*Hydrobiologia* **264**: 85–99, 1993.



# Bacterioplankton interactions with Daphnia and algae in experimental enclosures

Ružena Markošová & Josef Ježek [1]
*Department of Hydrobiology, Charles University, Viničná 7, CS-128 44 Prague 2, Czech Republic;*
[1] *Department of Applied Mathematics and Computer Science, Charles University, Albertov 6, CS-128 43 Prague 2, Czech Republic*

Received 19 May 1992; in revised form 10 December 1992; accepted 14 January 1993

*Key words:* bacterioplankton, morphometric analysis, Daphnia, algae, factor analysis, manipulated ecosystems

## Abstract

Development of bacterioplankton was studied by manipulation of planktivorous fish and/or nutrients in experimental enclosures in a fish pond. Grazing pressure exerted by large zooplankton (*Daphnia galeata* and *Daphnia pulicaria*) strongly influenced the counts and size distribution of bacterial populations. Morphometric analyses by scanning electron microscope revealed a shift in size distribution from larger mainly rod-type bacteria under low grazing pressure towards smaller mainly coccus-type under strong grazing pressure. The metabolic activity of bacteria measured as glucose uptake was higher under strong grazing pressure. After removal of large daphnids, the increase in bacterial density was probably the result of two additive factors: low grazing pressure and high level of dissolved organic matter (DOM) due to photosynthetic activity of more abundant algae. Composition of bacterial populations shifted toward larger, rod-type bacteria, and their metabolic efficiency measured by uptake, was lowered. The basic dimensionality of the system and interactions between variables was describe by R-mode factor analysis. The manipulated enclosures were relate with factor score.

## Introduction

Planktonic heterotrophic bacteria process more than half of the organic carbon produced in aquatic system. Because they are the only populations control the movement of the major part of organic matter and capable of significantly altering both DOM and POM, their recycling processes affect composition and trophic interactions in plankton systems (Overbeck *et al.*, 1990). The new concepts on the connections between microbial loop and classical food chain are presented by Porter *et al.* (1988).

Grazing activity by daphnids is an important factor influencing the size and quality of bacterial populations and has been well documented (Riemann, 1985; Lampert *et al.*, 1986; Pace *et al.*, 1990 and others). The filtering apparatus of many Cladocera is morphologically equipped to retain even small bacteria (Brendelberger, 1985; DeMott, 1985) and the fine filtering structures of *Daphnia galeata* are responsible for considerable

bacterial losses during clearwater phase (Geller & Müller, 1981; Lampert *et al.*, 1986). If large daphnids are reduced or eliminated by fish predation, protozoa quickly take over the control of bacterial populations (Riemann, 1985). The pelagic food webs are more complicated because bacteria can be grazed by protozoans and metazoans, including mixotrophic algae, heterotrophic flagellates, ciliates and zooplankton (Pace, 1988; McManus & Fuhrmann, 1988). The plankton system is very variable in time and its structure is a result of interactions between all components at each trophic level. The fate of bacterial production is dependent on consumers-daphnids and flagellates (and other loss factors, for example lysis by viruses). Direct predation on bacteria by daphnids can transferred a significant fraction of bacterial carbon up the food web.

On the other hand, bacterial populations are connected by positive interactions with phytoplankton. A close coupling between algae and bacteria has generally been found during the period of an algal bloom (Riemann *et al.*, 1982; Billen & Fontigny, 1987; Garnier & Benest, 1990; Overbeck *et al.*, 1990). Close interactions between bacteria and algae did not appear so clearly during the course of a seasonal cycle, probably because sources of dissolved organic matter are multiple and cannot be distinguished from each other (Pedrós-Alió & Brock, 1983; Simon & Tilzer, 1987). Phytoplankton excretion or algal decay may be an important source of dissolved organic matter (DOM): results have shown that extracellular organic matter originated from algae can sustain the major part (32–99%) of bacterial activity (Søndergaard *et al.*, 1985; Overbeck *et al.*, 1990).

Here, we report the effects of the fish stocks and concentrations of nutrients on natural plankton communities in experimental enclosures. Our purpose was to assess the fate of the bacterial component under different densities of *Daphnia* and algae in a eutrophic pond. The experiment was run for several months (June–October) and included the whole water column from the surface to the bottom to allow undisturbed movement of migrating plankters.

## Materials and methods

### *The study site*

Experiments were carried out during the period June–October 1985 in a shallow, eutrophic pond, Velký Pálenec, situated in southwest Bohemia 100 km from Prague, Czech Republic. Pond Velký Pálenec has been under limnological investigation over the last 40 years. Its basic limnological characteristics are given in Table 1.

*Table 1.* Limnological characteristics of the pond Velký Pálenec from last 20-years period May–October.

| Parameter | Number/Range |
|---|---|
| Surface area | 31.0 ha |
| Maximum depth | 3.3 m |
| Mean depth | 1.4 m |
| Water temperature | 10–24 °C |
| Oxygen | 0.1–15 mg $O_2$ $l^{-1}$ |
| pH | 7.5–10 |
| Transparency | 0.4–3.2 m |
| Phosphate $PO_4$-P (reactive) | 1–700 $\mu$g P $l^{-1}$ |
| Phosphate $PO_4$-P (non-reactive) | 10–60 $\mu$g P $l^{-1}$ |
| Total phosphorus (soluble + particulate) | 30–300 $\mu$g $l^{-1}$ |
| Nitrate nitrogen | Undetectable levels |
| Ammonia nitrogen ($NH_3$-N) | 1–1000 $\mu$g $l^{-1}$ |
| Chlorophyll-*a* | 1–179 $\mu$g $l^{-1}$ |
| Phytoplankton biomass | 0.130–22.942 $mm^3$ $l^{-1}$ |
| $BOD_1$ | 1.0–1.5 mg $O_2$ $l^{-1}$ |
| $BOD_5$ | 3.1–9.1 mg $O_2$ $l^{-1}$ |
| Bacterial direct counts | 1.27–5.24 × $10^6$ cells $ml^{-1}$ |
| Bacterial cell biovolume | 0.180–0.320 $\mu m^3$ |
| CFU-mesophilic bacteria (Beef-pepton Agar, 20 °C) | 12–3100 cells $ml^{-1}$ |
| CFU-coliform bacteria (Endo-Agar, 37 °C) | 0–200 cells $ml^{-1}$ |
| CFU-fecal streptococci (Slanetz-Bartley Agar, 37 °C) | 0–10 cells $ml^{-1}$ |
| Uptake of $^{14}$C-glucose | |
| $V_{max}$ | 0.50–4.20 $\mu$g glucose $l^{-1}$ $h^{-1}$ |
| $V_{max}$ specific | 0.16–2.23 $\mu$g glucose $h^{-1}$ for $10^9$ cells |
| Zooplankton numbers total | 0–568 ind $l^{-1}$ |
| numbers large daphnids (>850 $\mu$m size) | 0–261 ind $l^{-1}$ |
| Zooplankton dry biomass total | 0–5.1 g $l^{-1}$ |
| dry biomass large daphnids | 0–3.2 g $l^{-1}$ |
| Fish production (Cyprinus carpio) (1978–1984) | 600–900 kg $ha^{-1}$ $y^{-1}$ |

*Experimental design*

Enclosures were used to manipulate fish predation and concentration of nutrients. Four circular plastic enclosures (diameter 10 m, depth 1.5 m and approximate volume 110 $m^3$) were constructed from polyethylene sheet anchored at the bottom and floating on the surface through the collar filled by styrofoam. Walls were fastened to the poles to ensure a rigid circular shape. Natural bottom was a normal part of experimental enclosures.

