ECOLOGIA BALKANICA
2016, Vol. 8, Issue 2 December 2016 pp. 1-11

Study on the Changes of Biotic Variables, Their Influence on the Primary Productivity and the Effect of Manuring of Fish Ponds

Doychin I. Terziyski[1*], Roumen K. Kalchev[2], Hristina V. Kalcheva[2], Vesselin P. Alexandrov[3]

1 - Institute of Fisheries and Aquaculture, 248 V. Levski Str., 4003 Plovdiv, BULGARIA
2 - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, BULGARIA
3 - Medical University Plovdiv, 15-A Vasil Aprilov Blvd., 4002 Plovdiv, BULGARIA
*Corresponding author: doichint@abv.bg

Abstract. The experiment was carried out in the period 2004-2006 on seven fish ponds of the Institute for Fishery and Aquaculture in Plovdiv city, Bulgaria with a single area varying from 1.8 to 3.9 daa. Powered fertilization with cattle manure on dose 3000 kg.ha^{-1} was applied. The aim of the experiment was to study the relations between the biotic factors (zooplankton, phytoplankton, zoobenthos, chlorophyll *a*, macrophytes, bacterioplankton, percent of energy utilization by primary productivity (PEU), their influence on the primary productivity and the effect of manuring on the fish ponds. The bigger part of the biotic factor variation was defined by the differences between the monthly samplings compared to the changes between the ponds within the period of investigation (2004/05/06). There was a difference between the first and last months concerning the biotic factors. This great seasonal variability decreases the opportunity for revealing the differences between the manured and the control ponds. The biotic factors as the PP (primary productivity), respiration and PEU in the manured ponds were higher compared to the control ponds during 2005. More macrophytes and higher PP/respiration and PP/chlorophyll *a* ratios were detected in the control ponds. Increased biotic variable values of the PP, respiration and chlorophyll *a* were found in the manured ponds during 2006. The derived relationships might contribute for enhanced productivity of carp ponds and for improvement of existing management practices in the view of better water quality in fish farming.

Key words: primary productivity, carp fish ponds, biotic environmental factors.

Introduction

Despite the fact that fishponds are not distinguished to support high species diversity, their organisms sustain the main trophic levels. The abiotic environmental factors and biotic components determine the behavior and vital activity of the living organisms in these ponds. The bio-productivity in the fishponds is one of the main biotic characteristics of pond water being a natural source of food in the fishponds. There are a lot of intermediate stages, biological and biochemical processes between the bio-productivity and the final production of the fishpond – the fish (NORIEGA-CURTIS, 1979). The fishpond is a specific artificial aquatic ecosystem, where these interactions take place. Photosynthesis is the basic source for the increasing of oxygen concentration and saturation in the aquatic ecosystems. Its contribution is comparable to the atmospheric aeration. At „bottom-up“ manipulations (i.e. manuring) a positive correlation is observed between

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

nutrient concentrations on one side and phyto- and zooplankton on the other, which indicates that high nutrient concentrations caused high primary productivity and finally high fish yield (DELINCE, 1992).

The aim of the study was investigation of the relations between the biotic factors, their influence on the primary productivity and the effect of manuring on fish ponds. In this context basic environmental factors and biotic components in manured and control ponds were studied.

Materials and Methods

The institute of Fishery and Aquaculture, Plovdiv is located in the western part of the Upper Thracian valley. The region is characterized by a transitional-continental climate. The study was carried out in the Plovdiv basis during three consecutive years (2004, 2005 and 2006). The ponds are supplied with water from "Maritsa" River by means of "Eni-Ark" irrigation canal. Seven earthen ponds were involved in the experiment, which individual size varies between 1.8 and 3.9 daa (Table 1). According to ZHANG *et al.* (1987) the ponds of this size are among the most productive and easy for management. Their bottom is silty, but the periphery and the shallowest parts of some of them have a strip of 1-2 m width with increased content of sand.

The pond size, their shallowness, prevailing vertical and horizontal homogeneity are part of the preconditions for choosing them for model object.

About 3000 $kg.ha^{-1}$ mineralized manure once in April each year was applied to ponds No 6, 12 and 17. The ponds No 8, 15, 16 and 18 were used as control ponds without manuring. Additionally to the natural food grain forage was given to the fish according to a scheme related to their seasonal growth rate. The periodical examination did not reveal any fish diseases.

The applied polycultural technology (NIKOLOVA *et al.*, 2008a, b) included mixed breeding of 30 individuals of daa one-year old bighead carp (T_1), (*Aristhichthys nobilis* Richardson, 1845), 50 individuals of daa one-year old common carp (K_1), (*Cyprinus carpio* Linnaeus, 1758) and 10 individuals of daa grass carp (one and two-year old) ($A_{1/2}$), (*Ctenopharyngodon idella* Valenciennes, 1844).

The samples were taken from a station localized 1-2 m away from the shore before the outlet device (savak) of each fishpond. The sampling was carried out fortnightly between 8:30 and 11:00 a.m. from May to September (2004, 2006) and from June to September (2005). The final sampling was carried out in the last decade of September. Due to the large number of investigated characteristics some of the samples were taken with one to three days difference. All the samples were taken from the surface layer (0.3-0.5 m depth) according to the Bulgarian and European standards (e.g. EU Water Framework Directive 2000/60/EC).

