

## **BACTERIOPLANKTON DYNAMICS AND ITS RELATIONS TO THE PRODUCTIVITY IN CARP FISH PONDS**

H. KALCHEVA<sup>1</sup>, L. NIKOLOVA<sup>2</sup>, D. TERZIYSKI<sup>2</sup>, A. STOEVA<sup>2</sup> and R. KALCHEV<sup>1</sup>

<sup>1</sup>*Institute of Zoology, Bulgarian Academy of Sciences, , BG - 113 Sofia, Bulgaria*

<sup>2</sup>*Institute of Fisheries and Aquaculture, BG - 4000 Plovdiv, Bulgaria*

### **Abstract**

KALCHEVA, H., L. NIKOLOVA, D. TERZIYSKI, A. STOEVA and R. KALCHEV, 2008. Bacterioplankton dynamics and its relations to productivity in carp fish ponds. *Bulg. J. Agric. Sci.*, 14: 186-194

This study investigates dynamics of bacterioplankton in four carp fish ponds in Bulgaria during June-September period in 2005. Plankton gross primary production (GPP), chlorophyll a, phaeopigments, nutrients and environmental physico-chemical variables were registered simultaneously. The aim was to search for differences between treated and non-treated with organic manure ponds by comparing bacterioplankton abundance and in its relationships between the above listed variables on one hand, and numbers, biomass or percentages of bacterial morphological groups on the other by means of statistical analyses. The differences between bacterioplankton numbers of manured and non-manured ponds although insignificant, were close to the significance border. The bacterial biomass of control ponds tended to be higher during the first half of the experiment while during the second the tendency reversed. Oxygen saturation was the major environmental factor with significant influence on both number and biomass of bacteria in seasonal aspect. There were a positive relation ( $R=0.495$ ,  $P=0.0046$ ) of oxygen saturation to the number of free-living cocci - the most numerous part of bacterioplankton community, and a negative one ( $R=-0.519$ ,  $P=0.0023$ ) to on detritus attached bacteria. Free-living rods were weakly influenced by oxygen saturation. GPP was positively related to abundance while hydraulic retention time (RT) to biomass of free-living bacterioplankton. The temporal variations of factors mentioned above (GPP and RT) had opposite effects on development of bacteria attached on detritus. Chlorophyll a and nutrients (phosphorus, nitrogen) did not correlate to the development of bacteria neither in spatial nor in temporal aspect.

*Key words:* bacterioplankton, microbial loop, gross primary productivity, relationships, fertilization, carp fish ponds

*Abbreviations:* BSS - Bulgarian state standard, GPP - gross primary production, HNF - heterotrophic nanoflagellates, N – inorganic soluble nitrogen, P – phosphate phosphorus (soluble reactive phosphorus) RDA - redundancy analysis, RP - Pearson parametric correlation, RT - hydraulic retention time.

### **Introduction**

Bacterioplankton plays a central role in utilization of dissolved organic carbon and takes part in the

trophic interactions between microbial communities and phytoplankton-zooplankton grazing chain in pelagial, according to the “microbial food web” conception (Azam et al., 1983). The trophic role of mi-

crobal communities (microbial loop) weakens when the lake trophic state increases from oligotrophic to eutrophic (Porter et al., 1988, Straskrabova et al., 1999). A negative relationship exists between energy flux and recycling of nutrients (mainly phosphorus) in lakes (Begon et al., 1999, Boulion, 2002). Microbial loop accelerates recycling of nutrients, maintaining the primary productivity on high level, but decreases the efficiency of energy flux because of prolongation of trophic chain. Top-down control (predation) by cladocerans on bacterioplankton leads to shorter food chain (prevalence of grazer chain) but to slower nutrient recycling and instability of primary productivity, while indirect control of copepods on bacteria leads to longer trophic chain and energy loss, but high recycling of limiting nutrients. It was shown that both nutrient availability (bottom-up control) and predatory pressure (top-down control by HNF, ciliates and zooplankton) were important in the regulation of bacterioplankton development (Jurgens and Matz, 2002).

