience with live animals, children's attitudes and knowledge improve considerably (Yore & Boyer 1997).

Attitudes have been defined as feelings, based on our beliefs, which predispose our reactions to objects, people and events (Myers 2007).

According to a tripartite model, attitudes are based on three different sources: cognitive, affective and behavioural, which are not always consistent. As a person's attitude changes, so do his or her actions (Spielberger 2004). An attitude based on a direct experience is more likely to affect an individual's behaviour than an attitude formed on the basis of an indirect experience (Fazio & Zanna 1981).

In recent decades there has been considerable emphasis on the significance of researching attitudes toward contemporary environmental issues which also includes attitude toward organisms (Kellert

1985, 1996, Leming & al. 1995, Bogner 1998). Killerman (1998) points out research conducted among 6th graders in which in one case live invertebrates (earthworm, darkling beetle and spider) were used in the classroom and, in the other, only visual materials and models were presented. The results showed that the students who were taught using live animals developed a much better attitude to the organisms than those who had not been in direct contact with the animals. The former also exhibited a greater increase in their knowledge.

In research by Tomkins and Tunnicliffe (2001) 12-year-old pupils observed a bottle ecosystem of brine shrimps without any prior instruction. Their results indicate that pupils, while observing animals notice their salient anatomical and behavioural features, and those observations may provide a base for clearer hypothesis making when formal teaching and investigations begin.

In addition to one's attitude, one's knowledge also affects one's behaviour toward organisms. A person's positive attitude does not necessarily mean that once they are in contact with an organism, they would treat it in a way which would not harm it. Barney & al. (2005) find, for example, that only well-educated college students who have benefited from direct instruction exhibited the most knowledge about and appropriate attitudes toward dolphins.

It is the level of involvement (LOI) and amount of information (AOI) which help to build a balance between one's attitude and knowledge (Morgan 1992).

Biology teachers have the responsibility of creating conditions in which students meet various organisms (Yore & Boyer 1997). They should, however, take note that intrapersonal barriers, which significantly affect the quality of experience, play an important role in an individual becoming acquainted with an organism (Bixler & Floyd 1999). Negative feelings, such as fear and disgust, also affect the transformation of attitudes towards these animals.

Expressed fear of animals mostly functions as a response to perceived or immediate threat of physical injury. Disgust toward animals, on the other hand, may act as a protective agent against possible contamination (Davey & al. 1998, Davey & al. 2003).

Studies of people's attitudes toward and knowledge (familiarity) of different organisms often focuses on larger and/or 'charismatic' animals, such as sharks (Thompson & Mintzes 2002), dolphins Barney & al. (2005) and primates (Lukas & Ross 2005, Ross & al. 2008), but also invertebrates (Killermann 1998, Looy & Wood 2006). However, the effects of the use of animals such as amphibians are rarely studied as part of educational research (Randler & al. 2005, Yen & al. 2005).

It is well known that amphibians are endangered, and that their numbers have been declining in recent years. There are many possible causes for this, including human actions, which work in a synergistic way (Blaustein & Kiesecker 2002, Beebee & Griffith 2005).

As for the effects that the use of live amphibians in a pedagogical process has on students' attitude and knowledge are relatively poorly researched, and in view of the rising awareness of amphibian species decline, we set out to find:

1. how many 7th graders have already had direct experience with any common local amphibian species,
2. how they perceive their attitudes toward individual amphibian species before and after a classroom lesson, depending on whether they have had direct experience (before instruction) with live amphibians or not, and
3. how instruction with or without live animals affects their knowledge.

Method

The research was conducted in the school year 2004/2005. It included 21 7th grade classes (n = 487) from 10 Slovenian primary schools. The final sample included only students who attended a pre-test, participated in classroom lessons and attended all three post-tests (n = 392). In the school year 2004/2005, all respondents changed from an 8-year to a reformed 9-year primary school programme,

which meant that they progressed from the 5th to 7th grade. The reason 7th graders were included in the research is because they had not been taught about animals in science classes in the 5th grade, whose curriculum focuses mainly on plants. They would, however, have met live amphibians outside school or in the first four years of their elementary education. The age of respondents was 11 to 12 years. This age, according to KELLERT (1985), marks a period when pupils acquire knowledge in the form of information, which follows their development of emotional concern and sympathy for animals. As a result, the research included testing the students' attitude toward and knowledge about amphibians before and, three times, after instruction.