We studied the following biotic factors: PP, zooplankton, phytoplankton, zoobenthos, chlorophyll *a*, macrophytes, bacterioplankton, PEU (Table 2). The total solar radiation in MJ m^{-2} was recorded by pyranometer type M 80 M manufactured in the former Soviet Union. The PP ($g.O_2.m^{-2}.24\ h^{-1}$) was determined by a light/dark bottle technique in its oxygen modification (VOLLENWEIDER *et al.*, 1969).

Table 1. Schedule of the ponds included in the experiments 2004, 2005 and 2006 year (pond number and area in daa is shown in the brackets).

Year	Variants of breeding	
	Manured ponds	Control ponds
2004	6 (3.8) 17 (2.6)	8 (3.8) 16 (2.7) 15 (3.1) 18 (1.8)
2005	12 (3.9) 17 (2.6)	8 (3.8) 16 (2.7)
2006	12 (3.9) 17 (2.6)	8 (3.8) 16 (2.7)

Table 2. The methods applied for studying biotic variables in the current study.

Zooplankton	The zooplankton numbers were calculated by the method of DIMOV (1959). The biomass quantity was obtained by the volume-weight method of PRIKRYL (1980).
Phytoplankton	The phytoplankton was qualitatively determined, quantitatively counted and biovolume calculated afterwards (LAUGASTE, 1974).
Zoobenthos	The zoobenthos was first dried on filter paper and then weighted in order to obtain the biomass.
Chlorophyll *a*	The chlorophyll *a* was extracted and measured after ISO 10260 (1992).
Macrophytes	The macrophyte cover was visually estimated as percentage of total aquatic area.
Bacterioplankton	The bacterioplankton was microscopically determined by direct counting after RAZOUMOV (1932) in its contemporary modification of NAUMOVA (1999), and biomass after STRASKRABOVA *et al.* (1999).
PEU,%	The Percent of solar Energy Utilized by phytoplankton gross primary production was calculated as ratio of released oxygen converted into joules after ABAKOUMOV (1983) to measured solar energy multiplied by 100.

Firstly, the pond water was taken and homogenized in a 10 l plastic bucket and then the bottles were filled with water. Three pairs of light dark and initial bottles were used for each sample. The determination of the exposure period of the bottles was calculated as the light part of the day was separated in 5 equal time intervals. The bottles were exposed in 0.1, 0.3 and 0.5 m depth layers for a period including the second and third part of the above mentioned five time intervals. Within this period about 55-60% of total daily production was synthesized according to VOLLENWEIDER *et al.* (1969). The exposure depths depended on the measured Secchi transparency (S) and they usually were in the range 0.25.S - 3.S approximately.

Due to the high productivity of fishponds we frequently had to shorten the exposure time. In fact, the exposure time took one hour in most of the cases. In order to avoid the problem with oversaturation of water sample with oxygen and bubbles appearance in the bottles an original author's methodology was developed. The water sample was transferred to an empty plastic bottle double bigger volume than the sample. The bottle was pressed by hand until the liquid reached the bottleneck. Then the bottle was tightly closed and vigorously shaken. The elastic bottle walls tried to return to its normal position by creating reduced pressure insight the bottle. This drove the excessive dissolved oxygen to convert into a gaseous phase. Finally, the oxygen concentration in the water was lower than the saturation value under the instant atmospheric pressure and there were no bubbles released in the bottles during the exposure. The obtained productivity values were calculated for 1 m^2.

The diverse characteristics of fishponds presented by a great number of measurements allowed applying statistical methods. The application of statistical package Canoco for Windows 4.55 (TER BRAAK & SMILAUER, 2002) provides the opportunity to generalize the relative power and interactions of the whole multitude of factors in the presented study by means of principal component analysis.

Results and Discussion

Principal Component Analysis (PCA) – biotic factors (2004)

Spatial changes between the ponds. There was no separation between the manured and the control (without manure added) ponds concerning the biotic factors within 2004 (Fig. 1). The control ponds were more than the manured ponds and this probably increased the heterogeneity. Thus, according to the analysis performed there was no clear difference due to the applied manuring presented by all measured biotic factors.

The first main axis explains 23.1% of the variability in the biotic factors. This is the biggest part from the general spatial variation of the analyzed biotic variables. The changes in the zooplankton number and the zooplankton biomass together with the changes in the benthos biomass were the main contributors for the first main axis formation. The first and the second axes explain cumulatively 44.7% (Table 3) from the total spatial variation. Concerning the second axis, the gross primary productivity (GPP), percentage of the solar energy utilization (PEU), respiration and the GPP/respiration ratio were the main factors explaining the bigger part of the variation. The explained cumulative spatial variation significantly increases with the adding of the third axis (60.0%) and the fourth axis (73.5%). The last two axes add considerably lower percentage of the explained variability compared to the first two axes.

