

REGULATION OF SPECIES AND SIZE COMPOSITION IN PHYTOPLANKTON COMMUNITIES *IN SITU* BY N:P RATIO

A.P. LEVICH and N.G. BULGAKOV

*Chair of Vertebrate Zoology and General Ecology, Biological
Faculty of Moscow University, Moscow 119899, Russia*

ABSTRACT. To regulate the species and size structure of phytoplankton, nitrogenous and phosphate fertilizers in the form of NH_4NO_3 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ were applied during three vegetative seasons in fish-rearing ponds situated in the Lower Volga region (Astrakhan district). Weight nitrogen-phosphorus ratio (N:P) in fertilizers introduced into experimental ponds was 25-50:1. Fertilizers were introduced 2 times a week from April to September. In the control ponds, N and P were added in a 4:1 ratio every ten days. Biomass of Protococcales and Chlorophyta was higher in the experiment than in the control, while the opposite situation was observed for cyanobacteria. The biomass of algae belonging to intermediate weight classes (0.3 to 1 ng and 1 to 3.2 ng) was higher in the experiment than in the control. Biomass values for weight classes less than 0.1 ng, 0.1 to 0.3 ng, 3.2 to 10 ng and more than 10 ng did not differ both in the control and in the experiment. It was shown that N:P ratio is an important factor affecting the phytoplankton community structure. An increase in dissolved oxygen content and yield of herbivorous fish by 19 % was recorded in experimental ponds.

Key words: natural phytoplankton community, mineral nitrogen and phosphorus, requirement in biogenic elements.

The ratio between nutrients in the water can be directly used to control the species and size composition of phytoplankton community. For instance, experiments with batch cultures of laboratory strains of four Protococcales species showed (Levich and Bulgakov 1993) that at N:P ratio = 3.5:1 *Clorella vulgaris* dominates, while at N:P ratio = 20:1, the maximum biomass is attained by *Scenedesmus quadricauda*. In experiments with simultaneously cultured *Scenedesmus quadricauda* and *Ankistrodesmus falcatus*, the former species dominates at N:P ratio = 1.3:1, and the latter at N:P ratio = 57:1.

The results of experiments on batch culturing of pond algal community *in vitro* (Levich et al. 1991) proved that as the ratio between initial N:P concentrations in the medium changes (N:P ratios = 2:1, 5:1, 12:1, 16:1, 20:1, 50:1, 100:1 were tested), the algal community composition also changes. Green algae (Chlorophyta) attain their maximum biomass at the N:P ratios = 20:1 and 50:1. Cyanobacteria and diatoms (Bacillariophyta) have an optimum at N:P ratio = 2-5:1. The replacement of dominant cyanobacteria by Bacillariophyta in lake multispecies culture was observed by Sommer (1983) in the case of artificially increased ratio of silicon to phosphorus in hemostatic medium. The studies of species composition of lake phytoplankton in dependence on N:P concentration ratio (Schindler 1977,

Smith 1986) indicated that, at the atomic N:P ratio less than 25:1, the cyanobacteria prevail in the phytoplankton, meanwhile the increase of this ratio up to 25:1 and more leads to predominance of green and diatom algae.

The influence of nutrient ratio on the algal community structure can also be demonstrated (Levich and Bulgakov, 1993) in experiments with batch culturing: the optimum for particular species initial ratio between nutrients is equal to quota ratio for the same nutrients. The quota is the threshold nutrient content in a cell below which the growth of culture is stopped at the zero concentration of this nutrient in the environment.

In this paper, the authors present the results of their attempts to regulate the species and size structure of pond phytoplankton using the intentional changes in N:P ratio in the environment. The algal community composition was optimized in respect to food requirements of pond herbivores: fish (white and motley silver carps) and zooplankton. Chlorophyta algae are the most preferable food item for these organisms, while cyanobacteria cells are usually not consumed by fish and zooplankton because of their low food value or toxicity. To increase the availability of phytoplankton as a food for herbivores, it is necessary to decrease the share of too large (3.2 to 10 ng or more than 10 ng) as well as too small

References

- Baranov, I.V. 1968. Standards on lime and nitrogenous-phosphate fertilizers for humic ponds. *Izvestija GosNIORKH* 67: 321-332 (in Russian).
- Bothe, H. 1982. Nitrogen fixation. Pages 87-104 in N.G. Carr and B.A. Whitton, editors, *The biology of cyanobacteria*. Oxford, Blackwell Scientific Publications.
- Bryceson, I., and P. Fay. 1981. Nitrogen fixation in *Oscillatoria (Trichodesmium) erythraea* in relation to bundle formation and differentiation. *Marine Biology* 61: 159-166.
- Bulgakov, N.G., A.P. Levich, and T.V. Salomatina. 1988. Hydrobiological condition of fish-breeding ponds as a consequence of frequent use of mineral fertilizers. *Sbornik Nauchnykh Trudov GosNIORKH* 280: 143-149 (in Russian).
- Carpenter, E.J., and C.C. Price. 1976. Marine *Oscillatoria (Trichodesmium)*: explanation for aerobic nitrogen fixation without heterocysts. *Science* 191: 1278-1280.
- Chesnokov, S.V. 1982. Determinatsionnyj analiz sotsial'no-ekonomicheskikh dannykh. (Determination analysis of socio-economic data). Moscow, Nauka (in Russian).
- Daua, A. 1982. Conditions de développement du phytoplancton. Etude comparative du comportement de huit espèces en culture. 2. Rôle des nutriments: assimilation et stockage intracellulaire. *Annales de Limnologie* 18(3):263-292.
- Gaedeke, A., and V. Sommer. 1986. The influence of the frequency of periodic disturbances on the maintenance of phytoplankton diversity. *Oecologia* 71: 25-28.
- Levich, A.P. 1989. Phytoplankton requirements for environmental resources and the ways of phytoplankton community structure controlling. *Zhurnal obshchej biologii* 50(3): 326-328 (in Russian).
- Levich A.P., V.L. Alekseev, and S.U. Rubakova. 1993. The optimization of structure of ecological communities: a model analysis. *Biophysika* (in press) (in Russian).
- Levich, A.P., and V.I. Artukhova. 1991. Measurement of phytoplankton requirement for substratum environmental factors. *Izvestija AN SSSR, ser. Biologija* 1: 114-123 (in Russian).
- Levich, A.P., and N.G. Bulgakov. 1993. On the possibility of regulation of species structure of laboratory algocenosis. *Izvestija RAN, Ser. Biologicheskaja* (in press) (in Russian).
- Levich, A.P., A.A. Khudoyan, N.G. Bulgakov, and V.I. Artukhova. 1991. On the possibility of regulation of species and size community structure in experiments on natural phytoplankton in vitro. *Biologicheskij zhurnal Armenii* 44(3): 186-192 (in Russian).
- Levich, A.P., and E.G. Lichman. 1992. Model study of possibility of directional changes in phytoplankton community structure. *Zhurnal obshchej biologii* (in press) (in Russian).
- Levich, A.P., N.V. Revkova, and N.G. Bulgakov. 1986. "Consumption-growth" process in microalgal culture and cell requirements for components of mineral nutrition. Pages 132-139 in V.N. Maksimov, editor, *Ecologicheskij prognoz (Ecological prediction)*. Moscow, MGU (in Russian).
- Muller, H. von. 1972. Wachstum und Phosphat-bedarf von *Nitzschia actinastroides* (Lemn.) V. Goor. in statischer und homokontinuierlicher Kultur unter Phosphat-limitierung. *Archiv fur Hydrobiologie, Supplement* 38: 399-484.
- Reynolds, C.S. 1980. Phytoplankton assemblages and their periodicity in stratifying lake systems. *Polarctic Ecology*. 3: 141-159.
- Rhee, G-Yull, and I.J. Gotham. 1980. Optimum N:P ratios and the coexistence of planktonic algae. *Journal of Phycology* 16: 486-489.
- Schindler, R. 1977. Evolution of phosphorus limitation in lakes. *Science* 195: 260-262.
- Smith, V.N. 1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. *Canadian Journal of Fisheries and Aquatic Sciences* 43: 148-153.
- Sommer, U. 1983. Nutrient competition between phytoplankton species in multispecies chemostat experiments. *Archiv fur Hydrobiologie* 96(4): 399-416.
- Stroganov, N.S., and N.S. Buzinova. 1980. *Prakticheskoe rukovodstvo po gidrokhimii*. (Practical manual on hydrochemistry). Moskva, MGU.
- Suttle, C.A., J.G. Stockner, and P.J. Harrison. 1987. Effects of nutrient pulses on community structure and cell size of a freshwater phytoplankton assemblage in culture. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1768-1774.
- Zamolodchikov, D.G., N.G. Bulgakov, A.G. Gursky, A.P. Levich, and S.V. Chesnokov. 1992. On the method of application of determination analysis. *Biologicheskie nauki* (in press) (in Russian).

