Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 9, 2017: 171-180

# Influence of *Dreissena polymorpha* (Pallas, 1771) (Mollusca: Bivalvia) on Phytoplankton and Physicochemical Characteristics of Bulgarian Reservoirs

***Michaela Beshkova[1], Detelina Belkinova[1,2], Roumen Kalchev[1], Hristina Kalcheva[1], Rumen Mladenov[2,3] & Plamen Stojanov[2,3]***

[1] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria; E-mail: beshkova_m@yahoo.com, mbeshkova@zoology.bas.bg
[2] Department of Botany and Methods of Biology Teaching, Faculty of Biology, University of Plovdiv 'Paisiy Hilendarski', 24 Tsar Asen Street, 4000 Plovdiv, Bulgaria
[3] Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 120 Bratya Bukston Blvd. 4000 Plovdiv, Bulgaria

**Abstract:** The total numerical abundance of phytoplankton, expressed both in individuals and cells, the total biomass, number of species and species diversity, as well as the abundance of the major taxonomic groups were assessed in 18 Bulgarian reservoirs sampled in the summer and autumn of 2016. Eight of those reservoirs have been infested by the zebra mussel, *Dreissena polymorpha*. The main phytoplankton taxonomic groups of both infested and non-infested reservoirs were investigated by multivariate partial detrended corresponded analysis (DCA) and redundancy analysis (RDA). The presence/ absence of *D. polymorpha* explains a statistically significant part of the spatial variation in the mentioned taxonomic groups. All phytoplankton quantitative variables (number of individuals, cells and biomass), divisions and species diversity have statistically significant higher values in the non-infested than infested reservoirs. The variations in the phytoplankton taxonomic structure between the two different samplings were considerably smaller than the variations between the studied reservoirs.

**Key words**: Phytoplankton, *Dreissena polymorpha*, freshwater reservoirs

# Introduction

The invasion of zebra mussel, *Dreissena polymorpha* (Pallas, 1771), to reservoirs has become an increasingly important problem because of the species rapid spread, the significant impact on the whole aquatic ecosystems and the damages the mussel causes on the dam facilities. The relationship between *D. polymorpha* and phytoplankton is a basic issue because the phytoplankton development is a key element in assessing of ecological status, trophic status and water quality of reservoirs. Many literature sources have shown the significant impact of *D. polymorpha* on the phytoplankton abundance and composition (Vanderploeg et al. 2001, Nicholls et al. 2002, Naddafi et al. 2007, Higgins et al. 2010, De Stasio et al. 2014). This impact mainly consists in the clearance effect of the species, which leads to an increase in water transparency. The studies on the interaction between *D. polymorpha* and phytoplankton (Beshkova et al. 2014), and on some physicochemical variables (Kalchev et al. 2013, 2014, 2016) in reservoirs and lakes in Bulgaria also conform with these observations and show that *D. polymorpha* affects the functioning of the phytoplankton in different aspects.

The aims of the present work were: to compare the composition (on division level) and abundance of phytoplankton in infested and non-infested reservoirs, to relate them to environmental variables, and to assess to what extent the existing differences are associated with the presence of Dreissena polymorpha.

# Materials and Methods

### Sample collection

A total of eighteen freshwater reservoirs were sampled in August – beginning of September and October 2016. The visual inspection of the dams and shore facilities showed that eight of the reservoirs were infested by *D. polymorpha.* The reservoirs are scattered throughout the territory of Bulgaria (Fig. 1) and differed by their geographical, morphometric, physical and chemical characteristics (Table 1). Water temperature, conductivity, pH, water column transparency (Secchi disk depth), dissolved oxygen concentration and saturation were measured *in situ,* in most of the cases at sites near the dams. Integrated samples, i.e. pooled and well-mixed equal volumes of water, taken at different depths (obtained by multiplying of the value of measured Secchi depth by 0.1, 0.3, 0.5, 1.0, 2.0 and 3.0, respectively), were collected in the deepest lake zone by means of a *Ruttner bathometer.* In the shallow polymictic reservoirs (<3.0 m), the whole water column formed these integrated samples. For the phytoplankton analysis a volume of 0.5/ 1 L was taken and fixed with Lugol's or formaldehyde solution. For the chlorophyll-*a* analysis the mixed water was filtered through 0.7 μm glass fibers filters, added to 10 ml of 90% ethanol in a glass tube and transported in liquid nitrogen to the laboratory. There, after cooking at 75$^{o}$C and centrifugation, the chlorophyll-*a* extract was measured by spectrophotometer according to standard ISO 10260. The Bulgarian Ministry of Environment and Water provided data about the nutrients: total nitrogen and phosphorus (Ntot, Ptot), nitrite and nitrate nitrogen, ammonium nitrogen and phosphate phosphorous ($NO_3$-N, $NO_2$-N, $NH_4$-N, and $PO_4$-P) measured in conformity to the adopted standards: ISO 7150/1 for ammonium nitrogen, EN 26777 and ISO 7890-1 for nitrite and nitrate nitrogen, EN ISO 11905-1 for total nitrogen, EN ISO 6878 for phosphate phosphorus, EN ISO 6878 for total phosphorus, and ISO 7027 for turbidity.

### Phytoplankton analysis

The taxonomic composition of the phytoplankton was determined by a light microscope Amplival (400x) on preserved specimens. The total numerical abundance (both in cells and individuals per unit of volume) was estimated by counting in sedimentation and/or Bürker hemocytometer chambers under an inverted (Utermöhl 1958) and upright microscope, respectively. For most abundant species, at least 100 individuals were counted (Lund et al. 1958). The phytoplankton biovolume was calculated using the formulas for geometric shapes (Hillebrand et al. 1999). The transition between biovolume and fresh biomass was performed according to Wetzel & Likens (2000). The average individual volume (AIV, $\mu m^3$), the total number of species, and the Shannon index of species diversity were also calculated and analysed.

### Statistical analysis

All the morphometric, physical, chemical, biological characteristics and abundances of main algal taxonomic groups were analysed statistically by one way ANOVA and by partial multivariate analyses DCA and RDA (CANOCO 4.5 software) (ter Braak & Šmilauer 2002). This analysis was made in order to reveal the spatial and seasonal differences between the reservoirs infested and non-infested by *D. polymorpha*, as well as the relations of the differences to the environmental variables.

