THE EFFECTS OF NUTRIENTS, FATHEAD MINNOWS, AND SUBMERSED MACROPHYTES ON THE INVERTEBRATE COMMUNITY AND HABITAT QUALITY OF DELTA MARSH

BY

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A thesis
Submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements
for the Degree of

MASTER OF SCIENCE

Department of Zoology
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

The effect of nutrient addition, macrophyte removal and fathead minnow addition on the invertebrate community and habitat quality of Delta Marsh was assessed using *in situ* enclosures in the Blind Channel. Factors important in determining the stable state of the marsh were chosen as treatments (nutrient addition, submersed macrophyte removal, and fathead minnow addition). The clear water stable state, characterized by low turbidity, low phytoplankton biomass and abundant submersed macrophytes, is most likely when nutrient loading is low, macrophytes are abundant, and top-down control from planktivorous fish is low. The turbid water state, characterized by high turbidity, high phytoplankton biomass and few submersed macrophytes, is most likely when nutrient loading is high, submersed macrophyte biomass is sparse, and top-down control is high.

Inorganic nutrient addition (N and P) was found to cause phytoplankton blooms, and thus turbid conditions when submersed macrophyte biomass was relatively low. However, nutrient addition did not cause phytoplankton blooms or turbid conditions when submersed macrophytes were abundant. Addition of fathead minnows resulted in decreased densities of microinvertebrates, and thus a greater biomass of phytoplankton, due to decreased grazing pressure via the trophic cascade. Submersed macrophytes did not provide a refuge for zooplankton from predation by planktivorous young of the year fathead minnows.

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CHAPTER 1 - GENERAL INTRODUCTION

Shallow water bodies often exhibit a clear water stable state or a turbid water stable state. Scheffer et al. (1993) described a two stable state model in which a clear water state has abundant submersed macrophytes, low phytoplankton biomass and low turbidity, and a turbid water state has few submersed macrophytes, high phytoplankton biomass and high turbidity (Scheffer et al. 1993). Each stable state (or 'alternative equilibria') is resistant to change to the other state, and requires some threshold to be surpassed or disturbance to cause a switch to the alternative stable state. The basic concept of the model is that as nutrients and turbidity increase, submersed macrophytes persist until a critical nutrient level is reached where macrophytes cannot buffer all the excess nutrients available and a dramatic shift to the turbid water state occurs. However, both states can occur over a wide range of nutrient concentrations (Scheffer et al. 1993, Moss 1990, Beklioglu and Moss 1996a) due to stabilizers of each state (Irvine et al. 1989) that are essentially negative feedback loops which resist change to the alternative state.

Density of submersed macrophytes and density of planktivorous fish in addition to nutrient loading, are important factors in determining stable state. Submersed macrophyte density is important as macrophytes potentially compete with phytoplankton for nutrients, and provide habitat for algal grazing biota (zooplankton). Density of planktivorous fish can play a role in phytoplankton biomass and turbidity via the trophic cascade (Carpenter et al. 1985), i.e. top-down control ob fish through zooplankton to phytoplankton.

Macrophytes and associated epiphytes stabilize the clear water state by: 1) reducing wind action and re-suspension of sediments and thus reduces turbidity (Scheffer et al. 1993); 2) uptake of excess nutrients (Irvine et al. 1989, van Donk et al.

1993, Scheffer 1998), which decreases the availability of nutrients to phytoplankton; 3) inhibition of phytoplankton via allelopathy (Wium-Andersen et al. 1982, 1983); 4) providing habitat for zooplankton which graze phytoplankton and epiphyton (Timms and Moss 1984, Stansfield et al. 1997, Irvine et al. 1989); and 5) providing a refuge for zooplankton from predation by planktivorous fish (Timms and Moss 1984, Stansfield et al. 1997, Irvine et al. 1989).

The density of planktivorous fish is also important in this model. A high density of planktivorous fish results in a high predation pressure on zooplankton (Schriver et al. 1995, Zimmer et al. 2000) and thus loss of grazing pressure on phytoplankton. The interaction between planktivores and zooplankton is also affected by the presence of macrophytes as a refuge (Stansfield et al. 1997, Crowder and Cooper 1979). Macrophytes provide a refuge for zooplankton because of the increased structural complexity, and reduced available to visually feeding planktivorous fish (Crowder and Cooper 1979, Timms and Moss 1984).

