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VARIABILITY OF BIOLOGICAL CHARACTERISTICS AND THEIR RELATIONS TO ENVIRONMENTAL VARIABLES IN FISH PONDS STOCKED WITH CARP FISH LARVAE IN TWO DIFFERENT PROPORTIONS

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Abstract

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The top-down effect of densities of common carp (*Cyprinus carpio* L.) and bighead carp (*Aristichthys nobilis* Rich.) larvae applied in proportions 3:1 and 1:3 on biological fish pond characteristics like plankton primary production, respiration, abundances of bacterio, phyto and zooplankton, chlorophyll etc. was tested. The experiment lasted 4 months (May to September), was repeated in two consecutive years (2007, 2008) included four ponds in year 2007 from two different localities and five ponds in 2008 from one and the same locality. The measured biological variables showed statistically significant differences between means of localities, years and stocking variants. The sources of variations between localities and years have their ground in objective circumstances accompanying the experiment implementation but could not override the top-down effect of fish stocking variants. The variant 1 (3:1 common carp: bighead carp densities) which proved to be more effective was distinguished by higher phytoplankton and lower zooplankton abundances. Its primary production and respiration were also lower than in variant 2 (1:3 densities of common carp: bighead carp).

Key words: biological characteristics, carp ponds, water ecology, fish larvae

Introduction

Polyculture compared to monoculture is superior method for fish rearing originating from Asia where it is a traditional for long times (Biro, 1995; Kestemont, 1995). The Polyculture practice has extended to many countries with the introduction of Chinese carps for control of water quality and increase of fish production. In many countries, also in Bulgaria the three carp species (common, bighead and grass carps) are among the most frequently used to compose a fish polyculture in freshwater ponds.

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The approach to cultivate in the same pond different carp species is of a great interest not only in terms of available food utilization but also with respect to the utilization of all the ecological niches available in the pond ecosystem, as surface feeder, column feeder or bottom feeder (Kestemont, 1995; Milstein et al., 2006; Kadir et al., 2007). The optimization of spatial and nutrient resources niches utilization also is achieved by manipulating fish densities in wide ranges (Mattson, 1999; Lu et al., 2002; Rahman et al., 2006; Yong-Sulem et al., 2006).

Therefore the goal of the presented study is to test the effect of two stocking proportions of common and bighead carp larvae on biological elements of the pelagic food chain in fish ponds freshwater ecosystem. Beside a selection of proper stocking densities this study also will help to understand the mechanisms of interactions and probably will reveal the most important elements limiting or enhancing the fish growth under the tested conditions.

Materials and Methods

The effects of two breeding variants of common carp larvae (CC) and bighead carp larvae (BC) in two different proportions on biological variables in fish ponds were recorded. The experiment includes two earthen fish ponds stocked with CC and BC larvae by numeric ratio 3:1 (variant 1) and further two fish ponds of the same type with ratio 1:3 in favor of BC (variant 2). To each of the two stocking variants we added one and the same number of grass carps larvae and 1+ or 2+ aged common carps. As a rule each stocking variant was repeated every year in two ponds, however, in 2008 the variant 2 was exceptionally experimented in 3 ponds. The Table 1 summarizes the number of individuals from each fish species, ponds, sites and years included in the experiment.

Several physical, chemical and biological variables were monitored during the investigated period: total solar radiation (by pyranometer in MJ m^{-2}), water temperature (°C), water column transparency (cm, by Secchi disk), pH (measured electrochemically), hydraulic retention time (or flushing rate, RT, liter. $min^{-1}.0.1ha^{-1}$), oxidability by $KMnO_4$ (mg $O.l^{-1}$, Bulgarian state standard (BSS)), dissolved oxygen ($mg.l^{-1}$, measured electrochemically by oxygen-meter type WTW Oxi 315i/SET), oxygen saturation (%), gross primary production (GPP, $g.m^{-2}.24h^{-1}$, by the light and dark bottle technique in its oxygen modification after Vollenweider, 1969), plankton chlorophyll a and phaeopigments ($\mu g.l^{-1}$, ethanol extraction according to ISO 10260), percentage of pond area covered by macrophytes (visual estimation), ammonium and ni-

