REGULATION OF THE PHYTOPLANKTON COMMUNITY IN FLATHEAD LAKE BY NUTRIENTS AND ZOOPLANKTON

FINAL REPORT

PREPARED FOR THE SOAP AND DETERGENT ASSOCIATION

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AN INEXPENSIVE, DEEP-WATER LIMNOCORRAL THAT COMPENSATES FOR WAVE ACTION

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Running Head Deep water limnocorral

SUMMARY. 1. A large reusable limnocorral was developed, suitable for use in water bodies with substantial wave action. The clear plastic enclosure is 1m in diameter, 25 m deep, lightweight (~50 kg), and inexpensive ($350 USA).

2. The lower portion of the limnocorral is designed to hang motionless below the water surface, while an expandable floating collar section moves freely up and down with any wave action.

3. Two limnocorrals, equipped with removable sedimentation traps at the bottom, were field tested successfully for 6 weeks in Flathead Lake, Montana withstanding wave heights up to one meter. Chlorophyll levels and profiles of various physical-chemical parameters measured inside the limnocorrals were comparable to Flathead Lake values.

4. Despite their large size, the limnocorrals are relatively easy to transport, install and retrieve.

Key word index. enclosure, limnocorral, reusable, nutrients, algae, zooplankton, fish

Introduction

Limnologists and oceanographers commonly utilize enclosures to study *in situ* manipulations of food webs, nutrients, and other parameters. Two types of enclosures generally have been used: 1) small inexpensive enclosures, suitable for use in small lakes with minimal wave action (see review by Lundgren, 1985), and 2) large elaborate enclosures used in oceans and large lakes often with thick opaque sidewalls and equipped with floating docks and/or steel girders designed to withstand rigorous wave conditions (see reviews by Grice and Reeve 1982; Lundgren, 1985). While the first type of enclosure has been used extensively, the use of large enclosures in lakes with significant wave action has been limited by their expense, and/or the difficulties of handling these large structures.

Previous attempts using large limnocorrals in Flathead Lake, a 350 km^2 lake located in northwest Montana, ended in failure due to destruction of the structures by wave action (Stuart, 1983; Stanford, personal communication).

Similar problems with water currents and wave action led researchers working on Lake Tahoe, Nevada, USA to utilize a "kelp sac" design in which the large enclosure is submerged completely below the lake surface (Goldman and Carter, 1965; Goldman, personal communication). While the "kelp sac" design overcomes wave and water current problems, it has no air-water interface, thereby preventing gaseous exchange with the atmosphere as well as hindering sample collection and measurement of in situ profiles such as temperature or light. The following structure was developed out of a need for an inexpensive, transparent enclosure which could extend from the surface to deep depths, withstand significant waves and currents, and be handled easily.

Materials and Methods

The new enclosure (Figure 1) is 1m in diameter and 25 m deep. The sidewalls are made of Canvex II, a semi-transparent, low density polyethylene impregnated with a high density polyethylene ribbon mesh (Raven Industries, Sioux Falls, SD, USA). A large sheet of Canvex II (30.5 m x 3.2 m) was sewn into a 1m diameter tube with a heavy-duty sewing machine using dacron thread and a zig-zag stitch. The sewn seam was reinforced on the outside with Griff-tape (Griffolyn Products Co., Houston, TX, USA), a heavy-duty outdoor tape with a low temperature adhesive which bonds well to polyethylene and can even be applied underwater if necessary. This seam showed no weakening during field tests, and probably was overengineered. Alternatively, the Canvex II could be manufactured into a tube at the factory (Raven Industries) using a fin seam, which could be strengthened if necessary with Griff-tape.

The Canvex II is supported by a frame structure (Figure 1), constructed with plastic pipe, available from most plumbing and construction supply companies. The frame consists of a long, multiple-section lower unit, and an upper, expandable collar unit which includes an external stabilizer frame. The lower unit consists of hoops (1 m diameter) made of flexible polypipe (19 mm diameter, ASTM-D-2239), interconnected with four vertical rods made of CPVC, HOT-COLD water pipe (13 mm diameter, ASTM-D-2846) which extend from the bottom of the limnocorral up to the top collar unit. Individual hoops,

spaced 1.5 m apart along the length of the frame, are made from four equal lengths of polypipe joined into a hoop with press fitted, cross connectors (Figure 1, insert).

After the hoops are assembled, the open ends of each cross connector serve as sleeves, through which the vertical CPVC rods are fitted and held in place with hose clamps (Figure 1, insert). Tee connectors are used on the top and bottom hoops instead of cross connectors, and the vertical CPVC rods are secured to the tee connectors using bolts. The cross and tee connectors must be drilled out slightly in order for the 19 mm CPVC pipe to slide through. In order to allow escape of air from the pipes during submergence, small holes (5 mm diameter) should be drilled in the CPVC pipe every 1-2m.

After the frame is assembled, the Canvex II tube is stretched out inside the pipe frame and taped to the frame with Griff-tape. An early prototype limnocorral was assembled by stretching the enclosure tube over the outside of the frame, thereby eliminating the need for taping the tube to the frame. However, I chose to have the frame structure on the outside of the enclosure tube to eliminate internal surfaces which might serve as refuges for organisms or trap materials that might otherwise sink to the bottom. The Canvex II tube is attached to the top and bottom hoops by wrapping it around the hoop and clamping the material in place with another polypipe hoop, split in half and then screwed to the top or bottom hoop.

The bottom of the frame is attached to a plywood assembly which serves several functions (Figure 1). The plywood provides the bottom seal for the limnocorral, and it contains a hatch which may be removed during initial submergence and later retrieval of the limnocorral. The bottom unit also may be fitted with sediment traps for measuring sedimentation rates inside and outside the limnocorral.

This bottom unit is made of exterior grade plywood (19 mm thick), with a hole cut in the center (0.94 m diameter), and fitted with a plywood hatch (1.3m diameter). The bottom hoop of the limnocorral tube is bolted to the plywood with lag bolts. Large countersunk holes should be drilled halfway through the hoop, to allow insertion of a socket wrench for tightening the lag bolts. The hatch is bolted to the underside of the bottom unit with lag bolts and large wing nuts which are convenient for rapid attachment and removal

underwater. Two large ring gaskets, made from a foam sleeping pad (13 mm thick), are stapled onto the plywood to form a watertight seal for the hatch as well as for the joint between the board and the bottom hoop.

Removable sediment traps, attached to the bottom of the limnocorral (Figure 1), are made using clear jars with plastic lids. Each lid has a large hole drilled in the center, which is aligned with a similar hole drilled through the hatch. For accurate sedimentation rates, the height:diameter ratio of the jars should be 5:1 (Scavia and Fahnenstiel, 1987). The plastic lids are attached permanently to the bottom of the hatch with small screws, such that the jars may be unscrewed and easily replaced during limnocorral experiments using SCUBA. In order to make comparisons with more natural sedimentation rates occurring outside the corrals, similar external sediment traps may be attached to a long board bolted to the bottom of the limnocorral (Figure 1).

The top unit of the limnocorral has a separate frame and Canvex II tube section (Figure 1). The tube (2 m long) has polypipe hoops at the top and bottom which are attached to the enclosure tube in the same fashion described for the lower unit. The top hoop rides on top of a standard tractor tire innertube (designed to fit a narrow 0.96m tractor tire rim). The innertube and top hoop allow the upper enclosure bag to move up and down with any wave action. The innertube is constrained within a square stabilizer frame (Figure 1), which is 2 m tall and made of rigid pvc pipe (51mm diam.). The stabilizer frame maintains the innertube in alignment with the lower portion of the limnocorral, while allowing free vertical movement of the innertube. There are no vertical CPVC pipes attached directly to the top hoop. Instead, four 2 m long sections of CPVC pipe (13 mm diam.) are bolted to the top of the stabilizer frame and act as spreader bars to keep the top enclosure tube open (Figure 1). The top hoop rides just outside these spreader bars.

The stabilizer frame and spreader bars are attached to a plywood board which is submerged below the water surface (Figure 1). The board has a 0.91m hole cut in the center, and the openings of the top and bottom enclosure tubes are lined up with this hole and attached to board using foam gaskets and lag bolts as described for the bottom unit.

A key to the durability of the limnocorral is proper adjustment of the float, anchor, and weighting system which permits the top board to remain submerged below the depth of intense wave disturbances. The top board was submerged 1 m below the surface during the Flathead Lake experiments. Since the top Canvex II tube was 2 m long, approximately 1 m of "excess" bag was present during calm conditions, folded up like an accordion (Figure 1). During wave activity, the top bag could expand or contract approximately 1 meter. This configuration could accommodate waves up to two meters high, from trough to crest.

The length of the top bag and/or the submergence depth of the top board may be altered for use in other water bodies, to accommodate the maximum expected wave height. When the limnocorral is deployed properly, the lower section of the enclosure together with the top board and stabilizer frame remain stationary, largely unaffected by waves, while the innertube and the upper enclosure section float freely up and down with any wave action.

A cement ballast weight (25kg) is attached to the bottom of the limnocorral (Figure 1) which keeps it submerged and helps counteract any subsurface currents which could cause the limnocorral to tilt. The ballast weight is counterbalanced by floats attached with lines to the corners of the top board (Figure 1). Cylindrical boat bumpers (~20 x 50 cm) work well as floats since they are extremely durable and have reinforced grommets for easy line attachment. The submergence depth of the top board can be adjusted easily by altering the length of the float lines.

The entire limnocorral floats freely in the open water (Figure 1), and should be moored to the bottom with anchors. Three danforth anchors were used for each limnocorral in Flathead Lake, set in triangular fashion around the limnocorral, with anchor lines attached to the top board. Since the limnocorral is free floating, it is suitable for use in water bodies with changing water levels. The water level in Flathead Lake dropped approximately 0.5 m during these experiments, and this water level change was accommodated simply by shortening the anchor lines to remove the slack.

Limnocorral deployment Final assembly of the limnocorrals should take place as close as possible to the lakeshore. Although the completed limnocorrals are relatively lightweight, at least 8 people are required to lift and

carry the long limnocorrals without dragging them on the ground. The top frame section may be placed on the stern of a small rowboat, and towed slowly out into the lake. The remainder of the frame can be floated on three large innertubes, spread equidistant along the length of the limnocorral, with the last innertube supporting the bottom board.

To submerge the enclosure, the bottom hatch must be removed. Then, the ballast weight is clipped onto the bottom board and the enclosure is submerged slowly into place. The limnocorral frame is quite flexible, readily bending into a 90^{0} angle over a ten foot length at the water surface as it is submerged. The limnocorral goes down almost vertically, thus trapping an intact column of water. After deployment, the bottom hatch may be reattached using SCUBA.

It took approximately two hours to transport and submerge each limnocorral at a site located 1 km offshore. Total dive time required to attach each hatch at a depth of 25 meters was approximately 9 minutes, using two divers. Periodic replacement of sediment traps took two divers a similar length of time.

Limnocorral retrieval The limnocorrals can be retrieved, floated to other field sites, or removed from the water for later reuse. Removal of the limnocorrals is accomplished by reversing the installation procedure. The hatch is unbolted and the cement weight is unclipped from the bottom of the enclosure using SCUBA. Then, the limnocorral is hoisted vertically by four people, located in two boats, positioned on either side of the limnocorral. As the limnocorral is pulled up out of the water, the flexible frame is tilted on its side and floated on innertubes. The removal procedure took approximately two hours per limnocorral, which included towing and storage on shore.

Results and Discussion

Two limnocorrals were set up in Flathead Lake for six weeks in the Fall of 1987. They survived several storm events accompanied by high winds and wave heights up to one meter, and showed no visible damage upon removal from the water. Landers (1979) suggested that polyethylene enclosures deteriorate rapidly under prolonged exposure to UV radiation from the sun; however, I found no such evidence during the Flathead Lake experiments. The manufacturer (Raven Industries) suggests that degradation of Canvex II may

occur after 6 months to one year of continuous exposure to surface sunlight. The upper section of the limnocorral is most exposed to solar radiation, and could be replaced between experiments if deterioration is a problem. Alternatively, other more expensive side-wall material could be used, which is more resistant to deterioration from UV radiation. Alternative materials includes P 450, a cross-laminated polyethylene which contains an additive to retard UV degradation (Raven Industries), and Tu-Tuf-XR, a cross-laminated polyolefin (Sto-Cote Products Inc., Richmond, Ill).

Profiles Temperature, pH and dissolved oxygen profiles measured in a control limnocorral remained very similar to those measured in Flathead Lake (Figure 2). Photosynthetically active radiation (PAR) was reduced less than 20% throughout most of the limnocorral, compared to the lake due to reduced transmission of light through the semi-transparent side walls; however, the large black innertube at the surface produced considerably more shading in the top two meters of the limnocorral. Chlorophyll a concentrations, measured on integrated water column samples (0-25m) collected from a control limnocorral and in Flathead Lake, were similar throughout the 36 day field test (Figure 3).

This limnocorral design is suitable for various types of experiments including trophic level manipulations (zooplankton and fish), and manipulation of chemical and physical parameters such as nutrients or light. The limnocorrals are useful for experiments that require development of thermal stratification. In addition, they are well suited to the study of organisms which undergo significant vertical migrations. The limnocorrals described here were used to study trophic interactions and vertical migration of *Mysis relicta* (Spencer, in prep).

