



Interrelations between *Dreissena polymorpha* colonization and autotrophic periphyton development – a field study in a temperate floodplain lake

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With 6 figures and 1 table

Abstract: Temporal changes in autotrophic periphyton (algae and cyanobacteria) in relation to the colonization of *Dreissena polymorpha*, an invasive species, were investigated in situ using artificial substrata (glass slides) from April to August of 2008 in a Danubian floodplain. The colonization of cyanobacteria and planktonic diatoms firmly attached to the substrata followed by the development of apical pad forming diatoms and stalk forming diatoms, resulted in the rapid formation of a three-dimensional structure of periphyton. Increasing water temperatures, together with the well developed autotrophic periphyton, supported the colonization of *Dreissena*. A total of 16 size classes of *Dreissena* were found during the gradual mussel community development. The community most likely formed from the initially settled larvae which dominated the first period of aggregation, while adult individuals dominated the later phase, contributing to the increase in habitat complexity. High competition for space and nutrients on small-size substrata limited the autotrophic periphyton to achieve the “climax community” characterized by filamentous green algae. Consequently, algal abundance decreased and diatoms firmly attached to the substrata prevailed. The decrease in mussel abundance at the end of the colonization experiment enabled a gradual increase in diatom abundance and redevelopment of a three-dimensional autotrophic periphyton structure. Altogether, our results showed that dense coverage of hard surfaces by mussels may have negatively influenced the algal community causing a significant decrease in algal abundance and a shift to the dominance of species resistant to the disturbance.

Key words: Floodplain, Invasive species, Zebra mussel, Periphyton, Algae.

Introduction

As a complex community of bacteria, fungi, cyanoprokaryotes, algae and invertebrates, associated with detritus, periphyton develops on substrates immersed in waters and creates a habitat for various organisms (Vandeboncoeur & Steinman 2002, Azim et al. 2005). The functioning and development of this complex community can be regulated by different abiotic and biotic factors, such as light intensity (Hillebrand et al. 2004), nutrients (Scott et al. 2009, Mulholland &

Webster 2010), competition and grazing (Liboriussen et al. 2005).

In floodplains, the most productive and diverse ecosystems on the earth (Tockner & Stanford 2002), flooding dynamics provides a high level of habitat heterogeneity and supports intensive periphyton development (Rodrigues & Bicudo 2001, Gottlieb et al. 2006). A periphyton colonization study showed that nutrient rich floodplain waters with well developed bacterioplankton and phytoplankton, offer ideal conditions for rapid colonization of autotrophic periphy-

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ton (Mihaljević & Žuna Pfeiffer 2012). Diatoms were the most successful species during the colonization in spring conditions. Colonization of planktonic species and horizontally positioned diatoms followed by stalk-forming to vertically positioned diatoms resulted in the formation of a three-dimensional autotrophic periphyton structure (Mihaljević & Žuna Pfeiffer 2012). It is known that seasonality of physical factors (water temperature, light intensity) can significantly influence progress and structure of autotrophic communities and green filamentous algae usually characterize the mature periphyton in the summer (Hoagland et al. 1982). Periphyton development and production are strongly dependent on the assemblage of autotrophic and heterotrophic species that exist in the community.

Zebra mussel (*Dreissena polymorpha* Pallas, 1771), a Ponto-Caspian invasive species (Qualls et al. 2007) can be abundant in periphyton. It has become one of the most dominant bivalves in many European freshwater rivers, lakes and floodplains (Mihuc et al. 1999, Padilla 2005, Bogut et al. 2010, Lajtner & Crnčan 2011). *D. polymorpha* has a complex life cycle that includes two larval forms (trochophore and veliger) and a few mussel forms (plantigrade, juvenile and adult) differing in size and age stages (Ackerman 1995). Known as “ecosystem engineers” (Jones et al. 1997), mussels influence both the structure and function of the environment they invade (Karatayev et al. 2002, Mayer et al. 2002). Thus, mussels affect benthic macroinvertebrate communities by enhancing conditions for deposit-feeding organisms and small predatory invertebrates (Ricciardi et al. 1997). Especially important is their influence on algal communities. Dreissenids can be dominant consumers of phytoplankton resulting in a decrease in phytoplankton abundance and an increase in water clarity. As a consequence of greater light availability, benthic algae and macrophytes can develop more intensively and a shift from phytoplankton- to macrophyte-dominated state could be established (Pillsbury et al. 2002, Vanderploeg et al. 2002). Spatially complex aggregations of abundant dreissenid shells provide habitat for diverse algal assemblages (Makarevich et al. 2008). Furthermore, dreissenids may affect nutrient availability, and consequently algal composition. Their selective feeding is an important selective force favoring dominance of colonial cyanobacteria in summer (Vanderploeg et al. 2002).

The aim of this study was to determine short-term changes in the autotrophic periphyton community during the colonization on artificial substrates in floodplain waters of Kopački Rit Nature Park, one of the

largest floodplains of the Danube River. *D. polymorpha* has recently been found in the floodplain habitats on different types of substrates including sandy silt sediment (Bogut & Vidaković 2002) and different macrophyte species (Bogut et al. 2010). Thus, the specific goals were to find out how the colonization of *D. polymorpha* affects autotrophic periphyton community development, and to provide a deeper insight into the size structure of the *D. polymorpha* community.

Material and methods

Study site

The colonization experiment was carried out in the floodplain area of Kopački Rit Nature Park (Croatia) situated between river 1,383 and 1,410 km of the Danube (Fig. 1). The dominant wetland types are permanent freshwater marshes/pools, ponds, marshes, and swamps on organic soils, with emergent vegetation waterlogged during most of the growing season. The area is part of the Pannonian biogeographic region and has a typical continental climate with wide annual fluctuations of air temperatures and precipitation distribution, as well as four distinctive seasons. The study site, Lake Sakadaš, is located in the western part of the floodplain (close to the embankment) and in direct hydrological connection with the Danube main channel through two channels (Csonakut and Hulovo channels, total length ca. 10 km). The experimental site was located in the eastern part of the lake, close to the mouth of Csonakut channel. The average depth of the lake is 5 m and the surface water area is approximately 0.15 km². During the past few decades, the lake has been in eutrophic/hypertrophic state according to the OECD system (OECD 1982), with an annual average total phosphorus concentration higher than 100 µg l⁻¹, water transparency less than 1.5 m, and maximum phytoplankton chlorophyll-*a* concentration higher than 50 µg l⁻¹ (Mihaljević & Stević 2011).

Study design and sampling

Artificial substrates, microscope glass slides (37.5 cm²), mounted into modified plastic box holders and oriented vertically in the water column were placed at a depth of 0.2 m in the lake. In total, 4 plastic box holders were fastened to a frame that floated on the water surface and was anchored with rope to stone blocks on the lake bottom (for details, see Palijan 2010). Periphyton was sampled at weekly intervals (7–8 days) during the spring-summer period, from 22 April (day 7) to 19 August 2008 (day 126). Thus, the colonization study comprised of 18 sampling occasions in total. On each sampling occasion three slides for fauna analyses, three slides for autotrophic periphyton community analyses, three slides for chlorophyll analyses and three slides for periphyton biomass analyses were taken randomly. Samples for fauna analysis were transported to the laboratory in bottles containing a 4 % solution of formaldehyde. Samples for chlorophyll analyses, autotrophic species analyses and total periphyton biomass determination were placed separately into glass bottles with sterile water. In order to minimize cell death or dehydration, periphyton samples were transported to the laboratory within 1 hour after sampling in a dark plastic