Table 1
Stocking variants with larvae of common carp (CC_0), bighead carp (BC_0), grass carp (GC_0) and 1 or 2 year old common carp (CC_{1+}/CC_{2+} with 250g average body weight) reared in years 2007 and 2008 in fish ponds at two sites (Plovdiv and Trivoditsi)

Fish species groups	Variants of fish stocking			
	Variant 1, Ind ha^{-1}		Variant 2, Ind ha^{-1}	
CC_0	60000		20000	
BC_0	20000		60000	
GC_0	20000		20000	
CC_{1+}/CC_{2+}	500		500	
Years, Sites, Pond numbers	Variant No1		Variant No2	
2007 sites	Plovdiv	Trivoditsi	Plovdiv	Trivoditsi
2007 Pond No and area in brackets, ha	8 (0.38)	6 (0.25)	9 (0.46)	10 (0.20)
2008 sites	Trivoditsi		Trivoditsi	
2008 Pond No and area in brackets, ha	6 (0.25), 8 (0.20)		3 (0.20), 9 (0.20), 10 (0.20)	

trate nitrogen(N, μg.l^{-1},colorimetrically with Nester's reactive, BSS No 3587-79, respectively BSS No 3758-85) and phosphate phosphorus (P, μg.l^{-1}, colorimetrically with molybdenum reactive, BSS No 7210-838). The numbers and biomasses of the three main components of the pelagic food chain (bacterio, phyto and zooplankton) were also quantitatively investigated. The sampling, determining and counting procedures of bacterio, phyto and zooplankton were described by Kalcheva et al. (2008) and Terziyski et al. (2007).

The multivariate redundancy analysis (RDA) in its partial variant separating temporal and spatial variations (RDA_{time} and RDA_{ponds} taking into account the temporal and spatial structure of the data), by means of statistical software Canoco for Windows 4.5 (Braak and Smilauer, 2002), was applied. All measured biological variables were included in RDA as dependent (response) variables, while other environmental factors, aquatic chemistry variables, stocking variants, pond sites and years were treated as independent (explanatory) variables. The RDA is carried out with data of each year separately and with pooled data from the two years. In the second analysis some of the samples like first sampling date of June 2007 and samples of pond 3 in 2008 were excluded in order to obtain a temporal sequence of sampling close to a time series row. We also applied Mann and Whitney nonparametric test for testing the differences between means of stocking variants, pond sites and years of single biological variables. The resulted unbalanced design of the experiment (inclusion of three instead of two ponds for stocking variant 2 in year 2008) and some deviations in dates of sampling in 2007 made the application of variance analyses for all available data impossible.

Results and Discussion

As shown on the Table 2 the experiment of year 2007 is clearly distinguished by strong differences between the two different localities from each two ponds were applied in the stocking experiment. The different origin of water supplied to the ponds is the reason for these differences as already extensively discussed by Terziyski et al. (2010), this volume. The higher concentration of nitrates in ground water from

Table 2

Biological variables, for which statistically significant differences were discovered by Mann and Whitney test between means of different localities and two stocking variants within data set of single years; applied abbreviations: Pl (Plovdiv town) Tr (Trivoditsi village) V1 V2 (stocking variant 1, 2), PP plankton primary production, N_{bac} number of bacterioplankton, BM_{bac} – biomass of bacterioplankton, N_{zp}, BM_{zp} number and biomass of zooplankton

Variable	2007		2008	
	Sites, Variants	P ≤	Sites, Variants	P ≤
PP_{gross}	Pl < Tr	0.0058		
	V1 = V2	0.086	V1 = V2	0.19
N_{bac}	Pl > Tr	0.0056		
	V1 = V2	0.71	V1 = V2	0.54
BM_{bac}	Pl > Tr	0.0037		
	V1 = V2	0.73	V1 = V2	0.55
N_{zp}	Pl < Tr	0.000009		
	V1 = V2	0.44	V1 = V2	0.25
BM_{zp}	Pl < Tr	0.00014		
	V1 = V2	0.12	V1 > V2	0.033

Trivoditsi village than in the surface water from Maritsa River in Plovdiv town exercised a strong bottom up effect influencing all trophic levels recorded and overriding the top down effect of two stocking variants in this study during year 2007.