(less than 0.1 ng and from 0.1 to 0.3 ng) species. Therefore, it is desirable to obtain a stable increase in the green alga share in phytoplankton community as well as a decrease in cyanobacteria by means of increasing N:P weight ratio in applied fertilizers.

Material and methods

The experiments were conducted in 1987-89 on experimental fish ponds situated in the Lower Volga region (Astrakhan district). The area of ponds was 2-3 ha, the depth was about 1.5 m. The phosphate and nitrogenous fertilizers were introduced into the ponds in certain proportions under two schedules: experimental and control ones. There were one experimental and one control pond in each growing season, the roles of both control and experimental ponds being interchanged so that none of the ponds remains the control or the experimental during more than one year. The differences in the methods of fertilization are presented in Table 1. In the control ponds, the lump doses of fertilizers were the same throughout the season and corresponded to 2 mg/l of nitrogen and 0.5 mg/l of phosphorus concentration in the water. In the experimental ponds, the doses of fertilizers varied depending on the phytoplankton production observed in certain time of vegetative season. Thus, the doses were correlated with the dynamics of biomass of herbivores, with their daily food intake as well as with phytoplankton requirements for nitrogen and phosphorus.

Due to the shortage of ponds, more than one variable was varied during an experimental season. Although the pattern of experiment was changed several times from year to year, N:P ratio was in all cases higher in the experimental pond than in the control one.

The nitrogenous and phosphate fertilizers

were applied in the form of ammonium nitrate NH_4NO_3 and superphosphate $\text{Ca}(\text{H}_2\text{PO}_4)_2$. The ammonium nitrate and superphosphate were simultaneously introduced into the ponds from a boat, but they were not mixed and were distributed evenly over the whole pond area. The nitrate was poured into water at once, while the superphosphate was at first soaked and then poured into pond in the watery consistence.

The control and experimental ponds were stocked by young of carp (*Cyprinus carpio*), white silver carp (*Hypophthalmichthys molitrix*), and motley silver carp (*Aristichthys nobilis*). During the season fish grew on the average from 30 to 500-700 g. The fish population density was approximately the same in the control and experimental ponds and varied from season to season within the limits of 0.5 to $4 \cdot 10^3$ individuals per ha for white silver carp, 0.3 to $1 \cdot 10^3$ individuals per ha for motley silver carp, and 2 to $6 \cdot 10^3$ individuals per ha for carp. Fish stocking in ponds was performed in the middle of April. In 1988-1989, the fertilization of experimental ponds began two weeks before the fish stocking to provide the necessary phytoplankton biomass by the stocking time. During two weeks in 1987 and during a month in 1988, in the beginning of the season, fertilizers with N:P ratio 4-6:1 were applied.

Phytoplankton samples were taken from April to September in all ponds once in every ten days. At the same dates, the oxygen concentration as well as concentration of mineral forms of nitrogen and phosphorus were evaluated. The samples were obtained from a depth of 1 m by a sampler. Phytoplankton samples were poured into 0.5 l bottles and preserved by formalin (20 % formaldehyde solution neutralized by CaCO_3 or NaHCO_3) in such a way that for each 100 ml of sample there were 2-4 ml of formalin. The samples were

Table 1. Fertilization pattern in experimental and control ponds

Elements of fertilization system	1987		1988		1989	
	exper	control	exper	control	exper	control
Nitrogen quantity applied in a season, kg/ha	563	195	382	134	450	267
Phosphorus quantity applied in a season, kg/ha	16	48	39	34	11	67
N:P weight ratio in applied fertilizers averaged over a season	35	4	10	4	41	4
N:P weight ratio in fertilizers before the fish stocking	—	—	4	—	23	—
Interval between inputs of fertilizers, days	8	12	4	11	4	10
Beginnig of fertilization	April 17	April 17	April 1	May 10	April 4	April 17

Remarks. — Fertilization was begun after the fish stocking

settled for 7-10 days and, after seston settlement, the upper layer of water was poured out with the use of siphon, while remaining concentrated volume (30-50 ml) was used for quantitative and qualitative analysis of phytoplankton.

The cell quantity and individual cell volumes were estimated in Najotte camera using dissecting microscope. Cell volumes were calculated by approximation to the nearest simple geometric solid (sphere, cylinder, ellipsoid, etc.) after measurement of no less than 50 cells of each species. The average individual mass for every species was obtained by multiplying the cell volume value by density of 1 g/cm^3 . Then the total biomass of phytoplankton species, genera, orders and divisions were estimated. The species and genera with biomass comprising no less than 20 % of the total phytoplankton biomass in at least 5 arbitrary sampling dates (out of 15-20 samples in a season) were regarded as leading ones, and only these species were analyzed.

To analyze the size community structure, all phytoplankters were categorized according to their individual size (in mass units). The biomass in samples was calculated for the following size classes: less than 0.1 ng; 0.1-0.3 ng; 0.3-1 ng; 1-3.2 ng; 3.2-10 ng; more than 10 ng.

The comparison of biomass values of particular taxa was performed on data averaged over the total growing season as well as over such periods as April-May, June-July and August-September.