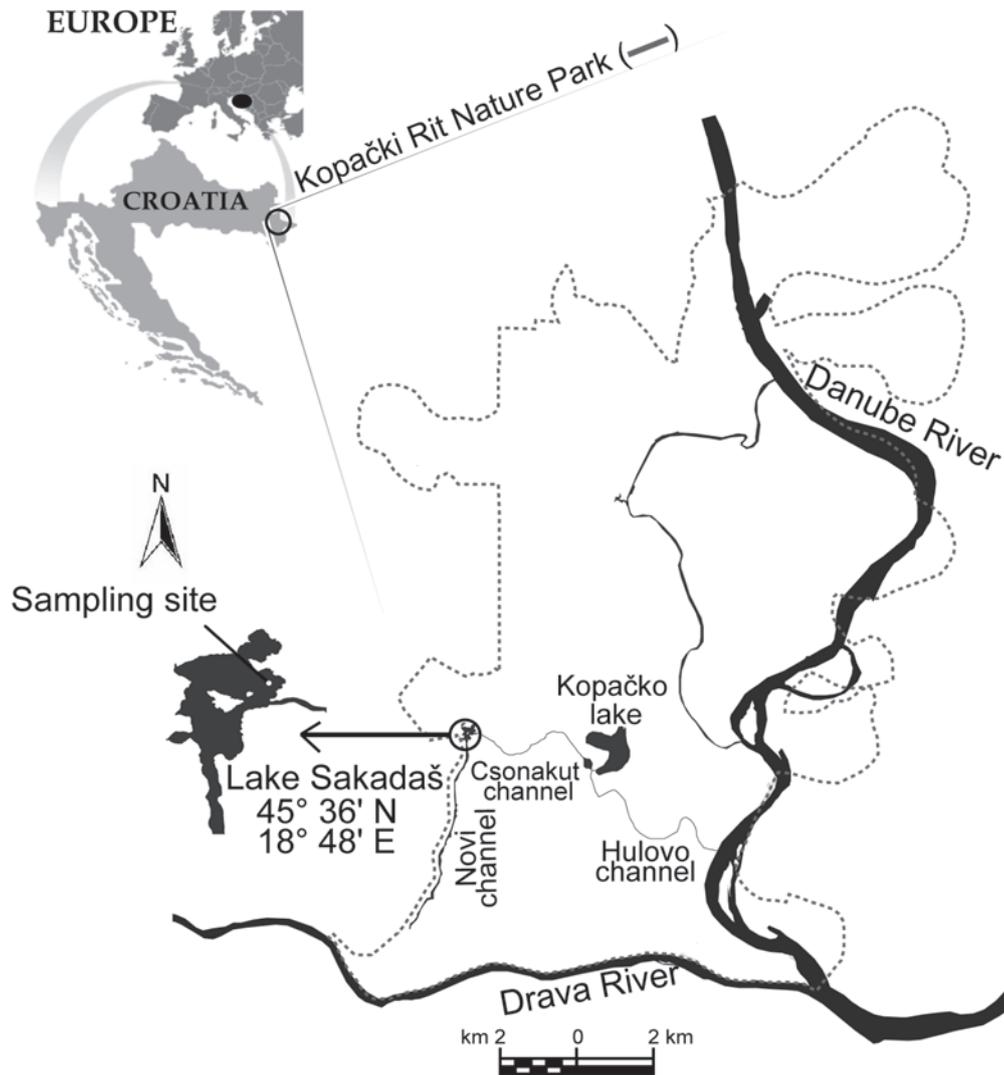


Fig. 1. Study area: Lake Sakadaš, a part of the Danubian floodplain area of the Nature Park Kopački Rit.

box at low temperature. Simultaneously with periphyton sampling, water samples for chemical analyses were taken just beneath the water surface (approximately at 0.2 m depth) at the study site.

Physico-chemical analyses

Ammonium, nitrate, nitrite, total nitrogen and total phosphorus were analyzed according to APHA (1985). Water temperature, pH, dissolved oxygen concentration, and conductivity were measured in the field using the Multi 340 i/set. Water depth and transparency were measured with a Secchi disc. For chlorophyll concentration, water samples and periphyton scraped from the glass slides were filtered through a Whatman GF/C glass fibre filter and extracted with acetone. Concentrations of phytoplankton chlorophyll-*a* and periphyton chlorophyll-*a* were determined according to SCOR-Unesco (1966) and Strickland and Parsons (1968). Periphyton biomass (dry weight, DW, and ash-free dry weight, AFDW) were analyzed according to APHA (1985).

Autotrophic periphyton community analyses

Autotrophic periphyton species (cyanobacteria and algae) were identified at least to species level using a microscope (Carl Zeiss Jena) at multiple magnifications (400×, 1000×) and standard literature for species determination. For quantitative analysis, the individuals of each species (cell, filament or colony) were counted (Sekar et al. 2004, Szabó et al. 2008) after sedimentation in a counting chamber with a millimeter grid with an area of 1 cm² and using the light microscope (Carl Zeiss Jena). Only diatom taxa were counted as a group. To identify the diatoms, periphyton subsamples were cleaned in distilled water, H₂O₂ and HCl treated (Szabó et al. 2008). In each cleaned sample, 300–400 valves were counted and identified to species level. The total number of each diatom species was calculated as a ratio between the number of diatom valves counted on samples embedded in Canada balsam and the total number of diatoms counted on a millimeter grid. Autotrophic periphyton species were expressed as the number of individuals per substrate area (cm²) (Stilinović & Plenković-Moraj 1995). Only species that

contributed with a minimum of 5% to the total abundance were considered to be dominants. The periphyton from each glass slide was analyzed separately, and average values of three slides were used for statistical analysis.

Dreissena polymorpha analyses

In the laboratory, samples of periphyton were scraped off with a razor, rinsed with tap water and sieved through a 24 µm mesh aperture sieve. All material retained on the sieve was examined under a dissecting microscope (Carl Zeiss Jena and Olympus SZX9) under different magnifications (1.5–20×). *D. polymorpha* individuals were separated, counted and measured lengthwise (mm), and placed into size classes according to their shell length. In total, 16 size classes were defined: individuals which measured less than 0.5 mm belonged to the size class I, between 0.5 and 1 mm to size class II, and then every subsequent size class was determined for the individuals 1 mm bigger than in the previous category (Smit et al. 1992, Strayer & Malcom 2006). Size class I, size class II and size class III include pediveliger and early plantigrade stage, late plantigrade stage, and juvenile stages, respectively. Adult mussels were classified in size classes IV to XVI. Mussels from each glass slide were analyzed separately, and average values of three slides were used for statistical analysis. Zebra mussel abundance is expressed as the number of individuals per substrate area (cm²).

Statistical analyses

Redundancy analysis (RDA) was performed using Canoco 4.5 (Biometrics-Plant Research International, the Netherlands) in order to allow identification of physico-chemical variables correlated with the abundance of the *Dreissena polymorpha* size classes and dominant autotrophic species abundance on the glass slides. RDA was selected according to an initial detrended correspondence analysis (DCA) with gradient length <3.0, which suggested a linear ordination model (Lepš & Šmilauer 2003). Abundances of *D. polymorpha* and autotrophic periphyton species were logarithmically transformed ($\log_{10}(x+1)$) to obtain a normal distribution. The statistical significance of the RDA axes was tested by Monte Carlo permutation tests. The importance of each variable was assessed using forward selection.

Hierarchical cluster analysis was used to determine similarity among samples, linking the samples in hierarchical

groups based on similarity and represented by a dendrogram using Primer 5.0 software (Clarke & Warwick 2001). Similarities were calculated for every pair of samples using the Bray-Curtis similarity index computed on the abundance of dominant autotrophic periphyton species and on the *D. polymorpha* abundance separately. Environmental variables (except pH and conductivity), abundances of *D. polymorpha* and dominant autotrophic periphyton species were logarithmically transformed ($\log_{10}(x+1)$) for hierarchical cluster analysis.

Results

Environmental parameters and periphyton biomass

Limnological conditions at the sampling site are summarized in Table 1. Water depth of the lake did not fall under 6.5 m due to the frequent influx of floodwaters into the lake during the spring and summer. Water transparency was the highest (3.2 m) in the middle of May, while it was very low (0.7 m) in July and August. A significant negative correlation between transparency and phytoplankton chlorophyll-*a* concentration ($r = -0.73$, $p = 0.001$) was found. Water temperature increased above 20.0 °C in the middle of May, and peak values were measured in June (28.8 °C) and July (27.7 °C). Dissolved oxygen concentration was high (7.8–13.8 mg l⁻¹) during the colonization experiment indicating a well-oxygenated upper layer of water. pH values (7.5–8.7) indicated low alkaline conditions. In spite of significant variations in nutrient concentrations, the floodplain lake retained high nutrient concentrations (mean values 193 µg l⁻¹ of total phosphorus and 954 µg l⁻¹ of total nitrogen).

Periphyton biomass (expressed as DW and AFDW) gradually increased from the beginning of the colonization experiment towards day 119 (Fig. 2). A substantial decrease in biomass was recorded on day 126.

Table 1. Mean, minimum and maximum values of environmental parameters recorded during April – August 2008 (N = 18).

