Research Article

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Bacterioplankton and its Relations to Environmental Factors and the Presence of Invasive Mussels *Dreissena* spp. in Three Bulgarian Reservoirs

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Abstract: Bacterioplankton was studied in three Bulgarian reservoirs, two infested and one non-infested by the invasive mussels *Dreissena* spp., in the period 2009-2011, with the aim to compare and detect interactions with environmental factors and presence of *Dreissena polymorpha*. The total number and biomass of bacteria in the infested reservoirs Ogosta and Zhrebchevo were higher at the ecotone stations in spring and at the depths around the thermocline in summer. The bacterioplankton in the non-infested Koprinka Reservoir increased in autumn at the surface layer or near the bottom. The smallest bacterial size class was dominant in all reservoirs. Positive relations existed between bacteria and the nutrients nitrogen and phosphorus, Fe ions, turbidity, chemical oxygen demand and phytoplankton. The relations to temperature, transparency, pH and Ca^{2+} were negative. The quantities of *D. polymorpha* showed slightly negative correlations with the total number and biomass of bacterioplankton, and positive correlations with the quantities of rods, larger and attached bacteria, probably because of more organics by excreta of *D. polymorpha*.

Key words: Bacterioplankton, reservoirs, abiotic factors, *Dreissena polymorpha*

Introduction

The invasive mussels of the genus *Dreissena*, *D. polymorpha* and *D. bugensis* (Bivalvia: Dreissenidae), are of Ponto-Caspian origin, but currently have been found in many lakes and reservoirs in Europe and North America (Strayer 1991, Cotner et al. 1995, Karatayev et al. 1997), including the inland waters of Bulgaria (Trichkova et al. 2008, 2009, Kozuharov et al. 2009). The two mussels are known as successful aquatic invaders that have great potential to impact directly or indirectly the biodiversity and ecosystem functioning. Due to their ability to filter the seston in large volumes of water, they cause huge ecosystem changes summarised as a shift of energy flow from pelagic to benthic food chain (Karatayev et al. 2002, Millane et al. 2008). The investigation of the effect of *D. polymorpha* on reservoir ecosystems is very important, especially for countries as Bulgaria, where the main freshwater resources are in reservoirs built on rivers (Kalchev et al. 2013). The studies on the ecological role of bacterioplankton in the pelagial of standing waters in Bulgaria are numerous (Kalchev et al. 2004, Beshkova et al. 2008, Kalcheva 2011, etc.), but they have not been conducted in long-term studies with inclusion of all trophic levels in reservoirs infested by *D. polymorpha*. Such a three-year experiment has been implemented and some of the results for abiotic factors, phytoplankton, zooplankton, benthic macroinvertebrates, nematodes, fish, *Dreissena* spp. quantities and their relations have been published (Stoichev & Danova 2012, Kalchev et al. 2013, 2014, Trichkova et al. 2013, Beshkova et al. 2014, Kenderov et al. 2014, Kozuharov & Stanachkova 2015, Stanachkova et al. 2015, Kalcheva et al. 2016). However, the results for bacterioplankton were presented only partially (Kalcheva et al. 2010, 2016, Kalcheva 2011).

The aim of this study was to compare the annual, seasonal and spatial bacterioplankton dynamics in the reservoirs Ogosta and Zhrebchevo, infested by *Dreissena* spp., and in the non-infested Koprinka Reservoir and to determine its relations to environmental factors and the presence of *D. polymorpha*.

Materials and Methods

The study was conducted in the period 2009-2011 in three reservoirs in Bulgaria (Fig. 1). Ogosta Reservoir is built on the Ogosta River, belonging to the Danube River catchment, while the reservoirs Koprinka and Zhrebchevo are on the Tundzha River from the Aegean Sea catchment. The invasive species *D. polymorpha* has been established in the reservoirs Ogosta and Zhrebchevo, and *D. bugensis* only in Ogosta Reservoir (Trichkova et al. 2008, 2009). The geographic coordinates, morphometric characteristics, trophic state, and the number of samples for each reservoir are given in Table 1. A total of 133 samples for bacterioplankton were taken in three seasons: in summer 2009 (August-September), in spring (April-May) and autumn 2010 (October) and in summer 2011 (July). The samples were collected from 3 to 5 stations at each reservoir (Fig. 1), divided from the wall to the tail of the reservoir, at depths from 0.3 m under the surface to the maximum depth of each station, but 1 m above the bottom.

The number of bacteria was determined by the updated Razumov's method of a direct count (Naumova 1999, Grudeva et al. 2006), with a phase-contrast microscope at a magnification of 1600x after preliminary fixation with 2% formalin and staining with erythrosine, described in details in Kalcheva et al. (2008) and Kalcheva (2011). The biomass was calculated in carbon content by Norland's formula (Straškrabova et al. 1999) after determination of the mean cell volume (MCV). Bacterioplankton was counted separately both for cells freely dispersed on the filter (0.2 µm pore sized) and for particles associated with detritus, since the morphological groups were provisionally divided into four groups (free cocci and rods and attached cocci and rods). The sizes of bacteria were divided into size classes (Pernthaler et al. 1996, Kalcheva et al. 2008, Chróst et al. 2009). The morphological index (Mindex) was calculated (M=%rods/ %cocci), which allows to determine the extent of pollution and self-purification in the reservoirs, because the increase in the quantity of rod-shaped cells, especially with the relatively large sizes is an indicator of increased organic content in the water (Pernthaler 2005). The number of detritus particles with attached bacteria was also counted.

