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Nutrient enrichment and zooplankton effects on the phytoplankton community in microcosms from El Andino reservoir (Venezuela)

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Abstract

To quantify the effects of nutrient enrichment (N and P) and zooplankton grazing on the phytoplankton community structure of El Andino reservoir (Venezuela), *in situ* microcosms were installed for 6–7 days. Microcosms consisted of polyethylene bags (42 cm × 71 cm, non-cylindrical shaped) filled with 10 l of filtered epilimnetic water. Experiments were carried out on a monthly basis from January to December 1993. The lack/addition of nutrients was cross-classified with the absence/presence of zooplankton, resulting in an experimental design of four treatment levels: (1) no nutrient addition, zooplankton absent (**C**); (2) nutrient addition (150 NH_4Cl μmol ml^{-1} and 10 KH_2PO_4 μmol ml^{-1}; 1 ml per l of sample), zooplankton absent (**N**); (3) no nutrient addition, zooplankton present (collected from the reservoir water column using a 6-m vertical tow with a 80-μm plankton net) (**Z**); and (4) nutrient addition (as in [2]), zooplankton present (as in [3]) (**NZ**). Treatments were triplicated, and samples were collected at the start and end of each experiment. Significant differences between treatments were determined using a two-way ANOVA at $p<0.05$. Nutrient enrichment caused an increase in phytoplankton biomass, with the increase of all algal groups, except Pyrrhophyta. In spite of this, relative proportions of Cyanobacteria decreased in most cases. Chlorophyta and Bacillariophyta increased, probably due to their greater competitive abilities for phosphorus. After enrichment, *Scenedesmus* was the dominant species from January to June, while from July to December, *Dactylococcopsis* and *Lyngbya* dominated in the enriched microcosms. Zooplankton affected the phytoplankton community in microcoms through grazing and nutrient (mainly P) regeneration. Cladocerans (*Ceriodaphnia cornuta, Moina micrura* and *Diaphanosoma* sp.) mainly grazed on diatoms, although particulate material was present in almost all the gut contents analyzed. Particulate material probably consisted of micro-algae, detritus, bacteria, triturated algae and mineral particles. Ostracoda mainly fed on *Peridinium* and particulate material, whereas *Thermocyclops* sp. and rotifers (*Brachionus* spp. and *Keratella* spp.) mainly ingested particulate material. On the other hand, zooplankton excretion caused a slight increase in phytoplankton biomass and P concentrations in microcosms with the animals present. The effects of nutrient and zooplankton did not interact in most cases. Experimental results suggest that, at the initial stages of a eutrophication process, phytoplankton could increase their abundance and biomass, but might not change its community structure. Since there was a strong correlation between phosphorus and chlorophyll-*a* (bottom-up control), it is suggested that eutrophication could be avoided by controlling P input to the reservoir.

Introduction

Many experiments have been carried out to assess effects of nutrient enrichment and zooplankton grazing on phytoplankton communities (González & Ortaz, 1998). Many of them use phytoplankton populations isolated in microcosms (e.g. Henry & Tundisi, 1982; Henry et al., 1985; Bergquist & Carpenter, 1986; Tundisi & Henry, 1986; Elser & Goldman, 1991; Elser, 1992; Oliveira, 1992; Queimaliños & Mondenutti,

1993; Grover et al., 1994; Dos Santos & Calijuri, 1997; González & Ortaz, 1998).

Nutrient enrichment causes a rapid increase in chlorophyll-*a* and phytoplankton cell number (Edmondson, 1957; De Costa et al., 1983; Vanni, 1987; Pollingher et al., 1988; Elser & Goldman, 1991; Pérez-Martínez & Cruz-Pizarro, 1993; Yasuno et al., 1993; Mazumder, 1994b; González & Ortaz, 1998). Fertilization tends to enhance the growth of specific algae (Yasuno et al., 1993), depending particularly on N:P ratios, and on the frequency and intensity of nutrient pulses (e.g. Stockner, 1981; Stockner & Shortreed, 1985; Neill, 1988). Nutrient supply often increases net phytoplankton, including Cyanobacteria (Yasuno et al., 1993), over nano-phytoplankton. This might result in the blockade of nutrients flow to higher trophic levels.

While nutrient enrichment can cause a rapid increase in phytoplankton biomass, herbivorous zooplankton have two contrasting effects on phytoplankton (Porter, 1977; Carpenter et al., 1985; Bergquist & Carpenter, 1986; Elser & Goldman, 1991): directly via grazing and indirectly via nutrient regeneration. Yasuno et al. (1993) and Köthe et al. (1997) stated that zooplankton grazing cannot be ignored, because it can control the dynamics of edible autotrophic biomass, therefore influencing the primary production. Zooplankton grazing may also have a positive effect on phytoplankton, because it can stimulate the growth of non-consumed algae (Bergquist & Carpenter, 1986). In this research, zooplankton grazing was considered by analysis of gut content of the specimens; nutrient regeneration was not considered here.

In Venezuela, there is little information regarding the effects of nutrient enrichment in water bodies (González & Ortaz, 1998). Besides, studies on the diet of zooplankton in South American water bodies are scarce (Infante, 1978a; Cisneros et al., 1991; González, 1998), particularly those using the microcosm approach. Therefore, the aims of this study were to experimentally assess the effects of artificial nutrient enrichment and zooplankton presence on the phytoplankton community in microcosms from El Andino reservoir. Experiments were intended to mimic the eutrophication and biomanipulation (zooplankton exclusion) processes, respectively. Then, the two main goals of this paper are: (1) quantify the main and combined effects of nutrient addition and zooplankton presence on the phytoplankton community and nutrient characteristics of a tropical reservoir, and (2) analyze the grazing preference (diet) of various herbivorous zooplankters, both along a 1-year cycle.

Study site

El Andino reservoir is located on the eastern part of Venezuela (9° 32′ N, 65° 09′ W), and was constructed for irrigation and flood control purposes (Ginez & Olivo, 1984). The main reservoir features are: catchment area 35 km^2, surface area 1.8 km^2, volume 1.4×10^{-2} km^3, mean depth 6.8 m, with a retention time of 167 days. The reservoir can be classified as warm monomictic, with vertical mixing between February and May (Infante et al., 1995). The reservoir remains stratified the rest of the year. Wind velocity drives vertical mixing. The reservoir was classified as oligo-mesotrophic using the Salas & Martinó (1991) index (Infante et al., 1995).

Materials and methods

In situ microcosms were isolated for 6–7 days near the dam in El Andino reservoir. Microcosms consisted of polyethylene bags (42 cm diameter and 71 cm depth, non-cylindrical shaped) filled with 10 l of epilimnetic reservoir water (filtered through a mesh size of 80-μm), excluding zooplankton organisms that could interfere with the experimental design. Experiments were performed in triplicate each month (January–December, 1993). Nutrient lack/addition was cross-classified with zooplankton absence/presence. It resulted in an experimental design of four treatment levels: 1. no nutrient addition, zooplankton absent (**C**); 2. nutrients addition (150 NH_4Cl μmol ml^{-1} and 10 KH_2PO_4 μmol ml^{-1}; 1 ml per l of sample), zooplankton absent (**N**); 3. no nutrient addition, zooplankton present (collected from the reservoir water column using a 6-m tow with a 80-μm mesh plankton net) (**Z**); and 4. nutrient addition (as in [2]), and zooplankton present (as in [3]) (**NZ**). Plastic bags were washed with 10% HCl, tap water and reservoir water before experiments, to eliminate impurities from the polymerization process. Enrichment conditions were taken from Elser & Goldman (1991).