Parameters	Mean	Min.	Max.
Water depth (WD, m)	7.7	6.5	8.3
Transparency (Secchi depth – SD, m)	1.4	0.7	3.2
Water temperature (WT, °C)	23.0	16.4	28.8
Dissolved oxygen (DO, mg l ⁻¹)	9.8	7.5	13.8
pH	8.2	7.5	8.7
Conductivity (Cond, µS cm ⁻¹)	463	332	803
Ammonium (NH ₄ , µg l ⁻¹)	193	4	2761
Nitrate (NO ₂ , µg l ⁻¹)	239	17	1067
Nitrite (NO ₃ , µg l ⁻¹)	26	2	227
Total nitrogen (TN, µg l ⁻¹)	954	104	3704
Total phosphorus (TP, µg l ⁻¹)	193	39	334
Phytoplankton chlorophyll- <i>a</i> (PhytoChl _a , µg l ⁻¹)	25.1	7.2	46.0

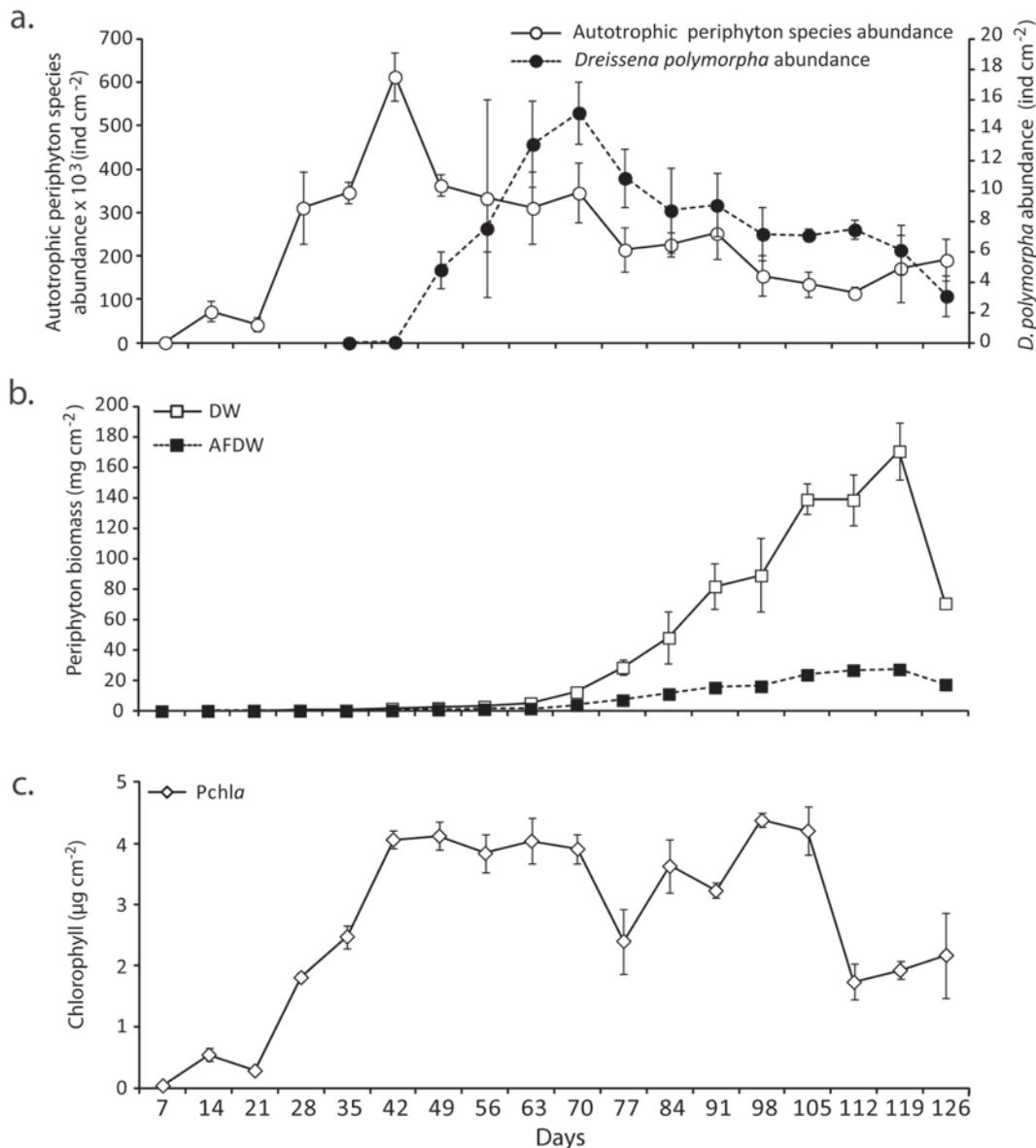


Fig. 2. Temporal variations of (a) autotrophic periphyton species and *Dreissena polymorpha* abundance, (b) periphyton dry weight (DW) and ash-free dry weight (AFDW) and (c) periphyton chlorophyll-*a* concentrations (PChla) during the research period (day 7 – day 126; N=3, ± SD).

Periphyton chlorophyll-*a* concentrations increased until day 42 (4.1 µg cm⁻²), the highest concentration (4.4 µg cm⁻²) was recorded on day 98, while lower values (1.7–2.2 µg cm⁻²) were recorded at the end of the colonization experiment (days 112–126).

Autotrophic periphyton community

A total of 199 autotrophic taxa were identified in the periphytic communities during the experiment. The number of taxa varied from 55 taxa on day 7 to 94 taxa on day 91. The total abundance of autotrophic pe-

riphyton taxa (Figs 2 and 3) increased from 1.7 × 10³ ind. cm⁻² (day 7) to 613.9 × 10³ ind. cm⁻² (day 42). Cyanobacterial species *Aphanocapsa delicatissima* W. West et G. S. West, *Gleocapsa punctata* Nägeli and *Phormidium mucicola* Hub.-Pest. et Naum were dominant only on day 7 and contributed up to 86% of the total abundance. The abundance of diatoms increased during the colonization experiment and they dominated from day 14 onwards. On days 14 and 21, the most abundant were *Fragilaria ulna* (Nitzsch) Lange-Bert., *Gomphonema acuminatum* Ehrenb., *G. olivaceum* (Lyngb.) Desm., *G. parvulum* (Kütz.) Kütz.,

