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# BACTERIA AS STRUCTURING AGENTS IN LAKES:
## Field Manipulations with Bacterioplankton

by

Ronald Newhook

A thesis
presented to the University of Ottawa
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Department of Biology

OTTAWA, Ontario, 1984

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## ABSTRACT

This study investigates the possible role played by bacteria in structuring lake plankton communities. It is already known that phytoplankton, zooplankton, and fish are each important determinants of community composition. Although neglected in this regard, bacteria have much potential as structuring agents. Many affect phytoplankton species differentially in culture; only microphagous zooplankton prey effectively on bacteria; certain bacteria are pathogens on planktivorous fishes. We do not know, however, whether this potential is realized in nature.

To investigate whether bacteria are important structuring agents in lakes, indigenous bacteria were added to enclosed plankton communities in a mesotrophic lake, and the community responses monitored. In early summer experiments, five 7800 L enclosures were enriched weekly with $1.4 \times 10^3$ cells/mL of Aeromonas hydrophila anaerogenes, while five served as controls. Late summer enrichments were the same, except that three enclosures were inoculated with A.h. anaerogenes, three with Serratia marcescens, and three were left unperturbed. Changes in nutrient, phytoplankton, and zooplankton levels with enrichment were followed for six weeks in each experiment.

The enrichment was lower than originally intended, increasing total *in situ* bacterial levels by an average of 5%. Total and active bacterial levels did not differ significantly between control and enriched enclosures. However, both species reproduced well in filter-sterilized lake water, suggesting that they were active in the enclosures. Zooplankton removal indicated that grazing did not control bacterial levels; lysis was the most likely regulator.

Bacterial enrichment produced strong increases in phytoplankton volume in all experiments, probably because the added bacteria increased supplies of limiting nutrients through mineralization. This stimulation was restricted almost exclusively to the net phytoplankton (longest axis> 30 μm) as the enrichment bacteria associated physically with these large algae. Diatoms were enhanced more consistently than other taxa, perhaps as the added bacteria interfered with allelopathic interactions directed by other algae at diatoms. These results suggest a role for bacteria in influencing phytoplankton succession in lakes where large cells replace small ones, or diatoms are replaced by other algae.

Zooplankton were affected by enrichment in only the late summer *Serratia* manipulations. Bactivorous genera increased, evidently in response to strong reproduction by *Serratia*. In turn, predaceous zooplankton levels were

increased in response to their bactivorous prey. Grazer composition was controlled by the nature of both their prey and invertebrate predators.

Total levels of phosphorus and carbon were elevated by enrichment, probably as mineralization of particulate organic matter by the added bacteria reduced nutrient losses through sedimentation. Epilimnetic oxygen concentrations increased, while those of ammonia decreased in enriched enclosures, apparently in direct response to the phytoplankton stimulation.

The specific organisms altered by enrichment often reflected the combined effects of several controlling factors. In the face of such complexity, manipulation is essential to test functional significance in all but the simplest of communities.

Bacterial effects were pronounced when the community was stable in terms of species composition, approximating a steady state. At other times, major shifts in phytoplankton biomass masked the impact of enrichment. Although bacterial structuring is limited to periods when the plankton is near equilibrium, this is also true of the effects of invertebrate predators and grazing zooplankton on their respective prey. Future studies of interactions among plankton should therefore consider the selective effects of specific bacteria on plankton composition.

## ACKNOWLEDGEMENTS

I am most grateful to Frédéric Briand, for providing a valuable and challenging learning experience, and for his patience.

This work benefitted considerably from discussions with Alain Baril and Phil Spear. I am also indebted to the many people who aided with technical aspects of this research, among them: Deborah Abramovitch, Alain Baril, Paul Brunon, Nino Cavacutti, Rama Chengalath, Ron Droste, Jacques Hélie, Betty Hurst, Peter Lomax, Michael McBurney, Wendy Moss, Colette O'Rourke, Ana Marie Pajor, Valerie Quinn, Dean Smith, Elizabeth Sullivan, George Ben-Tchavtchavadze, and Mike Wong.

Finally, personal thanks are extended to the Cubists and to my family for their support, and especially to Deborah, for being there.

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# Chapter I

## INTRODUCTION

Lakes exhibit a striking variety in the composition of their plankton communities. Some lakes are dominated by small flagellated phytoplankton, while others harbour mainly diatoms, and still others support dense growth and surface scums of blue-green algae for much of the year. Zooplankton assemblages are no less diverse: some are dominated by large cladocerans and calanoid copepods, others by small-bodied species, and some even contain only rotifers. Further, the contributions of these different groups vary predictably during the course of a year: for instance, spring diatom peaks are often succeeded by summer blooms of green and then blue-green algae (see Hutchinson 1967, Kalff and Knoechel 1978).

Early researchers focussed on abiotic factors in seeking to explain such patterns, suggesting that a given assemblage was a reflection of the prevailing temperature, light, mixing, pH or nutrient regimen (see Hutchinson 1967). While physical and chemical factors are certainly important in determining which organisms potentially inhabit a volume of water, the realization is growing that the biota are also major structurers of pelagic communities.

## 1.1 BIOTIC STRUCTURING AGENTS IN LAKES

The first biotic interaction to receive widespread attention in this regard was predation by vertebrate planktivores, primarily fish (see review by O'Brien 1979). Planktivorous fish are voracious predators which generally locate their prey by sight, and selectively consume the slower and more visible members of the zooplankton. As a result, in lakes with fish the zooplankton are dominated by small, evasive and cryptic forms, while in fishless lakes they are strikingly larger, less maneuverable and more highly pigmented (Brooks and Dodson 1965, Hutchinson 1971, Nilsson and Pejler 1973). Introduction or removal of fish produces the expected changes in the zooplankton size structure, respectively decreasing or increasing the abundance of the more visible prey (Hrbacek et al. 1961, Brooks and Dodson 1965, Galbraith 1967, Hall et al. 1970, Drenner et al. 1982).

In contrast, predaceous zooplankton selectively remove the smaller animals, presumably under constraints imposed by their own size. While they have lower consumption rates per individual than fish (see Hall et al. 1976), invertebrate predators are very abundant, with the result that they can be a major cause of zooplankton mortality (McQueen 1969, Confer 1971, Hillbricht-Ilkowska 1977, Lane 1979). Predation by invertebrates probably contributes to the predominance of large zooplankton species in the absence of

fish, although their structuring effects are in general weaker than those of fish (Hall et al. 1970, Neill 1981, Baril 1983, Baril and Briand, in press).

One trophic level lower, herbivorous zooplankton perform a similar role. In situ zooplankton removal reveals that phytoplankton vary considerably in their susceptibility to grazing (Porter 1973, Weers and Zaret 1975): small and naked algae are readily consumed by a variety of grazers, while large or filamentous phytoplankton are for the most part too large to ingest (Burns 1968, Porter 1973, Nadin-Hurley and Duncan 1976). When zooplankton are grazing a large proportion of the water column daily (eg. Haney 1973, Bogdan and Gilbert 1982), this selective grazing may direct the phytoplankton community from a predominance of small rapidly-growing algae to one of large inedible species (Porter 1973, Gliwicz 1975, Lehman 1980).

The reverse effect also occurs, whereby phytoplankton control zooplankton composition. Large and filamentous algae which cannot be ingested are rejected, and may clog the filtering appendages of grazers (Burns 1968, Porter 1977), reducing filtering rates, feeding rates and reproduction (Gliwicz 1977, 1980, Webster and Peters 1978). In general only large-bodied zooplankton are inhibited in this manner, as in smaller animals the narrow gap between the valves of their carapace does not permit these algae to enter (Gliwicz 1977, 1980). This selective inhibition may

drive the seasonal succession of zooplankton from a predominance of large-bodied species to one of small-bodied animals (Gliwicz 1977).

Notwithstanding a degree of resource partitioning unexpected in the relatively homogeneous water column (Wall and Briand 1979, 1980, Kerfoot and DeMott 1980, DeMott and Kerfoot 1982), competition is also emerging as a structuring agent in planktonic communities. Field experiments indicate that grazers with similar diets strongly depress and even exclude one another (Kerfoot 1977, Lynch 1978, Smith and Cooper 1982), while those with less dietary overlap have correspondingly weaker interactions (Kerfoot and DeMott 1980, DeMott and Kerfoot 1982). Parallel field studies with phytoplankton are yet to be done, but the close fit between competitive abilities of diatoms along an *in vitro* [Si]:[P] gradient and their distribution along this same gradient *in situ* is suggestive (Tilman *et al.* 1982).

Finally, some phytoplankton produce substances that control phytoplankton succession by inhibiting other algae. Keating (1977) described a succession of blue-green algae in a eutrophic pond, where species released chemicals inhibiting their predecessors. Natural bloom waters are often inhibitory to phytoplankton (Lefevre 1964, Vance 1965), which suggests that such allelopathy is widespread.

Our understanding of the function played by the biota in organizing plankton communities is thus considerable. In

contrast, very little attention has been given to the possible role of bacteria in this regard.

## 1.2 BACTERIAL POTENTIAL FOR STRUCTURING

This neglect is all the more remarkable considering that bacteria have much potential as structuring agents. While all phytoplankton require similar inorganic nutrients, a high surface area:volume ratio makes small algae better than large ones at taking up these nutrients (Friebele et al. 1978, Malone 1980). When bacterial mineralization supplies a major portion of phytoplankton needs (eg. Golterman 1973, 1976, Bloesch et al. 1977, Liao and Lean 1978, Axler et al. 1981) and grazing is heavy, nutrient regeneration undoubtedly permits the persistence of edible phytoplankton and the zooplankton they support. In this fashion, the bacteria contribute to a relatively high level of community metabolism, and counteract the selective removal of small algae by grazers.

Similarly, in instances when detritus is deficient in some mineral nutrient, bacterial decomposers will become net consumers of dissolved forms of this nutrient and compete with phytoplankton for them. If nutrient deprivation of phytoplankton increases as a result, small algae will be disadvantaged less than large ones by virtue of their greater surface area:volume ratio, and thus might increase their share of the total phytoplankton.

A variety of other bacterial influences affect algae differentially, suggesting a greater structuring potential for them. Thus, many phytoplankton are stimulated in culture by bacteria. Berland et al. (1970) observed that numerous marine algae grew better when bacterized than in axenic culture. A number of chlorophytes are stimulated to varying degrees by bacteria (Ukeles and Bishop 1975). Delucca and McCracken (1977) found that satellite bacteria from the blue-green alga Oscillatoria consistently enhanced the growth of their host, but were stimulatory, neutral, or inhibitory to other algae.

The mechanisms of such stimulation have only occasionally been addressed. Carbon dioxide production by bacteria can benefit algae when this nutrient is limiting (Lange 1967, 1970, 1971); Lange attributed the occurrence of blue-green algal blooms in productive waters to this interaction. Similarly, the vitamin requirements of algal auxotrophs can be satisfied by bacterial producers in culture (Haines and Guillard 1974). Correlations of vitamin levels and algal composition in lakes indicate that such auxotrophs may at times be limited by the supply of vitamins (Ohwada and Taga 1972, Cavari and Grossowicz 1977). As well, bacteria associated specifically with the heterocysts of blue-green algae have been shown to increase rates of nitrogen fixation (Paerl 1976, 1978, Paerl and Kellar 1978): bacterial respiration reduces oxygen levels in a microzone around the

heterocysts, thereby decreasing inhibition of nitrogenase by oxygen. When bacteria are net consumers of inorganic forms of nitrogen, they could further favour nitrogen-fixing algae by depleting supplies for phytoplankton.

Bacterial effects on phytoplankton may also be antagonistic. Thus, bacteria may inhibit algal growth in culture, or kill algae outright (Blasco 1965, Whitton 1973, Fitzgerald 1969, Berland et al. 1972, Darveau and Lynch 1977, Delucca and McCracken 1977). Inhibition is strongest from old bacterial cultures (Berland et al. 1972), perhaps reflecting a buildup of toxin. In general, satellite bacteria depress their host alga much less than other algae (Whitton 1973, Fitzgerald 1969, Delucca and McCracken 1977).

The mechanisms of antagonism are little known, but appear diverse. Serratia marcescens inhibits the growth of algae, especially blue-greens, by producing the pigment prodigiosin (Darveau and Lynch 1977). Lange (1976) speculated that under nutrient-poor conditions bacteria break down the sheath of their blue-green hosts, thereby reducing the ability of the algae to concentrate limiting nutrients. There are numerous reports of bacteria which lyse algae in culture by means of surface-bound or extracellular enzymes: while some attack a wide spectrum of algae (Stewart and Brown 1969), others appear specific to cyanophytes (Shilo 1970, Daft and Stewart 1971, Granhall and Berg 1972, Burnham

et al. 1976), and some strains attack only a single algal species (Coder and Starr 1978).

Bacteria may also be important in organizing zooplankton communities. They are a productive component of the seston of many lakes, particularly in eutrophic waters (Straskraba and Straskrabova 1969, Winberg 1972, Godliewska-Lipowa 1974a, 1974b), and as a result make up a major fraction of zooplankton diets (Gliwicz 1969a, Hillbricht-Ilkowska 1977). For instance, on the basis of experiments in a series of Polish lakes, Gliwicz (1969a) estimated that bacteria make up 70-85% of the zooplankton diet in eutrophic lakes and at least 30% in less productive ones. Further, only a subset of zooplankton species are capable of ingesting single cells (see reviews by Gliwicz 1969b, Hillbricht-Ilkowska 1977). Among rotifers, species with a finely dissected mastax and a ciliated buccal funnel ingest bacteria readily, while species with a robust mastax and an aciliate buccal funnel do not (Pourriot 1977, Starkweather et al. 1979, Bogdan et al. 1980, Bogdan and Gilbert 1982). As for crustaceans, single cells are accessible to those with a fine-meshed filtering apparatus, particularly the cladocera (Manuilova 1958, Monakov and Sorokin 1961, 1972, Saunders 1969, Tezuka 1971, Haney 1973, Peterson et al. 1978, Pedros-Alio and Brock 1983), and the naupliar stages of copepods (Malovitskaya and Sorokin 1961, Saunders 1969, Monakov 1972, Gophen et al. 1974, Pedros-Alio and Brock 1983). In

contrast, bacteria are generally not ingested by calanoid copepods, which possess a coarser filtering apparatus (Malovitskaya and Sorokin 1961, Kibby 1971, Monakov and Sorokin 1972, Sorokin 1972, Sorokin and Paveljeva 1972, Haney 1973), nor by the raptorial cyclopoid copepods (Sorokin 1972, Sorokin and Paveljeva 1972, Pedros-Alio and Brock 1983), unless the bacteria are clumped or attached to particles. When food limits zooplankton production, differing abilities to ingest individual bacteria may control zooplankton composition by altering the competitive balance among species.

Finally, bacterial pathogens on fishes could exert an indirect structuring effect by removing planktivorous fish species. Certain aeromonad pathogens selectively attack such important planktivores as salmonids (Bullock et al. 1971) and percids (Ross et al. 1960). Reduced vertebrate predation intensity would shift the zooplankton size structure toward larger-bodied species, and the annual reproduction of most fishes would ensure that this impact would persist for many months.

## 1.3 STRUCTURING POTENTIAL REALIZED?

However, it is not clear whether the potentials of bacteria as a structuring agent are expressed in situ. Effects of bacterial mineralization or nutrient depletion on phytoplankton composition have yet to be investigated.

Similarly, the role of cyanophyte sheaths in concentrating nutrients is still highly speculative (Lange 1976).

While bacteria might very well supply carbon dioxide or vitamins to algae, these nutrients may so seldom limit algae that they are not important in determining phytoplankton composition. The strongest evidence for carbon limitation comes from phosphorus-rich cultures, and such atypical water bodies as sewage lagoons and experimentally fertilized lakes (see Paerl 1982). Field enrichments indicate that vitamins are not limiting in moderately productive lakes (Smith et al. 1984).

Other bacterial effects on phytoplankton may be artifacts of studying such effects in culture. Antagonistic and stimulatory interactions are generally studied in rich cultures, where the densities of bacteria are orders of magnitude higher than in situ: lower natural levels may preclude many interactions which are effected by chemical means, or rely on chance encounter. As well, turbulence in the lake probably prevents most bacterial lysis of algae, as many lytic bacteria require prolonged contact with an alga to successfully attack it, and as a result are ineffectual in a liquid milieu (Shilo 1970, Daft and Stewart 1973, Coder and Starr 1978). Further, some interactions are suspect inasmuch as they depend on the method of assay: bacteria are frequently ascribed stimulatory or inhibitory properties on the basis of disc assay, but filtrates of the same

cultures often produce no effect (Ukeles and Bishop 1975, Delucca and McCracken 1977). In one instance, enhancement of algae was traced to bacterial hydrolysis of the agar on which the algae were grown (Ukeles and Bishop 1975).

In addition, individual algae vary considerably in their susceptibility to lysis (Granhall and Berg 1972, Coder and Starr 1978) or chemical inhibition (Whitton 1973, Berland et al. 1972, Delucca and McGracken 1977), and often develop resistance to such inhibition (Wu et al. 1968, Whitton 1973). Phytoplankton may be similarly resistant in situ, particularly if bacterial antagonism is an important selective force: it is probably telling that some bacteria producing antibiotics which are active against cyanophytes are also epiphytes on these same phytoplankton (Jones 1982).

As for the zooplankton, there is some question concerning the relative numbers and activity of the free-living versus attached bacteria in natural waters (see review by Paerl 1980). If bacteria associated with particles are much more abundant or active than freely dispersed cells, their availability to a wider range of grazers would eliminate any differential effect of bacteria on microphagous as opposed to macrophagous zooplankton.

Finally, it is not known how important bacterial pathogens are in controlling natural fish populations. Substantial disease-related mortality in the field is seldom reported, but this could reflect either a low incidence of

infection, or a rapid loss of diseased fish to predators, or sinking.

## 1.4 A MANIPULATIVE APPROACH

How can the uncertainty over whether bacteria structure plankton communities be resolved? Certain artifacts arising as a result of high cell densities or inappropriate media could be corrected *in vitro*, by choosing culture conditions more representative of those in lakes. However, the array of organisms present in a given environment is rarely, if ever, reproduced in a laboratory system. This is a serious problem, as the dynamics arising from an interaction between two species can be altered by other organisms. For example, whether limiting nutrients are ultimately converted into small or large algal biomass depends on the intensity of grazing (Gliwicz 1975). By extension, a network of interacting species may yield community dynamics that cannot be understood by studying species or pairs of species in isolation. Bacterial structuring must therefore be tested *in situ*, where the full web of biotic interactions is preserved.

In addition, other known structuring agents are correlated with bacteria seasonally, or along a trophic gradient. For instance, bacterial abundance and production increase in progressively more eutrophic lakes (Straskraba and Straskrabova 1969, Winberg 1972, Godliewska-Lipowa

1974a, 1974b), a pattern also exhibited by net phytoplankton (Pavoni 1963, Gliwicz 1967, 1975, Hillbricht-Ilkowska et al. 1972), planktivorous fish (Larkin and Northcote 1969, Nilssen 1978), and invertebrate predators (Hillbricht-Ilkowska 1977). These latter three factors are major organizers of zooplankton communities. In addition, some of them are probably interdependent: for example, the decomposition of inedible net phytoplankton undoubtedly contributes to bacterial production, and fish predation can produce increases in net phytoplankton through selective removal of large grazers (Hrbacek et al. 1961, Andersson et al. 1978). Distinguishing cause and effect among several factors such as these cannot be based solely on observation. An active approach, employing in situ experimental perturbation, is therefore essential to determine what role bacteria play in structuring the plankton. This study will investigate the response of freshwater plankton communities to controlled manipulations of indigenous bacterial abundance.

Various lines of evidence indicate that the densities of heterotrophic bacteria, which are the majority in most lakes, are frequently near a carrying capacity limited by supplies of readily assimilable organic matter. For instance, indigenous bacteria are stimulated by natural inputs of organic matter: bacterial numbers and activity track closely the extracellular release of DOM by healthy

phytoplankton (Saunders and Storch 1971, Tanaka et al. 1974, Overbeck 1975, Nalewajko et al. 1980, Coveney 1982, Straskrabova and Fuska 1982), and by scenescent ones (Jones 1971, 1976, Overbeck 1975, Coveney et al. 1977). Also, while DOM levels in lakes range from approximately 1 to 30 mg/L (Wetzel 1975), the simple organic molecules which are readily available to bacterial heterotrophs are usually present in µg/L quantities (Wright and Hobbie 1966, Allen 1968, Wetzel 1975, Saunders 1976) reflecting the heavy demand for them.

If the bacteria are at carrying capacity, as the preceding evidence suggests, then one would grossly overload the ecosystem by adding them in large numbers. Ideally, manipulation should reduce, not increase, the density or activity of bacteria. Unfortunately, reduction is not possible at present. Antibiotics have been used to inhibit bacteria in situ, but are inconsistent in their impact, because they affect only certain species and growth phases of bacteria (Berland and Maestrini 1969, Yetka and Wiebe 1974, Jones 1977). In addition, antibiotics inhibit both procaryotic and eucaryotic phytoplankton (Yetka and Wiebe 1974, Jones 1977). Filters are commonly used to fractionate plankton according to size, but this is also problematical, as bacteria overlap in size with the smallest phytoplankton. Further, a sometimes considerable fraction of the bacteria in lakes is attached to particles (Paerl 1980), and so cannot be separated by filtration.

As an alternative, this study investigates the response of plankton communities to *in situ* additions of cultured bacteria originally isolated from a lake, to determine whether bacterioplankton are important structuring agents in these communities. Since the activity and composition of bacteria and other plankton vary seasonally, enrichment experiments were conducted in both early and late summer. In addition, because bacterial effects on phytoplankton often vary with the bacterium concerned (Lange 1971, Ukeles and Bishop 1975, Delucca and McCracken 1977, Baker and Herson 1978, Berger *et al.* 1979), I carried out separate late summer enrichments with two different species of bacteria.

The monitoring protocol, in which the communities studied were regularly censused, did not directly explore the mechanisms underlying community responses to bacteria. Instead, I intend this as a pilot project, with the potential to identify interactions and suggest causal relationships which might not emerge from more conventional studies.

# Chapter II

# MATERIALS AND METHODS

## 2.1 STUDY LAKE

The field experiments were conducted during the summers of 1980 and 1982 in Heney Lake, Quebec (46° 22' N, 75° 55' W), a moderately productive lake located approximately 100 km north of Ottawa. This hardwater lake (pH range: 7.8-8.8) has a surface area of 1250 ha, a maximum depth of 25 m, and an average depth of 10 m. Nutrient levels are low, with $PO_4^{3-}$ concentrations typically less than 1 µg/L (Briand et al. 1978). The entire water column remains well-oxygenated throughout the summer (Table 1; McCauley 1978).

The phytoplankton succession in Heney Lake, typical of northern temperate mesotrophic lakes, has been described by Wall (1980). It is characterized by spring and fall diatom peaks dominated by Asterionella formosa, and a late summer blue-green maximum dominated by Aphanizomenon flos-aquae and Anabaena spp. Chlorophytes, chrysophytes, cryptophytes and dinoflagellates are present as minor components of the plankton.

