THE EFFECT OF *DREISSENA POLYMORPHA* ON BACTERIOPLANKTON, NEMATODE FAUNA AND THEIR RELATIONS TO ENVIRONMENTAL FACTORS IN OGOSTA RESERVOIR (DANUBE BASIN)

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ABSTRACT

Spatial, seasonal, and annual bacterioplankton dynamics in recently infested by the species *Dreissena polymorpha* Ogosta Reservoir were studied for the first time during three year period. Bacterioplankton total number was higher in spring in ecotone zones, than in summer at thermocline. NH_4-N, PO_4-P, turbidity, dissolved oxygen, COD and chlorophyll-a correlate positively, while transparency and Ca^{2+} negatively with bacteria. Nematode species composition, included 22 species studied (13 rarely found and *Rhabditis brevispina* new for Bulgaria) belonging to nine families. The *D. polymorpha* impact is positive on nematodes and phytoplankton, negative on zooplankton and bacterioplankton, but weak positive on larger bacteria, rods and attached bacteria.

RESUMEN: Efecto de *Dreissena polymorpha* en el bacterioplancton y la fauna de nemátodos y su relación con factores ambientales en el Reservorio Ogosta.

Por primera vez se estudia la dinámica espacial y estacional del bacterioplancton durante una invasión reciente de la especie *Dreissena polymorpha* en el Reservorio Ogosta, a lo largo de tres años. En términos numéricos, el bacterioplancton fue más abundante en los ecotonos durante la primavera y durante el verano en la termoclina. Los NH_4-N, PO_4-P, la turbidez, oxígeno disuelto, COD y la clorofila-a mostraron una correlación positiva con la abundancia de bacterias, en tanto que la transparencia y el Ca^{2+} se correlacionaron negativamente. La composición de especies de nemátodos, que también estudiada por primera vez, incluye 22 especies (13 raramente encontradas y *Rhabditis brevispina*, una especie nueva para Bulgaria) pertenecientes a nueve familias. El impacto de *D. polymorpha* es positivo sobre los nemátodos y el fitoplancton, pero negativo sobre el bacterioplancton y el zooplancton; se encontró una relación débil con las bacterias de gran tamaño y con aquellas que tienden a adherirse.

REZUMAT: Efectul speciei *Dreissena polymorpha* asupra bacterioplanctonului, faunei de nematode și relațiilor acestora cu factorii de mediu în lacul de acumulare Ogosta.

A fost studiată în trei ani, dinamica spaţială, sezonieră şi anuală a bacterioplanctonului în recentul invadat bazin Ogosta de către specia *Dreissena polymorpha*. Numărul total al bacterioplanctonului a fost mai mare primăvara în zonele de ecoton, decât vara la termoclină. NH_4-N, PO_4-P, turbiditatea, oxigenul dizolvat, CCO şi clorofila-a s-au corelat pozitiv, în timp ce transparenţa şi Ca^{2+} s-au corelat negativ cu bacteriile. Compoziţia speciilor de nematode, studiate pentru prima oară, include 22 de specii (13 rare şi *Rhabditis brevispina* nouă pentru Bulgaria) aparţinând la nouă familii. *D. polymorpha* are un impact pozitiv asupra nematodelor şi fitoplanctonului, negativ asupra zooplanctonului şi bacterioplanctonului, dar uşor pozitiv asupra bacteriilor mai mari, a bacililor şi a biofilmului.

INTRODUCTION

The investigation of *Dreissena polymorpha* (Pallas, 1771) effect on reservoir ecosystems is very important, especially for countries like Bulgaria, where main freshwater resources are in reservoirs built on rivers (Kalchev et al., 2013). Dreissena ssp. (*D. polymorpha* and *D. bugensis*, Bivalvia: Dreissenidae) are known as successful aquatic invaders that have great potential to directly or indirectly impact the biodiversity and ecosystem functioning. Due to their ability to filter the seston in large volumes of water, they cause huge ecosystem changes summarized as a shift of energy flow from pelagic to benthic food chain (Karatayev et al., 2002). Factors that limit the growth and spread of the invasive species *D. polymorpha* include low and high temperatures (below 10ºC or above 26-32ºC), low concentration of calcium ions (Ca^{2+}), and very high or very low production in lakes (Strayer, 1991). In Bulgaria the species is found at depths between three m to 10 m in reservoirs with moderate amounts of nutrients (Trichkova et al., 2008; Kozuharov et al., 2009).