Total nitrogen (TN) and total phosphorus (TP) (Valderrama, 1981), phytoplankton and zooplankton abundance (Wetzel & Likens, 1991), and phytoplankton biomass as chlorophyll-*a* (Nusch & Palme, 1975) were determined before and after the experiments.

Two-way ANOVA was used to identify significant differences between treatments at the end of incuba-

tion period ($p<0.05$) (Sokal & Rohlf, 1979). Significant linear correlations between variates were determined (Sokal & Rohlf, 1979). Kendall's concordance test (Siegel, 1988) was applied in search for significant differences between phytoplankton community structures in natural and microcosm conditions (ranked by numerical abundance). A Student's *t*-test ($p<0.05$) was applied to identify significant differences between initial and final conditions (differences after 6–7 days of incubation period) in non-enriched microcosms.

Zooplankton were anesthetized by adding a few ml of carbonated water to prevent regurgitation (Infante, 1978b) and, within 1–2 min, preserved in 4% formalin (final concentration). In the laboratory, specimens were cleared with Hoyer mounting medium for detailed examination of gut contents (González, 1998). Results are expressed as appearance frequency (%). Spearman coefficient rank test (Siegel, 1988) was applied in search for significant differences between dry (January–April and November–December) and rainy (May–October) season diets.

Results

El Andino reservoir features (initial conditions)

In El Andino reservoir waters, TP varies between 14.4 μg l^{-1} (March) and 37.5 μg l^{-1} (October), with a mean value of 25.6 $\pm$ 7.1 μg l^{-1}. TN ranges from 102.3 μg l^{-1} (January) to 2191.4 μg l^{-1} (February), with a mean value of 1350.3 $\pm$ 501.7 μg l^{-1}.

Infante et al. (1995) conducted a parallel study (from January to December 1993) in El Andino reservoir. They reported the following chemical features for this water body: P–PO_4 ranged between 0.0 μg l^{-1} (November) and 4.3 μg l^{-1} (February), with a mean value of 2.4 $\pm$ 1.2 μg l^{-1}. Nitrates, nitrites and ammonia were variable: nitrates varied between 0.0 μg l^{-1} (December) and 19.6 μg l^{-1} (January), with a mean value of 2.5 $\pm$ 5.4 μg l^{-1}; nitrites varied 0.0 μg l^{-1} (August to December) and 3.5 μg l^{-1} (May), with a mean value of 1.2 $\pm$ 1.3 μg l^{-1}; ammonia ranged between 12.8 μg l^{-1} (May) and 273.9 μg l^{-1} (February), with a mean value of 86.8 $\pm$ 76.2 μg l^{-1}. Ortophosphates were always lower than 10 μg l^{-1}, indicating that P-PO_4 could be the limiting nutrient in the reservoir (Sas, 1989).

Chlorophyll-*a* varied between 7.7 μg l^{-1} (November) and 89.4 μg l^{-1} (August), with a mean value of 26.1 $\pm$ 21.8 μg l^{-1}, whereas phytoplankton abundance ranged between 2864 cells ml^{-1} (June) and 9560 cells ml^{-1} (December), with a mean value of 5264 $\pm$ 1944 cells ml^{-1}. Cyanobacteria (mainly *Dactylococcopsis acicularis* and *Cylindrospermopsis raciborskii*) was the dominant phytoplankton group over the study period, except during April and June, when Cryptophyta (*Cryptomonas erosa*) dominated (González, 1998). *Cryptomonas erosa* were codominant.

Zooplankton densities ranged between 12 ind. l^{-1} (January) and 609 ind. l^{-1} (May), with a mean value of 282 $\pm$ 193 ind l^{-1}. Zooplankton were dominated by cyclopoid copepods (*Thermocyclops* sp.) almost all the year, except from September to November, when rotifers (*Brachionus* spp.) dominated (González, 1998). Cladocerans (*Ceriodaphnia cornuta, Diaphanosoma* sp. and *Moina micrura*) remained at low densities over the study period, as well as ostracods. Calanoid copepods were scarce.

Nutrient enrichment effects

Microcosm mean TN:TP ratios, measured as μg N l^{-1}: μg P l^{-1} (according to Salas & Martinó, 1991), were: (C) 92.9 $\pm$ 49.7 > (Z) 59.5 $\pm$ 23.6 > (N) 20.1 $\pm$ 4.4 > (NZ) 18.7 $\pm$ 5.0. Nutrient enrichment and probably zooplankton excretion lowered initial N:P ratios (mean value of 56.0 $\pm$ 23.6). Generally, phosphorus limitation prevailed before and after fertilization, as TN:TP ratios were > 9:1 in all experiments (Salas & Martinó, 1991).

In the microcosms, the nutrient enrichment caused a significant increase in phytoplankton biomass, measured as chlorophyll-*a* (**Figure 1**). Abundance of each algal groups increased, except Pyrrhophyta (**Figure 2**). In spite of these changes, relative proportions of Cyanobacteria decreased in most cases. Chlorophyta and Bacillariophyta increased (**Figure 3**). The significance of results is shown in **Table 1**.

After enrichment, *Scenedesmus* was the dominant species from January to June, while from July to December *Dactylococcopsis* and *Lyngbya* dominated in the enriched microcosms (N and NZ). *Nitzschia* increased its abundance in most microcosms at the end of the 6–7 days period.

A Kendall's concordance test showed no significant differences ($p<0.05$) between the community structure of phytoplankton in natural and microcosm conditions (**Table 2**). Because experimental values of X^2 were greater than a critical value of X^2, then there was a significant concordance ('coincidence') between

Table 1. Significant *F*-values ($p<0.05$) from Two-Way ANOVA for the enrichment treatment

Months	TP	TN	Chl-a	Phytop	Cya	Chl	Bac	Eug	Pyr	Cry
J	157.1	NS	166.21	1000.00	513.51	540.77	15.55	NS	NS	NS
F	21.98	45.67	59.22	64.04	189.55	20.83	22.14	1000.00	8.22[a]	NS
M	21.63	17.47	14.53	60.85	25.76	35.93	24.45	36.80	NS	27.50
A	5.44	NS	NS	7.83	5.40	NS	62.40	17.13	16.53	10.10
M	152.47	723.78	1000.00	60.15	39.67	509.01	25.33	69.54	16.55	NS
J	17.57	17.27	8.23	10.73	12.37	NS	NS	37.72	NS	NS
J	1000.00	28.81	183.63	61.10	21.74	56.72	9.96	86.76	30.11[a]	47.14[a]
A	103.72	78.18	34.86	10.61	5.37	13.44	49.14	35.83	NS	173.70
S	1000.00	277.57	413.56	269.45	78.56	53.61	80.31	79.55	NS	50.66
O	1000.00	713.87	394.04	234.21	149.49	224.96	38.58	112.14	NS	11.06
N	1000.00	441.15	60.51	204.05	126.20	121.47	7.32	368.34	NS	80.21
D	673.99	1000.00	66.79	59.02	67.05	28.77	68.68	252.46	NS	146.94

TP= Total phosphorus, TN= Total nitrogen, Chl-a= Chlorophyll-*a*, Phytop= Total phytoplankton, Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta, NS= Non-significant results, [a]= Lower at the end of the experiments.

rank order of the compared treatments during the study period.