The mineral phosphorus was measured spectrophotometrically after addition of ammonium molybdate, sodium antimonate and ascorbic acid. Ammonium nitrogen was also estimated spectrophotometrically after yellow color appeared as a result of Nessler's reagent and Segnette salt addition. The quantity of nitrate nitrogen was estimated through visual colorimetry (comparison with standard solutions) after reaction with sodium-potassium tartrate and metallic zinc (Stroganov and Buzinova 1980). An oxygen content was estimated using Winkler titration.

The determination analysis was used for statistical treatment of experimental data. The quantitative description of fish ponds includes a huge amount of data on physical, chemical and biological variables. The determination analysis allows to unravel efficiently such a great number of relations between variables and to select the most essential ones (Chesnokov 1982, Zamolodchikov et al. 1992). This is quite important when the experimental and control ponds differ in a lot of parameters. Each analyzed variable included 20 values corresponding to different ponds in various years of observations independently from the fertilization schedule. Besides the data obtained in 1987-89, this set contained also data

of 1973, 1977 and 1986 on ponds fertilized in the same way as the control ones (Table 1). The whole set of 20 numerical values of each analyzed variable was divided into three conventional classes: "low", "middle" and "great".

The determination analysis make it possible to calculate the frequencies which are responsible for the correspondence between different values of variables. Let us use an example to illustrate the mechanism of determination analysis action. Assume that it is of interest under which conditions the variable "green alga biomass" (explainable variable) may be placed in the "low" class. It is known that there are 10 such values out of 20 existing ones. Let us determine, for example, the values of variable "quantity of phosphate fertilizers applied in a year" (explanatory variable) such that the green alga biomass belongs to the class "low".

Explanatory variable		Explainable variable "low"
"low"	7	1
"middle"	9	7
"great"	4	2

So, for 7 points with low values of explanatory variable there is one value of explainable variable belonging to the "low" class, etc. Thus, in 78 % of cases (7 out of 9), the low value of explainable variable coincides with the mean value of explanatory variable. This comprises 70 % (7 out of 10) of the total number of cases of explainable variable in the "low" class. The first frequency (78 %) is termed the accuracy of determination, the second one (70 %) is designated the completeness of determination. Determinations with both accuracy and completeness in excess of 50 % are proved to be essential.

In the present paper, an explainable variable "green alga biomass" and explanatory variables "quantity of nitrogen introduced with fertilizers", "nitrogen-phosphorus ratio in fertilizers", "concentration of inorganic phosphorus in the water", and "ratio between concentrations of inorganic nitrogen and inorganic phosphorus in the water" have been studied. For each of these variables, the averaging was considered both over the whole season and over periods of April-May, June-July and August-September.

Results

Concentration of phosphates and inorganic nitrogen was constantly higher in experimental ponds (the averaging over a season and over two-month intervals for some hydrochemical characteristics is given in Table 2). N:P ratio

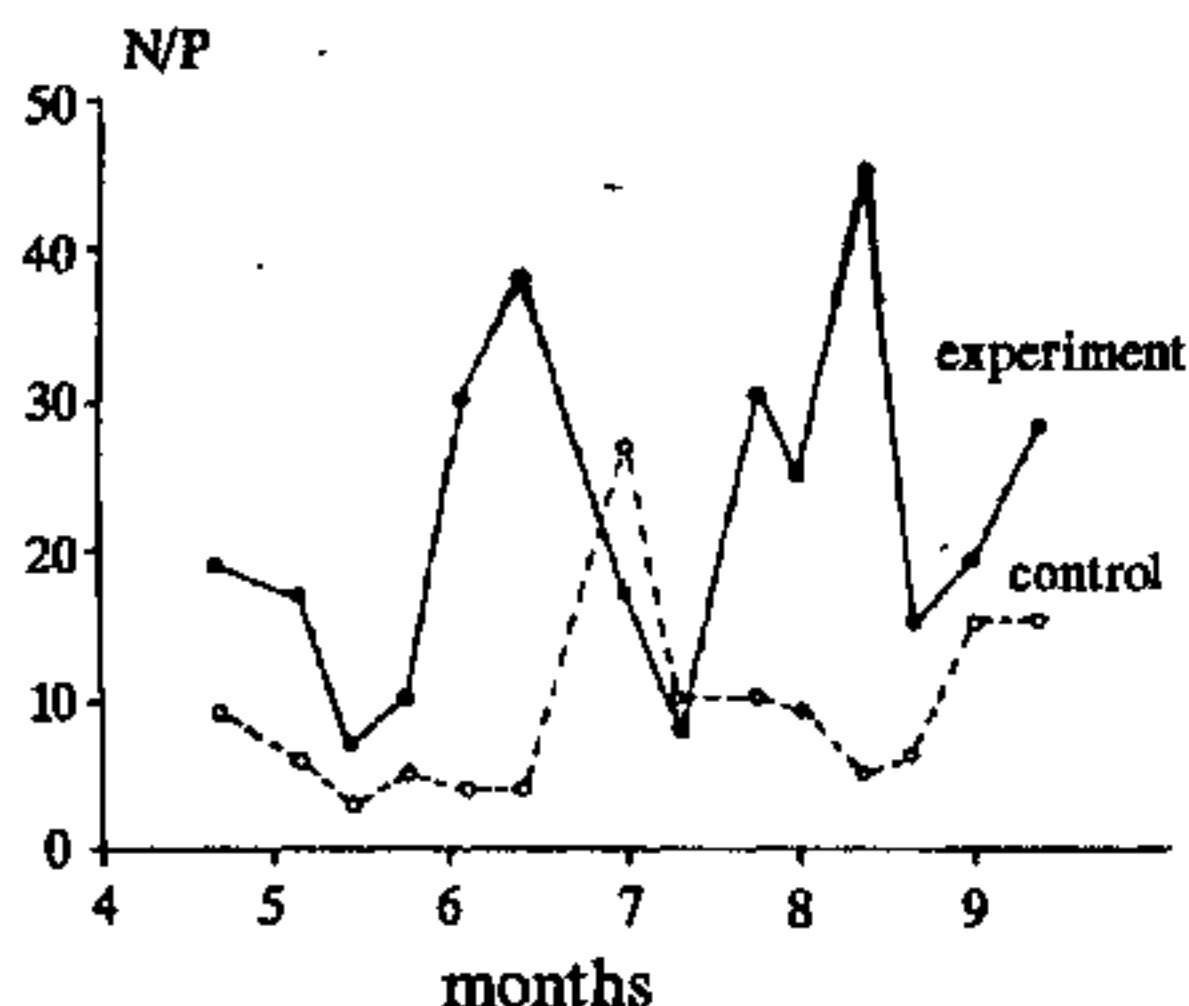


Figure 1. N:P ratio dynamics in the experiment and in the control in 1987.