The high numerical and biomass abundance of bacterioplankton in Plovdiv ponds might be due to low number or lack of bacteria in ground waters and their usual availability in considerable quantities in surface river waters. The prevalence of bacterial numbers and biomasses in ponds of Plovdiv town mainly is caused by high numerical abundance of two bacerioplankton morphological groups: the free cocci and free rods. The high total numerical and biomass abundance of zooplankton in Trivoditsi ponds is caused by high abundances of rotifers and copepods.

For data of year 2008 the only statistically significant difference between two experimented stocking variants is discovered for zooplankton biomass in favor of variant 1. It is caused by high biomass of copepods.

However, when we analyze the pooled data from the two years the highest number of significant differences emerges between the years, while between stocking variants there are only few differences.

The statistically significant differences between the two stocking variants proved by Mann and Whitney test were discovered only for plankton functional characteristics – respiration and primary production (Figure 1). The derived variable for percentage of energy utilization by plankton primary production showed significant differences for both – between years and between variants. These differences coincide with the reported higher fish yield for common carp larvae and higher survival rate of bighead carp larvae for V1 than for V2 (Terziyski et al., 2009).

Unfortunately all other discovered significant differences for the pooled data set of biological variables are based on between years comparison. Almost all significant annual differences were in favor of year 2008. The only exception was the BM_{zp}, which was higher in 2007 than in 2008 (Figure 2).

The bacterioplankton numbers and biomasses delivered the strongest between-year differences i.e. with

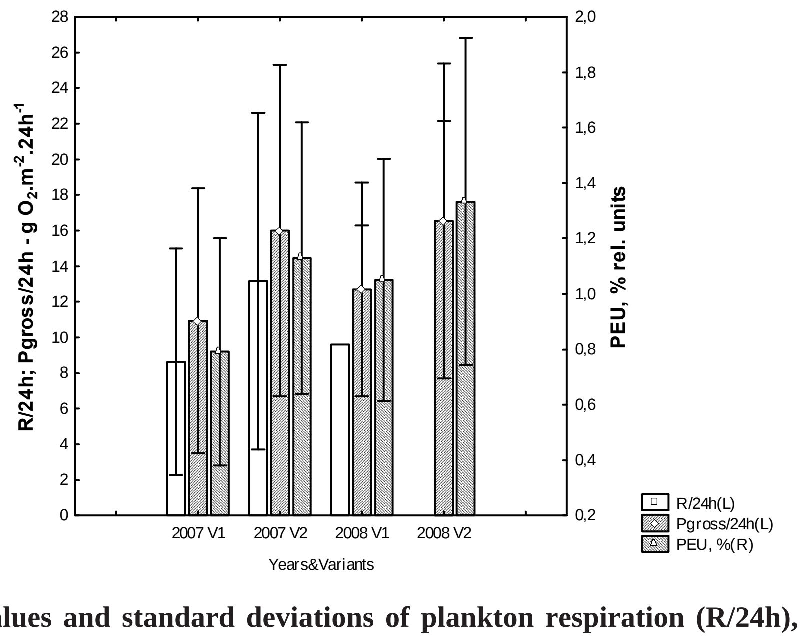

Fig. 1. Mean values and standard deviations of plankton respiration (R/24h), plankton gross primary production (Pgross/24h), percentage of energy utilization by plankton primary production (PEU) from stocking variants 1 and 2 (V1, V2) and years 2007 and 2008. The following differences are statistically significant: R/24h V2 > V1 for P<0.011; Pgross/24h V2 > V1 for P<0.024; PEU V2 > V1 for P<0.01 and 2008>2007 for P<0.045