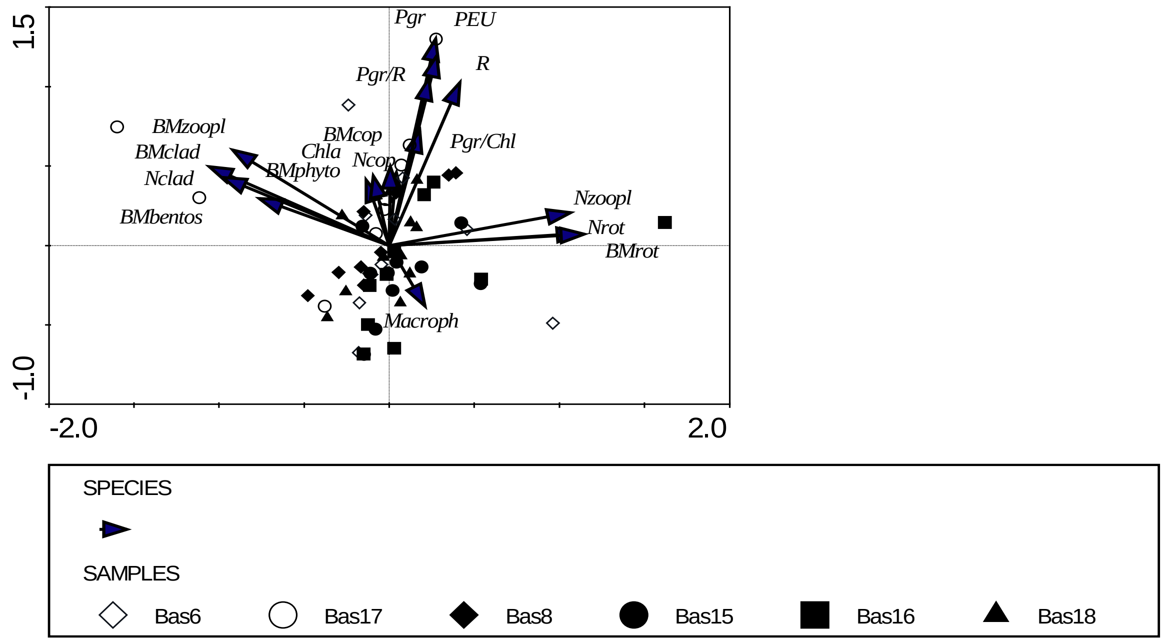

Fig. 1. PCA of the spatial changes between ponds No 6, 17 (manured) and No 8, 15, 16, 18 (control) of the biotic factors (2004).

Table 3. Variation distribution among the main axes for the spatial changes between the ponds of the biotic factors (2004).

Factor	**Main axes, No**				**Total variation**
	1	2	3	4	
Eigenvalues	0.167	0.156	0.111	0.097	1.000
Cumulative variability, %	23.1	44.7	60.0	73.5	
Total Eigenvalues					0.723

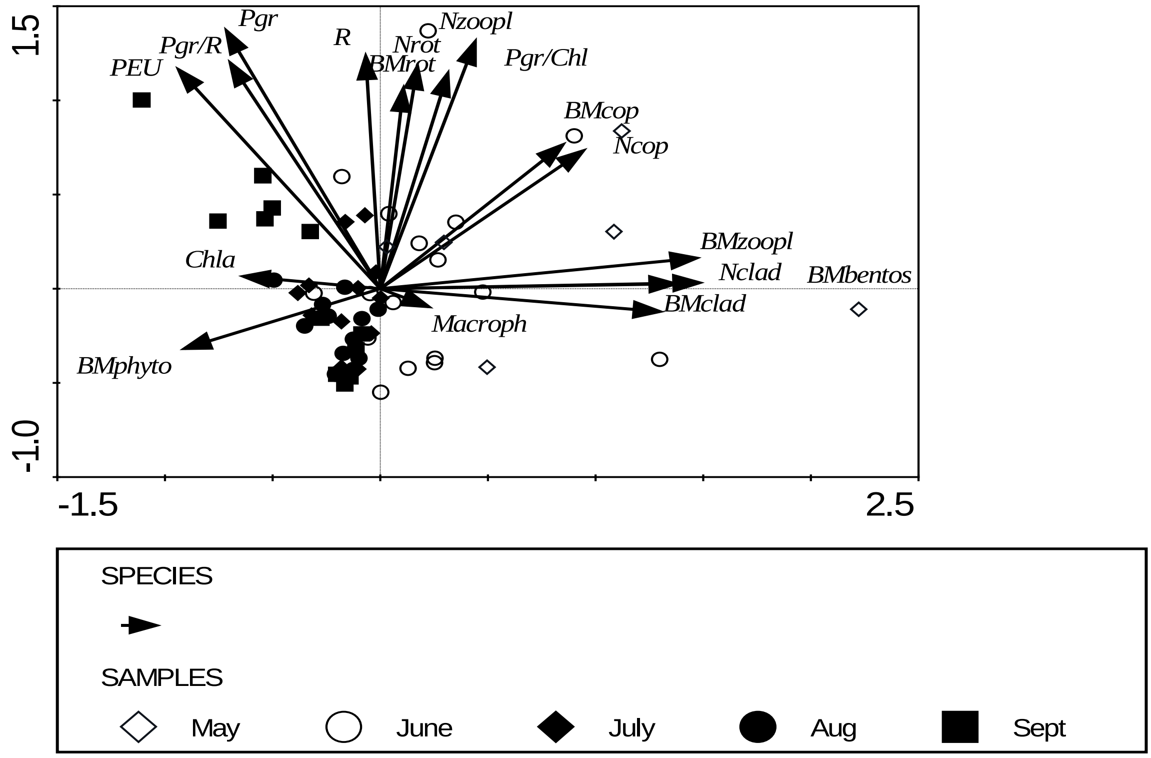

Fig. 2. PCA of the temporal changes (monthly) of the biotic factors (2004).