The fertilization of fish ponds is aimed to increase plankton primary productivity, which might result in higher fish production. Unfortunately till now the role of bacterioplankton in process of manure as well as in general in fish ponds is underestimated and poorly investigated. The aim of this study was to examine bacterioplankton development and its possible relationships with primary productivity and factors related to it as well as to look for differences between manured and control ponds, using statistical analyses.

## Materials and Methods

This investigation was carried out at the Institute of Fisheries and Aquaculture in Plovdiv (42° 9' N, 24° 45' E, elevation - 185 m), Bulgaria, from June 9 till September 27 2005. The vegetation period in these carp fish ponds, however, lasted from April (ponds were fertilized and stocked with fishes) until the beginning of November (fish production was estimated). Water samples, 32 in number, were collected biweekly from two fertilized (No 12 and No 17) and two non-fertilized, control, (No 8 and No 16) fish ponds. The

ponds were eutrophic, polymictic, shallow (depth – 0.6 – 0.9 m), small in surface area (0.26 – 0.39 ha) and stocked with 500 one-year old common carps (*Cyprinus carpio* LINNAEUS, 1758), 300 bighead carps (*Aristichthys nobilis* (RICHARDSON, 1846)) and 100 grass carps (*Ctenopharyngodon idella* (VALENCIENNES, 1844)) per ha. The applied dose of fertilizer of cattle origin was 3000 kg. ha<sup>-1</sup>.

Bacterioplankton sampling strategy and determination

Water samples were taken by a bucket from the 20-30 cm surface layer and filled in clean plastic bottles of 100-150 cm<sup>3</sup> in volume. The bottles were washed three times with the sampled water and then about 100 cm<sup>3</sup> were preserved with prefiltered (through a membrane filter with a pore size of 0.45 µm) formaldehyde to 2 % final concentration.

To determine the number, biomass, morphological and size structure the method of a direct microscopic count of bacteria, stained with erythrosine (Razoumov 1932, in its contemporary modification, Naumova 1999), was applied. An aliquot of 10 ml of the water sample was filtered under vacuum through a membrane filter (Sartorius) with a pore size of 0.2 µm. Then the filter was stained with 3 % carbolic erythrosine, decolorized to pale pink and after drying it was examined, using immersion, under phase-contrast microscope (Zeiss) with total magnification of 1600x. For ensuring significant results 600 to 1000 bacterial cells (free-living and attached on detritus particles) from 20 randomly determined fields were counted, using eyepiece grid. Further simultaneously 250 to 400 cells were measured, using an eyepiece micrometer. Distilled water was filtered through control filters, which were stained and counted in the same way. Volumes were estimated by assuming cocci as spheres and rod-shaped bacteria as cylinders. The biomass was indirectly calculated from mean cell volumes and converted in carbon content, according to the allometric relationship after Norland (1993), as cited by Straskrabova et al. (1999).