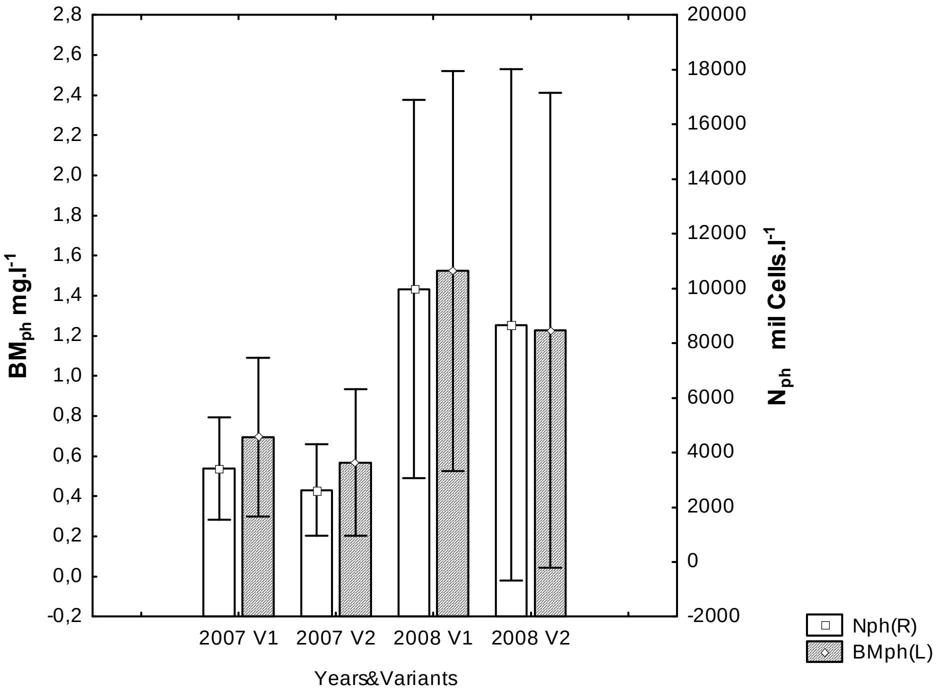

Fig. 2. Mean values with standard deviations of phytoplankton numerical (N_{ph}) and biomass (BM_{ph}) abundances of stocking variants 1 and 2 (V1, V2) and years 2007 and 2008. The annual differences are statistically significant: N_{ph} 2008>2007 for P<0.00004 and BM_{ph} 2008>2007 for P<0.023

highest level of significance (Figure 3). The next are coming the phytoplankton and finally the zooplankton characteristics according to significance strength of their annual differences. There are two possibilities in question for explaining the strong annual differences. The first and more probable one is application of manure of different origin in each year. This hypothesis is supported by the observed strong bacterioplankton effect, which slows down towards phyto- and zooplankton annual differences along the food chain. However, the absence of significant annual differences for nutrients reported by Terziyski et al. (2010), this volume, counteracts this explanation. Moreover, such nutrients like NH_4-N whose difference between annual means was on the significance border tended to be higher in 2007 than in 2008, which was the opposite of observed bacterio, phyto and zooplankton differences. On the other hand the detailed consideration of bacterioplankton data by their morphological, free and attached to detritus groups shows that the number of attached cocci and rods responsible for decomposition of organic substances deliver the strongest level of significance for differences between years. This fact suggests higher amounts of organic matter in year 2008 than in 2007.

The accidental occurrence of crucian carp in both years in some of experimental ponds also might be considered a reason for observed annual differences. However, the comparison of fished quantities of crucian carp at the experiment end does not show significant differences between years.

The strong between-year differences for many recorded biological variables and the distinct pattern of significant differences within each of the two years force us to apply multivariate analyses not only for pooled data but also separately for each year.