The clear water stable state is preferred over the turbid state for waterfowl (Hanson and Butler 1994) and many macroinvertebrates. Macrophytes provide habitat for many invertebrates that are important as food for waterfowl (Baldassarre and Bolen 1994). A high density of planktivorous fish (more likely to be present in the turbid state) may be a cue to invertebrate density for waterfowl (Mallory et al. 1994). Others have found a positive relationship between invertebrate densities and waterfowl abundance (Murkin et al. 1982, Murkin and Kadlec 1986).

Zooplankton are important grazers of phytoplankton and thus tend to stabilize clear water conditions. Zooplankton can respond quickly to edible phytoplankton blooms because of their large filtering capacity, and the ability of cladocerans to increase their numbers quickly due to parthenogenetic asexual reproduction (Pennak 1989). Copepods reproduce sexually, and their life cycle takes much longer than cladocerans.

Thus cladocerans are also more effective at controlling phytoplankton due to their reproductive strategy.

These three important factors (nutrient loading, density of submersed macrophytes, and density of planktivorous fish) have influenced, or have the potential to impact Delta Marsh, one of the largest freshwater wetlands in North America.

Nutrient addition

The Delta Marsh, located along the south shore of Lake Manitoba, is adjacent to a large area of agricultural land from which it receives runoff via a number of small creeks. The amount of runoff from this land is potentially high in the spring, and/or after heavy rainfall. Nutrient loading from runoff is probable as fertilizers are used in the production of oil seed and cereal crops in this area. The marsh also indirectly receives nutrient input periodically from the Assiniboine River diversion that empties into Lake Manitoba. The amount of fertilizer used per hectare of land in the Assiniboine basin doubled between 1971 and 1991, and the amount of land fertilized increased 4-fold over that time (Canada, Statistics Canada, 1998). Water entering the lake via the diversion probably has a high nutrient load as extensive filamentous algal blooms have been noted in the spring when the diversion flows (K. Sandilands pers. obs., Goldsborough pers. comm.). Because most of the turbidity in Delta Marsh is caused by phytoplankton, not suspended material (Goldsborough, pers. comm.), thus the potential impact of added nutrients to the marsh may augment turbidity by promoting phytoplankton blooms.

Loss of submersed macrophytes

There has been a decline in submersed macrophytes in Delta Marsh in recent years. Wrubleski and Anderson (IN PRESS) found that the surface area of submersed macrophytes in the marsh declined by 46% between 1973 and 1997 in the east Delta Marsh with some areas losing as much as 85% (e.g. Riley Bay). Carp, introduced to the

marsh in the late 1940's (Wrubleski and Anderson IN PRESS) are believed to have caused the decline in submersed macrophytes (Wrubleski and Anderson IN PRESS) by uprooting vegetation and increasing turbidity during feeding and spawning activities (King and Hunt 1967, Crivelli 1983). Submersed macrophytes increased when carp were excluded briefly from the marsh (Wrubleski and Anderson IN PRESS). The loss of submersed macrophytes and the refuge they provide may also have contributed to the loss of large-bodied cladocerans, which were once present in the large bays at densities of up to 1,000 ind./L (Collias and Collias 1963).

Planktivorous fish

The fathead minnow (*Pimephales promelas*) is the most abundant planktivorous fish in the marsh (Kiers and Hann 1995), and can have a large impact on the invertebrate community in the marsh, particularly zooplankton (Held and Peterka 1974, Zimmer et al. 2000). The fathead minnow was not introduced to Delta Marsh, however, it is abundant and often introduced into wetlands for rearing as a bait fish (Carlson and Berry 1990).

I examined these three important factors in the context of the two stable state model. It is necessary to examine these factors, so that their potential impact on the marsh can be assessed, especially what levels potentially cause a shift in stable state.

These are not the only factors seriously affecting Delta Marsh. Lake Manitoba water levels have been regulated since the installation of the Fairford dam at the outflow in 1961. The marsh no longer has large fluctuations in water level, disrupting the wet-dry cycle needed for healthy marsh habitat (Murkin et al. 2000). As a result, some areas of the marsh are filling in with dominant emergent vegetation (Goldsborough 1983).