All field studies were conducted in a protected bay, 12 m in depth, at the north end of the lake (Figure 1).

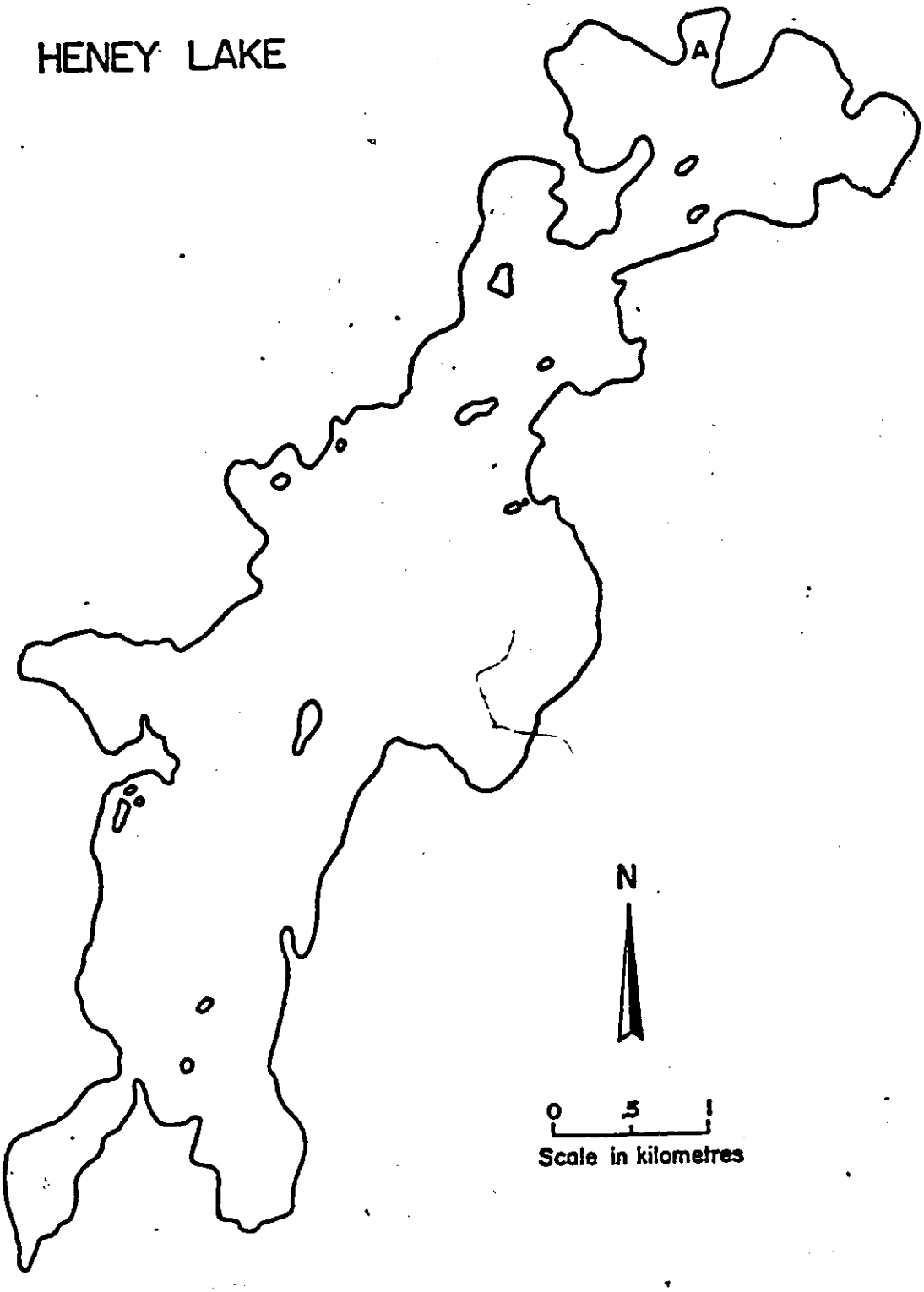
Table 1

Epilimnetic (2 m) and hypolimnetic (8 m) dissolved oxygen levels (mg/L) in Heney Lake during the summer of 1980.

| DATE | DEPTH | |
|---|---|---|
| | 2 m | 8 m |
| June 11 | 10.8 | 10.7 |
| June 18 | 10.2 | 10.1 |
| June 25 | 9.2 | 9.2 |
| July 2 | 9.25 | 9.6 |
| July 9 | 9.1 | 9.3 |
| July 16 | 8.8 | 8.85 |
| July 23 | 8.75 | 8.7 |
| July 31 | 8.6 | 8.3 |
| August 7 | 8.3 | 8.15 |
| August 14 | 8.65 | 8.7 |
| August 21 | 8.7 | 7.9 |
| August 28 | 8.65 | 6.3 |
| September 4 | 8.9 | 7.65 |
| September 11 | 9.1 | 8.9 |

Figure 1

Map of Heney Lake, Quebec. "A" marks the study area.


HENEY LAKE
A
N
0
.5
1
Scale in kilometres

## 2.2 BACTERIAL ISOLATION AND SELECTION

Bacteria were isolated from the study site prior to the experiments. Subsurface water samples were taken with a sterile hand-held bottle and refrigerated on their way to the laboratory. Subsamples were then serially diluted in sterile distilled water, and 0.1 mL of each dilution spread-plated onto 0.1% Bacto-peptone (Difco) agar, 1.0% Bacto-peptone agar, and 0.5% Bacto-peptone plus 0.5% Yeast Extract (Difco) Acetate agar (Skerman 1968). Plates were incubated at 37 (±1) °C in the dark for 36 hours. Strains of bacteria were distinguished by colony morphology, and several of the more common types transferred to 1.0% Bacto-peptone plates, on which they were maintained throughout the study. Transfer and incubation were performed repeatedly to ensure purity of the strains.

Batch culture in a rich liquid medium was chosen as a suitable method to yield the quantity of cells necessary for enrichment. Accordingly, several of the more abundant bacterial strains isolated were screened for their ability to grow well on the medium used (1.0% Proteose Peptone No. 3 (Difco) in distilled water) at the range of temperatures encountered in the cultures and in the lake (10-37 °C). On this basis, indigenous strains of Aeromonas hydrophila anaerogenes and Serratia marcescens (an unpigmented form, at least under the culture conditions) were chosen as the enrichment bacteria. In common with many limnetic bacteria,

these cosmopolitan species are motile, facultatively anaerobic, Gram negative rods with a generalized heterotrophic metabolism (Rheinheimer 1981).

Bacterial identifications were based on standard morphological and biochemical tests, primarily from the API 20E system (Analytab Products, Plainview, New York). The test results are summarized in Table 2.

Table 2

Results of test used in identifying enrichment bacteria.

"a" indicates test spoiled

"b" indicates that test was not done

"c" indicates a result contrary to Bergey's Manual of Determinative Bacteriology, 7th edition

"d" indicates that Bergey's reports that aberrant strains do occur

| Diagnostic Character | *Aeromonas hydrophila anaerogenes* | *Serratia marcescens* |
|---|---|---|
| Morphology | rod | rod |
| Gram test | -ve | -ve |
| Oxidase | + | - |
| Lactose fermentation | + | + c |
| Arginine dihydrolase | + | - |
| Lysine decarboxylase | - c | + |
| Ornithine decarboxylase | - | + |
| Citrate | - c | + |
| H2S | - | - |
| Urease | - | - |
| Tryptophane deaminase | - | - |
| Indole | - c,d | - |
| Acetoin (Voges-Proskauer) | + | + |
| Gelatinase | + | + |
| Glucose | + | + |
| Mannitol | a | + |
| Inositol | - | + |
| Sorbitol | + | + |
| Rhamnose | - | - |
| Sucrose | + | + |
| Melibiose | - | - |
| Amygdalin | - | + |
| Arabinose | - c | + c |
| Motility | + | + |
| Nitrate reduction | + | + |
| Lactose Fermentation Broth | acid, no gas | acid, no gas c |
| Moeller's Broth | - | - |
| Mannitol fermentation | acid & gas | acid & gas |
| Sucrose fermentation | acid & gas | acid & gas |
| Glycerol fermentation | no acid or gas | acid & gas c |
| Ammonium Phosphate Broth | - c,d | - |
| Litmus milk | acid & curd late proteolysis | acid & curd late proteolysis |
| Glucose Fermentation Broth | acid, no gas | acid & gas |
| Koser's Citrate Broth | b | + |
| Phenylalanine deaminase | b | - |

## 2.3 BACTERIAL HARVESTING

Bacteria were batch-cultured each week at 37 °C in the dark in the Proteose Peptone medium. As cultures approached stationary phase, cells were harvested by centrifugation for 15 minutes at 4000X g. When all of the medium had been centrifuged, the bacteria were twice resuspended in fresh filter-sterilized (0.2 μm pore size) lakewater to remove growth medium, after which the suspension was transported to the lake for the manipulations.

Preliminary experiments indicated that these procedures did not introduce serious sources of error. Cell loss as a result of the harvesting *per se* was not detectable (Total cells before harvesting (mean±one s.d.) = $1.06^{13} \pm 0.686X10^{13}$--- after = $1.21X10^{13} \pm 0.358X10^{13}$, Mann-Whitney U-test, $p > 0.10$, $n=10$). After their final suspension in filter-sterilized lake water, densities of bacteria declined by approximately 20% within six hours, presumably as a result of lysis, but were then stable for at least 36 hours (Figure 2). During this period, the fraction of cells actively respiring (determined as outlined in "Bacterial Enumeration") also declined, from more than 80% to approximately 30%, a level similar to that observed *in situ*. Finally, levels of soluble reactive phosphorus, total phosphorus, ammonia, nitrate, nitrite, dissolved organic carbon and dissolved inorganic carbon added along with the enrichment suspension were two to five orders of magnitude below ambient levels (Table 3).

Figure 2

Mean bacterial density (± one s.d.) over time in enrichment suspension.

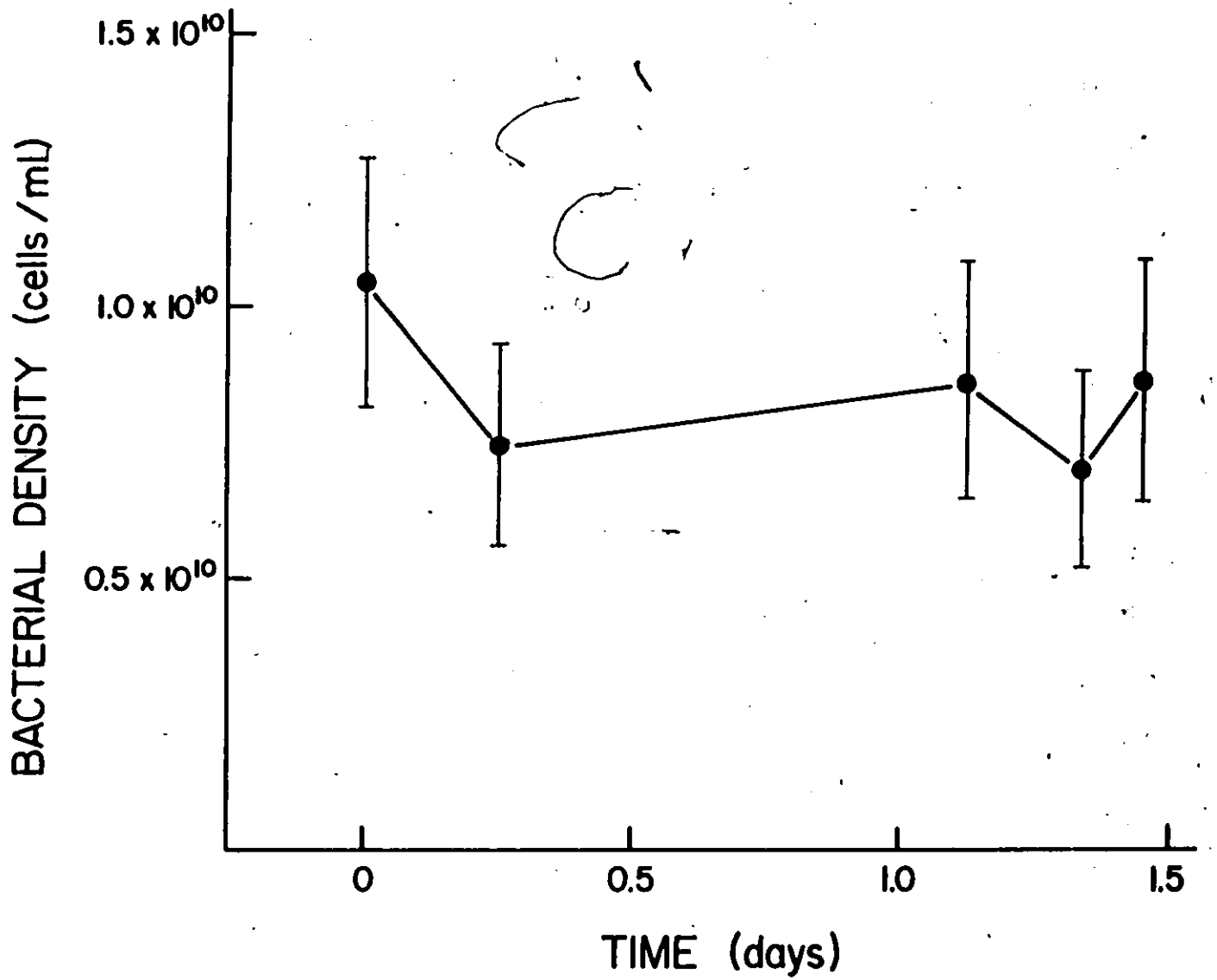

1.5 x 10^10
1.0 x 10^10
0.5 x 10^10
BACTERIAL DENSITY (cells/mL)
0
0.5
1.0
1.5
TIME (days)

Table 3

Levels of dissolved nutrients added along with bacterial suspension, compared to average ambient levels over the summer of 1980. All measurements are given in mg/L. Estimates are based on adding 150 mL of suspension to a 7800 L enclosure. "nd" indicates that a chemical species was not determined.

| | | Total Phosphorus | Soluble Reactive Phosphorus | Ammonia | Nitrate | DOC | DIC |
|---|---|---|---|---|---|---|---|
| Ambient levels (grand mean) | | $1.98 \times 10^{-3}$ | $1.78 \times 10^{-3}$ | $1.35 \times 10^{-3}$ | $7.37 \times 10^{-4}$ | 4.34 | 10.82 |
| Levels added as dissolved nutrients | Aeromonas (run 1) | $3.2 \times 10^{-3}$ | $8.8 \times 10^{-4}$ | $9.4 \times 10^{-3}$ | 0 | nd | nd |
| | Aeromonas (run 2) | $8.6 \times 10^{-4}$ | $2 \times 10^{-7}$ | $2.5 \times 10^{-3}$ | $9.6 \times 10^{-4}$ | $1.5 \times 10^{-4}$ | $4.2 \times 10^{-3}$ |
| | Serratia | $2.8 \times 10^{-3}$ | $3.3 \times 10^{-4}$ | $1.1 \times 10^{-4}$ | $9.6 \times 10^{-4}$ | $1.8 \times 10^{-4}$ | $7.3 \times 10^{-3}$ |

## 2.4 MANIPULATION PROTOCOL

The communities studied were contained in polyethylene tubes 1 m in diameter and 10 m deep, with a capacity of some 7800 L. These were sealed at the bottom, and held open at the top by an aluminum ring to permit gas exchange with the atmosphere. The filling procedure involved submerging the tubes while collapsed, and then drawing the open end up through the water column. Filling was completed with a gasoline-powered pump. The lower end of each enclosure was anchored with cement blocks and a steel bar sealed into the plastic hem, while the top was floated with four carboys and styrofoam floats mounted on the ring. After launching, the enclosures were left unperturbed for one week to allow the communities to return to equilibrium.

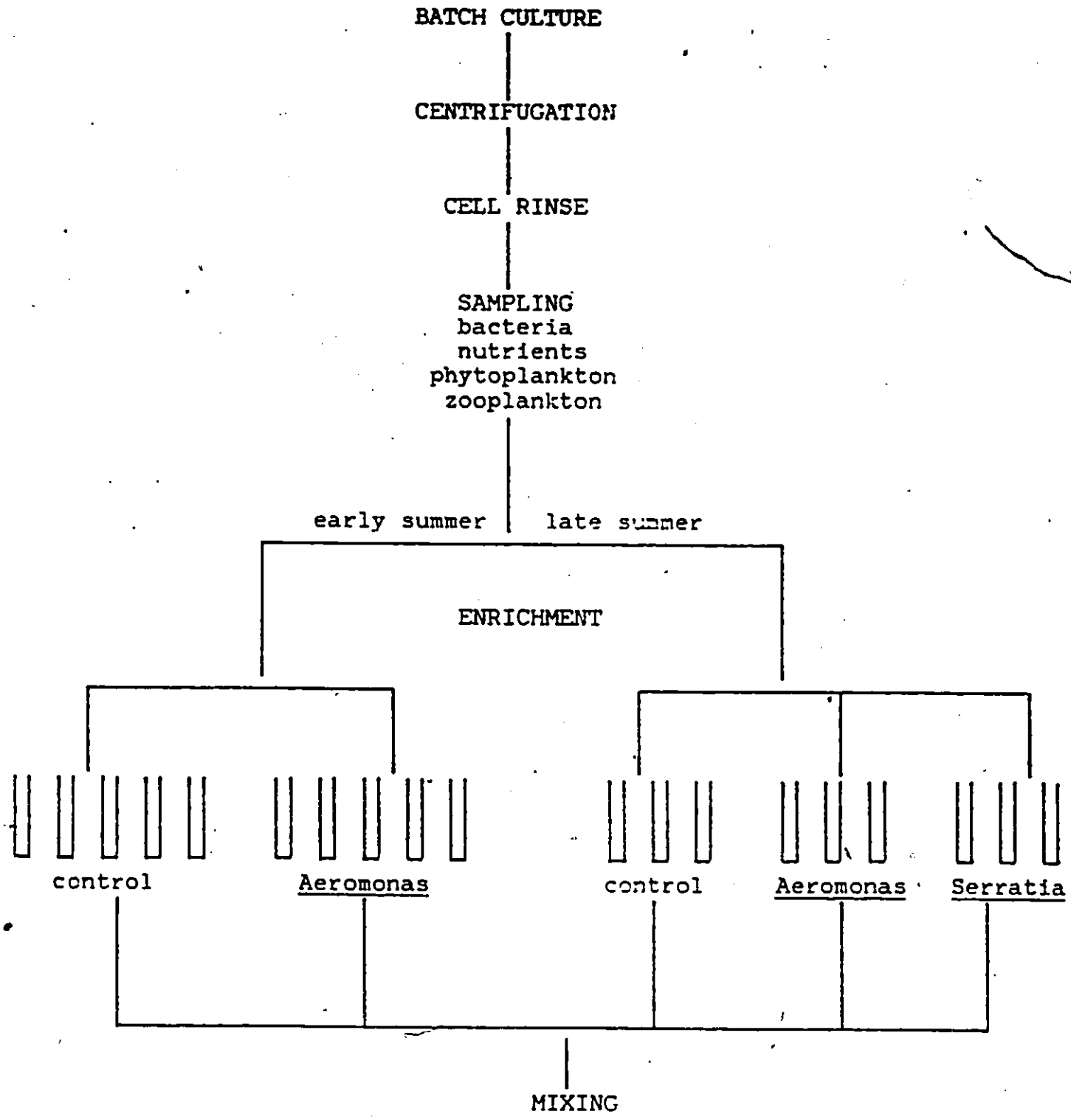
The major enrichment experiments lasted six weeks each. A general outline of the weekly protocol is presented in Figure 3. In the early summer experiment (June 11-July 23), five enclosures were left unenriched as controls, while five were enriched weekly with $1.4 \times 10^5$ Aeromonas cells/mL. In late summer enrichments (July 31-September 11), the protocol was the same except that three enclosures served as controls, three were enriched with Aeromonas, and three with Serratia. Enrichment was performed with a Van Dorn sampler, which was modified to allow the bacterial suspension to trickle out when drawn up the enclosure. To distribute the added cells uniformly in the water column, the enclosures

were thoroughly mixed by several rapid passes of the sampler. Control enclosures were mixed in same fashion.

Prior to each weekly enrichment, epilimnetic (2 m) and hypolimnetic (8 m) dissolved oxygen levels were measured with a YSI dissolved oxygen meter. Water samples were taken for nutrient analysis, phytoplankton censusing, and a total count of bacteria, using a 9 m Tygon tube sampler. To minimize bacterial contamination, sample bottles were heated overnight in a muffle furnace at 550°C to combust any attached cells. In addition, the tubing was rinsed before sampling with several volumes of distilled water, unless particle-free surgical tubing was used. Zooplankton were sampled by a bottom-to-surface vertical haul of an 80 μm mesh conical tow-net.

Figure 3

Outline of protocol for weekly cell production, harvesting, sampling and enrichment.


BATCH CULTURE
CENTRIFUGATION
CELL RINSE
SAMPLING
bacteria
nutrients
phytoplankton
zooplankton
early summer
late summer
ENRICHMENT
control
Aeromonas
control
Aeromonas
Serratia
MIXING

## 2.5 SHORT-TERM ENRICHMENTS

If bacterial changes following enrichment are short-lived, weekly monitoring would then be too infrequent. This would be the case if bacterial levels are increased by enrichment, but they are then heavily grazed by zooplankton, or lyse during the course of the week. (Sedimentation, although a major source of phytoplankton loss from the epilimnion, is not important for free bacterial cells, as they sink very slowly (Jassby 1975)). Further, even if enrichment did not enhance total bacterial levels, it might affect the abundance of the active bacteria, which may be a minor fraction of the total (Stevenson 1978).

Accordingly, a short-term (six day) *in situ* experiment was conducted using *Aeromonas*, to investigate more closely changes following enrichment. Three sets of enclosures were set up in duplicate: (a) controls, (b) enriched enclosures with the grazing community intact, and (c) enriched enclosures with grazers removed. Enrichment techniques and levels were the same as in the six-week experiments. Zooplankton were removed with a Unicomb filtering apparatus (model WYTF 18) fitted with 10 µm W19R10A filter tubes, connected to a gasoline-powered pump. Enclosures were sampled for bacteria using a tube sampler, once prior to addition, then every four hours for the first day, and daily for the next five. Density and volume (estimated from cell dimensions) of both total and actively respiring bacteria were monitored.

Added bacteria were similar morphologically to indigenous ones, which would complicate interpretation of any enrichment effect on bacterial levels. Consequently, a parallel one-week experiment was run in the laboratory, to examine the growth of added cells alone. Total bacterial abundance, and the proportion of actively respiring cells were monitored on the same schedule as in the short-term in situ experiments, in duplicate 500 mL volumes of filter-sterilized lakewater at in situ temperature. Lakewater was filtered at <200 mm Hg vacuum pressure to minimize filtration damage to plankton. A non-inoculated control series was also run, as a check against contamination during sampling, which proved to be nonexistent. All glassware was muffle-furnaced to combust attached organic matter, and all reagents were filter-sterilized. Other apparatus were disposable and particle-free.