Planktonic bacteria are important for nutrient remineralisation and participate as a key trophic source in pelagic food webs (Straškrabova et al., 1999; Cotner and Biddanda, 2002; Jürgens and Matz, 2002; Pernthaler, 2005). The necessity of studying bacterioplankton development and its interaction with environmental factors, including some invasive species like zebra mussel (*D. polymorpha*) found in many lakes in the world, but also in Eastern Europe and in some Bulgarian reservoirs (Cotner et al., 1995; Karatayev et al., 1997; Trichkova et al., 2008; Kozuharov et al., 2009; Kozuharov and Stanachkova, 2015) is an indication of understanding the biogeochemical processes in aquatic ecosystems in the period of global warming and climate changes (Häder et al., 2007). It is found that the sizes and morphology of aquatic species are essential for trophic relationships between them (Havens, 1998), and bacterioplankton is without exception (Cotner et al., 1995; Cole, 1999; Hahn and Höfle, 2001; Pernthaler, 2005). Research for the ecological role of bacterioplankton in standing waters in Bulgaria is numerous (Beshkova et al., 2008; Kalcheva, 2011; etc.), but has not been performed in long-term experiments with inclusion of different trophic levels in reservoirs with the invasive species *Dreissena polymorpha*.

Free-living Nematoda play a main ecological role, as primary consumers displaying saprophagous or bacterivorous feeding habits, and take part in the nutrient cycling control. Data about Nematoda in Bulgaria was given by Valkanov (1934), Russev (1979) and Stoichev (1996, 1998). This study gives the first information about the free-living freshwater nematode of the Ogosta Basin and its relation to the environmental factors and *Dreissena polymorpha*.

This study establish the spatial, seasonal, and annual bacterioplankton dynamics and free-living nematode species diversity and to determine, the influence of environmental factors on them and the effect of *Dreissena polymorpha* occurrence on their development.

MATERIAL AND METHODS

The study was conducted in the period 2009-2011 in Ogosta Reservoir in Bulgaria, infested with the invasive species *Dreissena polymorpha*. The Ogosta Reservoir (Fig. 1) is situated in North-West Bulgaria close to the town of Montana at an altitude of 203 m a.s.l. (43°22’31” N, 23°10’56” E). It is built on the river Ogosta, a direct tributary of the Danube River and gathers the waters of other two rivers, Zlatitsa and Barzeya. The reservoir area is 2,360 ha, its length by diagonal is 14 km and the total water volume is 506 million m^3 (Kenderov et al., 2014). A total of 58 samples were taken for bacterioplankton in late summer of 2009 (in beginning of September), spring (April) and autumn (September/October) of 2010 and in summer (July) of 2011 from five stations (Fig. 1), divided from the wall (station 1) to the tail (station 5) of the reservoir and from different depth horizons starting at 0.3 m to maximum depth of each station, but one m above the bottom (35 m in station 1, the wall).

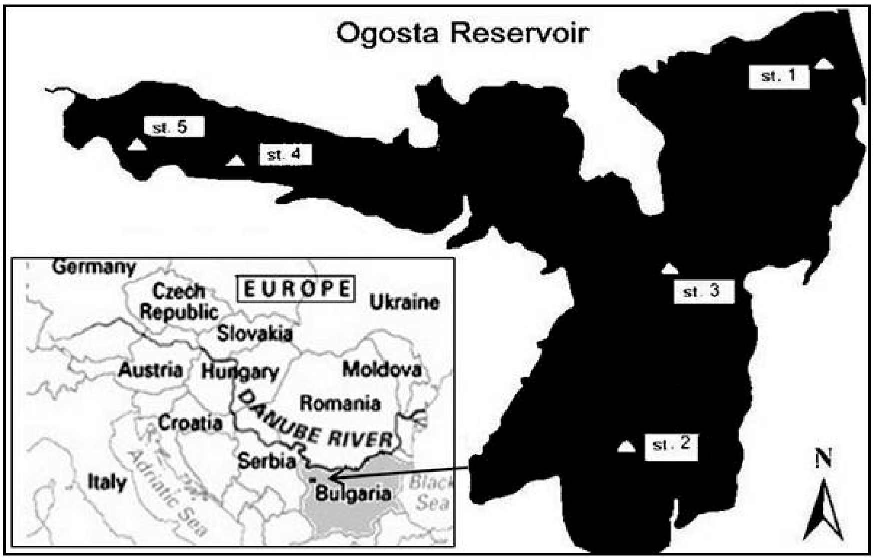

Figure 1: Overview of the study area in Bulgaria as a part of the Danube River basin countries in Europe and localisation of the measuring points (stations 1-5) in Ogosta Reservoir.