Temporal changes between the monthly sampling. According to the monthly sampling May and June differed from the rest of the months included in the vegetation season. The Cladocera's number and Cladocera's biomass were higher in this period. Cladocera composed the main part of the total zooplankton biomass, thus the mentioned trend is valid for the biomass of all zooplankton groups (Fig. 2). There was a similar trend for the zoobenthos biomass as well. Usually during the mentioned months the zooplankton and the zoobenthos had a biomass peak. This peak rarely could be influenced by the presence of the ichthyofauna species in the ponds, because the fish in the beginning of the vegetation period are too small in size and have less nutrition needs (TAKAMURA *et al.*, 1995; CAREY & WAHL, 2011). The chlorophyll *a* concentration and the phytoplankton biomass have increased during both August and September. Production factors as PP, respiration, Percent of solar Energy Utilized (PEU), assimilation number (AN), zooplankton number, rotatorian's number and biomass did not contribute for separation between months. The first main axis clearly separate the variation between the months of sampling – 29.2%, (Table 4). The bigger part of the variation is explained by the differences between the months of sampling (0.859) compared to the changes of the investigated factors between the different ponds (0.723), (Tables 3 and 4). The climatic variables explain 58-70% of the variation according to KIPKEMBOI *et al.* (2010).

Spatial changes between the ponds. During 2005 (Fig. 3) the manured ponds showed an increased level of PP, respiration, PEU%, and chlorophyll *a*. Similarly to our results, KIPKEMBOI *et al.* (2010) also reported a high chlorophyll *a* level in manured ponds. There was a trend to higher rotifer numbers and total zooplankton number in this type of ponds. The control ponds had higher PP/respiration and PP/chlorophyll *a* ratios. Macrophyte covering also increased. The biggest part of variation is explained by the first main axis – 34.8%. The next axes explained about 10% each (Table 5).

Principal Component Analysis (PCA) – biotic factors (2005)

Temporal changes between the sampling months. Concerning the first main axis (the abscissa) there was separation between samplings of two vegetation periods: June-July and August-September (Fig. 4). The first two months had a higher zoobenthos and zooplankton (all groups) biomass and a higher chlorophyll *a* values. During August the PP/respiration and PP/chlorophyll *a* ratios increased and there was an increase (a little bit lower) of the bacterioplankton biomass and PEU of the phytoplankton (see also JANA, 1979). The variation between the months of sampling (temporal component, 0.892) is considerably higher compared to the variation between the ponds (spatial component, 0.429) (Table 5 and 6).

Table 4. Main axes distribution of the temporal changes (monthly) of the biotic factors (2004).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.251	0.178	0.135	0.092	1.000
Cumulative variability, %	29.2	50.0	65.6	76.3	
Total Eigenvalues	29.2	50.0	65.6	76.3	0.859