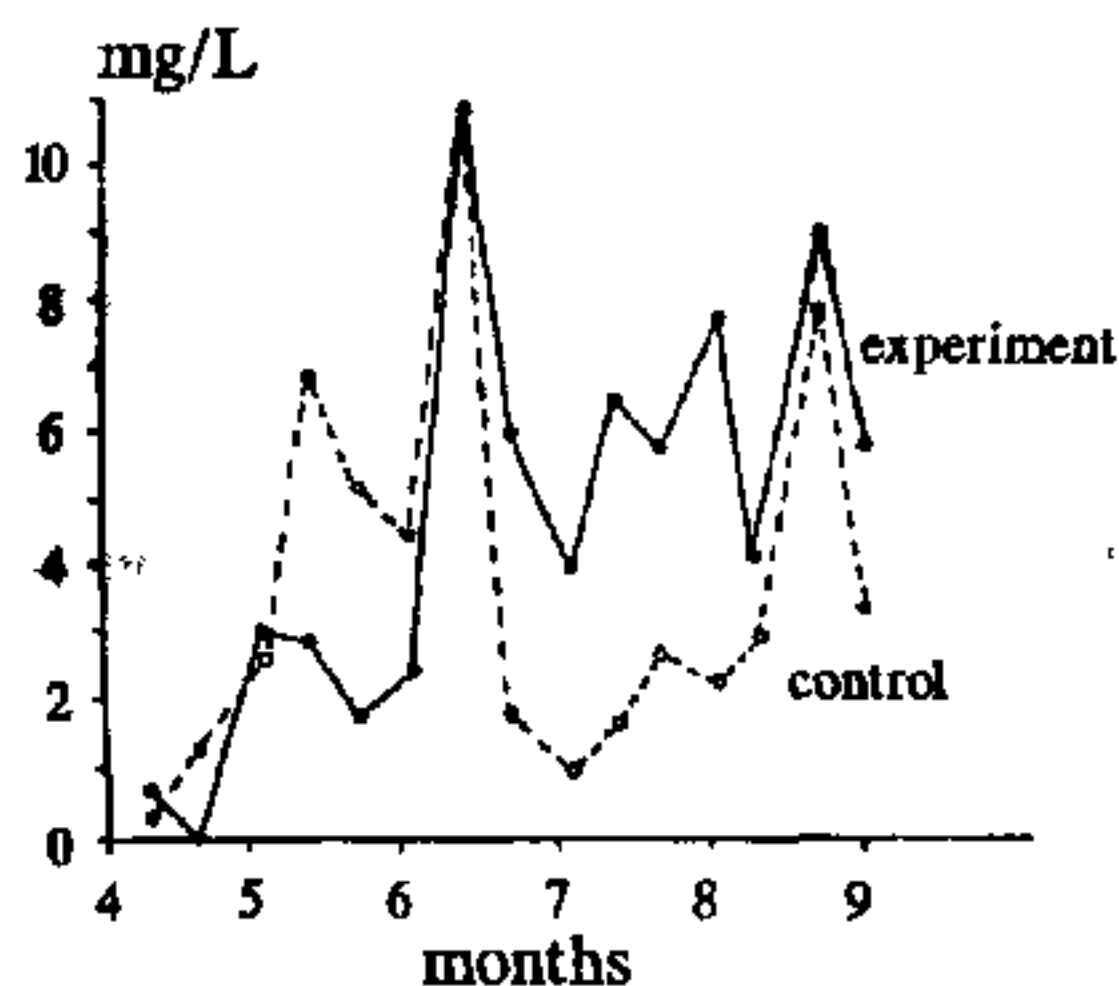


Figure 3. Green alga biomass dynamics in the experiment and in the control in 1989.

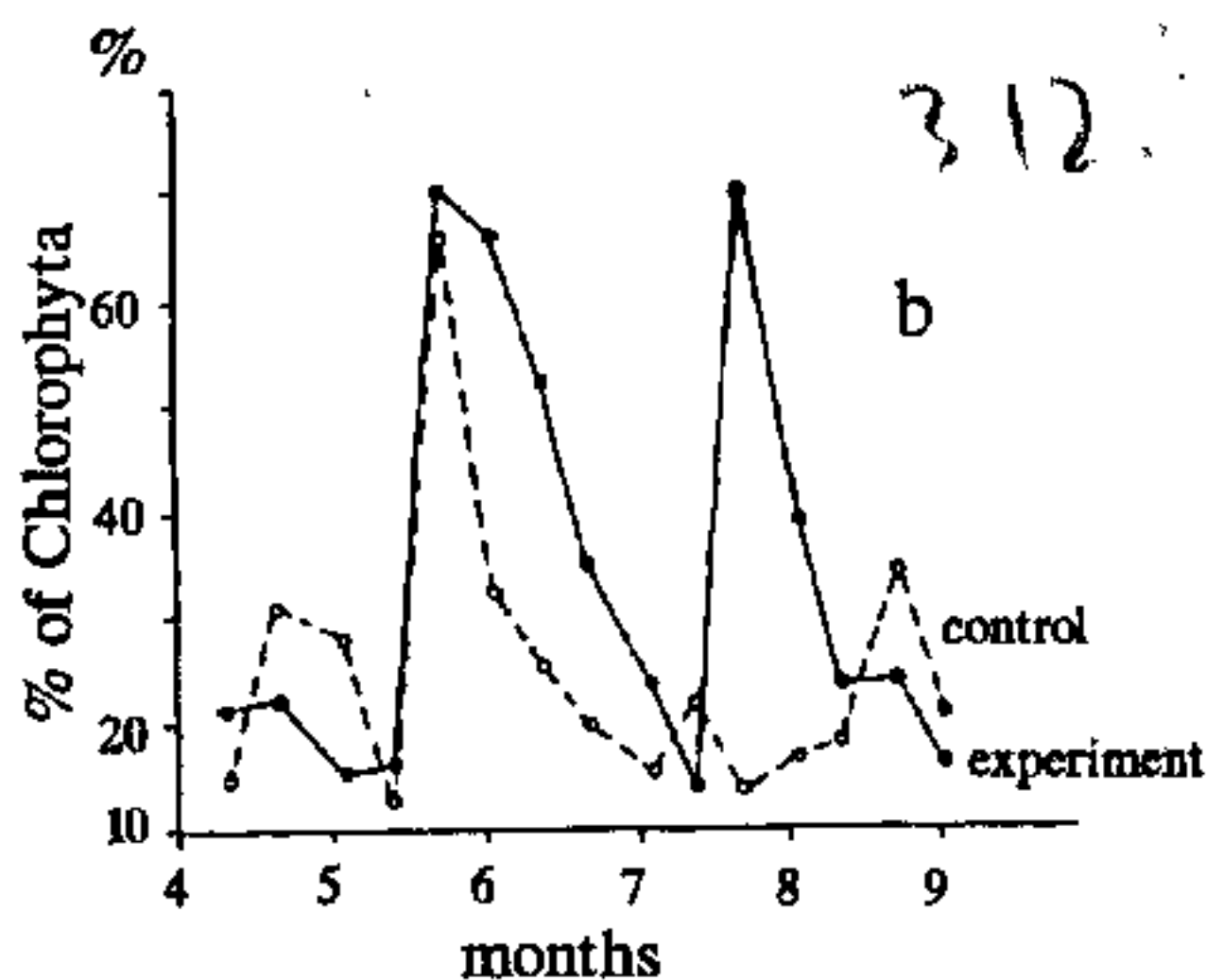
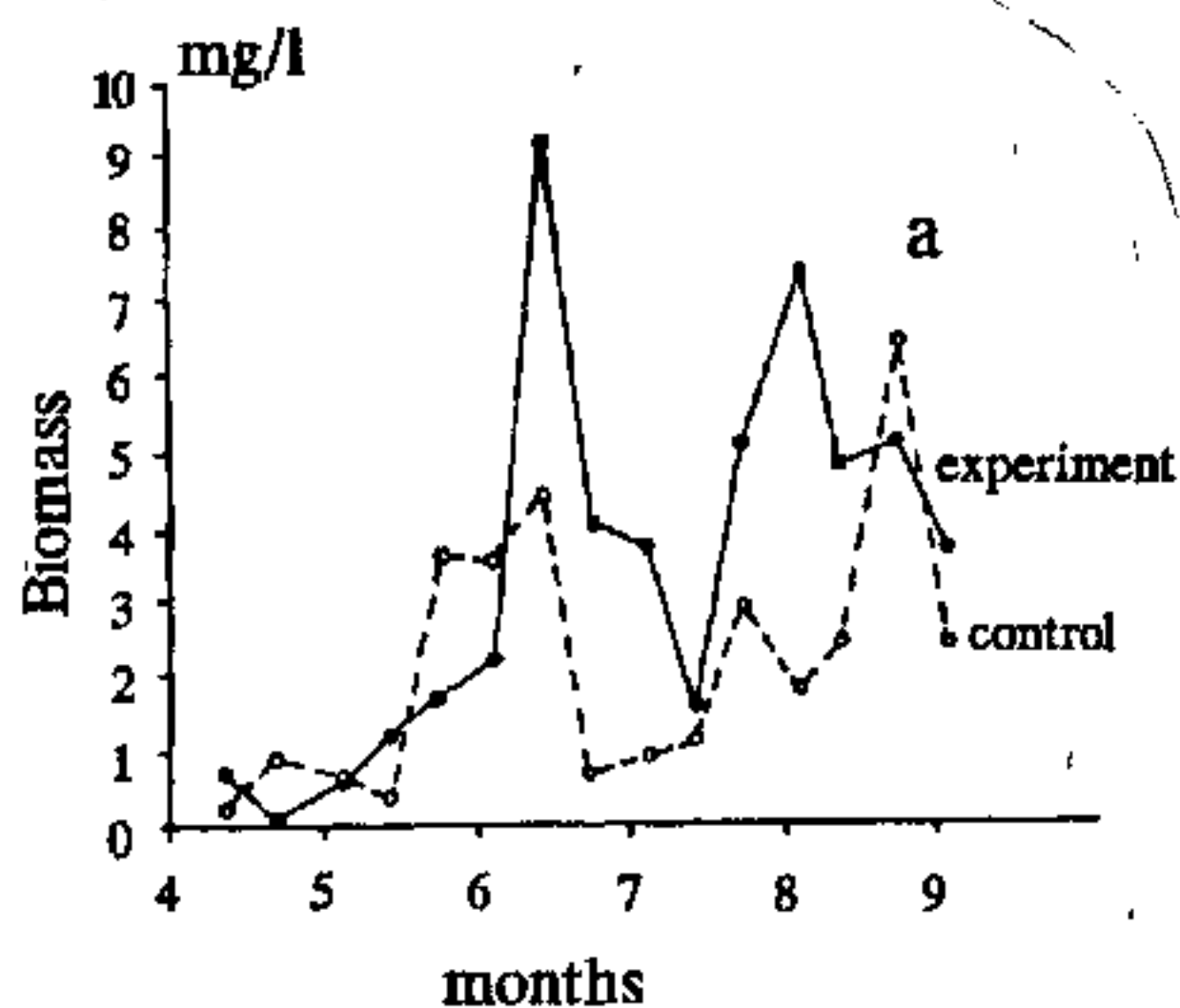