## 2.6 BACTERIAL ENUMERATION

Bacterial samples were preserved with 0.1 mL of filter-sterilized 37% formaldehyde per 10 mL of water (Zimmerman et al. 1978), and stored at 4°C in the dark. Before fixation, samples for the one-week field and laboratory experiments were incubated for 20 minutes with 1.0 mL of a 0.2% solution of the tetrazolium dye INT. The electron transport system of actively respiring bacteria

reduces this to INT-formazan, which is deposited as opaque intracellular spots (Zimmerman et al. 1978).

Bacteria were enumerated using the acridine orange INT-formazan reduction technique (Zimmerman et al. 1978). First a suitable volume of the preserved sample was filtered through a 0.1 µm Nuclepore polycarbonate membrane which had been stained for 24 hours in a 1:50,000 solution of Sudan Black B in 50% ethanol. While still in the filter holder, the membrane filter was stained for 3 minutes with 3 mL of a 1:10,000 phosphate-buffered (6.6 mM KHPO4, pH 6.7) acridine orange solution. This was then vacuumed through the filter, after which the filter was removed, air dried and mounted on a clean microscope slide between a drop of Cargille Type A immersion oil and a cover slip. All reagent solutions were filter-sterilized daily.

The stained filter was examined at 1562.5X on a Leitz Ortholux microscope equipped with 3 mm BG 12 and BG 38 transmission filters, a high pressure mercury vapour lamp (HBO 200W), and a Ploempak as a condenser (Daley and Hobbie 1975). Bacteria within an ocular grid (10X10 mm net micrometer, Leitz No. 519902) were counted in twenty randomly-selected fields to a minimum of 400 cells. Total bacterial counts were done with 410 nm light, under which the cells fluoresced orange. Actively respiring bacteria, which were enumerated in the one-week experiments only, displayed INT formazan deposits under transmitted light.

For the one-week experiments, where bacterial biomass was estimated in addition to density, large (volume = 1.0 $\mu m^3$) and small (0.1 $\mu m^3$) cells were recorded separately. Blanks (including all reagents, but excluding lakewater) were run routinely as a check against contamination, which proved negligible.

## 2.7 PHYTOPLANKTON ENUMERATION

Phytoplankton samples were fixed in the field with Lugol's solution and concentrated by sedimentation for 24 hours in a 50 cc sedimentation chamber (Lund et al. 1958). Phytoplankton were counted on a Zeiss inverted microscope at 400X under phase contrast: a minimum of 400 organisms were identified, usually to species, and tallied. Raw counts were converted to volumes (as $\mu m^3/mL$) using standard geometric shapes (Kovala and Larrance 1966) or published cell volumes (Wetzel 1975).

## 2.8 ZOOPLANKTON ENUMERATION

Zooplankton samples were preserved in the field in a 4% formalin and 4% sucrose solution buffered with 3% Na-beta-glycerophosphate. In the laboratory, samples were diluted with tapwater, briskly agitated to randomly distribute the zooplankton, and subsampled with a widemouthed pipette. A minimum of 400 animals, including rotifers, crustaceans, and water mites, were identified to

genus and counted on a Wild M7 stereomicroscope at 30X. Copepods were further subdivided into adults and juveniles. All nauplii were grouped into one category. For large species, the entire sample was counted.

Raw counts were converted to biomass (as µg dry weight/100 L) using estimates compiled from a number of sources (Richman 1958, Comita and Schindler 1963, Cummins et al. 1969, Moshiri and Cummins 1969, Burgis 1975, Dumont et al. 1975, Smyly 1973, Persson and Ekbohm 1980).

## 2.9 NUTRIENT ANALYSIS

Samples for chemical analysis were transported on dry ice to the laboratory, and stored frozen at -20°C. Levels of soluble reactive phosphorus, total phosphorus, nitrite, nitrate, ammonia, dissolved organic carbon, and dissolved inorganic carbon were determined for every second week (weeks 0, 2, 4, and 6). Analyses were performed on an Autoanalyzer II (Technicon Limited) as detailed in Ontario Ministry of the Environment (1975).

In addition, total carbon levels were estimated as the sum of assayed levels in solution and the amounts present in bacterial (Kuznetsov 1970, Horstman and Hoppe 1981), phytoplankton (Vallentyne 1974), and zooplankton biomass (Beers 1966, Baudoin and Raviera 1972). Total amounts of elements added as suspension were estimated from measured cell dimensions, dissolved nutrient levels, and published

values of bacterial density (Jassby 1975) and chemical composition.

## 2.10 STATISTICAL ANALYSIS

Statistical analyses were conducted using SPSS procedures (Nie et al. 1975). Two-factor (enrichment level and week) ANOVA was used to test enrichment effects. Both enrichment and interaction terms were tested in identifying significant responses: unless otherwise indicated, results of ANOVA reported refer to enrichment terms. Other statistical tests used are indicated where appropriate. All variables were log10(x+1)-transformed prior to analysis, as a precaution against non-normality and heterogeneity of variances (Green 1979).

# Chapter III

# RESULTS

## 3.1 BACTERIA

### 3.1.1 In situ experiments

I had originally intended to approximately double ambient bacterial levels by enrichment. These were estimated, based on an early epifluorescence technique (Zimmerman and Meyer-Riel 1974), at slightly more than $1X10^9$ cells/mL. However, during the course of the actual experiments, I discovered that these initial estimates were low by more than an order of magnitude: using an improved epifluorescence technique (Zimmerman et al. 1978), I determined that total bacterial densities usually ranged 2 and 4 million cells/mL (Figure 1). (Evidently background fluorescence had concealed many cells in the preliminary counts). This meant that the additions had increased total indigenous bacterial levels by only about 5%, which is less than the coefficient of variation for counts of replicate fields (grand mean = 10.5%). By the time this was discovered, the experiments were well advanced, and already indicated from oxygen and zooplankton patterns that these bacterial additions actually had a profound impact on the community. This accidental finding was so remarkable in

light of the low degree of enrichment that I decided to complete the experiments under the same conditions, in spite of the attendant difficulties in directly monitoring the added cells.

Densities of total bacteria in the enclosures were fairly stable throughout the season, usually falling between 2X10⁶ and 5X10⁶ cells/mL (Figure 4). These levels are similar to those reported for mesotrophic and eutrophic lakes (Ramsay 1978). Bacterial levels were decidedly more variable at the beginning and end of the summer than in midsummer.

Probably as a consequence of the low addition, bacterial concentrations were not significantly affected by enrichment in either the early or late summer six-week experiments (Figure 4 -- two-factor ANOVA: early summer Aeromonas, $p=0.382$; late summer Aeromonas, $p=0.625$; late summer Serratia, $p=0.138$). Cell numbers added averaged 5.1% of indigenous levels (early summer=4.9%, late summer=5.2%), and ranged from 2.2 to 16.3%.

Similarly, in the six day in situ experiments, bacterial levels were not significantly altered by enrichment: bacterial total density (two-factor ANOVA, $p=0.095$), total volume ($p=0.719$), active density ($p=0.771$), and active volume ($p>0.679$) were all quite similar in control and enriched enclosures (Figures 5-8). Active bacteria were increased in the middle of the first day after enrichment (peak increases are 23% of numbers, and 24% of volume), but this was not sustained.

Grazer removal was extremely effective, removing on average 98.7% of the total zooplankton biomass. However, this reduction had a negligible effect on bacterial levels, which were quite similar in enriched grazer-intact and enriched grazer-reduced enclosures (Figures 5-8).

3.1.2 Laboratory experiments

Following a lag of about eight hours, both bacteria grew rapidly in filter-sterilized lake water for about one to one and a half days (Figure 9). Numbers remained at peak densities for one to two days, and declined gradually over the next several. Serratia reproduced at a faster maximal rate ($r=6.32$ and 5.69 $days^{-1}$) than Aeromonas ($r=3.49$ and 4.55 $days^{-1}$); these growth rates correspond to average doubling times of 0.12 and 0.17 days respectively.

Cell volumes decreased significantly (Mann-Whitney U-test, $p<0.05$ in each of four flasks) over time in all flasks, suggesting that the bacteria might be using stored reserves to reproduce rather than assimilating material for growth from lakewater. Such residual reproduction is commonly observed in bacteria transferred from a rich culture to a starvation regime (eg. Morita 1982). However, in this instance cell volumes decreased by only 27-46%, while numbers multiplied 26- to 48-fold, resulting in an 18- to 25-fold increase in total bacterial volume (Table 4). This indicates that multiplication by the added cells was based on substances dissolved in the lake water, rather than

cellular reserves: bacteria "stepped down" to a starvation regimen divide without growing, producing no change in total biomass (Novitsky and Morita 1978). Instead, average cell volumes were probably reduced because bacterial multiplication was slowed in the lake water relative to the peptone medium: in general, cell volume is inversely related to growth rate at steady state (Echer and Schaechter 1962, Wright and Lockhart 1965, Dean and Rogers 1967, Sheehata and Marr 1971).

Changes in numbers were paralleled by those in activity. After the lag period, the fraction of INT-reducing microorganisms increased markedly from roughly 30-40% to 74-89%, peaking between sixteen hours and two days post-inoculation (Figure 9). Thereafter, the proportion of active cells generally declined over the next several days. The reduction in activity was greater for Serratia than for Aeromonas, but this difference may be artifactual: as the experiment progressed INT deposits in Aeromonas remained easily discernible, while those in Serratia became increasingly diffuse.

Strictly speaking, multiplication of the enrichment bacteria in filter-sterilized lake water cannot be quantitatively extrapolated to their activity in the lake. Both indigenous bacterial competitors for, and algal and zooplankton producers of organic substrates are absent from the laboratory system-- it is difficult to evaluate the net

effect of their removal on the added cells' reproduction. However, in all likelihood growth of the enrichment bacteria *in vitro* is qualitatively similar to that *in situ*, and may be virtually identical during the early stages of incubation before the added cells alter the levels of organic solutes.

Figure 4

Mean total bacterial density (± one s.e.) in control (●——●), Aeromonas -enriched (○- - - -○), and Serratia -enriched (△·····△) enclosures during long term in situ experiments.

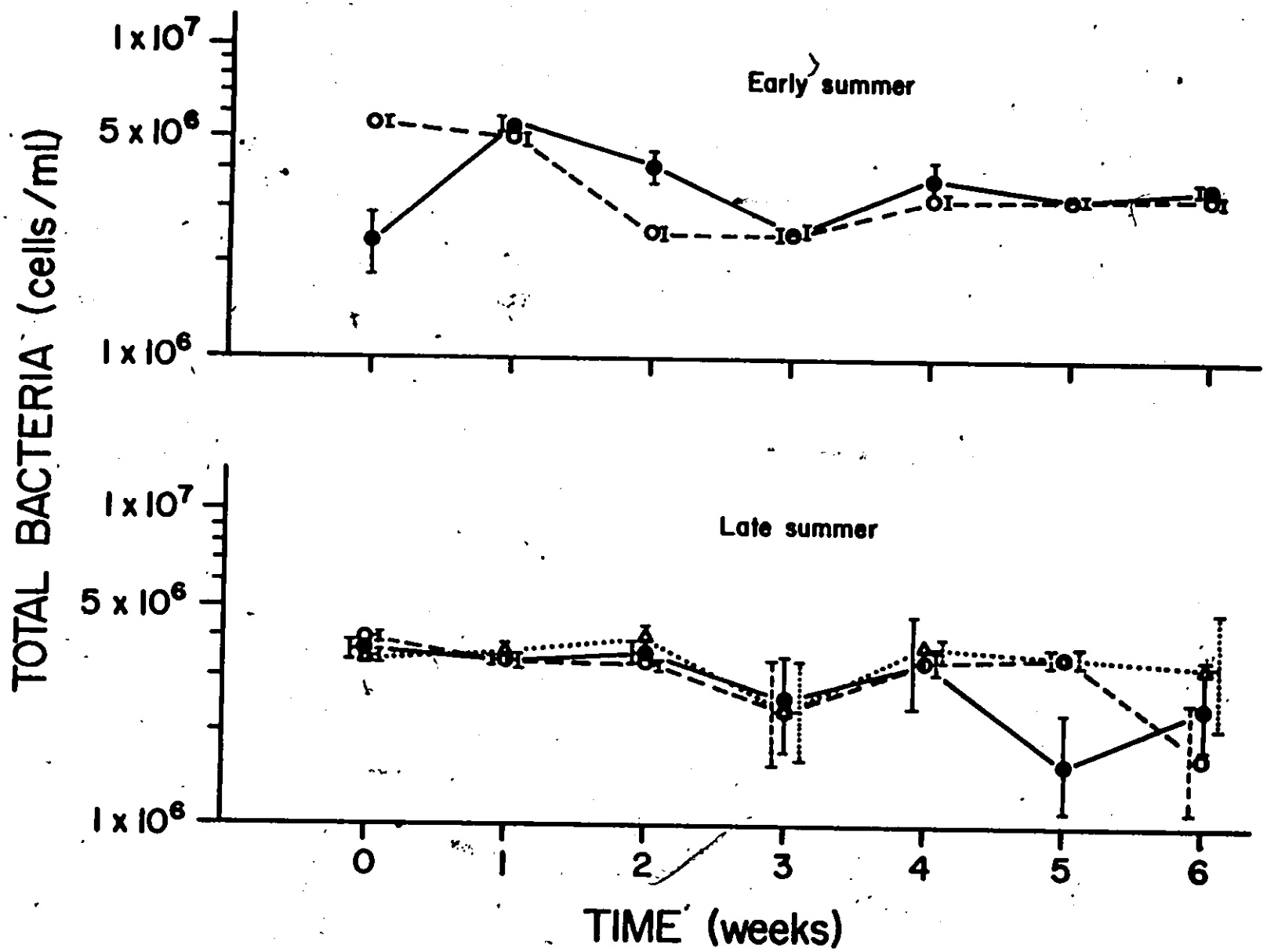

1 x 10^7
5 x 10^6
1 x 10^6
Early summer
Late summer
TOTAL BACTERIA (cells/ml)
0
1
2
3
4
5
6
TIME (weeks)

Figure 5

Mean total bacterial density (± one s.e.) in control (●——●), Aeromonas -enriched grazer intact (○- - - -○), and Aeromonas -enriched grazer-reduced (□-·-·-□) enclosures during short term in situ experiments.

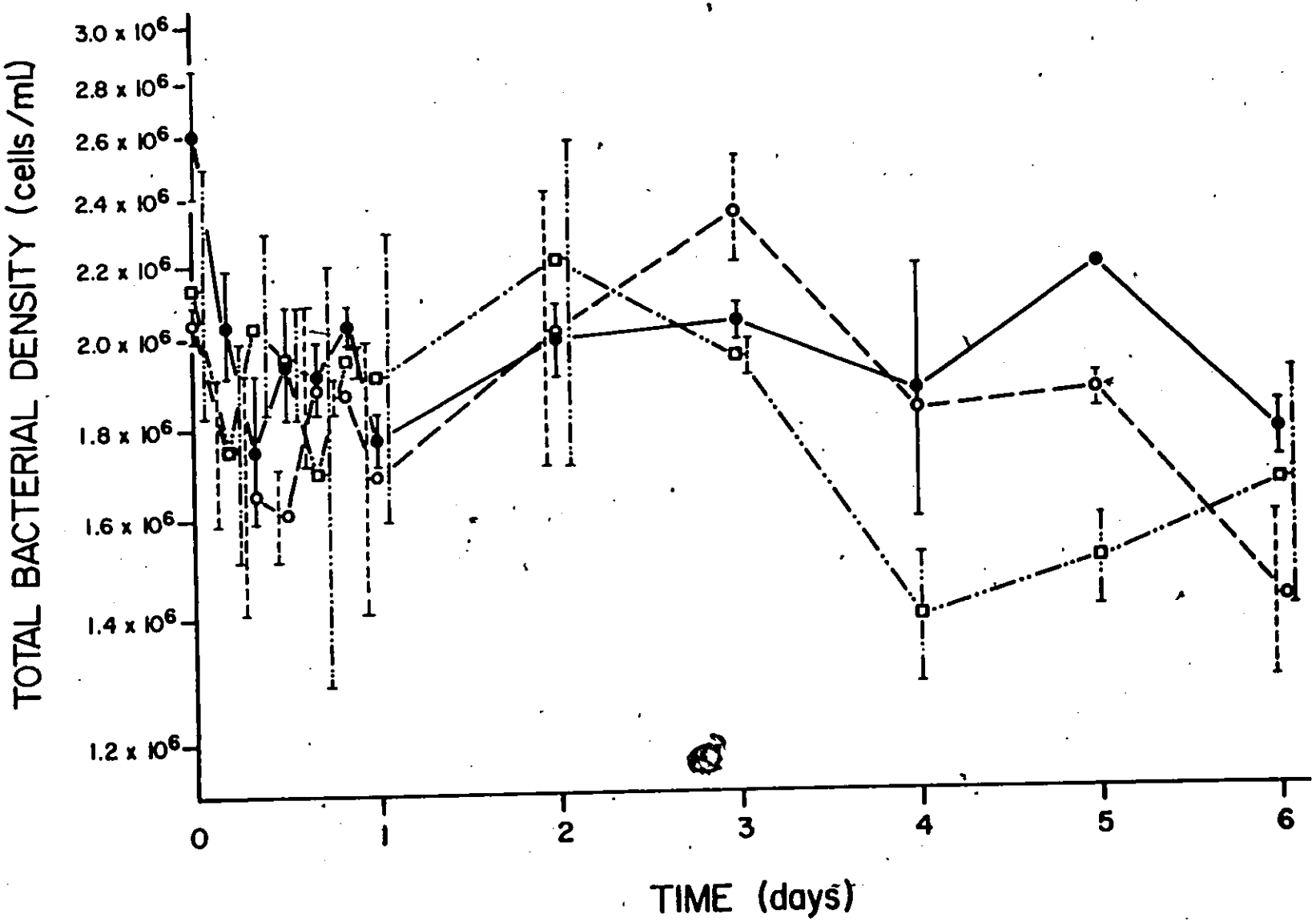

TOTAL BACTERIAL DENSITY (cells/mL)
3.0 x 10^6
2.8 x 10^6
2.6 x 10^6
2.4 x 10^6
2.2 x 10^6
2.0 x 10^6
1.8 x 10^6
1.6 x 10^6
1.4 x 10^6
1.2 x 10^6
0
1
2
3
4
5
6
TIME (days)

Figure 6

Mean total bacterial volume (± one s.e.) in control (●——●), Aeromonas -enriched grazer intact (○- - -○), and Aeromonas -enriched grazer reduced (□-·-·-□) enclosures during short term in situ experiments.

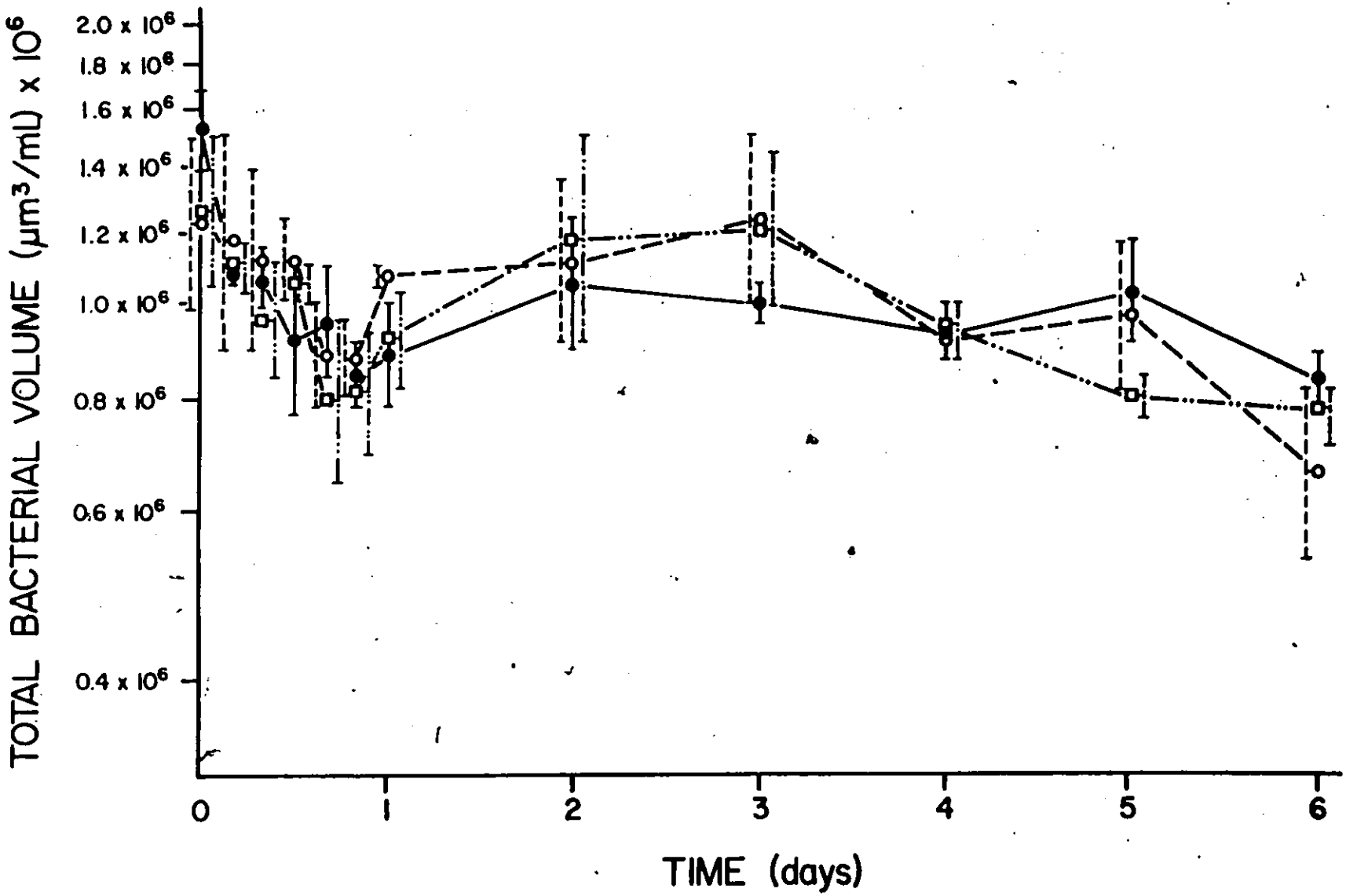

TOTAL BACTERIAL VOLUME (μm³/mL) x 10⁶
2.0 x 10⁶
1.8 x 10⁶
1.6 x 10⁶
1.4 x 10⁶
1.2 x 10⁶
1.0 x 10⁶
0.8 x 10⁶
0.6 x 10⁶
0.4 x 10⁶
0
1
2
3
4
5
6
TIME (days)

Figure 7

Mean active bacterial density (± one s.e.) in control (●——●), Aeromonas -enriched grazer intact (○– – –○), and Aeromonas -enriched grazer reduced (□—·—·—□) enclosures during short term in situ experiments.