Instruction

The following animals were used for instruction: the common toad (*Bufo bufo*), the green frog (*Pelophylax sp.;* formerly *Rana*), the European treefrog (*Hyla arborea*), the European fire salamander (*Salamandra salamandra*), the alpine newt (*Triturus alpestris*), the Italian crested newt (*Triturus carnifex*), and the cave salamander or olm (*Proteus anguinus*). With the last species, we did not use live animals, as the olm is a strictly protected species.

Two types of instruction were used. The first (**Kla**; n = 127), used in the control group, was frontal instruction, which involved presenting amphibians to students with the use of tertiary teaching objects (transparencies and pictures). By talking with the students about animals and giving them information about animals, we sought to change their attitudes to the species in question and provide a correct picture of them.

The second type of instruction (**Exp**; n = 265) involved live animals. After reassuring the students of their and animal well-being, they could first experience an animal (observe its behaviour and appearance, and touch them). Next, we talked about the animals, their characteristic features and the fact that they are endangered. We actively encouraged the students to hold an animal. If they were very reluctant to do so, we encouraged them to at least gently touch it. In neither case, however, did we force any student to make physical contact with an animal. The Exp-type instruction included two teaching forms. The first involved students forming a circle in which the teacher presented them with one amphibian species at the time. The second involved students in groups of three, with a maximum of two animal species per group. They would rotate every 8–10min, moving from one designated point to another. The first group of students was given on the spot instructions on what to do with the animals, while the second also received written instructions. As both methods involved handling live animals and we sought to find a connection with direct experience, we combined both groups in our statistical analysis of results. Neither of the two groups undergoing Exp-type instruction had shown any statistically significant differences pertaining to their attitude toward and knowledge about amphibians either before or after the lesson (Mann-Whitney U; all $p > 0{,}05$).

The influence of different teachers was considered, so all 21 classes were taught and supervised during evaluations by the author, who is a biology teacher by profession. The lesson was of standard duration (45 min).

Instrument

The tests were administered one week before instruction (pre-test) and three times after instruction (post-tests 1, 2, 3). The first post-test was administered one week, the second two months and the third four months after the lesson.

The pre-test consisted of two parts. The first required students to answer two open-ended questions about which animals they are afraid of, and which four they like the most (affection). They also had to explain their answers. The follow-up consisted of 10 questions testing the students' knowledge about

amphibians. Seven were multiple choice, two required them to provide missing information, and one required that they identify animals in a picture.

The second part of the pre-test included a self-report scale, which required students to rate their attitude toward three amphibian species (green frog, toad and salamander) on a five-point scale and state whether or not they had had any direct experience with these animals (yes/no statements). We had presumed that the students at least knew the species names of the selected animals before the lesson, as they are quite common locally. Students rated their fondness for individual amphibian species according to the following scale: 1 = 'I don't want anything to do with this animal'; 2 = 'I don't like this animal'; 3 = 'I do not have any special feelings toward this animal'; 4 = 'I like this animal'; 5 = 'I like this animal very much'.

In this part of the questionnaire students also assessed their fear of and disgust toward a selection of 20 animals, which also included the green frog, the toad and the salamander (*considered for publication elsewhere*). Here students had to list for the second time whether they had any direct experience with amphibians. Students' answers on prior direct experience with three amphibian species from this section of the questionnaire were used to test the reliability of their answers. Test-retest reliability for the green frog was modest ($r = 0{,}614$; $p < 0{,}001$). Reliabilities for the toad ($r = 0{,}918$; $p < 0{,}001$) and the salamander ($r = 0{,}834$; $p < 0{,}001$) were high.

Statistical Analysis

Basic descriptive statistics was used to obtain the average values and frequencies of each data component or group. Nonparametric tests were used to determine statistically significant differences between and within the experimental and control groups on attitude ratings and knowledge test scores. All the data was analysed with the SPSS statistical programme, version 15.0.0.