# Results and Discussion

### Physicochemical variables of infested and non-infested reservoirs

The main geographical, morphometric, physical and chemical characteristics of the studied reservoirs are shown in Table 1. Some of the reservoirs (Christo Smirnenski, Trakiets, Skala1, Krichim, and Kovachitsa) visually looked highly infested by *D. polymorpha*, while in the rest of the infested reservoirs only single specimens of the mussel were observed. The more precise quantitative assessment of *D. polymorpha* density in reservoirs is very complicated and hardly possible because the species inhabits the upper layers 2-4 m deep, which frequently dry as a result of the strong variations in the water level and thus the mass extinction of the mussel population may happen within a few weeks.

The partial RDA analysis shows that the main factors responsible for the spatial difference in the physical and chemical variables between reservoirs were the presence of *D. polymorpha* and the maximal depth, which acted in the same direction (Fig. 2). Excepting water transparency, which showed a highly positive relation with the presence of *D. polymorpha*, and the depth, all other physical and chemical variables were negatively related both to *D. polymorpha* and the depth. The phosphorus concentrations ($P0_4$-P, P-tot) did not correlate with *D. polymorpha* presence, while the total nitrogen

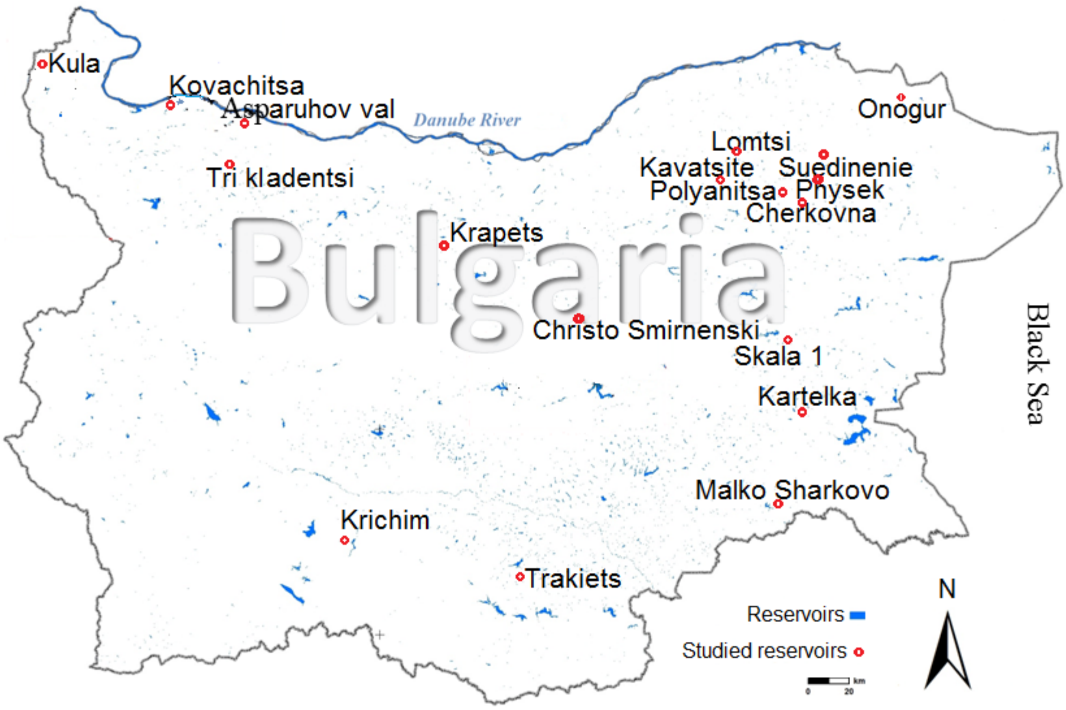


**Fig. 1**. A map with locations of the studied reservoirs

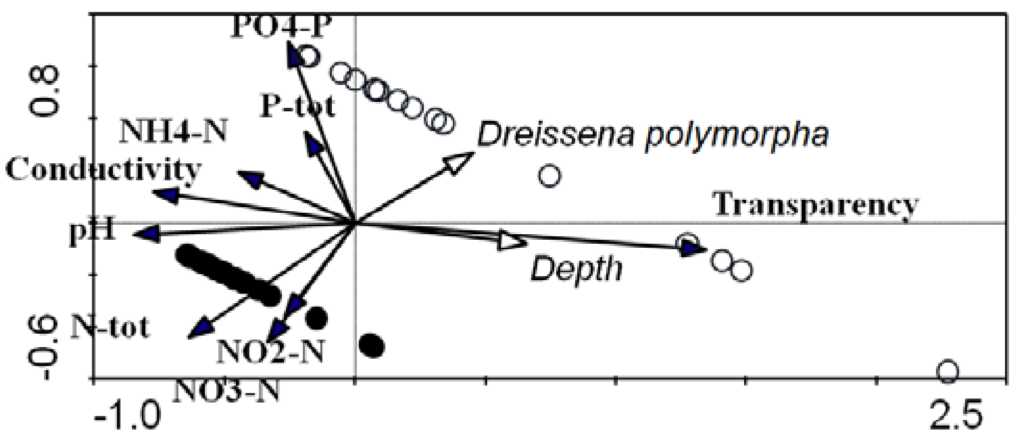


**Fig. 2**. A tri-plot diagram of partial RDA presenting spatial variations (between reservoirs) of physicochemical characteristics as response variables and presence or absence of *Dreissena polymorpha* and maximal depth as explanatory variables; the eigenvalue of the first axis is EV=0.200, statistically significant for P=0.002, the sum of all eigenvalues is EV=0.975, and the trace of all canonical axes is 0.218, statistically significant for P=0.002. The percentage of spatial variation explained by the presence of *D. polymorpha* and depth is 22%. ● reservoirs without *D. polymorpha*; ○ reservoirs with *D. polymorpha*; –► response variables; –> explanatory variables

(N-tot) and nitrate nitrogen ($NO_3$-N) showed highly negative correlations.