Figure 2. Dynamics of biomass and percentage of Protococcales in the total phytoplankton biomass in the experiment and in the control in 1989. a) biomass dynamics, b) % of Protococcales in the total biomass

was also usually higher in ponds fertilized in the same way as experimental ones (Fig. 1). In 1988, the ratio was the same in compared ponds with the exception of spring period.

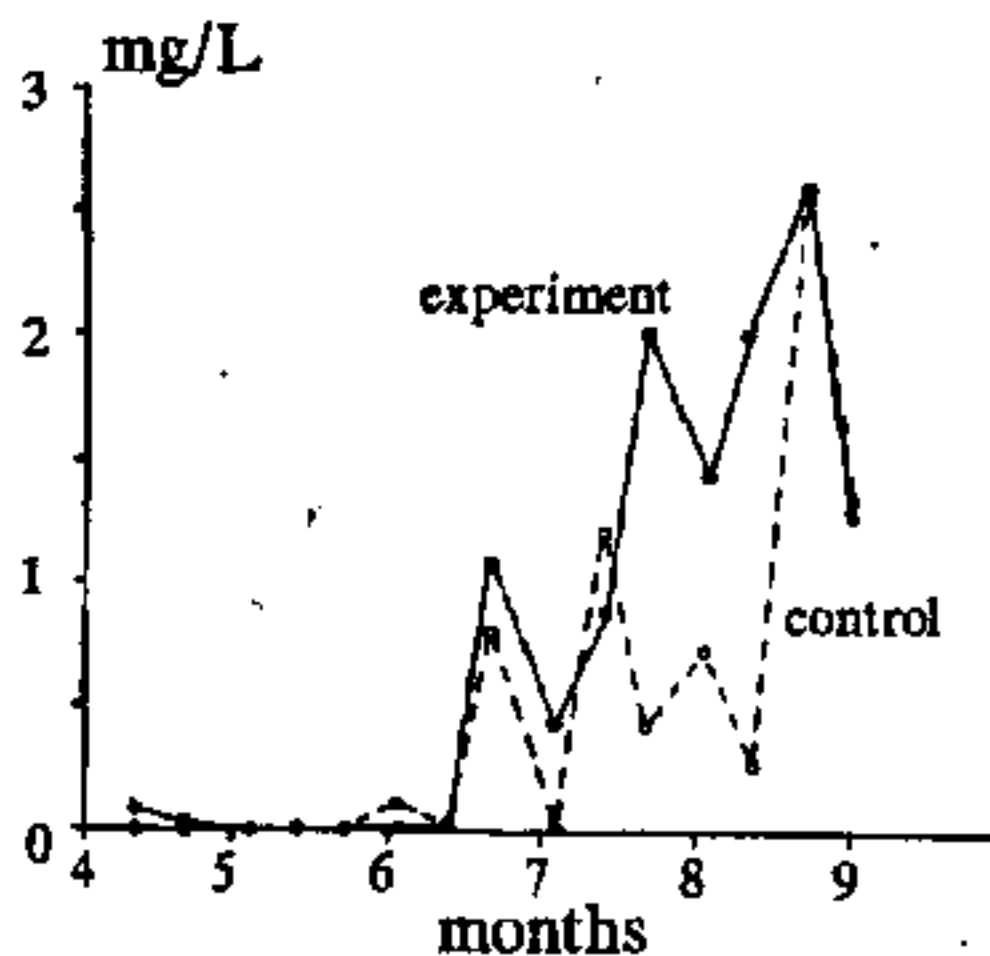


Figure 4. *Melosira* sp. biomass dynamics in the experiment and in the control in 1989.