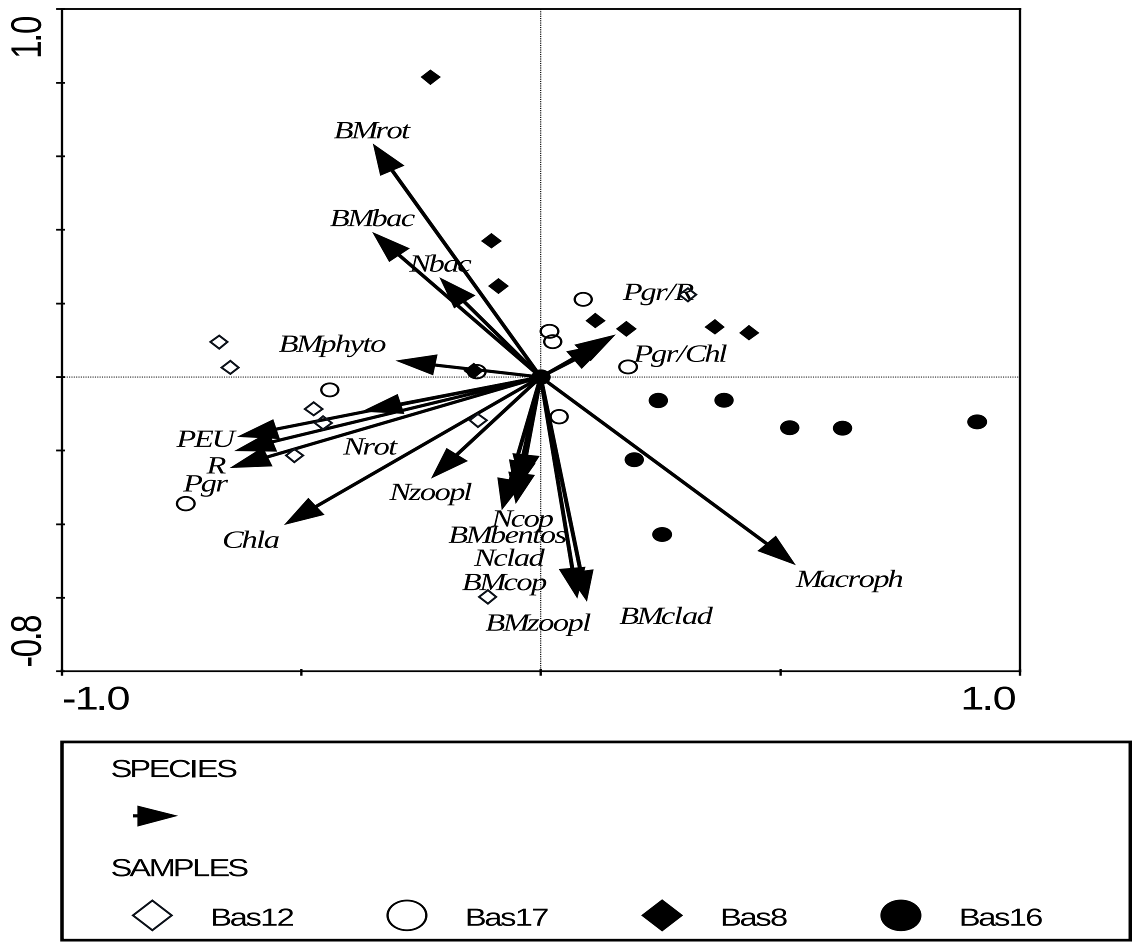

Fig. 3. PCA of the spatial changes between ponds No 12, 17 (manured) and No 8, 16 (control) of biotic factors (2005).

Table 5. Variation distribution among the main axes for the spatial changes of the biotic factors (between the ponds 2005).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.149	0.057	0.049	0.039	1.000
Cumulative variability, %	34.8	48.2	59.6	68.7	
Total Eigenvalues					0.429

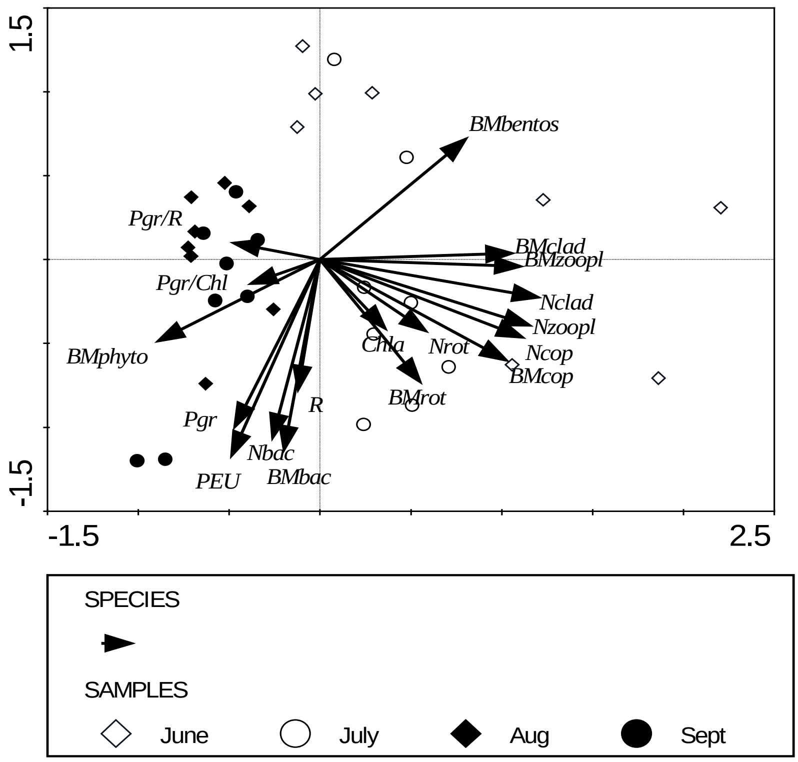

Fig. 4. PCA of the temporal changes (monthly) of the biotic factors (2005).

Table 6. Variation distribution among the main axes for the temporal changes of the biotic factors (2005).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.339	0.186	0.090	0.090	1.000
Cumulative variability, %	38.0	58.9	69.0	77.2	
Total Eigenvalues					0.892

Principal Component Analysis (PCA) – biotic factors (2006). Higher values of the GPP, respiration and chlorophyll *a* were detected during the most of the samplings from both of manured ponds (especially the pond No 12) (Fig.5). The positive relation of the manuring with the GPP and chlorophyll *a* was reported also by other authors (HEPHER, 1962; NORIEGA-CURTIS, 1979; KAGGWA *et al.*, 2009). Using different schemes of fertilizing by organic and inorganic fertilizers DIANA *et al.* (1991) came to the following conclusions: the high doses of fertilizing lead to similar levels of PP and chlorophyll *a* in all ponds. The fish growth and the yield are significantly higher in the ponds fertilized with organic fertilizers compared to the ponds with inorganic fertilizers used. The control ponds differ with an increased level of macrophyte growth, PP/respiration and PP/chlorophyll *a* ratios. In fact, the separation manured/control ponds by the biotic factors was not highly expressed. The bigger part of the dispersion is explained by the first main axis – 36.1%, the other axes explain about 10 – 16.4%, (Table 7).