By one-way ANOVA we tested for significance the differences of all physicochemical variables between the reservoirs with and without *D. polymorpha* (Fig. 3, Fig. 4). The water transparency showed higher values in the infested reservoirs with very high level of significance (Fig. 3).This cleaning effect of *D. polymorpha* is well known and reported by many authors (Yu & Culver 2000, Karatayev et al. 2002, Higgins et al. 2008). The effect is also confirmed by our recent investigations (Kalchev et al. 2013, 2014). The pH values and conductivity were statistically lower in the infested reservoirs (Fig. 3). However, these variables were highly related to the depth as well (Fig. 2). Since pH is highly dependent on the intensity of photosynthesis it could be supposed that its lower values registered in the epilimnion of the infested reservoirs may be caused by *D. polymorpha* pressure on phytoplankton.

**Table 1**. Main geographical, morphometrical and physicochemical characteristics of the studied reservoirs. Alt – altitude; MD – maximal depth; SD – Secchi disk depth; T – surface temperature; Con – conductivity; * – reservoirs infested by *Dreissena polymorpha*

| Name of reservoir | Date of sampling | Alt, m a.s.l | MD, m | SD, m | T, °C | pH | Con, µS/cm | NH4 – N, mg/l | NO3 – N, mg/l | NO2 –N, mg/l | N –tot, mg/l | P – tot, mg/l | PO4 – P, mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Asparuhov Val | 17.8.2016 | 99 | 5.0 | 1.30 | 26.60 | 8.72 | 899 | 0.05 | 0.02 | 0.01 | 1.39 | 0.04 | 0.02 |
| | 7.10.2016 | 99 | 3.5 | 0.80 | 17.80 | 8.75 | 966 | 0.03 | 0.24 | 0.01 | 0.97 | 0.03 | 0.02 |
| Kavatsite | 13.8.2016 | 212 | 7.0 | 0.60 | 26.00 | 8.30 | 395 | 0.26 | 0.02 | 0.01 | 1.36 | 0.07 | 0.06 |
| | 14.10.2016 | 212 | 6.0 | 0.40 | 16.90 | 8.60 | 427 | 0.40 | 0.93 | 0.04 | 2.1 | 0.09 | 0.03 |
| Kartelka | 28.8.2016 | 111 | 8.0 | 0.60 | 25.40 | 8.63 | 706 | 0.22 | 0.41 | 0.07 | 2.29 | 0.09 | 0.05 |
| | 26.10.2016 | 111 | 5.0 | 0.50 | 16.50 | 8.08 | 758 | 1.09 | 2.50 | 0.88 | 4.63 | 0.07 | 0.01 |
| Kovachitsa* | 16.8.2016 | 111 | 10.0 | 4.00 | 25.60 | 8.49 | 806 | 0.11 | 0.01 | 0.01 | 1.1 | 0.10 | 0.05 |
| | 7.10.2016 | 111 | 10.0 | 2.00 | 19.30 | 8.65 | 808 | 0.16 | 0.44 | 0.01 | 1.04 | 0.04 | 0.01 |
| Krapets | 11.8.2016 | 408 | 12.0 | 2.00 | 26.50 | 8.15 | 318 | 0.02 | 0.23 | 0.01 | 0.56 | 0.07 | 0.05 |
| | 13.10.2016 | 408 | 17.0 | 3.15 | 17.50 | 7.80 | 329 | 0.01 | 0.06 | 0.01 | 0.53 | 0.01 | 0.01 |
| Krichim* | 9.9.2016 | 418 | 40.0 | 4.70 | 22.00 | 7.89 | 192 | 0.26 | 0.34 | 0.02 | 0.50 | 0.01 | 0.01 |
| | 29.10.2016 | 418 | 60.0 | 7.00 | 15.40 | 7.25 | 191 | 0.04 | 0.30 | 0.02 | 0.50 | 0.18 | 0.01 |
| Kula | 16.8.2016 | 209 | 17.0 | 2.00 | 24.20 | 8.59 | 328 | | | | | | |
| | 6.10.2016 | 209 | 17.0 | 2.00 | 19.8 | 8.30 | 357 | 0.02 | 0.77 | 0.01 | 1.23 | 0.04 | 0.01 |
| Lomtsi | 13.8.2016 | 202 | 5.0 | 1.00 | 25.00 | 8.35 | 676 | 0.05 | 4.68 | 0.26 | 8.13 | 0.09 | 0.002 |
| | 14.10.2016 | 202 | 4.0 | 0.40 | 16.50 | 8.55 | 736 | 0.03 | 10.3 | 0.07 | 11.2 | 0.04 | 0.00 |
| Malko Sharkovo* | 29.8.2016 | 249 | 15.0 | 4.00 | 25.60 | 8.30 | 361 | 0.29 | 0.02 | 0.002 | 0.50 | 0.06 | 0.002 |
| | 27.10.2016 | 249 | 10.0 | 3.50 | 16.70 | 7.80 | 370 | 0.04 | 0.03 | 0.003 | 0.25 | 0.02 | 0.02 |
| Onogur | 25.8.2016 | 99 | 1.0 | 0.05 | 24.30 | 9.06 | 576 | 0.45 | 0.19 | 0.05 | 9.09 | 0.49 | 0.01 |
| | 17.10.2016 | 99 | 1.0 | 0.10 | 15.50 | 8.55 | 633 | 0.33 | 0.24 | 0.02 | 3.51 | 0.23 | 0.10 |
| Polyanitsa* | 26.8.2016 | 242 | 13.0 | 1.00 | 25.60 | 8.12 | 286 | 0.03 | 0.02 | 0.01 | 0.77 | 0.06 | 0.05 |
| | 15.10.2016 | 242 | 12.0 | 1.15 | 17.60 | 7.27 | 332 | 0.45 | 0.38 | 0.01 | 0.9 | 0.2 | 0.03 |
| Skala1* | 27.8.2016 | 470 | 4.0 | 0.70 | 24.60 | 8.95 | 177 | 0.08 | 0.12 | 0.02 | 1.89 | 0.31 | 0.04 |
| | 19.10.2016 | 470 | 4.0 | 1.10 | 14.10 | 8.20 | 240 | 0.05 | 0.02 | 0.01 | 1.2 | 0.07 | 0.05 |
| Saedinenie* | 27.8.2016 | 171 | 8.0 | 1.70 | 24.50 | 8.70 | 762 | 0.64 | 0.26 | 0.03 | 1.41 | 0.03 | 0.03 |
| | 18.10.2016 | 171 | 7.0 | 1.10 | 16.90 | 8.50 | 785 | 0.68 | 0.82 | 0.04 | 1.8 | 0.07 | 0.03 |
| Trakiets* | 8.9.2016 | 244 | 25.0 | 1.70 | 24.70 | 8.54 | 269 | 0.32 | 0.07 | | 1.3 | 0.04 | 0.01 |
| | 28.10.2016 | 244 | 16.0 | 1.80 | 16.80 | 6.44 | 303 | 0.26 | 0.13 | 0.06 | 1.0 | 0.11 | 0.10 |
| Tri Kladentsi | 15.8.2016 | 159 | 4.0 | 0.30 | 23.00 | 8.54 | 322 | 0.18 | 0.05 | 0.01 | 1.46 | 0.05 | 0.02 |
| | 8.10.2016 | 159 | 3.0 | 0.25 | 17.20 | 8.30 | 366 | 0.06 | 0.92 | 0.02 | 1.79 | 0.03 | 0.02 |
| Fisek | 26.8.2016 | 181 | 2.0 | 0.40 | 25.00 | 8.71 | 567 | 0.35 | 2.36 | 0.09 | 4.7 | 0.03 | 0.03 |
| | 18.10.2016 | 181 | 3.0 | 0.90 | 15.00 | 8.55 | 622 | 1.1 | 0.27 | 0.01 | 1.77 | 0.08 | 0.03 |
| Christo Smirnenski* | 12.8.2016 | 536 | 37.0 | 6.00 | 25.00 | 8.11 | 243 | 0.03 | 0.06 | 0.01 | 0.2 | 0.04 | 0.01 |
| | 14.10.2016 | 536 | 42.0 | 5.60 | 18.90 | 7.30 | 258 | 0.02 | 0.21 | 0.01 | 0.25 | 0.01 | 0.01 |
| Cherkovna | 24.8.2916 | 290 | 5.0 | 0.40 | 23.10 | 8.32 | 409 | 0.61 | 0.13 | 0.03 | 1.23 | 0.03 | |
| | 16.10.2016 | 290 | 2.5 | 0.70 | 15.50 | 8.10 | 431 | 0.83 | 0.09 | 0.01 | 0.93 | 0.09 | |