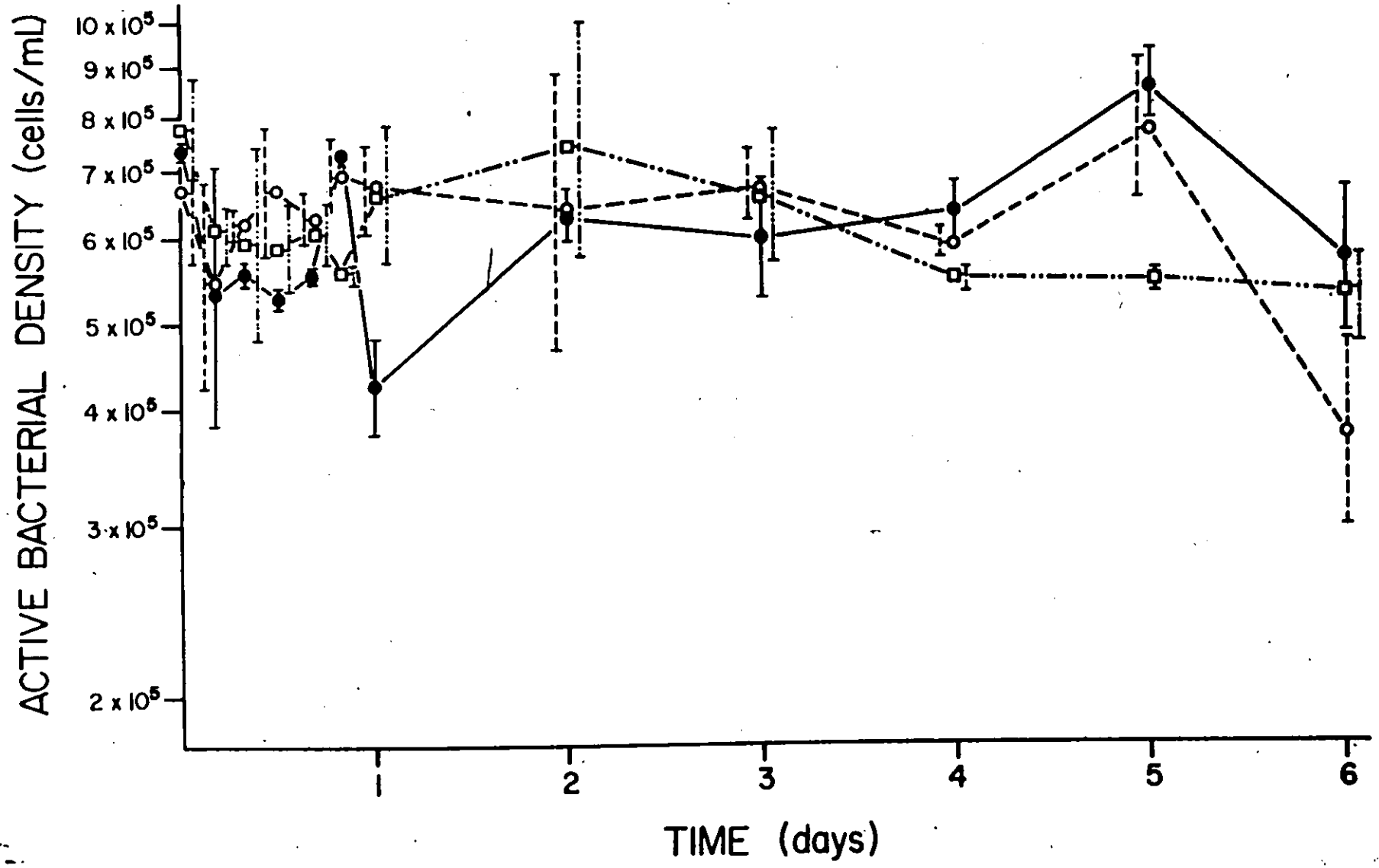

ACTIVE BACTERIAL DENSITY (cells/mL)
10 x 10^5
9 x 10^5
8 x 10^5
7 x 10^5
6 x 10^5
5 x 10^5
4 x 10^5
3 x 10^5
2 x 10^5
1
2
3
4
5
6
TIME (days)

Figure 8

Mean active bacterial volume (± one s.e.) in control (●——●), Aeromonas -enriched grazer intact (○- - - -○), and Aeromonas -enriched grazer reduced (□—·—□) enclosures during short term in situ experiments.

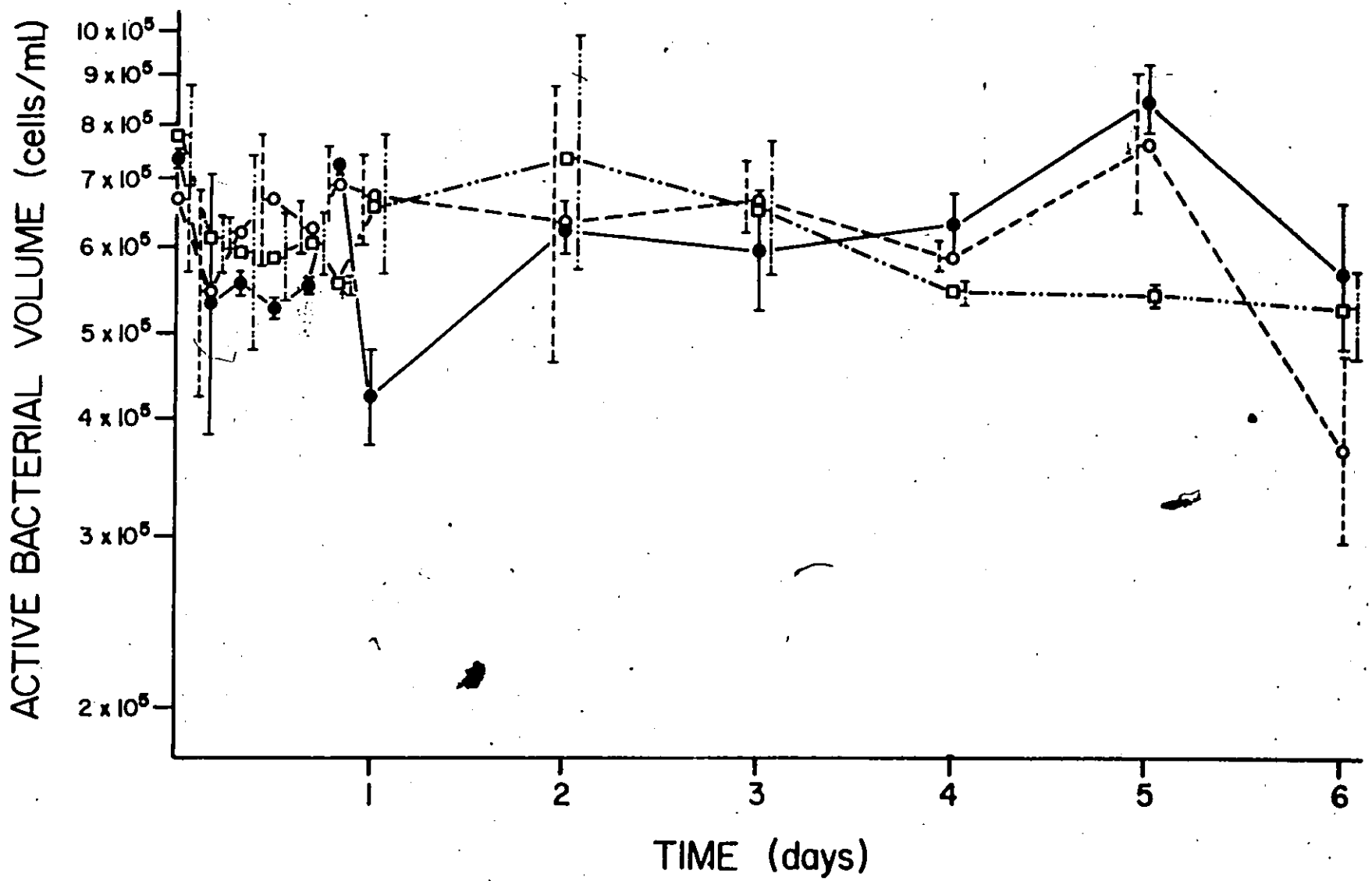

ACTIVE BACTERIAL VOLUME (cells/ml)
10 x 10^5
9 x 10^5
8 x 10^5
7 x 10^5
6 x 10^5
5 x 10^5
4 x 10^5
3 x 10^5
2 x 10^5
1
2
3
4
5
6
TIME (days)

Figure 9

Growth and activity of Aeromonas (○————○) and Serratia (△·····△) in filter-sterilized lake water during short term laboratory experiments. Each experiment was run in duplicate; results for individual flasks are shown.

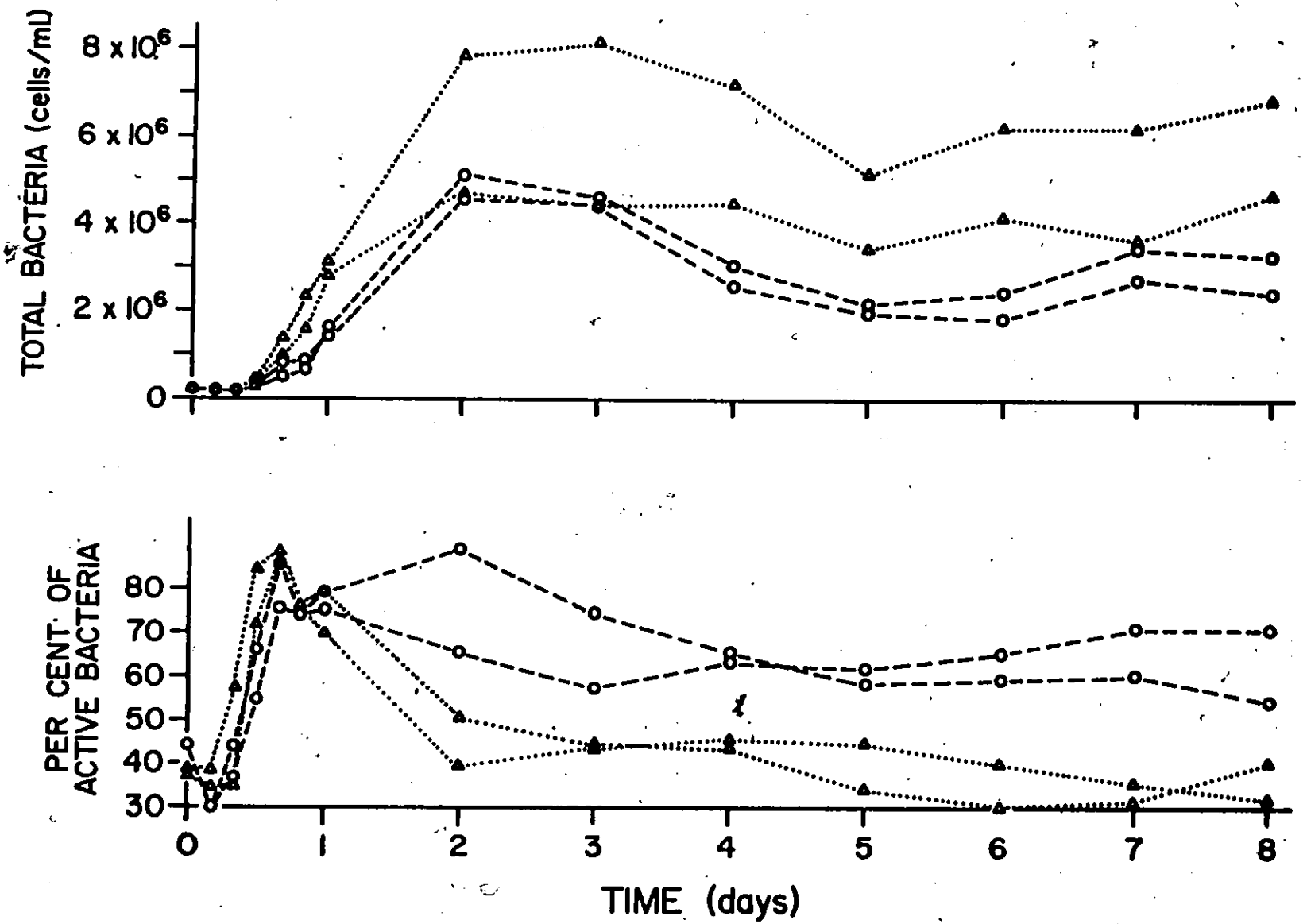

TOTAL BACTERIA (cells/mL)
8 x 10^6
6 x 10^6
4 x 10^6
2 x 10^6
0
PER CENT OF ACTIVE BACTERIA
80
70
60
50
40
30
0
1
2
3
4
5
6
7
8
TIME (days)

Table 4

Comparison of bacterial increase to cell volume decrease in short term laboratory experiments.

| | Peak Density/ Initial Density | Peak Cell Volume/ Initial Cell Volume | Peak Total Bacterial Volume/ Initial Total Bacterial Volume |
|---|---|---|---|
| Aeromonas | 31.2 | 0.604 | 18.8 |
| Aeromonas | 25.7 | 0.731 | 18.8 |
| Serratia | 48.2 | 0.543 | 26.2 |
| Serratia | 31.5 | 0.570 | 18.0 |

## 3.2 PHYTOPLANKTON

The phytoplankton identified included diatoms, green algae, blue-green algae, chrysophytes, cryptomonads and dinoflagellates. Enclosed community biomass was dominated throughout the early summer summer by large species of the diatoms Synedra and Nitzschia, with numerous small flagellates like Chrysochromulina parva as co-dominants during the first two weeks. The filamentous blue-greens Aphanizomenon flos-aquae and several species of Anabaena were not dominant, but comprised a substantial fraction of the biomass.

Diatoms were a minor component of the phytoplankton during late summer experiments. These communities were instead dominated for the first three weeks by the chrysophyte Rhodomonas minuta and the blue-greens Aphanizomenon flos-aquae and Anabaena spp., while in the last half of the experiments the green alga Mougeotia was prevalent.

For analysis, phytoplankton of the same taxonomic division, size and morphology were combined into functional groups, based on evidence that organisms within these groups have similar nutrient uptake rates, growth rates, and photosynthetic characteristics (Malone 1980, Tilman et al. 1982), as well as similar sinking rates (Hutchinson 1967), and vulnerabilities to grazing (Porter 1973).

Bacterial addition increased total phytoplankton volume in all experiments by the second week (Figure 10 -- two-factor ANOVA: early summer Aeromonas, p=0.008; late summer Aeromonas, p=0.083; late summer Serratia, p=0.002). In early summer, volume increased in all enclosures for 3-4 weeks and subsequently declined: Aeromonas -enriched enclosures peaked earlier than controls, producing up to a threefold difference between treatments. Late summer enriched enclosures were in phase with controls, but had as much as two (Aeromonas) to two and a half (Serratia) times the phytoplankton biomass.

These patterns in total volume were almost entirely attributable to a severalfold enhancement of large algae (longest axis $> 30$ μm) by manipulation (Figure 11-- two-factor ANOVA: early summer Aeromonas, p=0.004; late summer Aeromonas, p=0.094; late summer Serratia, p=0.002). Because the volume of large phytoplankton was generally much greater than that of the other size classes, their dynamics were quite similar to those just described for all algae combined. The small ($< 10$ μm) and medium (10-30 μm) phytoplankton were relatively unaffected, except that small cells were approximately doubled in the early summer enrichments (two-factor ANOVA, p=0.039).

When phytoplankton were subdivided along taxonomic lines, diatoms were found to be most strongly affected (Figure 12 -- two-factor ANOVA: early summer Aeromonas, p=0.002; late

summer Aeromonas, p=0.094; late summer Serratia, p=0.001). Total diatom volume was consistently elevated in mid-experiment by enrichment, attaining levels from 3 to 9 times those in controls. While diatoms were not alone in being affected by enrichment, they were the only group to respond consistently across experiments. Total blue-green volume was increased up to 3 or 4 times in early summer Aeromonas manipulations (p=0.010). Green algae were enhanced 6- to 7-fold in the Serratia manipulations (p=0.021).

The responses of individual phytoplankton functional groups are summarized in Table 1. Where particular genera account for much of the group response, they are specified in the table. For the most part the results reflect the enhancements of large algae and diatoms which were identified in the preceding analyses. In addition, early summer manipulations increased the abundance of medium flagellates (Figure 13 -- almost exclusively Rhodomonas), and decreased that of desmids (Figure 14).

It is noteworthy that, although enrichment effects appeared within the first two to three weeks and were often quite strong, they were never sustained to the end of the experiments. Their disappearance generally coincided with directional shifts in algal volume, suggesting that other factors controlling phytoplankton were overriding bacterial enrichment at these times.

Figure 10

Mean total phytoplankton volume (± one s.e.) in control (●———●), Aeromonas -enriched (○– – – –○), and Serratia -enriched (△·····△) enclosures during long term in situ experiments.

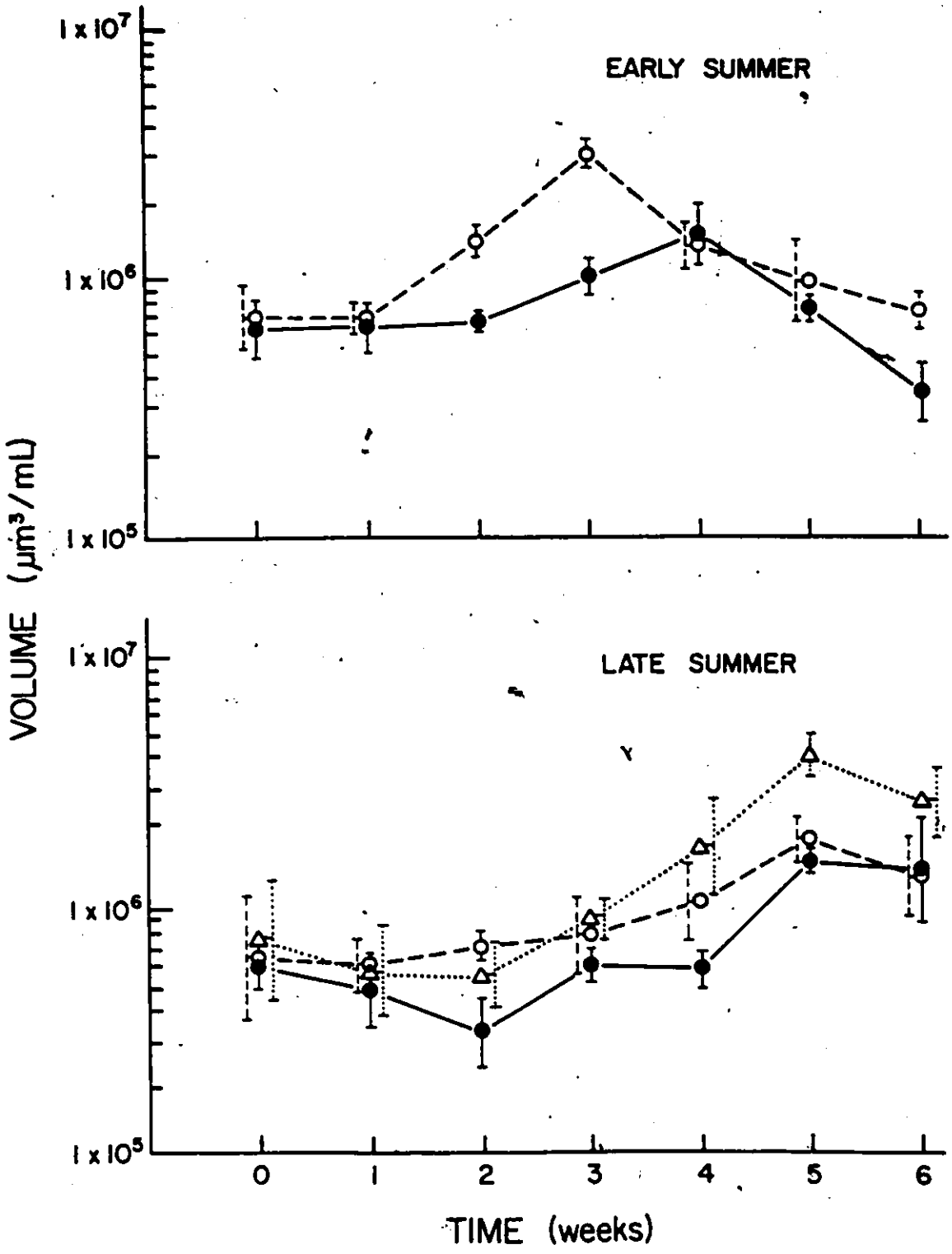

EARLY SUMMER
LATE SUMMER
VOLUME (μm³/mL)
1 x 10⁷
1 x 10⁶
1 x 10⁵
0
1
2
3
4
5
6
TIME (weeks)

Figure 11

Mean volume (± one s.e.) of small, medium and large phytoplankton in control (●——●), Aeromonas -enriched (○– – –○), and Serratia -enriched (△·····△) enclosures during long term in situ experiments.