Parallel measurements by standard methods were made for 15 abiotic parameters and three biotic factors: chlorophyll-a (ISO 10260 1992), phytoplankton in 2009 only (Beshkova et al. 2008, 2014) and *D. polymorpha* (Trichkova et

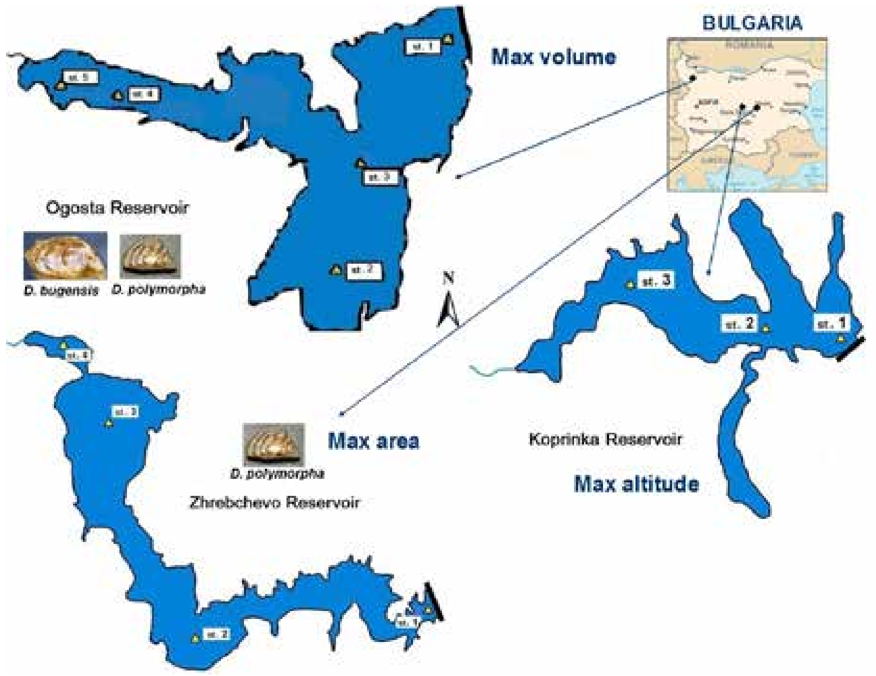

Fig. 1. Schemes and location of the studied reservoirs Ogosta, Zhrebchevo and Koprinka, with indicated sampling stations and infestation by *Dreissena* spp.; Max – the maximum value of the corresponding parameter among the three reservoirs

Table 1. Geographic coordinates, morphometric characteristics, trophic state and the number of samples analysed in 2009-2011 in the reservoirs Ogosta, Zhrebchevo and Koprinka

Reservoirs	**Geographic coordinates**	**Altitude, m**	**Surface area, ha**	**Maximum volume, $10^6 m^3$**	**Maximum depth, m**	**Trophic state**	**Water samples**
Ogosta Reservoir	N 43°23’ E 23°12’	203	2360	506	56	mesotrophic	58
Zhrebchevo Reservoir	N 42°63’ E 25°86’	250	2500	440	35	mesotrophic	43
Koprinka Reservoir	N 42°61’ E 25°28’	390	1120	140	28	eutrophic	32

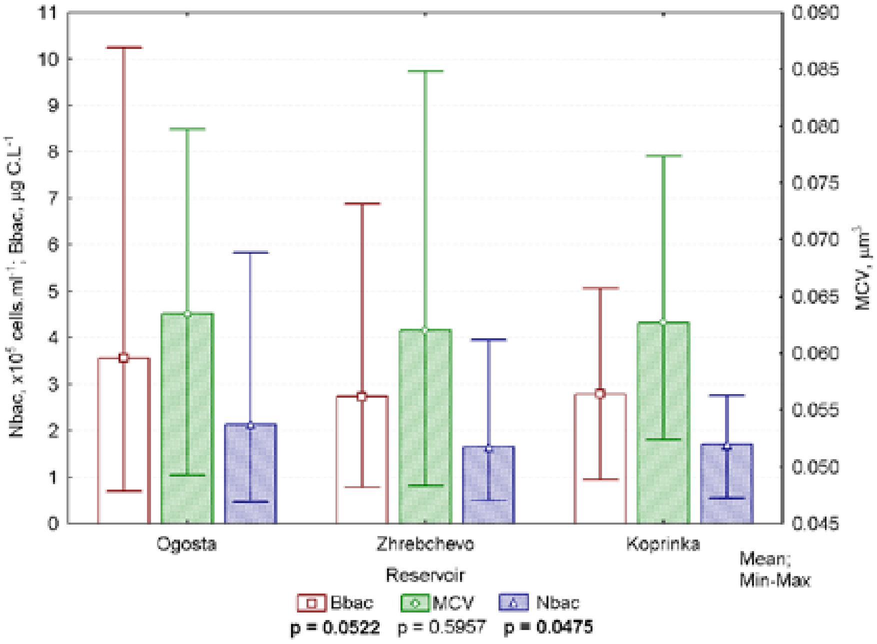

Fig. 2. Total number (Nbac), biomass (Bbac) and mean cell volume (MCV) of bacterioplankton and F-test (ANOVA) in the reservoirs Ogosta, Zhrebchevo and Koprinka during 2009-2011; Mean, minimum (Min) – maximum (Max) values

al. 2008, 2009). The temperature, pH, dissolved oxygen and oxygen saturation were measured *in situ* by pH- and oximeters type WTW 315i/SET, the transparency was measured by a Secchi disk, and the Ca^{2+} ions were determined in a laboratory at the University of Innsbruck. The other factors, NH_4-N, NO_3-N, NO_2-N, PO_4-P, total nitrogen (TN), total phosphorus (TP), Fe, Si and chemical oxygen demand (COD) were analysed with kits and by a spectrophotometer Spectroquant® NOVA60 and a thermoreactor (Merck, Bulgaria). The quantitative results about *Dreissena polymorpha* were assessed in five categories (absent, only shells, small, middle and high quantities) at each station and sampling depth. The data about *Dreissena bigensis* presence in Ogosta Reservoir were not used in the present study.