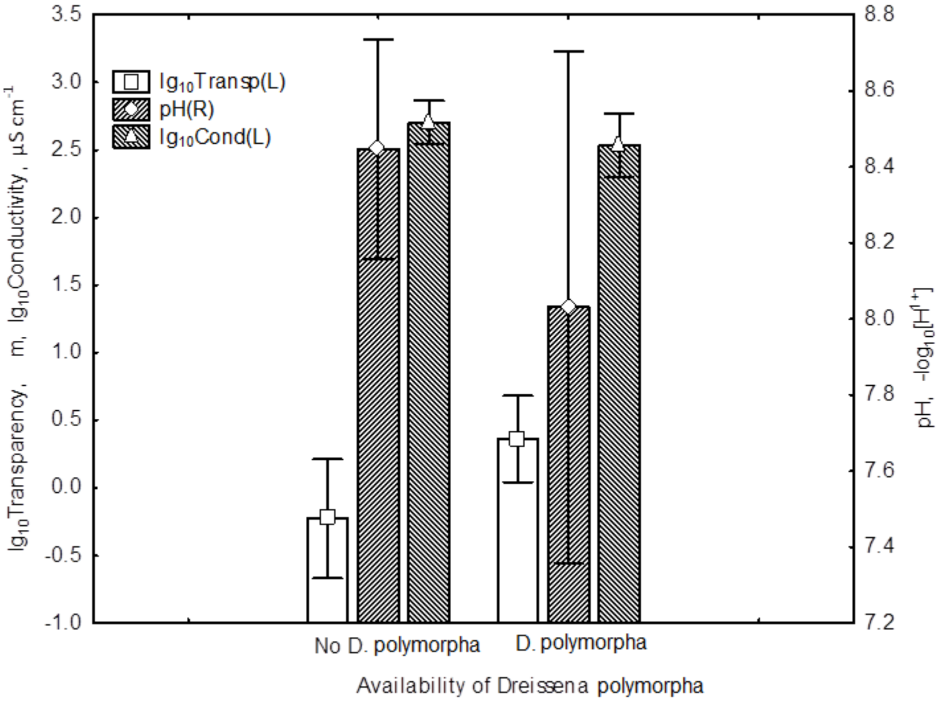


**Fig. 3**. Mean values and standard deviations of pH and logarithm base 10 of water column transparency (lg10Transp) and conductivity (lg10Cond) values in infested and non-infested by *Dreissena polymorpha* reservoirs; the F-test for one-way ANOVA delivered the following levels of significance for differences between infested and non-infested reservoirs: lg10Transp: 0.0001, pH: P≤0.02, and lg10Cond: P≤0.015