The design could be modified to allow a sediment interface at the bottom by removing the board at the bottom of the limnocorral, and anchoring the bottom hoop of the frame into the sediments with spikes or a heavy steel chain (Istvanovics et al 1986; Post & McQueen 1987). Although the limnocorrals described here are 25 m long, the length may be modified easily by adding or removing sections of the frame.

Acknowledgements I thank Joel Tohtz for helping build and monitor the limnocorrals, Mark Lorang, Carolyn Bauman, and Richard Hauer for SCUBA assistance, Joseph Shapiro for suggestions on bag and tape material, and Charles Goldman for early discussions on the limnocorral design. Support for this work was provided by the Soap and Detergent Association, and the Montana Water Resources Research Center.

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Figure Legends

Figure 1. Limnocorral design, (anchor lines not shown).

Figure 2. Profiles measured on September 30, 1987 in Flathead Lake (solid symbols) and inside the limnocorral (open symbols) of temperature (▲), pH (■), dissolved oxygen (●), and photosynthetically active radiation (PAR,◆).

Figure 3. Chlorophyll a concentrations measured on integrated 0-25m samples collected during the Fall 1987 experiments.

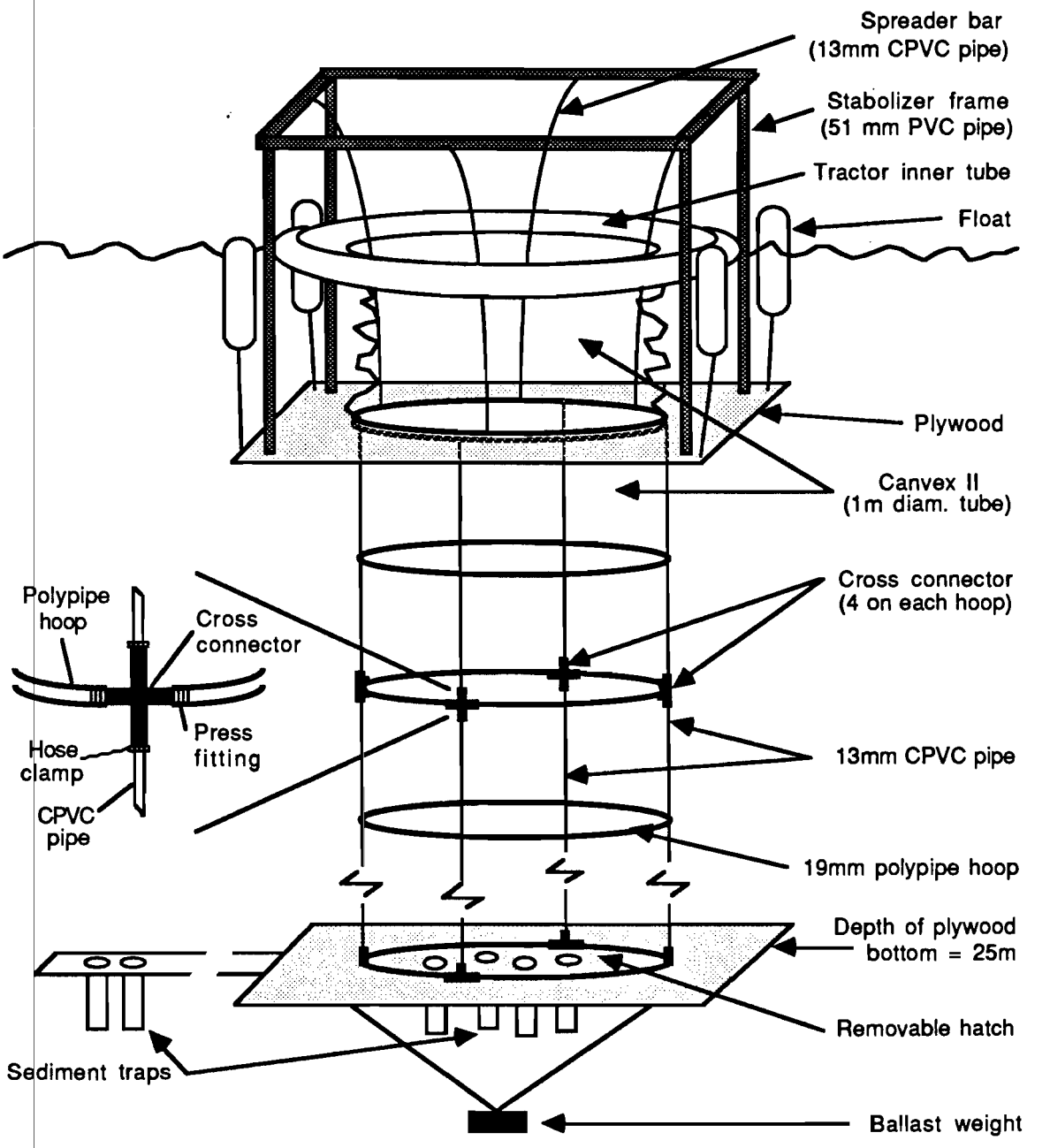

Spreader bar
(13mm CPVC pipe)
Stabolizer frame
(51 mm PVC pipe)
Tractor inner tube
Float
Plywood
Canvex II
(1m diam. tube)
Cross connector
(4 on each hoop)
Polypipe hoop
Cross connector
Press fitting
Hose clamp
CPVC pipe
13mm CPVC pipe
19mm polypipe hoop
Depth of plywood bottom = 25m
Removable hatch
Sediment traps
Ballast weight

Fig 1

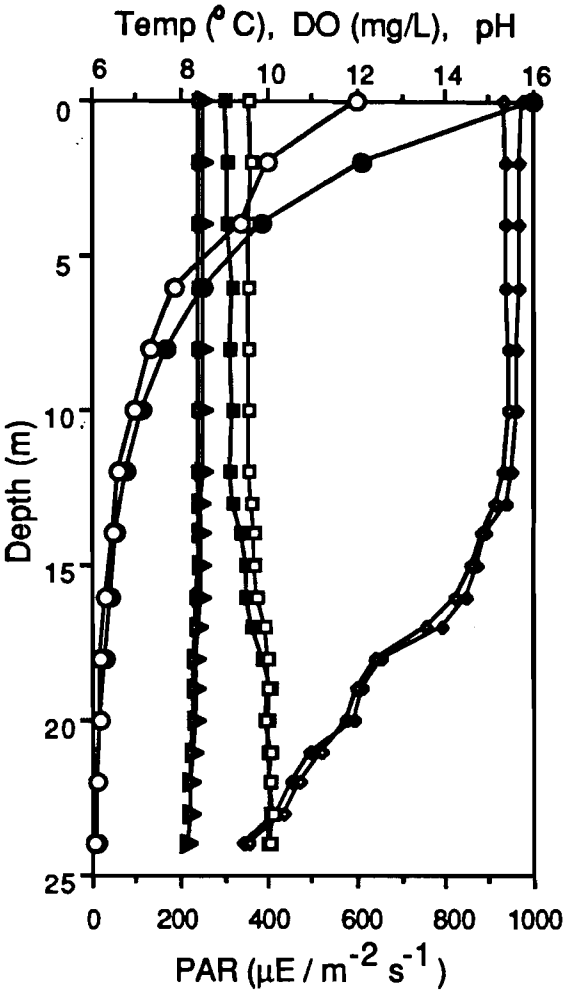
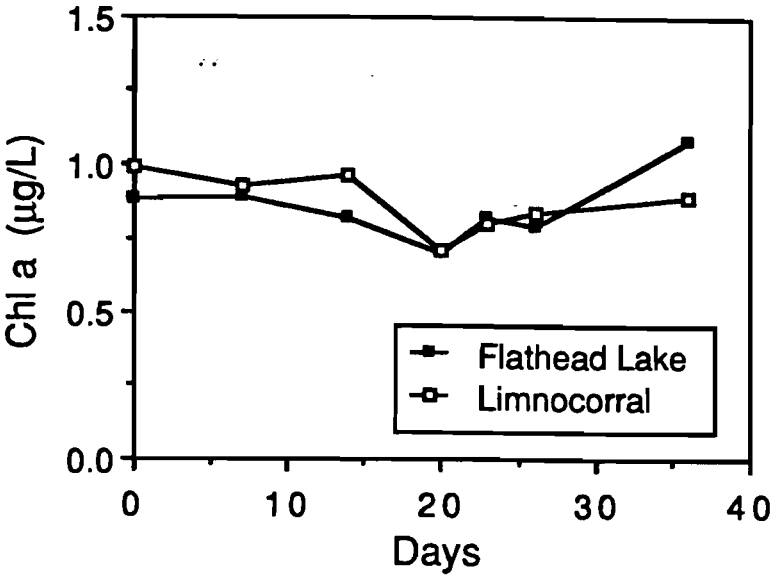

Temp (° C), DO (mg/L), pH
6
8
10
12
14
16
0
5
10
15
20
25
Depth (m)
0
200
400
600
800
1000
PAR (µE / m-2 s-1)

Fig 2.


1.5
1.0
0.5
0.0
Chl a (µg/L)
0
10
20
30
40
Days
Flathead Lake
Limnocorral

Co-limitation by phosphorus and nitrogen, and effects of zooplankton mortality, on phytoplankton in Flathead Lake, Montana, U.S.A.

CRAIG N. SPENCER AND BONNIE K. ELLIS

With 1 figure in the text

Introduction

Mean annual primary production has increased 6% over the last ten years in Flathead Lake (STANFORD & ELLIS 1988), a large oligotrophic lake located in Northwest Montana, U.S.A. Several factors may have contributed to the observed increase in primary production. Nutrient inputs have increased as a result of human population growth in the watershed. In addition, introduction of the exotic crustacean *Mysis relicta* in 1981 has produced major changes in the food web of Flathead Lake, including reductions in zooplankton abundance (BEATTIE & CLANCEY, 1989; SPENCER et al. 1989). The present study was designed to evaluate the importance of nutrients and zooplankton in regulating phytoplankton abundance in Flathead Lake.

Methods

Growth experiments were conducted in 20 L collapsible polyethylene carboys. Water was collected from the middle of Flathead Lake at depth of 5m (depth of maximum primary production in Flathead Lake) using a Van Dorn sampler (15L). The water was transferred to large polyethylene containers (120L), gently mixed with a plastic paddle, and siphoned into carboys. In order to minimize light shock to the phytoplankton, the entire process of water collection and transfer was carried out under black plastic shades.

Nutrient and zooplankton additions were made immediately after the carboys were filled. Twelve different treatments were set up, in duplicate, including 3 different zooplankton levels, 2 levels of P (6 and 60 ugP/L), 2 levels of N (30 and 140ugN/L), and P+N together (60 and 140ug/l respectively). The ambient zooplankton treatment consisted of straight lake water, while the low zooplankton levels was obtained by filtering the lake water through a small 280 um mesh bag as the lake water was siphoned into the carboys. This mesh size was selected to minimize removal of large

phytoplankton. Although many zooplankton, including some adult copepods, passed through the mesh, initial macrozooplankton densities (Cladocera and Copepoda) were reduced 50% by this treatment. The high zooplankton treatment was established by concentrating zooplankton from the lake with a plankton net (280 um mesh) and resuspending the zooplankton into the carboys with a wide-mouthed plastic syringe (turkey baster) yielding an initial macrozooplankton concentration of 6X ambient. There was no significant difference between initial phytoplankton densities in the three zooplankton treatments, student's t-test, $p<0.05$). Phosphorus was added as KH_2PO_4 while nitrogen was added as NH_4NO_3. Nutrient concentrations were monitored throughout the experiments, and nutrients were added periodically as required to maintain the concentrations within 20% of the initial concentrations.

Carboys were suspended in Flathead Lake at a depth of 5 m from a floating platform on October 29, 1986. Carboys were mixed daily by pulling up slightly on the suspension ropes. Chlorophyll a (corrected for pheopigments) was analysed by fluorometry using acetone extractions (STRICKLAND & PARSONS, 1972). Phosphorus and nitrogen were analysed using EPA approved methods on a Technicon Autoanalyser II.

There was no significant difference in the growth response with the two different P levels or the two N levels, therefore data from these treatments were combined. We initially anticipated that these experiments would last 1 to 2 weeks; however, the growth response was much slower than expected. As the experiments progressed, several additional manipulations were carried out including spiking some carboys with alternate nutrients.

Results

Chlorophyll concentrations declined in all treatments during the early stages of the experiments, even in the presence of abundant nutrients (Fig. 1). Part of the decline may be explained by a natural decline in the phytoplankton which occurred in Flathead Lake over the same time period (Fig. 1a), as commonly occurs at this time of year when temperature and light conditions become less favorable for algal growth. Additional initial stress on phytoplankton in the carboys presumably was the result of handling. Over time however, phytoplankton populations recovered in the carboys and showed variable growth responses depending on the treatment.

Nutrient limitation

Phytoplankton growth was significantly enhanced ($p<0.01$) with the simultaneous addition of phosphorous and nitrogen, as chlorophyll concentrations increased 40 fold over the initial levels (Fig. 1a). Addition of micronutrients (KEVERN & BALL 1965) to companion carboys did not increase the growth response (SPENCER, unpub. data) , therefore we conclude that phosphorus and nitrogen were the only two nutrients limiting phytoplankton growth in our experiments. There was slight growth enhancement with the addition of phosphorus alone in comparison to the ambient nutrient treatment, particularly during the middle of the experiment ($p<0.05$). However, by the end of the experiment, there was no significant difference between this treatment and the control. There was no stimulation of growth with addition of nitrogen alone.

