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Investigation on some biotic factors in carp fish ponds

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Abstract. Three years studies (2004 – 2006) on the main biotic parameters (chlorophyll-a, phytoplankton biomass, zooplankton biomass and bacterioplankton biomass) in carp fish ponds were carried out. The aim of the study was to investigate the biotic factors and the effect of manuring on the fish ponds. The relative changes in these factors in case of fertilization with manure 3000 kg.ha⁻¹ or without fertilization were determined. The impact of fertilization as bottom-up melioration on some biotic factors was proven by means of paired non-parametric Wilcoxon test with following significant differences: higher levels of chlorophyll-a and higher phytoplankton biomass in fertilized ponds. Zooplankton biomass was higher in fertilized ponds, but the differences were statistically insignificant. Bacterioplankton biomass was higher in the fertilized ponds, which is an indication that the applied melioration does not lead to overfood of organic matter in the ponds.

Keywords: carp fish ponds, manure, phytoplankton biomass, chlorophyll-a, bacterioplankton biomass, zooplankton biomass

Introduction

Biotic factors play a very important role in the production processes in fish ponds, which determines the attention that they need to be studied in fish farms. It is necessary to find the most reliable pathways to regulate the production by autotrophs and purposeful formation of the desired biological regime in the fish pond. Proper interpretation of biotic parameters and the factors that define them are extremely important preconditions for forecasting and rational search of ways to increase the productivity of the fish ponds, their management and finally for regulation of fish production (Bouillon, 1983). Anumber of studies have been devoted to the study of abiotic and biotic factors in fish ponds, some of them are related to complex influence of factors and disclosure of their complicated relationships each other (Duarte and Agusti, 1998; Kaggwa et al., 2009; Bhatnagar and Singh, 2010; Paria et al., 2011). For some factors, such the role of zooplankton in the processes of transformation of nutrients and energy from producers to consumers, studies are limited, but regarding the fish ponds are several (Carney, 1990; Pechar et al., 2002; Potužák et al., 2007).

Nowadays in hydrobiological research chlorophyll content is used as an indicator by which the biomass and the production of phytoplankton are determined. The dependence between chlorophyll-a and primary productivity and the increase of the level of the green pigment was studied in fertilized fish ponds (Abbás, 2000; Tabinda and Ayub, 2010). Positive relationships between chlorophyll a and bacterial abundance and biomass were also found (Simon et al., 1992). In traditional fish ponds, phytoplankton is the most important source of energy for fixing and its addition later in the food chain (Mistleå et al., 2003).

The use of organic manure in fish farming is based on the assumption that the manure is utilized through two pathways (Pekár and Olah, 1998). The manure organic matter provides dissolved and particulate substrates for bacteria and the bacterial laden particles provide food to the filter-feeding and detritus-consuming animals, while the mineralized fraction of the manure stimulates phytoplankton productivity similarly to the action of inorganic fertilizers. Bacterial biomass forms an important link between the various trophic levels in a pond ecosystem. Increasing the destruction of organic matter twice by microorganisms determines the increase in the phytoplankton production by 20% (Shtur, 2006).

The aim of the study was to investigate the biotic factors and the effect of manuring on the fish ponds. In this context basic biotic factors have been studied in manured and control ponds.

Material and methods

The Institute of Fishery and Aquaculture, Plovdiv is located in the western part of the Upper Thracian valley, Bulgaria. The region is characterized by a transitional-continental climate. The study is carried out in the experimental base in Plovdiv 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 are involved in the experiment, which individual areas vary between 1.8 and 3.9 da (1ha=10 da). According to Zhang et al. (1987) ponds of this area are among the most productive and easy for management. Their bottom is silt 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 area of ponds, their shallowness, vertical and horizontal homogeneity are part of the preconditions for choosing them for model objects.

About 3000 kg.ha⁻¹ mineralized manure once in April each year was applied to ponds No 6, 12 and 17. Additionally to the natural food grain forage was given to fishes according to a scheme related to their seasonal growth rate. The periodical examination did not reveal any fish diseases.