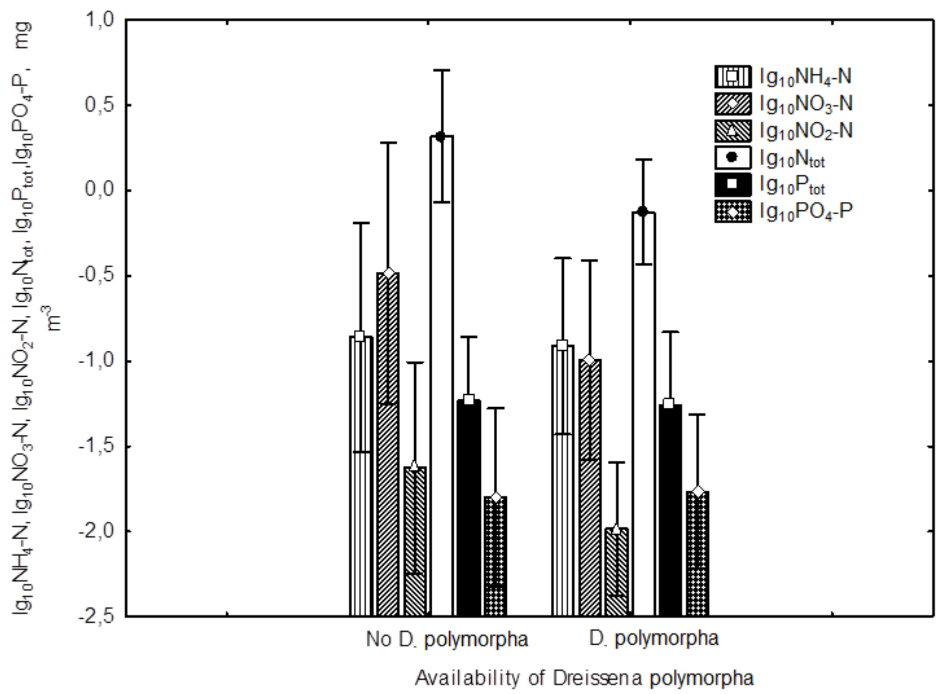


**Fig. 4**. Mean values and standard deviations of logarithm base 10 of concentrations of NH4-N, NO3-N, NO2-N, Ntot, Ptot, and PO4-P in infested and non-infested by *Dreissena polymorpha* reservoirs; the F-test for one-way ANOVA delivered the following levels of significance for differences between concentration values of infested and non-infested reservoirs: lg10NH4-N: P≤0.70, lg10NO3-N: P≤0.04, lg10NO2-N: P≤0.06, lg10Ntot: P≤0.0008, lg10Ptot: P≤0.85, and lg10 PO4-P: P≤0.80

The indirect influence of *D. polymorpha* on some physical parameters is more pronounced in the deeper and stratified reservoirs where the increase in the water transparency, caused by *D. polymorpha,* leads to the increase in the photic zone and creates good light conditions for the phytoplankton development in the deeper water layers. In turn, this results in an increase in the oxygen concentration and saturation, and in pH of the hypolimnion during summer stratification (Kalchev et al. 2013, 2014). This effect, however, strongly depends on the fluctuations in the water level, because, as mentioned above, the mussels inhabit the upper layers 2-4 m deep and may die during the frequent decrease in the water level (Kalchev et al. 2013, 2014).

From the chemical variables, only the total nitrogen (N-tot) and nitrate nitrogen ($NO_3$-N) concentrations differed statistically significantly between reservoirs with and without *D. polymorpha,* with lower values in the infested reservoirs. On the contrary, the phosphorus and the reduced forms of the nitrogen did not show statistically significant differences (Fig. 4). The mean N/P atomic ratio in both infested and non-infested reservoirs was high, indicating a phosphorus limitation. However, in the infested reservoirs this was less pronounced (on average 42) than in the non-infested reservoirs (on average 123), due to lower nitrogen concentrations in the non-infested reservoirs. It seems that *D. polymorpha* may affect the nutrient concentrations in the colonised water basins which leads to decrease in the ratio between nitrogen and phosphorus (directly by excretion and indirectly by the impact on zooplankton), especially in the epilimnion. Some authors also stated that the small-sized mussels may contribute to the decrease in the N:P ratio (Arnott &Vanni 1996, Wojtal-Frankiewicz & Frankiewicz 2011).

There was no relationship between the presence of *D. polymorpha* and phosphorus. At the same time, clear relation of transparency and phytoplankton abundance to *D. polymorpha* existed, which is in accordance with the previous observations, which indicated that *D. polymorpha* affects the interactions between phosphorus and chlorophyll-*a*, phytoplankton biomass and Secchi

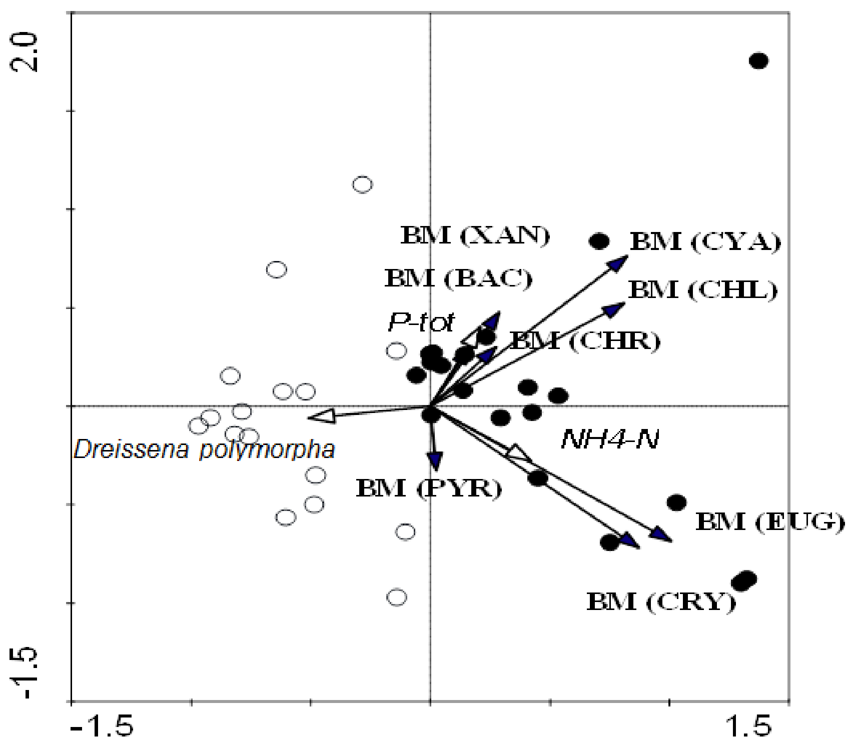


**Fig. 5**. A tri-plot diagram of partial RDA presenting spatial variations (between reservoirs) in biomass (BM) of main phytoplankton taxonomic groups as response variables and presence or absence of *Dreissena polymorpha*, total phosphorus (P-tot) and ammonia (NH4-N) concentrations as explanatory variables; the eigenvalue of the first axis is EV=0.170, statistically significant for P=0.002, the sum of all eigenvalues is EV=0.956, and the trace of all canonical axes is 0.262, statistically significant for P=0.002. The percentage of spatial variation explained by the presence of *D. polymorpha*, P-tot and NH4-N is 27%; ● reservoirs without *D. polymorpha*; ○ reservoirs with *D. polymorpha*; –► response variables; –> explanatory variables; CYA=Cyanophyta; CHL=Chlorophyta; BAC=Bacillariophyta; CHR=Chrysophyta; CRY=Cryptophyta; PYR=Pyrrhophyta; EUG=Euglenophyta; XAN=Xanthophyta

depth (Dzialowski & Jessie 2009, Beshkova et al. 2014, Kalchev et al. 2016).