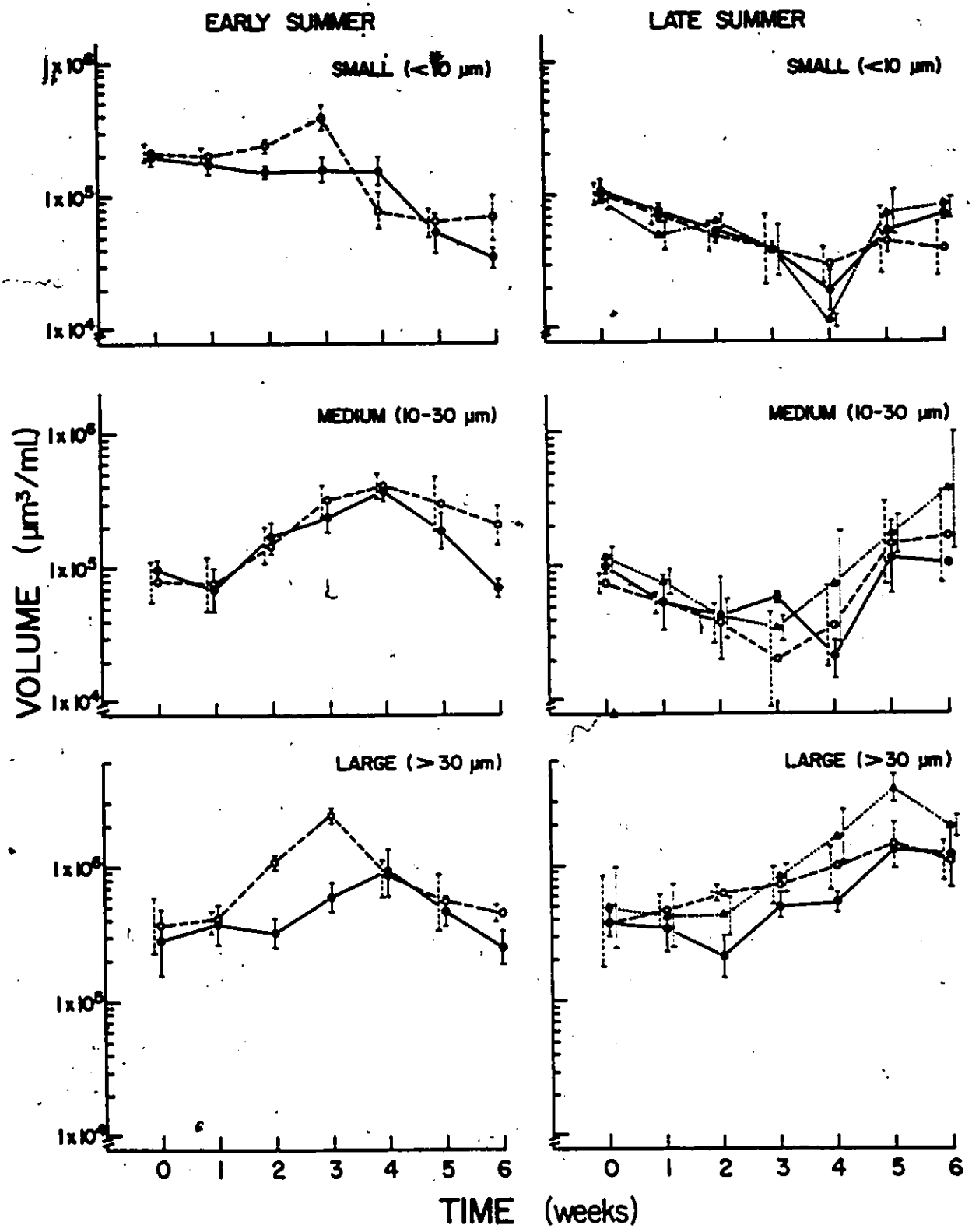

EARLY SUMMER
LATE SUMMER
SMALL (<10 µm)
SMALL (<10 µm)
MEDIUM (10-30 µm)
MEDIUM (10-30 µm)
LARGE (>30 µm)
LARGE (>30 µm)
VOLUME (µm³/mL)
1x10⁶
1x10⁵
1x10⁴
0 1 2 3 4 5 6
TIME (weeks)

Figure 12

Mean volume (± one s.e.) of blue-greens, diatoms, and green algae in control (●——●), Aeromonas -enriched (○– – –○), and Serratia -enriched (△·····△) enclosures during long term in situ experiments.

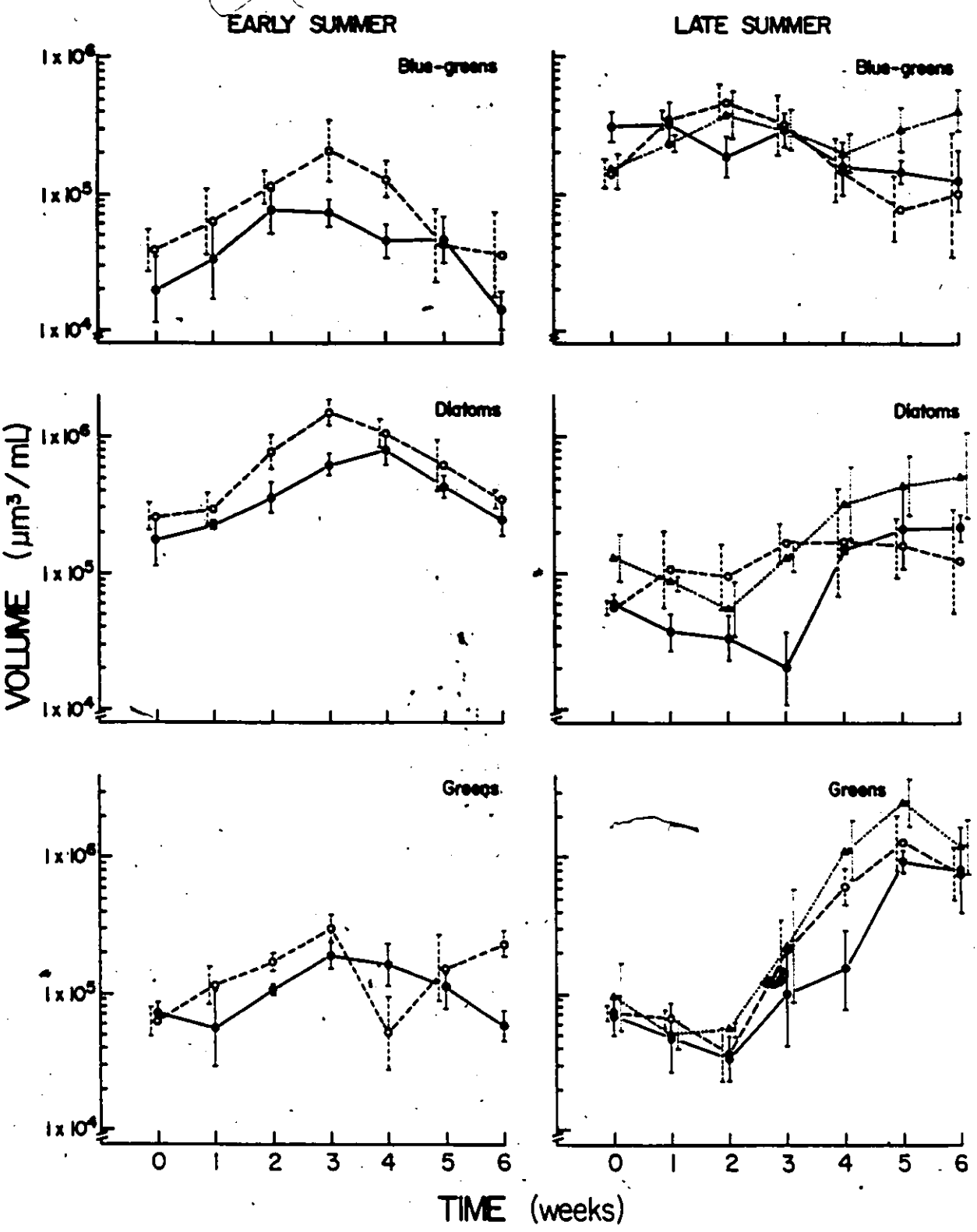

EARLY SUMMER
LATE SUMMER
Blue-greens
Blue-greens
Diatoms
Diatoms
Greens
Greens
1 x 10⁶
1 x 10⁵
1 x 10⁴
VOLUME (µm³/mL)
0 1 2 3 4 5 6
TIME (weeks)

Table 5

Summary of ANOVA results for phytoplankton groups. Arrows indicate the direction of response to enrichment. If the arrow is not enclosed in parentheses, $p<0.05$: if enclosed, $0.05 < p < 0.20$ but results are consistent between experiments. Subscript "i" indicates that only the F-ratio for interaction is significant.

| ALGAL GROUP | EXPERIMENT | | | PRINCIPAL GENERA RESPONDING |
|---|---|---|---|---|
| | Early summer Aeromonas | Late summer Aeromonas | Late summer Serratia | |
| Large colonial blue-greens | | | | |
| Filamentous blue-greens | (↑) | | (↑) | Aphanizomenon, Anabaena |
| Colonial blue-greens | ↑ | | | |
| Unicellular blue-greens | | | | |
| Large pennate diatoms | ↑ | ↑ | ↑ | Synedra, Nitzschia |
| Small pennate diatoms | (↑) | ↑ | ↑i | |
| Large centric diatoms | | | | |
| Small centric diatoms | | | | |
| Filamentous greens | | | | |
| Large desmids | ↓ | | | |
| Small colonial greens | | | | |
| Small unicellular greens | | | | |
| Dinoflagellates | | | | |
| Large flagellates | | | | |
| Medium flagellates | ↑ | | | Rhodomonas |
| Small flagellates | | | | |
| Assorted chrysophytes | | | | |

Figure 13

Mean volume (± one s.e.) of medium flagellates in control (●——●) and Aeromonas -enriched (○– – –○) enclosures during early summer in situ experiments.

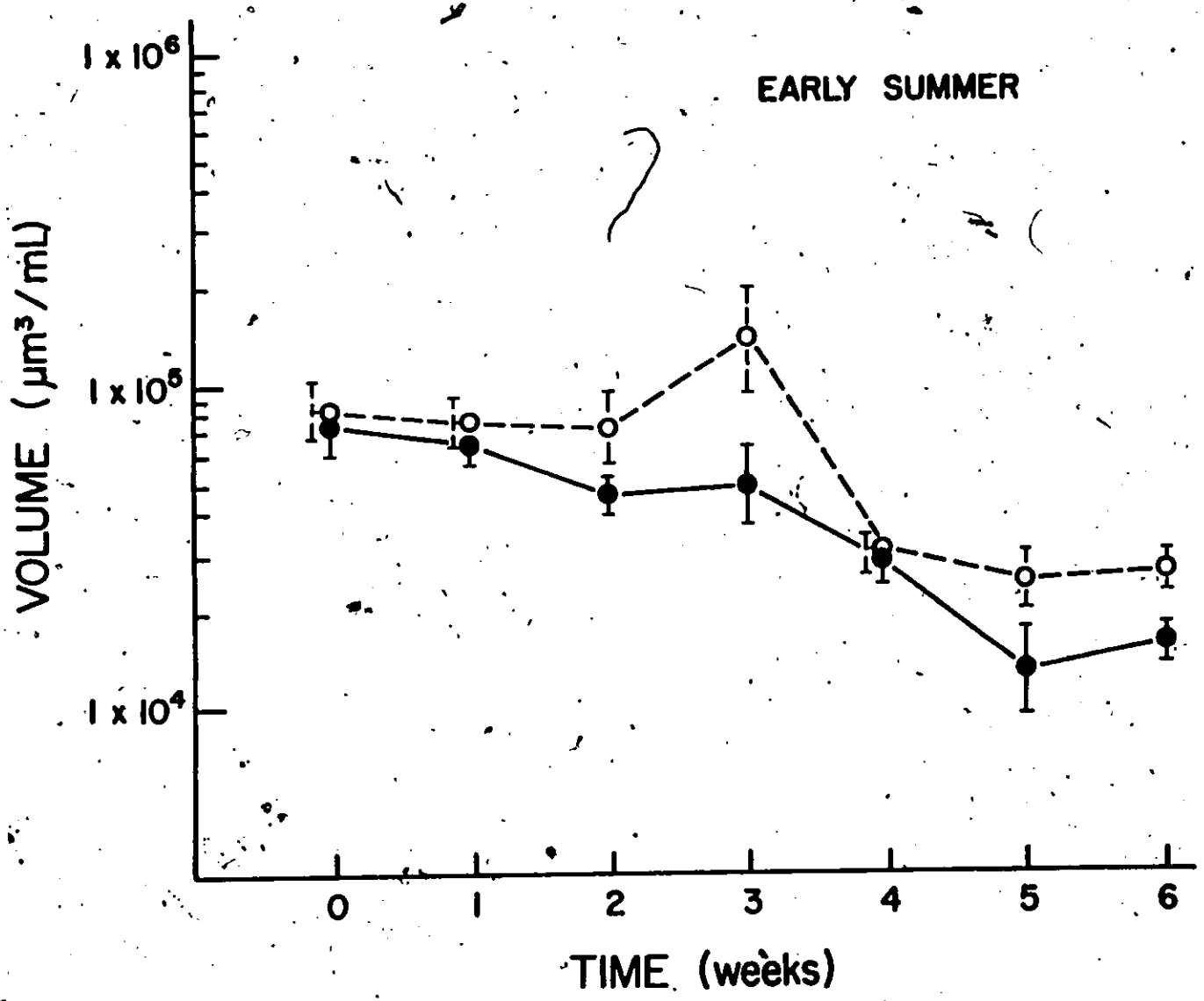
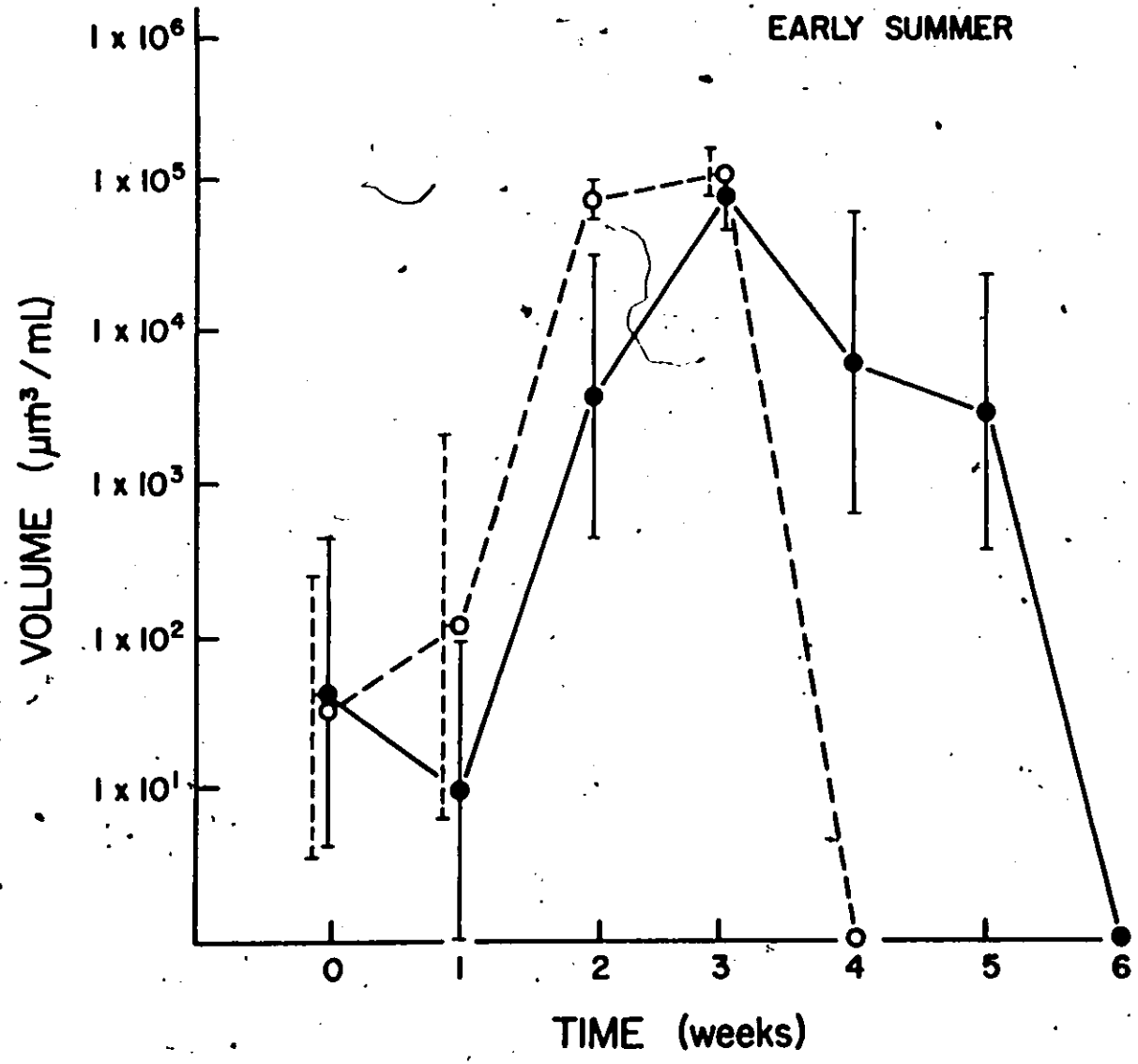

EARLY SUMMER
1 x 10⁶
1 x 10⁵
1 x 10⁴
VOLUME (μm³/mL)
0
1
2
3
4
5
6
TIME (weeks)

Figure 14

Mean volume (± one s.e.) of desmids in control (●——●) and Aeromonas -enriched (○- - -○) enclosures during early summer in situ experiments.


EARLY SUMMER
1 x 10⁶
1 x 10⁵
1 x 10⁴
1 x 10³
1 x 10²
1 x 10¹
VOLUME (μm³/mL)
0
1
2
3
4
5
6
TIME (weeks)

## 3.3 ZOOPLANKTON

A total of 23 zooplankton genera were identified (Table 5), including 8 rotifers, 9 cladocera, 5 copepods, a water mite and a midge larva. In the early summer experiments, the zooplankton was dominated for the first three weeks by juvenile stages of Acanthocyclops, and for the last three by large cladocera as Sida populations increased. Rotifers (mostly Kellicottia) and small cladocera (Bosmina, then Chydorus) were numerous throughout, although subdominant in terms of biomass. Among the carnivores, Asplanchna predominated at first, but was succeeded by carnivorous stages of Acanthocyclops and the water mite Piona in turn.

Late summer enclosed communities were dominated throughout by large cladoceran biomass, first Holopedium and then Sida. All herbivorous copepod genera were important sub-dominants early in the experiments, while the small cladoceran Chydorus became abundant near the end. Piona and the carnivorous stages of Mesocyclops were the most common carnivores throughout.

Patterns in herbivorous and carnivorous zooplankton biomass with manipulation are presented in Figure 15. Their levels were not significantly affected by enrichment with Aeromonas, but both herbivore (two-factor ANOVA, $p=0.002$) and carnivore fractions ($p=0.018$) were increased by late summer Serratia manipulation. Herbivore levels in controls were relatively stable, while in Serratia -enriched

enclosures they increased severalfold until mid-experiment, and then declined to control levels by the final week. At its peak, average herbivore biomass was four times greater in manipulated enclosures than controls. Carnivore levels increased for the first week and then declined during the rest of the experiment: enrichment slowed this decline, eventually producing a fourfold difference between Serratia -enriched and control enclosures.

The herbivore response was the result of changes in cladocera only. Levels of large cladocerans, primarily Sida and to a lesser extent Holopedium, were up to three times as high in enriched enclosures as controls (Figure 16 -- two-factor ANOVA, $p=0.057$). Small cladocerans, mostly Chydorus, were increased four times over controls in the final weeks of the experiment (Figure 17 -- $p=0.068$). The elevated carnivore levels with Serratia manipulation reflected increases in large carnivores ($p=0.035$), particularly the water mite Piona, which was elevated threefold in enriched enclosures during the last three weeks (Figure 18).

The responses of individual genera to manipulation are summarized in Table 7. For the Serratia manipulation these primarily reinforce the increases in cladocerans and carnivores just described. Other patterns were more consistent across experiments. Thus, although most rotifers were unaltered by enrichment, those genera that did respond

(Euchlanis and Bipalpus) were both enhanced. In contrast, a number of copepod genera, particularly Diaptomus, were depressed with enrichment.

Table 6

List of zooplankton distinguished during the study, and their feeding type.

| | HERBIVORES | CARNIVORES |
|---|---|---|
| **ROTIFERS** | | |
| *Asplanchna* | | + |
| *Bipalpus* | + | |
| *Euchlanis* | + | |
| *Kellicottia longispina* | + | |
| *Keratella* spp. | + | |
| *Ploesoma* | + | |
| *Trichocerca cylindrica* | + | |
| *Trichocerca multicrinis* | + | |
| **CLADOCERANS** | | |
| *Bosmina longirostris* | + | |
| *Ceriodaphnia lacustris* | + | |
| *Chydorus sphaericus* | + | |
| *Daphnia longiremis* | + | |
| *Daphnia retrocurva* | + | |
| *Daphnia galeata mendotae* | + | |
| *Diaphanosoma leuchtenbergianum* | + | |
| *Holopedium gibberum* | + | |
| *Leptodora kindtii* | | + |
| *Polyphemus pediculus* | | + |
| *Sida crystallina* | + | |
| **COPEPODS** | | |
| CALANOIDS | | |
| *Diaptomus* spp. | + | |
| *Epischura lacustris* | | + |
| CYCLOPOIDS | | |
| *Acanthocyclops vernalis* | | |
| Copepodites I-III | + | |
| Adults, cop.IV-V | | + |
| *Mesocyclops edax* | | |
| Copedodites I-III | + | |
| Adults, cop.IV-V | | + |
| *Tropocyclops prasinus* var. *mexicanus* | + | |
| Nauplii | + | |
| **INSECTS** | | |
| *Chaoborus* | | + |
| **WATER MITES** | | |
| *Piona constricta* | | + |

Figure 15

Mean biomass (± one s.e.) of herbivorous and carnivorous zooplankton in control (●——●), *Aeromonas* -enriched (○– – –○), and *Serratia* -enriched (△·····△) enclosures during long term *in situ* experiments.

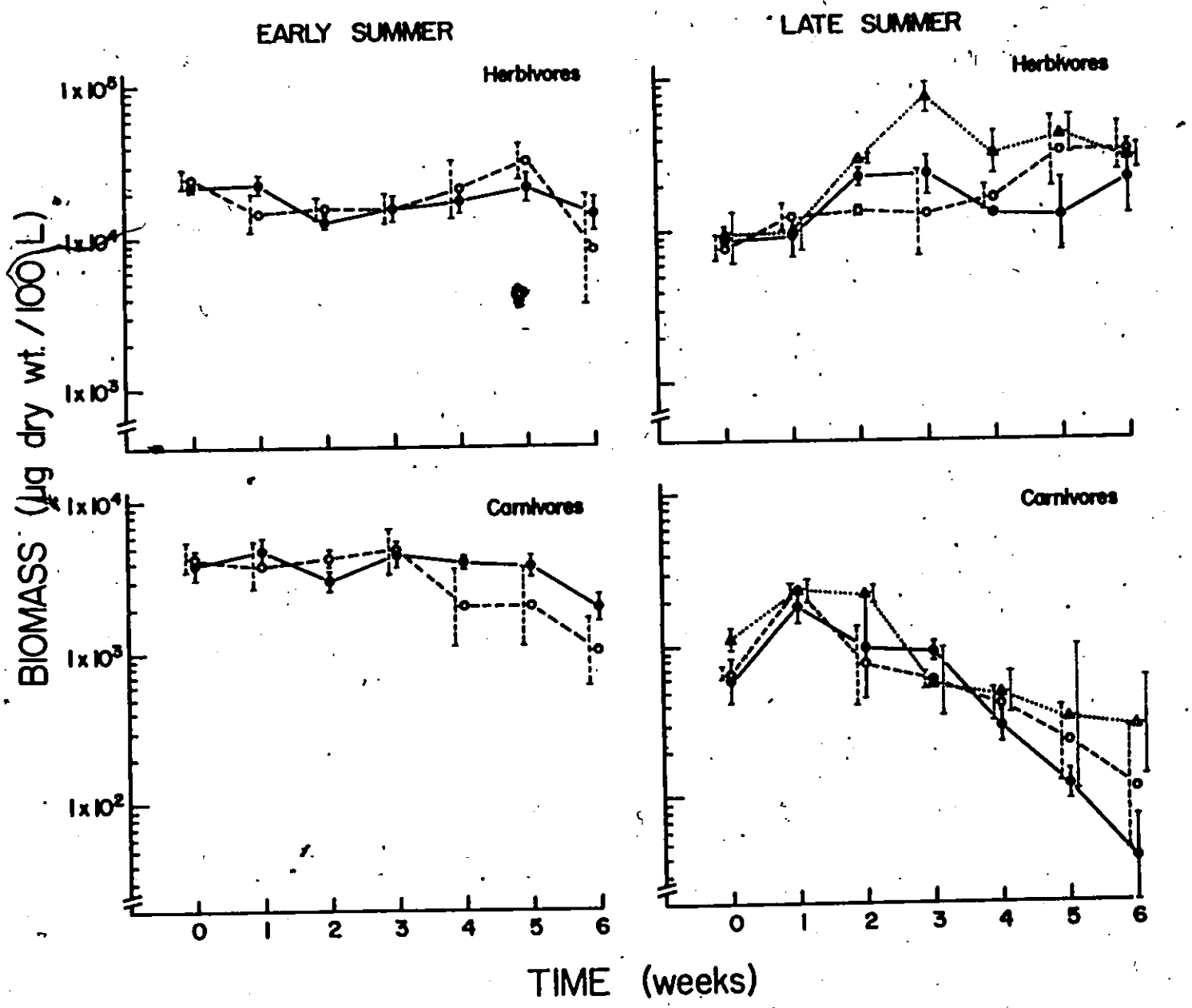

EARLY SUMMER
LATE SUMMER
Herbivores
Herbivores
Carnivores
Carnivores
1x10⁵
1x10⁴
1x10³
1x10⁴
1x10³
1x10²
BIOMASS (µg dry wt. / 100 L)
0
1
2
3
4
5
6
TIME (weeks)

Figure 16

Mean biomass (± one s.e.) of large cladocera in control (●——●) and Serratia -enriched (△·····△) enclosures during long term late summer in situ experiments.