Ambient concentrations of PO_4^{3-}, NO_3^- and NH_4^+ were all very low (below detection limits of 1, 5, and 10 ug/L respectively) in Flathead Lake at the start of the experiment, thus it is not surprising that these nutrient co-limited growth of phytoplankton in the carboys. Although past studies suggest that only one nutrient can limit primary production at any one time (SCHINDLER 1977) recent studies demonstrate co-limitation of primary production by nitrogen and phosphorus from a variety of other lakes (LANE & GOLDMAN 1984, WHITE et al. 1985, SUTTLE & HARRISON 1984).

Zooplankton treatments

Mean initial macrozooplankton densities in the low, ambient and high zooplankton treatments were 5, 12 and 75/L respectively. Over 90% of the zooplankton consisted of two copepods species (*Leptodiaptomus ashlandi*, and *Diacyclops thomasi*). The remaining macrozooplankton species consisted of *Bosmina longirostris, Daphnia thorata*, and *Epishura nevadensis* .

As a result of the initial decline in phytoplankton abundance, zooplankton populations were stressed due to food limitation during the experiment, resulting in high mortality. At the end of the experiment, macrozooplankton densities in all carboys were low, ranging from <1organism/L to 4/L. Zooplankton mortality problems are frequently encountered during enclosure experiments (DODSON; LEHMAN pers. comm.); however, the effects of such mortality has not been adequately addressed. Recently, THRELKELD (1987) reported that accidental fish mortality, and subsequent decay and

nutrient release, may confound the results of enclosure experiments designed to evaluate factors controlling phytoplankton growth.

Similarly, our experiments demonstrate that zooplankton mortality may significantly alter the outcome of enclosure experiments. The high zooplankton treatment showed significantly increased growth over the two lower density zooplankton treatments under three different nutrient regimes (Fig. 1b-d). Since phytoplankton growth was limited only by the availability of nitrogen and phosphorus, we suspected that zooplankton mortality in the high zooplankton treatments released these two nutrients in a form available for phytoplankton uptake. The timing and extent of growth enhancement in the high zooplankton carboys varied by treatment. Enhanced growth was noted in the +P treatment prior to the +N treatment (Fig. 1c,d), suggesting that nitrogen was mobilized more rapidly than phosphorus from the zooplankton. However, the stimulatory effect was greater in the +N treatments indicating that the proportion of phosphorus released from the zooplankton was higher than nitrogen, relative to algal needs. Water temperatures ranged from 3-9 ^{o}C during these experiments. When temperatures are warmer, we expect that the release and uptake of these nutrients would occur much more rapidly.

Further evidence that zooplankton mortality contributed to the available nutrient pool is borne out by direct measurements of nutrient concentrations. NH_4^+ concentrations increased up to 16 ug/L in the ambient nutrient/high zooplankton treatment on day 20, while remaining below detection levels in the ambient and low zooplankton treatments. PO_4^{3-} remained below detection limits in the high zooplankton treatments, indicating rapid uptake by the microbial community. P and N appeared to be recycled from zooplankton into phytoplankton since total N and P values were significantly higher in the high zooplankton treatments at the end of the experiments (Table 1) . The N and P was not in soluble forms (Table 1), but rather was in particulate forms. This particulate P and N was incorporated primarily into the phytoplankton as well as microbial and detrital pool, as zooplankton and their remains were largely absent at the end of the experiments. Total P was increased 75% compared to the ambient treatment, while total N increased 36%, supporting the hypothesis that more P than N was released by the zooplankton, relative to algal needs.

Table 1. Water chemistry parameters measured at the end of the experiment, mean (standard deviation). * indicates significant difference from ambient ($p<0.01$).

Zooplankton Treatment	Total P (ug/L)	Soluble P (ug/L)	Total N (ug/L)	Soluble N (ug/L)
Low	4.9 (0.3)	2.95 (0.5)	89 (1.4)	56 (0.1)
Ambient	5.2 (0.1)	2.5 (0.1)	86 (3.0)	62 (8.5)
High	9.1 (0.9)*	3.0 (0.2)	117(7.8)*	51 (4.6)

Nutrient spikes

Final evidence to support the theory that zooplankton mortality stimulated phytoplankton growth via release of limiting nutrients, comes from results of nutrient spikes added mid-way through the experiments. On day 49, phosphorus was added to two of the +N, ambient/low zooplankton carboys. At the same time, nitrogen was added to two of the +P, ambient/low zooplankton carboys. By the end of the experiment, algal growth had been significantly enhanced in both of the spiked treatments, reaching levels comparable or slightly below the high zooplankton treatments (Fig. 1c,d). The response was slower in the +N treatment (Fig. 1d) than in the +P treatment (Fig. 1c), however the algal biomass was lower in the former treatment at the time when the additional nutrient spikes were made. Thus the proportional response observed by the end of the experiment was similar between these two spike treatments.

Conclusions

Growth of Flathead Lake phytoplankton was co-limited by phosphorus and nitrogen. Evidence is presented which suggests that phosphorus was slightly more limiting than nitrogen. Accidental mortality of zooplankton during enclosure experiments can confound nutrient effects as the pool of nitrogen and phosphorus available for algal uptake may be increased significantly.

Acknowledgements

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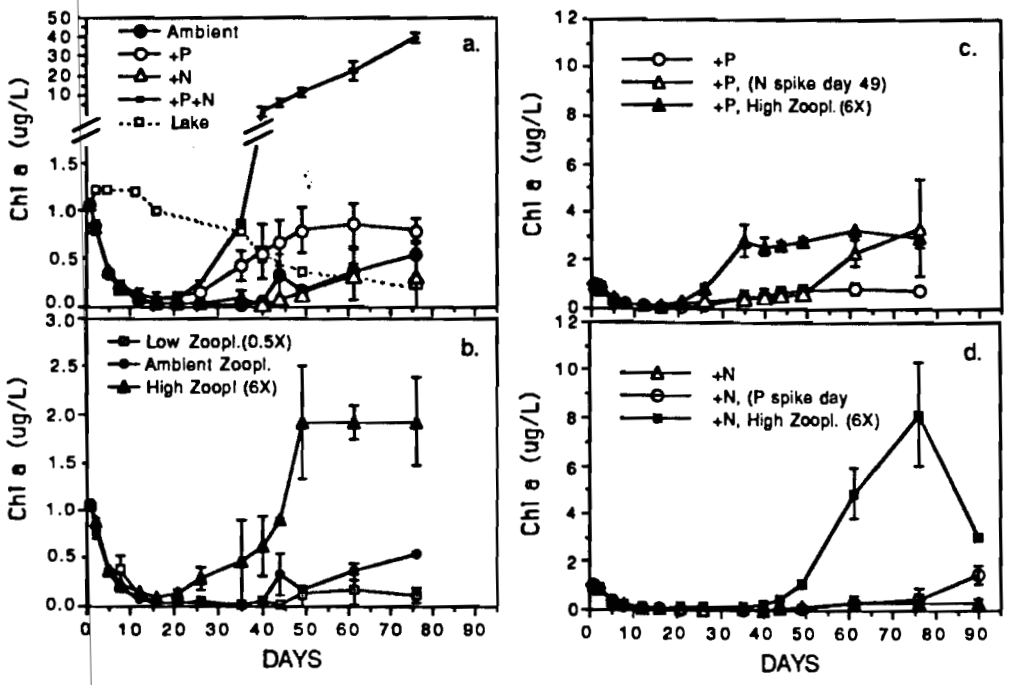

Figure 1. Time course measurements of chlorophyll a in carboys with the following treatments: (a) various additions of N and P, low zooplankton, (b) ambient nutrients with various levels of zooplankton, (c) phosphorus addition, and (d) nitrogen addition.

Role of phosphorus and nitrogen in regulating phytoplankton and bacterioplankton in Flathead Lake, Montana, USA

INTRODUCTION

Recent studies indicate a small but significant increase in primary production in oligotrophic Flathead Lake over the last 10 years, amounting to a 6% increase over this time period (Stanford and Ellis, 1988). This increase has led to concerns that continued growth of human populations in Flathead basin may stimulate lake productivity further through increased nutrient loadings to the lake. Previous studies of other lakes have implicated phosphorus as the primary nutrient limiting productivity (Vollenweider, 1968; Schindler, 1977), while other studies report that nitrogen also may be important in limiting primary production in some freshwater lakes (Goldman, 1978; Priscu and Priscu, 1984). In this study we examine the role of both phosphorus and nitrogen in regulating the phytoplankton community in Flathead Lake. Since planktonic bacteria play an important role in nutrient uptake and recycling in lakes (Currie and Kalff, 1981; Porter et al. ,1988) we also studied the role of nutrient availability in controlling bacterial densities in Flathead Lake.

METHODS

Nutrient bioassay experiments were conducted on five different occasions in 20L collapsible polyethylene containers. Lakewater was collected from the middle of Flathead Lake from a depth of 5m (the depth of maximum primary production), using a 15L Van Dorn sampler for the first two experiments, and a displacement sampler (Dodds and Priscu, 1988) for the latter experiments. Lake water was transferred to large polyethylene containers (120L), gently mixed and quickly siphoned into the carboys. This entire process was carried out in the evening, under opaque plastic shades in order to prevent exposure of the phytoplankton to potentially inhibiting surface light intensities. Carboys were resuspended in Flathead Lake from a floating platform.

Bioassays were conducted in June, July and October 1987, and May and August 1988. The July and August experiments were conducted during thermal stratification of the lake while the other experiments took place during turnover or incomplete stratification (June).

Nutrient treatments were conducted in triplicate and consisted of controls. The first two bioassays were amended with 140 ugN /L as NH_4NO_3(+N), 60 ugP/L) as

KH_2PO_4 (+P) and simultaneous additionsNH_4NO_3 + KH_2PO_4 (N+P). Final nutrient concentrations in these experiments were within 20% of the initial concentrations. The latter three experiments were amended with NH_4Cl instead of NH_4NO_3 after it was determined that there was no difference between these two treatments (unpublished data). Ambient nutrient concentrations in Flathead Lake during each experiment are shown in Table 1.

Chlorophyll *a* and pheopigments were analyzed by fluorometry using acetone extractions (Strickland & Parsons, 1972). Phosphorus and nitrogen were analyzed using EPA approved methods on a Technicon Autoanalyser II. Photosynthetic $^{14}CO_2$ incorporation was determined on 100 ml subsamples from each carboys, which were inoculated with [^{14}C]-$NaHCO_3$ and incubated in situ from 1000-1400 h. The incubations were terminated by filtering through Whatman GF/F filters, washed 5X with 5mL deionized water and counted by standard liquid scintillation spectrometry.

Water from the carboys was filtered onto pre-combusted Whatman GF/F filters for analysis of particulate carbon (PC) and particulate nitrogen (PN) using a Carlo-Erba 1106 elemental analyzer, and particulate phosphorus (PP) by dry oxidation (Solorzano and Sharp 1980). Bacteria were enumerated in the experiments conducted in October 1987 and May 1988 via epifluorescence microscopy using diamidino phenylindole DAPI (Porter and Feig, 1980). Samples were preserved in 4% formalin, filtered onto prestained Nucleopore filters (0.2μm pore size). Filters were stored in dark at 4 ^{o}C and subsequently enumerated using a Leitz Laborlux D fluorescence microscope.

RESULTS

Chlorophyll a

Changes in chlorophyll *a* biomass were noticeable within several days after fertilization, as observed in time course measurements made during initial experiments in June, 1987 (Figure 1). Chlorophyll *a* levels in the nitrogen plus phosphorus treatment increased 3-fold above the controls by day 5.5, and continued to increase through day 7.5 when the experiment was terminated. There was no significant growth response ($p<0.05$) with the addition of nitrogen or phosphorus alone. Since the response to fertilization was obvious by day 5, detailed analyses during subsequent experiments were concentrated within the first 5 days.

A total of five experiments were conducted during 1987 and 1988, and summary chlorophyll *a* results from these experiments (Figure 2) were similar to those observed during the initial experiment (Figure 1). There was a consistent stimulation of algal

growth with the addition of nitrogen plus phosphorus. The effects of fertilization with nitrogen or phosphorus alone were much less pronounced, and varied between experiments. We observed a significant increase in chlorophyll *a* with a single nutrient addition in only one experiment, during October 1987, with the addition of nitrogen (Figure 2). In May 1988, chlorophyll levels appeared to increase with single nutrient additions of phosphorus and nitrogen, as chlorophyll biomass increased from as much as 150% over the controls, however these increases were not significantly different from the controls ($p<0.05$).

$^{14}CO_2$

Analyses of $^{14}CO_2$ uptake also indicated significant algal stimulation with the simultaneous addition of nitrogen and phosphorus, as shown in typical time course measurements during the May, 1988 experiment (Figure 3). On day 2.5, $^{14}CO_2$ uptake also increased slightly with the addition of nitrogen or phosphorus alone; however, by day 4.5 there were no measurable differences between these treatments and the controls. Comparable $^{14}CO_2$ measurements from all five experiments are summarized in Figure 4 and also show generally increased productivity with simultaneous addition of phosphorus and nitrogen. Although the magnitude of the $^{14}CO_2$ response shown in Figure 4 was comparable to chlorophyll response (Figure 2), the $^{14}CO_2$ measurements were more variable, thus the significance levels for differences between treatments were reduced. In most cases, there was no significant stimulation of $^{14}CO_2$ uptake with single nutrient additions. As with the chlorophyll measurements, the only single nutrient addition treatment which stimulated carbon uptake occurred with the addition of nitrogen during the October, 1987 experiments.