**Phytoplankton characteristics in infested and non-infested reservoirs**

The partial RDA analysis revealed the presence of *D. polymorpha*, ammonia and total phosphorus concentrations as factors explaining statistically significant part of spatial variability in main phytoplankton divisions (Cyanophyta, Chlorophyta, Bacillariophyta, Chrysophyta, Cryptophyta, Pyrrhophyta, Euglenophyta, and Xanthophyta) between the reservoirs (Fig. 5). Among these three factors, the presence of *D. polymorpha* was the most important (its vector is the closest to the first main axis). These results are in accordance with our previous findings concerning the temporal differences of phytoplankton (Beshkova et al. 2014) and nutrients (Kalchev et al. 2013) before and after invasion of *D. polymorpha* in Zhrebchevo Reservoir. There, after invasion, the $PO_4$-P remained unchanged in both the epi- and hypolimnion, while the $NO_3$-N was not changed in the epilimnion and increased in the hypolimnion. This is a clear indication that the decrease in phytoplankton is not caused by the decrease in nutrients after invasion.

As may be seen, all phytoplankton groups (except Pyrrhophyta) were negatively related to the presence of *D. polymorpha*, with Cyanophyta, Chlorophyta, Cryptophyta, and Euglenophyta showing higher variability between reservoirs (longer vectors in Fig. 5).

In addition to the close relation to *D. polymorpha*, the phytoplankton divisions also showed a strong correlation with the ammonium nitrogen and total phosphorus. In this respect, two distinctive groups were formed. The first consisted of Pyrrhophyta, Cryptophyta, and Euglenophyta (all three of flagellated forms), which showed positive correlation with ammonium nitrogen (Fig. 5). The second group consisted mainly of non-flagellated species (except Chrysophyta) and correlated positively to total phosphorus. Thus, the motility (possession of flagella) of the algae appeared to play an important role in the complex of interactions in the aquatic systems invaded by *D. polymorpha*. The literature data about the selectivity of *D. polymorpha* grazing on the phytoplankton are rather controversial. Some of the authors argued that *D. polymorpha* can strongly influence the phytoplankton composition

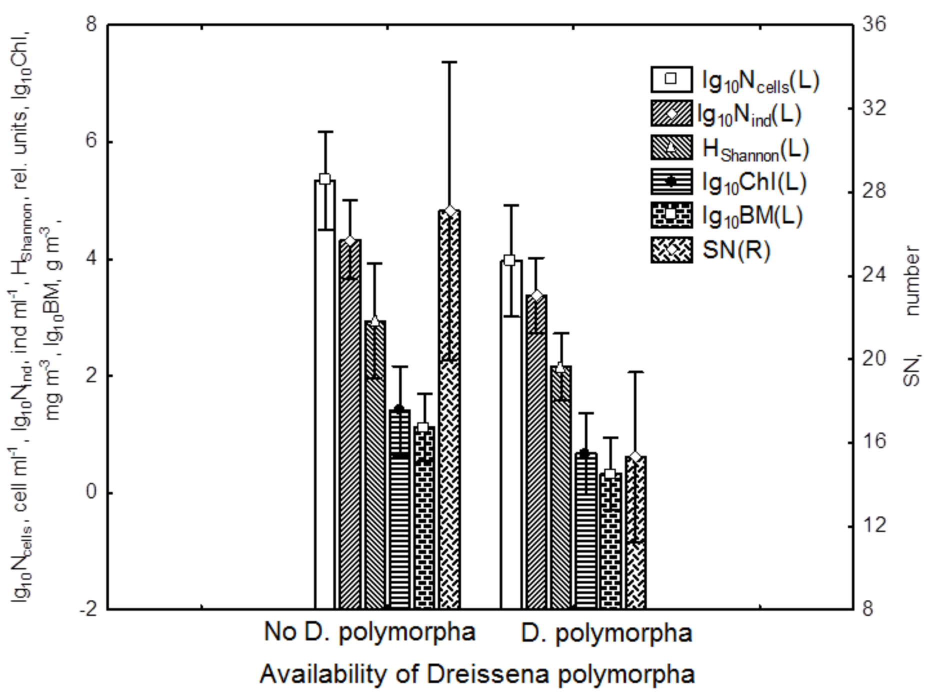


**Fig. 6**. Mean values and standard deviations of logarithm values base 10 of phytoplankton numerical abundance of cells (lg10Ncells) and individuals (lg10Nind), of chlorophyll-*a* concentration (lg10Chl), biomass (lg10BM), species diversity after Shannon (HShannon), and species number (SN) in reservoirs infested or non-infested by *Dreissena polymorpha*. The differences between infested and non-infested reservoirs are statistically significant for the mean values of lg10Ncells, P≤0.00005, lg10Nind, P≤0.00013, lg10Chl P≤0.005, lg10BM P≤0.0003, HShannon P≤0.0007, and SN P≤0.000 001 by F-test for one-way ANOVA