Zooplankton effects

Apparent zooplankton mortality, measured as the difference between initial and final densities, was high in microcosms. Copepods were most affected by confinement, with a mean mortality of all experiments of 85.5% and 94.7% in microcosms Z and NZ, respectively, followed by cladocerans (65.0% and 70.5%) and rotifers (67.1% and 61.8%). In microcosms, copepod relative proportions always decreased during experiments, whereas cladoceran and rotifer contributions increased (**Figure 4**).

In microcosms where zooplankton were present (Z and NZ), an increase of chlorophyll-*a* and TP, and thereby a lowered TN:TP ratios, were found (**Table 3**). TN did not follow the same pattern in these experiments. Significant differences due to zooplankton are shown in **Table 4**.

Nutrient regeneration rates by zooplankton were not measured in microcosms, but at the end of each experiment, TP 'excesses' of 12.2 μg l^{-1} in microcosm Z (as compared with microcosm C) and 15.7 μg l^{-1} in microcosm NZ (as compared with microcosm N) were found; this could indicate daily regeneration rates of 2.0 μg l^{-1} and 2.6 μg l^{-1} in microcosms Z and NZ, respectively.

Combined effects of nutrients and zooplankton

Table 5 shows significant interactions ($p<0.05$) between nutrient enrichment and zooplankton effects in microcosms. In most of the cases, the combined effects of nutrients and zooplankton were non-significant, indicating that the nutrient enrichment acted on phytoplankton independently from the zooplankton effects.

Initial versus final conditions

Non-enriched microcosms showed similar values compared to the initial conditions, and a Student's *t*-test was applied to identify significant differences between them. The results are shown in **Table 6** (initial versus final in microcosms C) and in **Table 7** (initial versus final in microcosms Z). In most of cases, there were significant differences between initial and final conditions.

Zooplankton diets

Figure 5 shows the diets of the main zooplankters in microcosm Z. A total of 562 specimens were examined, of which 42.2% had empty guts. On the other hand, **Figure 6** shows the diets of the main zooplankters in enriched microcosm (NZ). A total of 428 specimens were examined, of which 61.7% had empty guts. Besides particulate material (micro-algae, bacteria, fragments of algae, allochthonous organic matter in decomposition and mineral particles), the following

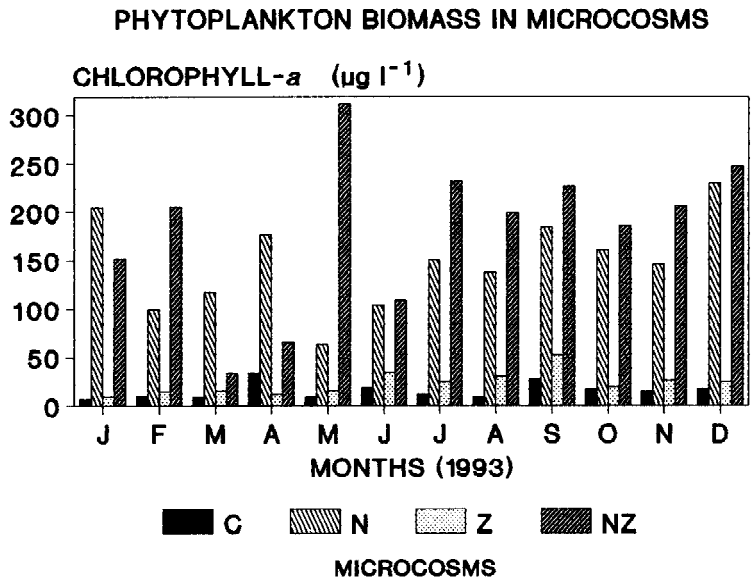

Figure 1. Mean final phytoplankton biomass (as chlorophyll-*a*) in microcosms for each treatment.

Table 2. Kendall's concordance test (W) results from comparisons between phytoplankton community structures in natural and microcosm conditions. S= Squares sum. Critical value: X^2 α= 0.05, n-1 d.f. (46 species – 1) = 30.6

Months	S	W	X^2
J	152852.7	0.757	172.4
F	152085.4	0.753	169.4
M	158123.0	0.783	177.5
A	140208.9	0.695	158.9
M	145817.2	0.722	165.2
J	150112.0	0.744	169.7
J	141753.3	0.702	160.4
A	166123.5	0.823	186.5
S	183612.4	0.910	205.7
O	175578.0	0.871	197.8
N	165094.7	0.818	185.6
D	176903.4	0.876	198.2

Table 3. Mean values of phytoplankton biomass (as chlorophyll-*a*), TN and TP concentrations in microcosms, and TN:TP ratios in microcosms

Microcosms	TN (μg l^{-1})	TP (μg l^{-1})	TN:TP	Chlorophyll-*a* (μg l^{-1})
C	2121.0 ± 1154.2	24.6 ± 9.0	92.9 ± 49.8	14.9 ± 8.1
N	4055.6 ± 1449.3	208.9 ± 54.7	20.1 ± 4.8	147.9 ± 47.6
Z	2076.1 ± 831.76	36.8 ± 11.2	59.5 ± 23.6	23.2 ± 12.0
NZ	3921.7 ± 1557.9	224.6 ± 85.6	18.7 ± 5.0	181.1 ± 79.2