### *Environmental variables*

Several physico-chemical and biological variables

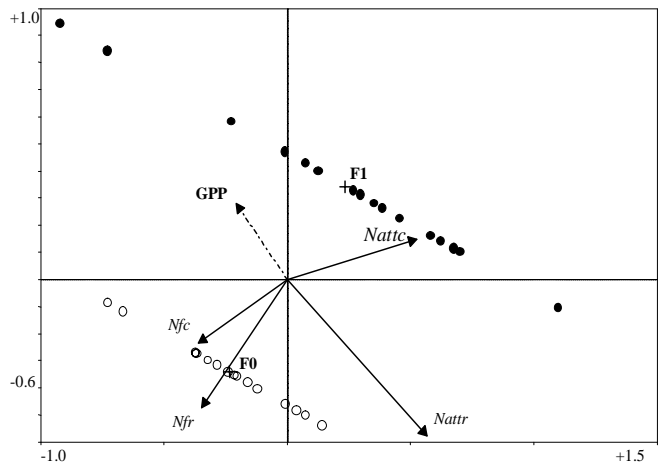
were monitored during the investigated period: water temperature ( $^{\circ}\text{C}$ ), water column transparency (cm, by Secchi disk), pH (measured electrochemically), hydraulic retention time (or flushing rate, RT, liter.min $^{-1}$ .0.1ha $^{-1}$ ), oxidability by  $\text{KMnO}_4$  (mg.0.1 $^{-1}$ , Bulgarian state standard), dissolved oxygen (mg.l $^{-1}$ , measured electrochemically by oxygen-meter type OXI 96), 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 ( $\mu\text{g.l}^{-1}$ , ethanol extraction according to ISO 10260), percentage of pond area covered by macrophytes (visual estimation), ammonium and nitrate nitrogen (N,  $\mu\text{g.l}^{-1}$ , colorimetrically with Nester's reactive, BSS 3587-79, respectively BSS 3758-85) and phosphate phosphorus (P,  $\mu\text{g.l}^{-1}$ , colorimetrically with molybdenum reactive, BSS 7210-838).

### Statistical analyses

The multivariate redundancy analysis (RDA) in its partial variant separating temporal and spatial variation (RDAtime and RDAponds), by means of statistical software Canoco for Windows 4.5 (ter Braak and Smilauer, 2002), were applied. Bacterioplankton variables were included in RDA as dependent (response) variables, while environmental factors and productivity characteristics were treated as independent (explanatory) variables. The Pearson parametric correlation ( $R_p$ ), Wilcoxon paired nonparametric test and simple linear regression were applied as well.

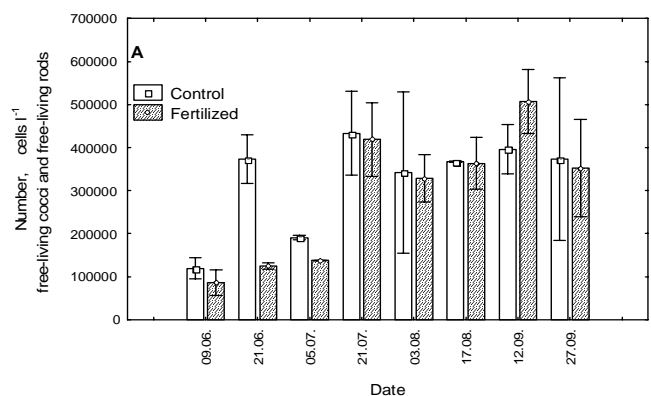
## Results and Discussion

The total number of bacterioplankton varied from 1.73 to 6.52 x 10 $^5$  cells ml $^{-1}$ . The values of the total biomass ranged from 2.1 to 17.2  $\mu\text{g C.l}^{-1}$ . The planktonic bacteria were divided into 4 fractions depending on their morphology (spherical - cocci and rod-shaped bacteria) and on their association to detritus: free-living cocci, free-living rods, attached cocci and attached rods. The spatial variations of abundances of these four fractions were explained significantly by fertilization effect (F0 and F1) and GPP as shown on

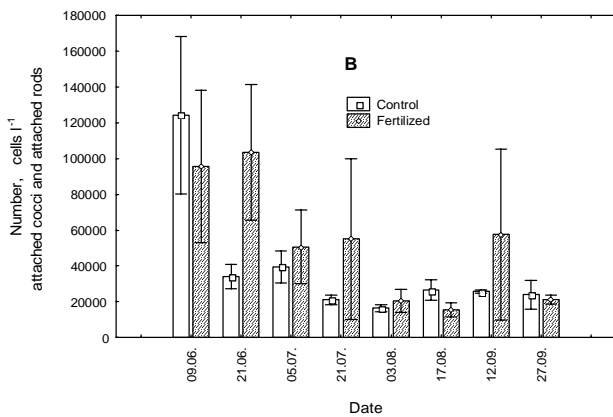


**Fig. 1. Results of partial RDA analysis for investigation of spatial variations (pond differences, RDAponds) with number (N) of bacterioplankton groups as response variables (Nfc – free cocci, Nfr – free rods, Nattc attached cocci, Natrr – attached rods) and F1 -fertilized, F0 – non-fertilized ponds and GPP – gross plankton primary production as selected explanatory variables. The explained part of variation is significant for  $P=0.01$**