Results

Direct experience

Before the lesson, few pupils stated that they had had previous experience with the selected animals (Tab. 1). In comparison with the experimental group (Exp), more students in the control group (Kla) stated that they had had previous direct experience with the green frog (Chi^2 (1, n = 383) = 3,674; $p = 0{,}055$). In both groups, it was the toad that the fewest pupils had previous direct contact with (Chi^2 (1, n = 377) = 0,231; $p = 0{,}631$), while the most knew the salamander first hand (Chi^2 (1, n = 379) = 0,775; $p = 0{,}379$).

Table 1: Descriptive statistics on students' prior direct experience with amphibians according to type of instruction.
Tabela 1: Deskriptivna statistika predhodne neposredne izkušnje učencev z dvoživkami glede na način pouka.

ANIMAL	Instr.	DIRECT EXPERIENCE					
		No		yes		no answer	
		n	*f (%)*	n	*f (%)*	n	*f (%)*
Green frog	Exp	184	69,4	73	27,5	8	3,0
	Kla	78	61,4	48	37,8	1	0,8
Toad	Exp	205	77,4	48	18,1	12	4,5
	Kla	103	81,1	21	16,5	3	2,4
Salamander	Exp	146	55,1	110	41,5	9	3,4
	Kla	76	59,8	47	37,0	4	3,1

Direct experience and attitude

Before the lesson, there were no statistically significant differences between the two groups (Exp and Kla) in rating their attitudes toward an individual amphibian species. (Mann-Whitney U; all $p > 0{,}01$).

Fig. 1 shows the average ratings of the students' attitudes towards the three amphibian species before and after both lessons, i.e. the one that included live animals and the one that did not. The ratings are shown in relation to students' previous direct experience with individual amphibian species. Statistically significant differences occurred only in ratings pertaining to the salamander, with students who had had previous direct experience with the animal. (Figure 1c; Kla1-Exp1; Mann Whitney U, $Z = -2{,}902$; $p = 0{,}004$). All graphs show that, on average, pupils with no prior direct experience with individual animals rated their attitude lower, which means that it was more negative. In contrast, pupils who had had previous direct experience with the animals on average rated their attitude higher. After the lesson which included the use of live animals (Exp), students with both higher and lower rated initial attitudes (Exp0 and Exp1) significantly changed their attitude toward the animals. In the Exp0 group, the differences between the initial and final ratings for individual animals were statistically significant (Wilcoxon Signed Ranks Test; all $p < 0{,}001$). The same holds true for the Exp1 group (Wilcoxon Signed Ranks Test; all $p < 0{,}001$).

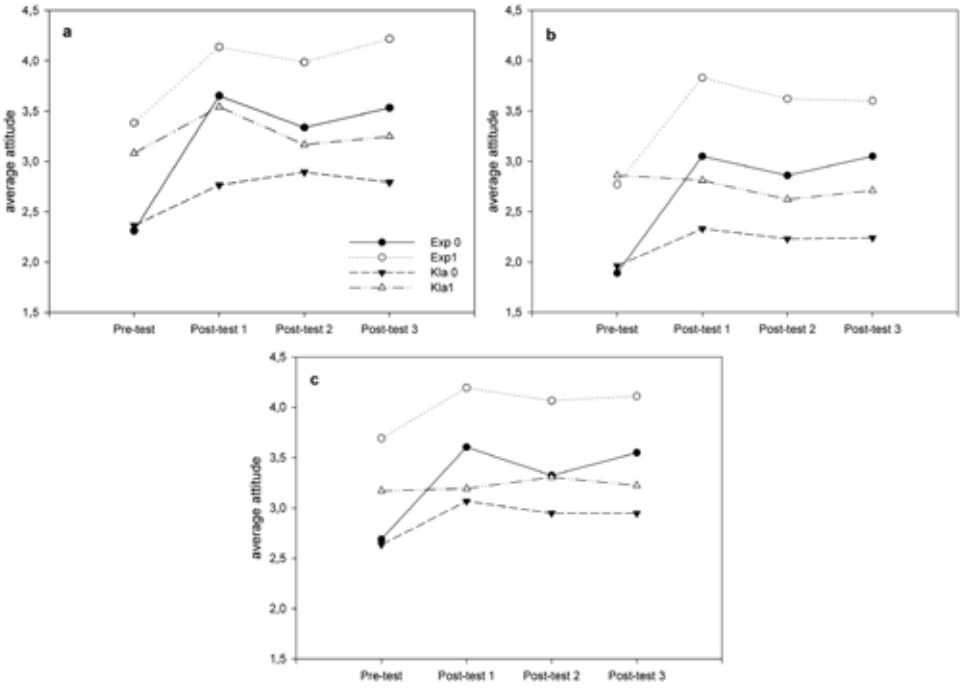

Figure 1: Average students' attitude ratings for individual amphibian species before and after instruction, according to prior direct experience and instruction type. Chart order: (a) green frog, (b) toad and (c) salamander. Exp0 and Kla0: students without prior direct experience with animals. Exp1 and Kla1: students with prior direct experience with animals.