The enclosures were manipulated in duplicates by additions of nutrients and fish. One enclosure was unmanipulated control, 2 contained fish *Cyprinus carpio* (600 small fish, 10 cm length), 2 enclosures contained nutrients (4 g $Pm^{-3}$). This arrangement enable to us to follow simple effect of one factor fish/nutrients or conjoint effect both factors. Fish and nutrients were added one month after the beginning of the experiment and the whole experimental period was four months (June–October) and covered completely summer period.

Changes in abiotic conditions, nutrients (phosphorus, nitrogen), phytoplankton, zooplankton, bacterioplankton and benthos were followed with sampling frequency not exceeding 14 days.

*Sampling*

Water temperature (measured by thermometer at 10 cm below surface), transparency (as Secchi depth), dissolved oxygen and pH (measured using Orion oxygen electrode) and major nutrients (N, P) were measured at 1–2-day intervals. Water samples were taken 10 cm below surface. Parameters of bacterioplankton (direct counts, glucose uptake, morphometric analysis of cells, $BOD_1$ and $BOD_5$) were assayed every fourteen days or less and were determining using standards methods as in the protocol of Markošová *et al.* (1990). Data on chlorophyll-*a*, phytoplankton biomass (by measuring biovolume) and zooplankton biomass (as dry weight) were obtained from Stuchliková (unpublished data), Prchalová (unpublished data) and Bytel (1986), respectively.

Sterile samples for bacteria were taken from 0.1 m depth into sterile 100 ml flasks with wire holders. Non-sterile samples and samples for other analyses were collected with 2-liter Friedinger bottle from 0.1 m depth. Bacteriological samples were transported within 20 minutes in cool boxes (+4 °C) to the field laboratory.

*Biochemical oxygen demand (BOD)*

Water samples were filtered through 40 $\mu$m size sieves and saturated bubbling by air for 1 hour at 20 °C. Two 250 ml bottles were filled with water and incubated for 1 and 5 days in dark at 20 °C. Concentration of oxygen was measured using an Orion oxygen electrode. Calculation and other details follow Markošová *et al.* (1990).

*Bacterial direct counts*

For direct microscopic counts, samples were fixed with 3% formaldehyde. 2–5 ml sample were filtered through a membrane filter with 0.1–0.3 $\mu$m pore size (Synpor 7, Synthesia, Czech Republic), stained with erythrosine, and dried and cleared with immersion oil. Two replicate filters were counted for each sample. The results are expressed as cells per ml (details in Markošová *et al.*, 1990).

*Glucose uptake*

Glucose uptake by bacteria was measured using the method of Hobbie & Wright (1965). The samples were filtered through 40 $\mu$m mesh size sieves to remove large organisms and particles. [U-$^{14}$C] glucose (UVVR, Czech Republic, sp. act. 0.1 mCi/mg) was added in ten concentrations (10–75 $\mu g l^{-1}$). Each concentration was prepared in triplicate. One bottle was fixed immediately with Lugol's solution (blank value); two others were incubated at the *in situ* temperature for 0.5–2 h depending on the season, fixed with Lugol's solution and filtered through membrane filters. After the filters were washed and dried, radioactivity was measured by liquid scintillation.

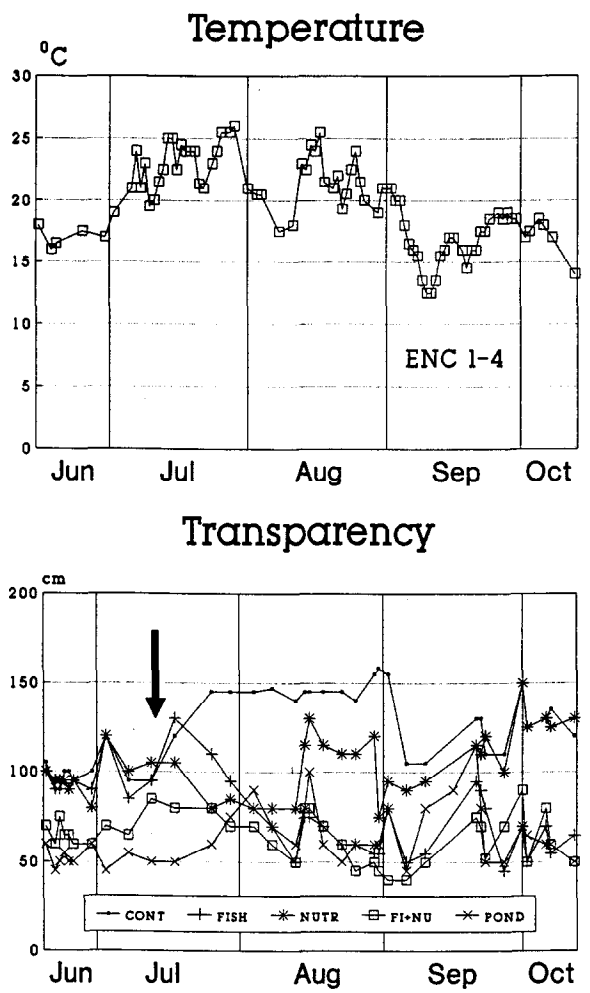



*Fig. 1.* Temperature (average values) and transparency for enclosure 1–4 and pond Velký Pálenec during experimental period June–October 1985. Arrow indicate onset of manipulation.

*Morphometric analyses on scanning electron microscope (SEM)*

Bacterial cell dimensions and biovolume were measured on SEM using microcomputer system (Krambeck *et al.*, 1981) which assists cell size measurements on images from scanning electron micrograph negatives They were projected on digitizer field, bacterial length and width were marked by a cursor. A special dialogue program organized and calculated biovolume of cells. The samples were analyzed at the MaxPlanck Institute of Limnology, Plön, Germany.

*Data analysis*

Data analysis was performed with the Statgraphics software version 4.2. The data have multivariate character and show strong time dependency. For the study of their inter-relations we started from the correlation matrix and further employed the method of factor analysis which creates a minimum number of new variables (factors) which are linear combinations of the original ones such that the new variables contain practically the same amount of information. For a description of factor analysis, see Afifi & Azen (1979) or Joreskog *et al.* (1976).

## Results

*Initial conditions in the system*

At the beginning of the experiment (13 June 1985) all enclosures contained the same initial plankton community.

Every enclosure was monitoring separately and the average data were; Temperature was 15 °C and transparency was 60–120 cm. Density of bacterioplankton was $2.8–3.2 \times 10^6$ cells $ml^{-1}$ and metabolic activity of bacteria (measured as glucose uptake) were in the range for $V_{max}$ 1.4–3.0 $\mu$g glucose $l^{-1}$ $h^{-1}$. Bacterial populations were comprised mostly rod-type cells. BOD value were 1.4 mg $O_2$ $l^{-1}$ (1 day) and 3.2 mg $O_2$ $l^{-1}$ (5 days). Chlorophyll-*a* concentration was 18 $\mu$g $l^{-1}$. The phytoplankton was dominated by *Oocystis marsonii* and phytoplankton biomass was 4.5–7.9 $mm^3$ $l^{-1}$. Zooplankton was composed of *Daphnia galeata* with density 135 ind $l^{-1}$ and biomass 2.37 g $l^{-1}$.

*Experimental conditions in the system*

Temperature in the enclosures tracked the values in surrounding pond (Fig. 1). During the experimental period temperature fluctuated in the range of 15–26 °C.

Oxygen concentration fluctuated over the range of 4.5–15 mg $O_2$ $l^{-1}$ with the exception of unma-

nipulated enclosure 1, where concentration of oxygen varied between 1–5 mg $O_2$ $l^{-1}$.

Transparency, measured by the Secchi disk reading (Fig. 1) ranged from 40–> 160 cm (bottom). The higher values developed in enclosure 1 (control) which had very high transparency from mid July through October.