Figure 1, however, the difference fertilized – control ponds was weak ( $P=0.07$ , by RDAponds). The same result (therefore not presented in the paper) was obtained for bacterioplankton number and biomass of

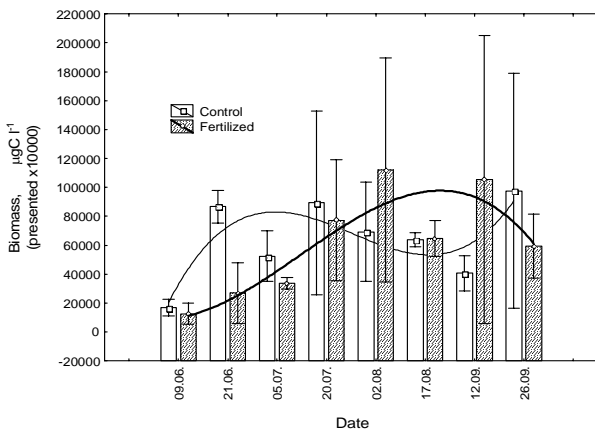


**Fig. 2A. Temporal changes (mean values and standard deviations) of percentages expressed by numbers of free-living cocci and free-living rods**



**Fig. 2B.** Temporal changes (mean values and standard deviations) of percentages expressed by numbers of attached cocci and attached rods

the four morphological groups expressed in percentages. Visually control ponds were distinguished by higher abundances of free-living cocci, free-living rods and attached rods, while the fertilized ponds tended to higher attached cocci abundance (Figure 1). No significant differences of these two groups between

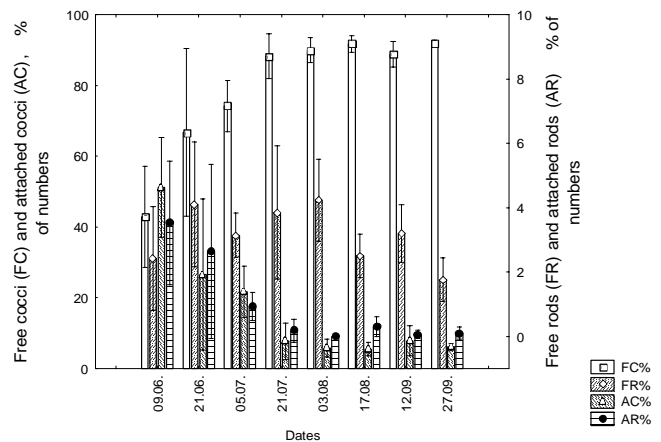


**Fig. 3.** Bacterioplankton biomass dynamics (mean values and their standard deviations) in control and fertilized fish ponds

fertilized and control ponds could be proved by mean of Wilcoxon paired test, however, abundance dynamics differed considerable between them (Figure 2A, 2B).

Dynamics differences also were observed between control and fertilized ponds by bacterial biomass variations (Figure 3).

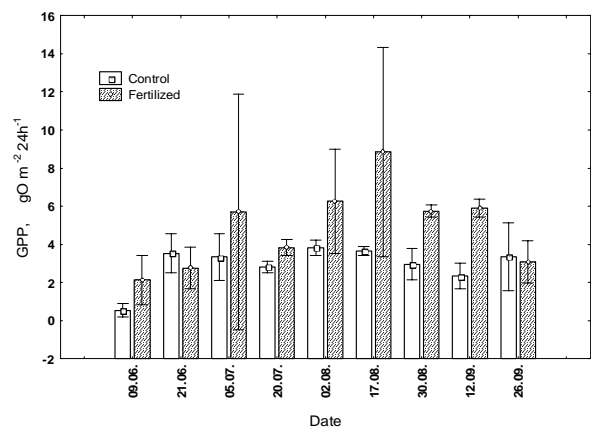
An overall comparison between bacterioplankton



**Fig. 4.** Bacterioplankton morphological groups - mean values and standard deviations of their numbers (in percentages). Abbreviations: FC-free cocci, FR-free rods, and AC-attached cocci, AR- attached rods, % - percentage

four morphological groups (presented by abundance percentage of pooled samples from control and fertilized ponds for each date) was shown in Figure 4.