The number of bacteria is determined by the method of a direct count with a phase-contrast microscope (Carl Zeiss, Jena, Germany) by the ocular grid at a magnification of 1,600x after preliminary fixation with 2% formalin and staining with erythrosine (Razumov's method, updated by Naumova in Grudeva et al., 2006), described in detail (Kalcheva et al., 2008; Kalcheva, 2011). Biomass is calculated in carbon content by Norland's formula (Straškrabova et al., 1999) after determination of the mean cell volume (MCV). Bacterioplankton is counted separately for cells (cocci and rods) freely dispersed on the filter (0.2 µm pore size) and for cells that are associated with detritus particles, since morphological groups are provisionally divided in four groups (free cocci, rods and attached cocci, rods). The sizes of bacteria are divided into size classes (Pernthaler et al., 1996; Kalcheva et al., 2008; Chróst et al., 2009). The number of detritus particles with attached bacteria is also counted.

The material for free-living nematodes was collected with Ekman-Birge grab in spring, summer, and autumn of 2010 and in the summer of 2011. A total of 25 samples were collected. The results were adjusted for one m^2. The nematode samples were rinsed on sieves, with mesh sizes of 500 µm and 50 µm. A careful heating up to 60ºC was performed, by which the nematodes become stacked and erected and thus convenient for measuring. Then the nematodes were fixed in 4% formalin. Nematodes were identified according to Gagarin (1981) and measured on the basis of the formula of De Man (1886). The monograph of Loof (1999) was also used to determine the nematodes.

Parallel measurements for the three year period were made for 15 environmental factors, in situ for some physicochemical factors and hydrochemical sampling for laboratory analyses, also for chlorophyll-a and *D. polymorpha*, but only in 2009 for phytoplankton and zooplankton. Abiotic factors temperature, pH, dissolved oxygen, and oxygen saturation are measured with oximeter type WTW 315i/SET, transparency by Secchi disc, Ca^{2+} by a standard method in a chemical laboratory at the University of Innsbruck. The other factors, NH_4-N, NO_3-N, NO_2-N, PO_4-P, TN, TP, Fe, Si, and COD are analyzed with kits of Merck (Germany) and by a spectrophotometer Spectroquant® NOVA60 and a thermoreactor (Merck, Bulgaria

EAD). Standard methods for phytoplankton (Beshkova et al., 2008), zooplankton (Kozuharov and Stanachkova, 2015), chlorophyll-a (ISO 10260, 1992) and *D. polymorpha* are used (Trichkova et al., 2008). Quantitative results about *Dreissena polymorpha* have not been presented and used in this study, but only the assessment of its development in five categories (absent, only shells, small, middle and high quantities) from the five stations and the sampling depths is performed for statistical analyses. Data of all environmental factors, abiotic and biotic, are also not presented, but only used in statistical analyses.

The multivariate statistical Redundancy Analysis (RDA) with the program CANOCO for Windows 4.5 (ter Braak and Smilauer, 2002), single factor analysis of variance (one-way ANOVA), nonparametric correlations of Spearman (R_{Sp}) and regressions (linear correlations) with the computer program STATISTICA 7.0 are used. Bacterioplankton variables are included in RDA as dependent (response) variables, while environmental factors (abiotic, biotic and the zebra mussel) as independent (explanatory) variables. Where RDA includes Nematoda species as dependent variables, bacteria are presented as independent. Statistical evaluations are performed using a level of significance P (probability) with 5% risk of error (α or $P < 0.05$).