by selective grazing (Vanderploeg et al. 2001, Nicholls et al. 2002, De Stasio et al. 2008, Higgins &Vander Zanden 2010). Other authors noted the absence of selectivity in *D. polymorpha* filtrating activity (Wilson 2003). With regard to Cyanophyta, Bacillariophyta and Chlorophyta, our data are in accordance with observations of Higgins & Vander Zanden (2010) who reported a negative relationship between these divisions and the occurrence of *D. polymorpha*. However, concerning Pyrrhophyta, Cryptophyta, and Chrysophyta (all flagellates) our data differ from the findings of the cited authors. In an experiment with isolated tanks, Bastviken et al. (1998) defined the gross and net clearance rate of *D. polymorpha* filtrating activity and its influence on the phytoplankton composition. The gross clearance rate characterises the non-selective removal of algae from the water, i.e. different taxa are removed at similar rates. The net clearance rate presents the re-suspension of not digested, survived algae and it highly differs between the taxa, which suggests that the impact of *D. polymorpha* may depend strongly on the mixing regime of the water column (Bastviken et al. 1998). Therefore, our results, showing a negative relation between *D. polymorpha* occurrence and all taxonomic groups (except Pyrrophyta), seem to confirm this non-selective gross clearance rate. Other issue of *D. polymorpha* – phytoplankton interactions is the size dependent selectivity. In our previous study, we found a decrease in phytoplankton average individual volume (AIV) in Zhrebchevo Reservoir after the invasion of *D. polymorpha* (Beshkova et al. 2014). On the other hand, in the present work we observed fairly higher AIV of the phytoplankton in the invaded reservoirs. The possible reason for this discrepancy appeared to be of methodological origin, because different counting chambers and microscopes were used for phytoplankton enumeration before and after the invasion of *D. polymorpha*. In this study, the differences in phytoplankton assemblages of the compared reservoirs could also be the reason for some discrepancies because not all algae, depending on their size, respond equally to the grazing of *D. polymorpha* (Naddafi et al. 2007). However, Bastviken et al. (1998) reported that the overall community changes do not seem to be size-related and that all taxa, even large filamentous and colonial algae, decrease in numbers as a result of the mussel grazing. More future research is needed for explaining these discrepancies

As observed before (Beshkova et al. 2014), the quantitative characteristics of the phytoplankton before and after invasion by *D. polymorpha* are clearer than the qualitative ones. Thus, we found that all abundance and structural characteristics

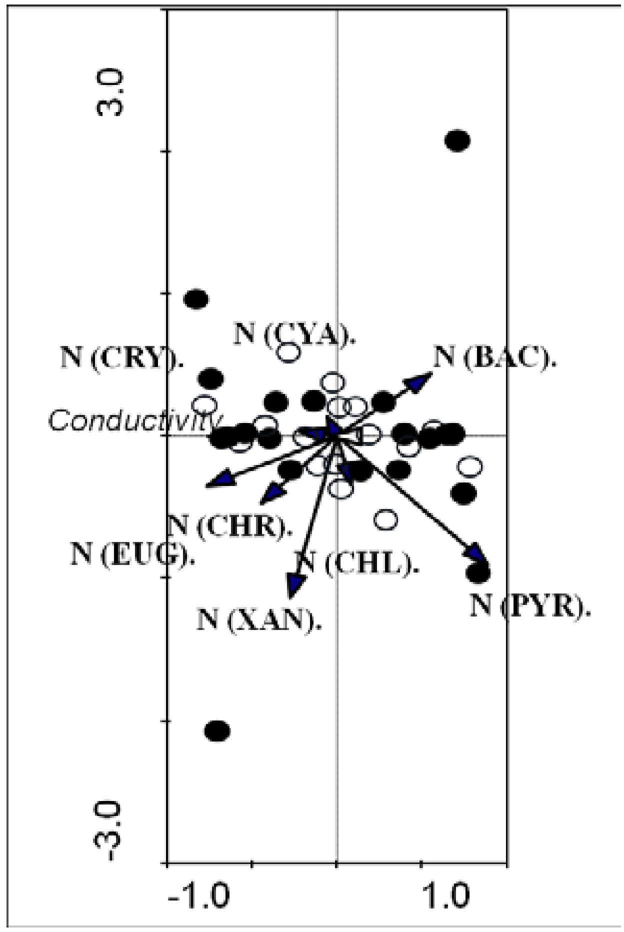


**Fig. 7**. A tri-plot diagram of partial RDA presenting seasonal variations (between summer and autumn) of numerical abundance expressed by cells per unit of volume (N) of main phytoplankton taxonomic groups as response variables and conductivity as explanatory variable; the eigenvalue of the first axis is EV=0.064 and statistically significant for P=0.002, and the sum of all eigenvalues is EV=0.316. The percentage of seasonal variation explained by the conductivity is 20%; ● reservoirs without *D. polymorpha*; ○ reservoirs with *D. polymorpha*; –► response variables; –> explanatory variable; for abbreviations of taxa see the legend of Fig. 5

(numerical abundance both in cells and individuals, biomass, chlorophyll-*a*, number of species, species diversity) showed significant differences between infested and non-infested reservoirs (Fig. 6). All these indices had lower values in the reservoirs invaded by *D. polymorpha*. The total number of species showed the highest level of significance for the difference between infested and non-infested reservoirs followed by the numerical abundance (both by cells and individuals), phytoplankton biomass and chlorophyll-*a*.

As a whole, the temporal variation in the phytoplankton taxonomic groups in months with stratification (August-September) and in months with mixing of water (October) was lower than the spatial variation (see the sums of eigenvalues of partial RDA analyses in the text under figures, Figs. 5 and 7). Only one environmental variable – conductivity or temperature (as they are closely correlated), passed the level of significance as a factor that possibly accounts for the seasonal distribution of the main taxonomic groups (Fig. 7).

Obviously, the presence/ absence of *D. polymorpha* could not explain the variations in phytoplankton composition between the months of the study, although these variations significantly differed depending on the presence or absence of stratification. Thus, our results do not confirm the influence of the mixing regime on the selective net clearance rate of *D. polymorpha* in phytoplankton suggested by Bastviken et al. 1998.

# Conclusions

The presence of *D. polymorpha* in the reservoirs was one of the major factors that explained the variations in the physicochemical parameters and phytoplankton divisions, abundance and diversity between the studied infested and non-infested reservoirs. Our results confirmed the clearance effect of *D. polymorpha* on the phytoplankton – statistically significant lower phytoplankton numerical abundance, biomass, and species diversity, as well as an increase in the water transparency in the infested reservoirs. The phytoplankton divisions were negatively related to *D. polymorpha*. This composition, however, depended on nutrients as well, since a clear separation was observed between the biomasses of flagellated and non-flagellated algae in relation to the ammonium nitrogen and total phosphorus, respectively.