Slika 1: Povprečna ocena odnosa učencev za posamezno vrsto dvoživk pred in po pouku glede na predhodno neposredno izkušnjo in način pouka. Vrstni red: (a) zelena žaba, (b) krastača in (c) močerad. Exp0 in Kla0: učenci brez predhodne neposredne izkušnje z živalmi; Exp1 in Kla1: učenci s predhodno neposredno izkušnjo z živalmi.

With the control group students who had had prior direct experience with the animals (Kla1), there were no statistically significant differences between their initial and final ratings concerning individual animals (Wilcoxon Signed Ranks Test; all $p > 0,05$). The rating of their attitude toward the toad (Fig. 1, b) fell somewhat, but not statistically significantly. The lesson which did not include live animals (Kla), on the other hand, had a positive effect on the attitude of pupils without prior direct experience (Kla0) of the animals. Differences in attitudes toward the toad and the green frog between the initial and final ratings in the Kla0 group were statistically significant (Wilcoxon Signed Ranks Test; both $p < 0,01$), while the attitudes toward the salamander were marginally significant (Wilcoxon Signed Ranks Test; $p = 0,055$).

Direct experience and knowledge

Students' knowledge about amphibians before and after the lesson did not differ significantly between the two groups (Tab. 2). The differences in achievement scores before and after instruction were rather small.

Table 2: Descriptive and inference statistics of average students' test scores before and after instruction, according to type of instruction.
Tabela 2: Deskriptivna in inferenčna statistika povprečnih rezultatov testov znanja učencev pred in po pouku glede na način pouka.

test	Instruction				sig.	
	Exp (n = 265)		Kla (n = 127)			
	Mean score (%)	*SD*	Mean score (%)	*SD*	Z	*p*
Pre-test	43,4	*15,6*	43,3	*14,5*	–0,491	*0,623*
Post-test 1	63,8	*13,1*	65,4	*13,2*	–1,135	*0,256*
Post-test 2	61,4	*14,2*	58,1	*19,0*	–0,798	*0,425*
Post-test 3	60,7	*14,7*	57,7	*15,8*	–1,724	*0,085*

Note: sig.: Mann-Whitney test.

After analysing the data about direct experiences with individual amphibian species before instruction, differences in knowledge between students became evident and statistically significant (Fig. 2). On average, pupils who attended a lesson which did not involve live animals (Kla) scored similarly on the first post-test as the Exp1 pupils. However, knowledge of the Kla0 and Kla1 pupils showed a more rapid decrease in subsequent testing in comparison to the pupils from Exp0 and Exp1 groups. In the first post-test, the Exp0 pupils achieved the lowest scores in the part which tested their knowledge; however, in subsequent tests, their knowledge showed a steadier decline than in the Kla group. In final testing, there were no statistically significant differences between the Kla0, Kla1 and Exp0 groups (Kruskal-Wallis test; all $p > 0,05$). Pupils who displayed the most knowledge were those who had direct contact with the animals before and during the lesson.

Discussion

Slovenian science curricula stipulate that at the beginning of their compulsory education students should encounter various living organisms. The results of our survey show that amphibians are rarely among them, as much as 55% or more of students had had no direct contact with individual amphibian species (Tab. 1). Moreover, the results suggest that students seldom encounter live amphibians outside the school environment (e.g. family influence).