RESULTS AND DISCUSSION

The total number of bacterioplankton in the period 2009-2011 varied in the range from 4.70×10^4 to 5.83×10^5 cells.ml^{-1} (Fig. 2). The biomass in carbon content varied from 0.69 to 10.24 μg C.L^{-1} and the mean cell volume (MCV) was in the range between 0.0493 and 0.0797 μm^3 (Fig. 3). The abundance was higher than in other studied reservoirs (Beshkova et al., 2008; Kalcheva, 2011). Abundance and biomass decreased towards 2011, but were higher in spring in stations close to inflows of the main and other two rivers (ecotone zones, 2 and 5), and in summer at the deeper layers with a maximum at thermocline (at a depth of 15 m) in the deepest station 1 (the wall) and at five m in other stations (Figs. 3 and 4), where the organic matter and biodegradation increased. The differences between the seasons were statistically significant (Fig. 3, ANOVA), between the years they were close to the level of significance ($p = 0.07$), while between the five stations they were not significant. The MCV was relatively low with the dominance of the smallest size class of 0.2-0.5 μm (49-83% of the total). This is typical for eutrophic waters (Šimek et al., 1997; Pernthaler et al., 1996; Chróst et al., 2009).

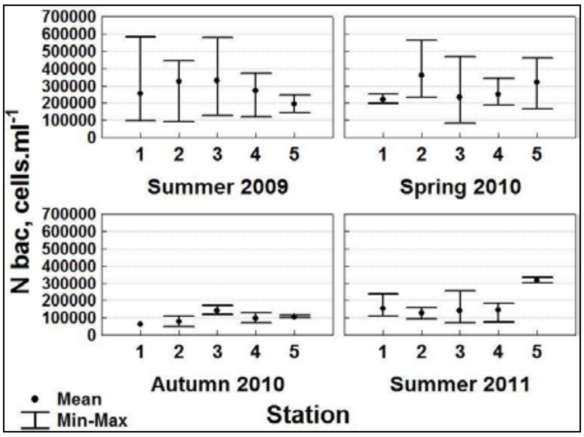

Figure 2: Spatial, seasonal and annual dynamics of bacterioplankton total number (mean, minimum and maximum) at sampling stations (1-5) in the Ogosta Reservoir during 2009-2011.

Free-living cocci were 24-59% and prevailed over the other morphotypes, but larger bacteria (< one μm) and bigger quantities of attached to detritus bacteria were found in 2009-2010 when the development of *D. polymorpha* was stronger than in 2011. The attached bacteria varied widely from three to 80% and were higher in summer. Most probably it is connected with the organic matter excreted by the zebra mussels, and also with their filtration of small sized seston including bacteria (Cotner et al., 1995; Dionisio Pires et al., 2004). The number of free-living large bacteria were higher in spring especially in stations 2 and 5, ecotone zones, where the inflows of the rivers (probably due to the rainfalls), had more organic matter and nutrients. The increase in the quantity of rod cells, especially with the relatively large sizes means, increased organic content in the water (Pernthaler, 2005). The detritus particles number was higher in 2009 summer in station 2 and at the depth layers between 0.3 m and 15 m. We assume that the reason, again, is excreted organic matter by *D. polymorpha*.

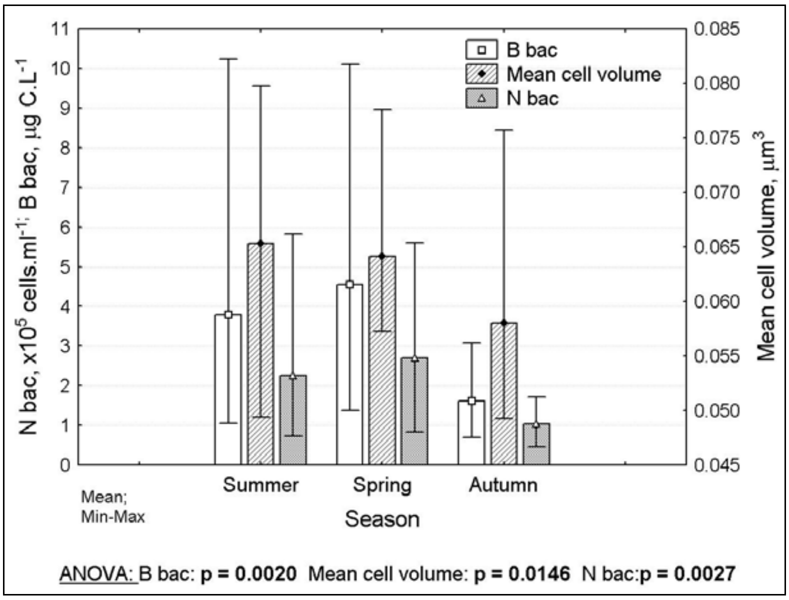

Figure 3: Seasonal dynamics of bacterioplankton total number, biomass and mean cell volume (mean, min-max) in Ogosta Reservoir for the three year period (2009-2011) and f-test with given p-values (ANOVA).