In contrast to the explicit evidence for the relationship between the presence of *D. polymorpha* and phytoplankton abundance and water transparency, the total phosphorus did not show such relations and differences between infested and non-infested reservoirs. This observation is another argument in favour of the previous findings that *D. polymorpha* affects the relationship between phosphorus and other trophic characteristics in standing aquatic ecosystems.

**Acknowledgements:** This work was funded by the Financial Mechanism of the European Economic Area (2009-2014), Programme BG03 Biodiversity and Ecosystem Services, ESENIAS-TOOLS Project, D-33-51/30.06.2015, and the Executive Environment Agency at the Ministry of Environment and Water of Bulgaria, Public Order No 260/19.07.2016.

**References**

Arnott D. L. & Vanni M. J. 1996. Nitrogen and phosphorus recycling by the zebra mussels (*Dreissena polymorpha*) in the western basin of Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 53: 646-659.

Bastviken D. T. E., Caraco N. F. & Cole J. J. 1998. Experimental measurements of zebra mussel (*Dreissena polymorpha*) impacts on phytoplankton community composition. Freshwater Biology 39 (2): 375-386.

Beshkova M., Kalchev R. & Kalcheva H. 2014. Phytoplankton in the Zhrebchevo reservoir (Central Bulgaria) before and after invasion of *Dreissena polymorpha* (Mollusca: Bivalvia). Acta Zoologica Bulgarica 66 (3): 399-409.

De Stasio B. T., Schrimpf M. B., Beranek A. E. & Daniels W. C. 2008. Increased chlorophyll a, phytoplankton abundance, and cyanobacteria occurrence following invasion of Green Bay, Lake Michigan by dreissenid mussels. Aquatic Invasions 3 (1): 21-27.

De Stasio B. T., Schrimpf M. B. & Cornwell B. H. 2014. Phytoplankton communities in Green Bay, Lake Michigan after invasion by dreissenid mussels: increased dominance by Cyanobacteria. Diversity 6: 681-704.

Dzialowski A. R. & Jessie W. 2009. Zebra mussels negate or mask the increasing effects of nutrient enrichment on algal biomass: a preliminary mesocosm study. Journal of Plankton Research 31 (11): 1437-1440.

Higgins T. M., Grennan J. M. & McCarthy T. K. 2008. Effects of recent zebra mussel invasion on water chemistry and phytoplankton production in a small Irish lake. Aquatic Invasions 3 (1): 14-20.

Higgins S. N. & Vander Zanden M. J. 2010.What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. Ecological Monographs 80 (2): 179-196.

Hillebrand H., Dürseken D., Kirschtel D., Pollingher U. & Zohary T. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403-424. doi: 10.1046/j.1529-8817.1999.3520403.x

Kalchev R., Beshkova M., Botev I., Kalcheva H., Kozuharov D. & Trichkova T. 2013. Effect of *Dreissena polymorpha* (Bivalvia: Dreissenidae) on physicochemical characteristics of Zhrebchevo Reservoir (Central Bulgaria). Comptes rendus de l'Academie bulgare des Sciences 66 (11): 1571-1578.

Kalchev R., Beshkova M., Botev I., Kalcheva H., Kenderov L., Kozuharov D. & Trichkova T. 2014. Relation of transparency, dissolved oxygen and pH to *Dreissena* spp. (Mollusca, Bivalvia) occurrence in Bulgarian standing waters. Acta Zoologica Bulgarica 66 (3): 389-397.

Kalchev R., Beshkova M. & Kalcheva H. 2016. Trophic relationships and status of reservoirs with and without occurrence of *Dreissena* spp. (Mollusca, Bivalvia) built on Bulgarian Danube River tributaries. Transylvanian Review of Systematical and Ecological Research 18.1 'The Wetlands Diversity': 13-22.

Karatayev A., Burlakova L. E. & Padilla D. K. 2002. Impacts of zebra mussels on aquatic communities and their role as ecosystem engineers. In: Leppäkoski E., Gollasch S. & Olenin S. (Eds.): Invasive aquatic species of Europe.

Distribution, impacts and management. Dordrecht: Kluwer Academic Publishers, pp. 433-446.

Lund J. W. G., Kipling C. & Lecren E. D. 1958. The inverted microscope method of estimating algal number and the statistical basis of estimating by counting. Hydrobiologia 11: 143-170.

Naddafi R., Pettersson K. & Eklöv P. 2007. The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. Freshwater Biology 52 (5): 823-842.

Nicholls K. H., Heintsch L. & Carney E. 2002. Univariate step-trend and multivariate assessments of the apparent effects of P loading reductions and zebra mussels on the phytoplankton of the Bay of Quinte, Lake Ontario. Journal of Great Lakes Research 28 (1): 15-31.

Utermöhl H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen der Internationale Vereinigung der theoretische und angewandte Limnologie 5: 567-596.

ter Braak C. J. F. & Šmilauer P. 2002. Canoco reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Wageningen and České Budějovice: Biometris, 500 p.

Vanderploeg H. A., Liebig J. R., Carmichael W. W., Agy M. A., Johengen T. H., Fahnenstiel G. L. & Nalepa T. F. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic Microcystis blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 58 (6): 1208-1221.

Wetzel R.G. & Likens G. E. 2000. Limnological Analyses, 3rd edition. New York: Springer-Verlag, New York Inc., 429 p.

Wilson A. E. 2003. Effects of zebra mussels on phytoplankton and ciliates: a field mesocosm experiment. Journal of Plankton Research 25 (8): 905-915.

Wojtal-Frankiewicz A. & Frankiewicz P. 2011. The impact of pelagic (*Daphnia longispina*) and benthic (*Dreissena polymorpha*) filter feeders on chlorophyll and nutrient concentration. Limnologica 41: 191-200.

Yu N. D. & Culver A. 2000. Can zebra mussels change stratification patterns in a small reservoir? Hydrobiologia 431: 175-184.