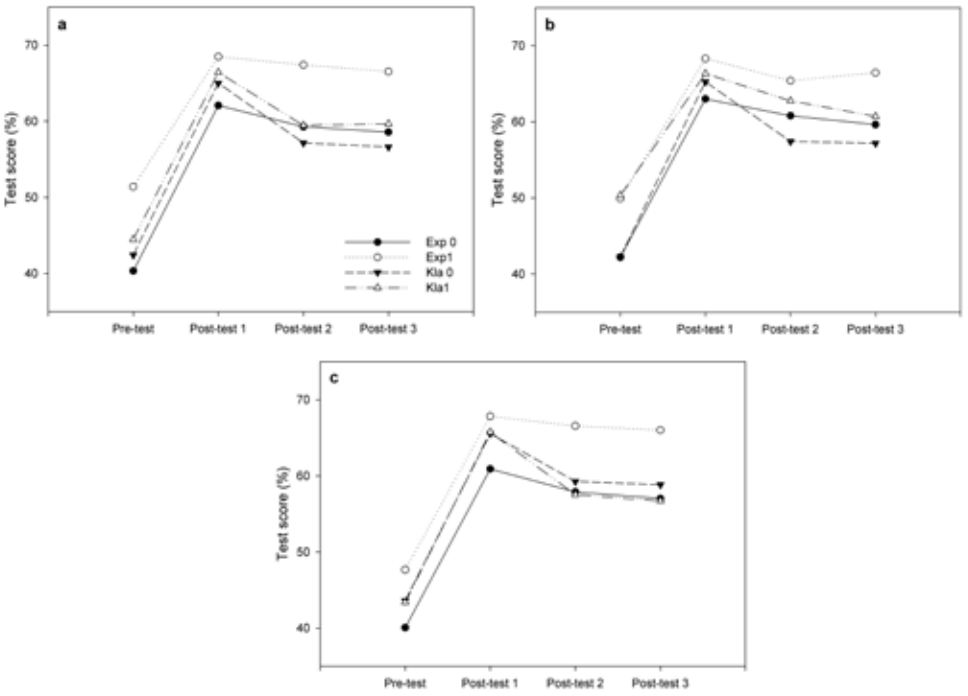

Figure 2: Average students' test scores before and after instruction in relation to students' prior experience of individual amphibian species. Chart order: (a) green frog, (b) toad and (c) salamander. Exp0 and Kla0: students without prior direct experience of animals. Exp1 and Kla1: students with prior direct experience of animals.

Slika 2: Povprečni rezultati testov znanja učencev pred in po pouku v povezavi z njihovimi predhodnimi izkušnjami s posamezno vrsto dvoživk. Vrstni red: (a) zelena žaba, (b) krastača in (c) močerad. Exp0 in Kla0: učenci brez predhodne neposredne izkušnje z živalmi; Exp1 in Kla1: učenci s predhodno neposredno izkušnjo z živalmi.

The pedagogical literature suggests several ways in which students could work with amphibians (Murphy & Fortner 2001, Green & Green 2005, Tomasek & al. 2005). However, there is little information about how this influences their attitudes toward and knowledge about these animals.

The pupils included in our survey who had had prior direct experience of amphibians showed better attitudes toward these animals before the lesson than pupils who had had no such experience (Fig. 1).

Kellert (1985) makes a similar point in his research, stressing that the focus with young students should be firstly on developing the emotional component of experiencing animals. This stage should be followed by acquiring information about them. Only when students are familiar with animals and have developed appropriate attitudes towards them (experiencing animals through direct contact and acquiring enough factual information) can they, according to Kellert, develop their own understanding of ecology and their ethical concerns for the welfare of animals (aged 13 or older).

Pupils who had neither been in contact with amphibians before the lesson nor encountered them during the lesson (Kla0) changed their attitudes more significantly than those who did not encounter amphibians during the lesson, but had previous direct experiences with them (Kla1, Fig. 1). With the Kla0 pupils, the teacher's role was probably greater, as he presented his own view of and experience with

the animals. Pupils who had no prior experience (Kla0) found it much easier, through information passed on to them by the teacher, to form a positive attitude towards the animals than the Kla1 pupils. Kla1 pupils' prior experience with the animals probably affected the change in their attitudes, since an attitude arising from direct experience shows more resistance to external influences (Fazio & Zanna 1981).

The students who gained the most were those who attended the lesson where live animals were used (Exp; Figure 1). Both those who had no prior direct experience (Exp0) and those who had prior direct experience (Exp1) rated their attitudes toward the animals higher than their peers who did not see live animals during the lesson (Kla). We believe that the teacher's guidance played a vital part in this, as the teacher would notice and immediately correct any false ideas pupils may have had about the animals. After the lesson, the attitude of the Exp0 pupils was at roughly the same level as that of the Exp1 students before the lesson. The latter, however, changed their attitudes so much that, on average, these could be rated as "I like this animal".