The diagram of year 2007 reveals only one variable (Flow, measure of water residence time in ponds) explaining significant share of spatial variations (Figure 4). The separation of samples belonging to the two variants of fish stocking is insignificant and the presented points of the variants on the diagram have an informative character only. The partial spatial variability (all EV=0.388) is lower in 2007 than in 2008

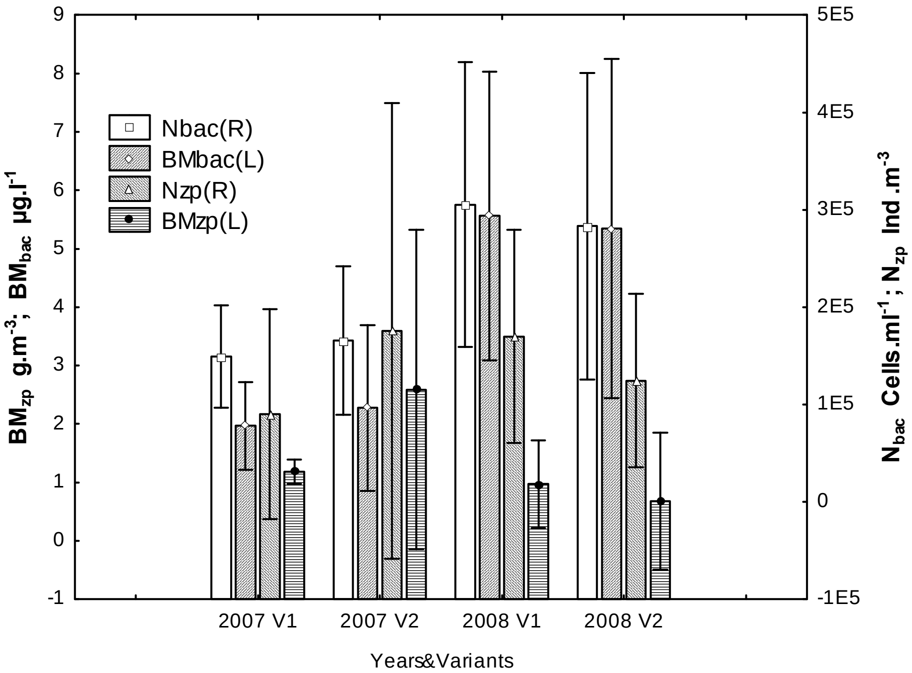

Fig. 3. Mean values and standard deviations of numerical and biomass abundances of bacterioplankton (N_{bac} and BM_{bac}) and zooplankton (N_{zp} and BM_{zp}) from stocking variants 1 and 2 (V1, V2) and years 2007 and 2008. The following differences are statistically significant: N_{bac} 2008>2007 for P<0.00002; BM_{bac} 2008>2007 for P<0.000001; N_{zp} 2008>2007 for P<0.04; BM_{zp}2008<2007 for P<0.0004

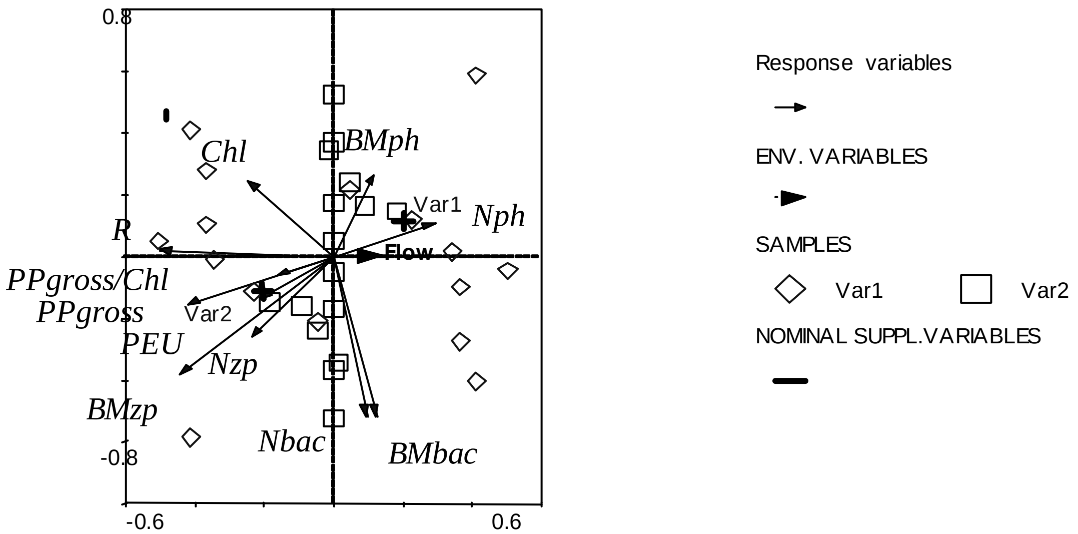

Fig. 4. A tri-plot diagram of parial RDA presenting spatial variations (between fish ponds) of biological variables data set of year 2007. The first axis EV= 0.067 with P=0.01, sum of all eigenvalues is 0.388. The plus sign indicates the place of supplementary (not involved in analysis) environmental variables i.e. V1 and V2