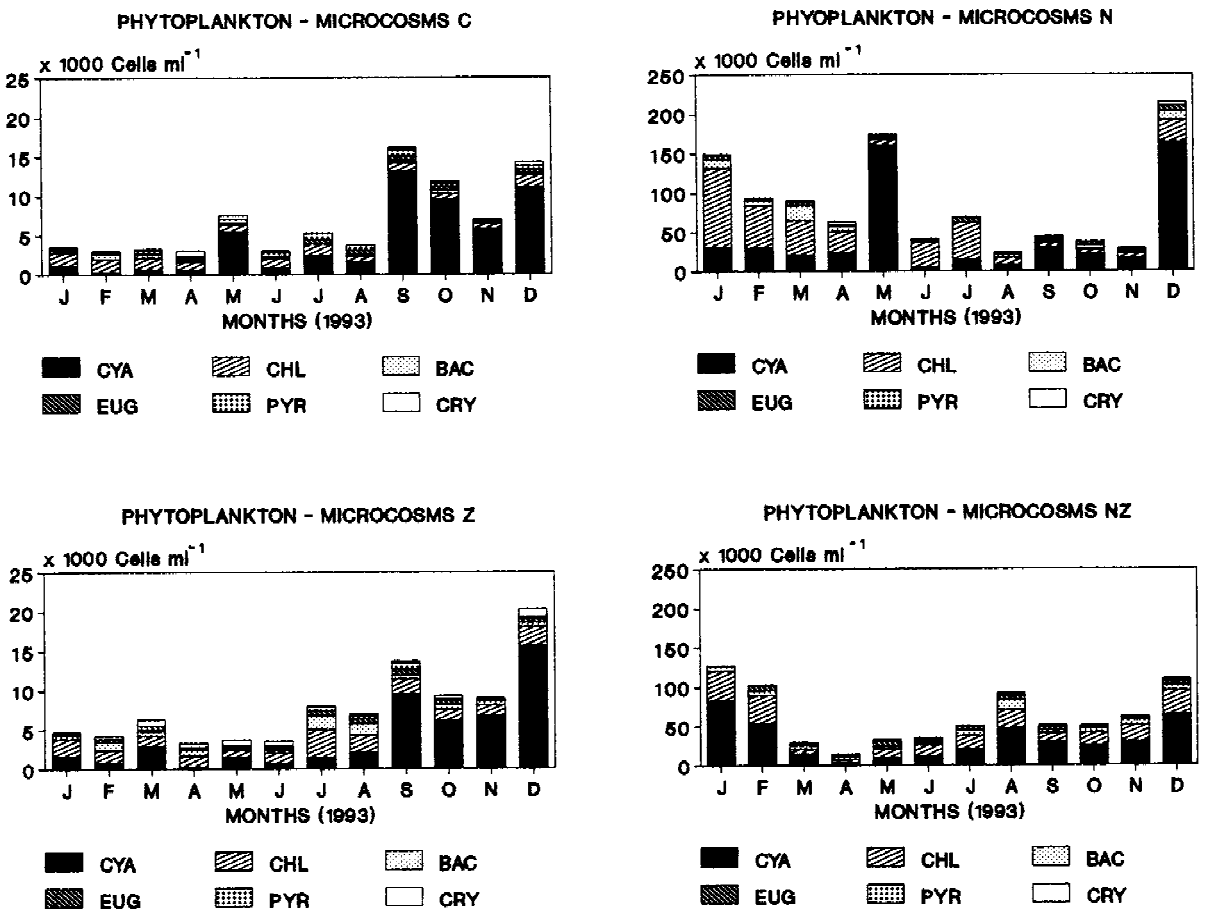

Figure 2. Mean final abundance of phytoplankton groups in microcosms for each treatment. Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta. Phytoplankton abundance scale in microcosms N and NZ are 10 times greater than in microcosms C and Z.

Table 4. Significant F-values ($p<0.05$) from Two-Way ANOVA for the zooplankton treatment

Months	TP	TN	Chl-a	Phytop	Cya	Chl	Bac	Eug	Pyr	Cry
J	NS	NS	NS	NS	108.45	110.12	NS	NS	NS	210.61
F	NS	NS	9.17	NS	19.48	NS	NS	295.89	NS	NS
M	NS	7.50[a]	5.45[a]	17.04[a]	NS	24.26[a]	10.82[a]	NS	NS	NS
A	NS	NS	NS	NS	NS	NS	NS	5.83[a]	NS	NS
M	10.77	23.09	1000.00	29.18[a]	36.12[a]	79.36	NS	21.21	159.51[a]	NS
J	NS	NS	NS	NS	NS	NS	NS	NS	NS	9.98
J	11.31	NS	13.64	NS	NS	13.93[a]	12.34	NS	NS	NS
A	NS	NS	NS	NS	NS	NS	24.68	5.83	NS	46.31
S	NS	NS	16.31	NS	NS	13.00	NS	NS	NS	NS
O	93.34	NS	NS	6.26	NS	68.87	NS	NS	9.03[a]	12.07
N	29.72	NS	NS	45.32	20.21	51.57	NS	NS	NS	NS
D	NS	23.10	NS	NS	14.20[a]	NS	NS	23.73	NS	NS

TP= Total phosphorus, TN= Total nitrogen, Chl-a= Chlorophyll-*a*, Phytop= Total phytoplankton, Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta, NS= Non-significant results, [a]= Lower at the end of the experiments.

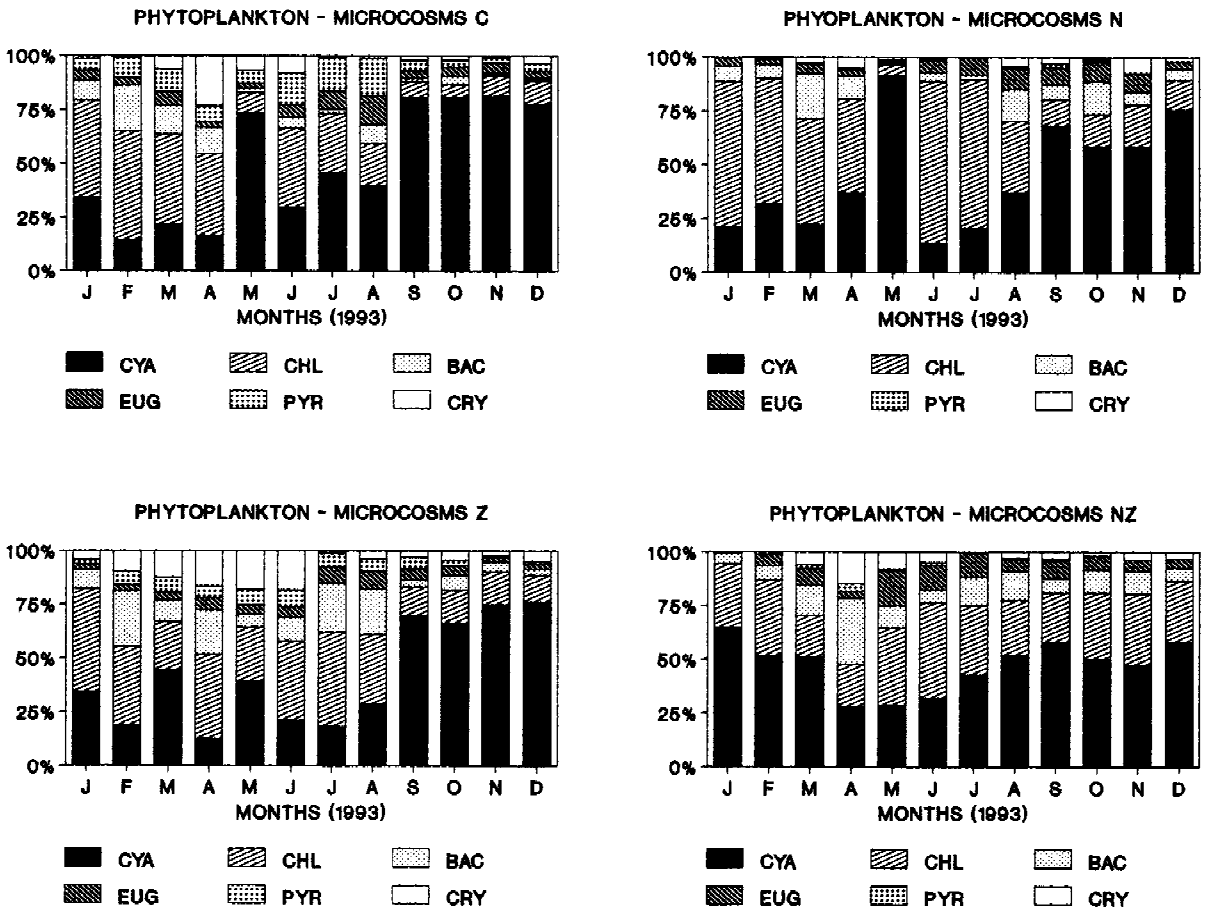

Figure 3. Mean final relative proportions of phytoplankton groups in microcosms for each treatment. Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta.

Table 5. Significant *F*-values ($p<0.05$) from Two-Way ANOVA for combined effects of nutrients and zooplankton

Months	TP	TN	Chl-a	Phytop	Cya	Chl	Bac	Eug	Pyr	Cry
J	NS	NS	NS	7.83	106.33	115.23	NS	NS	NS	237.15
F	NS	NS	NS	NS	18.47	NS	NS	296.69	NS	NS
M	NS	5.78	7.58	20.69	NS	24.35	11.34	NS	NS	NS
A	NS	7.33	NS	NS	NS	NS	NS	8.27	NS	NS
M	9.20	37.03	1000.00	24.30	30.87	79.86	NS	22.54	7.73	NS
J	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
J	NS	NS	7.15	NS	NS	18.16	NS	NS	NS	NS
A	NS	NS	NS	NS	NS	NS	14.63	NS	6.86	25.62
S	NS	NS	NS	5.76	NS	8.52	NS	NS	NS	NS
O	19.62	5.76	NS	13.45	7.33	53.87	NS	NS	NS	NS
N	16.97	7.43	NS	36.15	14.81	42.79	NS	9.09	NS	NS
D	NS	NS	NS	NS	16.75	NS	NS	24.08	NS	NS

TP= Total phosphorus, TN= Total nitrogen, Chl-a= Chlorophyll-*a*, Phytop= Total phytoplankton, Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta, NS= Non-significant results.