It follows from Table 2 that, starting with June, one can observe the higher biomass values of Protococcales in the experiment than in the control. In 1988, the effect of stimulation of Protococcales by experimental fertilization became apparent later (August and September), hence the average seasonal biomass appeared to be lower in the experiment than in the control. An increase in Protococcales biomass in the experimental ponds by the end of season as compared with control ones is confirmed by Fig. 2a. Simultaneously the percentage of Protococcales in the total biomass also increased in the experimental ponds (with the exception of 1988) sometimes reaching 60 % (Table 2, Fig.2b).

Among the "leading" Protococcales species, *Scenedesmus quadricauda* was most sensitive to experimental fertilization. By the end of season, this species usually had an increased biomass in the ponds with increased N:P ratio (Table 2).

Stimulation of Protococcales species led to an increase in the whole biomass of Chlorophyta

Table 2. Averaged chemical parameters in experimental and control ponds

Parameter	1987		1988		1989	
	experi- ment	control	experi- ment	control	experi- ment	control
Concentration of total inorganic nitrogen in water, mg/l						
averaged over April-May	1.27	0.48	1.61	0.61	1.01	1.20
averaged over June-July	2.17	0.52	1.23	0.84	1.35	0.75
averaged over August-September	2.74	0.89	1.30	1.18	3.50	1.73
averaged over the season	1.94	0.57	1.40	0.86	1.81	1.16
Concentration of inorganic phosphorus in the water, mg/l						
averaged over April-May	0.18	0.09	0.13	0.11	0.05	0.08
averaged over June-July	0.11	0.07	0.43	0.29	0.21	0.14
averaged over August-September	0.10	0.10	0.37	0.24	0.09	0.08
averaged over the season	0.13	0.08	0.30	0.21	0.13	0.10
N:P concentration ratio in the pond water						
averaged over April-May	7	5	12	6	20	15
averaged over June-July	20	7	3	3	6	5
averaged over August-September	27	9	4	5	39	22
averaged over the season	15	7	5	4	14	12
Protococcales biomass, mg/l						
averaged over April-May	3.47	3.87	0.58	4.09	0.90	1.17
averaged over June-July	3.08	2.47	2.71	3.92	4.26	2.26
averaged over August-September	5.13	5.01	11.18	9.39	4.92	3.13
averaged over the season	3.65	3.32	3.83	5.60	3.32	2.13
Chlorophyta biomass, mg/l						
averaged over April-May	3.69	4.39	47.49	4.98	1.74	3.25
averaged over June-July	3.95	3.03	3.40	4.26	5.77	3.85
averaged over August-September	8.24	8.02	14.53	9.66	6.34	4.09
averaged over the season	4.98	4.48	24.15	6.14	4.58	3.71
Cyanobacteria biomass, mg/l						
averaged over April-May	0.83	6.41	0	0	3.73	3.61
averaged over June-July	1.28	3.64	1.98	2.08	11.37	18.60
averaged over August-September	5.64	4.08	2.43	3.35	22.15	17.34
averaged over the season	2.30	4.16	1.27	1.60	11.70	13.27
Bacillariophyta biomass, mg/l						
averaged over April-May	4.48	10.00	0.91	11.68	3.38	1.22
averaged over June-July	2.36	3.86	0.76	1.34	1.79	1.30
averaged over August-September	2.89	8.25	8.16	2.10	4.37	2.71
averaged over the season	2.81	5.88	2.56	5.82	3.01	1.65
Euglenophyta biomass, mg/l						
averaged over April-May	6.71	8.78	5.61	3.32	0.12	0.14
averaged over June-July	4.25	7.22	1.07	0.83	1.46	0.51
averaged over August-September	8.12	2.84	4.27	3.64	4.93	1.55
averaged over the season	5.59	6.36	3.69	2.68	1.94	0.66
Protococcales biomass share in total biomass, %						
averaged over April-May	19	16	11	16	18	20
averaged over June-July	24	11	30	41	33	11
averaged over August-September	26	11	29	44	15	12
averaged over the season	24	12	22	31	23	14

Table 2. Averaged chemical parameters in experimental and control ponds (finished)

Parameter	1987		1988		1989	
	experi- ment	control	experi- ment	control	experi- ment	control
Chlorophyta biomass share in total biomass, %						
averaged over April-May	20	24	78	23	24	42
averaged over June-July	30	24	44	47	39	20
averaged over August-September	38	72	43	47	19	16
averaged over the season	30	41	58	37	29	26
Cyanobacteria biomass share in total biomass, %						
averaged over April-May	4	3	0	0	24	28
averaged over June-July	10	36	17	19	44	55
averaged over August-September	20	17	14	18	54	67
averaged over the season	11	21	9	11	40	49
Biomass of size class 0.3-1 ng, mg/l						
averaged over April-May	2.96	5.32	6.16	11.31	1.64	1.48
averaged over June-July	4.10	3.52	1.39	1.54	3.44	1.26
averaged over August-September	9.17	7.36	10.00	9.88	7.97	4.13
averaged over the season	5.20	4.75	5.38	8.02	4.05	2.10
Biomass of size class 1-3.2 ng, mg/l						
averaged over April-May	5.51	2.13	41.62	3.47	3.80	3.23
averaged over June-July	1.53	0.67	3.04	2.49	5.16	4.66
averaged over August-September	0	1.97	12.05	0.98	9.87	8.46
averaged over the season	1.74	1.22	21.05	2.45	5.96	5.20
<i>Scenedesmus quadricauda</i> biomass, mg/l						
averaged over April-May	0.32	0.74	0.01	0.53	0.36	0.12
averaged over June-July	0.93	0.65	0.20	0.27	1.28	1.02
averaged over August-September	2.66	1.61	0.54	0.42	1.25	0.36
averaged over the season	1.27	0.90	0.20	0.42	0.97	0.55
<i>Melosira</i> sp. biomass, mg/l						
averaged over June-July	—	—	0.28	0.20	0.70	0.42
averaged over August-September	—	—	5.39	0.19	1.79	1.20
averaged over the season	—	—	1.37	0.12	0.76	0.49
<i>Merismopedia</i> sp. biomass, mg/l						
averaged over June-July	—	—	0.01	0.09	—	—
averaged over August-September	—	—	0.04	0.21	—	—
averaged over the season	—	—	0.01	0.09	—	—
<i>Phormidium</i> biomass, mg/l						
averaged over June-July	—	—	—	—	0.39	15.16
averaged over August-September	—	—	—	—	6.94	10.67
averaged over the season	—	—	—	—	2.01	8.91

that can be recorded by the end of season. (Table 2, Fig. 3). Chlorophyta attained their rather high biomass (47.49 mg/l) in April-May, 1988 mainly due to high biomass of Volvocales species.