Concentrations of nutrients ($PO_4$, $NH_4$ and $NO_3$) followed the seasonal pattern typical for the locality (Table 1).

*Biochemical oxygen demand (BOD)*

The lowest values of $BOD_1$-labile fraction of DOM (Fig. 2) were found in the control system (enclosure 1). It corresponded with a very low density of phytoplankton (Fig. 4). The 'background' system pond Velký Pálenec had the second lowest value but in the range of the values determined in the long-term observations (Table 1). The strongest effect of manipulated parameters on $BOD_1$ were in enclosure 2 and 4 (Fig. 2), in correlation with the highest concentration of chlorophyll-*a* (Fig. 4).

The control system (enclosure 1) and 'background' pond Velký Pálenec (Fig. 2) had $BOD_5$ values (more resistant fraction of DOM) in the range typical for the locality (Table 1). Adding nutrients (enclosure 3) had a positive effect on $BOD_5$: its concentration increased from 3 to 6 mg $O_2$ $l^{-1}$ (Fig. 2). This level was maintained till the end of the experiment. In the enclosure 2 and 4, the presence of the fish stock had the strongest effect on the concentration $BOD_5$; addition of nutrient to enclosure 4 had negligible effect. The removal of large daphnids was followed by a fast development of phytoplankton (Fig. 4) which probably enriched the system with extracellular organic matter from photosynthetic process.

*Bacterial density*

The density and composition of bacterial populations were dependent on the character of food web present in the enclosures.

In the first group (enclosures 1 and 3) low fish stock caused development of the population large daphnids, especially *Daphnia galeata* (Fig. 5). This kept bacterial assemblages on a low level ($1.4–2.5 \times 10^6$ cells $ml^{-1}$) without significant oscillations. Situation in the 'background' system pond Velký Pálenec was very similar to these enclosures. Bacterial direct counts were ($1.1–2.4 \times 10^6$ cells $ml^{-1}$) in the year-round range (Table 1).

In the second group (enclosures 2 and 4) high fish stock resulted in low density of *Daphnia galeata* (Fig. 5). Absence of large daphnids enabled development of phytoplankton up to biomass 13.8 $mm^3$ $l^{-1}$ (enclosure 2) and 8.2 $mm^3$ $l^{-1}$ (enclosure 4). Bacterial direct counts increased very

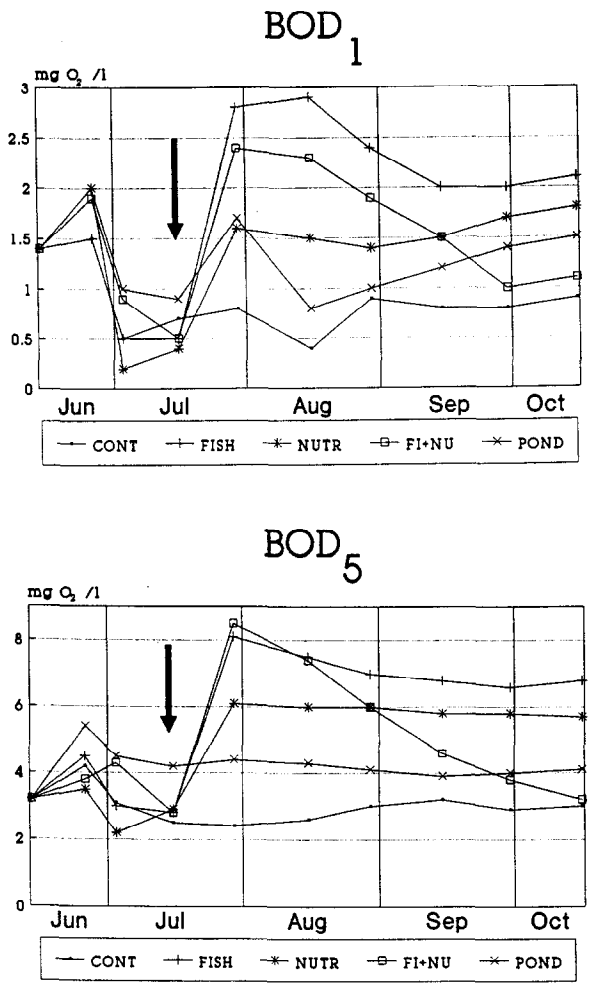
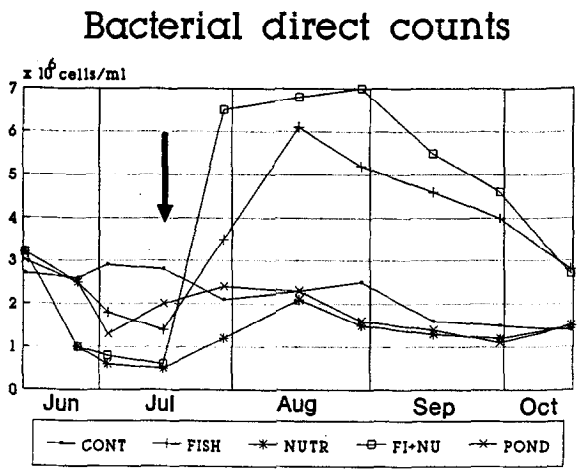
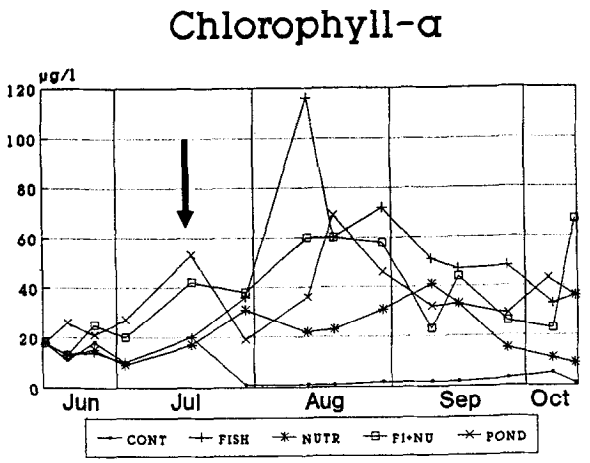
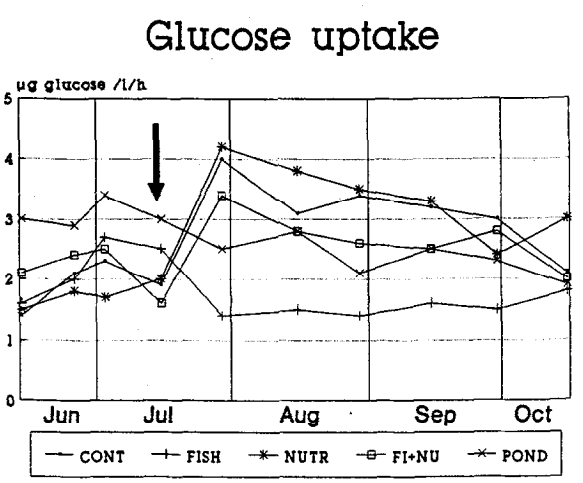



*Fig. 2.* Biochemical Oxygen Demand (BOD) measured as $BOD_1$ and $BOD_5$ for enclosure 1–4 and pond Velký Pálenec. Arrows indicate onset of manipulation.