The two groups (Exp and Kla) also differed in terms of their knowledge (Fig. 2). The Kla0, Kla1 and Exp0 pupils achieved similar scores in the final test, while the Exp1 group, which had prior direct experience of the animals and worked with live animals during the lesson, achieved a better score. The latter also showed better knowledge retention. One week after the lesson, the Exp0 students showed the weakest knowledge, which decreased evenly by the time of the final test. It is possible that these students scored poorer than the Exp1 group as a result of their emotional involvement during the lesson, for which reason they required more time to switch from emotionally experiencing an animal to becoming rationally involved in the lesson.

Although Slovenian curricula maintain that students should develop their relationship toward nature and the living organisms, there are often no clear guidelines about how teachers are supposed to achieve this. In their work teachers focus greatly and spend considerable amounts of time on passing information to their pupils about animals and their endangerment. A question arises here: will students who have never encountered a living organism be prepared to do something for them? Lindemann – Mathies (2005) finds that students who are in contact with organisms (even the more inconspicuous ones, such as plants) develop more sensitivity toward them. Moreover, they develop a liking for them and quite a special attitude. Furthermore, these students show greater knowledge of these organisms.

As Strgar (2007) notes, when students work with plants their interest in them increases and even more so after teacher intervention. Our experience about the interest and motivation of students in our survey is almost the same. Students who attended the lesson which used live animals were highly motivated at the end and ready to learn more about them (they did not wish to leave the classroom and they asked many questions).

Based on our research, we believe that prior direct experience with animals outside the classroom and relatively short exposure (45min) to live animals in the classroom have a positive effect on pupils' attitude toward amphibians.

If the educational process provided 'the right' experiences with living organisms, there would probably be fewer questions raised about potentially harmful behaviour toward animals as noted by Barney & al. (2005).

Conclusion

The results of our research show that:

1. enabling pupils brief direct experience significantly changes their attitude toward living organisms, which helps to reduce their intrapersonal barriers on contact with living organisms,
2. higher grade teachers could compensate for pupils' 'forgotten' or missed opportunities to emotionally experience animals in elementary classes,

3. students who gained the most knowledge and had the best attitude were those who had prior direct experience with animals and attended the lesson in which live animals were used; therefore, it would be sensible that, during the course of their education, students were given several opportunities to experience the same living organisms (e.g. in elementary school, secondary school).

Worth researching in the future would be the attitude of both future teachers and teachers of life science topics toward living organisms, as they have an important role and the responsibility to create such learning environment in which students can further develop their knowledge and attitudes.

In addition, it would be beneficial to study the extent to which a teacher's attitude toward living organisms can affect the attitude of students.

The lesson which involved live amphibians would make a good starting point for students' subsequent work, as once they were in direct contact with the animals, they also asked questions to which they could later find answers on their own.