Multivariate statistical Redundancy Analysis (RDA) with the computer program CANOCO for Windows 4.5 (TER BRAAK & SMILAUER 2002), single factor analysis of variance (one-way ANOVA), nonparametric correlation of Spearman (R_S) and parametric linear correlations with the program STATISTICA 7.0 (FOWLER et al. 1998) were applied. The bacterioplankton variables were included in RDA as dependent variables, while the environmental factors (abiotic and biotic) as independent variables. Because of incomplete data for environmental factors in one of the samples (station 3 in Ogosta Reservoir in spring 2010) it was excluded from the statistical analyses (n=132). The statistical evaluations were performed using a level of significance P (probability) with 5% risk of error (α or P<0.05).

Results and Discussion

Bacterioplankton dynamics

The total number of bacterioplankton in the period 2009-2011 in a total of 133 water samples varied in the range from 4.70×10^4 to 5.83×10^5 cells.ml^{-1} (Fig. 2). The minimum was found in Ogosta Reservoir in September 2010 and the maximum was in summer 2009 in the same reservoir, at a depth of 15 m. The biomass in carbon content varied from 0.69 to 10.24 µg C.L^{-1} and followed the total number distribution in the reservoirs (Fig. 2). The mean cell volume (MCV) was in the range of 0.0484 to 0.0848 µm^3, with slightly higher variations in the infested reservoirs Ogosta and Zhrebchevo (Fig. 2). This is a relatively low average volume, typical of eutrophic waters (Šimek et al. 1997) and water bodies with the domination of bacteria from the smallest size class, 0.2-0.5 µm (Pernthaler et al. 1996, Chróst et al. 2009). Significant differences in the total number and biomass between reservoirs were found (Fig. 2). MCV, although higher in Ogosta Reservoir, did not differ significantly between the reservoirs (Fig. 2). However, the differences between the years were statistically significant (P=0.048). The total number decreased towards 2009-2011 in Ogosta Reservoir, while in Zhrebchevo Reservoir increased (P=0.0004). A slight increase in Koprinka Reservoir was found. The total number of bacterioplankton in the infested reservoirs was higher than in the more trophic Koprinka Reservoir, but also than other studied non-infested stagnant water bodies (Beshkova et al. 2008, Kalcheva 2011). This may indicate an indirect impact of *Dreissena* spp. due to shifts in the other plankton communities participating in the microbial loop.

The bacterioplankton total number and biomass were higher in spring and summer in Ogosta Reservoir and in summer in Zhrebchevo Reservoir, while in Koprinka Reservoir the seasonal differences were negligible, with the maximum values in autumn (Fig. 3). Horizontal distribution by sampling stations from the last stations in the reservoir (3, 4 or 5 – the tail) to the first station (the wall), showed less or more well-defined decreasing gradient in the total number of bacterioplankton, which is an indication of ecotone effect and self-purification of the water (Fig. 3). Higher values existed in two ecotone stations (2 and 5) in Ogosta Reservoir, due to inflow of other two rivers at station 2, especially in spring, when the organics and biodegradation increased.

The total number and biomass (Fig. 4) varied at the depth horizons in summer when the reservoirs