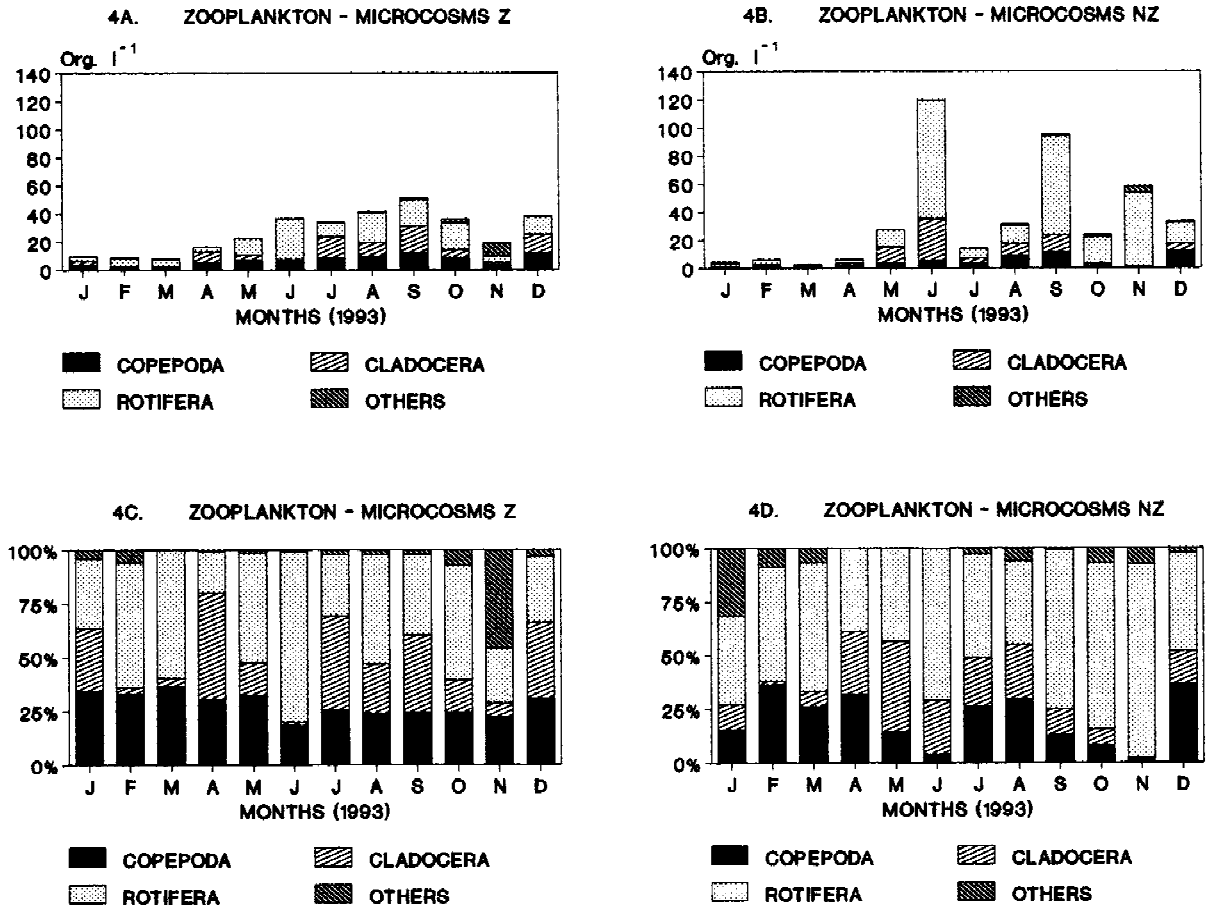

Figure 4. Mean final abundance of zooplankton groups in microcosm Z (4A) and NZ (4B), and mean relative proportions of zooplankton groups in microcosm Z (4C) and NZ (4D).

phytoplankton genera were identified in zooplankton gut contents from both microcosms: *Aulacoseira, Cyclotella, Navicula, Nitzschia, Rhizosolenia, Synedra, Cosmarium, Dictyosphaerium, Monoraphidium, Oocystis, Scenedesmus, Peridinium, Merismopedia, Oscillatoria, Synechococcus* and *Trachelomonas.*

In microcosm Z, cyclopoids (n=213) fed mainly on particulate material (over 80% of the cases), *Cyclotella, Peridinium, Monoraphidium*, and *Synechococcus*. A greater proportion of cyclopoids contained diatoms and *Monoraphidium* in the dry season (November–April), whereas *Cosmarium, Oocystis* and *Synechococcus* were more frequent in the gut contents during the rainy season (May–October). In microcosm NZ, cyclopoid copepods (n=166) presented particulate material over 80% during both dry and rainy seasons, *Cyclotella* were consumed only in the dry season. *Peridinium* were more frequent during the rainy season. *Cosmarium, Monoraphidium* and *Oocystis* were found in low frequencies.

Only particulate material appeared in nauplii (n=22) gut contents from microcosm Z during the dry season (not shown in figure), whereas particulate material (100%) and *Monoraphidium* (>30%) were present during the rainy season. In microcosm NZ, nauplii (n=9; not shown in figure) only contained particulate material during the dry period, whereas particulate material (100%) and *Monoraphidium* (>30%) were present in their gut contents during the rainy period.

Calanoids (n=35) from microcosm Z mainly contained particulate material. Diatoms were present only during the dry season, and *Cosmarium, Monoraphidium, Oocystis* and *Peridinium* were more frequent during the rainy season. Calanoids copepods (n=12) from microcosm NZ only fed on *Cosmarium* during