There were insignificant differences in percentages at the start of experiment while later a clear separa-



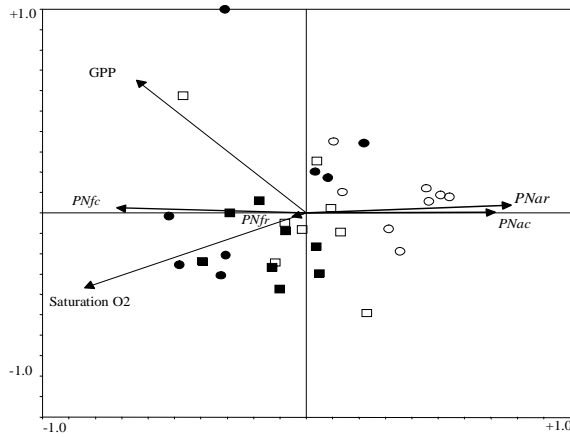
**Fig. 5.** Mean values and standard deviations of plankton gross primary production (GPP) in control and fertilized fish ponds by dates of sampling

tion emerged between free cocci, free rods and the attached forms. The differences have been persisting till the end of experiment. Free-living rods had weak variations during the experiment. The free-living cocci

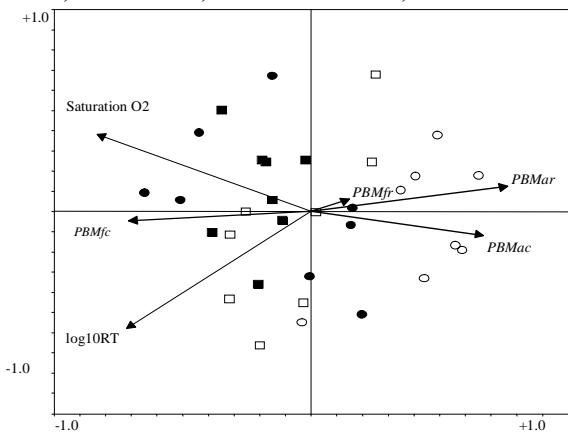
composing the smallest size group (0.2–0.5µm) dominated, with percentages from 87% to 100%. Mean values of GPP (Figure 5) showed statistically significant differences (Wilcoxon paired test,  $P=0.007$ ) in favor of fertilized ponds with a peak on August 17.

The results of partial multivariate analysis investigating temporal part of variation (RDAtime Figure 6) revealed a significant influence of oxygen saturation