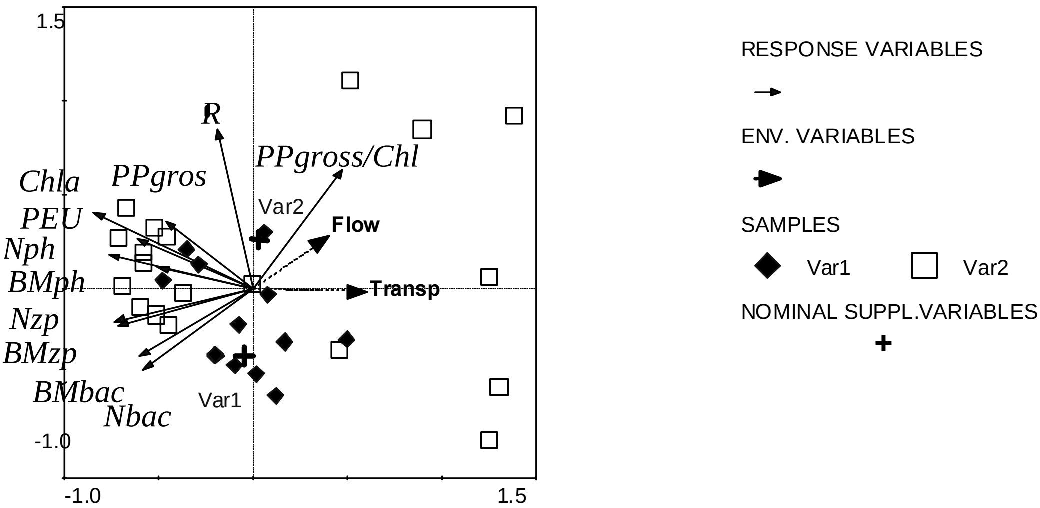

Fig. 5. A tri-plot diagram of partial RDA presenting spatial variations (between fish ponds) of biological variables data set of year 2008. The first axis has EV=0.143, for P=0.0020, the second axis EV= 0.030; all canonical axes trace = 0.174, for P=0.0020; the sum of all EV= 0.719. The plus sign indicates the place of supplementary (not involved in analysis) environmental variables i.e. V1 and V2

(all EV= 0.719).

The diagram of year 2008 beside the explanatory variable Flow adds also transparency (Transp) as a second explanatory variable, thus increasing substantially the share of explained partial spatial variability (Figure 5). In both years as a rule the quantity of water flow correlates negatively with the majority of analyzed biological variables most probably due to the wash away effect exercised on plankton organisms. However, again the stocking variants did not explain a significant deal of variability and their places on the diagram are only informative. All these results about the effect of stocking variants on multiple variations of biological characteristics are in accordance with the previously proved differences between variants for each single variable, i.e. there are no significant differences in both cases.

When we carry out partial spatial analysis with pooled data from the two years the Flow again appears as first significant explanatory variable together with NO_3-N, years and stocking variants (Figure 6). The effect of stocking variants as a single explanatory variable also is highly significant with EV=0.056 and level of significance P=0.006. Thus samples of variant 1 are distinguished by high values of phytoplankton number and biomass while variant 2 samples have high values for zooplankton biomass, plankton primary production and respiration. Thus the multiple variations extended the significant differences discovered by Mann and Whitney test for primary production and respiration with the differences between variants for phytoplankton variables and zooplankton biomass. These differences between variants for phytoplankton and zooplankton despite being close to significance boarder appeared insignificant in the previous analyses by the nonparametric test.