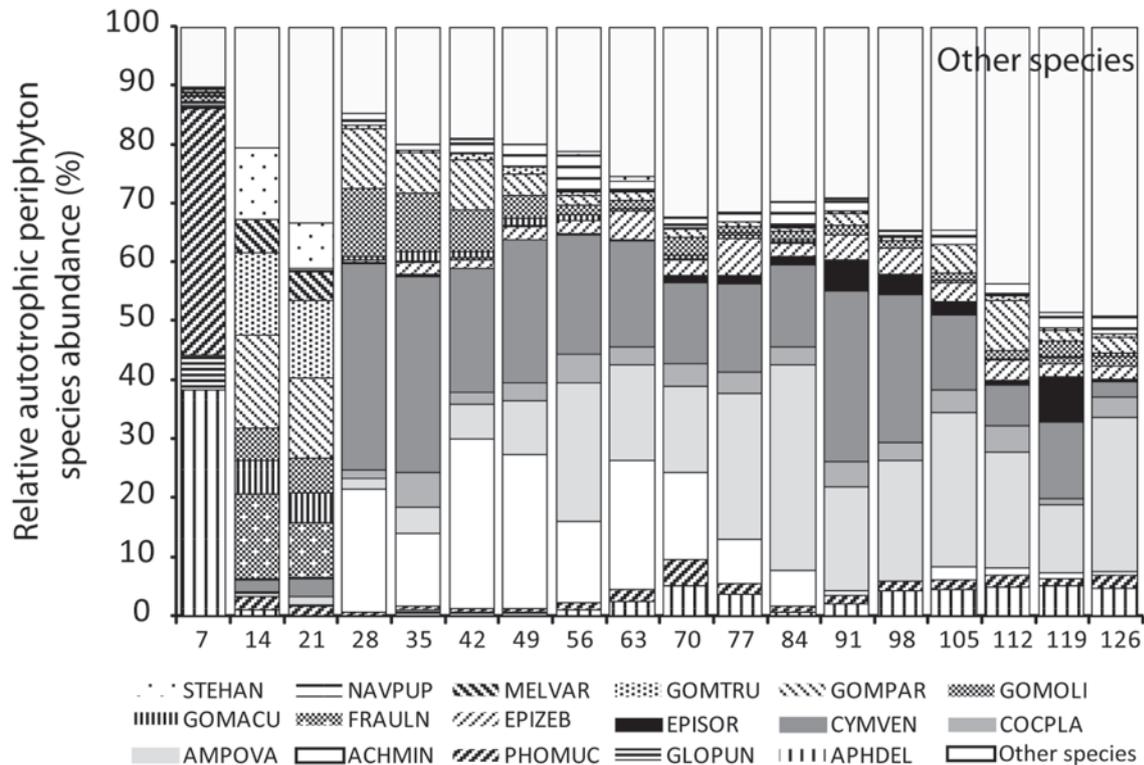


Fig. 3. Changes in the relative abundance of autotrophic periphyton species during the study period (day 7 – day 126). Legend: *Achnantheidium minutissimum* ACHMIN, *Amphora ovalis* AMPOVA, *Cocconeis placentula* COCPLA, *Cymbella ventricosa* CYMVEN, *Epithemia sorex* EPISOR, *Epithemia zebra* EPIZEB, *Fragilaria ulna* FRAULN, *Gomphonema acuminatum* GOMACU, *Gomphonema olivaceum* GOMOLI, *Gomphonema parvulum* GOMPAR, *Gomphonema truncatum* GOMTRU, *Melosira varians* MELVAR, *Navicula pupula* NAVPUP, *Stephanodiscus hantzschii* STEHAN, *Aphanocapsa delicatissima* APHDEL, *Phormidium mucicola* PHOMUC, *Gloeocapsa punctata* GLOPUN. Species which contributed with less than 5% of the total autotrophic periphyton abundance were grouped into “other species”. Data represent the mean value of three glass slides.

G. truncatum Ehrenb. and *Stephanodiscus hantzschii* Grun. After that, to the end of the research period, *Achnantheidium minutissimum* (Kütz.) Czar, *Cymbella ventricosa* Kütz. and *Amphora ovalis* (Kütz.) Kütz. were the most abundant.

***Dreissena polymorpha* abundance and size classes**

Zebra mussels started to colonize the substrate in May, on day 35 (Figs 2 and 4). Only a few individuals were found on days 35 and 42. Afterwards, the abundance increased more rapidly and the highest total abundance (15.9 ind. cm⁻²) was recorded on day 70. From day 56 to day 70 more than 10 ind. cm⁻² were found. After the maximum, the abundance of *D. polymorpha* decreased towards the end of the study (Fig. 2). Colonization of *Dreissena* began with a few pediveliger individuals (I) (Fig. 4). During the initial colonization period, the *Dreissena* community comprised only larval stages. Size class I and II individuals were present

in the periphyton community until July (days 77 and 91, respectively). The representatives of juvenile (III) and early adult stages (IV) appeared first in June, on day 56. On the last few sampling dates, size classes VIII, IX and X were most abundant. Size classes XV and XVI were found only in samples from the last two sampling dates (Fig. 4).

***Dreissena polymorpha* and autotrophic species in the environmental gradient**

The eigenvalues for RDA axis 1 (0.370) and axis 2 (0.212) explained 58.1% of the variance in the autotrophic periphyton species and *Dreissena* size classes. The species-environment correlation of RDA axis 1 and RDA axis 2 were 0.988 and 0.918, respectively, indicating a significant relationship between environment variables and 16 size classes of *D. polymorpha* and 17 dominant periphyton cyanobacterial and algal species. Most of the variance contained in the first and second RDA axes is described by the following environmen-

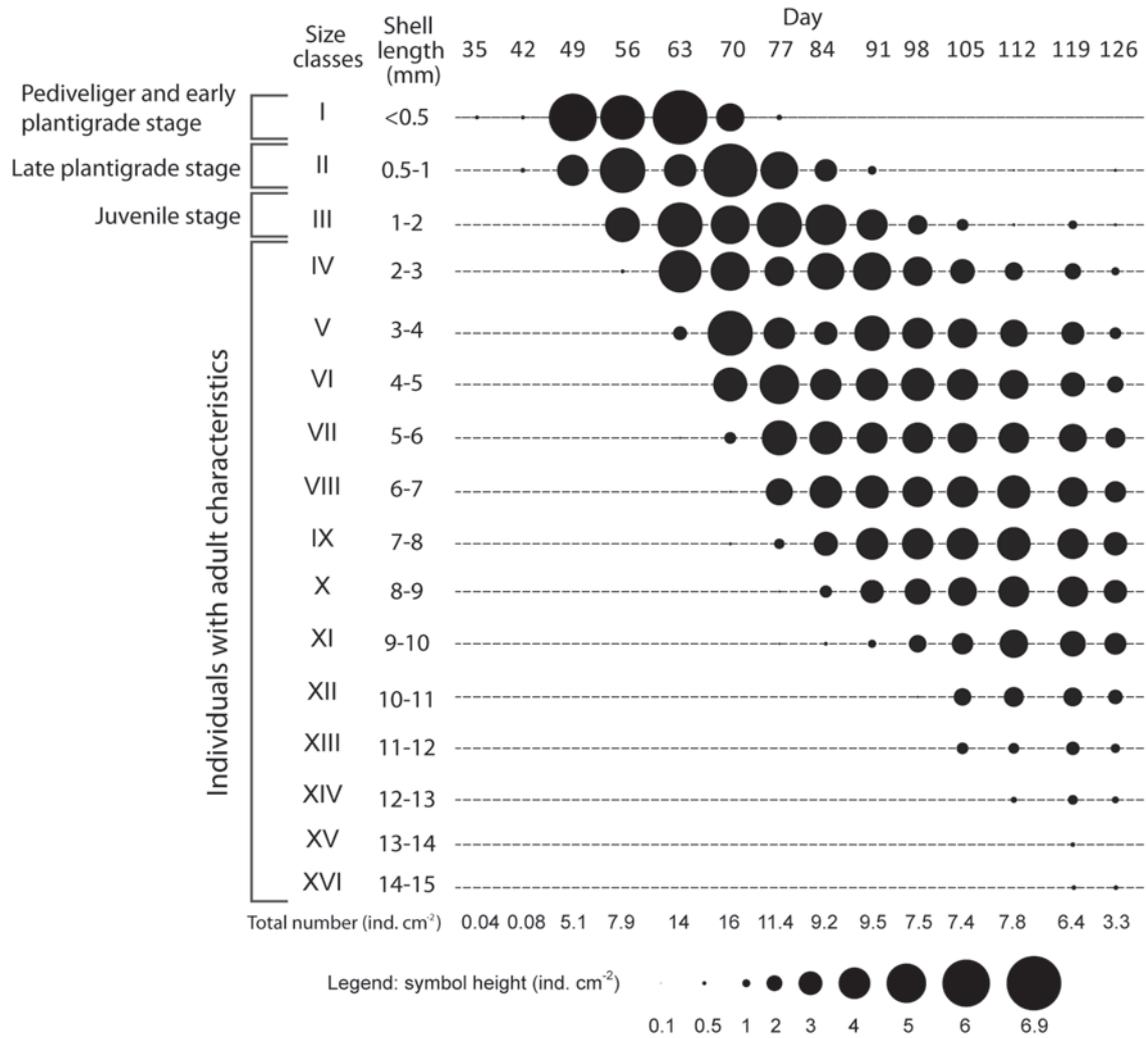


Fig. 4. Temporal variation of the abundance of *Dreissena polymorpha* size classes (I–XVI) during the research period (day 35 – day 126). Log₁₀(x + 1) transformed abundances of *D. polymorpha* were used for the plot. Maximum symbol height = 6.9 individuals per cm². Data represent the mean value of three glass slides.

tal parameters (Fig. 5): dissolved oxygen concentration ($r_{\text{RDAaxis1}} = +0.58$), conductivity ($r_{\text{RDAaxis1}} = +0.52$), periphyton chlorophyll-*a* concentration ($r_{\text{chlaRDA axis1}} = -0.66$), phytoplankton chlorophyll-*a* ($r_{\text{RDAaxis1}} = -0.50$; $r_{\text{RDAaxis2}} = -0.42$), periphyton biomass ($r_{\text{DW RDAaxis1}} = -0.53$, $r_{\text{DW RDAaxis2}} = -0.55$), water temperature ($r_{\text{RDAaxis2}} = -0.83$) and transparency ($r_{\text{RDAaxis2}} = +0.59$). RDA indicated that the early colonization phase was characterized by high transparency, dissolved oxygen concentration and conductivity (Fig. 5); presence of early developmental stages of *D. polymorpha* was characterized by high periphyton chlorophyll-*a* concentration, transparency and water temperature, while later developmental stages were associated with periphyton biomass and phytoplankton chlorophyll-*a*. Accord-

ing to RDA, nutrient concentrations in water were not identified as significant variables.