Pheopigments

We measured the percent of chlorophyll *a* pigments which had degraded to pheopigments as an indication of the health of phytoplankton in the various nutrient treatments. As shown in the June, 1987 experiments (Figure 5), initial pheopigment levels made up less than 2.5 % of the chlorophyll biomass, and this fraction remained low in the +N+P treatment indicating healthy phytoplankton throughout the experiment. By contrast, the pheopigment fraction increased 3-fold in the control and single nutrient treatments by day 5.5, presumably due to deterioration of chlorophyll pigments resulting from stress caused by nutrient limitation. Similar results were obtained in the other experiments as summarized in Figure 6. The percentage of pheopigments in the +N+P treatments were generally lower than the controls at the end of the experiments.

Pheopigment levels in the single nutrient addition treatments were more variable and in most cases were not significantly lower than the controls. The exception occurred in the +P treatment during May, 1988.

Taken together, analyses of chlorophyll *a* biomass, $^{14}CO_2$ uptake, and pheopigments, all indicate that the phytoplankton community in Flathead Lake was co-limited by the availability of both phosphorus and nitrogen. With the exception of October 1987 experiments, addition of nitrogen or phosphorus alone either produced no stimulatory effect, or stimulated the phytoplankton community for a short time, suggesting that both nitrogen and phosphorus were generally in short supply, and that the addition of one of these nutrients stimulated growth sufficiently to cause rapid depletion of the other nutrient. For example, during the May 1988 experiments, $^{14}CO_2$ uptake was slightly enhanced on day 2.5 with the addition of nitrogen and phosphorus individually, however by day 4.5, $^{14}CO_2$ uptake in these treatments was indistinguishable from the controls (Figure 3). Ambient ammonium and nitrate concentrations were between 4 and 6 ugN/L at the start of this experiment (Table 1) and declined below the detection limits during the course of the experiment. Ambient SRP levels were below detection levels at the beginning of this experiment, thus we can only speculate that the availability of this nutrient decreased further during the experiment. Similarly, during the June 1987 experiment, there was a slight enhancement of growth on day 2.5 in the phosphorus treatment, but no difference between this treatment and the control by day 5.5 (Figure 1). The ambient nitrate concentration was 17ugN/l at the beginning of this experiment while the ammonium concentration was below the detection limit (Table 1); however, inorganic nitrogen levels in the +P treatment rapidly declined below detection limits by day 5.5 due to algal uptake.

Particulate C/N/P ratios

Particulate C, N, and P were measured only during the October 1987 and May 1988 experiments, and these two experiments yielded similar results (Figures 7,8). C/P ratios declined almost 3-fold within 12 hours in all treatments fertilized with P, indicating rapid incorporation of P into particulate forms. Such luxury uptake of P by phytoplankton has been demonstrated in previous studies (Kuhl, 1974). The C/P ratio in the control and +N treatment increased during the 5 day experiments, especially during the October, 1987 experiment, indicating that the algal biomass was becoming increasingly impoverished with P in these treatments. Algal C/N ratios showed little change throughout the experiments in all treatments.

Bacteria

In the May 1988 experiment, bacterial abundance increased in the N+P treatment and the +P treatment ($P<0.1$). By contrast, growth of the phytoplankton community in the May 1988 experiments was not enhanced with the addition of P alone (Figures 2,4). In the October, 1987 experiments, phytoplankton growth was stimulated with the addition of N alone (Figures 2,4) while bacterial abundance in this same treatment appeared reduced, although not significantly different from the controls. These results suggest that the phosphorus and nitrogen requirements for the phytoplankton and bacterioplankton communities in Flathead Lake may be very different. In other lakes, Currie and Kalff (1981) showed that bacteria are very effective at competing with phytoplankton for phosphorus. Further research on phytoplankton/bacterioplankton interactions in Flathead Lake appears warranted, especially in light of recent studies highlighting the importance of microbial communities in food webs and nutrient cycles, particularly in oligotrophic lakes (Stockner and Porter, 1988)

DISCUSSION

This study presents comprehensive evidence that the phytoplankton community in Flathead Lake is generally co-limited by the availability of phosphorus and nitrogen. Similar conclusions have been drawn in other recent studies of Flathead Lake(Spencer and Ellis, 1990; Dodds et al. 1988). Various analyses were utilized to evaluate nutrient limitation in our nutrient bioassay experiments. Of the various procedures, simple determination of chlorophyll *a* biomass after 4.5 days of incubation yielded the lowest variability between replicates and greatest statistical separation between treatments. Although analyses of $^{14}CO_2$ uptake and pheopigments suggested similar trends in nutrient limitation, increased within-treatment variability in these analyses routinely reduced the significance levels for statistical comparisons between the various nutrient treatments. Increased variability in $^{14}CO_2$ uptake compared to chlorophyll *a* measurements likely are related to the fact that $^{14}CO_2$ uptake measurements were made over a four hour incubation, whereas, the chlorophyll *a* biomass measurements integrated the growth response over a 4-5 day period. The mechanisms responsible for increased variability in pheopigment measurements compared to chlorophyll *a* are unclear.

Use of particulate C,N, P ratios for predicting nutrient limitation in our experiments do not correspond with results from monitoring the algal growth response. For example,

the initial particulate N/P ratio in the October, 1987 bioassay was significantly lower than for the May, 1988 experiments (Figures 7,8). This could indicate that the phytoplankton community was more deficient in nitrogen, relative to phosphorus during October, 1987. Yet, results from the chlorophyll and $^{14}CO_2$ uptake analyses clearly indicate that nitrogen was more limiting for phytoplankton growth during the May, 1988 experiments. The usefulness of analyzing particulate C,N ,P ratios for evaluating nutrient limitation is likely compromised by the fact that the particulate analyses include many different particulates besides phytoplankton, such as detritus, bacteria, protozoans and other microheterotrophs. Thus the actual C/N/P ratios in the phytoplankton biomass may be obscured by non-algal material present in the watercolumn.

Finally, bacterioplankton abundance also appeared to be affected by the addition of phosphorus and nitrogen. However, the bacterial response to individual additions of N or P was not consistent with the phytoplankton response. Limited results from our experiments suggest that the phosphorus and nitrogen requirements for the phytoplankton and bacterioplankton communities in Flathead Lake may be very different. Further studies of phytoplankton and bacterioplankton interactions should be conducted to more fully elucidate the differential response of these communities to nitrogen and phosphorus availability.

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DATE	SRP (ugP/L)	NH4 (ugN/L)	NO3 (ugN/L)
Jun-1987	BDL*	BDL*	17
Jul-1987	BDL*	BDL*	BDL*
Oct-1987	0.4	4.5	BDL**
May-1988	BDL**	4.8	5.8
Aug-1988	0.5	4.9	BDL**

* Below detection limit : SRP=1.0, NH4=5.0, NO3=10
** detection limits lowered : SRP=0.4, NH4=2, NO3=2.4

TABLE 1. Ambient nutrient concentrations in Flathead Lake during each carboy experiment.

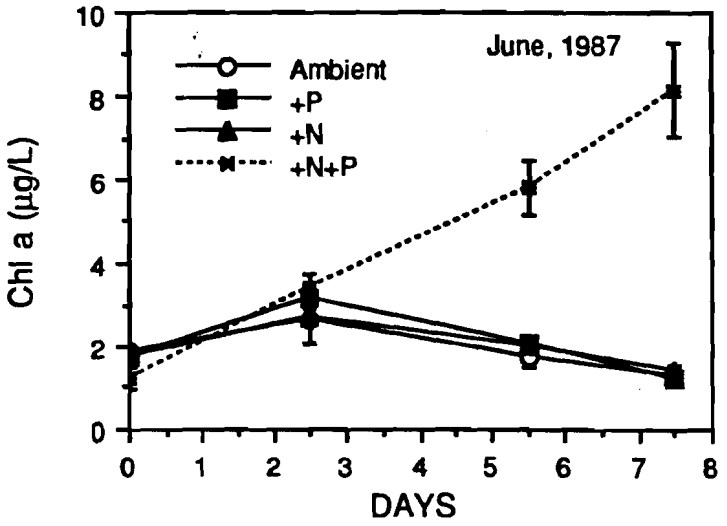

Figure 1. Time course measurements of chlorophyll *a* concentrations measured during the June, 1987 carboy experiments. Error bars represent one standard deviation from the mean.

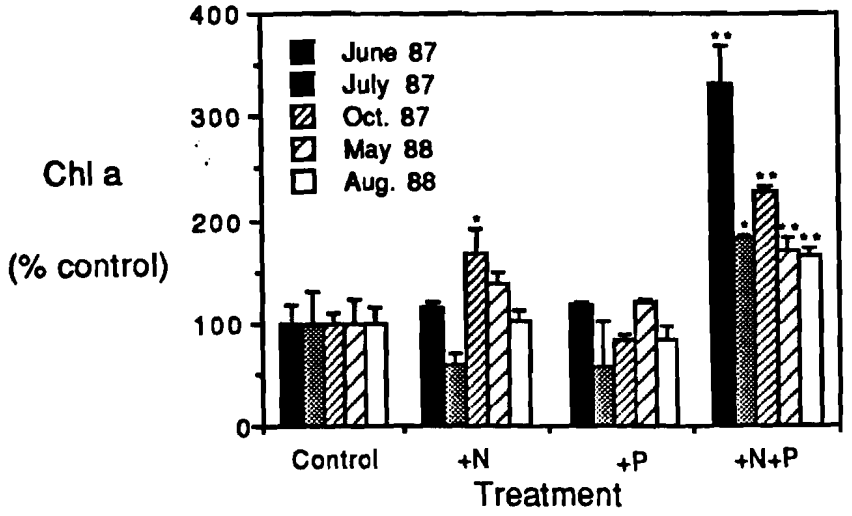

Figure 2. Mean chlorophyll *a* concentrations expressed at the percent of the control. Measurements were made after 4.5 days in all experiments except June, 1987 which were made after 5.5 days. Error bars represent one standard deviation from the mean. Asterisks indicate significant increases above the control (Student's t-test), ** indicates $p<0.01$, * indicates $p<0.05$.

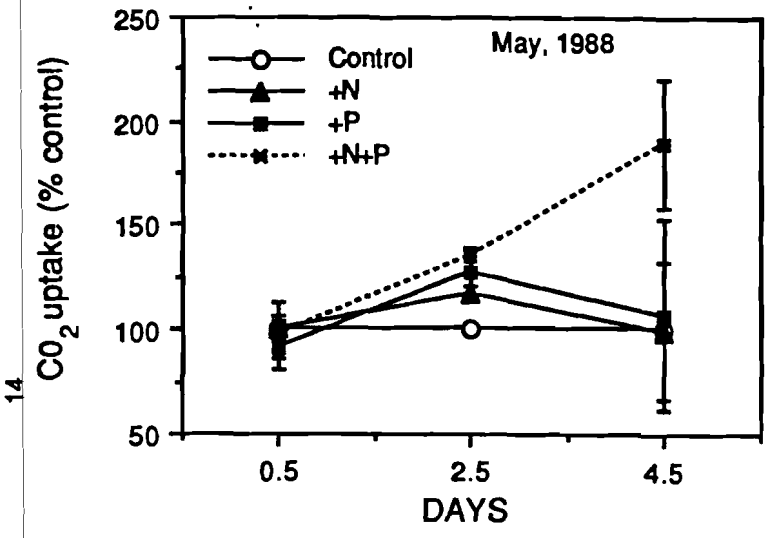

Figure 3. Time course measurements of $^{14}CO_2$ uptake measured during the May, 1988 carboy experiments. Error bars represent one standard deviation from the mean.

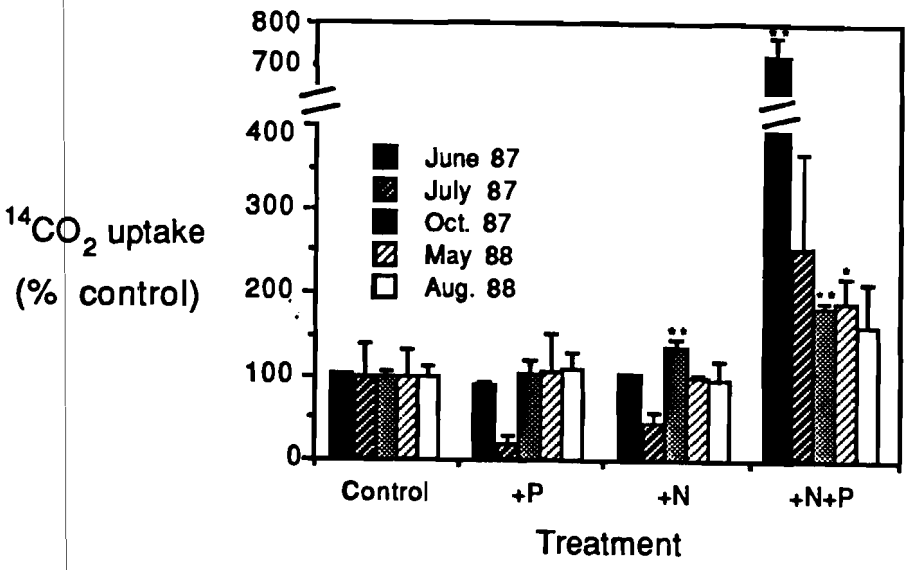

Figure 4. Mean $^{14}CO_2$ uptake measurements expressed at the percent of the control. Measurements were made after 4.5 days in all experiments except June, 1987 which were made after 8 days and July, 1987 after 9 days. Error bars represent one standard deviation from the mean. Asterisks indicate significant increases above the control (Student's t-test), ** indicates $p<0.01$, * indicates $p<0.05$.