Besides by higher phytoplankton number and biomass the ponds of variant 1 are distinguished also by higher yield of common carp larvae and higher survival rate of bighead carp larvae than ponds of variant 2 (Terziyski et al., 2009). This coincidence let suppose a stronger press on zooplankton under conditions of variant 1 and as a consequence larger phytoplankton quantity available than in ponds of variant 2. On contrary, under conditions of variant 2 the bighead carp fish larvae did not grow differently or they grew even worse than common carp in variant 1, leading to lower press on zooplankton, which is confirmed by higher zooplankton biomass available. The grazing effect of zooplankton on phytoplankton is stronger,

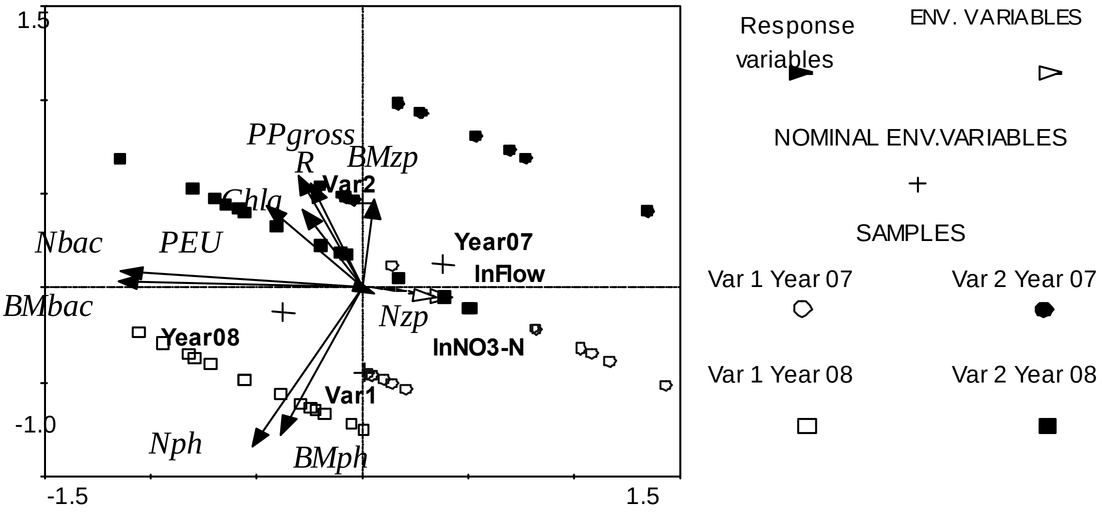

Fig. 6 A tri-plot diagram of partial RDA analyzing spatial variations (between fish ponds) of biological variables data set of year 2007 and 2008 pooled together. The first axis has EV=0.125, for P=0.0020, the second axis EV= 0.067; all canonical axes trace = 0.240, for P=0.0020; the sum of all EV= 0.777

resulting in low algal concentrations, but in more intensive metabolism (production and respiration).

Conclusions

The obtained results let conclude that due to objective reasons several differently directed influences are acting during the experiments in the highly manipulated fish pond ecosystems.

The strong statistically significant differences for many of recorded biological variables between experimental fish ponds belonging to Trivoditsi village and Plovdiv town seem to be caused by application of underground water in Trivoditsi and of surface river water in Plovdiv for ponds flooding and circulation. Similar strong significant differences were found between the two years of experimenting which seem to be caused by different quality of applied manure in each of the two years. These sources of variations reduced the effect of applied fish stocking variants. However, by combining the results of analyses of single and multiple variable differences we managed to reveal the effect of the two fish stocking variants on pond biological characteristics.

Thus besides the strong disturbances the weakened top-down influence caused by manipulating densities of common carp and bighead carp larvae could be reliably proved by considering data and analyses of all investigated elements of fish pond ecosystem – aquatic chemistry (Terziyski et al., 2010), primary production, respiration, bacterio, phyto and zooplankton abundances (this article) survival and fish yield of common and bighead carp larvae (Terziyski et al., 2009).

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