The objectives of this research were: 1) to determine if nutrient addition produces phytoplankton blooms and thus turbid conditions characteristic of the turbid water state, 2) to determine if the exclusion of submersed macrophytes causes the turbid state via

phytoplankton blooms, 3) to determine if fathead minnow addition produces the turbid state by indirectly increasing phytoplankton blooms, 4) to determine the effect of each of the three treatments on the densities and community structure of invertebrates, and 5) to determine if submersed macrophytes provide a refuge for invertebrates from predation by planktivorous fish.

CHAPTER 2

THE IMPACT OF NUTRIENTS AND SUBMERSED MACROPHYTES ON INVERTEBRATES IN A PRAIRIE WETLAND, DELTA MARSH, MANITOBA.

INTRODUCTION

Two stable states occur in shallow freshwater systems (Scheffer et al. 1993, Scheffer 1998, Moss 1990). The clear-water state is characterized by low turbidity and abundant macrophyte growth; the turbid state is characterized by abundant phytoplankton growth, few macrophytes, and high turbidity (Scheffer et al. 1993). The clear-water stable state is preferred by waterfowl (Hanson and Butler 1994) and other wildlife as macrophytes provide habitat for many invertebrates which are important to foraging waterfowl during reproduction (Baldassarre and Bolen 1994), and for growth and survival of ducklings (Cox et al. 1998).

Important factors determining the state of shallow, lentic systems are: 1) nutrient loading, 2) density of macrophytes, 3) density of zooplankton grazers, and 4) density of planktivorous fish. Above a critical level of nutrient loading, a shift to phytoplankton dominance is more likely due to increased nutrient availability (Scheffer et al. 1993). A high density of macrophytes (and associated epiphytes) maintains the clear-water stable state (Scheffer et al. 1993, Balls et al. 1989, Sand-Jensen and Borum 1991, Irvine et al. 1989, Timms and Moss 1984, Stansfield et al. 1997, Schriver et al. 1995). A high density of zooplankton (phytoplankton grazers), especially large cladocerans, may maintain effective control of phytoplankton blooms (Stansfield et al. 1997, Timms and Moss 1984). A large population of planktivorous fish will decrease the effectiveness of the refuge, making zooplankton more susceptible to predation (Stansfield et al. 1997). A combination of high levels of nutrient loading, sparse submersed macrophytes, high

density of planktivorous fish and low zooplankton density most favour the turbid state. A change in one factor (e.g. high nutrient loading) is unlikely to cause turbid conditions as either state can persist over a wide range of nutrient loading due to various mechanisms which maintain clear-water conditions (Moss 1990).

In other enclosure studies to examine nutrient addition to wetlands or shallow water systems relatively small (5 m diameter) enclosures have been used (Drenner et al. 1990, Campeau et al. 1994, Gabor et al. 1994). Large (8 m diameter x 3 m deep) *in situ* enclosures open to the sediments have rarely been used (Proulx et al. 1996). Large enclosure size reduces edge effects which may be a factor in small enclosure studies (Stephenson et al. 1984).

Macroinvertebrates may also have direct and indirect effects on the stable state (Diehl and Kornijów 1998). Herbivorous macroinvertebrates (e.g. snails) may control epiphyton growth on macrophytes, reducing shading by epiphyton (Brönmark 1989, Underwood et al. 1992, Thomas 1987), thus potentially facilitating growth and survival of macrophytes (Brönmark 1985). Predatory macroinvertebrates may also influence the density of zooplankton, and thus control of phytoplankton.

The objectives of my study were: 1) to determine if nutrient (N and P) addition, at levels used in this study, were sufficient to produce turbid conditions (defined as >80-100 µg Chl *a*/L based upon review of the literature) via phytoplankton blooms, 2) to determine if macrophyte exclusion could produce turbid conditions, 3) to determine if nutrient addition and macrophyte exclusion together produce turbid conditions even if either nutrient addition or macrophyte exclusion do not do so independently, 4) to determine the effectiveness of mechanisms (presence of submersed macrophytes and associated zooplankton grazers) which maintain clear-water conditions when nutrients are added, and 5) to examine densities of herbivorous and predatory macroinvertebrates to assess the possible impact on the stable state.

