

## **INVESTIGATION OF RELATIONS BETWEEN ABIOTIC VARIABLES, THEIR INFLUENCE ON THE PLANKTON PRIMARY PRODUCTIVITY AND THE MANURING EFFECT IN FISH PONDS**

D. TERZIYSKI<sup>\*1</sup>, R. KALCHEV<sup>2</sup>, H. KALCHEVA<sup>2</sup> and V. ALEXANDROV<sup>3</sup>

<sup>1</sup>*Agricultural Academy, Institute of Fisheries and Aquaculture, BG-4003 Plovdiv, Bulgaria*

<sup>2</sup>*Bulgarian Academy of Sciences, Institute of Biodiversity and Ecosystem Research, BG-1113 Sofia, Bulgaria*

<sup>3</sup>*Medical University Plovdiv, BG-4002 Plovdiv, Bulgaria*

### **Abstract**

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The experiment was carried out within three consecutive years (2004, 2005 and 2006). Seven earthen fish ponds (area varying from 1.8 to 3.9 da) located in the Institute for Fishery and Aquaculture, Plovdiv, Bulgaria were included in the investigation. A manure dose of 3000 kg.ha<sup>-1</sup> was used. The aim of this study was investigation of relations between the abiotic factors, their influence on the plankton primary productivity (PP) and the manuring effect in fish ponds. The bigger part of the abiotic factor variation was defined by the differences between the monthly sampling compared to the changes in the ponds within investigation period (2004/05/06). Concerning the abiotic factors there was a difference between first and last months. The great seasonal variability decreases the chance to find differences between the manured and the control ponds. The abiotic factors as the permanganate oxidability, oxygen content and pH value in the manured ponds had a higher level compared to control ponds during 2005. An increased level of the oxidability, phosphate phosphorus and pH value was found in the manured ponds during 2006.

**Key words:** primary productivity, carp fish ponds, abiotic environmental factors

### **Introduction**

The carp breeding and carp fishery are some of the most widespread fish yields in Bulgaria. It is important to know how different abiotic and biotic factors influence the primary productivity in carp fish ponds. There are a plenty of studies related to complementary influence of these factors and the complicated relationships between them. Especially the studies about the influence of abiotic factors on the primary productivity are limited. The role of some abiotic variables as permanganate oxidability, oxygen concentration and saturation, phosphorus

and nitrogen concentration, pH value is not fully investigated.

Therefore investigations concerning primary productivity and its correlations with the abiotic factors in carp fish ponds are of a great scientific interest and these would have an important practical application too.

### **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.

\*Corresponding author: doichint@abv.bg

The study was carried out within three consecutive years (2004, 2005 and 2006). The ponds were supplied with water from Maritsa river by "Eni-Ark" irrigation canal.

Seven earthen ponds were involved in the experiments. Their size varied between 1.8 and 3.9 da (Table 1). The bottom of some ponds was silty with a sand strip (1–2 m width) in the peripheral shallowest parts. After Zhang et al. (1987) ponds of this size are among the most productive and easy for management.

**Table 1**  
**Schedule of the ponds included in the experiments 2004, 2005 and 2006 year**

Year	Variants of breeding	
	Manured ponds	Control ponds
	Pond No and area (in da)	
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)

Some of the features as size, shallowness, vertical and horizontal homogeneity of the ponds make them an appropriate model object for the experiments.

The ponds No 6, 12 and 17 were treated with mineralized organic manure (appr. 3000 kg.ha<sup>-1</sup>) once per year (each April). The ponds No 8, 15, 16 and 18 were used as control ponds without manuring. Additionally the fish was fed with the natural grain feeds according to the seasonal growth rate. A periodical examination of fish healthy conditions was performed and no fish diseases were revealed.

The applied polycultural technology (Nikolova et al., 2008a, b) included mixed breeding of 30 individuals per da<sup>-1</sup> one-year old bighead carp (*T<sub>1</sub>*), (*Aristichthys nobilis* Richardson, 1845), 50 individuals per da<sup>-1</sup> one-year old carp (*K<sub>1</sub>*), (*Cyprinus carpio* Linnaeus, 1758) and 10 individuals per da<sup>-1</sup> grass carp (one and two-year old) (*A<sub>1/2</sub>*), (*Ctenopharyngodon idella* Valenciennes, 1844).

The samples were taken from a station localized 1–2 m away 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 Bulgarian and European standards (e.g. EU Water Framework Directive 2000/60/EC). The most of the samples were processed immediately after sampling.