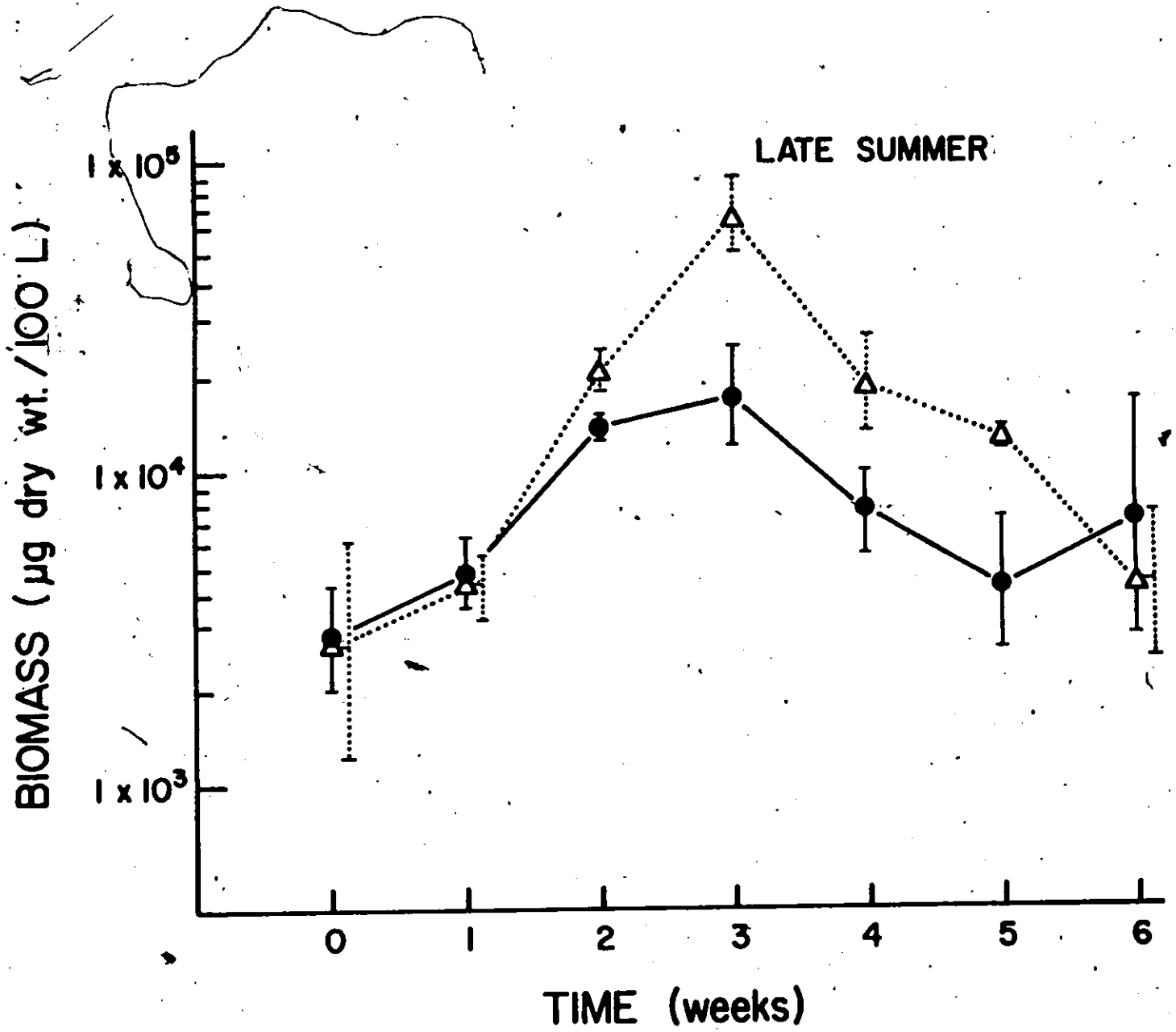
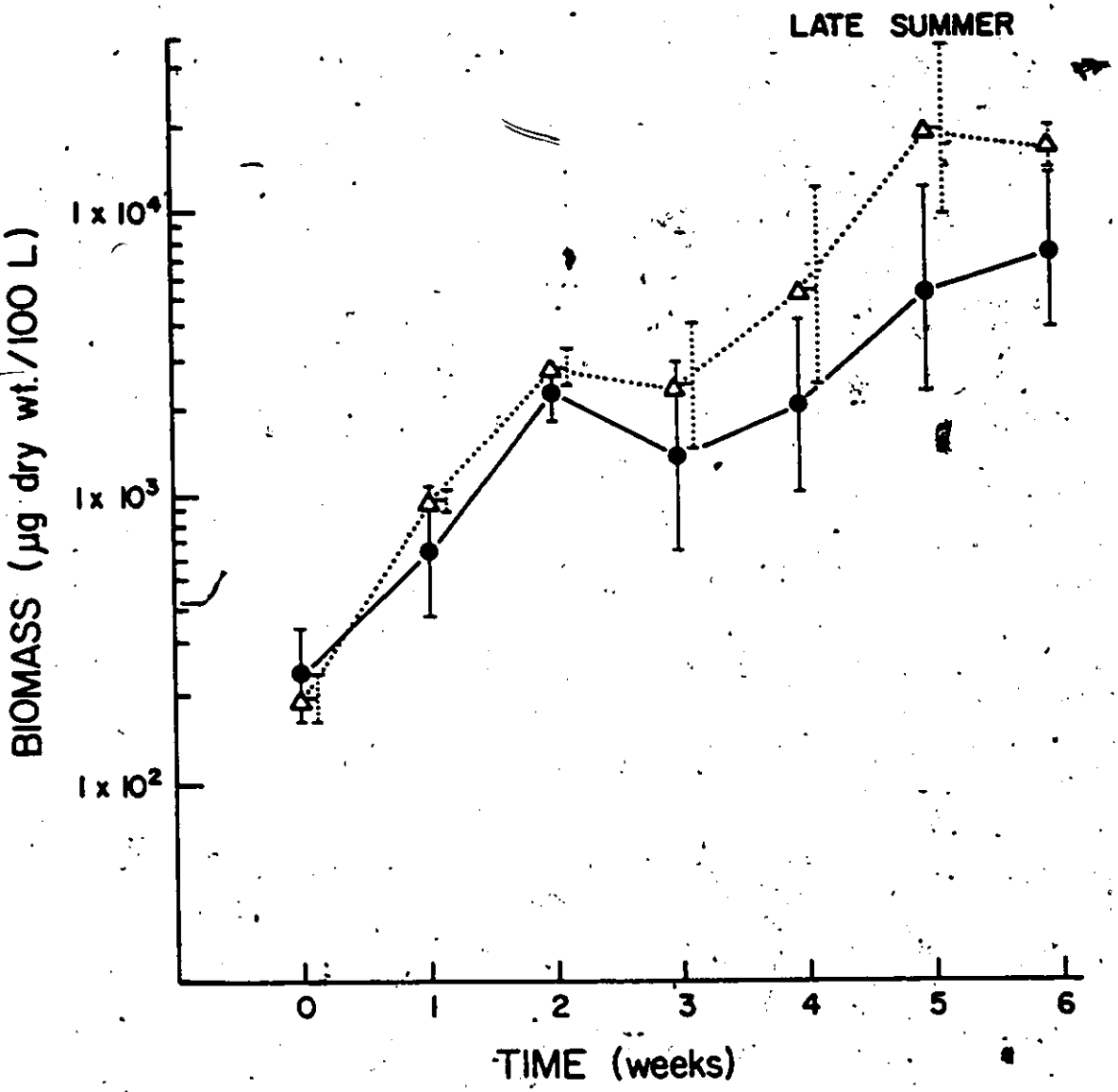

LATE SUMMER
BIOMASS (µg dry wt./100 L)
1 x 10⁵
1 x 10⁴
1 x 10³
0
1
2
3
4
5
6
TIME (weeks)

Figure 17

Mean biomass (± one s.e.) of small cladocera in control (●——●) and Serratia -enriched (△·····△) enclosures during long term late summer in situ experiments.


LATE SUMMER
BIOMASS (µg dry wt./100 L)
1 x 10⁴
1 x 10³
1 x 10²
0
1
2
3
4
5
6
TIME (weeks)

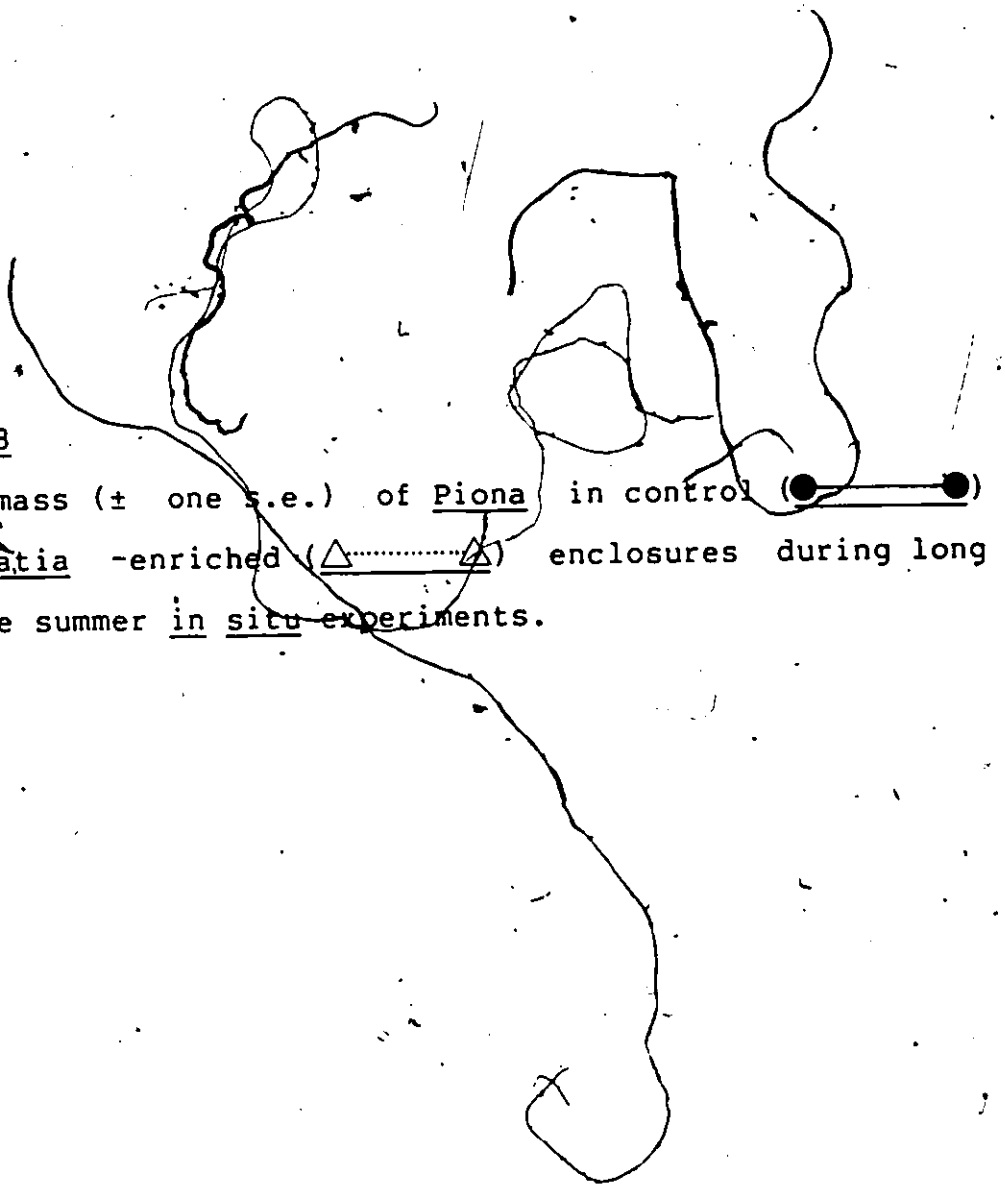


Figure 18

Mean biomass (± one s.e.) of Piona in control (●——●) and Serratia -enriched (△·····△) enclosures during long term late summer in situ experiments.

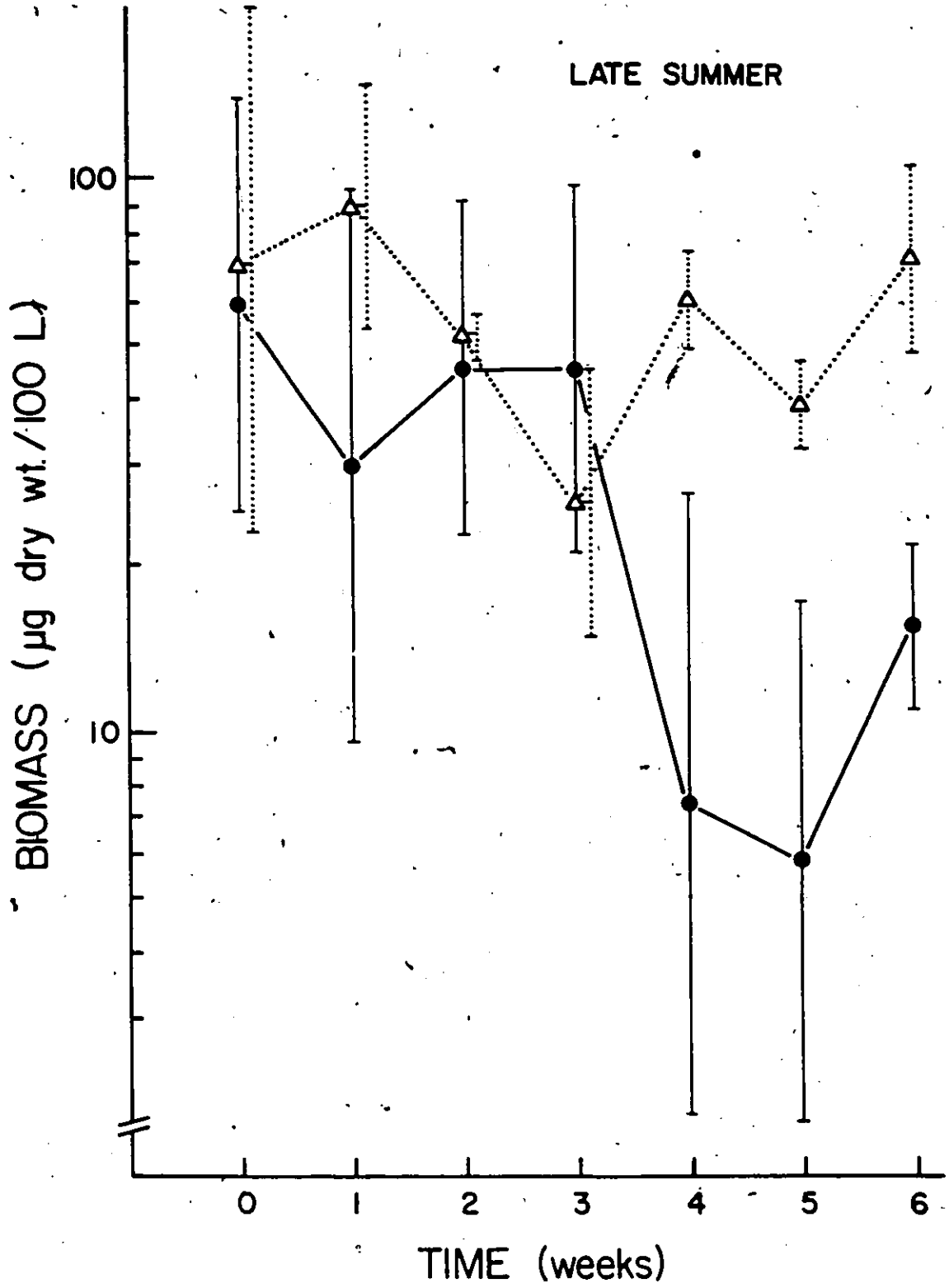

LATE SUMMER
100
10
BIOMASS (µg dry wt./100 L)
0
1
2
3
4
5
6
TIME (weeks)

Table 7

Summary of ANOVA results for zooplankton genera. Arrows indicate the direction of response to enrichment. If the arrow is not enclosed in parentheses, $p < 0.05$; if enclosed, $0.05 < p < 0.10$, but results are consistent between experiments. Subscript "i" indicates that only the F-ratio for interaction is significant.

| ZOOPLANKTER | EXPERIMENT | | |
|---|---|---|---|
| | EARLY SUMMER *Aeromonas* | LATE SUMMER *Aeromonas* | LATE SUMMER *Serratia* |
| HERBIVOROUS ROTIFERS | | | |
| *Bipalpus* | | ↑ | |
| *Euchlanis* | ↑ | | (↑) |
| *Kellicottia* | | | |
| *Keratella* | | | |
| *Ploesoma* | | | |
| *Trichocerca cylindrica* | | | |
| *Trichocerca multicrinis* | | | |
| Other rotifers | | | |
| SMALL CLADOCERA | | | |
| *Bosmina* | | | |
| *Ceriodaphnia* | | | |
| *Chydorus* | | | |
| LARGE CLADOCERA | | | |
| *Daphnia* | | | |
| *Diaphanosoma* | | | |
| *Holopedium* | | | ↑ |
| *Sida* | | | ↑ |

HERBIVOROUS COPEPODS

- Acanthocyclops cop. I-III
- Diaptomus
- Mesocyclops cop. I-III
- Tropocyclops
- Small cyclopoids
- Nauplii

SMALL CARNIVORES

- Acanthocyclops cop. IV+V, adults
- Asplanchna
- Mesocyclops cop. IV+V, adults

LARGE CARNIVORES

- Chaoborus
- Epischura
- Leptodora
- Piona
- Polyphemus

## 3.4 NUTRIENTS AND OXYGEN

Total phosphorus concentrations were consistently elevated in manipulated enclosures by the second to fourth week (Figure 19 -- two-factor ANOVA: early summer Aeromonas, $p=0.001$; late summer Aeromonas, $p=0.201$; late summer Serratia, $p=0.047$). Levels were more than doubled by early summer enrichments, while late summer additions of Aeromonas and Serratia increased total phosphorus by as much as 20% and 80% respectively.

Ammonia concentrations were depressed by up to 60% in early summer enrichments (Figure 20 -- two-factor ANOVA, $p=0.019$). Soluble reactive phosphorus, nitrate, dissolved organic carbon, and dissolved inorganic carbon were not significantly altered by manipulation.

Epilimnetic dissolved oxygen was elevated in enriched enclosures (Figure 21 -- two-factor ANOVA: early summer Aeromonas, $p=0.009$; late summer Aeromonas, $p=0.073$; late summer Serratia, $p=0.008$), while hypolimnetic levels were unaffected.

Figure 19

Mean total phosphorus concentration (± one s.e.) in control (●———●); *Aeromonas* -enriched (○– – –○), and *Serratia* -enriched enclosures (△·····△) during long term *in situ* experiments.

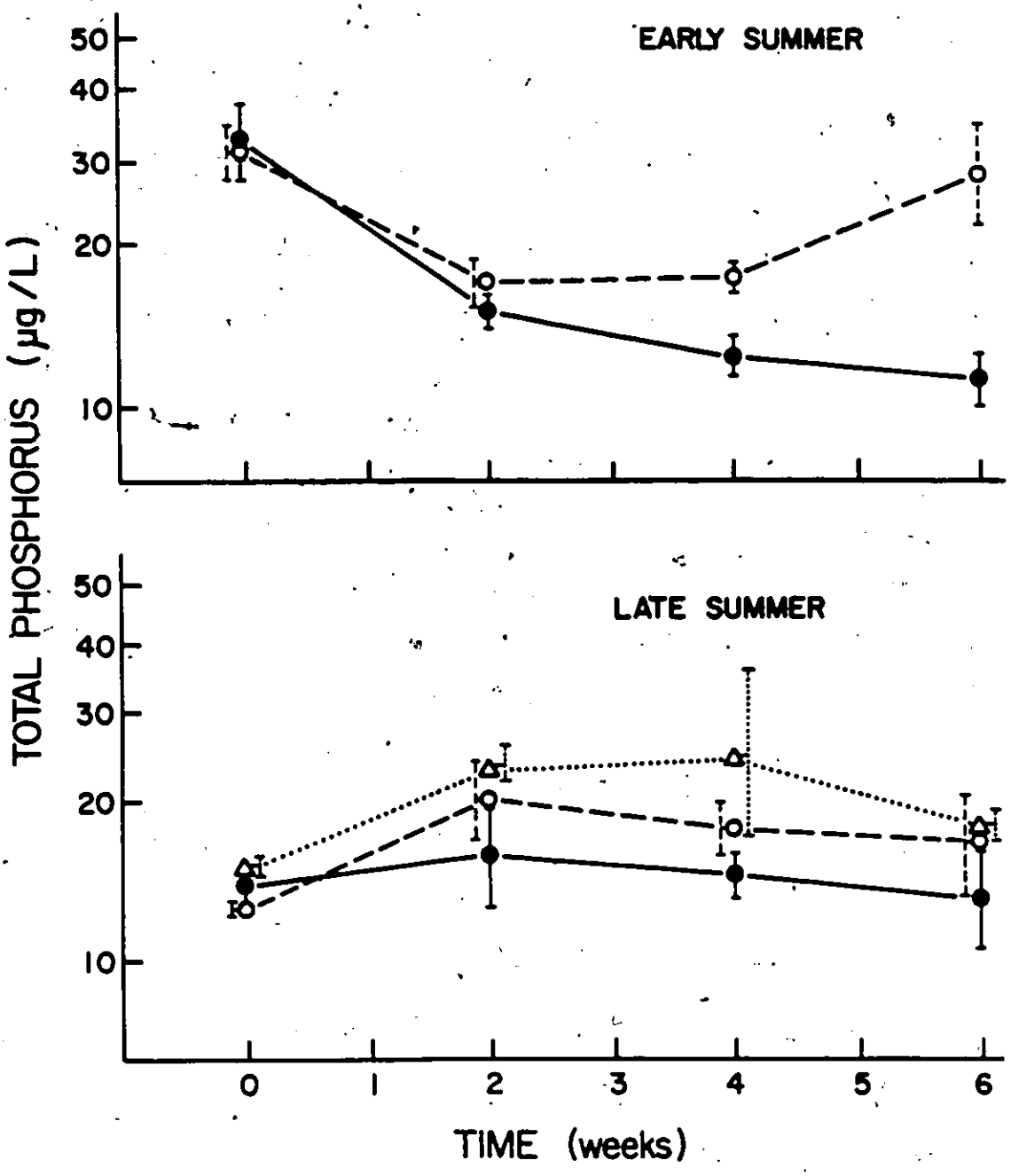

EARLY SUMMER
LATE SUMMER
TOTAL PHOSPHORUS (µg/L)
50
40
30
20
10
0
1
2
3
4
5
6
TIME (weeks)

Figure 20

Mean ammonia concentration (± one s.e.) in control (●——●) and Aeromonas -enriched (○– – –○) enclosures during long term in situ experiments.