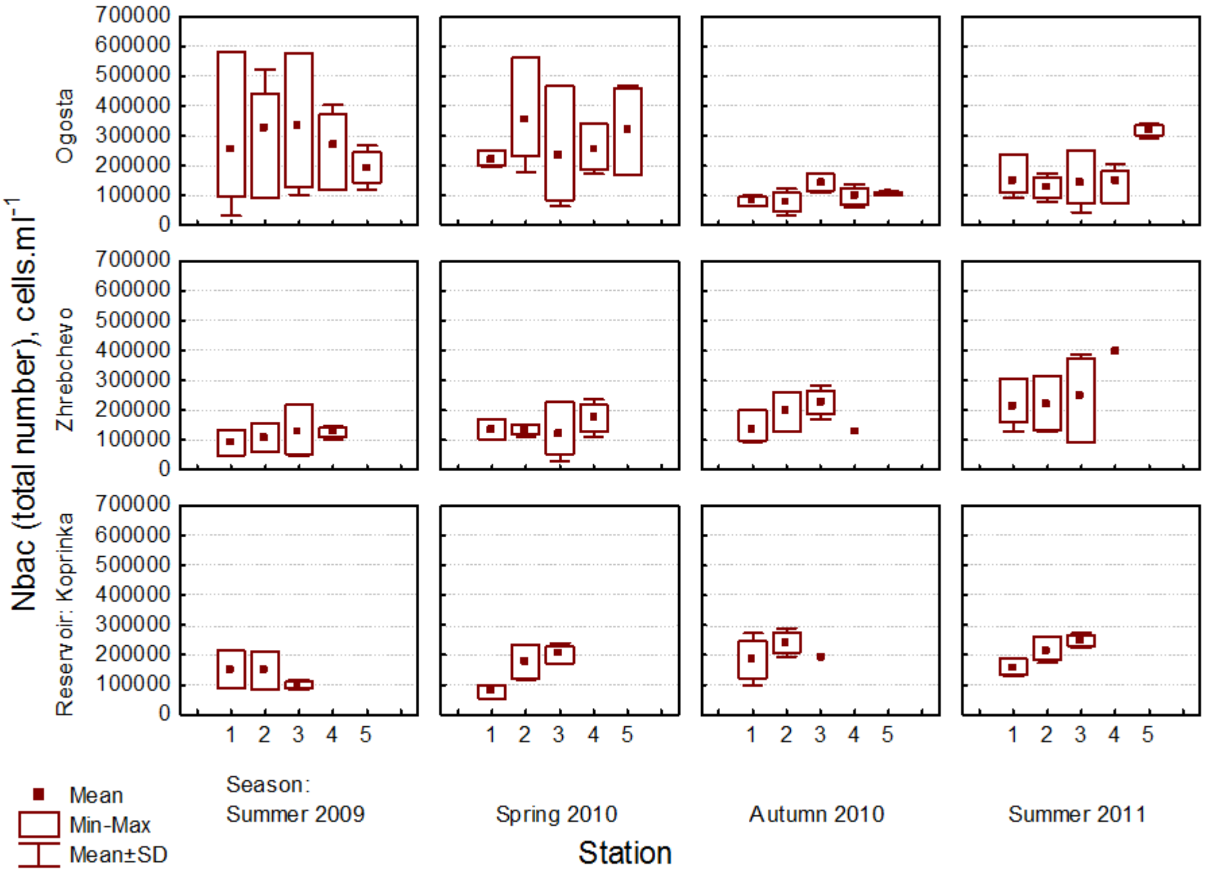

Fig. 3. Seasonal dynamics in the total number of bacterioplankton (Nbac) at sampling stations (1-5) in the reservoirs Ogosta, Zhrebchevo and Koprinka during 2009-2011; Mean, minimum (Min) – maximum (Max) values and standard deviations (SD)

were stratified, due to temperature differences in the water column, while the changes in the vertical distribution of bacterioplankton were weak in spring (except in Ogosta Reservoir, probably due to *Dreissena* spp. infestation) and in autumn, during homothermy. The differences were not significant, but higher values were found in summer at depths of 10-15 m at Station 1 in infested reservoirs, and at 5 m in Koprinka Reservoir. These depths were determined as thermocline layers (Kalchev et al. 2014). Bacterioplankton at the other stations increased in surface layers up to 5 m, and also near the bottom in Koprinka Reservoir. In the infested reservoirs *Dreissena* spp. were found at depths from 0.30 to 30 m, most often up to 10 m (Trichkova et al. 2008, 2009); the higher bacterioplankton biomass in summer was found at the same depths.

The free-living cocci dominated over the other three morphological groups and differed significantly between the reservoirs (P=0.01). The attached bacteria varied widely (3-67%) with summer maximum and higher values in the reservoirs Ogosta and Koprinka. The rods and attached bacteria increased with depth. The number of detritus particles was higher in summer of 2009 at the depth layers between 0.3 m and 15 m in Ogosta Reservoir. Most probably there are different reasons for the increase in the number of attached bacteria in the two reservoirs: in Ogosta Reservoir this may be due to increased organic matter from *Dreissena* spp. excreta in large quantities (Cotner et al. 1995), while in Koprinka Reservoir – because of the strong predatory pressure of bacterivores, consuming free-living bacteria, which are included in the microbial loop. Attached bacteria are involved in the detritus food chain and nutrient remineralisation (Pekàr & Olah 1998). The morphological index was low and below 1, showing easy degradable organics in the reservoirs, but varied in summer and had higher values in spring and autumn. The smallest size class of bacteria, 0.2-0.5 µm (49-83%) dominated in all reservoirs. Chróst et al. (2009) found in a mesotrophic lake that 52 to 82% of bacteria were with sizes 0.2-0.5 µm. Domination of bacteria