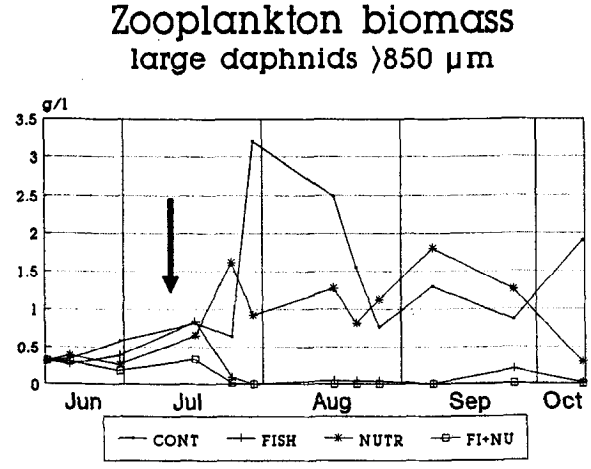




*Fig. 3.* Bacterial direct counts and $^{14}$C-glucose uptake expressed as $V_{max}$ for enclosure 1–4 and pond Velký Pálenec. Arrows indicate onset of manipulation.

*Fig. 4.* Chlorophyll-*a* concentration (Z. Stuchlíková data) in enclosures 1–4 and pond Velký Pálenec. Zooplankton biomass of large daphnids >850 µm (J. Bytel data) in enclosures 1–4. Arrows indicate onset of manipulation.

rapidly from 0.5–1.5 × $10^6$ cells $ml^{-1}$ to 6.1–7.0 × $10^6$ cells $ml^{-1}$ (Fig. 3).

*Glucose uptake*

In manipulated systems of enclosures 1 and 3 (low fish stock) the highest values of glucose uptake were observed (Fig. 3). Under high density of large daphnids (*Daphnia galeata*) glucose uptake increased from $V_{max}$ 1.37 to 4.2 µg glucose $l^{-1}$ $h^{-1}$.

A different development pattern was observed in the second group (enclosures 2 and 4, high fish stock) containing low density of daphnids (Fig. 3), where the lowest metabolic activity of glucose uptake (1.4 µg glucose $l^{-1}$ $h^{-1}$) was recorded. Weak grazing pressure by large daphnids and high density of phytoplankton (Fig. 4) in coincidence with high concentration of BOD (Fig. 2) resulted in bacterial populations (especially in enclosure 2) with low metabolic activity (1.4–1.8 µg glucose $l^{-1}$ $h^{-1}$).

*Morphometric analysis of bacterial cells*

Mean cell volume under conditions of high density of daphnids and low density of phytoplank-

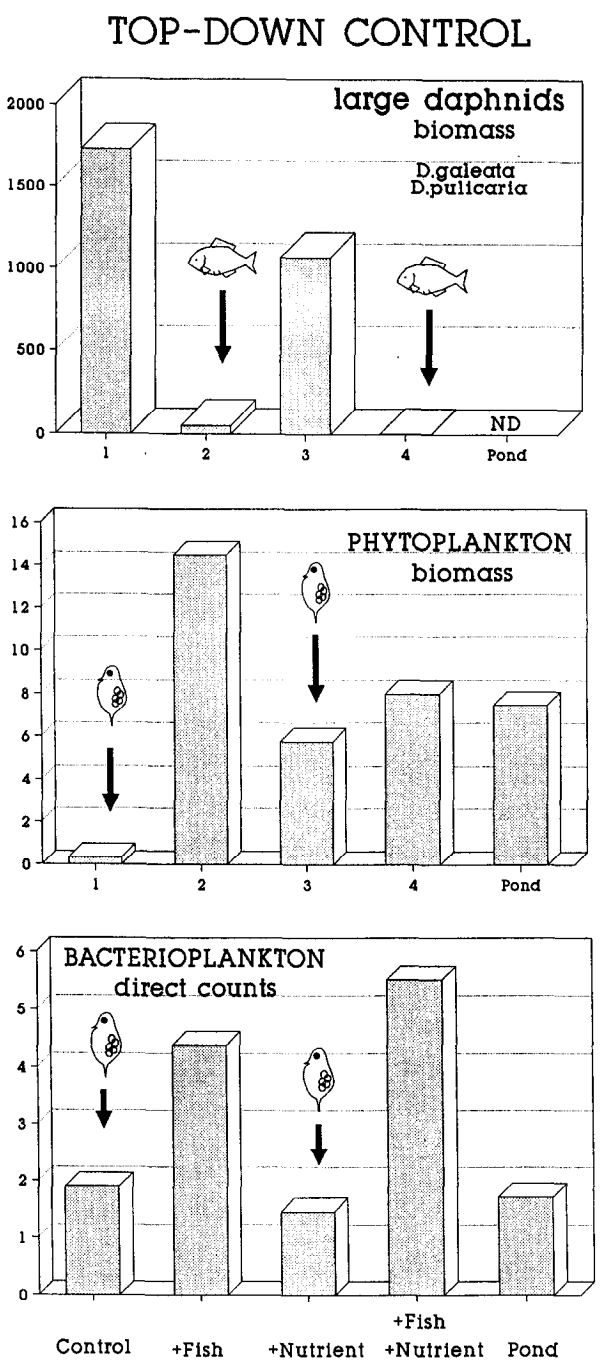



*Fig. 5.* Zooplankton biomass, phytoplankton biomass (measured as biovolume-M. Prchalová data) and bacterioplankton in the experimental systems during period 15 July–15 October 1985 (after manipulation). Bars represents average values for this period (15.7–15.10). (ND = no data). Legends on the bottom of figure indicate experimental treatments. (Zooplankton biomass in g $l^{-1}$ and phytoplankton biovolume in $mm^3$ $l^{-1}$ and bacteria in b $ml^{-1} \times 10^6$.)

ton (enclosures 1 and 3) had the lowest value from 0.10–0.15 $\mu m^3$ $cell^{-1}$ (Fig. 6). Mean bacterial cell volumes were lower in these enclosures than in the pond Velký Pálenec (up to 0.17 $\mu m^3$ $cell^{-1}$). In the enclosures 2 and 4, without large daphnids and with dense phytoplankton (Fig. 5), the mean cell volume increased to the value 0.25 $\mu m^3$ $cell^{-1}$ (Fig. 6).

In the enclosures 1 and 3 with strong grazing pressure by *Daphnia galeata* 81% (enclosure 1) and 67% (enclosure 3) of the bacterial population were represented by cocci. On the other hand, only 10% (enclosure 4) and 27% (enclosure 2) of bacterial population were present as coccus and most cells were in form of rods.

*Results of statistical analysis*

Using Statgraphics software we calculated correlation coefficients for bacterial direct counts (BAC), bacterial glucose uptake (BAG), large daphnids (DAPH), biovolume of phytoplankton (PHYTO), chlorophyll-*a* (CHLA), dissolved organic matter-labile ($BOD_1$) and dissolved organic matter-more resistant ($BOD_5$). We analyzed every enclosure separately and all enclosure together as one system.

Starting from the correlation matrix in Table 2 the factor analysis was performed. The results of the factor analysis were following: the first two principal components accounted for 95% of the variance examined and thus two factors were judged to be sufficient to form an adequate model of the problem. These factors were rotated by the varimax method. Table 3 shows rotated factor loadings as well as the communalities of the variables. The communalities give a part of the variance of a variable which is explained by the model.

