INVERTEBRATES IN *Myriophyllum spicatum* L. STANDS IN LAKE SAKADAŠ, CROATIA

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Abstract

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The invertebrates associated with *Myriophyllum spicatum* L., were studied during the vegetation period in 2004, at three stations I, II and III, in lake Sakadaš within Kopački rit floodplain, Croatia. A total of twenty one taxa were recorded, with the dominance of the insect larvae representatives. Family Chironomidae had the greatest abundance and represented 67% of all recorded invertebrates, while as nematodes and oligochaetes were especially abundant at the third station. Significant differences in invertebrate abundances were recorded between months of sampling – July and August as well as July and September. Also, stations I and II showed statisticaly important differences in relation to station III, located near lake's dam. Physicochemical data, including trophic state indices, as well as invertebrate community composition were indicative for eutrophic state of water with a tendency to hypertrophy at all stations in lake Sakadaš.

Key words: submerged macrophytes, eutrophication, chironomids, nematodes, oligochaetes

Introduction

In relatively small and shallow lakes, the littoral flora has a very important role in productivity of the lake and can regulate and dominate metabolism of the entire lake ecosystem (Wetzel, 2001). Macrophytes in shallow waters provide shelter for invertebrates from predators and disturbance (Dvořák, Best, 1982; Tessier et al., 2004). Aquatic plants with different architecture of leaves support different biomass, taxonomic composition as well as size structure of invertebrate fauna (Soszka, 1975; Hann, 1995; Cheruvelil et al., 2002). Plant surface is ideal for the colonisation of periphytic algae, which are a source of food for many of invertebrates (Dvořák, Best, 1982; Monahan, Caffrey, 1996; Zirk, Goldsborough, 1996). Due to the importance of the Kopački rit Nature Park as a large floodplain area and a nesting area for many birds as well as a habitat for a variety of invertebrate and vertebrate fauna, there have been constant research projects conducted since 1979 in a ways of monitoring the water quality and a composition of aquatic invertebrates. Kopački rit is on the RAMSAR list of wetlands of international importance since 1993 (RAMSAR, 2005). Published data about invertebrate fauna in water-bodies of the Kopački rit (lake Sakadaš, Čonakut channel, Kopačko lake and Hulovo channel) mainly describe the composition of the benthic invertebrates (Bogut, Vidaković, 2002; Vidaković, Bogut, 2004), and the research of invertebrate fauna associated with macrophytes started in 2001 on several macrophyte stands including different species and types of plants in the Čonakut channel (Bogut et al., 2007a), which connects lake Sakadaš with the Danube. Although it has been known to appear in Kopački rit, *Myriophyllum spicatum* L. or Euroasian water milfoil, appeared for the first time in lake Sakadaš in 2004. In Canada and North America, this macrophyte has been known as an invasive species (Couch, Nelson, 1985; Smith, Barko, 1990) but it is quite common in Croatian inland waters. One other invasive species in lake Sakadaš was found: in association with *M. spicatum* in samples from 2004, one individual of species *Limnomysis benedeni* (Czerniavsky, 1882) (Mysidacea), and according to Bogut et al. (2007b) it came to lake Sakadaš during high water-level with Danube waters.

The purpose of the investigation presented in this paper was to establish the stability of macrophyte stands, composition and the colonization of the invertebrates on *Myriophyllum spicatum* in a floodplain eutrophic lake Sakadaš.

Materials and methods

Study site

Kopački rit Nature Park is a floodplain situated in the north-east Croatia, in-between the rivers Drava and the Danube and covers the area of approximately 100 km^2 (Mihaljević et al., 1999). Lake Sakadaš is the deepest water depression in the Kopački rit, in average 7 meters deep, oval shaped (Vidaković et al., 2001). The lake covers an area up to 9 ha, depending on the water-level, directly influenced by the Danube water-level, which also influences the composition of flora and fauna and the state of water-bodies of the entire Kopački rit. Besides the Danube, there is also an influence of the river Drava, but in much smaller rate in the means of water inflow (Mihaljević et al., 1999). Lake Sakadaš is an open water system, there is a water inflow into the lake from the Čonakut channel in the east which brings water from the Danube, from the Novi channel in the south and there is a constant exchange of water through the dam in the west (Fig. 1). According to Bogut et al. (2003) and Vidaković et al. (2002), there has been, during the years, a shift of tropic state of lake Sakadaš, from mesoeutrophy to hypertrophy.