The results of hierarchical clustering (Fig. 6a) based on the abundance of autotrophic periphyton species at Bray-Curtis similarity level of approximately 80% revealed five distinctive groups. The first three groups encompassed the phases of gradual colonization of autotrophic species to the formation of a three-dimensional community structure. The fourth group represented a disrupted autotrophic periphyton community (species firmly attached to the substrate), while the fifth group encompassed the phases of gradual redevelopment of a periphyton community. The results of hierarchical clustering (Fig. 6b) based on abundance of *D. polymorpha* size classes at Bray-Curtis similarity



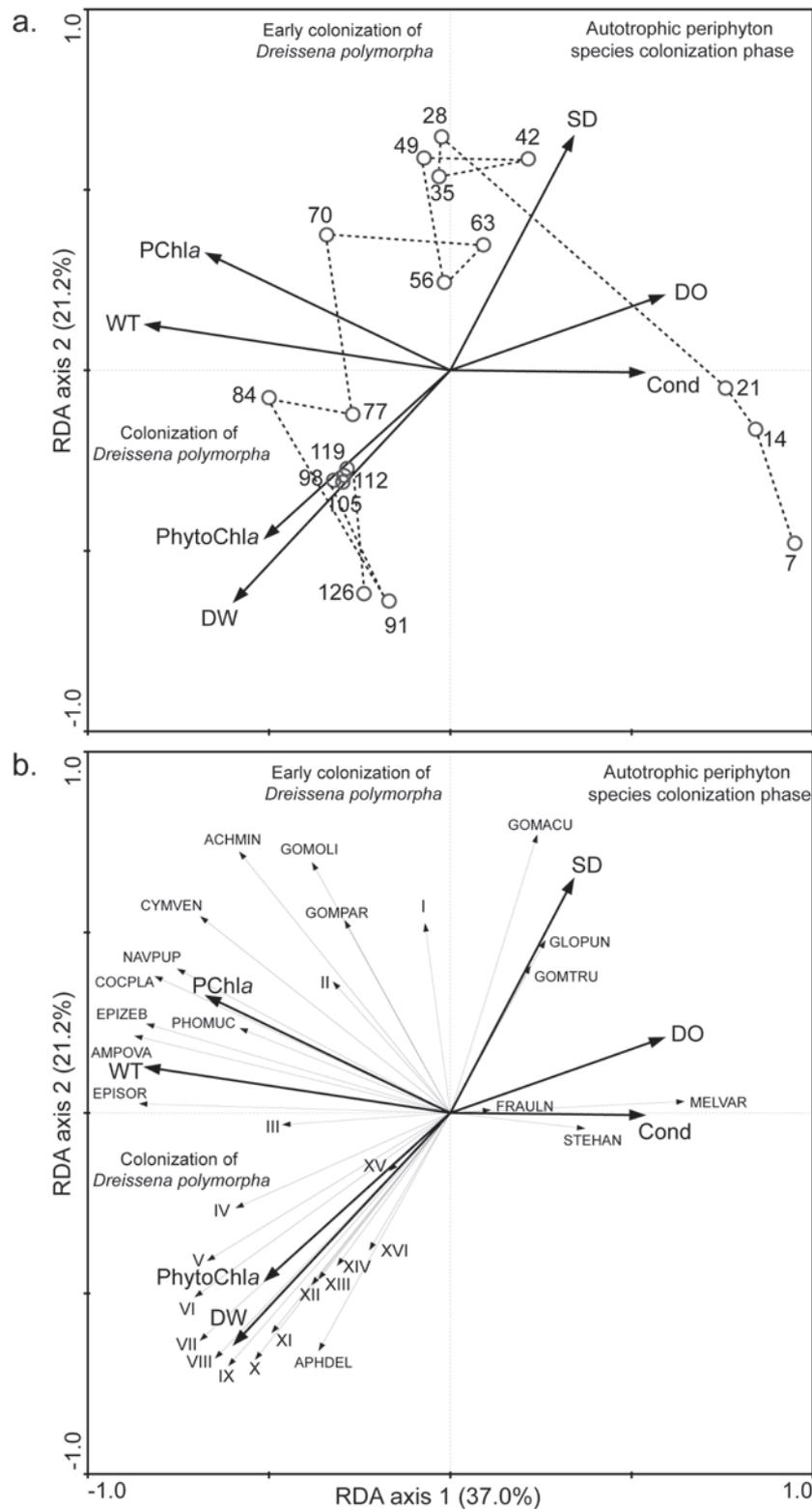


Fig. 5. Redundancy analysis based on the dominant autotrophic periphyton species, size classes of *Dreissena polymorpha* and environmental variables during the study period (day 7 – day 126) in 2008 in Lake Sakadaš. **(a)** ordination of the samples and environmental variables; **(b)** ordination of the autotrophic periphyton species and size classes of *D. polymorpha* and environmental variables. Only significant environmental variables are presented in the plot; see the legend of Fig. 3 for species code and Table 1 for environmental variables; Arabic numbers represent size classes of *D. polymorpha*, sample dots are connected with a dashed line showing the time trajectory.