The water column transparency was measured by the Secchi disk method. A total of 1120 samples for determination of 8 parameters were analyzed (Table 2).

**Table 2**

**Standard methods applied for determination of physico-chemical parameters of water samples**

No	Parameters	Applied standards
1	Water temperature	BSS 17.1.4.01-77
2	pH	BSS 3424-81, ISO 10523,1994
3	Dissolved oxygen, mg.l <sup>-1</sup>	BSS EN 25814-2002
4	Oxygen saturation, %	BSS EN 25814-2002
5	Permanganate oxidability, mg.l <sup>-1</sup>	BSS EN ISO 8467, 2001
6	Ammonium nitrogen, mg.l <sup>-1</sup>	BSS 3587-79, ISO 5664
7	Nitrate nitrogen, mg.l <sup>-1</sup>	ISO 7890-3, 1998
8	Orthophosphate, mg.l <sup>-1</sup>	BSS EN ISO 6878-1:2004

The PP (g.O<sub>2</sub>.m<sup>-2</sup>.24 h<sup>-1</sup>) was determined by a light/dark bottle technique in its oxygen modification. First 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 depth layer. 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 or 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 has been synthesized according to Vollenweider (1969). The exposure depths depended on the measured Secchi transparency and they usually were in the range 0.25.S – 3.S approximately.

Due to the big productivity of fishponds we frequently had to shorten the exposure time. In fact, the exposure time has taken one hour in many of the cases. In order to avoid the problem with oversaturation of water 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 with a volume twice the sample volume. The bottle was pressed by hand till the liquid reaches 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 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 are calculated for 1 m<sup>2</sup>.

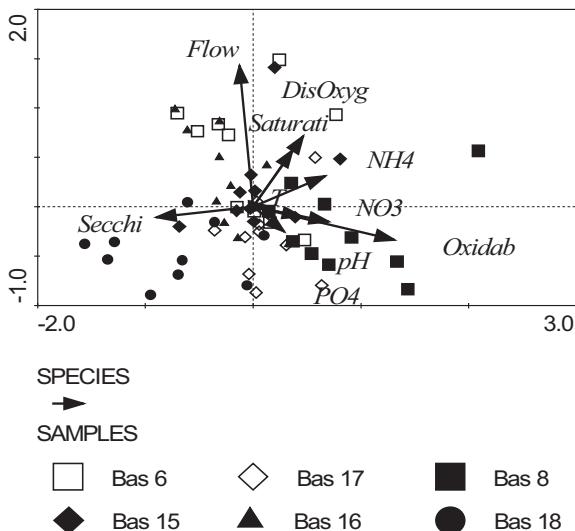
The diverse characteristics of fishponds presented by a great number of measurements were analyzed by statistical methods. The difference between manured and control ponds was tested by Wilcoxon paired rank test. The multiple relations between the variables and sample ordination were revealed by partial principal component analysis (PCA) with statistical packages Statistica 7 (Sokal and Rohlf, 1997; Fowler et al., 1998; McGarigal et al., 2000) and Canoco 4.55 – after ter Braak and Šmilauer (2002).

## Results and Discussion

### Partial Principal Component Analysis (PCA) – abiotic variables (2004)

#### *Spatial changes (between the ponds)*

There were no significant differences of abiotic variables between the manured and the control (without manure added) ponds (Figure 1). However, oxidation and pH, nitrate nitrogen and water transparency values in the control group of ponds were bigger than the mentioned abiotic variables in the manured ponds. The first main axis explains the biggest

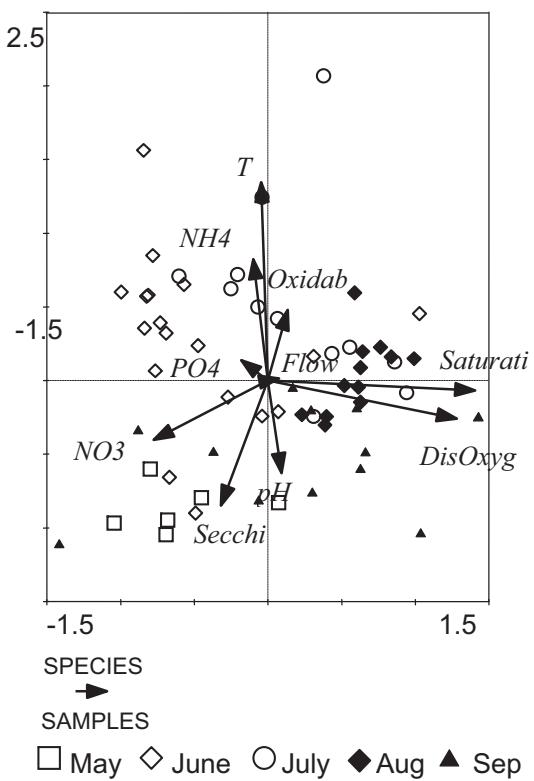


**Fig. 1. Partial PCA of the spatial changes (between the ponds) of the abiotic variables (2004). Filled black symbols – control, empty symbols – manured ponds**