The research stations have been chosen after surveying the entire lake area and evaluating the state and appearance of macrophytes. There have been several species found in the lake Sakadaš during the research period: *Potamogeton gramineus* L., *Ceratophyllum demersum* L., *Myriophyllum spicatum* L., *Trapa natans* L., *Nymphoides peltata* (S. G. G m e l.) O. K u n t z e, *Lemna* sp., *Polygonum amphybium* A l l. and *Spirodella* sp. *Myriophyllum spicatum* formed rather permanent and large stands, on a good location in a sense of water depth and an area that didn't dry out during the summer season, and lasted in lake Sakadaš during whole vegetation season in 2004. Also, some of the species were represented with a few plants sporadically located in the lake. Due to those reasons *M. spicatum* was selected for the investigation. *M. spicatum* is a submerged plant with dissected leaves and according to Tessier et al. (2004) and other authors (Soszka, 1975; Dvořák, Best, 1982; Zirk, Goldsborough, 1996; Pinowska, 2002) those dissected leaves provide good shelter and a food source for most invertebrates.

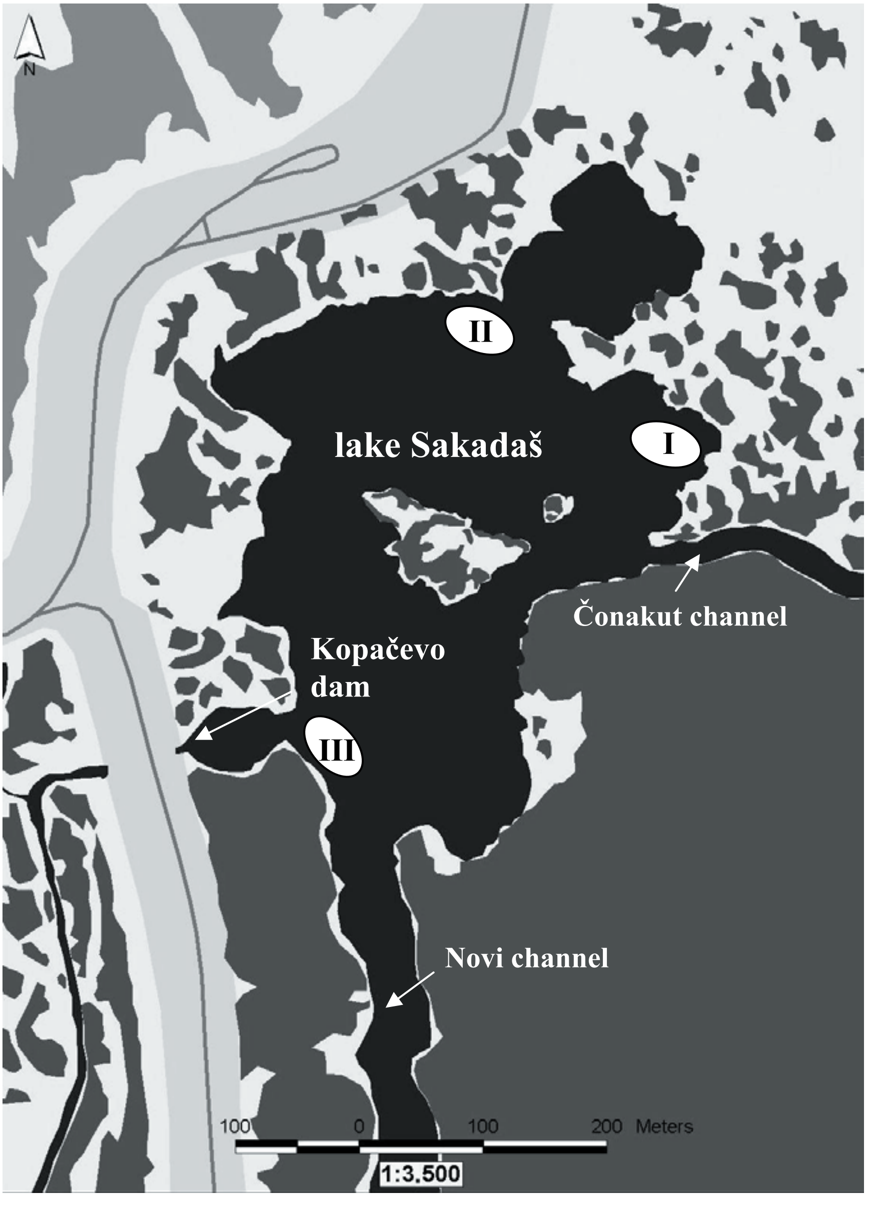

Fig. 1. Map of the lake Sakadaš with marked positions of the research stations.