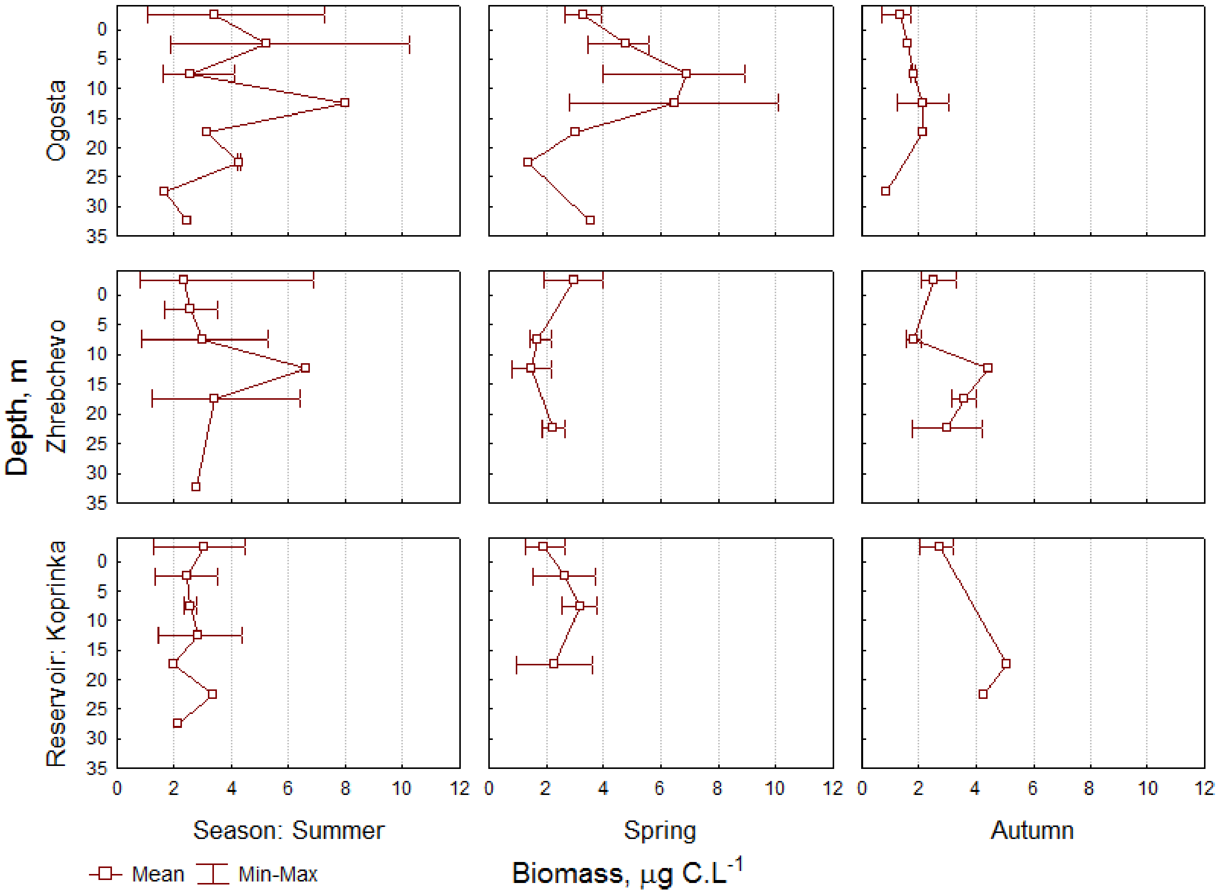

Fig. 4. Vertical distribution (at different sampling depth horizons) of bacterial biomass in seasonal aspect (summer, spring, autumn) in the reservoirs Ogosta, Zhrebchevo and Koprinka during 2009-2011; Mean, minimum (Min) – maximum (Max) values

from the smallest size class has been reported for stagnant freshwater bodies with different trophic status (Pernthaler et al. 1996, Šimek et al. 1997, Cole 1999, Straškrabova et al. 1999, Hahn & Höfle 2001, Jürgens & Matz 2002, Chróst et al. 2009), and it is considered normal due to nutrient limitation, abiotic factors (temperature, pH, etc.) that are beyond the optimum, increase in bacterivores or inactive state (Pernthaler 2005). The number of bacteria of larger size (0.5-4.2 µm) in the reservoirs with the presence of *Dreissena* spp. was greater than that in Koprinka Reservoir: in summer 2009 and spring 2010 in Ogosta Reservoir and in autumn 2010 and summer 2011 in Zhrebchevo Reservoir. The largest bacteria increased towards 2010-2011. Most probably this was connected with the organic matter excreted by the invasive mussels, but also with their filtration of small-sized seston, including bacteria (Cotner et al. 1995, Dionisio Pires et al. 2004). Competition for inorganic forms of phosphorus with phytoplankton, which in eutrophic waters is better developed and more competitive than bacteria (Heath et al. 2003), probably was another reason for the low number of bacteria with sizes above 0.5 µm in Koprinka Reservoir. In the infested reservoirs, a greater part of detritus and bacteria with sizes up to 1 µm were most likely filtered by *D. polymorpha*, compared to the non-infested Koprinka Reservoir, and therefore, the number of the smallest bacteria there was lower. This assumption is related to the findings of Dionisio Pires et al. (2004) for preferential filtration of seston with sizes of 0-1 µm and 30-100 µm by *D. polymorpha*.

Relations of bacterioplankton to environmental factors and *D. polymorpha* presence

We found positive correlations of the attached morphotypes of bacterioplankton with TP (r=0.56) and NH_4-N (r=0.42 in 2009, r=0.37 in 2010-2011). The ratio of the nutrients N and P, especially P, is very important for bacterioplankton development (Vadstein et al. 2003). The correlation of bacteria with another major macroelement, Fe, also was positive (r=0.40, in 2010-2011). Negative relations were found with pH, temperature, transparency and Ca^{2+} (r=-0.33). Enhanced transparency implies less suspended matter and probably less dissolved organic carbon (DOC) for bacteria or DNA damage from UV-B rays (Carrillo et al. 2006). High concentrations of Ca^{2+} in the water increase the permeability of the cells and the likelihood of entry of foreign