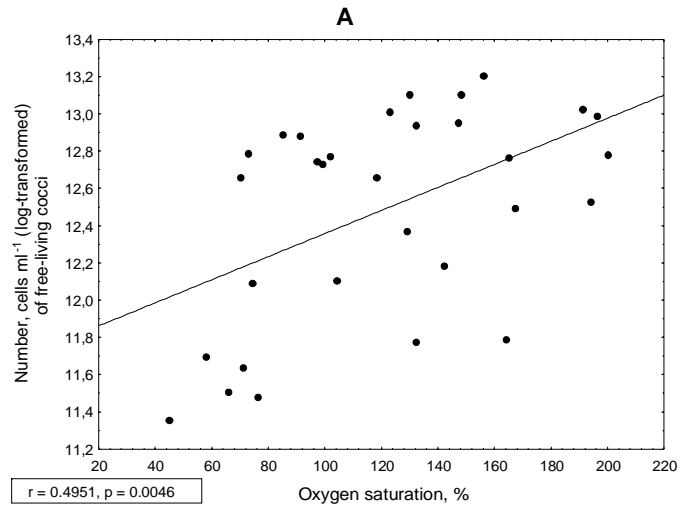
and



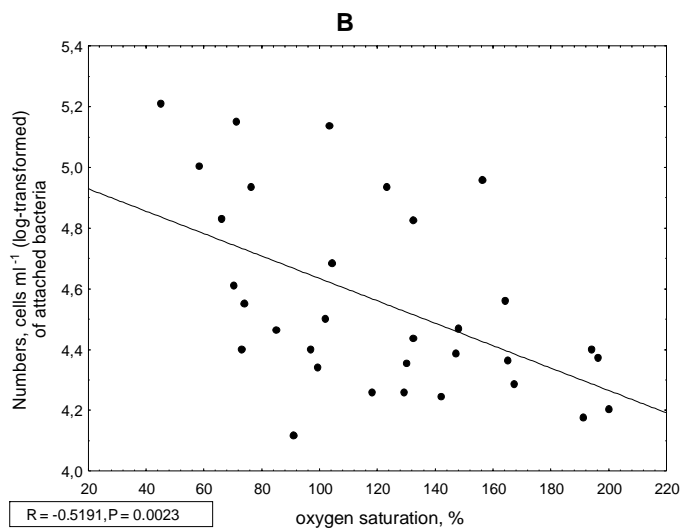
**Fig. 6A.** Correlation and ordination plot of partial RDA analysis investigating dependence of temporal variations (RDAtime) of bacterioplankton group percentages presented by numbers on environmental factors. In both parts the explained part of temporal variations was significant for  $P=0.005$ . The samples were coded as follows: months: ○ June □ July ● August ■ September; bacteria: P- percent, N- number, BM-biomass, fc-free cocci, fr-free rods, ac-attached cocci, ar- attached rods



**Fig. 6B.** Correlation and ordination plot of partial RDA analysis investigating dependence of temporal variations (RDAtime) of bacterioplankton group percentages presented by biomass on environmental factors. In both parts the explained part of temporal variations was significant for  $P=0.005$ . The samples were coded as follows: months: ○ June □ July ● August ■ September; bacteria: P- percent, N- number, BM-biomass, fc-free cocci, fr-free rods, ac-attached cocci, ar- attached rods



**Fig. 7A.** Linear regressions and correlations of oxygen saturation as independent variable and logarithmic values of bacterioplankton numbers as dependent variable for free-living cocci calculated without one outlier



**Fig. 7B.** Linear regressions and correlations of oxygen saturation as independent variable and logarithmic values of bacterioplankton numbers as dependent variable for attached bacteria

**Table 1**  
**Parametric correlation coefficients after Pearson ( $R_p$ ) with corresponding level of significance (P) between bacterioplankton morphological groups expressed in numbers and oxygen saturation in percentages**

Bacterioplankton morphological groups	Control fish ponds		Fertilized fish ponds	
	R	P	R	P
Free-living cocci	0.5	0.016	0.56	0.022
Free-living rods	0.31	0.22	0.198	0.48
Attached cocci	-0.715	0.002	-0.53	0.036
Attached rods	-0.68	0.0036	-0.72	0.0015

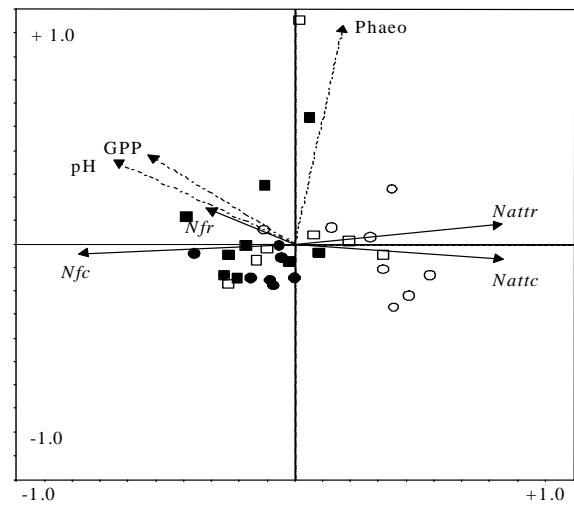
and GPP on percentages of the four morphological groups by numbers (Figure 6A), while percentages by biomasses were influenced by oxygen saturation and RT (Figure 6B).