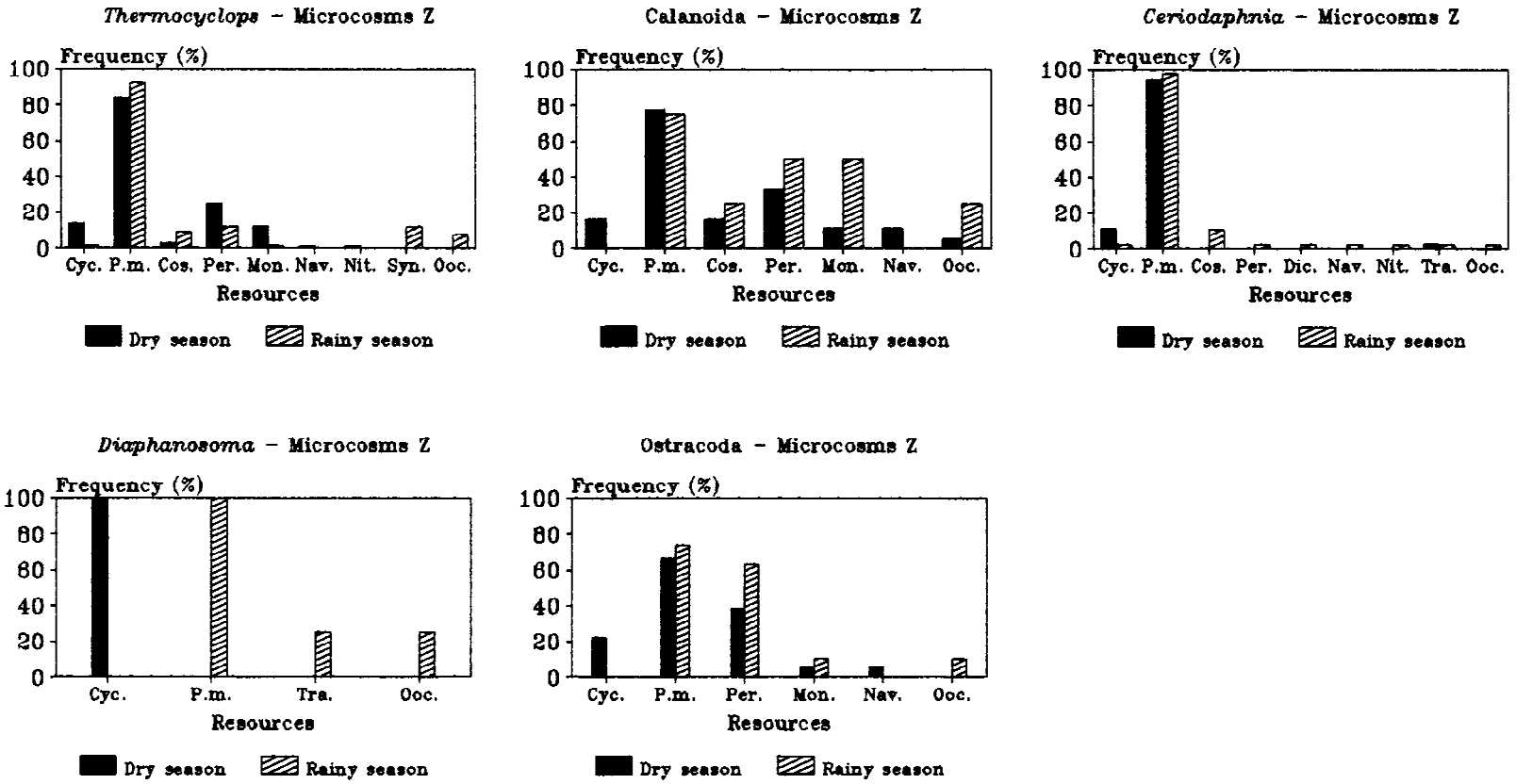

Figure 5. Mean diet of the main zooplankters in microcosms Z during the dry and rainy seasons. Cyc.= *Cyclotella*, Nav.= *Navicula*, Nit.= *Nitzschia*, Cos.= *Cosmarium*, Dic.= *Dictyosphaerium*, Mon.= *Monoraphidium*, Ooc.= *Oocystis*, Per.= *Peridinium*, Syn.= *Synechococcus*, Tra.= *Trachelomonas*, and P.m.= Particulate material.

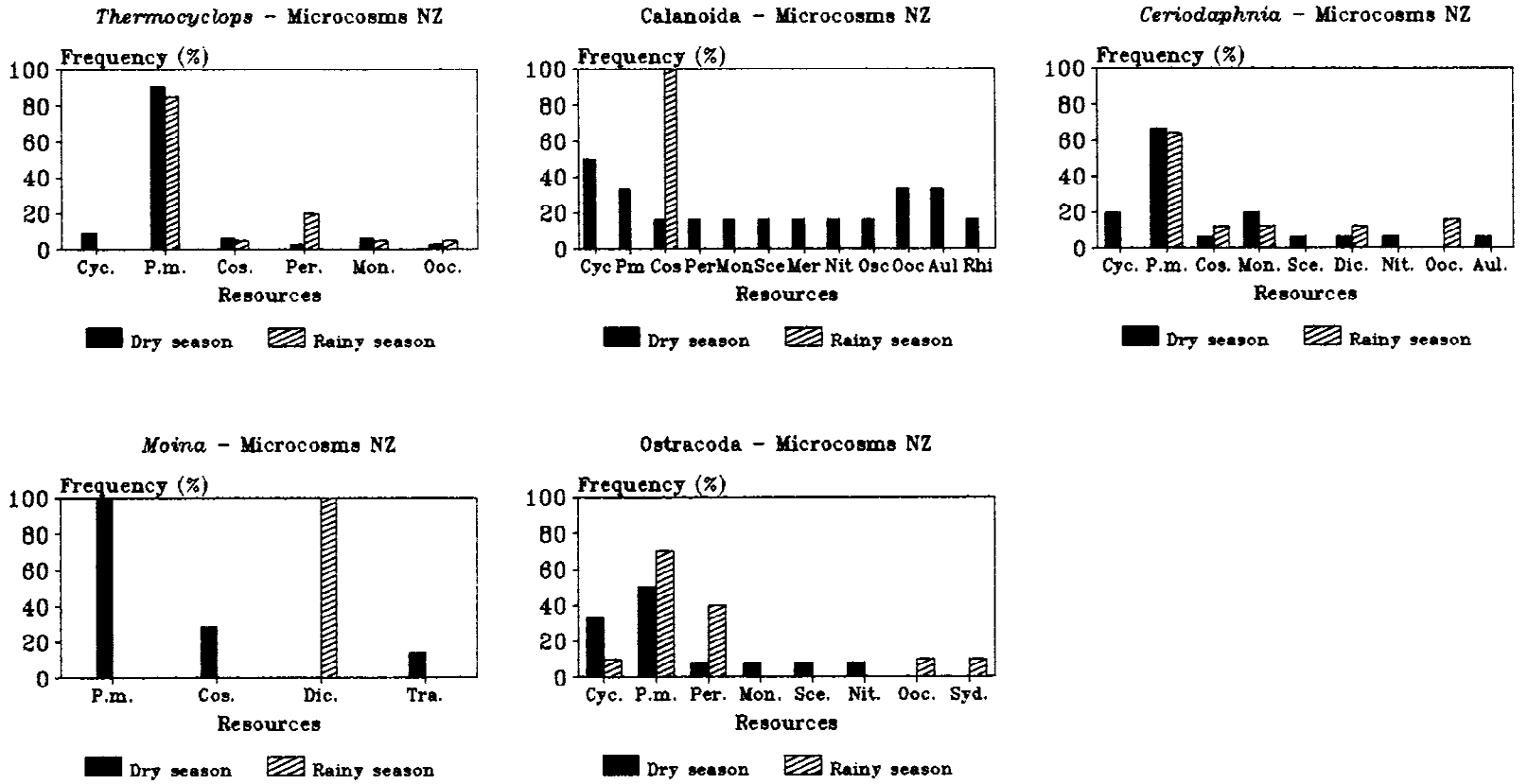

Figure 6. Mean diet of the main zooplankters in microcosms NZ during the dry and rainy seasons. Aul.= *Aulacoseira*, Cyc.= *Cyclotella*, Nav.= *Navicula*, Nit.= *Nitzschia*, Rhi.= *Rhizosolenia*, Syd.= *Synedra*, Cos.= *Cosmarium*, Dic.= *Dictyosphaerium*, Mon.= *Monoraphidium*, Ooc.= *Oocystis*, Sce.= *Scenedesmus*, Per.= *Peridinium*, Mer.= *Merismopedia*, Osc.= *Oscillatoria*, Syn.= *Synechococcus*, Tra.= *Trachelomonas*, and P.m.= Particulate material.