METHODS

Study Site

The study was conducted in the Blind Channel at Delta Marsh, Manitoba, Canada (50º11'N, 98º12'W). Delta Marsh is one of the largest (22,000 ha) freshwater marshes in North America, located on the south shore of Lake Manitoba and connected to it by several channels. Delta Marsh receives run-off directly from agricultural land and indirectly from the Assiniboine River (drainage area of 152,000 km^2) via a diversion into Lake Manitoba. The use of fertilizers in the Assiniboine River watershed increased 8-fold between 1971 and 1991 (Canada. Statistics Canada 1998). Vegetation in the marsh varies spatially with areas dominated by either emergent macrophytes (*Typha glauca*, *Scirpus acutus*, *Phragmites australis*), or submersed macrophytes in open water (*Ceratophyllum demersum*, *Potamogeton pectinatus*, *P. zosteriformis*, *Myriophyllum spicatum*, and *Utricularia vulgaris*). Fathead minnows (*Pimephales promelas*) and brook sticklebacks (*Culaea inconstans*) are the most abundant planktivorous fish in the spring. Adult fish migrate out of the marsh into L. Manitoba as water temperatures rise and oxygen concentrations decrease throughout the summer (Kiers and Hann 1996). Young of the year fish use the marsh habitat as they grow throughout the summer. The major components of the food web in the marsh are shown in Figure 2-1.

Experimental Design

Experimental enclosures (5 m x 5 m) consisted of contiguous floating wooden platforms (to accommodate fluctuations in water level) from which impermeable polyethylene curtains extended through the water column and at least 20cm into the sediments, anchored with iron bars to seal the inside water from the Blind Channel. Enclosures were installed on June 11, 1996. Macrophyte exclusion treatment was from