In both cases, the derived explanatory variables correlated positively with free cocci and negatively with attached cocci and attached rods. The relations between these explanatory variables and number and biomass of free-living rods were weakly expressed. The linear regression analyses confirmed once more that the correlations between oxygen saturation on one hand and free cocci abundance (Figure 7A,  $R_p = 0.495$ ,  $P = 0.0046$  without one outlier) and attached bacteria abundance (Figure 7B,  $R_p = -0.519$ ,  $P = 0.0023$ ) on the other were highly significant.

A summary of parametric correlation coefficients after Pearson ( $R_p$ ) of relationships between numbers of the four morphological groups and oxygen saturation was given in Table 1. The four group abundances correlated with oxygen saturation in the same manner in control and fertilized ponds.

After excluding the strong influence of oxygen saturation the partial RDAtime analysis, choose other three explanatory variables (GPP, pH and phaeopigments) with significant influence on bacterial numbers (Figure 8).

Free-living bacteria correlated positively with GPP and pH, while their connection with phaeopigments was of negligible character. The attached bacteria had



**Fig. 8. Partial temporal analysis (RDAtime) between percentages of bacterial groups expressed by numbers and environmental variables after excluding the influence of oxygen saturation. The part of explained variation was significant for  $P=0.005$ . The samples were coded as follows: months: O June □ July • August ■ September; bacteria: P- percent, N-number, fc - free cocci, fr - free rods, ac - attached cocci, ar - attached rods, phaeo-phaeopigments, GPP – gross primary production**

opposite correlations with these factors exactly as it was observed with oxygen saturation on Figure 7. Obviously the oxygen saturation was supplemented to a great extent by GPP and pH as immediate results from it. They both appear to be redundant in the presence of oxygen saturation in the analysis. One additional feature of all three presented partial RDAtime analyses (Figure 6A, B, Figure 8) is the almost clear separation of samples from the first and second halves of the experiment, which was confirmed by other figures too (e.g. Figure 4).

Soluble reactive phosphorus, nitrate, ammonium nitrogen and chlorophyll a, were not selected by analyses as factors significantly influencing bacterioplankton development in spatial or temporal aspect.

The purpose of pond fertilization was to enhance primary production by higher concentrations of nutrients in order to obtain higher fish yield. However, ac-

According to the results of an analogous experiment in the previous year (2004) the fertilization caused no increase in primary productivity but in solar energy utilization by plankton primary production (Terziyski et al., 2007). Slightly higher concentrations of phosphate, nitrate and ammonium in fertilized than in control ponds for a level of significance varying between 0.05-0.1 were reported (Kalchev et al., 2006). Despite the statistically higher plankton primary production in the experiment during the year 2005 the bacterioplankton abundance of the same year showed a weak, close to significance border difference between fertilized and control ponds. One possible explanation would be the assumption that the bacterial loop contribution to energy transfer in fertilized and control ponds did not differ substantially. Another interpretation might be offered by Boulion (2002), who in his supplement to the conception for the “trophic cascade” (Carpenter et al., 1985) considered the quantity of nutrients as determining only the potential productivity of the lake, while its utilization might depend on the communities’ structure and on the relationships of “predator-prey” type. Our results seem to confine to this concept and suggest that both nutrient availability (bottom-up control) and predatory pressure (top-down control, Kalcheva et al., in press) were important in the regulation of bacterioplankton development in carp fish ponds, as was found in other experiments (Jürgens et al., 1999, Jürgens and Matz, 2002, Muylaert et al., 2002).