part (27.4%) of the total spatial variation of the abiotic variables analyzed (Table 3).

#### *Temporal changes (between the months of sampling)*

According to the monthly sampling, a highest water transparency and increased nitrate nitrogen content have been observed during May. Usually it happens frequently during the filling of the ponds (Figure 2). There was a weak increasing of phosphate ions concentration during the June sampling. In July, the highest temperature, oxidability and increased ammonium ions concentrations were detected. The results from August showed increased oxygen concentration and increased percentage of oxygen saturation in the water. There was an increased pH value of the water detected during the last vegetation month



**Fig. 2. Partial PCA of the temporal changes (monthly) of abiotic variables (2004)**

**Table 3**

**Variation distribution of the four main axes for the spatial changes (between the ponds) of the abiotic variables (2004)**

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.166	0.100	0.090	0.075	1.000
Cumulative variability, %	27.4	43.9	58.7	71.1	
Total Eigenvalues					0.606

**Table 4**

**Variation distribution among the four main axes for the temporal changes of the abiotic factors (2004)**

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.194	0.167	0.123	0.089	1.000
Cumulative variability, %	23.5	43.8	58.7	69.5	
Total Eigenvalues					0.824

(September) as well. There is an expressed trend for separation of the first three months from the last two months included in the investigated period on the first main axis. The first main axis explains the main part (23.5%) of the total temporal variation of the abiotic variables analyzed (Table 4).

The bigger part of the variation (Tables 3 and 4) has been defined by the differences between the sampling

months (0.824) compared to the changes between the ponds (0.606).

#### **Partial Principal Component Analysis (PCA) – abiotic variables (2005)**

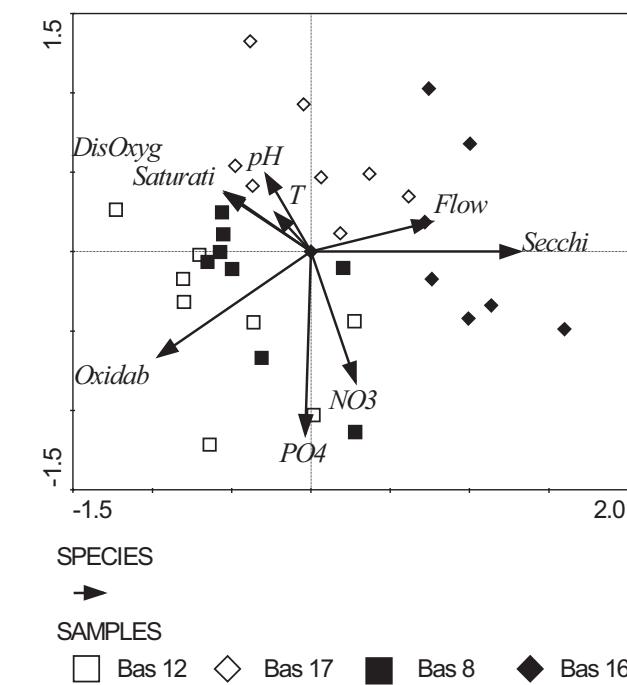
##### **Spatial changes (between the ponds)**

A trend to higher values of the oxidability, dissolved oxygen and pH in the manured ponds than the control pond No 8 within 2005 has been observed (Figure 3). There is a positive correlation between the pH value and the oxygen content and a negative correlation with the transparency (Milstein et al., 2003). The synergism between both of the factors in manured fish ponds has been established by other authors (Garg, Bhatnagar, 2000; Jha et al., 2006). The transparency and the flow rate in the control pond No 16 were increased. The biggest part of the variation is explained by the first main axis – 37.6%. The next axes explain between 10 and 21% of the variation (Table 5).