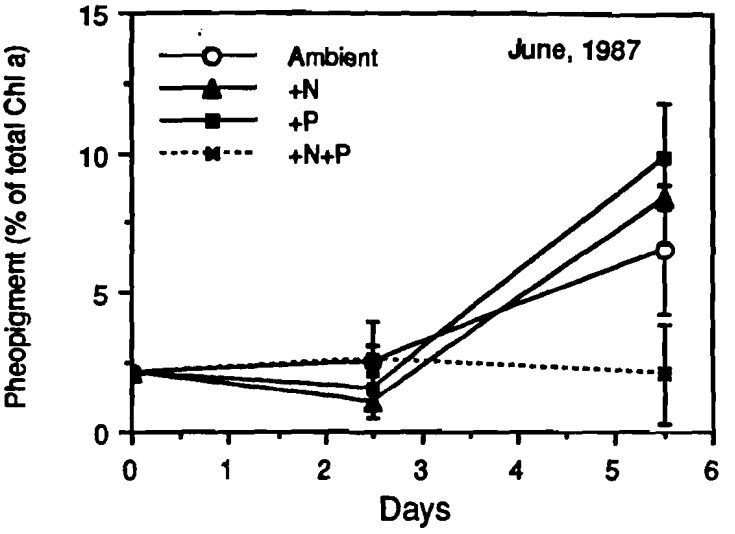

Figure 5. Time course measurements of pheopigments (expressed as the % of total chlorophyll pigments) measured during the May, 1988 carboy experiments. Error bars represent one standard deviation from the mean.

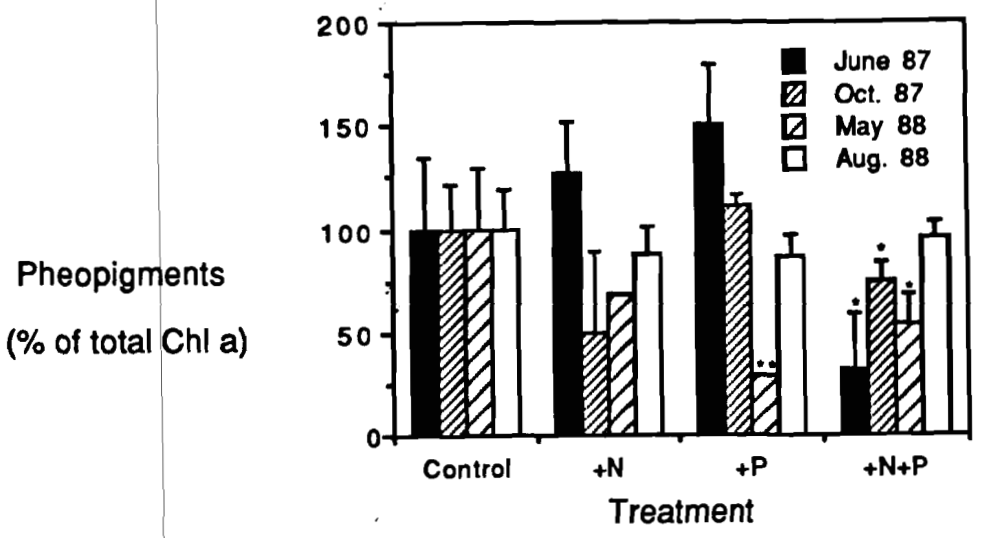

Figure 6. Mean pheopigment concentrations (expressed as the % of total chlorophyll pigments) shown at the percent of the control. Measurements were made after 4.5 days in all experiments except June, 1987 which were made after 5.5 days. Error bars represent one standard deviation from the mean. Asterisks indicate significant increases above the control (Student's t-test), ** indicates $p<0.01$, * indicates $p<0.05$.

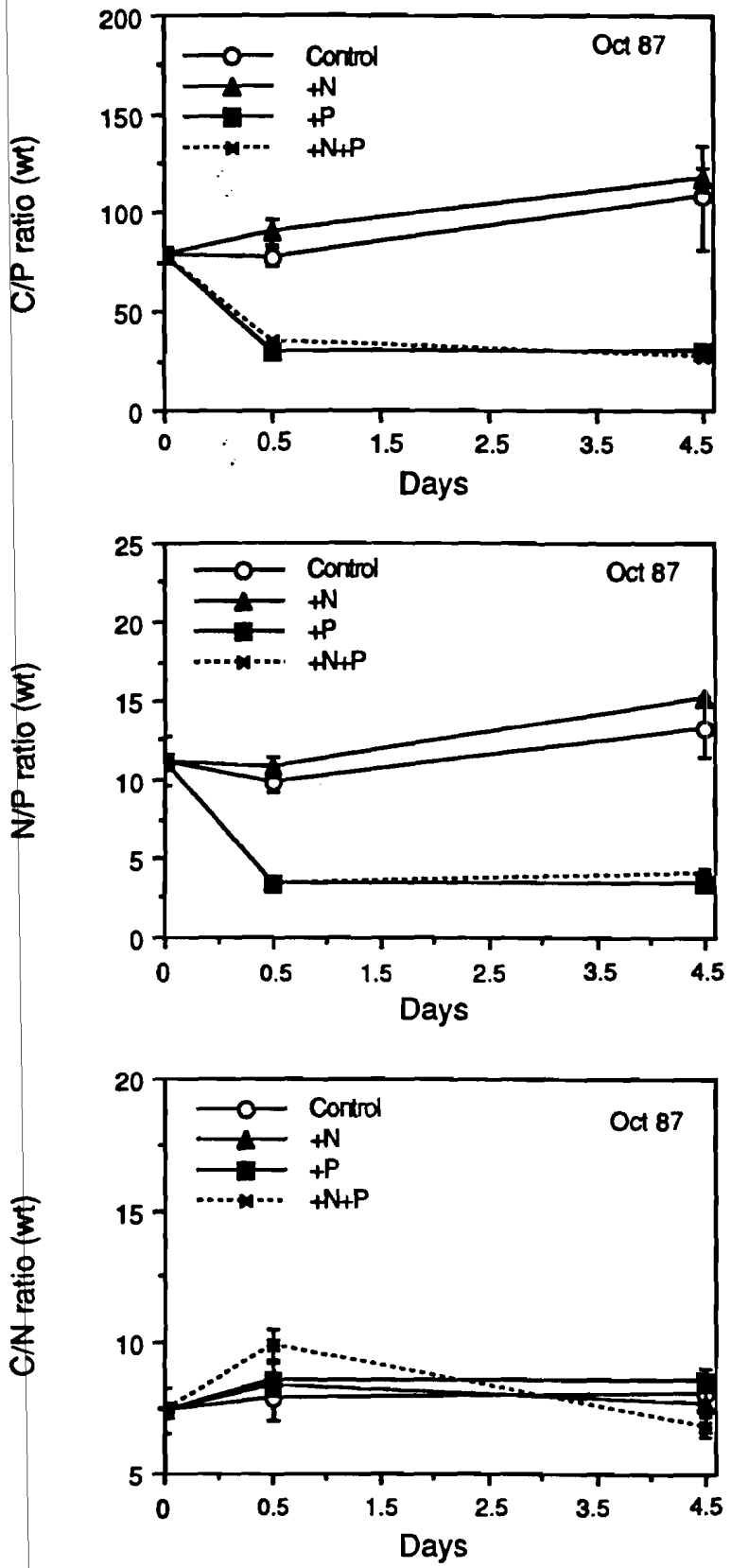

Figure 7. Time course measurements of particulate C,N, and P ratios (by weight) during the October, 1987 carboy experiments. Error bars represent one standard deviation from the mean.

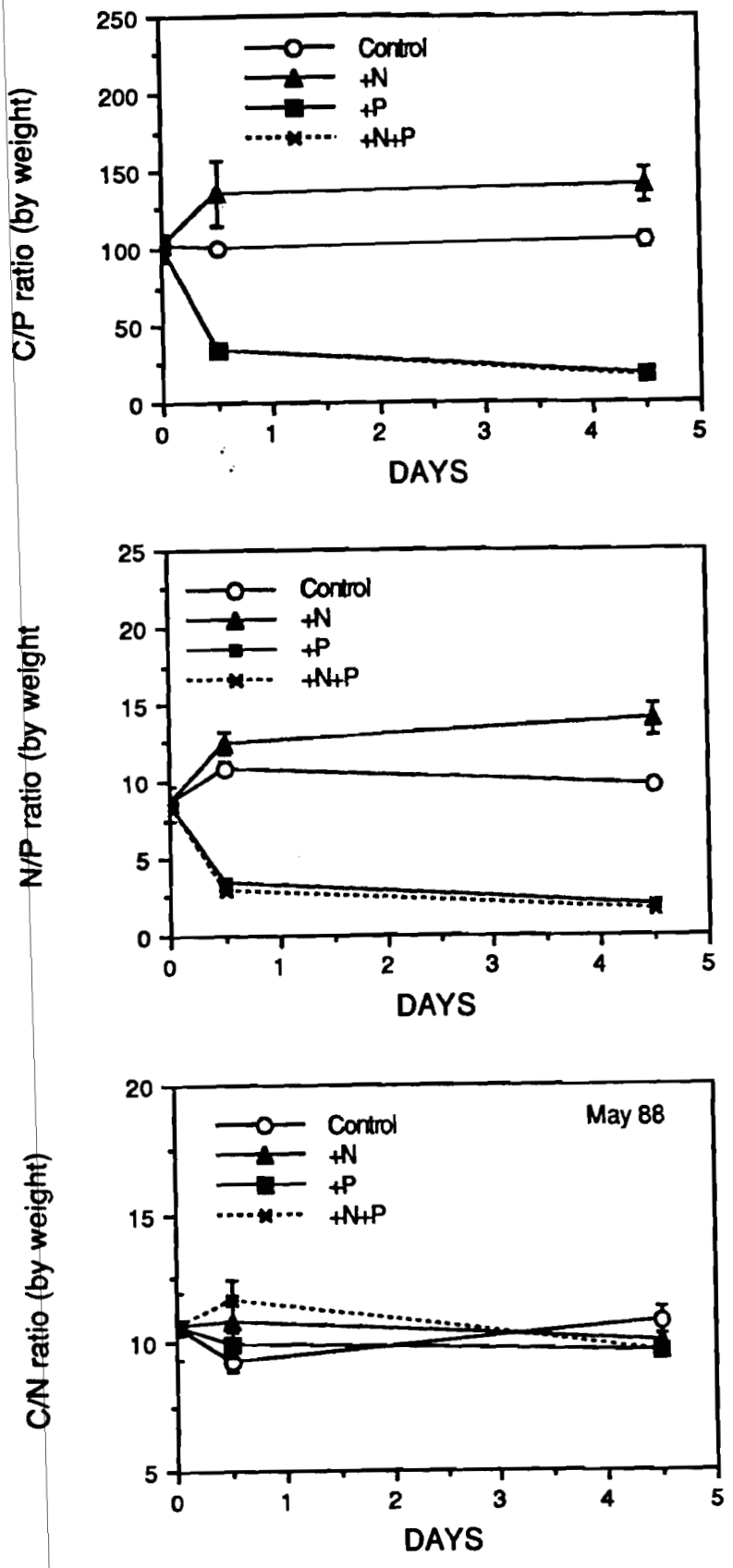

Figure 8. Time course measurements of particulate C,N, and P ratios (by weight) during the May, 1988 carboy experiments. Error bars represent one standard deviation from the mean.

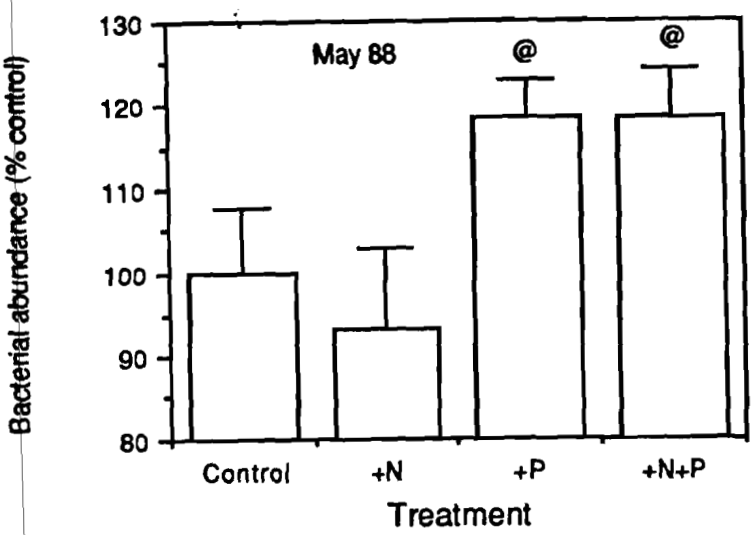

Figure 9. Mean bacterioplankton abundance (% of controls) after 4.5 days in the May, 1988 carboy experiments. Error bars represent one standard deviation from the mean. Asterisks indicate significant increases above the control (Student's t-test), ** indicates $p<0.01$, * indicates $p<0.05$, @ indicates $p<0.1$.