The increase of N:P ratio most often did not cause the acceleration of Bacillariophyta and Euglenophyta growth if the total biomass of these divisions is considered. An increase of biomass in the experiment was recorded for Euglenophyta in 1988 and for Bacillariophyta in 1989 only (Table 2).

When the averaged seasonal biomass and growth dynamics of such a mass Bacillariophyta species as *Melosira* sp. is considered (Fig. 4, Table 2), it can be noted that its biomass increased in experimental ponds in 1988-89. In 1987, this species did not belong to "leading" ones.

The decline in biomass and relative abundance of cyanobacteria since the mid-season was a permanent consequence of experimental ponds fertilization (Fig. 5, Table 2). As it is shown in Table 2, this was connected with the biomass lowering in "leading" Cyanobacteria genera *Merismopedia* and *Phormidium* within June to September period.

An increase in the cell biomass in the intermediate size classes 0.3-1 and 1-3.2 ng in the experimental ponds is an evidence of shift in size structure.

The determination analysis showed (Table 3) that low biomass values of Chlorophyta in late and mid-season correlate with low N:P weight ratio in applied fertilizers. Decline of Chlorophyta biomass is also determined by lowering in inorganic nitrogen content both in applied dry fertilizers and in the environment. The increase of phosphorus content is a factor of biomass suppression. On the other hand, the same effect can be achieved by lowering of phosphates content in the environment.

Discussion

The regulation of algal community structure can be performed for different goals. In our case, the community composition was optimized for food requirements of pond consumers — phytoplankton-feeding fishes (white and motley silver carps) and herbivorous zooplankton. Besides increased N:P ratio, the experimental schedule of fertilization differed from the control one in increased nitrogen content and decreased content of phosphorus, in earlier (before stocking with fish) beginning of this manipulation as well as in more frequent fertilization.

The fact that availability of renewed environmental resources along with N:P ratio can be the controlling parameter for algal communities was demonstrated by experimental data of various

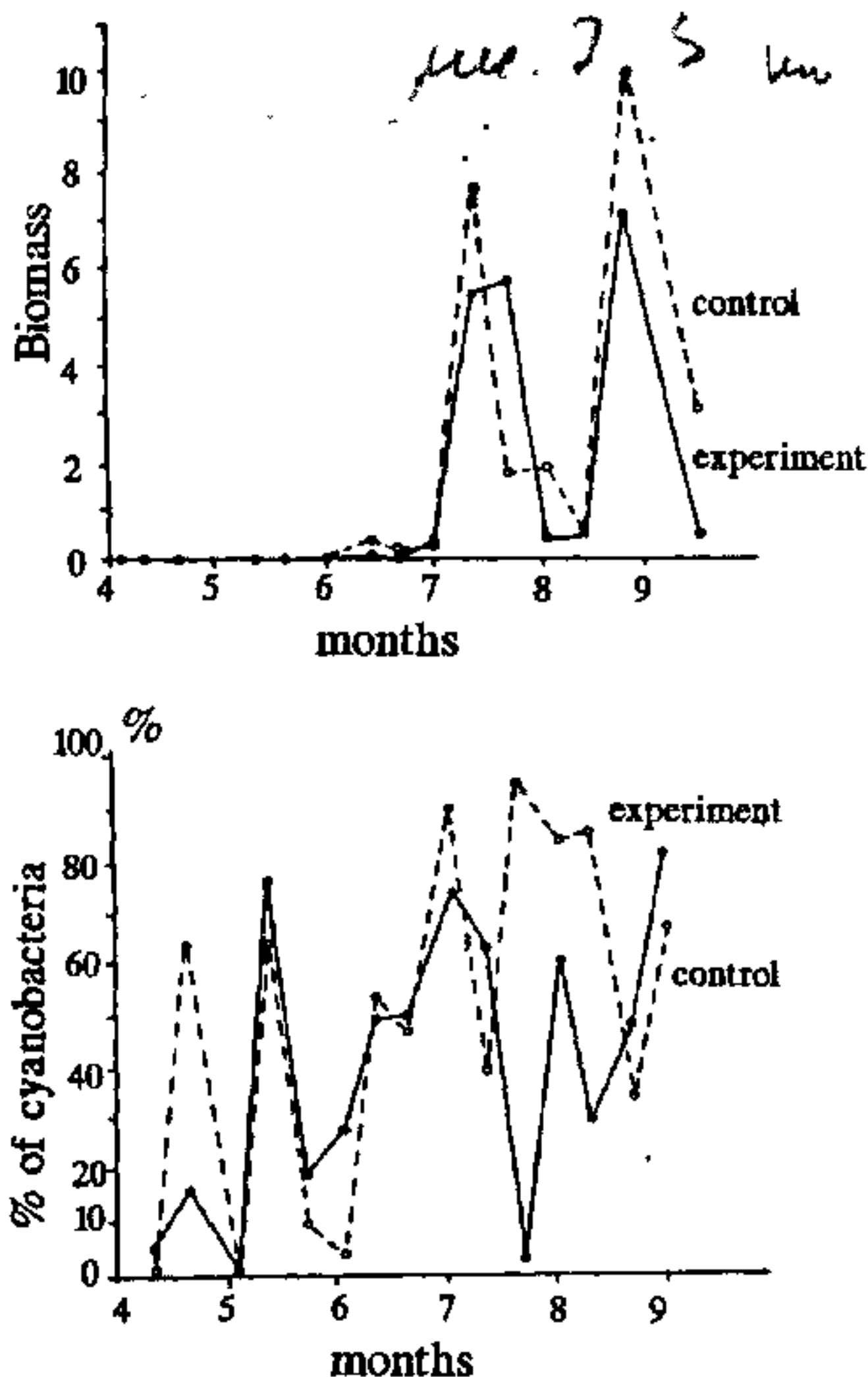


Figure 5. The dynamics of cyanobacteria biomass and percentage of cyanobacteria in the experiment and in the control in 1989. a) biomass in 1988, b) % in total biomass in 1989.

authors. The shorter the intervals between nutrient inputs are, the greater are the advantages of fast-growing species (Gaedeke and Sommer 1986) and species with high rate of nutrient intake in the period of several days. When inorganic fertilizers were introduced into ponds every two days, the biomass of Protococcales, Euglenophyta and Bacillariophyta increased in comparison with biomass of these divisions in ponds fertilized once in ten days (Bulgakov et al. 1988). At the same time, an increase in the cyanobacteria biomass has not been recorded.

Nevertheless, the data obtained in our experiments do not allow to decide whether there was an influence of more frequent fertilization on the phytoplankton community structure. For example, some elements of controlling the phytoplankton community structure such as an increase in Chlorophyta biomass and decrease in that of cyanobacteria were recorded in the experiment in 1987 despite the fact that the intervals between applying nutrients in the experimental and control ponds differed insignificantly (Table 1).

Table 3. Essential determinations for low biomass of green algae.