##### **Temporal changes between the monthly sampling**

Concerning the monthly sampling June differs from the other months by an increased water transparency and elevated phosphate phosphorus concentration. (along the first main axis, Figure 4). An increased water temperature and oxidability have been observed during July and August. Probably the more intensive metabolic processes could cause that (Antipchuk, 1971; Albrecht, 1977, Carvalho et al., 2009). The pH value and the nitrate nitrogen increased in September.

The bigger part of the variation (Tables 5 and 6) is due to the differences between the monthly sampling (0.763) compared to the changes between the ponds of the investigated variables (0.531).

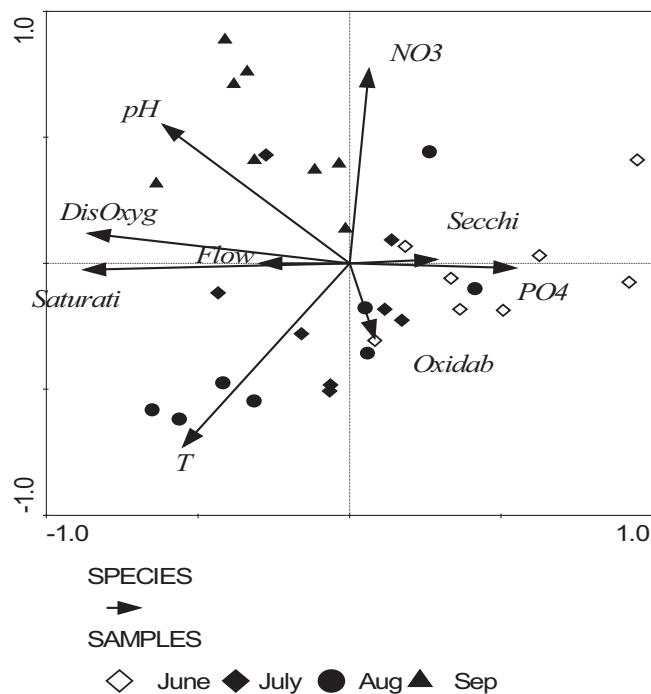


**Fig. 3. Partial PCA of the spatial changes (between the ponds) of the abiotic variables (2005). Filled black symbols – control, empty symbols – manured ponds**

**Table 5**

**Distribution of the variation axes of the PCA for the spatial changes (between the ponds) of the abiotic factors (2005)**

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.200	0.113	0.082	0.048	1.000
Cumulative variability, %	37.6	58.8	74.2	83.2	
Total Eigenvalues					0.531

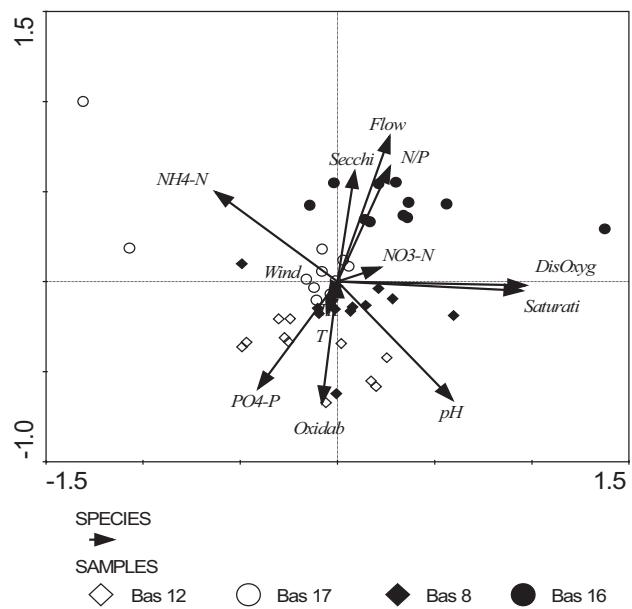


**Fig. 4.** Partial PCA of the temporal changes (monthly) of abiotic factors (2005)

#### Partial Principal Component Analysis (PCA) – abiotic factors (2006)

##### *Spatial changes (between the ponds)*

The manured ponds usually have higher oxidability, phosphate phosphorus concentration and pH value (Figure 5). There is a positive correlation available between the pH value and the oxygen content (Garg, Bhatnagar,



**Fig. 5.** Partial PCA of the spatial changes (between the ponds) of abiotic factors (2006). Filled black symbols – control, empty symbols – manured ponds

2000; Milstein et al., 2003; Jha et al., 2006). The control ponds have an increased flow rate and transparency, higher N/P ratio and more ammonium nitrogen. Kipkemboi et al (2010) reported a lower N/P ratio and a higher phosphate concentration during manuring.