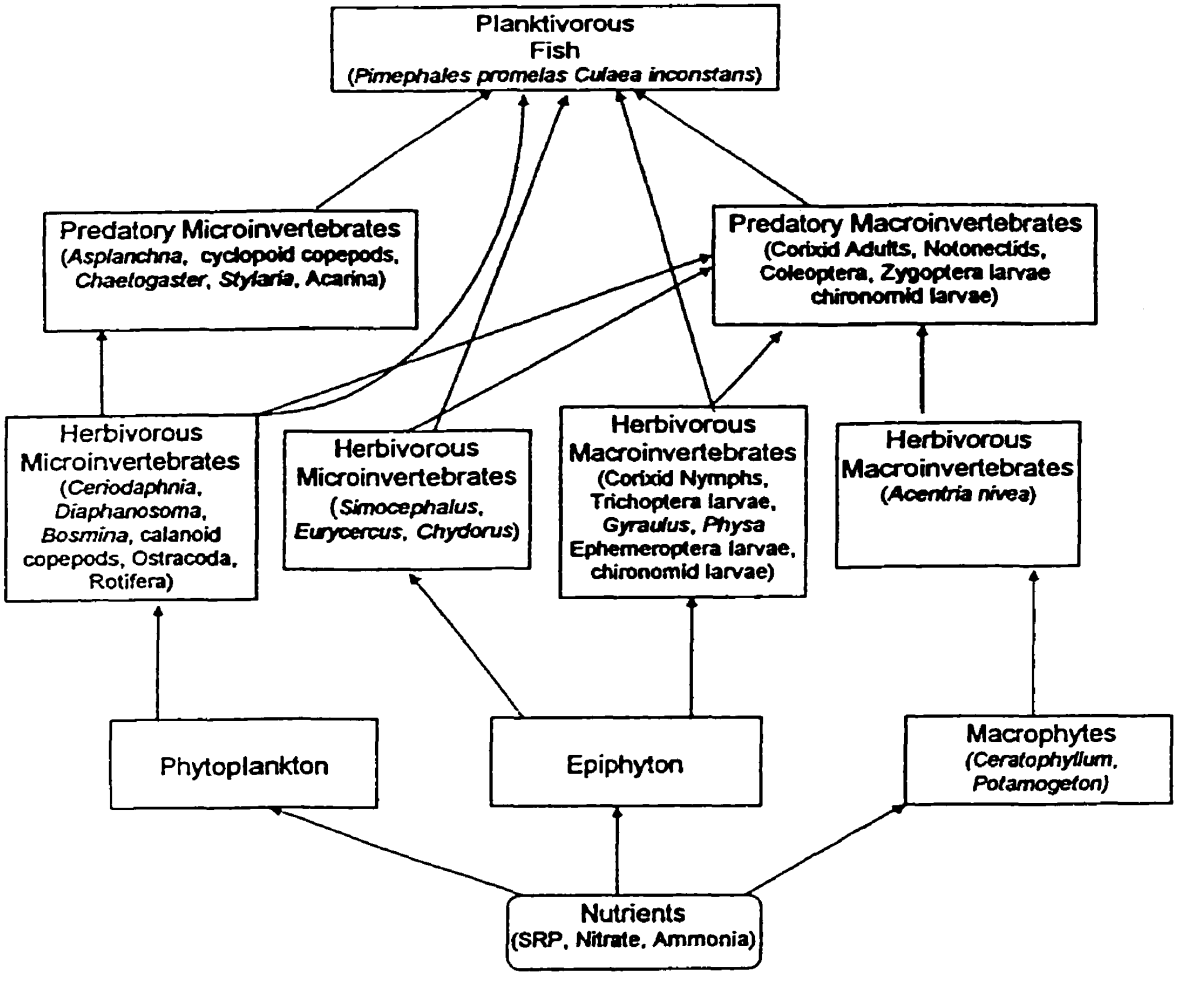

Figure 2-1. Food web diagram representative of major groups found in Delta Marsh

June 12 to August 28 (11 weeks), and the nutrient addition treatment from July 3 to August 28 (9 weeks).

The experiment was a factorial design with two main factors, inorganic nutrient addition, macrophyte exclusion, and their interaction. Two replicates of each of the experimental treatments were assigned randomly to enclosures (Figure 2-2). Treatment combinations were: 1) macrophytes present, nutrients added, 2) macrophytes excluded, no nutrient addition, and 3) macrophytes excluded, nutrients added. Control enclosures (3 replicates) were not manipulated, macrophytes were present, and no nutrients were added.

Inorganic nutrients were added to mimic input from agricultural land. Nitrogen (N) as $NaNO_3$, and phosphorus (P) as $NaH_2PO_4 \cdot 2H_2O$, were added three times a week to produce a cumulative loading of 103 g/m^2 for N and 13.5 g/m^2 for P. Nutrients were dissolved in 1L of carbon filtered water which was mixed with water from the appropriate enclosure and sprinkled evenly over it.

Submersed macrophytes were excluded on June 12 by installing a black, permeable, woven polypropylene fabric (Dewitt pro-5 Weed Barrier) on the sediment surface to block light needed for germination and growth. Slits were made in the material at regular intervals to allow movement of invertebrates and release of gases from sediments. The few macrophytes that did grow (mostly around the edges) were removed by hand.

Water Chemistry

Water samples were collected twice weekly at ~30 cm depth from each enclosure throughout the study period. Soluble reactive phosphorus (SRP), nitrate + nitrite ($NO_3 + NO_2$), and ammonia (NH_3) were determined using methods described in Stainton et al. (1977) and APHA (1992). Water samples were taken weekly for turbidity