The applied polyculture technology includes mixed breeding of 30 individuals da⁻¹ one-year old bighead carp (Aristichthys notatus).
Table 1. Scheme of the experiments in years 2004, 2005 and 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Variants of breeding</th>
<th>Pond No. area (da)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertilized ponds, numb.da⁻¹</td>
<td>Control ponds, numb.da⁻¹</td>
</tr>
<tr>
<td>2004</td>
<td>6 (3.8)</td>
<td>17 (2.6)</td>
</tr>
<tr>
<td></td>
<td>8 (3.8)</td>
<td>15 (3.1)</td>
</tr>
<tr>
<td></td>
<td>16 (2.6)</td>
<td>18 (1.8)</td>
</tr>
<tr>
<td>2005</td>
<td>12 (3.9)</td>
<td>17 (2.6)</td>
</tr>
<tr>
<td></td>
<td>8 (3.8)</td>
<td>16 (2.7)</td>
</tr>
<tr>
<td>2006</td>
<td>12 (3.9)</td>
<td>17 (2.6)</td>
</tr>
<tr>
<td></td>
<td>8 (3.8)</td>
<td>16 (2.7)</td>
</tr>
</tbody>
</table>

nobilis Rich.), 50 pieces da⁻¹ of carp (K.), (Cyprinus carpio L.) and 10 pieces da⁻¹ grass carp (one and two-year) (A.,) (Clitorhynchus idella Val.) according to Nikolova et al. (2008a, b). The scheme of the experiment is presented in Table 1.

Each fish pond was sampled on one station localized 1 – 2 m from the shore before the outlet device (savak). The sampling was carried out biweekly between 8:30 and 11:00 a.m. in the period May-September of years 2004 and 2006 and June-September in 2005. The final sampling was carried out in the last decade of September. Some of the samples were taken with one to three days difference due to the large number of investigated characteristics. The collection of water samples was carried out from the 0.3 – 0.5m surface layer according to Bulgarian and European standards (e.g. Water Framework Directive 2000/60/EC). The majority of the samples were processed immediately after sampling.

For the analysis of biotic factors in the study were used the following methods:

Chlorophyll-a concentration in phytoplankton (μg.l⁻¹). The required amount of sample (fixed volume) is filtered through a glass fiber filter (GFA Millipore or GF1 Chm) with a pore size diameter of 0.7 – 1.2 μm, followed by extraction with 90% ethanol, acidification of a part of the filtrate and measurement using spectrophotometer Model 6105 (Jenway, UK) at 665 and 750 nm (including turbidity correction also) according to ISO-1/1980 and ISO 5667-2/1991. In the analyzed period were made 140 samplings.

Quantitative and qualitative composition of phytoplankton. The analysis of phytoplankton is implemented by the method of Laugaste (1974) using a chamber of Burker. The identification book of Cox (1996) is used. The samples were immediately preserved after sampling with formalin to a final concentration of 4%. In 2006, the starting levels in phytoplankton biomass were calculated using the concentration of chlorophyll-a by Vinberg (Fedorov, 1979):

\[
Bc = 15 \times \text{Chla}/1000
\]

where 15 is the factor of recalculation, Bc is biomass in mg.l⁻¹, and Chla is the concentration of chlorophyll-a. The relevant date was divided to 1000 to convert from μg.l⁻¹ into mg.l⁻¹. 140 samplings in the analyzed period were taken.

Quantitative and qualitative composition of zooplankton. The number was determined using the methodology of Dimov (1959) and the biomass (g.m⁻³) by volume-weight method (Pnkril, 1980). In this case, a representative number of individuals of each species are distributed by size classes and the biomass of each class is calculated. The samples were immediately preserved after sampling with formalin to a final concentration of 4%. Using hand trawling method during sampling, 60 μm mesh size plankton net was trawled horizontally ten times to capture zooplankton. 140 samplings were taken.

Bacterioplankton. Bacterioplankton number is determined by the Razumov's method of a direct microscopic count in its contemporary interpretation (Naumova, 1999) and the biomass (μgC.l⁻¹) is calculated according to Straskrabova et al. (1999) after the conversion of the mean cell volume in carbon content (Noric, 1993). The samples were immediately preserved after sampling with formalin to a final concentration of 2%. 80 samplings were taken, except in 2004, when the indicator was not included in the research.

The diverse characteristics of fish ponds presented by large number of measurements offer the possibility to apply statistical methods. Additionally the difference between fertilized and control ponds was tested by means of Wilcoxon rank paired test with statistical package STATISTICA 7.0 (Sokal and Rohlf, 1997; McGarigal et al., 2000).