Explanable variable		Explanatory variable		Characteristics of determination, %	
term	value	term of variable	value	accuracy	completeness
Green algae biomass averaged over the season	low	Quantity of nitrogenous fertilizers applied in April-May	middle	78	70
		Quantity of nitrogenous fertilizers applied in August-Sept.	low	56	90
		Quantity of phosphate fertilizers applied in June-July and in a season as a whole	great	78	70
		N:P ratio in fertilizers in August-September	low	50	70
		Concentration of inorganic nitrogen in June-July	low	75	60
		Concentration of inorganic nitrogen averaged over a season	low	71	50
		Concentration of inorganic phosphorus in April-May	low	78	70
		Concentration of inorganic phosphorus in August-September	low	69	90
Green algae biomass averaged over August-September	low	Concentration of inorganic phosphorus averaged over a season	low	86	60
		Quantity of nitrogenous fertilizers applied in April-May	middle	56	62
		Quantity of phosphate fertilizers applied in June-July	great	67	75
		Concentration of inorganic nitrogen in April-May	low	64	88
		Concentration of inorganic nitrogen in June-July	low	62	62
		Concentration of inorganic nitrogen in August-September	low	67	50
		Concentration of inorganic phosphorus in April-May	low	56	62
Green algae biomass averaged over June-July	low	Concentration of inorganic phosphorus in August-September	low	54	88
		Quantity of phosphate fertilizers applied in June-July	great	75	54
		N:P ratio in fertilizers in April-May	low	75	69
		Concentration of inorganic phosphorus in April-May	low	78	54
		Concentration of inorganic phosphorus in June-July	middle	75	69

At the same time, later beginning of fertilization in the experimental pond in 1987 did not influence the community. Therefore, the earlier beginning of fertilization can not be considered as a factor controlling the algal community structure.

It might be assumed that, when algae compete for phosphorus and nitrogen separately, the absolute quantities of these elements should be responsible for the dominance of some taxa and elimination of others. However, the differences in doses of NH_4NO_3 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ between experimental and control ponds are observed for the whole season. At the same time, the change in community structure becomes apparent only from mid-season when high N:P ratios become important for the community. In 1989, when N:P ratio in fertilizers was 25:1 from the very beginning, the change in the community structure did not occur; this can be explained by delay of phytoplankton response to inorganic nutrient

input. It should also be mentioned that, when N:P weight ratio in fertilizers and concentration ratio of these elements in the water were lower from June to September in 1988 than in other years, the effect of increase in *Protococcales* biomass and decline of cyanobacteria biomass appeared to be less distinct (Table 2).

It follows from the above analysis that out of all studied factors the N:P ratio predominantly determines the species relationship in the community. It should be stressed that this result was obtained not under controlled laboratory conditions but in such a multifactoral ecosystem as the fish-breeding pond, where none of biotic and abiotic factors obscured the effect of high N:P ratio.

Various microalgal taxa differently response to N:P ratio change. The reason is that the requirements for mentioned elements do not coincide in various species and consequently the ratio between these requirements differ as well.

If the requirement ratio (measured by cell quotas in the particular species) is equal or close to the ratio between respective nutrients in the environment, such an environment can be considered as optimum for this species (Levich 1989, Levich et al. 1993). Requirement ratio is a species-specific value, and it is constant within individual stages of growth (Levich 1989).

There are some other evidences of correspondence between optimum concentrations for the cell growth and cell requirements. For example, the optimum N:P ratios for several fresh-water Chlorophyta and cyanobacteria species based on requirement ratios are given by Rhee and Gotham (1980).

The optimum N:P ratio for Chlorophyta species found by the direct calculation of requirements or by experimentation with addition of nutrients (Rhee and Gotham 1980, Levich et al. 1986) were higher than for other taxa reaching 20-30. At such high N:P ratios, for example, the genus *Sphaerocystis* (Chlorophyta) dominates in natural community (Reynolds 1980).

It can be seen that, in the experimental ponds, the biomass of the whole division Chlorophyta increases. Nevertheless, it should be conceived very clearly that among green algae there are species with requirement ratio other than 20-30. That is why one can observe the promotion of not all the Chlorophyta species, but only of those, whose requirements for nitrogen and phosphorus correspond to the ratios between these elements in the environment. Among Chlorophyta, *Scenedesmus quadricauda* is the species, for which the ratio of nitrogen to phosphorus requirement varies just in the range from 20 to 30 (Muller 1972, Dauta 1982, Levich and Artukhova 1991).

The "leading" cyanobacteria genera such as *Merismopedia* and *Phormidium* grow worse when the N:P ratio increases. In this case, the biomass of the whole taxon of cyanobacteria declines. Requirements for nitrogen and phosphorus are unknown for these species. It is known for other cyanobacteria genera that they have a low ratio of nitrogen to phosphorus requirements. For example, this ratio is only 9:1 for *Microcystis* (Rhee and Gotham 1980). For cyanobacteria inhabiting small lakes, this ratio on the average is 10:1 (Baranov 1968).

There is another explanation for stimulation of cyanobacteria growth by low N:P concentration ratio. Besides the correspondence between requirement ratio and nutrient concentration ratio in the water, the growth of cyanobacteria is positively affected by their ability for nitrogen fixation. Some heterocystous cyanobacteria (e.g.

Anabaena and *Aphanizomenon*) as well as non-heterocystous *Oscillatoria* are able to assimilate dissolved atmospheric nitrogen (Carpenter and Price 1976, Bryceson and Fay 1981, Bothe 1982). Under conditions of low N:P ratio in the water, the limitation by nitrogen arises that promotes the competitive success of these taxa. However, in the ponds investigated, the relative biomass of these genera was not high. An increase in the cyanobacteria biomass in the control was achieved by the development of genera *Merismopedia*, *Phormidium*, *Aphanothece*, and *Microcystis*, which do not have N-fixing property.

The failure to demonstrate the reproducible dependence of Bacillariophyta and Euglenophyta total biomass on the increase of N:P ratio can be presumably explained by great range of nutrient requirements specific for different species of these divisions. Hence it follows that it is impossible to create conditions for dominance of these divisions as a whole. It is only possible to stimulate the individual species or genera (in our case — *Melosira* sp.).

An increase in biomass of size classes 0.3-1 and 1-3.2 ng in the experimental ponds can be probably explained by taxonomic composition of algae at mentioned classes. For instance, promoted species *S. quadricauda* and *Melosira* sp. belong to these size classes. At the same time *Merismopedia* and *Phormidium* which have higher biomass in the control belong to the classes less than 0.1 ng and 3.2 - 10 ng.

In the experimental ponds, the results important for fish-breeding were achieved. On the average the total fish production here was 16 % higher and the production of herbivorous fish (white and motley silver carps) 19 % higher than in the control. The authors think that the increase in fish production can be explained by optimization of edible for fish phytoplankton through intentional nutrient loading on ponds.

The experiment showed that it is possible to control over the composition of both laboratory and natural algal communities. Selecting appropriate N:P ratio, one can regulate the development of certain species and size groups of phytoplankton.

Acknowledgments

The authors are grateful to the staff of the Laboratory of Pond Fish Breeding of CaspNIRKH for providing the data on hydrochemical analysis, to V.N. Maksimov for his valuable help in the work, as well as to V.G. Artukhova, N.V. Revkova, N.Z. Mazilova, D.G. Zamolodchikov for participation in fertilization of ponds and identification of phytoplankton samples.