Temporal changes between the sampling months. There was an apparent difference between May as a sampling month and the rest of the sampling months within year 2006 (Fig. 6). The benthos biomass, PEU%, zooplankton components and the bacterioplankton biomass increased during May, while in June PP/respiration and PP/chlorophyll *a* ratios and particularly PP were higher (similar to JANA, 1979). There was an increased level of the respiration and the macrophyte covering. The first main axis explains 47.8% of the biotic factor changes month by month. The other axes explain about 8 – 10%, (Table 8).

The bigger variation part (Tables 7 and 8) is explained by the differences between the months of sampling (0.889) compared to the changes of the investigated factors between the ponds (0.594).

Regularly the fish were checked for health problems during the experiments. No diseases were found.

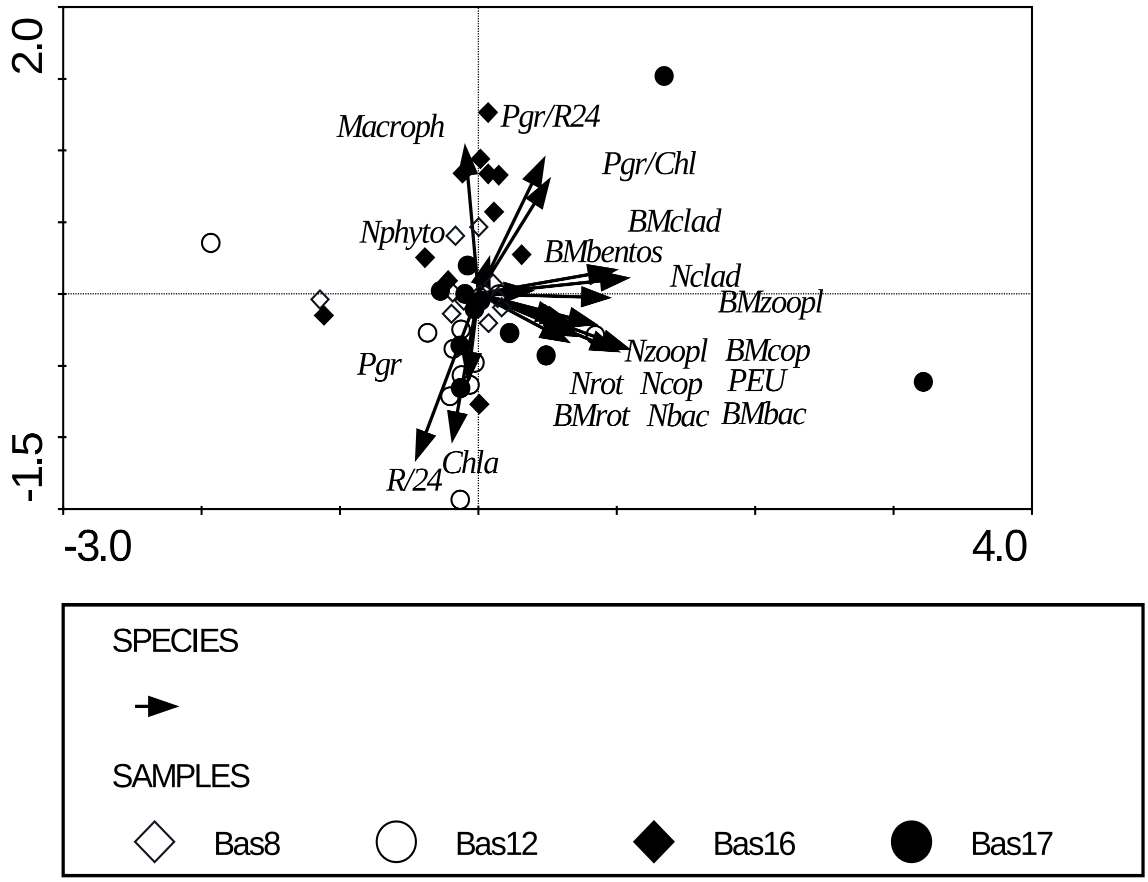

Fig. 5. PCA of the spatial changes between ponds, No 8, 12 (manured) and No 16, 17 (control) of the biotic factors (2006).

Table 7. Variation distribution among the main axes for the spatial changes (between the ponds) of biotic factors (2006).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.214	0.097	0.081	0.058	1.000
Cumulative variability, %	36.1	52.5	66.2	75.9	
Total Eigenvalues					0.594