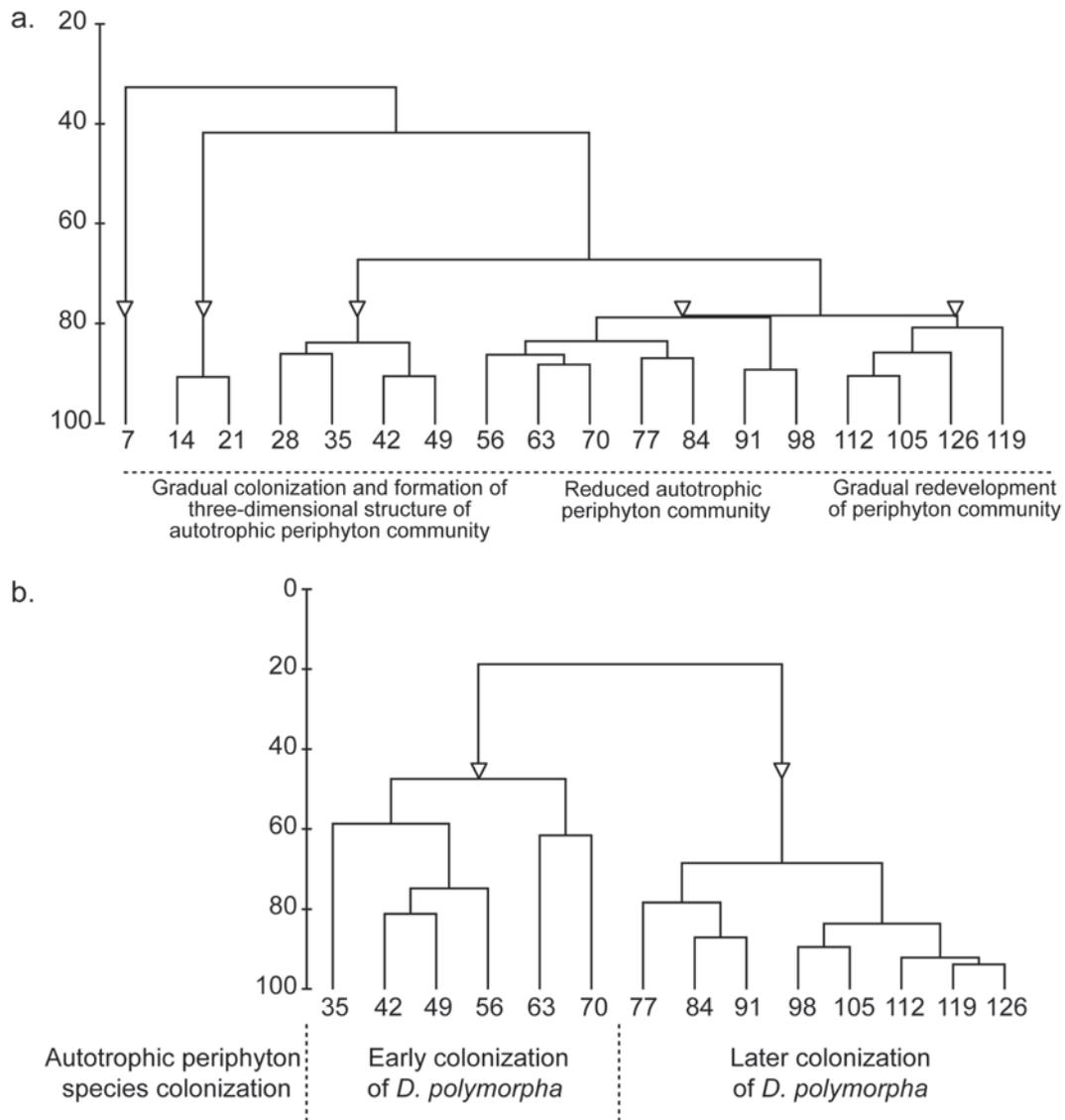


Fig. 6. Dendrograms based on the dominant autotrophic periphyton species (**a**) and the abundance of *Dreissena polymorpha* size classes (**b**) during the study period (day 7 – day 126) in 2008 in Lake Sakadaš.

level of approximately 50% revealed two distinctive groups. The first group included early developmental stages of *D. polymorpha*, while the second group encompassed the development of larger individuals of *D. polymorpha*.

Discussion

Periphyton development on artificial substrates in the investigated floodplain lake is a very dynamic process and the colonization of zebra mussels has a profound influence on the succession and community structure of the periphytic autotrophs. According to our statisti-

cal analyses, distinctive phases in periphyton development can be defined. From the beginning of the colonization experiment, gradual colonization of autotrophic species led to the formation of a three-dimensional community structure. Progressive settling and development of *D. polymorpha* caused the disruption of the autotrophic periphyton community, while the decrease in mussel abundance at the end of the colonization experiment probably enabled an increase in autotrophic species abundance and gradual redevelopment of the periphyton community.

High concentrations of organic matter in floodplain waters, high abundance of bacteria (Palijan 2010) and well developed phytoplankton communities, as was

shown by high phytoplankton chlorophyll-*a* concentrations, supported the formation of a “conditioning film” (Ács et al. 2007) which enabled rapid periphyton development (Hoagland et al. 1982). Cyanobacteria (*Aphanocapsa*, *Gloeocapsa*, *Phormidium*) and planktonic diatoms (*Stephanodiscus hantzschii*) are able to attach to the already developed biofilm (Ács et al. 2007) and were among the first species colonizing the artificial substrata. Further immigration of diatoms can be related to their morphological characteristics. Thus, from the second week, large-bodied, apical pad forming *Fragilaria ulna* and *Melosira varians* settled more rapidly to the surface of the substratum (Ács & Kiss 1993) and reached a high relative abundance probably supported by high conductivity. *F. ulna* was also found as a pioneer colonist of artificial substrates in the River Danube (Ács & Kiss 1993, Ács et al. 2000).

Apical pad forming diatoms were replaced by stalk forming *Gomphonema* and *Cymbella* species and by *Achnantidium minutissimum* which can have a different position in the periphytic community. During the colonization process, these adherence strategies are useful adaptations to avoid competition for nutrients and light (Bahulikar 2006).

The development of heterotrophic organisms in the periphytic communities generally depends on the production of autotrophic periphyton species (Haack & McFeters 1982). Thus, colonization of *D. polymorpha* began in the late spring (May) after the formation of the well developed autotrophic periphyton community. The colonization process of *Dreissena* species was correlated with increasing water temperature and decreasing conductivity, as was shown by the RDA. It is known that all stages of *Dreissena*'s life cycle have a strong dependency on water temperature. In most cases, 10–12 °C is the threshold temperature for growth of the mussel; at 30 °C growth stops and at 32 °C individuals die (Karatayev et al. 2006). Dreissenids have different modes of spreading during different stages in their life cycles, as planktonic larval stage or settling benthic stages (Son 2007). *Dreissena* eggs usually remain immature during the winter-early spring period. Also, the overwintering plantigrade larvae can represent a source for the development of a new population translocating from winter position to a plant or other new substrate (Claudi & Ackerman 1992). Pediveliger finds an adequate substrate for settlement and metamorphoses into postveliger or plantigrade stage within 18–90 days after fertilization, which can be prolonged if there are no suitable surfaces covered with algae (Ackerman et al. 1994). The suitable temperature range of 16 to 19 °C for

larval production (Sprung 1993) was reached at the beginning of the study period (16.6–20.4 °C). After the appearance of first individuals of size class I, we recorded the first peak after two weeks, and the second peak two weeks after that, while Nichols (1996) found that larval abundance is rather low at the beginning of the larval production and that a period of 4 to 8 weeks is required for the first of the two peaks to occur. A similar occurrence was found for size class II individuals. According to the two-phase model, the settlement of pediveliger and metamorphosis is the first phase; the second phase is translocation of plantigrade, juvenile and adult mussels (Ackerman et al. 1994). As the colonization progressed there was a shift from the predominance of smaller sized individuals and an increase in the higher size class abundance, indicating the development of the *Dreissena* community from the initial association, i.e. primary settled larvae and juveniles on the glass slides. If the adult mussels colonized the slides from the surroundings, there would be more large-shelled individuals recorded during the earlier phases of the colonization process. The adult mussels gradually grew, with maximum sizes reached on the last two sampling dates. Individuals were mostly arranged in clusters and often near the edges of the slides; however, as the periphyton community developed and mussel abundance rose, they filled all available spaces. Zebra mussels prefer edges and angles, and usually live in dense aggregations (Stańczykowska 1964, Kobak 2004) probably as a strategy to avoid predators (Kobak & Kakareko 2009). Furthermore, Lauer (1997) noticed that a newly created space in the centre of a *Dreissena* colony will be rapidly re-colonized by the mussels. Competition for space, related to competition for food and oxygen, could motivate the movement of mussels (Lauer 1997). Glass slides, being a firm surface, submersed into water near the surface, provide a very good substrate for the development of the abundant *Dreissena* community, which could be seen in a densely formed association in Lake Sakadaš. In shallow waters, zebra mussels can grow faster, possibly due to a more suitable temperature and more available food than at larger depths (Mikheev 1964, Garton & Johnson 2000). For example, Mikheev (1964) found that yearling mussels reached the size of 14 mm, at 1–1.5 m depth. The maximal shell length mussels reached during our colonization experiment was 15 mm, but most abundant of the adult mussels were those belonging to size classes VIII, IX and X, i.e. 6–9 mm in length. The size of the slides could have been a limiting factor for the abundance and size of the individuals, since

the slides were packed with *Dreissena* druzes towards the end of study.

Low abundance of mussels in early developmental stages (e.g. pediveliger, plantigrade stage) has not significantly influenced autotrophic periphyton development and an increase in periphyton chlorophyll-*a* concentration and abundance of autotrophic species was continued. As a result, a complex three-dimensional autotrophic periphyton structure supported by stalk-forming diatoms (*Gomphonema*, *Cymbella*) was formed. However, a significant decrease in the total autotrophic species abundance established on day 49 indicated that loss processes existed. Death, emigration and sloughing of periphyton organisms due to light and nutrients limitation in a quite developed periphyton community, as well as grazing pressure, are well known factors that may lead to a decrease in autotrophic species abundance (Biggs 1996). As a result, structural changes in algal communities occurred in the way that firmly attached, prostrate diatoms (*Cocconeis*, *Amphora*, *Epithemia*) became dominant. These species generate a high amount of mucus and are therefore more resistant to abrasion and grazing (Ferreira & Seeliger 1985, Pillsbury et al. 2002).