Factor loadings give the correlation coefficients between factors and a variables. In a rather simplified form we can say that the first factor includes the variables BAC, PHYTO, CHLA, $BOD_1$, $BOD_5$ and the second factor is composed mainly from the variables DAPH and BAG. A graph of the variables in relation to factor axes is

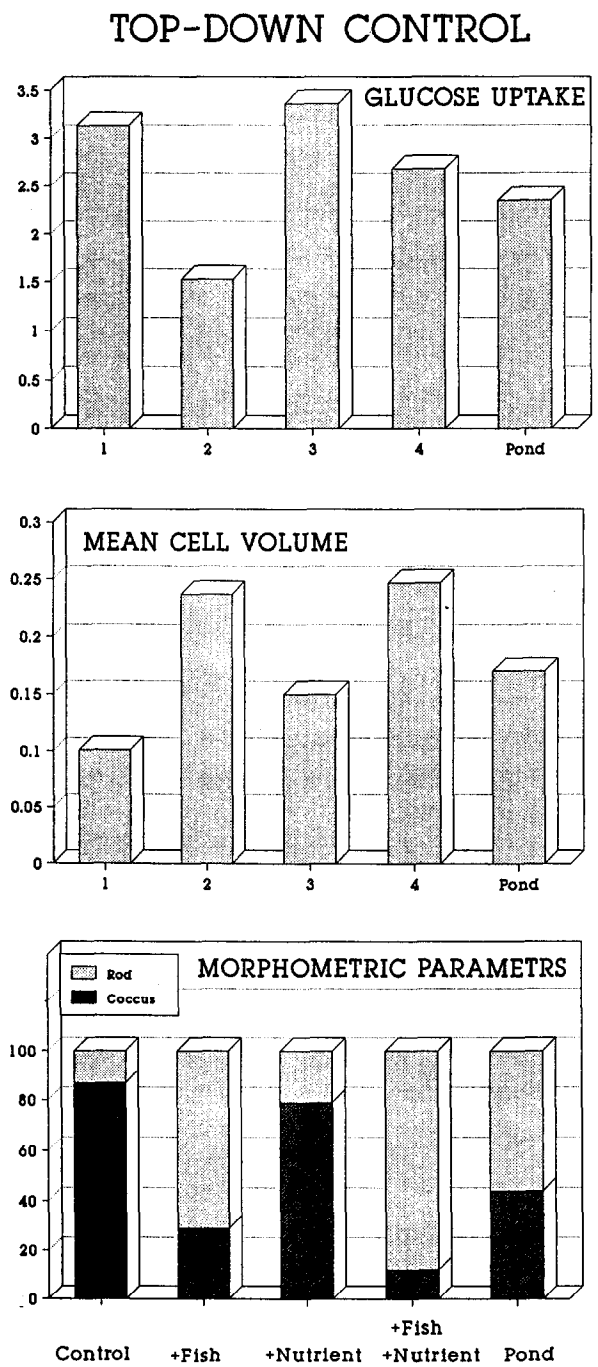



*Fig. 6.* Manipulation effect on bacterial parameters; metabolic activity as $^{14}$C-glucose uptake and morphometric parameters (mean cell volume and shape diversification) in the experimental systems during period 15 July–15 October 1985 (after manipulation). Bars represents average values of this period (15.7–15.10). Legends on the bottom of figure indicate experimental treatments. (Glucose uptake in $\mu$g glucose $l^{-1}$ $h^{-1}$ and bacterial cell volume in $\mu m^3$.)

a convenient way of illustrating the relationships between variables. Figure 9 shows such a plot for the rotated factor loadings.

The factor scores provides the information about the time distribution of the factor values. The plots of the factor scores corresponding to the first or the second factor are in Figs 7 and 8, respectively.

## Discussion

### *Experimental system with high densities of* Daphnia

Unmanipulated experimental system (enclosure 1) by excluding fish resulted in an extended clearwater phase. A period of clearwater was very probably caused by mass developments of *Daphnia* and is common in many meso- and eutrophic lakes (Lampert, 1985; Sommer *et al.*, 1986; Lampert *et al.*, 1986). In this system a clearwater phase was observed from the beginning of August until the end of October (Fig. 1). Absence of fish predation allowed development of high densities of large daphnids (up to densities 196 ind $l^{-1}$ and biomass 3.5 g $l^{-1}$, Fig. 4) and consequently a drastic reduction of phytoplankton (Fig. 5). This situation is typical in low-stock fish ponds (Fott *et al.*, 1980). Spring zooplankton peaks coincident with the period of clearwater as a direct consequence of grazing were also observed in Lake Schösee (Sommer *et al.*, 1986; Lampert *et al.*, 1986). Concentration of dissolved organic matter ($BOD_1$) in enclosure 1, (Fig. 2) was very low in coincidence with the low concentration of chlorophyll-*a* (Fig. 4) and bacterial density was low too. Perhaps *Daphnia* strongly reduced bacteria both; by direct consumption and by strongly depressing primary productivity, the major source of dissolved substrates. Both of these mechanismus are probably important in our experimental systems (Fig. 3). Some studies have shown that *Daphnia* and Metazoa in general, are relatively unimportant as bacterial consumers (Pace, 1988; McManus & Fuhrmann, 1988; Sanders *et al.*, 1989) or that *Daphnia* feeds on bacteria with a lower efficiency than it does on algae (Güde, 1988)

*Table 2.* Correlation matrix for the variables corresponding to enclosures 1–4 together as one system. BAC-bacterial direct counts, BAG-bacterial glucose uptake, DAPH-large daphnids, PHYTO-biovolume of phytoplankton, CHLA-chlorophyll-*a*, $BOD_1$-dissolved organic matter-labile, $BOD_5$-dissolved organic mater-more resistant.

| | Sample correlations | | | | | |
|---|---|---|---|---|---|---|
| | BAC | BAG | DAPH | PHYTO | CHLA | $BOD_1$ |
| BAC | | −0.142 | −0.433 | 0.484 | 0.660 | 0.594 |
| | | (40) | (40) | (40) | (40) | (40) |
| | | 0.383 | 0.005 | 0.002 | 0.000 | 0.000 |
| BAG | −0.142 | | 0.567 | −0.442 | −0.299 | −0.194 |
| | (40) | | (40) | (40) | (40) | (40) |
| | 0.383 | | 0.000 | 0.004 | 0.061 | 0.229 |
| DAPH | −0.433 | 0.567 | | −0.567 | −0.545 | −0.460 |
| | (40) | (40) | | (40) | (40) | (40) |
| | 0.005 | 0.000 | | 0.000 | 0.000 | 0.003 |
| PHYTO | 0.484 | −0.442 | −0.576 | | 0.763 | 0.632 |
| | (40) | (40) | (40) | | (40) | (40) |
| | 0.001 | 0.004 | 0.000 | | 0.000 | 0.000 |
| CHLA | 0.660 | −0.299 | −0.545 | 0.763 | | 0.654 |
| | (40) | (40) | (40) | (40) | | (40) |
| | 0.000 | 0.061 | 0.000 | 0.000 | | 0.00 |
| $BOD_1$ | 0.594 | −0.194 | −0.460 | 0.632 | 0.654 | |
| | (40) | (40) | (40) | (40) | (40) | |
| | 0.000 | 0.229 | 0.003 | 0.000 | 0.000 | |
| $BOD_5$ | 0.567 | 0.029 | −0.373 | 0.634 | 0.657 | 0.865 |
| | (40) | (40) | (40) | (40) | (40) | (40) |
| | 0.000 | 0.858 | 0.18 | 0.000 | 0.000 | 0.000 |

Coefficient (sample size) significance level.

*Table 3.* Varimax rotated factor loadings and estimated communality of variables. BAC-bacterial direct counts, BAG-bacterial glucose uptake, DAPH-large daphnids, PHYTO-biovolume of phytoplankton, CHLA-chlorophyll-*a*, $BOD_1$-dissolved organic matter-labile, $BOD_5$-dissolved organic matter-more resistant.

| Variable | Factor loadings | | Estimated communality |
|---|---|---|---|
| | Factor 1 | Factor 2 | |
| BAC | 0.653 | −0.226 | 0.478 |
| BAG | −0.004 | 0.779 | 0.607 |
| DAPH | −0.384 | 0.650 | 0.570 |
| PHYTO | 0.645 | −0.543 | 0.711 |
| CHLA | 0.735 | −0.423 | 0.720 |
| $BOD_1$ | 0.867 | −0.185 | 0.786 |
| $BOD_5$ | 0.935 | 0.008 | 0.874 |

and bacteria alone are a poor food for *Daphnia* (Pace *et al.*, 1983). Our data show that *Daphnia galeata* and *Daphnia pulicaria* grazed effectively on bacteria and strongly reduced bacterial populations (Fig. 5); correlation coefficient between large daphnids biomass and bacterial direct counts (for all enclosure together) was -0.433 (Table 2).