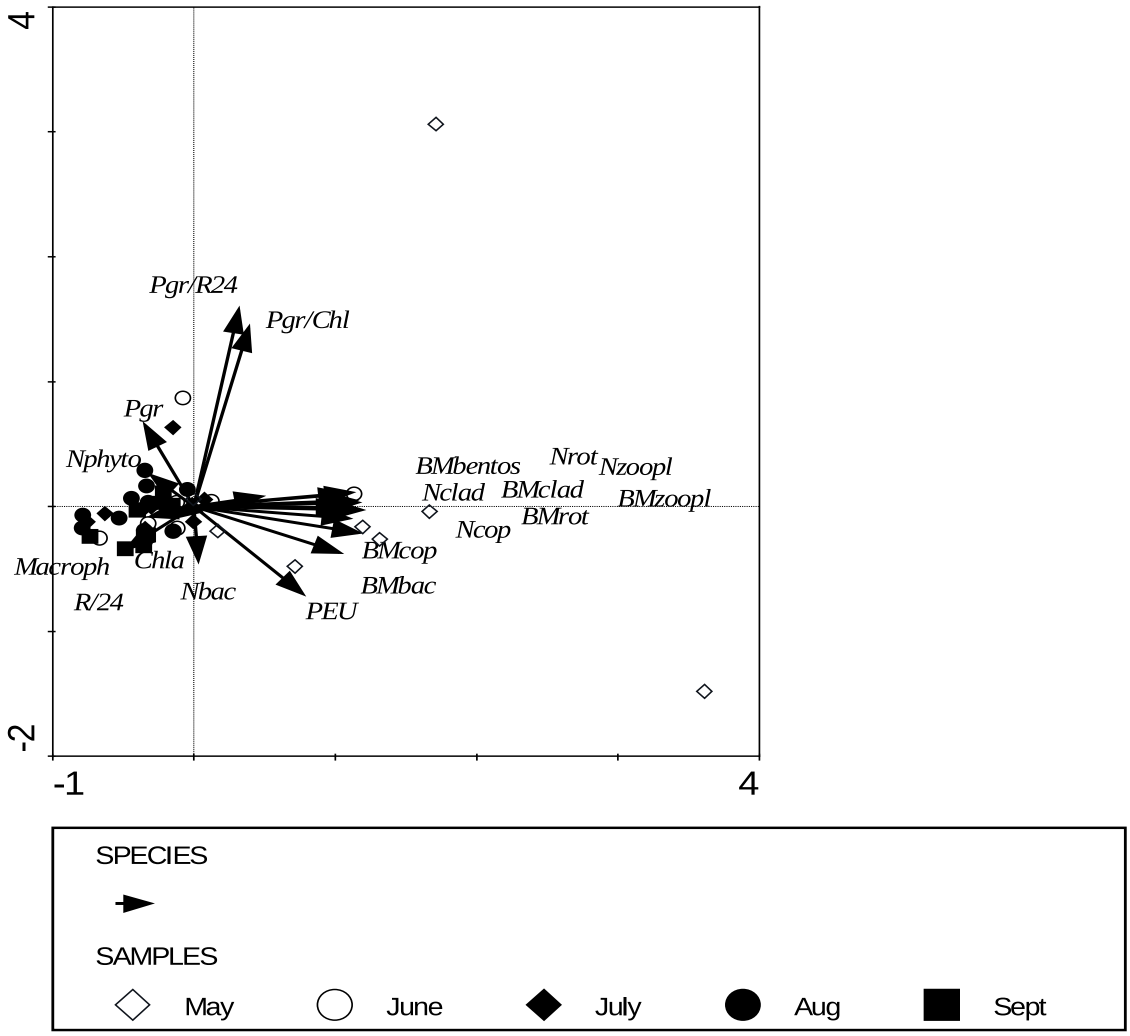

Fig. 6. PCA of the temporal changes (monthly) of the biotic factors (2006).

Table 8. Variation distribution among the main axes for the temporal changes of the biotic factors (2006).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.425	0.097	0.071	0.068	1.000
Cumulative variability, %	47.8	58.8	66.7	74.4	
Total Eigenvalues					0.889

Conclusions

1. Differences between the manured and the control ponds concerning the biotic factors observed were not found during 2004. Probably the reason, leading to increased dispersion, is the different number of the manured (2) and the control (4) ponds included in the experiment.

2. The bigger part of the biotic factor variation was defined by the differences between the monthly sampling compared to the changes between the ponds within the period of investigation (2004/05/06). There was a difference between the first and last months concerning the biotic factors. This great seasonal variability decreases the opportunity for revealing of the differences between the manured and the control ponds.

3. The biotic factors as the PP, respiration and PEU in the manured ponds showed higher values compared to the control ponds during 2005. More macrophytes and higher PP/respiration and PP/chlorophyll *a* ratios were detected in the control ponds.

4. An increased level of the biotic factors as the PP, respiration and chlorophyll *a* was found in the manured ponds during 2006. More macrophytes and higher PP/respiration and PP/chlorophyll *a* ratios were detected in the control ponds.

Acknowledgements. This study was possible with the financial support of Agricultural Academy within the following two projects: "Characterization, relationships and possibilities for management of ecological parameters of fishponds for thermopile fish breeding (2004-2006)" and "Exploration of possibilities for introduction of organic farming in thermopile fish species breeding in Bulgaria (2004-2006)."

References

ABAKOUMOV V.A. (Ed.). 1983. *Roukovodstvo po metodam gidrobiologicheskogo analiza poverkhnostnikh vod I donnikh otlozhenii, (A manual of methods for hydro-biological analysis of surface waters and bottom sediments)*. Leningrad, Gidrometeoizdat 239 p. (In Russian).