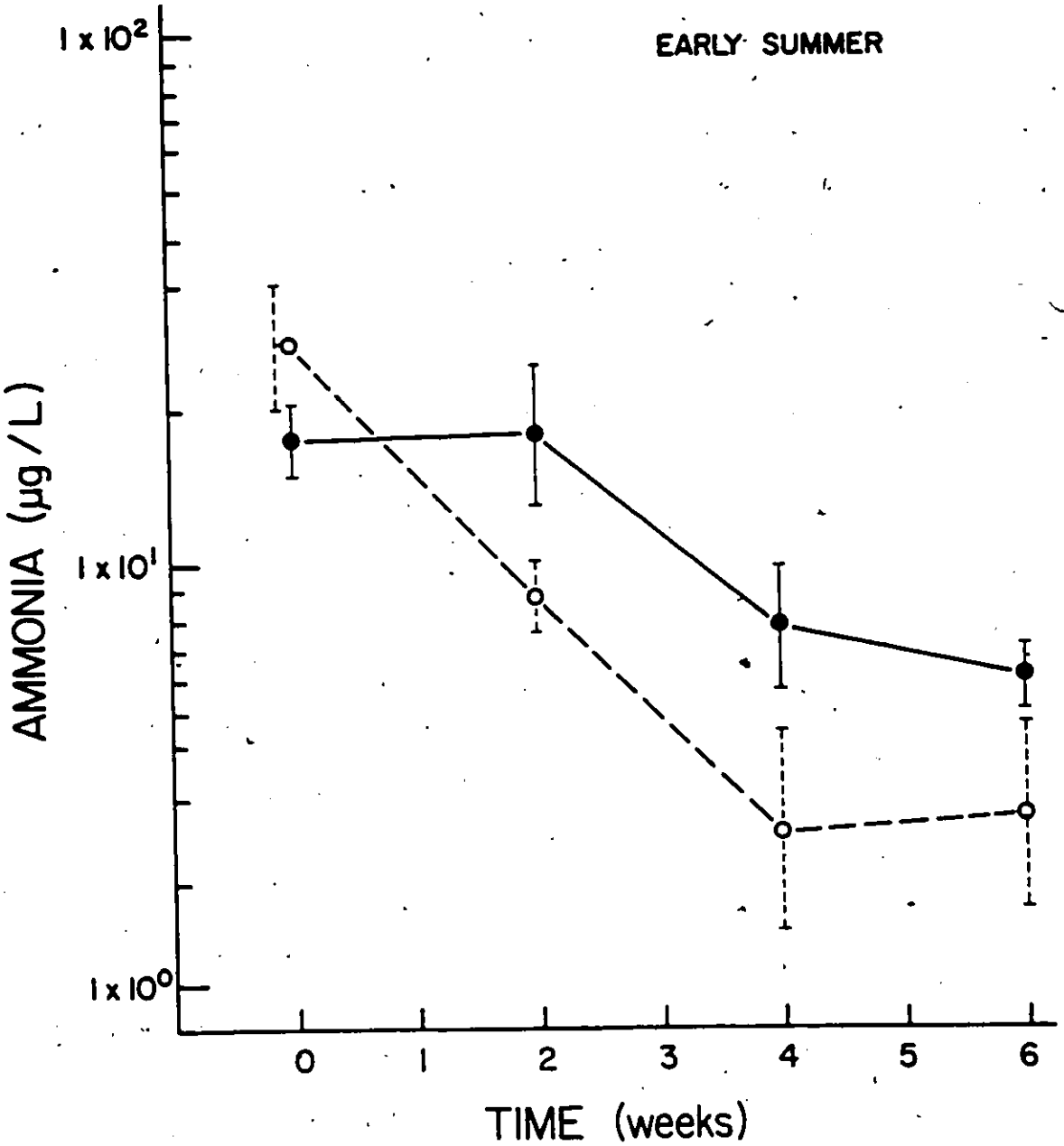
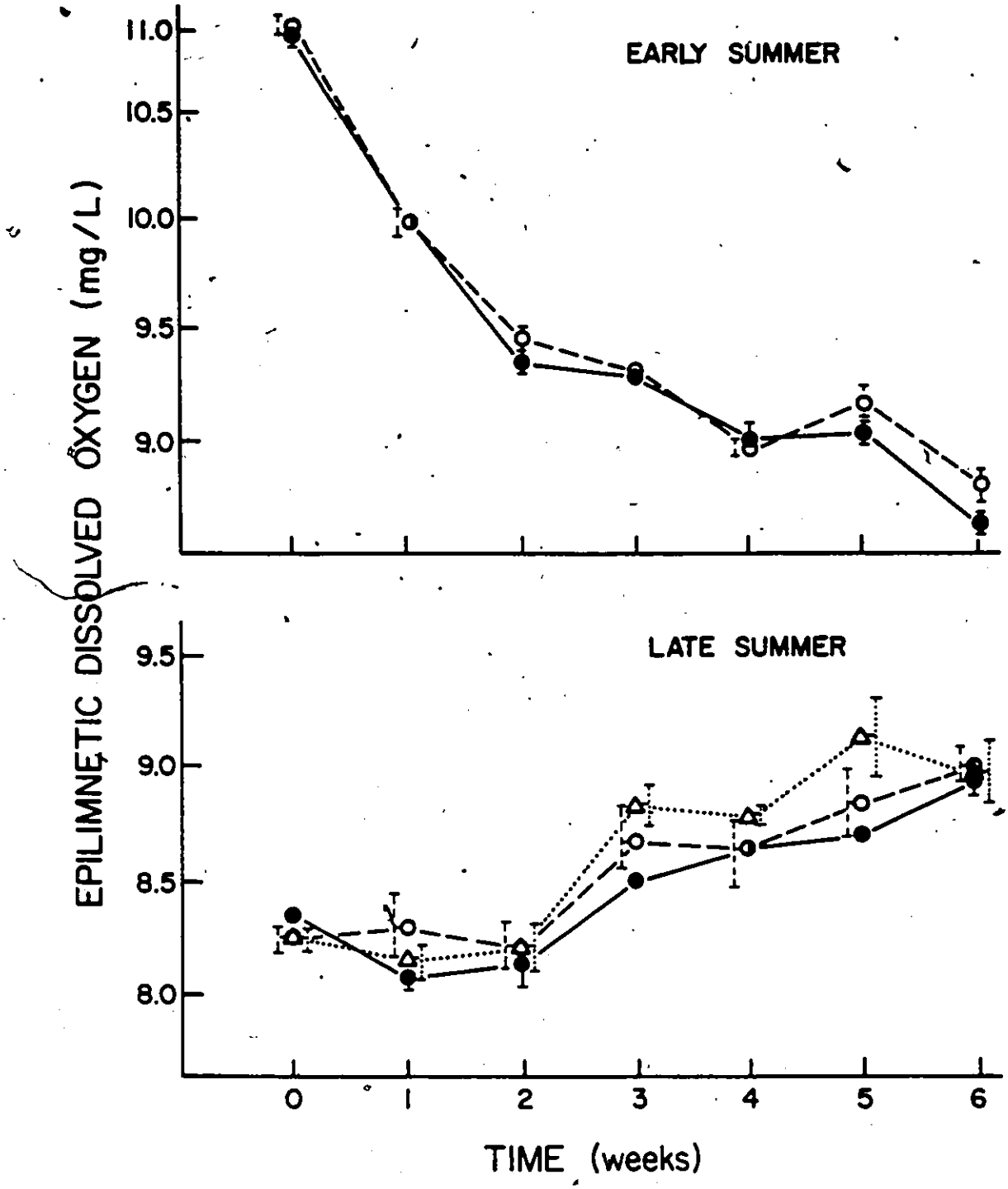

EARLY SUMMER
AMMONIA (µg/L)
1 x 10²
1 x 10¹
1 x 10⁰
0
1
2
3
4
5
6
TIME (weeks)

Figure 21

Mean epilimnetic dissolved oxygen concentration (± one s.e.) over time in control (●——●), Aeromonas -enriched (○—·—·—○), and Serratia -enriched (△·····△) enclosures during long term in situ experiments.


EARLY SUMMER
LATE SUMMER
EPILIMNETIC DISSOLVED OXYGEN (mg/L)
11.0
10.5
10.0
9.5
9.0
9.5
9.0
8.5
8.0
0
1
2
3
4
5
6
TIME (weeks)

# Chapter IV

# DISCUSSION

## 4.1 BACTERIAL FATE IN SITU

The rapid growth of both enrichment bacteria in filter sterilized lake water strongly suggests that they were active *in situ*. Bacterial activity *in vitro* could have been exaggerated if dissolved nutrient levels were elevated from filtration damage to plankton, or nutrient adsorption to vessel walls, but a low vacuum pressure was employed specifically to minimize these problems. As well, these extra nutrients would in all likelihood affect only the level, rather than the presence, of activity.

However, reproduction of added cells did not affect levels of total and active bacteria *in situ*, indicating that some factor was regulating bacterial abundance. Zooplankton removal demonstrated that grazing was not the controlling agent. Instead, the added cells may have been metabolizing but reproducing poorly, a moribund state recognized by Postgate (1976) and Morita (1982) on the basis that viable counts are often a small fraction of the numbers of metabolizing bacteria. This is a distinct possibility in the case of *Aeromonas hydrophila*, which multiplied relatively slowly in filter-sterilized lake water, and

reportedly reproduces poorly in natural waters (Fliermans et al. 1977, van der Kooij et al. 1980).

In contrast, Serratia marcescens probably reproduced well in situ, as it multiplied rapidly in vitro, and is reported to do so even in double-distilled water (Botzenhart and Kufferath 1976). In this instance, bacterial levels in the enclosures may have been controlled by lysis, a factor recently implicated as an important regulator of bacteria in lakes (Riemann et al. 1982). The metabolism of the added cells would certainly deplete supplies of limiting nutrients, and may have caused lysis of bacteria, as starvation renders bacteria susceptible to autolysis (Alexander 1971). Alternatively, the added bacteria may have produced heterolytic enzymes, an ability many species, including Aeromonas, possess (Coles and Gilbo 1967).

The zooplankton responses support these interpretations as to the reproduction of the added bacteria. Herbivorous zooplankton were increased by enrichment only in the Serratia manipulations, even though the peak phytoplankton enhancement was similar to that recorded in the early summer enrichments, approximately $1.5 \times 10^4$ $\mu m^3/mL$. Thus, the grazers were responding not to the phytoplankton, but more likely to the production of the added bacteria. It appears that Aeromonas multiplied poorly, and left grazing stocks unaltered, while strong reproduction by Serratia produced increased herbivore levels. In support of this explanation,

I note that only bactivorous zooplankton (Sida, Holopedium, and Chydorus) were increased by enrichment.

These interpretations are strengthened by the fact that alternative explanations for the fate of the added cells and for the enrichment effects are not tenable. One might hypothesize that the enrichment effects were the result of fertilization with nutrients derived from the added cells as they were grazed or decomposed. However, differences in total phosphorus and carbon between enriched and control enclosures exceeded the cumulative amounts added as suspension in eight of nine instances for phosphorus (Table 8; binomial test, $p=0.018$) and seven of nine for carbon ($p=0.070$), even under the conservative assumptions that losses to sedimentation were nonexistent, and mineralization complete. In addition, if added cells were completely mineralized, the levels of fertilization would be only 16% of those used to produce similar phytoplankton increases in the ELA lakes (Schindler et al. 1971), which are extremely oligotrophic. Thus, nutrients added as suspension were inadequate to produce the observed responses.

A similar analysis for nitrogen is not possible, as dissolved organic nitrogen was not assayed. However, since bacteria and algae have similar chemical compositions (Horstmann and Hoppe 1981) nitrogen fertilization would not have sufficed to produce the enrichment effects.

A second possibility is that the zooplankton grazed the added bacteria, and the resultant changes in zooplankton biomass or composition in turn gave rise to the other enrichment effects. However, zooplankton responses occurred only in the Serratia enrichments, and then did not precede but followed changes in the other variables.

I consider unlikely the possibility that the experimental effects reflected organic fertilization, through its stimulation of the activity of indigenous heterotrophs as they decomposed the enrichment bacteria. I estimate that the added cells, larger on average than the indigenous bacteria, increased the total mass of seston by 12.2% on average. Decomposition of this amount of biomass should have greatly enhanced bacterial activity and perhaps abundance, yet no such stimulation was observed. Further, the strikingly different impact of the two bacterial species-- which, as organisms of similar biochemical capability raised on the same medium, would probably have a like chemical composition -- in the late summer experiments argues against this view.

It thus appears that the added bacteria were active in situ, and that this activity was responsible for the enrichment effects. I will use this working hypothesis presently in interpreting the responses of other biota to the enrichment bacteria.

Obviously a more direct evaluation of the added bacterial activity and fate is necessary. The principal difficulty in this regard is to distinguish the added cells from indigenous microorganisms, as they are similar in morphology and, although the added cells are larger on average, show some overlap in size. However, species-specific identification and measurement of activity is feasible with a recently-developed technique which combines immunofluorescent staining with INT reduction as an indicator of electron transport activity (Baker and Mills 1982). Applying this technique to control and enriched enclosures, in conjunction with the use of membrane diffusion chambers (Fliermans et al. 1977) to exclude zooplankton while maintaining nutrient regeneration, would yield direct estimates of added bacterial net reproduction, activity and grazing losses.

Table 8. Total cumulative carbon and phosphorus added in suspension compared with average differences in total carbon and phosphorus between enriched and control enclosures. All estimates are given in mg/L.

| EXPERIMENT | WEEK | CUMULATIVE AMOUNT ADDED AS SUSPENSION | | WEEK | AVERAGE DIFFERENCE BETWEEN CONTROL + ENRICHED. | |
|---|---|---|---|---|---|---|
| | | P | C | | P | C |
| Early | 1 | $1.4X10^{-3}$ | $2.3X10^{-2}$ | 2 | $2.4X10^{-3}$ | 2.1 |
| Summer | 3 | $2.9X10^{-3}$ | $4.5X10^{-2}$ | 4 | $5.2X10^{-3}$ | $4.8X10^{-1}$ |
| Aeromonas | 5 | $4.3X10^{-3}$ | $6.8X10^{-2}$ | 6 | $2.0X10^{-2}$ | $-5.0X10^{-1}$ |
| Late | 1 | $1.4X10^{-3}$ | $2.3X10^{-2}$ | 2 | $4.0X10^{-3}$ | $-2.8X10^{-1}$ |
| Summer | 3 | $2.9X10^{-3}$ | $4.5X10^{-2}$ | 4 | $3.3X10^{-3}$ | $9.3X10^{-1}$ |
| Aeromonas | 5 | $4.3X10^{-3}$ | $6.8X10^{-2}$ | 6 | $3.7X10^{-3}$ | $2.1X10^{-1}$ |
| Late | 1 | $1.4X10^{-3}$ | $2.3X10^{-2}$ | 2 | $3.7X10^{-3}$ | 1.7 |
| Summer | 3 | $2.9X10^{-3}$ | $4.5X10^{-2}$ | 4 | $1.3X10^{-2}$ | 1.5 |
| Serratia | 5 | $4.3X10^{-3}$ | $6.8X10^{-2}$ | 6 | $4.4X10^{-3}$ | 1.2 |

## 4.2 PHYTOPLANKTON

### 4.2.1 Nature of Phytoplankton Response

The most striking effect of enrichment was the severalfold increase in phytoplankton biomass. It is axiomatic that bacteria are important nutrient regenerators; the phytoplankton response likely reflected this process. Elevated heterotrophic bacterial activity with enrichment would accelerate the breakdown of organic matter, directly through increased bacterial mineralization and indirectly as they were grazed or lysed, thereby increasing nutrient availability and phytoplankton production in turn. In addition, the activity of the added bacteria likely also produced the observed increases in total levels of phosphorus (Figure 19) and possibly of carbon (Table 8). The concentrations of these elements reflect a balance between their inputs (particulate loading from the atmosphere, diffusion from the hypolimnion, and atmospheric sources of carbon dioxide) and losses (sedimentation, and atmospheric losses of carbon dioxide). The only means by which the enrichment bacteria could increase total levels of phosphorus (and parsimoniously, of carbon) is by mineralizing particulate organic matter (POM) which would otherwise sediment out, thus reducing nutrient losses through sedimentation and increasing total nutrient levels as a result. Thus, mineralization by the added bacteria may have increased both the availability and total levels of nutrients critical to phytoplankton production.

What is known of decomposition in lakes supports this scenario. Zooplankton and phytoplankton, which dominated the POM in the enclosures, are readily decomposed by bacteria following their death (Golterman 1972, Frankland 1974, Saunders 1976). As a result, the majority of sedimenting POM is mineralized before it settles out of the epilimnion (Kleerekopper 1953, Lawacz 1969, Wetzel et al. 1972, Golterman 1976, Bloesch et al. 1977), particularly under the relatively warm temperatures prevailing throughout these manipulations (Lawacz 1969). Further, a growing body of evidence indicates that heterotrophic mineralization is a major supply of limiting nutrients in stratified lakes, and that bacteria alone can account for most nutrient regeneration (Golterman 1973, Bloesch et al. 1977, Axler et al. 1981), although zooplankton may also be important in this regard (Liao and Lean 1978, Lehman 1980, Axler et al. 1981).

Phosphorus and nitrogen are the nutrients most likely to have stimulated the phytoplankton. These are the most commonly limiting elements in temperate lakes (Paerl 1982). More importantly, in previous experiments, combined phosphate and nitrate additions to enclosures in Heney Lake produced dramatic increases in phytoplankton biomass, indicating that one or both of these nutrients limit Heney Lake phytoplankton (Briand et al. 1978).

Further, alternative hypotheses for the stimulatory effect of enrichment on phytoplankton appear unlikely. If phosphate or nitrate limit summer Heney Lake phytoplankton as fertilization experiments suggest (Briand et al. 1978), then other essential nutrients like carbon dioxide or vitamins obviously do not. Confirming this, in situ vitamin fertilization in Heney Lake did not substantially alter the phytoplankton community (Smith et al. 1984). Bacterial respiration is also known to stimulate nitrogen-fixing algae by removing oxygen, thereby protecting nitrogenase from inhibition (Paerl 1976, 1978, Paerl and Kellar 1978). This, however, was not the case here: although nitrogen-fixers were present, phytoplankton species incapable of nitrogen fixation, particularly diatoms, were stimulated first in the late summer experiments. Further, the response of the blue-green algae, dominated by the nitrogen-fixers Anabaena and Aphanizomenon, was relatively weak (Figure 12, Table 5).

Whether the mineral nutrients were mobilized more by bacterial mineralization or by grazer mineralization of added bacterial production cannot be determined on the basis of these experiments. The direct route most likely predominated: although bacteria and grazers are estimated to each account for a large fraction of nutrient regeneration during stratification (Golterman 1973, Liao and Lean 1978, Lehman 1980, Axler et al. 1981), nutrients regenerated by grazers include those derived from

phytoplankton biomass, which in this instance exceeded that of bacteria.

4.2.2 Size-related Effects

Enhancement occurred almost exclusively in the large phytoplankton, probably because the enrichment bacteria associated with the larger algae but not with smaller ones. Slow-growing (large) phytoplankton often have an epiphytic microflora which rapidly-growing (small) species do not possess (Campbell 1977). As well, Aeromonas is commonly isolated from net plankton tows (Caldwell 1977), where it is present in a greater proportion than in surrounding waters (Simidu et al. 1971). Further, the means by which the enrichment bacteria were originally isolated, on a rich organic medium, would select for those bacteria able to grow in a nutrient-rich microenvironment such as that in the vicinity of an algal cell.

Alternatively, one might hypothesize that the phytoplankton stimulation was more general, but appeared primarily in the large algae because zooplankton grazed selectively on smaller phytoplankton. Grazing is indeed selective in this manner (Porter 1973, 1977, Nadin-Hurley and Duncan 1976, Ross and Munawar 1981, Gulati et al. 1982). However, early summer Aeromonas and late summer Serratia enrichments produced comparable responses in large phytoplankton (Figure 11), yet the herbivores were increased

only in the latter experiments, and this response was limited to bactivorous genera. This hypothesis is thus untenable.

I did not enumerate bacteria associating with large algae during the course of the phytoplankton analysis, and did not observe any in passing. Bacteria were difficult to resolve at 400X magnification, and the fixation and agitation routinely employed when counting microalgae might well disrupt such associations, if they are fragile. To better assess whether the added bacteria are adjacent to large algae, one could survey the phytoplankton from control and bacteria- enriched enclosures, using high magnification and live, gently concentrated material. Further, to determine whether the enrichment bacteria selectively stimulate nutrient uptake by large algae, grazer-free lake water in control and enriched enclosures should be incubated with a dissolved organic compound which contains labelled phosphorus , perhaps a nucleic acid. Bacterial mineralization of the compound would release phosphate, and if the hypothesis is true, the difference in the amount of labelled phosphorus incorporated into phytoplankton biomass between enriched and control enclosures should be much greater for net than for nannophytoplankton.

The enhancement of large algae is interesting in light of the fact that in the seasonal succession of phytoplankton,

early summer species are frequently replaced by large inedible forms (Gliwicz 1975, Kalff and Knoechel 1978). It has been suggested (Porter 1973, Gliwicz 1975, Lehman 1980) that this transition is effected by the zooplankton through their selective predation on small algae. My results indicate that this size-related succession may be determined instead by the association of large algae with bacteria, whereby large cells gain an advantage over smaller ones because their bacterial flora, which the small algae lack (Campbell 1977), regenerates limiting nutrients. Competition experiments in chemostats could prove useful in evaluating the latter hypothesis. If, using axenic cultures, small phytoplankton outcompete large ones for limiting nutrients under steady state conditions -- as Malone (1980) has cogently argued should be the case -- changes in the relative competitive abilities of the two size classes upon introduction of suitable epiphytic bacteria would indicate whether algal-bacterial associations have the potential to cause size shifts in phytoplankton *in situ*.