Povzetek

V raziskavi, ki je potekala v šolskem letu 2004/2005, smo preverjali, kako neposredna izkušnja učencev z nekaterimi dvoživkami vpliva na spremembo njihovega odnosa do in znanja o dvoživkah. V raziskavo je bilo vključenih 392 učencev 21 sedmih razredov devetletne osnovne šole. Sodelujoči učenci so bili stari od 11 – 12 let. Po Kellertu (1985) je to obdobje, v katerem učenci intenzivno pridobivajo podatkovna znanja. To obdobje naj bi sledilo obdobju izgradnje čustvene skrbi in navezanosti otrok do živali. Zato smo raziskavo zasnovali tako, da smo pri učencih preverili njihov odnos do in znanje o dvoživkah pred poukom in izvedli tri zaporedna preverjanja po pouku. Statistična obdelava podatkov je upoštevala predhodne neposredne izkušnje učencev z dvoživkami. Da bi v raziskavi preprečili vpliv različnih učiteljev, je vsa preverjanja in pouk izvedel raziskovalec. Učenci so eno šolsko uro spoznavali dvoživke. Pouk je bil izveden na dva načina. Pri prvem načinu so učenci spoznavali dvoživke preko pogovora, razlage in uporabe terciarnih učnih virov (slikovnega materiala). Ta način pouka je služil kot kontrola. Pri drugem načinu pouka pa smo uporabili žive živali, kjer smo učence spodbujali k vzpostavitvi neposrednega stika z živalmi. Rezultati so pokazali, da večina učencev pred poukom še ni imela neposrednih izkušenj z dvoživkami. Učenci, ki niso imeli predhodne neposredne izkušnje s posamezno živaljo, so živalim v povprečju pripisali nižje ocene odnosa, kar pomeni, da je bil njihov odnos 'slabši' od učencev, ki so pred poukom že imeli neposredno izkušnjo. Po pouku so najboljši odnos izrazili učenci, ki so se pri pouku srečali z živimi živalmi. Pri pouku, kjer žive živali niso bile uporabljene, pa so v manjši meri spremenili odnos samo tisti učenci, ki niso imeli predhodnih neposrednih izkušenj. Pri slednji skupini učencev je bila verjetno prisotna večja moč informacij, ki jih je posredoval učitelj. Pri zadnjem preverjanju znanja se je izkazalo, da so učenci s predhodno neposredno izkušnjo in uporabljenimi živimi živalmi pri pouku izkazali najvišje znanje. Raziskava je pokazala, da s kratkotrajno neposredno izkušnjo pri učencih pomembno spremenimo njihov odnos do organizmov in tako pozitivno vplivamo na znižanje intrapersonalnih ovir, s katerimi se učenci srečajo ob stiku z organizmi. Z uporabo živali lahko nadoknadimo ″pozabljeno″ doživljanje le-teh v nižjih razredih šolanja. Na znanju so največ pridobili in imeli najboljši odnos učenci, ki so imeli predhodne neposredne izkušnje z živalmi in so doživeli živali pri pouku. Zato bi bilo smiselno, da bi se učenci v času šolanja večkrat srečali z istimi skupinami organizmov, začenši že v nižjih razredih šolanja otrok. V nadaljevanju bi bilo pomembno ugotoviti, ali so učitelji in bodoči učitelji usposobljeni za delo z živimi organizmi pri pouku, saj imajo učitelji pomembno vlogo in odgovornost, da ustvarjajo učno okolje, kjer lahko učenci pridobijo največ na znanju in odnosu.

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NAVODILA AVTORJEM

1. Vrste prispevkov

a) ZNANSTVENI ČLANEK je celovit opis originalne raziskave in vključuje teoretični pregled tematike, podrobno predstavljene rezultate z diskusijo in sklepe ter literaturni pregled: shema IMRAD (Introduction, Methods, Results And Discussion). Dolžina članka, vključno s tabelami, grafi in slikami, na 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, biokemije, genetike, mikrobiologije, ekologije itd.), ki ne vsebuje podrobnega teoretičnega pregleda. Njen namen je seznaniti bralca s preliminarnimi ali delnimi rezultati raziskave. Dolžina na 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, predstavljene 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. Zakjučki

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. Wieser 1992: Zur Flora und Fauna des Webersees. Carinthia II 182/102 (1): 159–173.

Trinajstić & 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. 1–7.

12. Format in oblika članka

Članek naj bo poslan v obliki Word dokumenta (doc) ali kot obogateno besedilo (rtf) 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. Uporabljene nomenklaturne vire navedemo v poglavju Metode. Tabele in slike so posebej priložene tekstu. Vse strani (vključno s tabelami in slikami) morajo biti oštevilčene. Glavnemu uredniku je potrebno oddati original, dve kopiji in elektronski zapis na disketi 3,5", na CD-romu ali kot priponko elektronske pošte (slednjega odda avtor po opravljenih strokovnih in jezikovnih popravkih).

13. Recenzije

Vsak 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 30 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, dendrograms, 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 vo-lume (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 Webersees. Carinthia II 182/102 (1): 159–173.

Trinajstić & 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. 1–7.

12. Format and Form of Articles

Articles should be send as Word document (doc) or Rich text format (rtf) 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 in the Methods section. Tables and illustrations shall accompany the texts separately. All pages including tables and figures should be numbered. The original manuscript, two copies, and an electronic copy (after all corrections) on a 3.5" computer diskette, on CD-ROM or by e-mail 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 thirty (30) 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 the editor-in-chief.