CAREY M.P., D.H. WAHL. 2011. Fish diversity as a determinant of ecosystem properties across multiple trophic levels. - *Oikos*, 120(1): 84-94. [DOI]

DELINCE G. 1992. *The Ecology of the fish pond Ecosystem with special reference to Africa*. Kluwer Academic Publishers.

DIANA J.S., C.K. LIN, PH.J. SCHNEEBERGER. 1991. Relationships among nutrient inputs, water nutrient concentrations, primary production, and yield of *Oreochromis niloticus* in ponds. - *Aquaculture*, 92: 323-341. [DOI]

DIMOV I. 1959. Improved quantitative method of plankton treatment. - *Proceeding of the Bulgarian Academy of Sciences*, 12(5): 427-430 (In Russian).

EU WATER FRAMEWORK DIRECTIVE 2000/60/EC. 2000. - *CELEX-EUR Official Journal* L, 327: 22.

HEPHER B. 1962. Primary Production in Fishponds and its Application to Fertilization Experiments. - *Limnology and Oceanography*, 7(2): 131-136. [DOI]

ISO 10260, 1992. *Water quality – Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration*, p. 1-4.

JANA B.B. 1979. Primary production and bacterioplankton in fish ponds with mono and polyculture. - *Hydrobiologia*, 62(1): 81-87. [DOI]

KAGGWA R.C., A.A. van DAM, J.S. BALIRWA, F. KANSIIME, P. DENNY. 2009. Increasing fish production from wetlands at Lake Victoria, Uganda using organically manured seasonal wetland fish ponds. - *Wetlands Ecology and Management*, 17: 257–277. [DOI]

KIPKEMBOI J., C.M. KILONZI, A.A. van DAM, N. KITAKA, J.M. MATHOOKO, P. DENNY. 2010. Enhancing the fish production potential of Lake Victoria papyrus wetlands, Kenya, using seasonal flood-dependent ponds. - *Wetlands Ecology and Management*, 18: 471–483. [DOI]

LAUGASTE R. 1974. The sizes and the weights of the most widespread algae of the Chudsko-Pskov and Vyrtjar lakes. -

Hydro-biological investigations, 6: 7-26 (In Russian).

NAUMOVA S. 1999. Total number of the microorganisms. - In: Peev D., S. Gerasimov (Ed.). *National programme for biomonitoring of Bulgaria*, Sofia, Geya-Libris.

NIKOLOVA L., L. HADJINIKOLOVA, K. DOCHIN, D. TERZIYSKI, R. ATANASOVA, A. STOEVA, G. GROZEV, E. PASKALEVA. 2008a. Carp fish rearing in autochthonous polyculture of one and the same age (*Cyprinus carpio* L., *Aristichthys nobilis* Rich. and *Ctenopharygodon idella* Val.). – *Bulgarian Journal of Agricultural Science*, 14(2): 133-138.

NIKOLOVA L., L. HADJINIKOLOVA, K. DOCHIN, D. TERZIYSKI, R. ATANASOVA, A. STOEVA, G. GROZEV, E. PASKALEVA. 2008b. Carp fish rearing in autochthonous mixed polyculture (*Cyprinus carpio* L., *Aristichthys nobilis* Rich. and *Ctenopharygodon idella* Val.). - *Bulgarian Journal of Agricultural Science*, 14(2): 139-144.

NORIEGA-CURTIS P. 1979. Primary productivity and related fish yield in intensely manured fishponds. - *Aquaculture*, 17(4): 335–344. [DOI]

PŘIKRYL I. 1980. Determination of zooplankton biomass on the basis of length–weight relations. – *Bull. VURH Vodnany*, 1: 13-18.

RAZOUMOV A. S. 1932. Pryamoi metod oucheta bakterii v vode. Sravnenie evo s metodam Kokha. [Method for direct counting of bacteria in water. Comparison with Koch's method]. *Microbiologiya* [*Microbiology*], 1(2): 112-131 (In Russian).

STRAŠKRABOVÁ V., C. CALLIERI, P. CARRILLO, L. CRUZ-PIZARRO, J. FOTT, P. HARTMAN, M. MACEK, J. M. MEDINA-SÁNCHEZ, J. NEDOMA, K. ŠIMEK. 1999. Investigations on pelagic food webs in mountain lakes - aims and methods. - *Journal of Limnology*, 58(2): 77–87. [DOI]

TAKAMURA N., X. ZHU, H. YANG, X. JIANG, J. LI, Z. MEI, Z. SHI, Y. TAN. 1995. Characteristics of plankton communities in Chinese integrated fish ponds: effects of excessive grazing by planktivorous carps on plankton communities. - *Hydrobiologia*, 315(3): 211-225. [DOI]

TER BRAAK C. J. F., P. SMILAUER. 2002. *CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5)*. New York. Microcomputer Power, Ithaca.

VOLLENWEIDER R. A., J. F. TALLING, D. F. WESTLAKE. 1969. *A manual on methods for measuring primary production in aquatic environments*. Oxford and Edinburgh. Blackwell Science Publication.

ZHANG F. L., Y. ZHU, X. Y. ZHOU. 1987. Studies on the ecological effects of varying the size of fish ponds loaded with manures and feeds. - *Aquaculture*, 60(2): 107-116. [DOI]

Received: 10.01.2016
Accepted: 10.07.2016