Table 6. Student's *t*-test results ($p<0.05$) from comparisons between initial and final conditions in microcosms C ('Control')

Months	TP	TN	Chl-a	Phytop	Cya	Chl	Bac	Eug	Pyr	Cry
J		I	D	D	D	I			D	D
F	D			D	D	I				D
M			D		D		I		I	D
A		I				I	I		I	D
M		I	D							
J						I	I			D
J			D			I			I	D
A			D	D			I			D
S		I		I	I	I				D
O			D	I	I		I			D
N		I								D
D		I	D	I	I	I				D

TP= Total phosphorus, TN= Total nitrogen, Chl-a= Chlorophyll-*a*, Phytop= Total phytoplankton, Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta, I= Increase significatively at the end of experiments, D= Decrease significatively greater at the end of experiments. No mark means non-significant differences.

the rainy season. Their dry season diet was more diverse and ingested particulate material, *Aulacoseira, Cyclotella, Navicula, Nitzschia, Rhizosolenia, Cosmarium, Monoraphidium, Oocystis, Scenedesmus, Merismopedia, Oscillatoria,* and *Peridinium*.

Ceriodaphnia cornuta (n=150) mainly ingested particulate material over the study period in microcosm Z. *Cyclotella* were ingested in greater proportions during the dry season, but *Cosmarium* were ingested only during the rainy season. Other items appeared with frequencies <2.2%. In microcosm NZ, *Ceriodaphnia cornuta* (n=81) ingested diatoms (*Aulacoseira, Cyclotella,* and *Nitzschia*) and *Scenedesmus* only during the dry season, and *Oocystis* only during the rainy season. *Dictyosphaerium* and *Cosmarium* were found more frequently during the rainy season rather than in the dry season, whereas the inverse situation was found for *Monoraphidium*. Particulate material appeared in frequencies >60% during both seasons.

In microcosm Z, specimens of *Diaphanosoma* sp. (n=11) only contained *Cyclotella* in their guts during the dry season, whereas particulate material (100%), *Trachelomonas* (∼25%) and *Oocystis* (∼25%) appeared in the gut contents during the rainy season. In microcosm NZ *Diaphanosoma* sp. (n=5; not shown in figure) only ingested particulate material during the dry season. All the specimens analyzed showed empty guts during the rainy season.

In microcosm Z, *Moina micrura* (n=8; not shown in figure), only ingested particulate material during the dry season, and particulate material (100%) and *Cosmarium* (25%) during the rainy season. In microcosm NZ, this species (n=10) only ingested particulate material (100%), *Cosmarium* and *Trachelomonas* during the dry season, and fed only on *Dictyosphaerium* during the rainy season.

In microcosm Z, ostracods (n=70) mainly contained particulate material, *Cyclotella* and *Peridinium* in the dry period, and particulate material and *Peridinium* during the rainy period. During the dry season in microcosm NZ, ostracods (n=92) mainly ingested particulate material and *Cyclotella*; *Monoraphidium, Scenedesmus* and *Nitzschia* were present too. Particulate material and *Peridinium* were mainly ingested during the rainy season; *Oocystis* and *Synedra* were present at lower frequencies.

Rotifers (not shown in figure) almost exclusively fed on particulate material over the study period in microcosm Z. *Cyclotella* was present in ∼30% of *Brachionus* spp. (n=22) gut contents during the dry season, whereas *Cosmarium* was ingested by ∼20% of *Platyias* spp. (n=12) during the rainy season. *Keratella* spp. (n=4) and *Lecane* spp. (n=15) only ingested particulate material. In microcosm NZ, rotifers (n=53; not shown in figure) fed almost exclusively on particulate material over the study period.

Table 7. Student's *t*-test results ($p<0.05$) from comparisons between initial and final conditions in microcosms Z

Months	TP	TN	Chl-a	Phytop	Cya	Chl	Bac	Eug	Pyr	Cry
J		I	D	D	D	I			D	D
F					D					
M									I	D
A										D
M							I			
J						I		D		
J	I			I	D	I	I	I	D	
A			D		D	I	I	D		
S	I	I	I	I	I	I				
O			D	I	I	I	I	I		
N		I				I	I			D
D		I		I	I	I	I		D	D

TP= Total phosphorus, TN= Total nitrogen, Chl-a= Chlorophyll-*a*, Phytop= Total phytoplankton, Cya= Cyanobacteria, Chl= Chlorophyta, Bac= Bacillariophyta, Eug= Euglenophyta, Pyr= Pyrrhophyta, Cry= Cryptophyta, I= Increase significatively at the end of experiments, D= Decrease significatively at the end of experiments. No mark means non-significant differences.

In microcosm Z, significant differences ($p<0.05$) were found between Cyclopoida, *Ceriodaphnia cornuta* and ostracod dry and rainy season diets, due to the greater diatom proportions ingested by these groups during the dry period rather than in the rainy season.

In microcosm NZ, *Scenedesmus* and *Nitzschia*, successful species after microcosm fertilization during part of the dry period, were selected by some of the analyzed specimens (calanoid copepods, *Ceriodaphnia cornuta* and ostracods).

As for microcosm Z, significant differences ($p<0.05$) were found between Cyclopoida, *Ceriodaphnia cornuta* and ostracod dry and rainy season diets, mainly due to the greater diatom proportions found in their gut contents during the dry period than in the rainy period.

Discussion

Responses to nutrient enrichment were similar to those obtained by De Costa et al. (1983), Bergquist & Carpenter (1986), Vanni (1987), Elser & Goldman (1991), Yasuno et al. (1993), Mazumder (1994a,b) and González & Ortaz (1998). These authors reported the increase of phytoplankton biomass and abundance after the nutrient enrichment.

TN:TP ratio may reflect nutrient source (Downing & McCauley, 1992). For instance, watersheds from agricultural activity have N:P ratios of 20:1, which were adopted for the microcosms. Therefore, the experiments carried out in El Andino reservoir would have reflected a hypothetical eutrophication process due to agricultural activities in the surrounding lands.

In enriched microcosms, Chlorophyta and Bacillariophyta increased their relative proportions, probably due to their greater competitive abilities for phosphorus (Margalef, 1983; Reynolds, 1984). *Scenedesmus* and *Nitzschia* were successful species in experiments. As pointed by Sommer (1983, 1988), these species are successful in the early stages of succession, whereas flagellates as *Cryptomonas* were unsuccessful. This could explain the increase of *Scenedesmus and Nitzschia* in enriched microcosms from El Andino reservoir.

Some Cyanobacteria species (*Dactylococcopsis* and *Lyngbya*) dominated enriched microcosms from July to December, and this could indicate that depending on the initial community structure of phytoplankton, different initial responses to a nutrient enrichment process (eutrophication) could occur.

The Kendall's concordance test did not show significant differences between the community structure of phytoplankton in natural and microcosm conditions. Perhaps a 6–7 days period was not long enough to observe changes in the phytoplankton community structure after fertilization with N and P in El Andino reservoir.