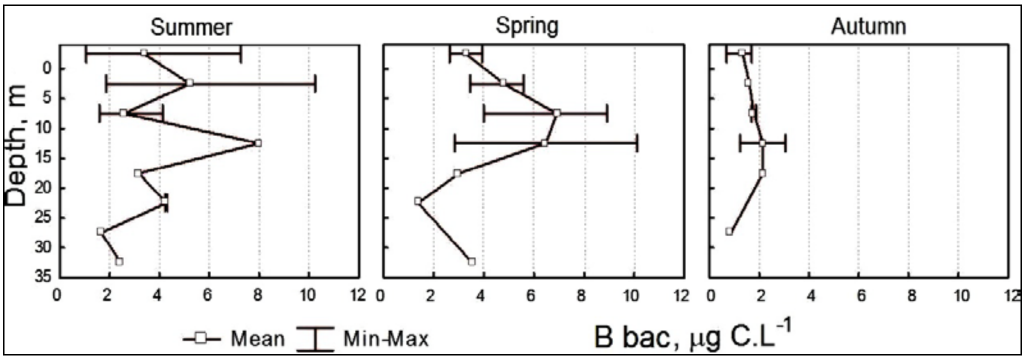

Figure 4: Seasonal dynamics of the bacterial biomass (B bac) in different depth layers in Ogosta Reservoir in the period 2009-2011.

22 species from 12 genera, nine families, and five orders were determined in Ogosta Reservoir (Tab. 1). The species *Monhystera stagnalis*, *Monhystera filiformis* and *Dorylaimus stagnalis* were found in all sites. One species (*Rhabditis brevispina*, marked by +) is new to the Bulgarian hydrofauna. The Nematoda diversity in Zhrebchevo Reservoir (Stoichev and Danova, 2012), 26 species, was close to the species number in Ogosta Reservoir, both infested by *Dreissena polymorpha*. While in the non-infested Koprinka Reservoir and Stambolijski Reservoir the species number is lower, 15 and 11 respectively (Stoichev, 1996). The number of species that we found is close to the data given by Traunspurger (2002), who reported that the species richness in general ranges between 30 and 70 in lakes and rivers. The results from the dominance analysis of the species (pF, DF and Dt in %) are shown in table 1. Beside species with high values of frequency of occurrence and range of dominance (*Monhystera stagnalis*, *Monhystera filiformis*, *Dorylaimus stagnalis*), are also species with high values of range of dominance, low frequency of occurrence, and frequency of dominance can be found (*Cylindrolaimus communis*, *Rhabditis filiformis*, *Prodesmodora circulata*).

In 2009-2011 for *D. polymorpha* and *D. bugensis* (Andrusov, 1897, the impact was not assessed in this study, see in Stanachkova et al., 2015), were found at a depth of 0.30 to 30 m. *D. polymorpha* more often was found at depths up to 15 m, while *D. bugensis*, which is deep-water species, at 24-30 meters (at station 1, the wall). According to the measured temperature (7-29ºC, but except the extreme values) and chl-a (0.7-8.9 $\mu g.L^{-1}$, mesotrophy), Ogosta Reservoir is appropriate for the development of *D. polymorpha* (Strayer, 1991).

Table 1: Nematoda species by stations and dominant analysis (pF%, DF% and Dt%).

Taxa	Stations					Dominant analysis		
	1	2	3	4	5	pF %	D %	Dt %
Monhysterida								
Monhysteridae								
Monhystera stagnalis Bastian, 1865	X	X	X	X	X	74.28	54.3	73.07
Monhystera filiformis Bastian, 1865	X	X	X		X	62.85	51.4	81.81
Monhystera sp.				X		2.85		
Dorylaimida								
Dorylaimidae								
Dorylaimus stagnalis Dujardin, 1845	X	X	X	X	X	88.57	62.8	70.96
Dorylaimus sp.			X			2.85		
Paradorylaimus filiformis (Bastian, 1896) Andrassy, 1969				X		5.71		
Paradorylaimus sp.		X				2.85		
Mesodorylaimus sp.			X			2.85		
Qudsianematidae								
Eudorylaimus carteri (Bastian, 1865) Andrassy, 1969	X		X		X	31.42	14.3	45.44

Table 1 (continued): Nematoda species by stations and dominant analysis (pF%, DF% and Dt%).