Grazing pressure by daphnids resulted in increasing metabolic activity of bacteria (Fig. 6): correlation coefficient between large daphnids and glucose uptake (for enclosures 1 and 3 with low fish predation) was +0.675. Zooplankton-induced stimulation of bacterial activity was described also by Riemann *et al.* (1986) and Simon & Tilzer (1987). Under strong grazing press by large daphnids, filamentous and rod-type bacte-

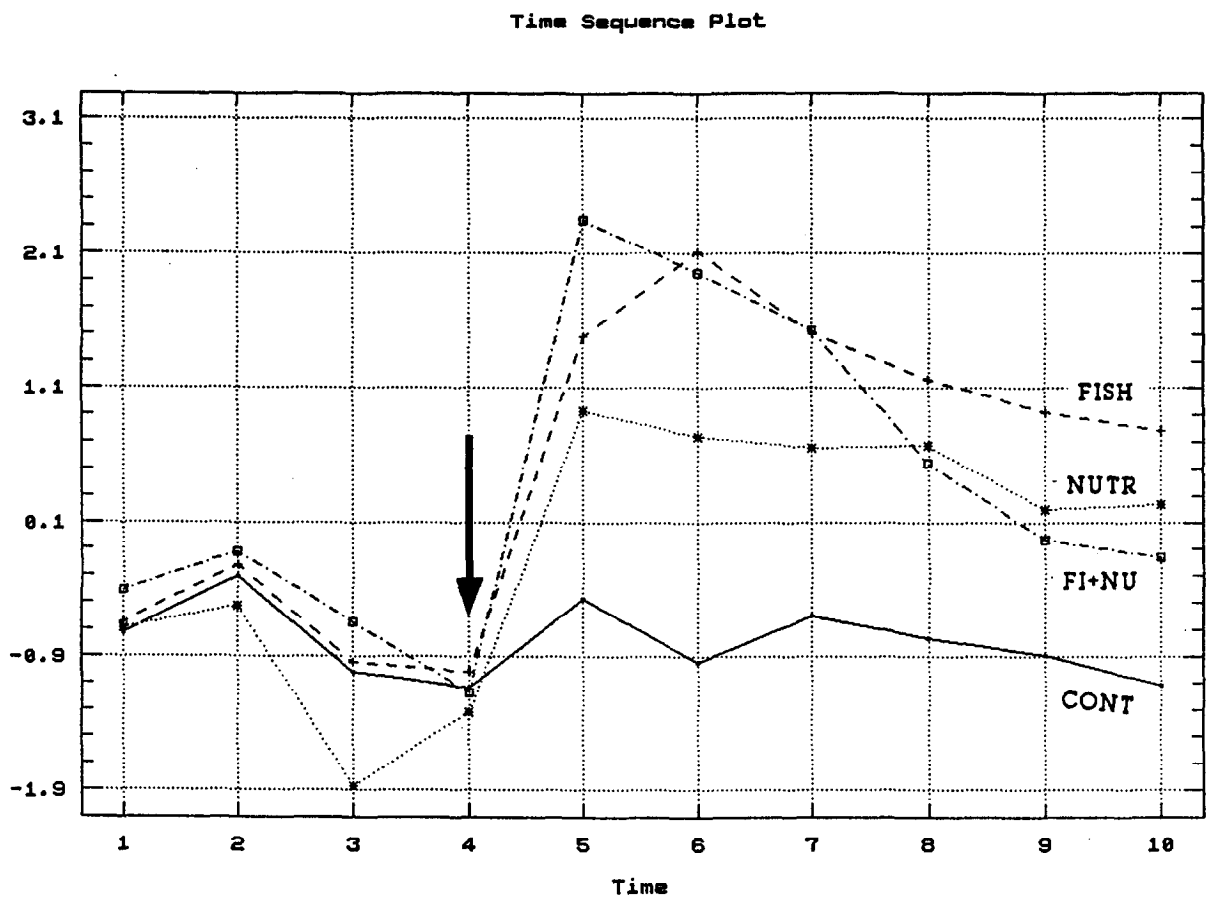



*Fig. 7.* Development of the first factor score in time (June–October) for experimental systems (enclosure 1–4). Arrow indicate onset of manipulation.

ria were preferentially removed with a concomitant decrease of mean bacterial cell volume to the lowest value 0.10 $\mu m^3$ (Fig. 6); bacterial population was composed predominantly from the cocci (up to 80% population, Fig. 6). Similar shift in bacterial morphology structure was observed by Simon (1987), Güde (1979) and Güde (1988); from inhomogenous composition towards well distributed small cells of the coccus-type.

The situation in enclosure 3 was similar to that in enclosure 1 with exception that adding nutrients and lower density of daphnids allowed better development of algae (Fig. 5). In experimental or natural systems, however, where dense population of daphnids develop, they can consume a substantial portion of the bacterial production as noted during seasonal maximum of *Daphnia* in several lakes (Pedrós-Alió & Brock, 1983; Lampert *et al.*, 1986) and in enclosure experiment with high density of *Daphnia* (Riemann, 1985). High densities of *D. longispina* (5–326 ind $l^{-1}$) could graze 3–48% of bacterial biomass daily (Kankaala, 1988). Grazing pressure by daphnids can resulted in modeling time-structure of bacterial production.

### *Experimental systems with low densities of* Daphnia

In the system with high fish predation (enclosures 2 and 4) the zooplankton density drastically decreased (Fig. 4) and its biomass approached nearly zero (Fig. 5). Removing large daphnids