D. polymorpha has a series of attributes that may influence abundance and structure of the autotrophic periphyton community. As efficient filter-feeders, mussels remove particulate organic matter from water thereby increasing water transparency that leads to an increase in benthic algal production (Pillsbury et al. 2002, Cecala et al. 2008). Furthermore, mussels excrete nutrients and their shells may increase habitat heterogeneity and three-dimensionality of heterotrophic periphyton, supporting the development of high taxonomic diversity and abundance of autotrophic species (Nalepa & Fahnenstiel 1995, Makarevich et al. 2008, Fortino 2010). Therefore, although physical and biological attributes of *D. polymorpha* probably have an important role, we believe that the abundant mussel aggregations on small-sized artificial substrates used for the investigation, and consequently the lack of the space necessary for the periphyton growth, were the most important factors controlling the changes in autotrophic communities. Since *D. polymorpha* comprised a largest portion of the total periphyton biomass, as it was shown by the significant correlation between the mussels' later developmental stages and total DW, competition for space was expressed and overgrowth of periphyton algae was limited. Furthermore, a correlation between the later developmental stages and phytoplankton chlorophyll-*a* indicate that mussels' high filtration activity influenced the phytoplankton

community. Thus, intensive development of green algae to achieve a periphyton "climax community" was probably prevented by the filtration activity of abundant mussels that may consume zoospores of the green algae floating in the water column (Reutter et al. 1993). Only *Aphanocapsa delicatissima*, a cyanobacteria with a mucilaginous sheath, was associated with the later developmental stages of *D. polymorpha* indicating that this species was not significantly affected by intensive development of zebra mussel (Heath et al. 1995). It has been suggested that *D. polymorpha* avoid filtering certain algal species, especially those with thick mucilaginous sheaths around the cells, probably due to the large size and shape of the colonies (Pillsbury et al. 2002, Dionisio Pires et al. 2005). The decrease in mussel abundance at the end of the colonization experiment enabled a gradual increase in the autotrophic community. Progress in periphyton development was supported by the development of stalk-forming diatoms (*Gomphonema* spp., *Cymbella*) resulting in redevelopment of the three-dimensional autotrophic periphyton structure.

Conclusions

Rapid development of the three-dimensional autotrophic periphyton community creates favorable substrata for the colonization of *Dreissena polymorpha*. Gradual growth and the formation of abundant mussel aggregations most likely suppressed further progress and overgrowth of autotrophic species. High competition for space on small-size substrata could be one of the main factors that limited the autotrophic species development and shifted the community to the dominance of species resistant to disturbance. Altogether, long-term monitoring of population dynamics of *D. polymorpha* is necessary to elucidate the consequences of the invasion of this species in dynamic and complex floodplain habitats.

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References

- Ackerman, J. D., 1995: Zebra mussel life history. – In: Proceedings of the Fifth International Zebra Mussel and Other Aquatic Nuisance Organisms Conference. – Toronto, pp. 1–8.



- Ackerman, J. D., Sim, B., Nichols, S. J. & Claudi, R., 1994: A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. – *Can. J. Zool.* **72**: 1169–1179.
- Ács, É. & Kiss, K. T., 1993: Colonization process of diatoms on artificial substrates in the River Danube near Budapest (Hungary). – *Hydrobiologia* **269/270**: 307–315.
- Ács, É., Kiss, K. T., Szabó, K. & Makk, J., 2000: Short-term colonization sequence of periphyton on glass slides in a large river (River Danube, near Budapest). – *Arch. Hydrobiol. Suppl., Algal. Stud.* **100**: 135–156.
- Ács, É., Borsodi, K. A., Kröpfl, K., Vladár, P. & Zárny, G., 2007: Changes in the algal composition, bacterial metabolic activity and element content of biofilms developed on artificial substrata in the early phase of colonization. – *Acta Bot. Croat.* **66**: 89–100.
- APHA, 1985: Standard methods for examination of water and wastewater. 16th ed. – American Public Health Association, Washington, DC.
- Azim, M. E., Verdegem, M. C. J., van Dam, A. A. & Beveridge, M. C. M., 2005: Periphyton. Ecology, exploitation and management. – CABI Publishing, Wallingford, Oxfordshire.
- Bahulikar, R. A., 2006: Diatoms from littoral zone of Lake Constance: Diversity, phylogeny, extracellular polysaccharides and bacterial associations. – Dissertation zur Erlangung des akademischen Grades des Doktors der Naturwissenschaften (Dr. rer. nat.) an der Universität Konstanz, Fachbereich Biologie, Konstanz.
- Biggs, B. J. F., 1996: Patterns in benthic algae of streams. – In: Stevenson, R. J., Bothwell, M. L. & Lowe, R. L. (eds): *Algal Ecology: Freshwater Benthic Ecosystems*. – Academic Press, San Diego, pp. 31–56.
- Bogut, I. & Vidaković, J., 2002: Littoral sediment meiofauna communities of Lake Sakadaš (Kopački Rit Nature Park, Croatia). – In: *Limnological Reports: Proceedings of the 34th IAD Conference*. Tulcea, pp. 3–9.
- Bogut, I., Čerba, D., Vidaković, J. & Gvozdić, V., 2010: Interactions of weed-bed invertebrates and *Ceratophyllum demersum* L. stands in a floodplain lake. – *Biologia* **65**: 113–121.
- Cecala, R. K., Mayer, C. M., Schulz, K. L. & Mills, E. L., 2008: Increased benthic algal primary production in response to the invasive zebra mussel (*Dreissena polymorpha*) in a productive ecosystem, Oneida Lake, New York. – *J. Integr. Plant Biol.* **50**: 1452–1466.
- Clark, K. R. & Warwick, R. M., 2001: Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. – Plymouth Marine Laboratory, Plymouth.
- Claudi, R. & Ackerman, J. D., 1992: Post-settlement movements of juvenile zebra mussels and their potential impact on plant systems. – In: *Corrosion '92: Proceedings of the 47th NACE Annual Conference*, National Association of Corrosion Engineers. – Houston, pp. 34111–34116.
- Dionisio Pires, L. M., Bontes, B. M., van Donk, E. & Ibelings, B. W., 2005: Grazing on colonial and filamentous, toxic and non-toxic cyanobacteria by the zebra mussel *Dreissena polymorpha*. – *J. Plankton Res.* **27**: 331–339.
- Ferreira, S. & Seeliger, U., 1985: The colonization process of algal epiphytes on *Ruppia maritima* L. – *Bot. Mar.* **28**: 245–249.
- Fortino, S., 2010: The effects of zebra mussel (*Dreissena polymorpha*) presence on rock substrate benthic algal concentrations in an inland lake system. – Biological Station, University of Michigan (UMBS).
- Garton, D. W. & Johnson, L. E., 2000: Variation in growth rates of the zebra mussel, *Dreissena polymorpha*, within Lake Wawasee. – *Freshw. Biol.* **45**: 443–451.
- Gottlieb, A. D., Richards, J. H. & Gaiser, E. E., 2006: Comparative study of periphyton community structure in long and short-hydroperiod Everglades marshes. – *Hydrobiologia* **569**: 195–207.
- Haack, T. K. & McFeters, G. A., 1982: Nutritional relationships among microorganisms in an epilithic biofilm community. – *Microb. Ecol.* **8**: 115–126.
- Heath, R. T., Fahnenstiel, G. L., Gardner, W. S., Cavaletto, J. F. & Hwang, S.-J., 1995: Ecosystem-level effects of zebra mussels (*Dreissena polymorpha*): An enclosure experiment in Saginaw Bay, Lake Huron. – *J. Great Lakes Res.* **21**: 501–516.
- Hillebrand, H., De Montpellier, G. & Liess, A., 2004: Effects of macrograzers and light on periphyton stoichiometry. – *Oikos* **106**: 93–104.
- Hoagland, K. D., Roemer, S. C. & Rosowski, J. R., 1982: Colonization and community structure of two periphyton assemblages with emphasis on the diatoms (Bacillariophyceae). – *Am. J. Bot.* **69**: 188–213.
- Jones, C. G., Lawton, J. H. & Shachak, M., 1997: Positive and negative effects of organisms as physical ecosystem engineers. – *Ecology* **78**: 1946–1957.
- Karatayev, A. Y., Burlakova, L. E. & Padilla, D. K., 2002: Impacts of zebra mussels on aquatic communities and their role as ecosystem engineers. – In: Leppkoski, E., Gollasch, S. & Olenin, S. (eds): *Invasive aquatic species of Europe: Distribution, impacts and management*. – Kluwer Academic Publishers, Dordrecht, pp. 443–446.
- Karatayev, A. Y., Burlakova, L. E. & Padilla, D. K., 2006: Growth rate and longevity of *Dreissena polymorpha* (Pallas): A review and recommendations for future study. – *J. Shellfish Res.* **25**: 23–32.
- Kobak, J., 2004: Recruitment and small-scale spatial distribution of *Dreissena polymorpha* (Bivalvia) on artificial materials. – *Arch. Hydrobiol.* **160**: 25–44.
- Kobak, K. & Kakareko, T., 2009: Attachment strength, aggregation and movement of the zebra mussel (*Dreissena polymorpha*, Bivalvia) in the presence of potential predators. – *Fundam. Appl. Limnol.* **174**: 193–204.
- Lajtner, J. & Crnčan, P., 2011: Distribution of the invasive bivalve *Sinanodonta woodiana* (Lea, 1834: Croatia. – *Aquat. Invasions* **6**: 119–124.
- Lauer, T., 1997: Space as a limiting resource among sessile benthic invertebrates: zebra mussels, freshwater sponges and bryozoans. – Dissertation, Purdue University, West Lafayette, Indiana.
- Lepš, J. & Šmilauer, P., 2003: *Multivariate Analysis of Ecological Data using Canoco*. – Cambridge University Press, Cambridge.
- Liboriussen, L., Jeppesen, E., Bramm, M. E. & Lassen, M. F., 2005: Periphyton-macroinvertebrate interactions in light and fish manipulated enclosures in a clear and a turbid shallow lake. – *Aquat. Ecol.* **39**: 23–39.
- Makarevich, T. A., Mastitsky, S. E. & Savich, I. V., 2008: Phytoperiphyton on the shells of *Dreissena polymorpha* (Pallas) in Lake Naroch. – *Aquat. Invasions* **3**: 283–295.
- Mayer, C. M., Keats, R. A., Rudstam, L. G. & Mills, E. L., 2002: Scale-dependent effects of zebra mussels on benthic invertebrates in a large eutrophic lake. – *J. N. Am. Benthol. Soc.* **21**: 616–633.