At the start of our experiment the attached forms dominated with a higher values in the fertilized than in control ponds within the first two months most likely due to imported organic fertilizer and its repeated re-suspension as detritus particles from the bottom by water currents, winds or feeding behavior of the common carps (Miller and Crowl, 2006). The subsequent strong dominance of smallest cocci (0.2 - 0.5  $\mu\text{m}$ ) after the start of experiment seems to be a natural phenomenon in bacterioplankton development, which might be explained with an inactive “dormant” state (Cole, 1999). The small size prevalence also might be an adaptation against phagotrophy. The lack of correlation between free-living rods and oxygen satura-

tion (Table 1) might be connected with facultative anaerobic respiration or indirect interactions not explained by variables involved in the statistical analyses.

Oxygen saturation was the major environmental factor with significant effect on both number and biomass of bacteria in seasonal aspect (Figure 6A, B). The oxygen saturation integrates many influences – phytoplankton and phytobenthos production, respiration of plankton, nekton and benthos organisms, solubility of oxygen depending on temperature and wind impact. Therefore the significant effect of oxygen saturation might be supplemented to great extent by GPP, which is also coupled with high pH. The results in Figure 7 and Table 1 confirm the significant character of the positive influence of high oxygen saturation on abundance of free-living cocci – the most numerous part of bacterioplankton community and of the corresponding negative relation to attached bacteria.

Jana (1979) found significant relationships in summer between biochemical oxygen demand, GPP and bacterioplankton number in fish ponds with mono- and poly-pisciculture. There were also some relationships of bacterioplankton reported in the literature and not found in our study such as: between chlorophyll a and bacterioplankton (Bell and Kuparinen, 1984, Lind et al., 1997); between nutrients and development of planktonic bacteria (Muylaert et al., 2002). As often encountered in statistical comparisons they might be a result of indirect relations.

## Conclusions

The application of organic fertilizer resulted in higher plankton gross primary production in treated than in control ponds, while the fertilization effect on bacterioplankton abundance was of dubious character. The weakening of manure effect on bacterioplankton might be a result of lower share of dissolved organic substances from total plankton primary production under eutrophic conditions, usual for fish ponds. The oxygen saturation variations as well as GPP alone influenced significantly both spatial and temporal changes of bacterial numbers. The temporal

variations of percentages of free-living cocci, expressed by numbers and by biomasses, always positively were correlated with oxygen saturation, GPP and RT while on detritus attached bacteria were negatively related to these factors. Chlorophyll a concentrations and nutrients P and N were not related to development of bacteria. A prevalence of bottom-up control is widely acknowledged in water bodies of high trophic and seems to be valid in our experiment too by the proved strong influence of GPP on bacterioplankton. As a result we have an increase of bacterioplankton number, long and weak grazer chain distinguished by energy losses, good expression of microbial loop, acceleration of nutrient recycling and stability of plankton community in both – manured and control fish ponds, because the temporal variations seem much stronger than those between ponds. Therefore, the shortening of the food chain seems to be one of the ways to increase the efficiency of fertilization in fish ponds. This might be achieved by decreasing the number of free living forms correlating with productivity and increasing the number of attached bacteria, either by applying larger quantities at the beginning (pond inundation) or by repeated introduction of manure in the middle of the experiment when the free cocci became unambiguously high and attached forms low in number. Thus, the fertilization effect might be prolonged and the efficiency increased at least from the point of view of established bacterioplankton relations in this study.

#### Acknowledgements

We would like to thank the leadership all the colleagues of the Institute of Fisheries and Aquaculture, Plovdiv for providing the opportunity to investigate bacterioplankton of fish ponds and the sampling assistance. The first author is especially grateful to Dr. Viera Straskrabova for her help with calibration of bacterioplankton data and Dr. Svetlana Naumova, for introducing in bacterial microscope technique.

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*Received February, 20, 2008; accepted for printing March, 15, 2008.*