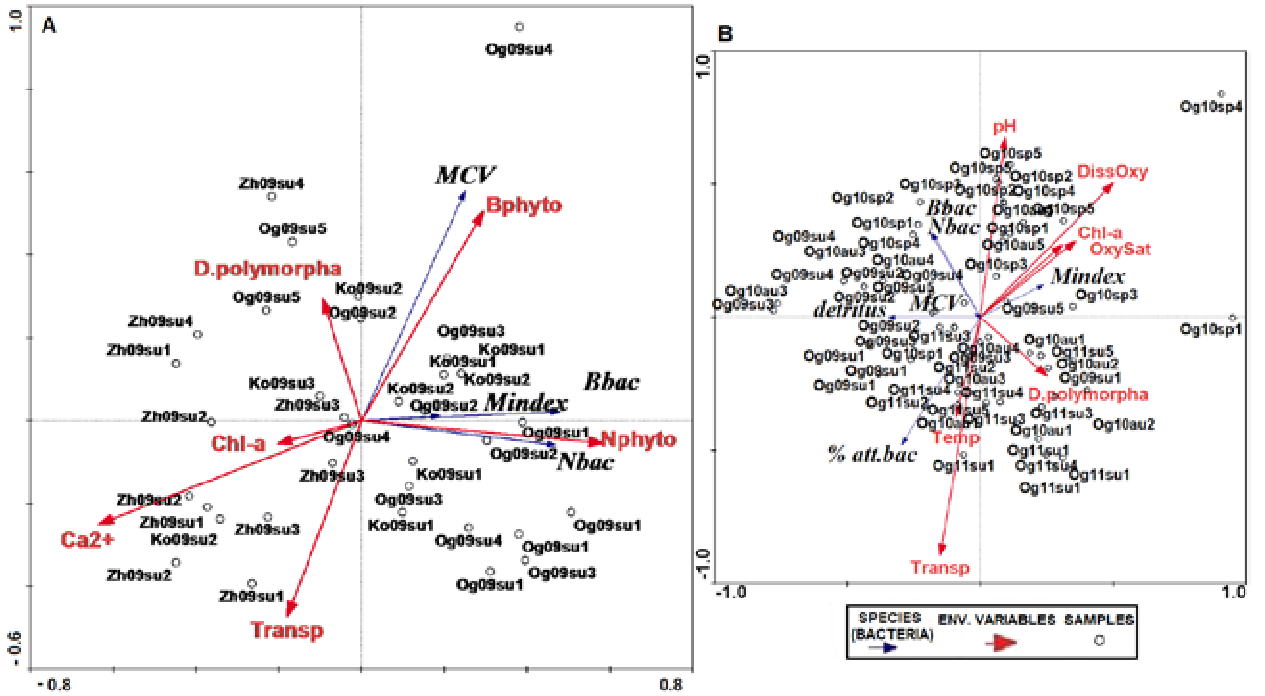

Fig. 5. RDA triplot of correlations of bacterioplankton (total number, biomass, mean cell volume and morphological index and/ or % of attached bacteria) with: (A) two abiotic environmental factors (transparency and Ca^{2+}), chlorophyll-a, number and biomass of phytoplankton and *Dreissena polymorpha* in summer 2009 in all reservoirs; and (B) five abiotic environmental factors (temperature, transparency, dissolved oxygen, oxygen saturation and pH), chlorophyll-a and *D. polymorpha* for the 3-year period in Ogosta Reservoir. Coding of the samples: the first two letters – reservoir name, the first two numbers – year, next two letters – season, next number – station. Statistics: (A) ΣAllEV=1.000, ΣCanEV=0.228 (23%), F-ratio=1.428, P=0.2280; (B) ΣAllEV=0.001; ΣCanEV=0.001, F-ratio=15.277, P=0.0020

DNA (Shemarova & Nesterov 2005). The number of phytoplankton correlated positively with the total number and biomass of bacterioplankton in 2009 (r=0.58). Such positive relation was found in other reservoirs (Beshkova et al. 2008). This is an indication of bottom-up effect of phytoplankton cells that release DOC extracellularly in the pelagial used by free-living bacteria.

The nonparametric tests showed that *D. polymorpha* correlated negatively with the bacterioplankton total number (R_S=-0.185) and biomass (R_S=-0.181) for the whole period (2009-2011), and this correlation was weak but significant. RDA analyses (Figs. 5, 6 and 7) demonstrated better possible positive and negative relations of bacterioplankton with environmental factors and *D. polymorpha* by years, seasons and stations.

The correlations of bacterioplankton total number and biomass with environmental factors were not significant in summer 2009 (Fig. 5A, P=0.23, n=36), but the trends were: negative with Ca^{2+}, transparency, chlorophyll-a and *D. polymorpha*, and positive with phytoplankton number (the biomass with MCV), as was demonstrated with linear correlations and R_S. The relation of *D. polymorpha* (which had the highest quantities at the last stations of the infested reservoirs) to the volume (MCV), however, was positive. The relations of zooplankton and *D. polymorpha* with phyto- and bacterioplankton in Ogosta Reservoir, in 2009, were negative (Kalcheva et al. 2016). *Dreissena polymorpha* showed the strongest negative impact on bacteria, except a weak positive effect on the attached bacteria, in Ogosta Reservoir for the whole 3-year period (Fig. 5B, P=0.02, n=57).

The nutrients nitrogen and phosphorus and the turbidity directly influenced the size structure of bacterial cells in spring 2010 (Fig. 6A, P=0.002, n=36). Bacteria probably were actively involved in the mineralisation of nutrients, received from the river. *Dreissena polymorpha* had no negative effect on smaller bacteria, and at the same time, influenced positively the larger bacteria and rods. Dionisio Pires et al. (2004) found preferential filtration by *D. polymorpha* of seston size of 0-1 µm, which we also proved by the nonparametric test with bacteria with sizes 0.2-1.2 µm, but the negative correlations were very weak (0.11 <R_S <0.13). The strongest relationships of bacteria with nutrients (PO_4-P, NO_2-N and NH_4-N), Fe, Si and with turbidity were in autumn 2010 (Fig. 6B, P=0.04, n=28). *Dreissena polymorpha* had a weak negative impact on bacteria, except on rods (Mindex). Bacteria correlated positively with TN, TP and turbidity, but the attached bacteria – with COD and Chl-a, while *D. polymorpha* affected negatively all bacterial morphotypes in summer 2011 (ΣCanEV=0.372, P=0.034, n=32).