Results and discussion

Chlorophyll-a

In 2004 a relatively highest levels in chlorophyll-a concentrations were registered in the two groups of fish ponds with maximum of 225 μg.l⁻¹ (29.06) in fertilized and 200 μg.l⁻¹ in the control group. In next year 2005 was the other extreme case with the lowest values, when maximum levels were 148 μg.l⁻¹ and 100 μg.l⁻¹ respectively, while 2006 had intermediate position. In typical summer months, June, July and August, the dynamics was seasonal and most often highest values of chlorophyll-a were recorded (Figure 1).

The prevalence of higher chlorophyll-a concentrations in the group of fertilized fish ponds in the three years of this experiment showed statistically significant (p = 0.045, Wilcoxon rank paired test) higher mean value (92 μg.l⁻¹) in them compared to the control group (79 μg.l⁻¹) (Figure 2). Fertilization with manure had positive influence on chlorophyll-a in each of the three years. Similar opinion was expressed by Garg and Bhatnagar (2002) and Kaggwa et al. (2009). Increasing the level of the green pigment in fertilized fish ponds was found by other authors (Tabinda and Ayub, 2010).

Phytoplankton biomass

During the three-year period average-seasonal minimum values of phytoplankton biomass in the fertilized fish ponds ranged from 0.61 mg.l⁻¹ to 1.10 mg.l⁻¹, in control ponds from 0.35 mg.l⁻¹ to 1.15 mg.l⁻¹. The maximum values were in the range 2.11 – 3.92 mg.l⁻¹. Overall, during initial samplings significant difference between the versions of the experimental set was not found. Obviously it takes time – about a month, so that the effect of fertilization to occur.

Changes in phytoplankton biomass during the three years revealed a clear seasonal turn. In late July and in early August there was intensive phytoplankton development in the two groups of fish ponds, but was better expressed in the fertilized group and reached its maximum at the end of August (Figure 3). In September a decrease trend was registered, better expressed in the control group of fish ponds.

Mean values of phytoplankton biomass were 18.18% higher in fertilized compared to control fish ponds (1.54 mg.l⁻¹ and 1.26 mg.l⁻¹ respectively) and with significant difference between them, p = 0.0001 (Figure 4). Fertilization with manure clearly influenced the
dynamics of phytoplankton biomass in these fish ponds compared with the control ones. Manure stimulated the phytoplankton organisms in dose of 3000 kg ha\(^{-1}\) even applied once at the beginning of the vegetation season. Our results give the evidence that the impact of manure on phytoplankton is continued almost until the end of September. For the three-year study period maxima in phytoplankton biomass (Figure 3) were registered in the summer months, which indicate that mainly the temperature, light and nutrient availability (bottom-up control) are important, but not the reduction by phytophagous fish and zooplankton (Fott et al., 1980). Fertilization with manure stimulates phytoplankton growth almost throughout the vegetation season. Kaggwa et al. (2009) and Kipkemboi et al. (2010) also found a positive response of phytoplankton to fertilization with manure.

Schroeder et al. (1990), in experiments with polyculture in earthen fish ponds, established, that during use of organic fertilization and analyses of gastric and intestinal contents of fish above 90% of yield was based on the carbon by the algae. For fish ponds Bulgakov et al. (1992) also found the role of fertilization on intensification in the processes of accumulation of phytoplankton biomass.

**Zooplankton biomass**

Overall, in the three-year research (Figure 5) the maximum in zooplankton biomass always was register at the beginning of the season, unfortunately with little time duration – about one/two weeks. The second characteristic peak in autumn is missing. From the middle/end of June zooplankton biomass was very low, less than 1 gm\(^{-2}\) in most cases and was insufficient as a trophic resource for farmed fish.

Comparison between mean values of zooplankton biomass for

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**Figure 1.** Chlorophyll-a concentrations (mean values and standard deviations) by months in fertilized and control fish ponds in 2004 – 2006

**Figure 2.** Chlorophyll-a concentrations (mean values and standard deviations) in fish ponds by groups (fertilized and control) in 2004 – 2006 (Wilcoxon paired test, p<0.05)
the period 2004 – 2006 showed higher mean value in fertilized (1.68 gm⁻³) than in control fish ponds (1.36 gm⁻³), without significant difference, \( p = 0.863 \) (Figure 6). Fertilization with manure increased the biomass of zooplankton in the relevant fish ponds, but only in rare cases at the beginning of the season mainly in 2004 and partially in 2005 (Figure 5), which was insufficient for significant difference throughout the whole period. Analyzing key indicator groups showed predominance of large cladocerans in the fertilized and copepods in the control fish ponds. In rotifers differences between the variants of fertilization were not found.