Taxa	Stations					Dominant analysis		
	1	2	3	4	5	pF %	D %	Dt %
Cylindrolaimidae								
Cylindrolaimus communis De Man, 1880		X				8.57	2.85	33.25
Cylindrolaimus sp.				X		2.85		
Plectidae								
Plectus cirratus Bastian, 1865			X	X		22.85	17.1	75.01
Plectus assimilis Bütschlii, 1873		X				5.71		
<u>Rhabditida</u>								
Rhabditidae								
Rhabditis filiformis Bütschlii, 1873	X	X				17.14	11.4	66.62
Rhabditis brevispina (Claus, 1862) Bütschlii, 1873			X			2.83		
<u>Enoplida</u>								
Tripylidae								
Tripyla glomerans Bastian, 1865	X		X	X		51.42	45.7	88.89
Tobrilus gracilis Bastian, 1865			X			11.42		
Tobrilus sp.			X			2.85		
Enoplidae								
Tobrilus gracilis Bastian, 1865			X			11.42		
Tobrilus sp.			X			2.85		
Enoplidae								
Enoploides fluviatilis Micoletzky (1923)	X	X		X		14.28	5.71	39.98
Enoploides sp.		X				2.85		
<u>Chromadorida</u>								
Microlaimidae								
Prodesmodora circulata (Micoletzky, 1913) Micoletzky, 1915		X	X	8.5 7	2.8 5	33.25		
Prodesmodora sp.				X		2.85		

Interactions of bacterioplankton and nematodes with environmental factors and the effect of *D. polymorpha* occurrence

From environmental factors, mainly abiotic, positive relationships exist between bacterioplankton and nutrients, NH_4-N (r = 0.38), PO_4-P (r = 0.34), TP, turbidity, dissolved oxygen, Fe ions, COD (Fig. 5B), phytoplankton biomass (r = 0.58), and chlorophyll-a. Negative relations are found with pH, transparency (Fig. 5A), and Ca^{2+} (r = – 0.33). The ratio of the nutrients N and P is very important for bacterioplankton development (Vadstein et al., 2003; Chróst et al., 2009). Temperature, in most cases, shows negative relation with bacteria except cell volume (sizes), but probably is very high and beyond the optimum under conditions of global warming and shallowness of the water bodies, which affects all organisms.

A negative correlation existed between *D. polymorpha* and the bacterial abundance (and biomass) in 2009, while in 2010-2011 it was positive, close to the level of significance with p-value = 0.08 and p-value = 0.07 respectively (RDA, Fig. 5A, B). Negative correlations were confirmed, also by nonparametric tests, for the whole period (2009-2011) as weak, but significant of *D. polymorpha* with bacterioplankton abundance (r_{Sp} = – 0.287) and biomass (r_{Sp} = – 0.298). The results are in agreement with previous findings reported in similar studies. We assume that the increase of larger sized free-living bacteria in Ogosta Reservoir is related to the findings of Dionisio Pires et al. (2004) about preferential filtration by *D. polymorpha* of two seston size groups, 0-1 μm and 30-100 μm. Filtration by zebra mussels of free-living bacteria with sizes under one μm leads to better transparency and that is pointed out with the positive relation of *D. polymorpha* with transparency, and negative with bacteria (Fig. 5A).

The relation of bacterioplankton with the other two plankton communities (Fig. 5A), determined in 2009, showed a positive correlation with phytoplankton biomass (due to extracellular release of DOC), but negative with zooplankton number (due to predation). The effect of *D. polymorpha* on the plankton was positive impact on phytoplankton number, but negative on zooplankton number, bacterioplankton number, and biomass (Fig. 5A).

Two important environmental factors, COD and oxygen saturation, correlated significantly to the spatial diversity of nematode species (RDA, p = 0.026, not shown). Bacterioplankton total number and COD (Fig. 5B) had higher values in stations 2 and 5. These stations represent ecotone zones, because of the inflow of the rivers Zlatitsa and Barzeya and respectively Ogosta, where the quantity of dead organic matter coming from the rivers to the reservoir, is higher and the processes of its degradation is more intensive.