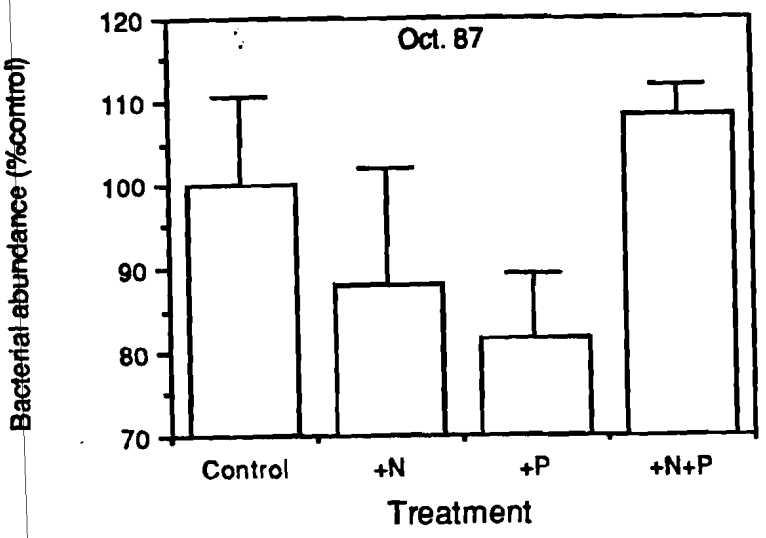

Figure 10. Mean bacterioplankton abundance (% of controls) after 4.5 days in the October, 1987 carboy experiments. Error bars represent one standard deviation from the mean. Asterisks indicate significant increases above the control (Student's t-test), ** indicates $p<0.01$, * indicates $p<0.05$.

Top-down verses bottom-up control of phytoplankton and bacterioplankton communities in a large oligotrophic lake

INTRODUCTION

Historically, limnologists have focused on nutrients (principally phosphorus) as the primary determinant of phytoplankton growth and biomass in lakes. Although such bottom-up controls (e.g. nutrients) remain a focus and have been expanded to include other important regulatory nutrients such as nitrogen, recent studies suggest that top-down controls via the upper trophic levels also may play an important role in regulating the phytoplankton community (see reviews in Carpenter et al. 1988). These studies demonstrate that shifts in the fish community which lead to changes in the abundance of zooplankton (the primary herbivores in lakes) may have a pronounced impact on the phytoplankton community in eutrophic lakes, to the extent that algal blooms may be produced or eliminated simply by manipulating the upper trophic levels (Shapiro, 1980; Spencer and King, 1984). However, there is some controversy over the relative importance of top-down verses bottom-up controls in regulating lake ecosystems, especially as one moves across the nutrient gradient from eutrophic to oligotrophic waters (Crowder et al. 1988). Some researchers suggest that grazer control of the phytoplankton community should be stronger in oligotrophic waters than eutrophic waters (McQueen et al. 1986) while results from other studies suggest the opposite (Vanni 1986).

To date, most of the top-down/bottom-up studies have been conducted in the more nutrient-rich end of the trophic gradient, stemming in large part from the fact that growth of phytoplankton in eutrophic lakes experiencing nuisance algal blooms has attracted more attention than in oligotrophic lakes. Thus few comparable studies of top-down versus bottom-up control have been conducted in oligotrophic lakes. Additional studies in the latter lakes are needed before accurate predictions can be made about the relative importance of top-down verses bottom-up forces across a gradient of nutrient enrichment.

In the present study, we focused our efforts on Flathead Lake, a large oligotrophic lake in northwestern Montana which has experienced changes in both upper trophic levels and nutrient loadings in recent years. The upper trophic levels in Flathead Lake have been altered dramatically over the last 5-10 years, due in large part to the

appearance of *Mysis relicta* in the lake (Spencer et al. 1990). This highly predacious crustacean has dramatically reduced the abundance of herbivorous zooplankton in the lake. At the same time, increased development and human habitation of the watershed has led to concerns that nutrient loadings to the lake are increasing.

There has been a small but significant increase in primary production in Flathead Lake over the last 10 years (Stanford and Ellis 1988). The purpose of this study was to study the relative importance of nutrients and zooplankton in regulating the phytoplankton community in Flathead Lake.

METHODS

Nutrient bioassay experiments were conducted on four different occasions in 20L collapsible polyethylene containers. Lakewater was collected from the middle of Flathead Lake from a depth of 5m (the depth of maximum primary production), using a 15L Van Dorn sampler for the first experiment, and a displacement sampler (Dodds and Priscu, 1989) for the latter experiments. Lake water was transferred to large polyethylene containers (120L), gently mixed and quickly siphoned into the carboys. This entire process was carried out in the evening, under opaque plastic shades in order to prevent exposure of the phytoplankton to potentially inhibiting surface light intensities.

Bioassays were conducted in July and October 1987, and May and August 1988. The July and August experiments were conducted during thermal stratification of the lake while the other experiments took place during turnover. We used a factorial experimental design with two levels of nutrients and three levels of zooplankton.

Treatments were conducted in triplicate. Ambient nutrient treatments consisted of no nutrient additions. Nitrogen and phosphorus were added to the other another treatment at levels in excess of algal needs. The first bioassay was amended with NH_4NO_3 and KH_2PO_4 (N+P). Nutrient concentrations were monitored daily and nutrients were added periodically to maintain initial concentrations. The latter three experiments were inoculated with higher nutrient concentrations (+N (140 ug/L , +P (60 ugP/L), in order to eliminate the need for laborious nutrient monitoring. Final nutrient concentrations in these experiments were within 20% of the initial concentrations. The latter three experiments were amended with NH_4Cl instead of NH_4NO_3 after it was determined that there was no difference between these two treatments (unpublished data). Ambient nutrient concentrations for each experiments are shown in Table 1.

The three zooplankton levels were established as follows. Ambient zooplankton treatments consisted of straight lakewater, while low zooplankton levels were obtained be filtering the lakewater through a small 280 um mesh bag as the lake water was siphoned into the carboys. Although many zooplankton , including some adult copepods, passed through the mesh, initial macrozooplankton densities were reduced 50% by this treatment. Higher zooplankton treatments were established by concentrating zooplankton from the lake with a plankton net (280 um mesh) and resuspending the zooplankton into the carboys with a wide-mouthed plastic syringe yielding initial zooplankton densities ranging from 2X to 15X ambient in the different experiments. These densities span the range of zooplankton densities present in Flathead Lake at various times of the year prior to the introduction of *Mysis relicta* in the lake.

Chlorophyll *a* and pheopigments were analyzed by fluorometry using acetone extractions (Strickland & Parsons, 1972). Phosphorus and nitrogen were analyzed using EPA approved methods on a Technicon Autoanalyser II. Photosynthetic $^{14}CO_2$ incorporation was determined on 100 ml subsamples from each carboys, which were inoculated with [^{14}C]-$NaHCO_3$ and incubated in situ from 1000-1400 h. The incubations were terminated by filtering through Whatman GF/F filters, washed 5X with 5mL deionized water and counted by standard liquid scintillation spectrometry.

Water from the carboys was filtered onto pre-combusted Whatman GF/F filters for analysis of particulate carbon (PC) and particulate nitrogen (PN) using a Carlo-Erba 1106 elemental analyzer, and particulate phosphorus (PP) by dry oxidation (Solorzano and Sharp 1980). Bacteria were enumerated in the experiments conducted in October 1987 and May 1988 via epifluorescence microscopy using diamidino phenylindole DAPI (Porter and Feig, 1980). Samples were preserved in 4% formalin, filtered onto prestained Nucleopore filters (0.2μm pore size). Filters were stored in dark at 4 ^{o}C and subsequently enumerated using a Leitz Laborlux D fluorescence microscope.

RESULTS

Chlorophyll a

Addition of N+P markedly stimulated the growth of phytoplankton in all carboy experiments as illustrated in the May, 1988 experiments (Figure 1, and the nutrient limitation manuscript in this report). These results, described in detail in the nutrient manuscript elsewhere in this report, suggest that phytoplankton growth in Flathead

Lake is limited by bottom-up controls, e.g. phosphorus and nitrogen availability, both of which are in short supply in Flathead Lake (Table 1).

Alteration of zooplankton abundance over a wide range of concentrations produced no significant changes in chlorophyll *a* biomass as illustrated in the May, 1988 experiments (Figure 2). However, alteration of zooplankton abundance in the presence of abundant N+P resulted in marked differences in chlorophyll *a* biomass as illustrated in the May, 1988 experiments (Figure 3). Similar results were obtained in the other three experiments as summarized in Figures 4-7. There was no significant relationship ($P<0.1$) between zooplankton abundance and chlorophyll *a* biomass in any of the ambient nutrient treatments. However, with the addition of N+P, there was a negative correlation between zooplankton abundance and chlorophyll *a* biomass by the end of each experiment. This correlation was significant at the $p<0.01$ level in the first three experiments. In the last experiment conducted in August, 1988, there also appeared to be a negative relationship between zooplankton and chlorophyll a biomass in the N+P treatment, however this relationship was not a statistically significant due to unusually high variability in chlorophyll *a* levels between replicates.

Two factor ANOVA's indicated a significant interaction between nutrients and zooplankton in controlling chlorophyll *a* biomass (Table 2-5). This interaction was significant at the $p<0.01$ in the middle two experiments, and at the $p=0.78$ and $p=0.11$ in the first at last experiments respectively.

These results suggest that top-down controls via zooplankton grazing have little impact on total phytoplankton biomass in Flathead Lake at the low nutrient concentrations that exist in the lake today. Thus it is unlikely that recent declines in zooplankton abundance in Flathead Lake following the introduction of *Mysis relicta* (Spencer et al. 1990) have altered the abundance of phytoplankton in the lake. Phytoplankton growth appears to be so limited by nutrient availability in this oligotrophic lake, that additional control of phytoplankton biomass via zooplankton grazing appear to be negligible.

However, with the addition of N+P, grazer control of phytoplankton biomass may become important. For example, 4-7 fold increases in zooplankton biomass appeared to be sufficient to reduce chlorophyll *a* abundance in Flathead Lake waters to ambient levels even in the presence of high concentrations of N and P. Spencer and King (1984) also demonstrated that phytoplankton abundance may be greatly reduced, even in nutrient-rich sewage lagoon, given sufficient zooplankton densities maintained in the presence of the appropriate fish community characterized by reduced densities of zooplanktivorous fish..

$^{14}CO_2$

Analysis of $^{14}CO_2$ uptake in these same experiments yielded comparable results as chlorophyll *a* biomass as illustrated in representative data taken from the May, 1988 experiments (Figure 8).

C-N-P ratios

Particulate C, N, and P were measured in the October, 1987 and May, 1988 experiments and the results from these two experiments were very similar (Figures 9,10). In the ambient nutrient treatments, both the C/N ratio and C/P ratio decreased in the presence of high zooplankton abundance in comparison to ambient zooplankton treatments. By contrast, C/N and C/P ratios appeared unaffected by zooplankton abundance in the N+P treatments. Taken together, these results suggest that nitrogen and phosphorus were more available for phytoplankton uptake in the high zooplankton treatments, presumably as a result of enhanced regeneration of nutrients by increased zooplankton grazing activity. Similar results have been reported elsewhere (Lehman, 1980). Such enhancement of N and P availability by zooplankton appear insignificant in the N+P treatments, as N and P were already available in quantities in excess of algal needs in these treatments. Thus, any additional enhancement of N and P availability by the zooplankton had negligible impacts of the phytoplankton.

Bacteria

The abundance of bacterioplankton was monitored in the October, 1987 and May, 1988 experiments (Figures 11,12). In the first experiment, there was a positive correlation between zooplankton abundance and bacterial abundance in the ambient nutrient treatments as well as the N+P treatments. Similar trends were observed in the May, 1988 experiments, although the significance levels were reduced. The impacts of zooplankton manipulations on bacterial abundance were in marked contrast to impacts on phytoplankton abundance in these same two experiments which showed no relationship with zooplankton abundance at ambient nutrient levels, and a significant negative relationship in the N+P treatment (Figure 5,6). Increased zooplankton abundance stimulated the bacterioplankton, but not through enhancement of N and P availability, otherwise there would have been no noticeable

effect in the N+P treatment. Increased zooplankton abundance may have increased the availability of dissolved and particulate carbon through excretion of zooplankton feces and DOC, thereby providing additional substrate for the heterotrophic bacteria. Lampert (1978) demonstrated that a significant amount of the algal carbon injested by zooplankton was lost immediately as dissolved organic carbon during feeding. Although zooplankton also may graze on bacteria, their stimulatory effect on bacterial growth appeared to outweigh any increased losses due to enhanced zooplankton grazing in our experiments.

Given the importance of interactions between phytoplankton and the microbial communities in lakes (Stockner and Porter, 1988), more study should be directed at the apparent contrasting effects of the zooplankton community on phytoplankton and bacterioplankton in Flathead Lake.

CONCLUSIONS

Results from our carboy experiments suggest that phytoplankton abundance in oligotrophic Flathead Lake is regulated primarily by the availability of nutrients (phosphorus and nitrogen). Recent alteration of the food web via introduction of *Mysis relicta* and subsequent declines in zooplankton abundance (Spencer et al. 1990) would appear to have had little impact on total phytoplankton abundance in Flathead Lake. Our results suggest that grazer control of phytoplankton abundance would likely be much more important under more nutrient-rich conditions. Although consumer control of phytoplankton abundance appears to be minimal when nutrient concentrations are low, zooplankton impacts may be quite important under more nutrient-rich conditions.