Often the high fish density greatly reduced the population of the zooplankton (Qin and Culver, 1996; Britton et al., 2010) as in our case, and relatively high levels were only at the beginning of the season (Figure 5). Zooplankton in the rest of the vegetation season was limited to small species such as nauplii, small crustaceans (from Cyclopoida and Cladocera, own unpublished data) and rotifers, but they are not such effective in terms of filtration of algae. The realization of this effect means weak efficiency in using primary production through zooplankton to fish (Potužák et al., 2007).

In fertilized fish ponds more than 50% of the biomass of zooplankton belonged to the group of Cladocera, but unfortunately organisms with size greater than 1 mm were observed only at the beginning of the season in insufficient numbers. In control fish ponds the ratio Cladocera/(Copepoda+Rotatoria) is approximately 45/55%, which is an indication of much worse utilization of primary productivity. Fertilization with manure in single dose of 3000 kg.ha⁻¹ in terms of zooplankton was not enough to maintain a high level of large organisms throughout the season regarding the applied stocking densities of fish polyculture.

In 50% or higher dominance of Daphnia sp. or similar species, with mean body size at least 1.5 mm, efficient transfer of matter and energy can be expected through planktonic food web. In many water
bodies, according to many authors (Seda and Duncan, 1994; Pechar, 1995) zooplankton with such structure is able to reduce the development of phytoplankton. Similar community structure (especially with large *Daphnia* sp.), according to Carney (1990), is crucial in mediating both bottom-up and top-down effects, therefore plays a key role in the transfer of matter and energy in grazer food chain.

**Bacterioplankton biomass**

At the beginning of the experiment bacterioplankton biomass showed a similar level in both fertilized and control fish ponds and in summer months a trend to increase was registered, more pronounced in 2005 with maximum in August in one of the fertilized fish ponds (Figure 7).

During the two-year experiment (2005–2006) bacterioplankton biomass was 10% higher in the group of control fish ponds compared to fertilized ones, due to the lower biomasses in 2006 in fertilized ponds, and consequently no significant difference among them was found, p = 0.325 (Figure 8). The results showed that the mean values of bacterioplankton biomass were higher in the control ponds, but ranged more (StDev) in fertilized ponds. Fertilization with manure had not negative effect (the dose is not “overdose”) on the dynamics of bacterioplankton biomass in the fertilized fish ponds.

Bacterioplankton biomass was higher in fish ponds with higher phytoplankton biomass and with prevalence of copepods as a part of zooplankton biomass. The zooplankton pressure on bacterioplankton by Cladocera (especially *Daphnia* sp.) in the first 1–2 months is the reason of lower bacterial abundance and biomass, because cladocerans are predators of all components of the microbial loop and transfer efficiently the matter and energy (from the applied manure via bacteria in our case) to grazer food chain, according to other studies (Jürgens and Jeppesen, 2000; Zöllner et

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**Figure 5.** Zooplankton biomass (mean values and standard deviations) by months in fertilized and control fish ponds in 2004–2006

**Figure 6.** Zooplankton biomass (mean values and standard deviations) in fish ponds by groups (fertilized and control) in 2004–2006 (Wilcoxon paired test, p>0.05)
(at the beginning of the experiment every year), and the autotrophic and heterotrophic picoplankton, including the bacterioplankton, becomes less important with increasing trophic state (Simon et al., 1992; Straškrabová et al., 1999; Jürgens and Jeppesen, 2000).

Sometimes the organic matter in fish ponds, although high, does not contain the optimum ratio of nutrients for bacterial growth (Jana et al., 2001; Hargreaves and Tucker, 2004) which is an explanation that in our study we find differences between bacterioplankton biomasses in fish ponds of one and the same group and in annual aspect.

**Conclusion**

Fertilization of fish ponds with manure in dose of 3000 kg ha⁻¹ creates the conditions for better development of phytoplankton by increasing the intensity of photosynthesis, which is expressed in the following significant differences: higher level of chlorophyll-a in fertilized compared to control fish ponds and higher phytoplankton biomass in fertilized compared to control fish ponds.

The experiment showed higher zooplankton biomass (by mean values) in fertilized fish ponds than in control ones, but the differences were statistically insignificant. No significant difference was found between control and fertilized fish ponds during the experiment regarding bacterioplankton biomass.
Acknowledgments
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