Three locations where stands of *M. spicatum* developed were selected as research stations, given in Fig. 1, where samples were weekly collected:

- station I – littoral site near the mouth of the Čonakut channel into lake Sakadaš, in the east part of the lake; stand was present from 14th of July till 4th of August;
- station II – littoral site near pier, in the north-east part of the lake; stand was present from 14th of July till 12th of August;
- station III – in front of the Kopačevo dam, in the south-western part of the lake, stand lasted constantly from the beginning of the vegetation season till the end (14th of July – 8th of September).

Environmental parameters

Chemical and physical water parameters: pH, conductivity, temperature, dissolved oxygen concentration and O_2 saturation, were measured weekly *in situ* using WTW Multi 340i / set. Transparency was measured with Secchi disc. Methods assigned by APHA (1985) regulations were used for analysis of the concentration of chlorophyll-a and the total phosphorus (T–P). Trophic state indices (TSI) were determined according to Carlson, Simpson (1996).

Vegetation and invertebrates

Triplicate samples (each replicate included a single plant) within every macrophyte stand, were taken from a boat, on a weekly basis with a plastic hand cylinder (43 cm high, covered surface area of 78.5 cm^2) and processed in the laboratory. After dislodging the invertebrates from the plant, water containing removed fauna was rinsed on the sieve with 60 μm mesh. Animals were preserved within the solution made of 585 ml 96% ethanol, 310 ml H_2O, 100 ml 4% formaldehyde and 5 ml of glycerine. Macrophytes were first weighted to determine wet weight, and then dried for 24 hours at 60 ˚C (Hann, 1995; Cattaneo et al., 1998) and weighted again to record plant's dry biomass. For determination and counting of the separated fauna, different magnifications of stereoscopic microscope Olympus SZX9 were used.

Invertebrate abundances are presented by a number of individuals per 100 g of plant dry weight and diversity indices: Shannon-Wiener diversity index H', Simpson index 1-D, and species richness as Margalef's d were calculated.

Statistical analysis

To test differences in invertebrate abundance and physicochemical parameters between the stations and sampling dates (months) we applied the non-metric multidimensional scaling (MDS) based on Bray-Curtis similarity applied to square root transformed data, and analysis of similarity (ANOSIM test) (PRIMER-e v5.2.9 software, Clarke, Warwick, 2001).

Results and discussion

The water temperature varied and followed the changes in air temperature, depending on weather conditions, which were usual for the summer season. From the beginning of the sampling onward, water depth was decreasing as expected due to the high temperatures and insufficient rain. Physicochemical parameters are given in Table 1, and there was no significant difference present among the stations but ANOSIM shows statistically important differences for recorded values of physicochemical parameters: for July and August ($R = 0.647$, $p < 0.001$) and July and September ($R = 0.739$, $p < 0.001$), so does the NMDS displayed on the Fig. 2. Chlorophyll-a ($F = 19.549$), depth ($F = 14.995$), Secchi depth (F

T a b l e 1. Physicochemical water parameters measured weekly from July till September 2004 at three stations in lake Sakadaš.