### 4.2.3 Nature of Diatom Stimulation

The selective enhancement of diatoms was even stronger relative to other phylogenetic divisions than their levels would indicate, as their high silica content causes them to sink faster than other algae. This specific stimulation may

be due to bacterial interference with toxic substances produced by blue-green algae against diatoms (Carmichael and Gorham 1977, Keating 1978). Increased bacterial activity in enriched enclosures could reduce the active levels of such substances, freeing diatoms from inhibition. I note as supporting evidence that the greatest differences in diatom levels between control and enriched enclosures occurred when blue-green biomass was maximal (Figure 12) and allelopathic effects presumably strongest. Further, some inhibitors produced by blue-greens are specific to diatoms: in Keating's (1978) study, levels of flagellates were not related to blue-green abundance, while diatom levels were. This hypothesis would predict that filtrates from enriched enclosures should be less inhibitory to test organisms than filtrates from control enclosures.

Other hypotheses for the mechanism of diatom stimulation are less plausible. There is preliminary evidence that diatoms are superior to green and blue-green algae at competing for low levels of phosphate (Tilman et al. 1982), so that diatoms would have an advantage in taking up any nutrients mobilized by the added bacteria. However, the effect of size on rates of uptake and growth (Malone 1980) is likely to override any competitive edge large diatoms may possess, as the differences in phosphate affinity between divisions are quite small. Alternately, the added bacteria may have associated preferentially with diatoms, but this

also seems unlikely. Large blue-green and green phytoplankton were also stimulated by enrichment, suggesting that physical association is nonspecific. As well, levels of small diatoms were altered by enrichment (Table 5), an unlikely result if the size-specific physical association with large algae holds. Regardless of the basis of diatom enhancement, it raises the intriguing possibility that bacteria can influence phytoplankton succession, slowing the seasonal transition from spring diatom communities to summer blue-green associations characteristic of this lake (Wall 1980) and many others (Hutchinson 1967, Kalff and Knoechel 1978).

### 4.2.4 Other Phytoplankton Responses

The remaining groups of phytoplankton responding to bacterial addition were obviously influenced by other aspects of enrichment. In the case of desmids, the only algae reduced by manipulation, a rise in pH following increased photosynthesis is likely responsible, as most desmids cannot grow under alkaline conditions (Brook 1981). The reason for the enhancement of medium flagellates, primarily Rhodomonas, is unclear. As small cells, they were probably not associated with bacteria. While Rhodomonas is auxotrophic for vitamin B12 (Provasoli 1958), which Aeromonas produces (Niewolak and Sobierajska 1971), it is not among the few phytoplankton species enhanced by in situ B12 fertilization (Smith et al. 1984).

4.2.5 Strength of Response

Although the tests used in identifying the bacteria (Table 2) showed that both strains had similar biochemical capabilities, Serratia produced a much stronger phytoplankton response than did Aeromonas in late summer enrichments. This was probably because of differences in the levels of activity of the two species. Serratia grew more rapidly than Aeromonas in batch culture, filter-sterilized lake water, and likely also in the lake, thus causing the stronger effect. Again, the zooplankton data reinforce this interpretation, as only Serratia manipulations altered grazing zooplankton levels.

For its part, Aeromonas produced a more striking response in early than late summer enrichments, perhaps reflecting greater losses to sedimentation early in the season when the phytoplankton was dominated by diatoms, and when cooler temperatures would have reduced decomposer activity. At this time the potential for added bacteria to affect nutrient levels by intercepting sedimenting particles would have been great relative to late summer, when higher temperatures and a predominance of algae with flotation mechanisms (blue-greens) or benthic habit (Mougeotia) would have increased recycling, and reduced sedimentation losses.

## 4.3 ZOOPLANKTON

### 4.3.1 Grazing Zooplankton

As outlined earlier, zooplankton were affected by enrichment only when added bacteria appeared to reproduce well. Bacterial effects on zooplankton were most likely restricted to direct grazing only because the large algae selectively stimulated are not consumed by grazers in any quantity (Burns 1968, Porter 1973, 1977, Nadin-Hurley and Duncan 1976, Ross and Munawar 1981). Thus, the potential for bacteria to indirectly affect zooplankton through the edible phytoplankton was not realized.

Only bactivorous zooplankton were stimulated, indicating that bacteria may be important in structuring zooplankton communities. If grazing zooplankton competed for food resources, bacteria could organize the zooplankton community by advantaging bactivorous species over others. Food was probably limiting: bactivorous genera were increased, and strong regressions of zooplankton biomass on nannoplankton biomass (McCauley and Kalff 1981, Pace 1983) suggest that such limitation is general. However, competition between bactivores and other grazers was weak or nonexistent, as the decrease in herbivorous copepods, which capture unattached bacteria poorly, occurred independently of any changes in bactivorous zooplankton levels with enrichment (Table 7).

The lack of competitive interactions between these two groups probably reflected their small degree of dietary

overlap. Cladocera, particularly small-bodied ones, consume mostly particles from less than one to several μm in diameter, and consequently eat bacteria, fine detritus and the smaller nannoplankton (Gliwicz 1969a). Copepods, on the other hand, generally consume few tiny particles, instead preying most effectively on those of 10 μm diameter or more (Gliwicz 1969a, McQueen 1970, Richman et al. 1980).

Nonetheless, the bacteria do appear to have a structuring influence on the zooplankton, through their contribution to the seston. This role is evidenced by patterns in zooplankton composition along a gradient from oligotrophic to eutrophic lakes (Gliwicz 1969a, Hillbricht-Ilkowska and Weglenska 1970, Hillbricht-Ilkowska et al. 1972, Beaver and Crisman 1982). The seston of oligotrophic lakes has a large fraction of nannophytoplankton, and the grazing zooplankton are dominated by macrophagous species. Lakes which are increasingly more productive have a seston correspondingly dominated by net phytoplankton, and the zooplankton are for the most part microphagous. In such productive lakes, the large share of net plankton in total phytoplankton both decreases the share of edible algae and increases the amounts of detritus and bacteria arising through the decomposition of large algae. These shifts in food size composition apparently in turn control zooplankton composition (Gliwicz 1969a, Hillbricht-Ilkowska 1977, Beaver and Crisman 1982).

Obviously, it is the spectrum of available food which seems to control grazer composition rather than the bacteria per se, but their roles in determining this spectrum are manifold. My experiments indicate that certain bacteria may cause the increase in net plankton which drives community dynamics in eutrophic lakes. Also, heterotrophic bacteria production is often a substantial fraction of primary production, and may exceed it when allochthonous inputs are large (eg. Kuznetsov 1970, Anderson and Dokulil 1977, Pedros-Alio and Brock 1982). Finally, the bacteria are intimately involved in controlling the supplies of other food types, by decomposing large inedible detritus, enriching detritus for grazers (Paerl 1974, 1975, Paerl and Lean 1976), and incorporating the soluble organic matter released by phytoplankton into a form available to grazers.

Since the bacteria added in my experiments were on average several times larger (roughly 1 $\mu m^3$ versus 0.1 $\mu m^3$) than indigenous cells, this might exaggerate their structuring impact on zooplankton by increasing their importance as food: in general, very small bacteria are captured inefficiently by even microphagous grazers (Gliwicz 1969a, Peterson et al. 1978). However, many of the indigenous cels were similar in size to the added bacteria, which indicates that this was not a serious source of error. Further, bacteria similar in size to cultured cells are common in eutrophic lakes, suggesting that their impact on

zooplankton composition would be strong in productive waters, in agreement with the patterns in grazer composition along a trophic gradient cited previously.

### 4.3.2 Carnivorous Zooplankton

Pulses in carnivorous zooplankton predictably followed those of their prey, with a lag of three to four weeks. It is interesting to note that those prey enhanced by enrichment are all resistant to invertebrate predation, by virtue of being large (Holopedium and Sida) or covered by a hard carapace (Holopedium, Sida, and Chydorus -- Kerfoot et al. 1980). Presumably more vulnerable prey, such as Bosmina or Ceriodaphnia, also consumed bacteria but were held in check by invertebrate predators. This interpretation is strengthened when one considers that Piona, the major predator increased, specializes on cladocera, and exhibits the following prey preference: Bosmina > Daphnia, Ceriodaphnia > Chydorus >> Diaphanosoma, Diaptomus > Mesocyclops (Riessen 1981 in Baril 1983). Thus, herbivorous zooplankton composition appears to be determined by the nature of both their prey and invertebrate predators. This concurs with recent experimental findings that herbivorous zooplankton dynamics are controlled primarily by food resources and secondarily by invertebrate carnivores (Neill 1981, Baril 1983, Baril and Briand, in press).

4.3.3 Other Zooplankton Responses

In a large part, the responses of individual zooplankton genera (Table 7) reflected the increases in bactivorous and predaceous zooplankton just discussed. From among the remaining genera, the elevated levels of Euchlanis most likely resulted from the stimulation of large phytoplankton, as this benthic species is known to make late summer forays into the plankton attached to filamentous bloom algae (Dumont 1977). The reason for the response of the omnivorous rotifer Bipalpus is unclear, as it occurred when the only other significant enrichment effect on zooplankton was a decrease in levels of Diaptomus, which is neither prey nor predator for Bipalpus.

The consistent reduction in herbivorous copepod genera with enrichment is also puzzling. A wide variety of invertebrates prey on copepods, but copepod decreases occurred independently of levels of any carnivores. Likewise, the abundance of nannoplankton, the principal food of herbivorous copepods, was unaltered by enrichment. Alternatively, large algae can decrease zooplankton feeding and reproduction by interfering with filtering (Gliwicz 1977, Webster and Peters 1978), but herbivorous copepods were unaffected in the Serratia manipulations, when algal biomass was highest and dominated by large phytoplankton. The reason for the patterns in herbivorous copepod abundance thus remains obscure.

### 4.3.4 Absence of Vertebrate Predators

A common criticism of enclosures is that they do not usually contain fish. As a result, the dynamics they reveal could be artifacts of exclosure, since fish predation on the more visible zooplankton has a major impact on zooplankton community structure. In my experiments, one might argue that the increases in large grazers in Serratia manipulations were such artifacts. This is likely true of Sida, which is a large and opaque plankter. In contrast, since Holopedium has a gelatinous sheath which reduces its apparent body size, it is eaten by fish much less frequently than its absolute size would predict (O'Brien et al. 1979), and consequently increases in abundance when fish are plentiful (Wells 1970). More importantly, the property that these specific responses reflect is bactivory, which is characteristic of both large zooplankton and the smaller ones (Chydorus) which would not be preyed upon by fish.

In a similar vein, exclusion of fish would reduce predation on the large invertebrate predators, and might consequently exaggerate the importance of invertebrate predation. However, carnivorous zooplankton abundance decreased throughout these experiments (Figure 15) for all genera except Piona, which is not even eaten by planktivorous fish because it is distasteful (Kerfoot 1982). I conclude that the lack of fish in the enclosures did not introduce major artifacts.

## 4.4 OXYGEN AND NUTRIENTS

Patterns in oxygen and nutrient levels were closely linked to phytoplankton responses. Epilimnetic oxygen was most likely elevated through increased photosynthesis by the stimulated algae. Alternative explanations appear unlikely. Water temperature and exchange with the atmosphere can influence oxygen levels, but temperature and mixing regimes were the same in all enclosures. While respiration uses up oxygen, heterotrophic activity was probably increased rather than decreased with enrichment.

As discussed earlier, the ultimate cause of the increases in total phosphorus was likely mineralization of particulate organic matter by the added bacteria. However, the sink for this increased supply was the phytoplankton biomass: total phosphorus in fresh waters is present mostly as organic matter (Wetzel 1975), of which the phytoplankton was the sole fraction consistently elevated in enriched enclosures. In addition, zooplankton undoubtedly contributed in the *Serratia* manipulations. The other major phosphorus pool, dissolved organic phosphorus, was not measured, but would likely parallel dissolved organic carbon, which was unaffected by the manipulation.

Ammonia reduction with enrichment was probably due to phytoplankton uptake. Other explanations seem unlikely. Grazers, which excrete ammonia, were unaltered when ammonia levels were reduced by enrichment (see Figure 15). Added

bacteria could assimilate ammonia, but elevated heterotrophic bacterial activity would likely increase its production through ammonification, depending on the nature of the compounds undergoing decomposition: ammonia is commonly produced during decomposition of phytoplankton cells (Grill and Richards 1964, Goltërman 1972, DePinto and Verhoff 1977). No data was collected on activity of nitrifying bacteria. In sum, phytoplankton incorporation seems the simplest explanation for reduced ammonia levels, although lack of a similar reduction in the Serratia manipulations where phytoplankton were stimulated suggests that levels were further modified by zooplankton grazing, or bacterial ammonification.

Although added bacteria would have increased demands on the assimilable DOC pool, levels of DOC were not affected by enrichment. This is most likely because the small organic molecules which are readily assimilable by bacteria are a minor fraction of the total DOC, which is instead dominated by refractory molecules (Gocke 1970, Wetzel 1975). Consequently, total levels of DOC are generally insensitive to phytoplankton release and bacterial use of organic molecules (Storch and Saunders 1978, Riemann et al. 1982), as they appear to have been here.

Similarly, although the stimulated phytoplankton certainly took up orthophosphate, soluble reactive phosphorus (SRP) was unaltered. Rigler (1966, 1968)

demonstrated from radiotracer turnover that orthophosphate is often a small fraction of SRP, which probably accounts for the fact that SRP was unaffected by enrichment. In light of this information, measurement of total DOC and soluble reactive phosphorus seems useful primarily as a rapid survey technique for distinguishing major lake types.

## 4.5 ENCLOSED COMMUNITIES AS LAKES

The results of these manipulations can be extrapolated to lakes only to the degree that the enclosed communities are representative of natural plankton assemblages. Working with a near-natural community is of course the rationale for using *in situ* enclosures: they initially contain a diverse community of bacteria, algae, and herbivorous and carnivorous zooplankton present in the lake, and maintain this community under field conditions. Such a technique obviously removes many of the artifacts which are unavoidable in any laboratory system.

Both the physical and chemical environments, and the composition and dynamics of the enclosed communities are similar to surrounding waters. Levels of nutrients measured in the lake were quite similar to those in enclosures (Table 9, Figures 19 and 20). I did not directly compare levels of organisms in the enclosures and the lake, but note that zooplankton and phytoplankton genera recorded in the enclosures were also present in the lake. Furthermore,

Baril (1983) reported that zooplankton levels in twelve similar enclosures were equal to or slightly lower than those in Heney Lake at the same time, and exhibited the same trends over time. As for the phytoplankton, studies using similar enclosures in marine systems show that phytoplankton composition and dynamics are similar in enclosures and adjacent waters (Takahashi et al. 1975, Oviatt et al. 1980, Reeve et al. 1982).

As the experiments progressed, the enclosures diverged from the lake, and the communities took on an increasingly benthic aspect. After several weeks, visible periphyton built up on the enclosure walls, corresponding to increases in benthic phytoplankton (particularly Mougeotia) and zooplankton (Sida). Similar changes led Baril (1983) to suggest that four weeks might be as long as this type of enclosure is representative of the lake. However, I note that both aforementioned genera are found in the lake plankton, albeit in low numbers. As well, while Mougeotia and Sida responded to enrichment, planktonic genera were also altered. Further, the properties that these responses reflect, large size in the case of Mougeotia and combined bactivory and resistance to predation for Sida, are more universal. These points suggest that the benthic members of the community at the end of the experiments do not preclude the extension of these results to the lake.

Enclosure reduces turbulence in the contained water columns, which may lead to a considerable fallout of algae (Davies and Gamble 1979, Steele 1979). This could exaggerate the importance of bacterial mineralization in intercepting sedimenting organic matter, by increasing sedimentation rates of phytoplankton. However, these two phenomena are already substantial in lakes: sedimentation losses can drive phytoplankton succession (Knoechel and Kalff 1975), and bacteria decompose the majority of POM before it leaves the hypolimnion (Kleerekopper 1953, Wetzel et al. 1972, Golterman 1976, Bloesch et al. 1977). In addition, many of the large phytoplankton stimulated by enrichment sink most rapidly, and are thus underrepresented. It seems unlikely that enclosure exaggerated bacterial effects on the community.

Finally, the use of replicate enclosures and classical statistics relies on the assumption that the enclosures are replicable. Although the variability in the data is often high, enrichment effects were detectable and sustained over consecutive weeks, indicating that the enclosures were similar within treatment groups relative to the differences between treatments. Other researchers studying enclosed communities in fresh water systems have in general neglected replication, but marine workers have addressed this question explicitly, concluding that the enclosures are highly repeatable (Takahashi et al. 1975, Davies and Gamble 1979, Oviatt et al. 1980, Reeve et al. 1982).

Table 9. Nutrient levels measured in Heney Lake, 1980. Carbon forms are given in mg/L; all other nutrients in µg/L.

| Date | Ammonia | Nitrate | Soluble Reactive Phosphorus | Total Phosphorus | DOC | DIC |
|---|---|---|---|---|---|---|
| June 11 | 30 | 0 | .0 | 36 | 3.6 | 11.3 |
| June 25 | 20 | 5 | 6 | 18 | 7.1 | 8.8 |
| July 9 | 38 | 5 | 3 | 17 | 3.9 | 9.6 |
| July 23 | 24 | 5 | 6 | 22 | 8.0 | 8.8 |
| July 31 | 12 | 5 | 3 | 18 | 5.4 | 10.0 |
| August 14 | 22 | 5 | 7 | 22 | 4.6 | 10.8 |
| August 28 | 14 | 0 | 4 | 22 | 4.0 | 10.6 |
| September 11 | 22 | 0 | 7 | 20 | 3.9 | 10.6 |

## 4.6 STRUCTURING AND MANIPULATION

The interdependence of all the response variables highlights the substantial degree of integration in the pelagic zone of lakes. Serratia addition in particular had impacts through several compartments, and the overall response to perturbation reflected the web of interactions rather than bacterial addition by itself. For example, herbivorous zooplankton patterns with enrichment apparently resulted from a combination of bacterial consumption, predation by invertebrate carnivores, and interference of large algae with filtering. Such complexity may preclude prediction of community dynamics solely on the basis of individual pairwise interactions (Levins 1975, Briand and McCauley 1978). Clearly field manipulations are essential to test functional significance in all but the simplest of communities.

## 4.7 BACTERIA AS STRUCTURING AGENTS

Are bacteria important structuring agents in lakes? They certainly appear to be on the basis of these experiments. Bacterial enrichment produced dramatic selective increases in diatoms and large phytoplankton, and in bactivorous zooplankton when added bacteria were evidently reproducing well.

4.7.1 Pelagic Community Sensitivity to Enrichment

Furthermore, the pelagic community seems quite sensitive to bacterial enrichment, as relatively low levels of addition produced strong responses. Of course, the production of the added bacteria was not measured: if the added cells were much more active than indigenous ones, this sensitivity could be apparent only. However, it seems unlikely that these batch-cultured cells should be better adapted to conditions in the lake than the suite of bacteria already present there.

This structuring role may be quite widespread among bacteria, as both enrichment species produced similar qualitative effects. However, it is unknown to what degree the original isolation was selective for a specific subset of indigenous bacteria. Plating techniques recover only a fraction of the total bacteria present in a lake, and it is possible that the isolation medium used supported primarily those bacteria able to grow in a rich microenvironment, such as that surrounding a phytoplankter. The ubiquity of structuring among bacteria is thus an open question.

This problem suggests another sense in which the sensitivity of the plankton to bacteria may be exaggerated: although I increased total and actively respiring bacterial levels but little, perhaps enrichment produced a substantial increase in a specific component of the bacterioplankton. For instance, censuses of numerous water bodies for

Aeromonas hydrophila reveal that its maximum levels approach 1X10⁴ cells/mL (Hazen et al. 1978, Rippey and Cabelli 1980). Enrichment would thus increase A. hydrophila densities by at least an order of magnitude, provided that the enumeration techniques used in these surveys did not greatly underestimate its abundance.

In actual fact, the sensitivity of the community to bacteria is most likely intermediate between the extremes that total and A. hydrophila densities would suggest. All isolation media are selective for a subset of microorganisms, and rich organic media like the one employed in this study select for heterotrophic bacteria with high growth rates and low substrate affinities -- the so-called zymogenous flora. This selection, combined with the qualitatively similar impacts of Aeromonas and Serratia on the phytoplankton suggests that it was the zymogenous fraction of the bacteria which was altered in the experiments. As I did not conduct viable counts, I cannot directly estimate the extent of enrichment, but the abundance of these saprophytes in productive waters may be as high as several hundred thousand colony forming units per milliliter (Rheinheimer 1981), so that enrichment increased zymogenous bacterial levels by perhaps fifty percent, and likely much more.

Since the zymogens are poor competitors for the low levels of organic substrates characterizing many water

bodies, these large enrichments might appear to be of questionable relevance. However, algal death, watershed runoff and organic pollution increase the levels of zymogenous bacteria, indicating that the experiments are an appropriate means of evaluating the role of these bacteria in productive waters.

### 4.7.2 Periods of Bacterial Structuring

Although the responses were strong, usually involving severalfold increases in abundance, enrichment effects were generally overshadowed when abrupt shifts in phytoplankton biomass occurred. For example, enhancement of large phytoplankton with treatment disappeared following peaks in the abundance of large algae, suggesting that locking nutrients into biomass had depleted available nutrient supplies and thereby caused the phytoplankton decline (Gliwicz 1975). In another instance, levels of large cladocera in enriched and control enclosures merged when the net phytoplankter Mougeotia was blooming, perhaps because it interfered with feeding by these large grazers (Gliwicz 1977, Webster and Peters 1978).

During the stable periods between major changes in plankton composition, the pelagic community approximates a steady state. At such times, the bacteria are important structuring agents, probably because they alter fluxes between pelagic compartments. For instance, it appears that

phytoplankton and zooplankton were stimulated as the added bacteria increased rates of mineralization and total bacterial production respectively. When major changes in community composition occurred, the steady state was evidently disrupted, net directional flows predominated, and the effects of bacterial enrichment were overwhelmed.

This restricted structuring role may reflect the fact that, while bacteria organize plankton communities, they also depend on these biota for nutrients and energy, which limits their potential impact on the plankton. In this regard, it is probably telling that the effects of other "internal" structuring agents appear similarly limited. Thus, major changes in phytoplankton mask the impact of invertebrate predators on herbivorous zooplankton (Neill 1981, Baril 1983) and reduce grazer effects on edible phytoplankton (Baril 1983). In contrast, "external" agents such as light, temperature, nutrient loadings and fish predation drive shifts in phytoplankton biomass, and so play a primary structuring role.

In conclusion, these studies point to a substantial, previously unidentified role for certain bacterioplankton in determining the composition of lake plankton communities. Future studies of biotic interactions among plankton should therefore consider selective effects of specific bacteria, in addition to the more general role -- as nutrient regenerators -- of the bacteria as a whole.

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