The bacterioplankton total number and biomass had a slightly negative correlation with *D. polymorpha*, as was established by the nonparametric test of

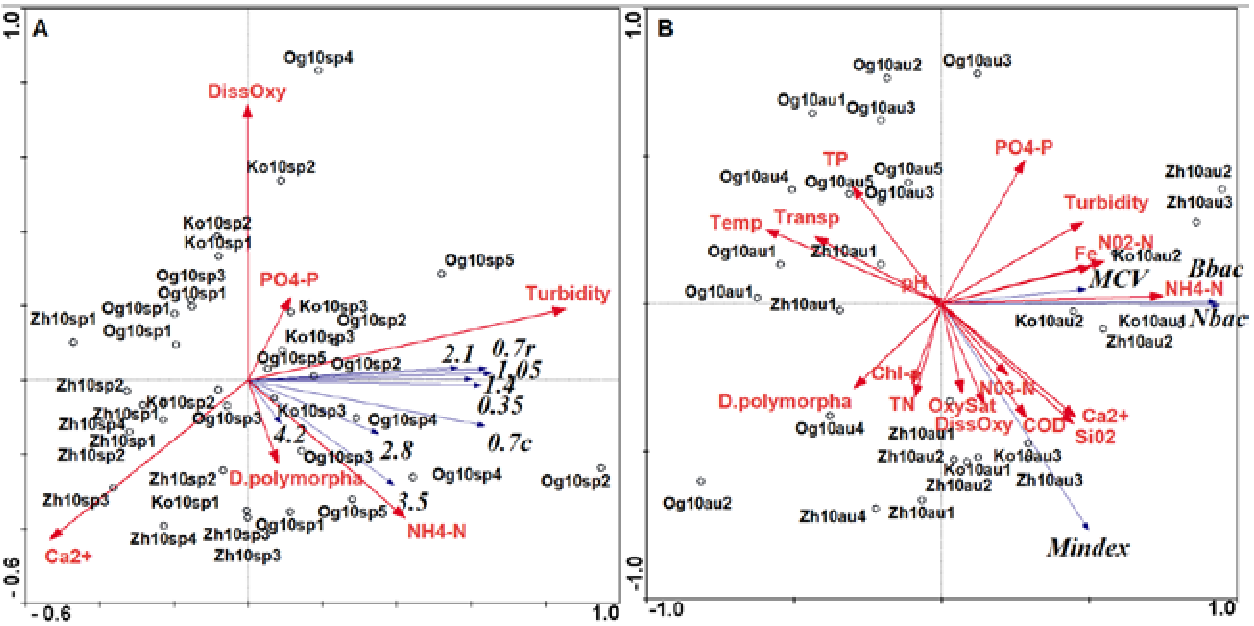

Fig. 6. RDA triplot of correlations of: (A) bacterioplankton (size structure, nine classes with mentioned medians) with five abiotic environmental factors, chlorophyll-a and *Dreissena polymorpha*, in spring 2010; (B) bacterioplankton (total number, biomass, mean cell volume and morphological index) with 15 abiotic environmental factors, chlorophyll-a and *D. polymorpha* in autumn 2010, in all reservoirs. Coding and symbols: see Fig. 5. Statistics: (A) ΣAllEV=1.000; ΣCanEV=0.838 (84%), F-ratio=4.871, P=0.0020; (B) ΣAllEV=1.000; ΣCanEV=0.873 (87%), F-ratio=3.445, P=0.0400