Table 8. Significant correlations ($p<0.05$) in microcosms

Microcosm C	
TP VS	
	Chlorophyll-*a*, *r*=0.827
	Cryptophyta, *r*=0.668
Microcosm N	
TN VS	
	Euglenophyta, *r*= 0.637
Microcosm Z	
TP VS	
	Chlorophyll-*a*, *r*=0.819
	Euglenophyta, *r*=0.802
	Pyrrhophyta, *r*=0.628
Chlorophyll-*a* VS	
	Rotifera, *r*=0.689
Euglenophyta VS	
	Total zooplankton, *r*=0.830
	Copepoda, *r*=0.863
	Cladocera, *r*=0.881
Total phytoplankton VS	
	Copepoda, *r*=0.696
	Cladocera, *r*=0.641
Microcosm NZ	
TN VS	
	Cyanobacteria, *r*=0.648
	Chlorophyta, *r*=0.609
Total phytoplankton, *r*=0.649	
TP VS	
	Chlorophyll-*a*, *r*=0.784
	Pyrrhophyta, *r*=−0.587
Chlorophyll-*a* VS	
	Euglenophyta, *r*= 0.669

In this study, zooplankton absence was used to mimic situations where high fish predation occurs. The absence of fish implies that, apart from predation effects, there is also absence of nutrient excretion from these animals (Arcifa et al., 1986; Vanni, 1987; Vanni & Findlay, 1990; Matveev et al., 1994).

Apparent mortality of zooplankton was high in microcosms probably due to the hauling stress and posterior isolation in plastic bags. Manipulations negatively affect zooplankters, because manipulations produce hyperactivity and reduce filtering activities (Chow-Fraser, 1986). Microcosms generate different conditions inside as compared to the outside environment, and prevent natural migratory movements (Havens & De Costa, 1986). Chow-Fraser (1986) found that copepods did not recover from stress conditions, even after a 24 h acclimatization period. This could explain the high mortality of this group in microcosms from El Andino reservoir. Rotifers seemed more tolerant to these conditions.

Zooplankton might have affected phytoplankton community in microcosms through grazing and probably through nutrient regeneration. Nutrient recycling by consumers can have substantial effects on phytoplankton community (Vanni & Layne, 1997). Zooplankton excretion may alter the balance of N and P supplied to algae (Carpenter et al., 1992). Zooplankton P-excretion may be a mechanism to explain the slight increase in phytoplankton biomass and TP in microcosms Z and NZ. Moegenburg & Vanni (1991) found in Lake Mendota (U.S.A.), that zooplankton excretion lowered nitrogen and phosphorus limitation for phytoplankton. According to Lenz et al. (1986), in tropical lakes, with warm waters and high zooplankton densities, nutrient regeneration by zooplankton could be an important feature.

Although the excess of TP in microcosms with zooplankton is not the best way to calculate nutrient regeneration, because other processes could occur (phytoplankton uptake, bacterial uptake, and detritus degradation), zooplankton 'daily regeneration rates' in microcosms coincide with the more carefully calculated values reported by Den Oude & Gulati (1988) in their laboratory experiments with zooplankton from the eutrophic lakes Breukeleveen and Loosdrecht (Netherlands); they measured daily P-regeneration rates ranging from 0.9 to 2.4 μg l^{-1}. The only difference between microcosms C and Z, and between microcosms N and NZ, was the presence of zooplankton, so the excess of TP in microcosms could be attributed to zooplankton excretion.

Apparently, grazing by zooplankton was ineffective to reduce phytoplankton biomass. Although the animals selected some phytoplankton species in microcosms, excesses of chlorophyll-*a* were detected where zooplankton were present. In tropical and subtropical lakes, large-bodied zooplankton are scarce and small-bodied filter-feeding species dominate (Gliwicz, 1990; Roche et al., 1993; Arcifa et al., 1995); these species are less efficient in controlling phyto-

plankton because of their lower filtering rates and their narrow food size spectra (Gliwicz, 1990). Thus, zooplankton community structure to manage algal biomass may be of limited value in many lakes (Pace, 1984).

Zooplankton diets in microcosms were similar to natural diets in El Andino reservoir, according to reported data from González (1998). Herbivorous zooplankton mainly grazed on diatoms, especially in the dry season months, when diatoms were a little more abundant (González, 1998), although particulate material was present in almost all the gut contents analyzed. Particulate material, probably associated with bacteria (Infante, 1978a; Gómez, 1984; González, 1998), seemed to be an important food source in El Andino reservoir, both in natural and microcosm conditions.

In mesotrophic to eutrophic lakes, net phytoplankton (>50 mm) is not extensively grazed by herbivorous zooplankton, and net phytoplankton are more efficiently utilized by bacteria (Gliwicz, 1969, 1977; Hillbricht-Ilkowska, 1977). Only after partial decomposition to tiny particles of 1–2 μm in size (detritus and bacteria suspension), net phytoplankton becomes an available food source for the herbivorous zooplankton (Gliwicz, 1969). Apparently, this is the case in El Andino reservoir (natural environment and microcosms).

Despite the incubation period applied, as was suggested by Ringelberg & Kersting (1978) and Havens & De Costa (1988), significant differences were found between initial and final conditions. Isolation of communities 'per se' alters environment inside microcosms, because they prevent nutrient incoming, mineralization and nutrient re-circulation (Ringelberg & Kersting, 1978; Havens & De Costa, 1988).

Carpenter (1996) pointed that microcosm experiments may exclude or distort important features of communities and ecosystems, because some processes and organisms change so rapidly that they reach unrealistic rates or population densities, such as nutrient regeneration, phytoplankton production and plankton communities. However, with appropriate spatial and time scales, microcosms provide an important tool for the analysis of ecological communities (Fraser & Keddy, 1997), and results could be similar to whole-lake experiments (Vanni et al., 1997).

Implications for El Andino reservoir water quality

Table 8 shows significant linear correlations ($p<0.05$) in El Andino microcosms. In three out of four microcosms (C, Z and NZ) a strong correlation between TP and phytoplankton biomass (as chlorophyll-*a* concentration) was observed. The bottom-up control was present in microcosms C, Z and NZ. The lack of correlation between TP and chlorophyll-*a* in microcosm N could be explained by the short time of incubation, and phytoplankton community may not have had enough time to attain an equilibrium with nutrient (Carpenter, 1996). TP showed significant correlations with Cryptophyta (microcosm C), Euglenophyta and Pyrrhophyta (microcosm Z). Significant correlations were present between TN and Euglenophyta (microcosm N), and between TN and phytoplankton, Cyanobacteria and Chlorophyta (microcosm NZ), indicating a control by nitrogen too.

Zooplankton correlated with phytoplankton groups only in microcosm Z. This fact could indicate that in El Andino reservoir, linkage between zooplankton and phytoplankton might not be weak at all. However, these correlations were not present in microcosm NZ (enriched), indicating that eutrophication could break this linkage (McQueen et al., 1986).

Conclusions

Nutrient enrichment (N and P) caused an increase in phytoplankton biomass and abundance, except for Pyrrhophyta in most of the experiments. Relative proportions of Cyanobacteria decreased in most of microcosms, while Chlorophyta and Bacillariophyta increased. From January to June, *Scenedesmus* was the dominant species after the enrichment, while from July to December, *Dactylococcopsis* and *Lyngbya* were dominant. Thus, depending on the season of the year, the available stock of algae could determine the initial response of phytoplankton community to a nutrient enrichment (eutrophication) process. Results suggest that at the initial stages of a eutrophication process, phytoplankton increase their biomass and abundance, but would not change their community structure in El Andino reservoir.

Herbivorous zooplankton in microcosms mainly grazed on diatoms and particulate material during the dry season, and fed on particulate material and other algae (mainly green algae) during the rainy season. Diatoms were slightly more abundant during the

dry period, but when they were scarce, zooplankton searched for other food resources.

Since there was a strong correlation between P and chlorophyll-*a* (bottom-up control), it is suggested that eutrophication could be avoided by controlling P input into the reservoir.

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