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FIGURE LEGENDS

Figure 1. Time course measurements of chlorophyll *a* concentrations in the May, 1988 carboy experiments. These treatments contained ambient zooplankton levels. Error bars indicate one standard deviation from the mean.

Figure 2. Time course measurements of chlorophyll *a* concentrations in the May, 1988 carboy experiments. These treatments contained ambient nutrient levels and various zooplankton levels. Error bars indicate one standard deviation from the mean.

Figure 3. Time course measurements of chlorophyll *a* concentrations in the May, 1988 carboy experiments. These treatments were amended with N+P and various zooplankton levels. Error bars indicate one standard deviation from the mean.

Figure 4. Chlorophyll *a* biomass (% control) measured after 4.5 days, as a function of zooplankton abundance in the July, 1987 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols).P values indicate the significance level of the linear relationship between zooplankton and chlorophyll *a*. Error bars indicate one standard deviation from the mean.

Figure 5. Chlorophyll *a* biomass (% control) measured after 4.5 days, as a function of zooplankton abundance in the October, 1987 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols).P values indicate the significance level of the linear relationship between zooplankton and chlorophyll *a*. Error bars indicate one standard deviation from the mean.

Figure 6. Chlorophyll *a* biomass (% control) measured after 4.5 days, as a function of zooplankton abundance in the May, 1988 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols).P values indicate the significance level of the linear relationship between zooplankton and chlorophyll *a*. Error bars indicate one standard deviation from the mean.

Figure 7. Chlorophyll *a* biomass (% control) measured after 4.5 days, as a function of zooplankton abundance in the August, 1988 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols).P values indicate the significance level of the linear relationship between zooplankton and chlorophyll *a*. Error bars indicate one standard deviation from the mean.

DATE	SRP (ugP/L)	NH4 (ugN/L)	NO3 (ugN/L)
Jun-1987	BDL*	BDL*	17
Jul-1987	BDL*	BDL*	BDL*
Oct-1987	0.4	4.5	BDL**
May-1988	BDL**	4.8	5.8
Aug-1988	0.5	4.9	BDL**

* Below detection limit : SRP=1.0, NH4=5.0, NO3=10
** detection limits lowered : SRP=0.4, NH4=2, NO3=2.4

TABLE 1. Ambient nutrient concentrations in Flathead Lake during each carboy experiment.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Nutrients (A)	1	8021.366	8021.366	6.101	.0331
Zooplankton (B)	2	3292.769	1646.385	1.252	.3271
AB	2	8724.872	4362.436	3.318	.0785
Error	10	13148.42	1314.842		

Table 2. Two-factor ANOVA table evaluating the impact of nutrients (N+P) and zooplankton on chlorophyll abundance measured after 4.5 days in the July, 1987 carboy experiments.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Nutrients (A)	1	32324.921	32324.921	149.424	.0001
Zoopl. level (X ambie...	2	21312.615	10656.308	49.26	.0001
AB	2	16676.466	8338.233	38.544	.0001
Error	12	2595.958	216.33		

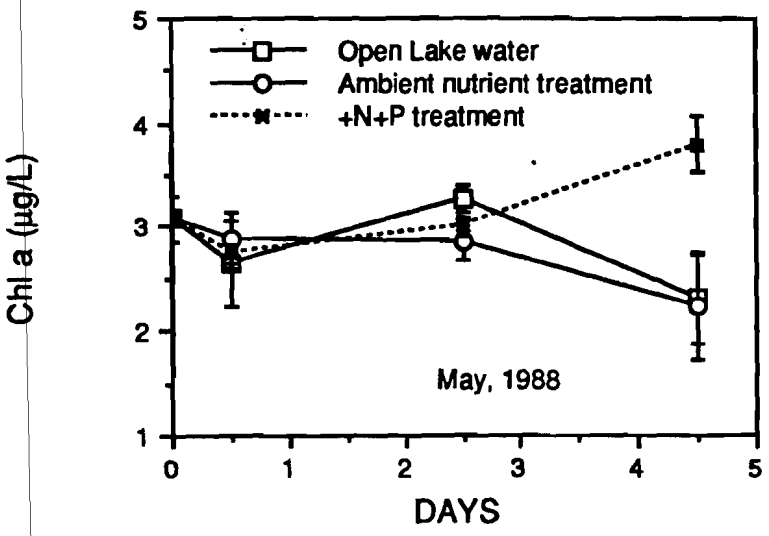
Table 3. Two-factor ANOVA table evaluating the impact of nutrients (N+P) and zooplankton on chlorophyll abundance measured after 4.5 days in the October, 1987 carboy experiments.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Nutrients (A)	1	7241.66	7241.66	54.626	.0001
Zoopl. level (X ambie...	2	2566.504	1283.252	9.68	.0031
AB	2	2651.955	1325.978	10.002	.0028
Error	12	1590.807	132.567		

Table 4. Two-factor ANOVA table evaluating the impact of nutrients (N+P) and zooplankton on chlorophyll abundance measured after 4.5 days in the May, 1988 carboy experiments.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Nutrients (A)	1	2921.537	2921.537	4.198	.063
Zoopl. level (X ambie...	2	238.007	119.003	.171	.8448
AB	2	3623.394	1811.697	2.604	.115
Error	12	8350.427	695.869		

Table 5. Two-factor ANOVA table evaluating the impact of nutrients (N+P) and zooplankton on chlorophyll abundance measured after 4.5 days in the August, 1988 carboy experiments.



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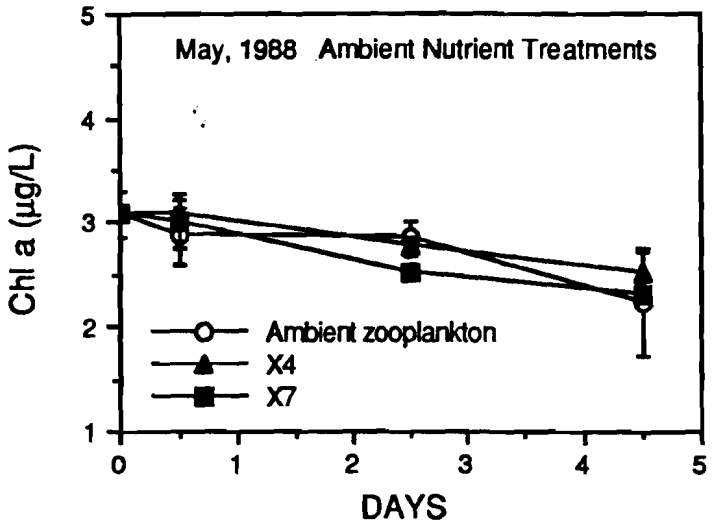

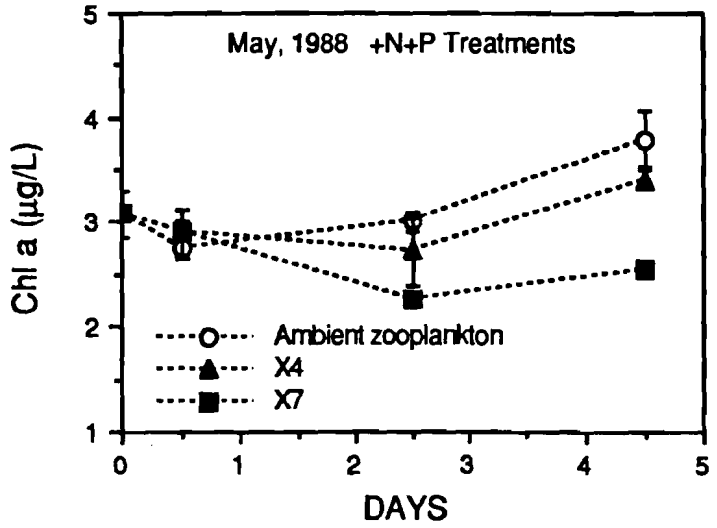

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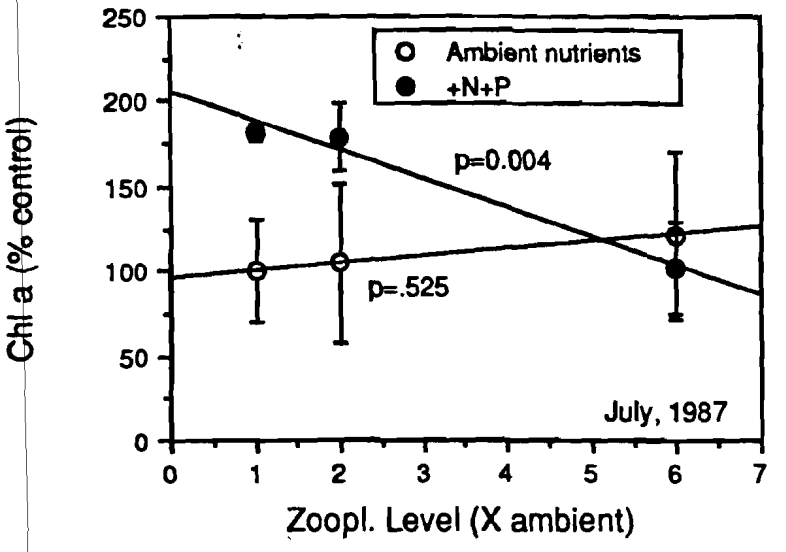

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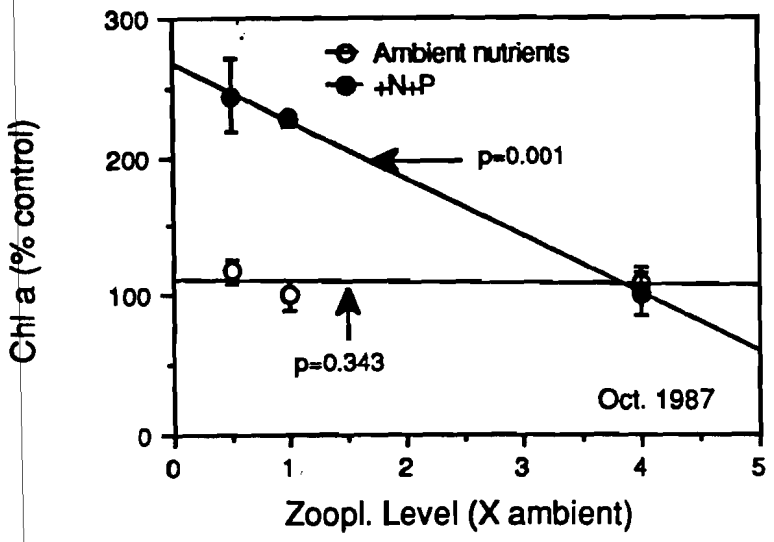

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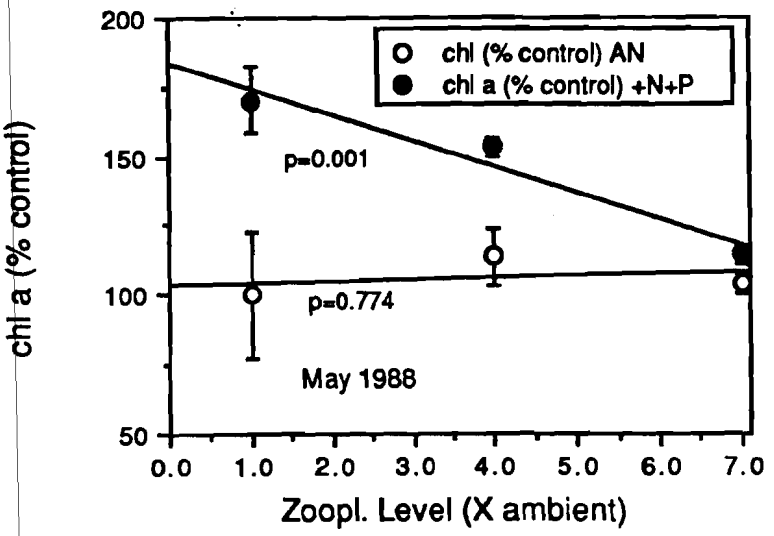

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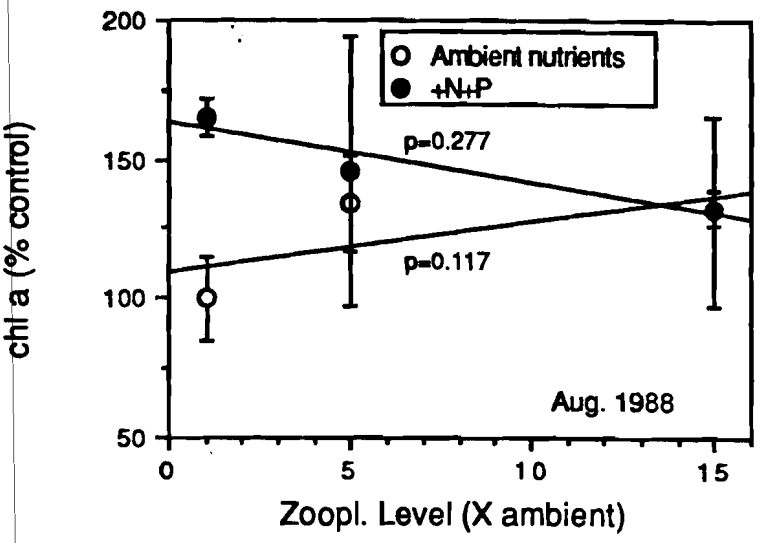

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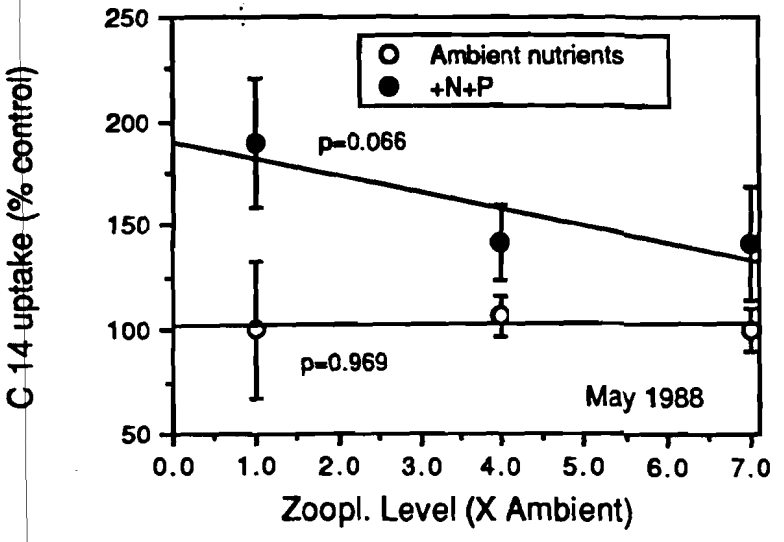

Figure 8. $^{14}CO_2$ uptake measured after 4.5 days, as a function of zooplankton abundance in the May, 1988 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols).P values indicate the significance level of the linear relationship between zooplankton and chlorophyll *a*. Error bars indicate one standard deviation from the mean.