- Mihaljević, M. & Stević, F., 2011: Cyanobacterial blooms in a temperate river-floodplain ecosystem: the importance of hydrological extremes. – *Aquat. Ecol.* **45**: 335–349.
- Mihaljević, M. & Žuna-Pfeiffer, T., 2012: Colonization of periphyton algae in a temperate floodplain lake under a fluctuating spring hydrological regime. – *Fundam. Appl. Limnol.* **180**: 13–25.
- Mihuc, T. B., Battle, J. M., Mihuc, J. R. & Bryan, C. F., 1999: Zebra mussel (*Dreissena polymorpha*) seasonal colonization patterns in a sub-tropical floodplain river. – *Hydrobiologia* **392**: 121–128.
- Mikheev, V. P., 1964: Linear growth of *Dreissena polymorpha* Pallas in some reservoirs of the European part of the USSR. – *Tr. Ins. Biol. Vnutr. Vod. Akad. Nauk. SSSR* **7**: 55–65.
- Mulholland, P. J. & Webster, J. R., 2010: Nutrient dynamics in streams and the role of J-NABS. – *J. N. Am. Benthol. Soc.* **29**: 100–117.
- Nalepa, T. F. & Fahnenstiel, G. L., 1995: *Dreissena polymorpha* in the Saginaw Bay, Lake Huron Ecosystem: overview and perspective. – *J. Great Lakes Res.* **21**: 411–416.
- Nichols, S. J., 1996: Variations in the reproductive cycle of *Dreissena polymorpha* in Europe, Russia, and North America. – *Am. Zool.* **36**: 311–325.
- OECD, 1982: Eutrophication of waters. Monitoring, Assessment and Control. – OECD Publications, Paris.
- Padilla, D. K., 2005: The potential of zebra mussels as a model for invasion ecology. – *Am. Malac. Bull.* **20**: 123–131.
- Palijan, G., 2010: Different impact of flood dynamics on the development of culturable planktonic and biofilm bacteria in floodplain lake. – *Pol. J. Ecol.* **58**: 439–448.
- Pillsbury, R. W., Lowe, R. L., Pan, Y. D. & Greenwood, J. L., 2002: Changes in the benthic algal community and nutrient limitation in Saginaw Bay, Lake Huron, during the invasion of the zebra mussel (*Dreissena polymorpha*). – *J. N. Am. Benthol. Soc.* **21**: 238–252.
- Qualls, T. M., Dolan, D. M., Reed, T., Zorn, M. E. & Kennedy, J., 2007: Analysis of the impacts of the zebra mussel, *Dreissena polymorpha*, on nutrients, water clarity, and the chlorophyll-phosphorus relationship in lower Green Bay. – *J. Great Lakes Res.* **33**: 617–626.
- Reutter, J., Monaco, M. E., Lorenz, R. C. & Herdendorf, C. E., 1993: The areal and vertical distribution of *Cladophora glomerata* in western Lake Erie and its interaction with the zebra mussel (*Dreissena polymorpha*). – A technical report submitted to The Ohio State University's Lake Erie Program.
- Ricciardi, A., Whoriskey, F. G. & Rasmussen, J. B., 1997: The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. – *Can. J. Fish. Aquat. Sci.* **54**: 2596–2608.
- Rodrigues, L. & de Bicudo, D. C., 2001: Similarity among periphyton algal communities in a lentic-lotic gradient of the upper Paraná river floodplain, Brazil. – *Revta brasil. Bot., São Paulo* **24**: 235–248.
- Scott, J. T., Lang, D. A., King, R. S. & Doyle, R. D., 2009: Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: evidence for differential nutrient limitation in stream periphyton. – *J. N. Am. Benthol. Soc.* **28**: 57–68.
- Sekar, R., Venugopalan, V. P., Nandakumar, K., Nair, K. V. K. & Rao, V. N. R., 2004: Early stages of biofilm succession in a lentic freshwater environment. – *Hydrobiologia* **512**: 97–108.
- Smit, H., Bij de Vaate, A. & Fioole, A., 1992: Shell growth of the zebra mussel (*Dreissena polymorpha* (Pallas)) in relation to selected physico-chemical parameters in the lower Rhine and some associated lakes. – *Arch. Hydrobiol.* **124**: 257–280.
- Son, M. O., 2007: Native range of the zebra mussel and quagga mussel and new data on their invasions within the Ponto-Caspian Region. – *Aquat. Invasions* **2**: 174–184.
- Sprung, M., 1993: The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. – In: Nalepa, T. F. & Schloesser, D. W. (eds): *Zebra mussels: Biology, impacts, and control.* – Lewis Publishers, CRC Press, Boca Raton, pp. 39–54.
- Stańczykowska, A., 1964: On the relationship between abundance aggregations and “condition” of *Dreissena polymorpha* Pall. in 36 Mazurian lakes. – *Ekol. Pol. Ser. A* **12**: 653–690.
- Stilinović, B. & Plenković-Moraj, A., 1995: Bacterial and phytoplanktonic research of Ponikve artificial lake on the island of Krk. – *Period. Biol.* **97**: 351–358.
- Strayer, D. L. & Malcom, H. M., 2006: Long-term demography of a zebra mussel (*Dreissena polymorpha*) population. – *Freshw. Biol.* **51**: 117–130.
- Strickland, J. D. H. & Parsons, T. R., 1968: A practical handbook of seawater analysis. – *Fish. Res. Bd. Can. Bull.* **167**: 311.
- Szabó, K. E., Makk, J., Kiss, K. T., Eiler, A., Ács, É., Tóth, B., Kiss, Á. K. & Bertilsson, S., 2008: Sequential colonization of river periphyton analysed by microscopy and molecular fingerprinting. – *Freshw. Biol.* **53**: 1359–1371.
- Tockner, K. J. & Stanford, A., 2002: Riverine flood plains: present state and future trends. – *Environ. Conserv.* **29**: 308–330.
- UNESCO, 1966: Determinations of photosynthetic pigments in seawater, Report of SCOR – Unesco working group 17, Monogr. Oceanogr. Methodol., Paris.
- Vandeboncoeur, Y. & Steinman, A. D., 2002: Periphyton Function in Lake Ecosystems. – *Sci. World J.* **2**: 1449–1468.
- Vanderploeg, H. A., Nalepa, T. F., Jude, D. J., Mills, E. L., Holeck, K. T., Liebig, J. R., Grigorovich, I. A. & Ojaveer, H., 2002: Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. – *Can. J. Fish. Aquat. Sci.* **59**: 1209–1228.

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