The first main axis explains 32% of the abiotic variable variation between the ponds. The others axes explain about 10–25% each (Table 7).

**Table 6**

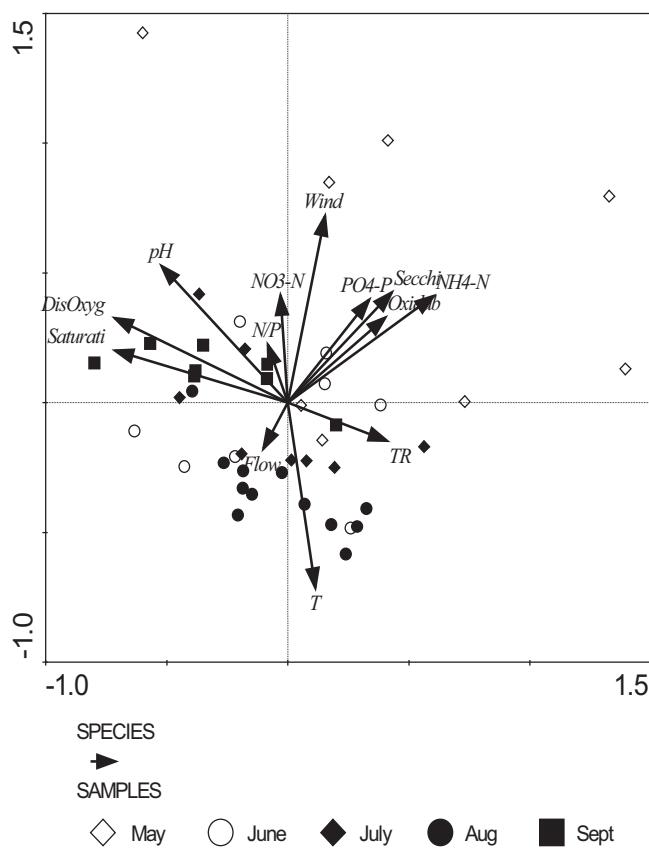
Variation distribution along the first four main axes for the temporal changes of the abiotic factors (2005)

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.295	0.169	0.094	0.072	1.000
Cumulative variability, %	38.7	60.9	73.2	82.6	
Total Eigenvalues					0.763

**Table 7**

Distribution of the variation axes for the spatial changes between the ponds of the abiotic factors (2006)

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.168	0.129	0.077	0.053	1.000
Cumulative variability, %	32.0	56.5	71.2	81.3	
Total Eigenvalues					0.524



**Fig. 6. Partial PCA of the temporal changes (between months) of abiotic variables (2006)**

#### Temporal changes between the monthly sampling

The transparency, wind strength, phosphate phosphorus, ammonium nitrogen concentration, and oxidability increased in May (Figure 6). There was no expressed trend of separation of June samples, while during July and August an increased temperature and an increased water flow rate have been observed. The oxygen concentration, saturation and pH value increased in September. The first main axis explains the highest rate of dispersion of temporal changes – 23.5%, the second axis – 19.9%, the other axes about 10–16% each (Table 8).

#### Table 8

#### Variation distribution between the four main axes of the temporal changes of the abiotic variables (2006)

Variables	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.195	0.166	0.132	0.084	1.000
Cumulative variability, %	23.5	43.4	59.2	69.4	
Total Eigenvalues					0.832

The bigger part of the total variation (Tables 7 and 8) has been defined by the differences between the sampling months (0.832) compared to the variable changes between the ponds (0.524). The manuring effect, which is a component of the spatial differences between experimental fish ponds, had also an influence to definition of this variation.

After three years, the investigations led to conviction that the season has a strong influence upon PP and abiotic variables. Kumar et al. (2004) showed the role of the climate for the productivity in manured fish ponds.

Regularly the fish has been checked for healthy problems in parallel to the experiments. No diseases are found.

#### Conclusions

No differences between the manured and the control ponds concerning the abiotic variables observed have been found during 2004 investigation period. Probably the different number of manured (2) and control (4) ponds included in the experiment is the reason, leading to an increased dispersion, which masked the differences.

The bigger part of the abiotic variable 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 regular difference between first and last months concerning the abiotic factors. This great seasonal variability decreases the opportunity for revealing the differences between the manured and the control ponds.

As a rule the abiotic variables like oxidability, oxygen content and pH value in the manured ponds had a higher level compared to the control ponds during 2005.

An increased level of the oxidability, phosphate phosphorus and pH value was found in the manured ponds during 2006.

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