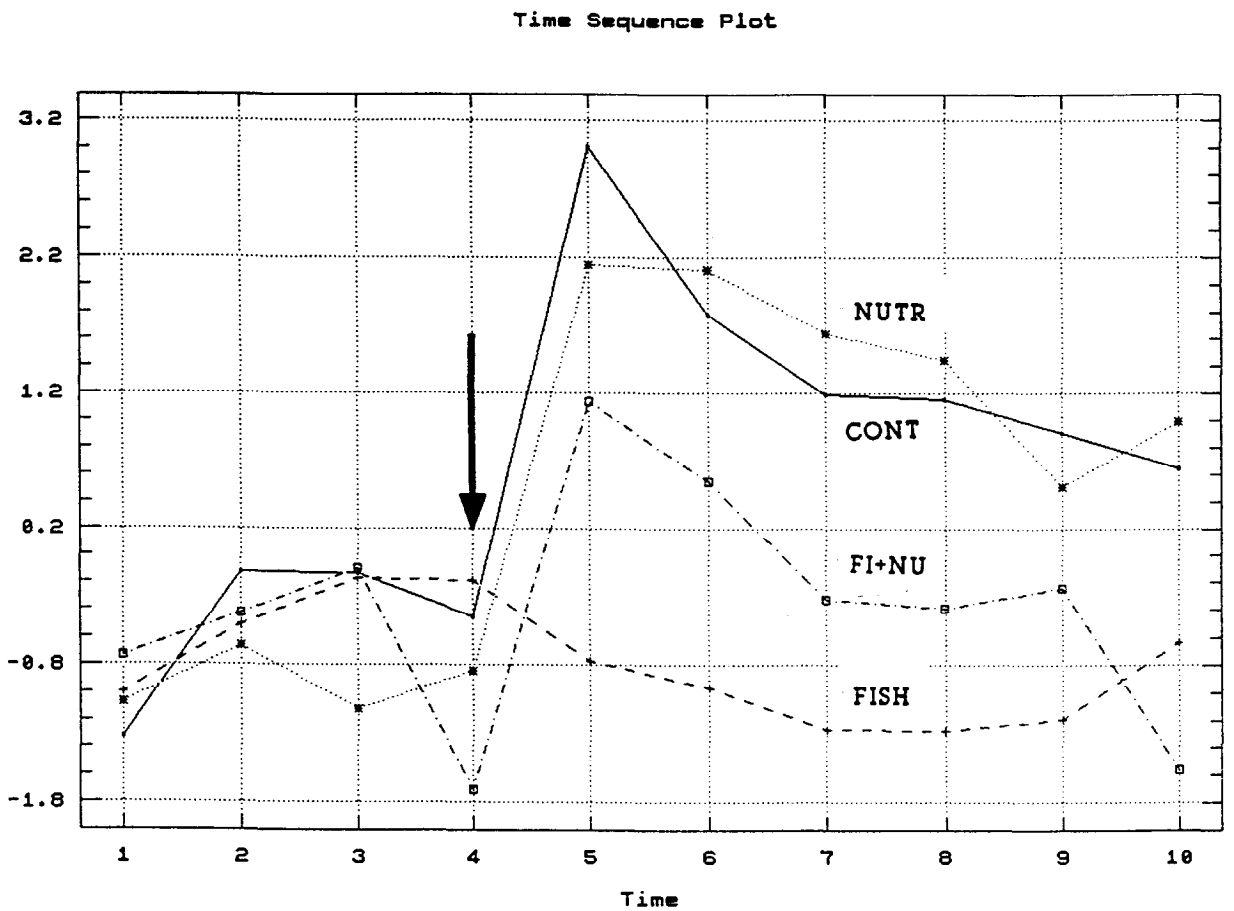



*Fig. 8.* Development of the second factor score in time (June–October) for experimental systems (enclosure 1–4). Arrow indicate onset of manipulation.

(*Daphnia galeata* and *Daphnia pulicaria*) allowed undisturbed development of phytoplankton with chlorophyll-*a* values as high as to 115 $\mu g\ l^{-1}$. Phytoplankton was identified as important contributor to the concentration of DOM pool in eutrophic ecosystems (Garnier & Benest, 1990, Markošová *et al.*, 1990); this paper contributes further evidence to support this idea (Fig. 2). Highest values of BOD were observed in the enclosures 2 and 4. The coefficient of correlation between chlorophyll-*a* and $BOD_5$ for all enclosure systems was +0.657 (Table 2). Absence of large daphnids in these systems resulted in fast increasing density of bacteria (Fig. 3). The experimental design with low density of *Daphnia* offered excellent conditions for development of bacteria (Fig. 3) supporting them by substrates and protecting them from grazing pressure of large daphnids (Fig. 4). Prokaryotic cells were probably well supported by sufficient concentration of DOM in the surroundings (coefficient of correlation for direct counts and $BOD_1$ was +0.828), therefore their cellular metabolism switched to lower efficiency, activity was 1.4–1.8 $\mu g$ glucose $l^{-1}\ h^{-1}$ and coefficient of correlation for BAG and $BOD_1$ was −0.883. Mean cell volume reached the highest values up to 0.250 $\mu m^3$ and bacterial populations were composed predominantly of rods (Fig. 6). Similar, Pedrós-Alió & Brock (1983) suggested that bacteria tended to be longer in eutrophic conditions.

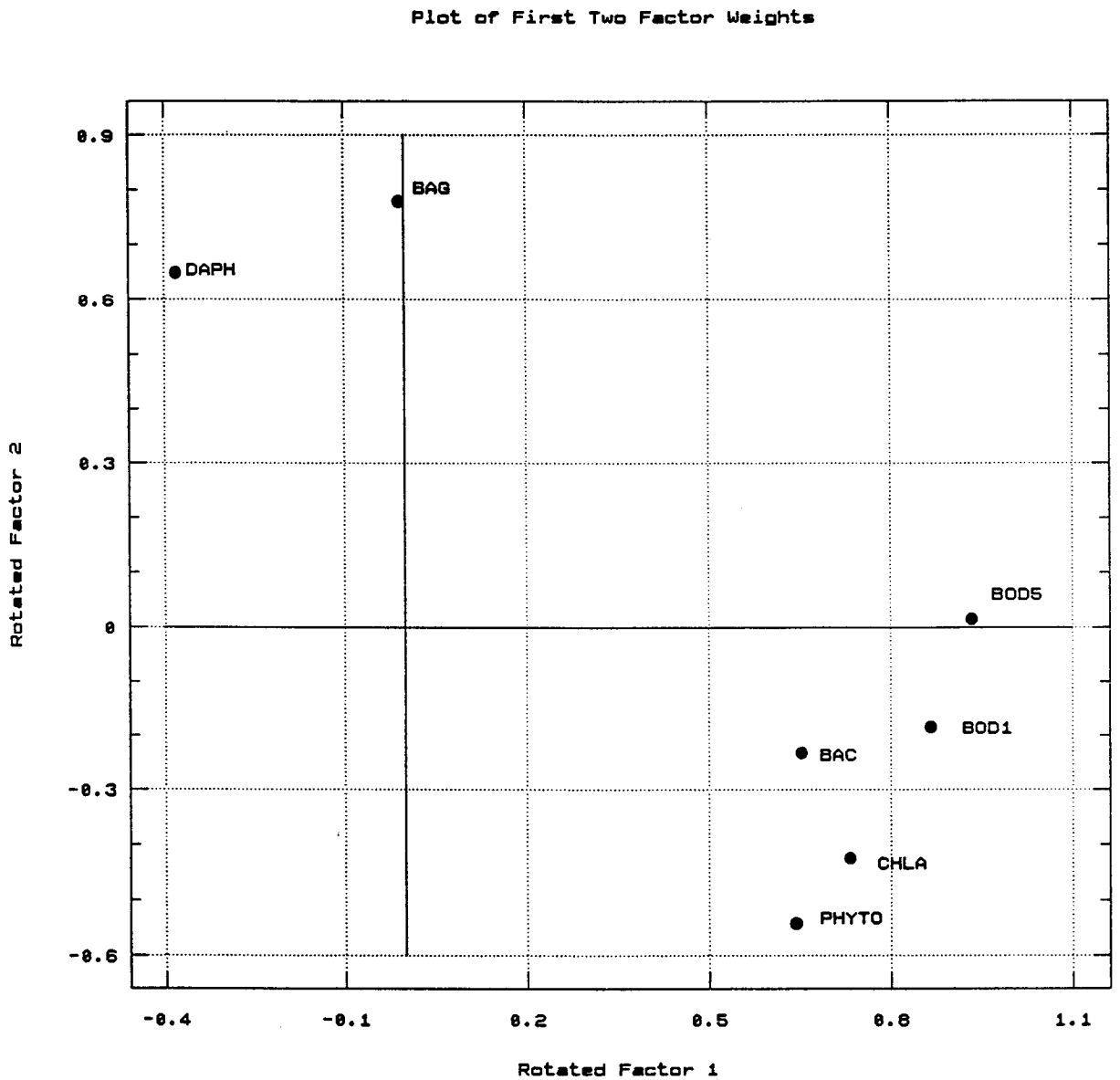



*Fig. 9.* First two factor weights corresponding to the original variables; (DAPH–large daphnids, BAG-bacterial glucose uptake, BAC-bacterial direct counts, $BOD_1$-dissolved organic matter-labile, $BOD_5$-dissolved organic matter-more resistant, PHYTO-biovolume of phytoplankton, CHLA-chlorophyll-*a*).