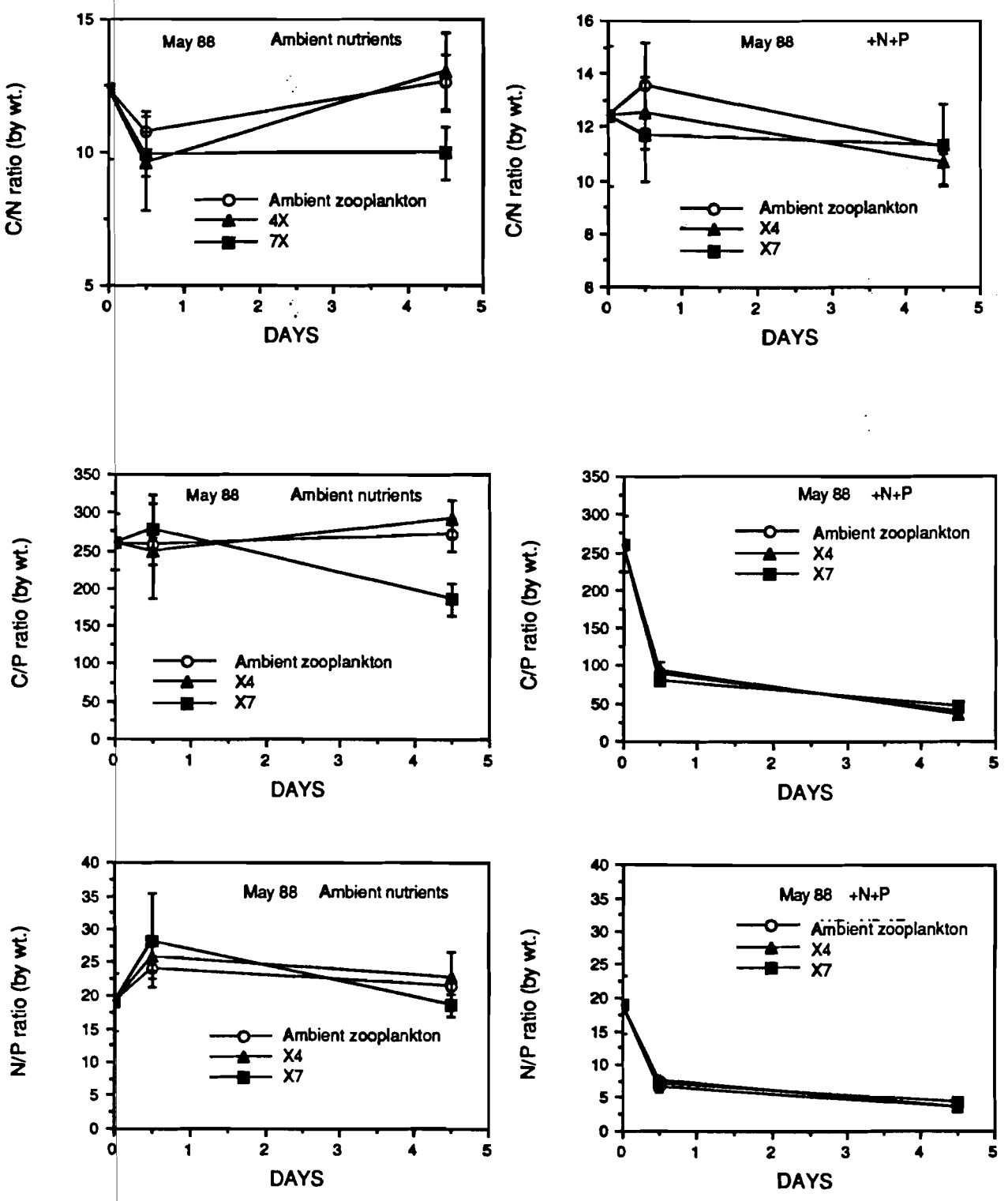

Figure 9. Time course measurements of particulate carbon (C), nitrogen (N), and phosphorus (P) ratios in the May, 1988 carboy experiments. The figures on the left are from the ambient nutrient treatments, while the figures on the right are from comparable carboys amended with N+P. Each graph contains data from the three different zooplankton treatments. Error bars indicate one standard deviation from the mean.

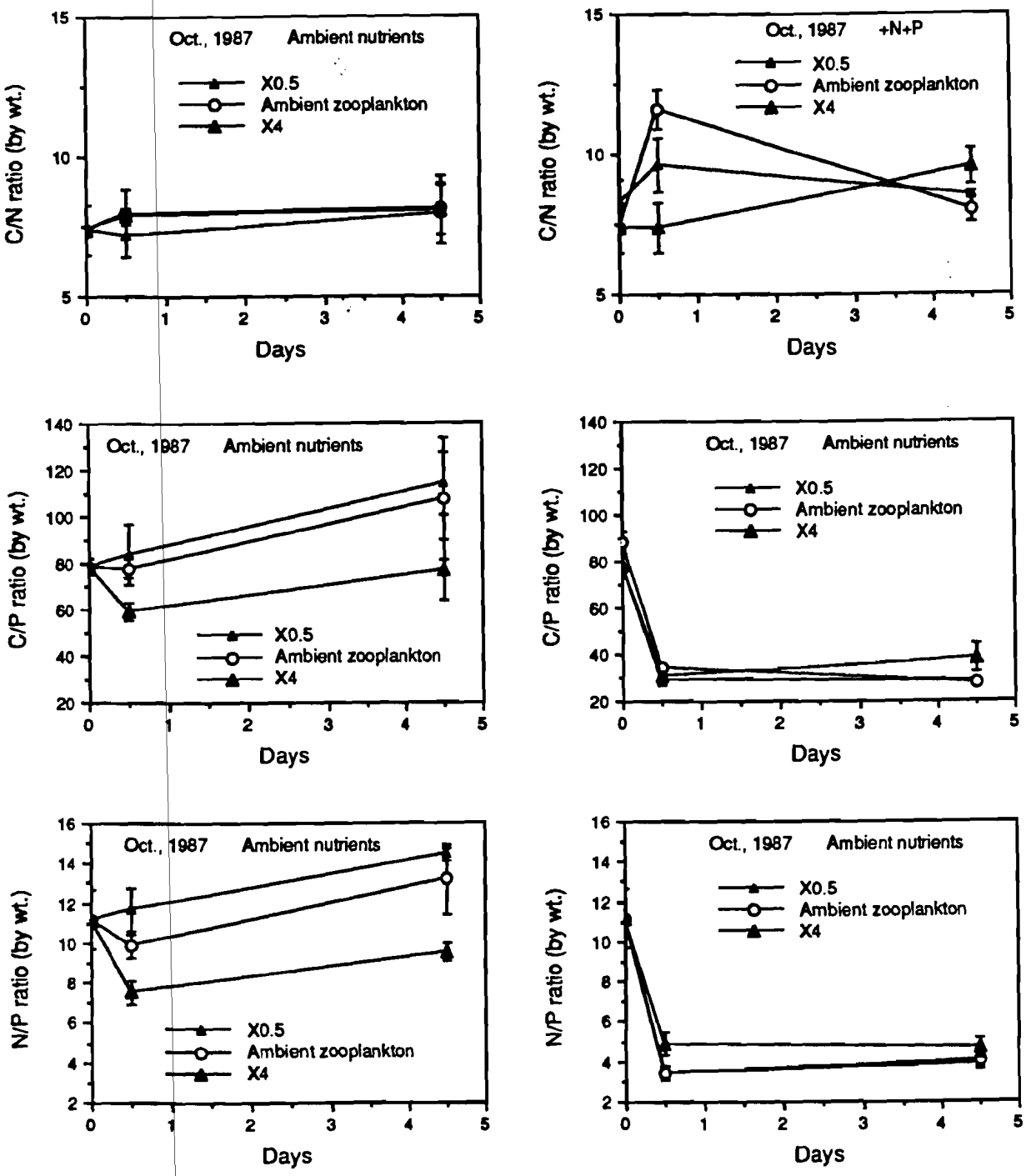

Figure 10. Time course measurements of particulate carbon (C), nitrogen (N), and phosphorus (P) ratios in the October, 1987 carboy experiments. The figures on the left are from the ambient nutrient treatments, while the figures on the right are from comparable carboys amended with N+P. Each graph contains data from the three different zooplankton treatments. Error bars indicate one standard deviation from the mean.

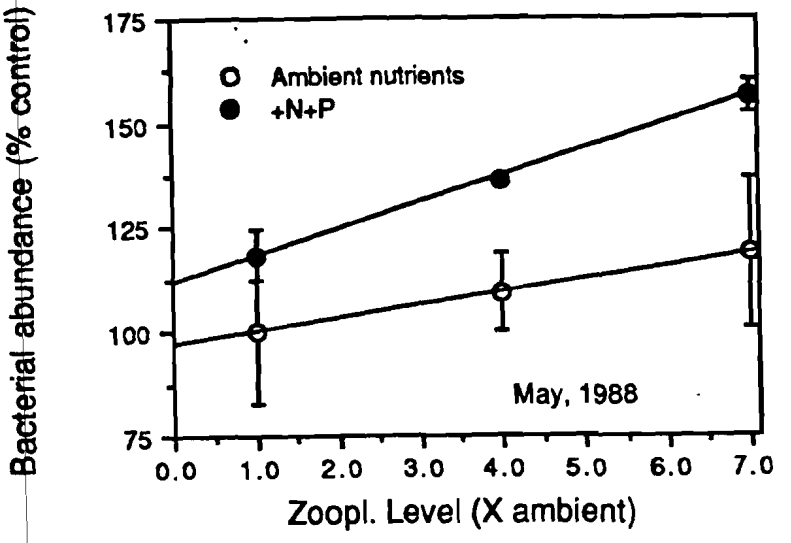

Figure 11. Biomass of planktonic bacteria (% control) measured after 4.5 days, as a function of zooplankton abundance in the May, 1988 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols). Error bars indicate one standard deviation from the mean.

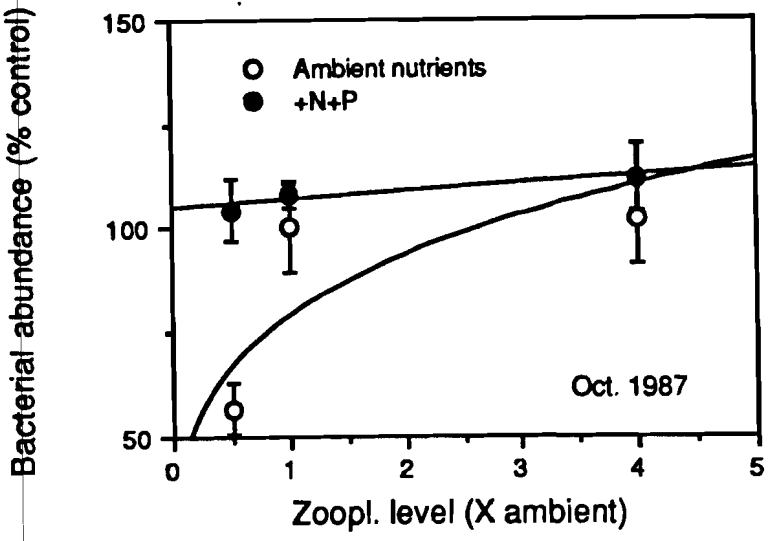

Figure 12. Biomass of planktonic bacteria (% control) measured after 4.5 days, as a function of zooplankton abundance in the October, 1987 carboy experiments. Results are presented for the ambient nutrient treatments (open symbols) and treatments amended with N+P (closed symbols).Error bars indicate one standard deviation from the mean.