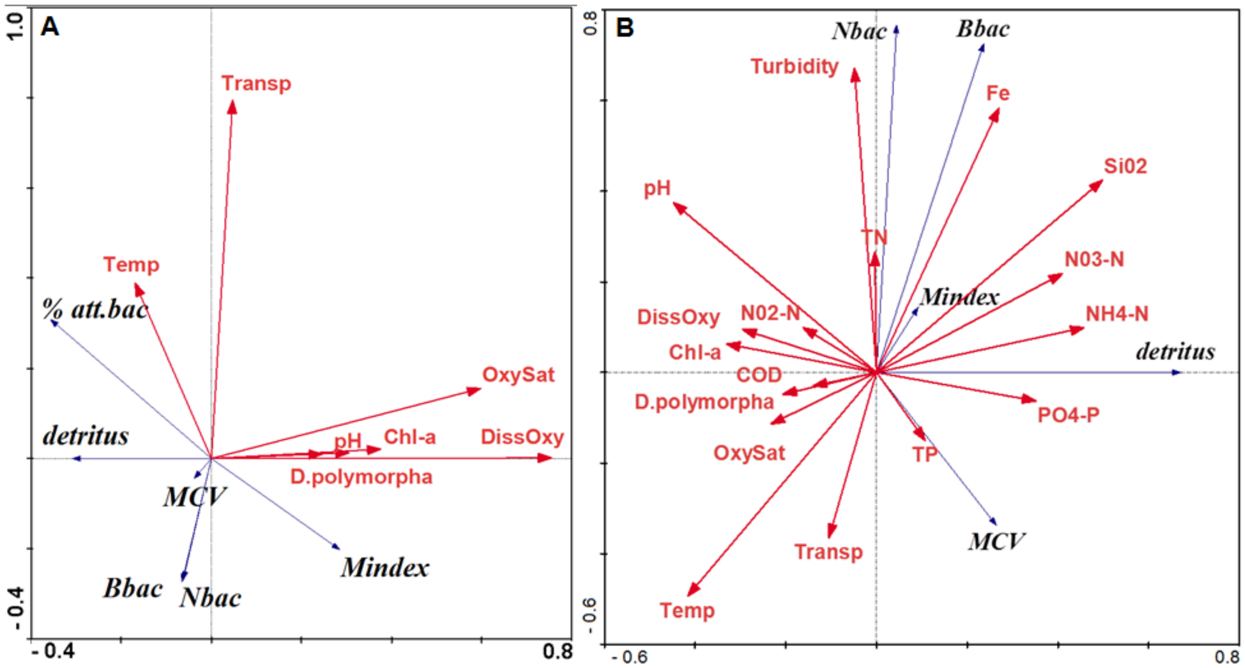

Fig. 7. RDA biplot of correlations of bacterioplankton (total number, biomass, mean cell volume, morphological index, detritus particles and/or % of attached bacteria) with: (A) five abiotic environmental factors (temperature, transparency, dissolved oxygen, oxygen saturation and pH), chlorophyll-a and *D. polymorpha* in all reservoirs for three years (2009-2011; n=132); (B) 15 abiotic environmental factors, chlorophyll-a and *D. polymorpha* for the period 2010-2011 (n=96). Symbols: see Fig. 5. Statistics: (A) ΣAllEV=1.000; ΣCanEV=0.094, F-ratio=2.151, P=0.0800; (B) ΣAllEV=1.000; ΣCanEV=0.449 (45%), F-ratio=3.740, P=0.0020

Spearman, but a positive with the ratio rods:cocci in all reservoirs for the 3-year period 2009-2011 (Fig. 7A, P=0.08, close to the level of significance, n=132). Bacterioplankton again correlated slightly negatively with *D. polymorpha* during the two year-period 2010-2011 (Fig. 7B, P=0.002, n=96). All forms of nitrogen, Fe, Si, and turbidity were positively related to bacteria. Correlations of bacteria with transparency and temperature were negative in both analyses (Fig. 7 A, B).

Temperature in most samples showed negative relation, except on cell volume and attached bacteria (Figs. 5B and 7), but probably was very high and beyond the optimum under conditions of global warming and shallowness of the water bodies, which affects all organisms (Häder et al. 2007). Chlorophyll-a had different relation to bacterioplankton development, from absence to weakly negative for the whole period (Fig. 7A, B), but negative in 2009 and 2010 (Figs. 5A and 6), and positive in 2011 and in Koprinka Reservoir for the whole period. The positive relation found in other non-infested reservoirs (Kalcheva 2011) was disturbed in these two infested reservoirs. The same disturbance could be assumed for the temperature, as well. Positive direct correlations are found between the number of bacterioplankton and phytoplankton biomass (Currie 1990) and also chlorophyll-a (Bird & Kalff 1984). With respect to abiotic factors, water temperature is the main factor that affects positively bacteria (Lindström et al. 2005), but also the phosphate phosphorus (Currie 1990, Vrede et al. 1999, Carrillo et al. 2006) and ammonium nitrogen (Vrede et al. 1999). On the other hand, the high pH values and low inflow affect negatively bacterioplankton (Lindström et al. 2005). Our results were in agreement with findings, mentioned above, except for the temperature and chlorophyll-a, which relations probably were disturbed due to the presence of *Dreissena* spp.

The published results for the same period and in the same reservoirs showed a decrease in phytoplankton (Beshkova et al. 2014) and zooplankton development, lower quantities (and even absence) of rotifers and higher quantities of cladocerans (Kozuharov & Stanachkova 2015), an increase in nematodes (Kalcheva et al. 2016) and other changes in macroinvertebrate and fish communities (Trichkova et al. 2013, Kenderov et al. 2014, etc.) in the infested reservoirs by *Dreissena* spp. compared to the non-infested reservoirs or before the infestation. Discussion of these results could explain only partially, but insufficiently the indirect impact of *Dreissena* spp. on bacterioplankton.

Conclusions

The annual, seasonal and spatial changes in the total number, biomass, morphotypes and size structure of bacterioplankton demonstrated greater variability in the reservoirs Ogosta and Zhrebchevo infested by the invasive mussels *Dreissena* spp., than in the non-infested Koprinka Reservoir. Positive relations existed with turbidity, nutrients N and P, COD, Fe and phytoplankton, and negative – with temperature, transparency, pH and Ca^{2+}. The positive relations with chlorophyll-a and temperature were disturbed in the infested reservoirs. Indirect positive impact of *Dreissena* spp. presence on bacterioplankton in the infested reservoirs was observed. The direct effects of *D. polymorpha* were expressed by the slightly negative correlations with the total number and biomass of bacterioplankton, and positive correlations with the rods, larger and attached bacteria. Further analyses with inclusion of available data on all biotic factors in the reservoirs Ogosta, Zhrebchevo and Koprinka, based on trophic interactions would explain better the bacterioplankton dynamics and its relations. Such analyses may help to assess all changes in the infested reservoir ecosystems compared to the non-infested.

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