Physicochemical parameters	station I min–max mean ± SD	station II min–max mean ± SD	station III min–max mean ± SD	July min–max mean ± SD	August min–max mean ± SD	September min–max mean ± SD
water temperature (˚C)	18–29 23.83 ± 1.68	16.5–29 23.94 ± 1.86	17–31 24 ± 1.60	23–27 24.83 ± 1.88	23–25.5 24.29 ± 1.02	21–22.5 21.83 ± 0.94
Secchi depth (m)	0.53–1.21 0.74 ± 0.22	0.23–1.17 0.62 ± 0.33	0.36–1.32 0.68 ± 0.32	0.7–1.32 1.01 ± 0.24	0.23–0.74 0.51 ± 0.1	0.38–0.63 0.53 ± 0.05
depth (m)	0.73–3 1.44 ± 0.84	0.23–3.74 1.19 ± 1.13	0.36–3.22 1.26 ± 0.95	1.13–3.74 2.31 ± 0.86	0.23–1.67 0.81 ± 0.44	0.38–1.02 0.74 ± 0.21
dissolved oxygen concentration (mgl^{-1})	3.77–12.55 7.78 ± 3.14	3.6–12.03 8.24 ± 2.96	3.95–12.08 7.59 ± 2.98	3.6–5.27 4.09 ± 0.31	7.28–12.55 9.49 ± 1.98	8.39–11.62 9.61 ± 0.74
chlorophyll-a (μgl^{-1})	14.26–57.83 36.77 ± 13.34	13.05–50.45 36.59 ± 11.33	8.63–56.82 40.55 ± 14.46	8.63–40.32 26.4 ± 13.36	24.53–50.45 40.27 ± 6.82	43.04–57.83 50.72 ± 3.09
total phosphorus (mgl^{-1})	0.04–1.16 0.22 ± 0.35	0.07–0.15 0.11 ± 0.03	0.01–0.16 0.10 ± 0.05	0.08–0.16 0.13 ± 0.02	0.05–0.13 0.09 ± 0.02	0.01–1.16 0.27 ± 0.31
fresh biomass * (gm^{-2})	1.4–6.4 3.9 ± 1.29	2.7–9.4 5.1 ± 2.0	2.3–26.1 6.3 ± 4.4	3.8–6.5 4.6 ± 0.8	0–14.8 4.1 ± 4.3	0–7.3 2.25 ± 3.5
dry biomass* (gm^{-2})	0.2–1.3 0.6 ± 0.3	0.4–2.2 1.0 ± 0.4	0.3–2.6 0.9 ± 0.4	0.42–1.37 0.8 ± 0.3	0–1.65 0.7 ± 0.6	0–1.27 0.4 ± 0.6
TSI-Chl-a	56.7–70.4 65.3 ± 4.2	55.8–69.1 65.3 ± 4	51.7–70.2 65.9 ± 5.6	51.7–66.9 61.6 ± 5.4	61.9–69.1 66.7± 2.1	67.5–70.4 69.1 ± 1.2
TSI-TP	58.1–105.8 73.79 ± 13.53	65.4–76.3 70.86 ± 3.88	35.8–77.3 68.25 ± 12.65	68.1–77.2 73.88 ± 0.84	61.7–74.2 69.24 ± 0.95	35.8–105.8 70.06 ± 12.81

* three replicates

Fig. 2. NMDS plot of physicochemical parameters at all three stations in lake Sakadaš during investigation period in 2004.

= 18.744) and concentration of oxygen (F = 36.894) have shown statistical differences between months at $p < 0.0001$ (df = 8.18; $F_{crit.} = 2.510$). Trophic state indices values ranged from 35.8 to 105.8, calculated based on the amount of total phosphorus (TSI-TP) and from 51.7 to 70.4 based on the concentration of chlorophyll-a (TSI-Chl-a) indicating eutrophy and hypetrophy during research period, what coincides with earlier research on water quality in water-bodies of Kopački rit (Bogut et al., 2003; Vidaković et al., 2002). The total of 21 taxonomic groups were found which belonged to the following main groups: insect larvae (Chironomidae, Ceratopogonidae, Ephemeroptera, Heteroptera, Zygoptera, Anisoptera, Plecoptera, Trichoptera, Muscidae, Dytiscidae), Crustacea (Cladocera, Copepoda including nauplii, Mysidae), Hirudinea, Oligochaeta, Nematoda, Mollusca (Bivalvia, Gastropoda), Hydracarina, Hydrozoa and Turbellaria. Taxonomic groups are presented in Table 2 by their abundance, those with percentage rate less than 0.5% are labeled as others. The highest abundance had chironomids larvae (34340 ind. 100 g^{-1} d.w.), they made 67% of recorded invertebrates, nematodes 12.5% (6367 ind. 100 g^{-1} d.w.) and oligochaetes (5330 ind. 100 g^{-1} d.w.) made 10.4%. The composition of invertebrates associated with *Myriophyllum spicatum* was as expected for the eutrophic state of water. In eutrophic waters very successful taxonomic groups, present in high abundance are oligochaetes according to Mackie (2001) and chironomids larvae (van der Berg, 1999) found in a greatest abundance during this research. Chironomids larvae are very often found as the most abundant group of macrofauna in most aquatic (fresh-water) habitats (e.g. Cranston, 1982; Kornijów, 1989; Epler, 2001), they feed on algae, detritus and associated microorganisms, macrophytes, wood debris, and other invertebrates of adequate size (Dvořák, 1996; Nazarova et al., 2004). According to Fagundes, Shimizu (1997), chironomids larvae followed by oligochaets and leaches have

T a b l e 2. Abundance of invertebrate fauna (number of individuals per 100 g dry weight) associated with *M. spicatum* in lake Sakadaš.