Negative correlations were found between bacteria and *Tripyla glomerans* (r = – 0.98, p = 0.002, T. glomerans = 36,0076-0,0001*x) and between detritus and *Enoploides fluviatilis* (r_{Sp} = – 0.88, $p < 0.05$). *Tobrilus* genus was found in the sampling station 3 and correlated negatively with the oxygen saturation. The individuals of *Tobrilus* are represented at a high density in the sediments of lenthic ecosystems, especially in eutrophic lakes (Traunspurger, 2002), and are considered as tolerant of low oxygen conditions (Vidakovic and Bogut, 2004). Positive correlations were found between *D. polymorpha* and *M. filiformis* and *R. filiformis* (Fig. 5B), most probably utilizing detritus particles excreted by the zebra mussels.

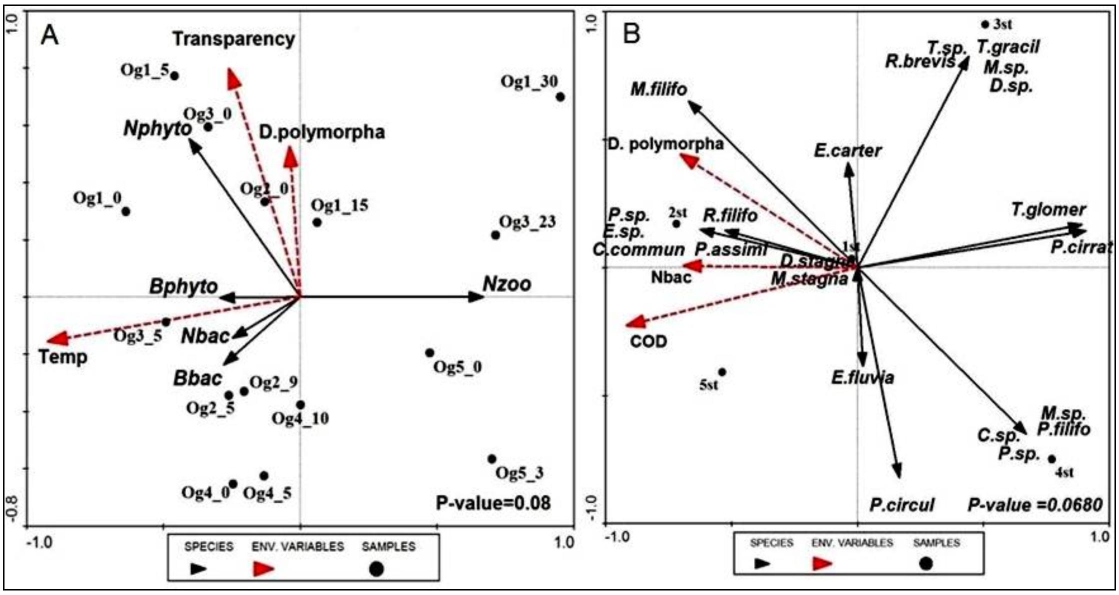

Figure 5: RDA triplots presenting the correlations (with given p-values) between:
(A) bacterioplankton, phytoplankton and zooplankton (dependent variables, species)
and environmental factors and *D. polymorpha* (env. variables)
by stations and depth horizons (samples) in 2009,
(B) Nematode species composition (species) and environmental factors, bacterioplankton and *D. polymorpha* (env. variables) by stations (samples) in 2010-2011. Abbreviations: N – number, B – biomass, bac – bacterioplankton, phyto – phytoplankton, zoo – zooplankton, COD – chemical organic demand, st – stations from 1 to 5; coding of Og2_5 = Og (Ogosta), 2 (the station number), 5 (from five m depth); for full names of Nematode species see in table 1.

CONCLUSIONS

The effect of *Dreissena polymorpha* on bacterioplankton in investigated Ogosta Reservoir is indirect, manifested with higher bacterial abundance, due to weakened zooplankton pressure. Direct effects are a slightly negative impact on the number and biomass (during the whole period), and a slightly positive impact on larger bacteria, rods, and attached bacteria.

The effect of *Dreissena polymorpha* on phytoplankton, transparency and COD, is positive, but leading to eutrophication and more organic matter, while it is negative on zooplankton, leading indirectly to lower fish production and diversity.

The Nematoda community in the ecosystem of Ogosta Reservoir is composed of species with high ecological valence as well as species with different level of specialization and adaptation to the environmental conditions, and also to the infestation of the invasive species *Dreissena polymorpha*. Nematode species richness is higher than in others studied, not in investigated reservoirs in Bulgaria, proving prosperity of detritivores in the invested reservoir. Nematodes species richness is higher than in others in Bulgarian reservoirs, proving prosperity of detritivores in this studied case.

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