## Conclusions

As we can conclude from the experimental systems microbial food webs may differ between systems with and without abundant populations of large *Daphnia*, which is in a good agreement with results of Stockner & Porter (1988). *Daphnia*, because of their broad feeding abilities, are able to 'break' the microbial loop and directly consume both algae and a broad spectrum of heterotrophic microbes (Porter *et al.*, 1979). Therefore the flow of carbon to higher trophic levels will be greater in systems with *Daphnia*, and lower in the systems without *Daphnia* (Pace *et al.*, 1990). The density of large daphnids controls the movement and direction of energy in the systems and influences the

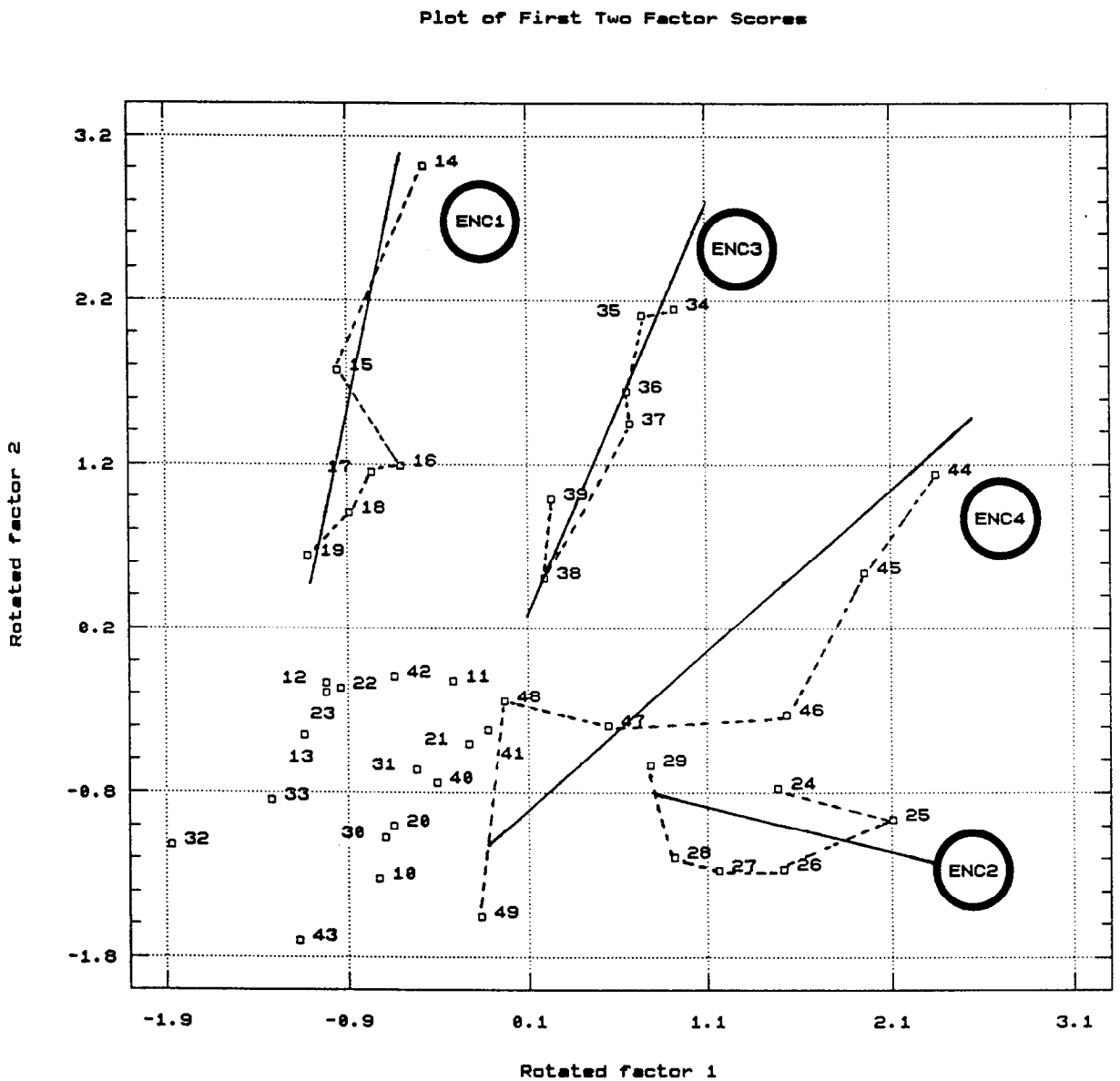



*Fig. 10.* Development pattern given by means of first two factor scores for experimental systems (enclosure 1–4):
Enclosure 1 – unmanipulated system
Enclosure 2 – manipulated system with fish
Enclosure 3 – manipulated system with nutrients
Enclosure 4 – manipulated system with fish and nutrients.

manner in what bacterial and algal carbon enter the food web. Biological interaction between zoo-phyto- and bacteria components seem to be important in creating the structure of food webs in plankton ecosystems.

*Simplified mathematical model of the experimental systems*

The results of the factor analysis enable a simplified description of the study systems. We concluded that the basic dimensionality of the prob-

lem can be seen as two and only two new variables-factor and can be used for a good description of the model. As a complement of Table 3, Fig. 9 shows the relationships between variables and the two factors. The biological meaning of the first and second factor is related to the two processes; phytoplankton control and *Daphnia* control. First factor (F-1) is composed from PHYTO, CHLA, $BOD_1$, $BOD_5$ and BAC. Parameters $BOD_1$ and $BOD_5$ have very similar correlation structure, what is in good agreement with their biological interpretation. BAC and CHLA have near their correlation structure in agreement with the ecological interpretation; algae produce extracellular DOM and bacteria take up and grow. Second factor (F-2) is composed of two parameters with similar factoral weight and correlation structure DAPH and BAG. Ecological implication is that grazing pressure by daphnids has positive effect on metabolic activity of bacteria.

The plots of the factor scores of the two factors in question (Figs 7, 8) convey the same amount of information as the seven corresponding time plots of the seven original variables BAC, BAG... Analyzing Figs 7 and 8 we can conclude, that the pattern of the first factor score from ENC 2 is close to that from ENC 4 and partly from ENC 3. In the case of second factor score ENC 1 and ENC 3 had very similar pattern.

In Fig. 10 the scores of the first versus second factor are plotted. For the simplicity the points are numbered so that the first cipher (1, 2, 3, 4) represents the number of enclosure, and the second cipher (0, 1...9) corresponds to the rank in time. The point related to the times after manipulation (second cipher from 4 to 9) are connected by dashed line. The full lines show the simplified character of the tendency of the separate enclosure to the return to the original state of ecosystem equilibrium. In this manner interesting classification schema emerges. Note that the situation in the enclosure 1 can be characterized as changing the second factor as well as the changes of the first factor determine the enclosure 2. The enclosures 3 and 4 lie between the two above mentioned.

In respect to the results we can conclude that the factor analysis should be an effective tool for biological data analysis.

**Acknowledgements**

We thank Z. Stuchliková, M. Prchalová and J. Bytel for providing data on chlorophyll-*a*, phytoplankton biomass and zooplankton. Professor W. Lampert kindly read the first version and offer to us helpful comments and suggestions. Part of this study (SEM analyses) was supported by Max-Planck Society postdoctoral grant to R. Markošová during her stay at the Department of Microbial Ecology, Max-Planck Institute of Limnology, Plön, Germany. The authors wish to thanks J. Hrabě for discussion concerning data processing.

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