Invertebrates	Station I min– max mean ± SD	Station II min– max mean ± SD	Station III min– max mean ± SD	July min– max mean ± SD	August min– max mean ± SD	September min– max mean ± SD
Odonata, Zygoptera	152– 2668 1095 ± 1105.6	106– 4923 1294.2 ± 2039.7	0– 1050 300.3 ± 324.7	0– 2668 532.1 ± 538.4	118– 4923 1363.5 ± 1135.7	211– 488 349.4 ± 196
Diptera, Chironomidae	17904– 93539 47258.8 ± 33157.6	19498– 167453 53886.4 ± 63825.6	3986– 54735 17739.9 ± 15123.8	8088– 167453 51514.4 ± 24358	3986– 30093 22001.5 ± 8119.3	6656– 18216 12436 ± 8174.5
Ceratopogonidae *Bezzia* sp.	0– 97 43.3 ± 50.7	39– 2708 814 ± 1079.4	0– 558 189.1 ± 175	0– 461 128.6 ± 110.2	0– 2708 979.6 ± 950.3	79– 175 126.7 ± 67.6
Ephemeroptera, Caenidae *Caenis* sp.	0– 709 382.5 ± 341.9	125– 787 338.6 ± 266.4	0– 242 71.1 ± 86.4	0– 709 288.2 ± 209.5	0– 787 160.7 ± 256.2	70– 184 126.8 ± 80.8
Crustacea, Cladocera	680– 2447 1326.8 ± 797.9	146– 2764 1272.6 ± 1054.5	723– 2952 1794.8 ± 759	146– 2764 1261.5 ± 392.9	1051– 2952 1889.8 ± 497.4	1428– 2684 2056 ± 887.5
Copepoda incl. nauplii	38– 3888 2133.7 ± 1619.4	195– 3104 1175.5 ± 1117.4	89– 4815 1433 ± 1439.4	38– 3104 1060 ± 456.3	89– 3888 2314.8 ± 1432.2	453– 4815 2634 ± 3084
Turbellaria	0– 98 43.5 ± 51	0 – 1383 790.8 ± 713.1	0– 1324 377.2 ± 437.1	0– 1383 175.7 ± 255.7	0– 1317 547.8 ± 655.3	593– 1324 958 ± 517
Nematoda	191– 1728 587.3 ± 760.8	197– 1116 694.2 ± 365.6	1112– 22283 12086.3 ± 9971.7	191– 5422 1345.1 ± 1649.4	787– 10285 4703.1 ± 5931.2	22283– 30651 26467.2 ± 5916.8
Oligochaeta, Naididae	0– 1367 686.5 ± 589.2	315– 5370 2014.4 ± 2088.5	2706– 25682 9235 ± 6687.5	0– 6586 2525.4 ± 2356.3	1367– 25682 5160.9 ± 6031.5	8682– 10693 9687.4 ± 1422
others	97– 551 274.5 ± 204.7	0– 886 302.8 ± 350	0– 1328 202.2 ± 426.2	40– 1328 343.7 ± 401.7	0– 886 191.2 ± 312.7	- -
Number of taxa, S	10	10	10	10	10	9
Number of individuals, N	53826	62578	43424	59171	39546	54837
Shannon's H'	0.59	0.69	1.43	0.62	1.54	1.39
Simpson's 1-D	0.23	0.26	0.71	0.24	0.71	0.68
Margalef's d	0.83	0.82	0.84	0.82	0.85	0.73

tendency to dominate if there is an influx from organic sewers and if levels of oxygen are low. There is no sewer inflow in lake Sakadaš but there are fields near the lake what could contribute to greater nutrient input into the water. Unlike at stations I and II, nematodes were present at station III in a very high percentage rate: 28% (12086 ind. 100 g^{-1} d.w.) and Oligochaeta were represented with 21% (9235 ind. 100 g^{-1} d.w.). According to Vidaković, Bogut (2007) there has been a shift from the dominance in nematode feeding groups from chewers in July to epistrate feeders towards September. The result of the shift in chironomids nematodes relation could be a result of a direct competition for food. Predators, Zygoptera larvae, had a good average percentage rate at stations I and II, approximately 2%, with abundance of 1095 ind. 100 g^{-1} d.w. and 1294 ind. 100 g^{-1} d.w. Most of the invertebrates found at the investigated stations belonged to the feeding (trophic) classes of predators (Prus et al., 2002), such as Ansioptera and Zygoptera, Ceratopogonidae, Heteroptera, Muscidae, Dytiscidae, Turbellaria, Hirudinea and Plecoptera. Oligochaetes, trichopters larvae and juveniles of snails are found plant-detritus feeders, while the taxonomic group belonging to filter-feeders was juveniles of bivalves. Prejs et al. (1997) propose that the predators such as Odonata and Heteroptera can regulate the density of chironomids larvae better than fish. We found no statistically important correlation between chironomids larvae and Zygoptera. Zygoptera and Anisoptera, sit-and-wait predators, according to Hann (1995), use the plant as a source of food in means of area providing food and shelter for other invertebrates, what can be also useful for avoiding their predators (Lombardo, 1997; Prejs et al., 1997; Rennie, Jackson, 2005). Rantala et al. (2004) found in samples from Lake Saarioisjärvi that larvae of *Aeshna viridis* seek shelter from fish predation in macrophytes.

As representatives of meiofauna, cladocerans (1465 ind. 100 g^{-1} d.w.) and copepodes (incl. nauplii) (1581 ind. 100 g^{-1} d.w.) were found in rather low percentages but still more than it was recorded for Hydrozoa, Turbellaria, Gastropoda juv., Bivalvia juv., Hirudinea juv. and Hydracarina. Research conducted by Mastrantuono, Mancinelli (2005) of macrophyta in an Italian lake as a result gave qualitative dominance of chironomids, among few other groups, but quantitatively microcrustaceans were dominant what indicates a good environmental status opposite to the state of water we recorded for lake Sakadaš. Meiofauna is generally not considered phytophilous fauna according to Tessier et al. (2004), but it is found in water column around the plants and has an important role in a food web (Linhart et al., 1998).

Abundances of recorded invertebrate taxa compared by ANOSIM, separated months of sampling: July and August ($R = 0.184$, $p < 0.05$), and July and September ($R = 0.462$, $p < 0.04$), what is presented in a form of a NMDS plot on Fig. 3. For invertebrate abundance ANOSIM detected statistically significant difference between stations I and III ($R = 0.709$, $p < 0.004$) and II and III ($R = 0.512$, $p < 0.004$), but there was no statistical difference between the stations I and II. Separation of station III is shown on Fig. 4 as NMDS plot. In addition, this station showed greater diversity than the first and second investigated stations, according to the diversity indices: Shannon-Wiener diversity index (H' = 1.43), Simpson index (1-D = 0.71) and species richness as Margalef's d (d = 0.84). Most likely, the fact that the stand at station III lasted the longest is the reason for separating of the third station concerning invertebrate abundance during all three month, plants in other two stands sank

Fig. 3. Multidimensional scaling of invertebrate abundances, associated with *Myriophyllum spicatum*, at all three stations in lake Sakadaš during investigation period in 2004.

Fig. 4. Multidimensional scaling of macrofaunal abundances, associated with *Myriophyllum spicatum*, at all stations in lake Sakadaš during investigation period in 2004.

to the bottom and sampling wasn't possible. The reasons for that could be that the third stand was the largest of all and covered a greater surface, also that part of the lake is less disturbed either by boats or water fluctuations - waves.

When compared with the previous research of the phytophilous fauna in Kopački rit, conducted in 2001 and 2002, some similarities can be found, since the Čonakut channel is a slow flowing channel, which can during low water-level (in the summer) be referred as a standing water (Bogut et al., 2007a). Results for invertebrate communities with *Ceratophyllum demersum* in that channel showed that there was a dominance of chironomids and oligochaetes as well as insect larvae, cladocerans and nematodes at all investigation stations.

This paper gives informations providing a base for the future research regarding plant – invertebrate relationship as well as bio-assessment of the ecosystem, considering that there are some human activities present. Also, we expect that the further research, combined with earlier published data on water quality status and phytophilous invertebrate composition in the water bodies of the Kopački rit, will help us in understanding and preservation of the floodplain, bearing in mind the importance and uniqueness of the Kopački rit Nature Park.

Conclusion

The trophic state of lake Sakadaš is consistent through years (from mesotrophy to hypertrophy in some periods) and the composition of invertebrates associated with macrophytes is characteristic for eutrophic type of lakes, what can be seen in rather high number of taxa groups found during sampling period and in predominance of chironomids larvae and oligochaetes.

Translated by the authors

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