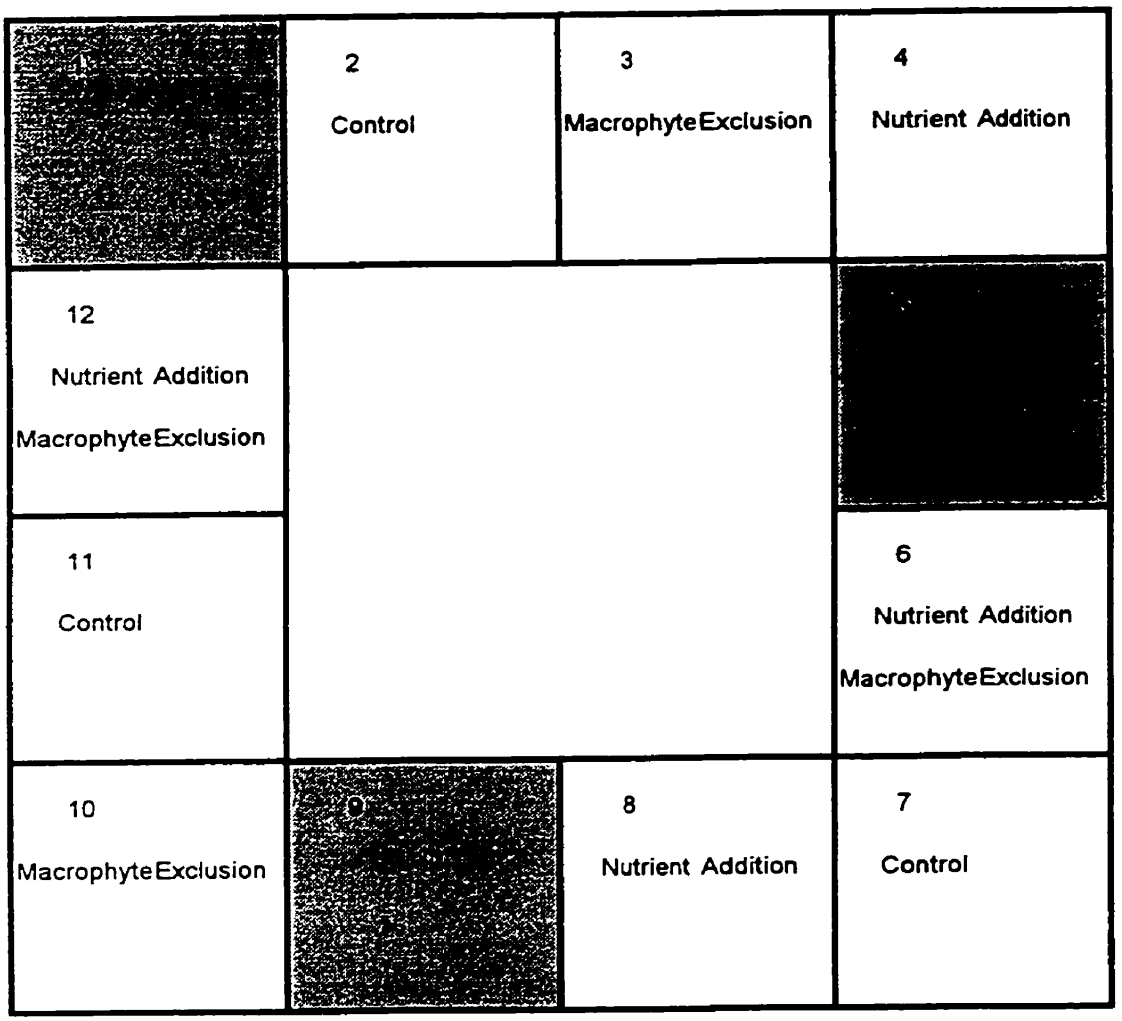

Figure 2-2. Schematic diagram of setup of experimental encloures in 1996. Gray enclosures not used in this experiment.

determination using a Hach turbidimeter (Model 2100A). Oxygen concentration and water temperature were determined weekly mid-morning at 10 cm depth in each enclosure using a YSI oxygen meter (Model # 51).

Biotic Sampling

Biota in the water column

Zooplankton (microinvertebrates) were sampled quantitatively (in triplicate) using a clear acrylic cylinder (5.5 cm diameter, 4 L) lowered through the water column in an area free of macrophytes. Contents of the cylinder were filtered through a 53 μm mesh net and preserved with 5% formalin. A phytoplankton sample was taken from all three water column samples from each enclosure, and biomass was determined as chlorophyll *a* (methods in McDougal et al. 1997).

Macroinvertebrates (e.g. aquatic insects, snails) in the open water column were sampled weekly using activity traps (Murkin et al. 1983, Ross and Murkin 1989). Activity traps consist of a glass jar (1 L) with a large funnel (10 cm diameter) in the opening, suspended vertically with the funnel opening about 25 cm below the water surface. Two traps were set overnight in each enclosure. Contents of the jar were poured through a 1 mm mesh to remove macroinvertebrates which were preserved in 5% formalin.

Biota associated with macrophytes

Phytophilous members of the microinvertebrate community were sampled at different heights within the macrophyte bed. Funnel traps sampled the lower 30 cm of the macrophyte bed, whereas the Downing Box (Downing 1986) sampled the upper 30 cm. Funnel traps consisted of 3 large funnels (10 cm diameter) mounted on a plate of plexiglass, with funnel stems extending into 3 small bottles where microinvertebrates were trapped. Funnel trap samples provided a semi-quantitative estimate of population

density based on vertical migratory behaviour of individuals (Whiteside and Williams 1975). Funnel traps were set overnight once a week, and their contents were filtered through a 53 µm mesh net and preserved in 5% formalin.

The Downing Box sampler, a 'plexiglass suitcase' (4 L), was lowered into the water and closed around approximately the top 30 cm of macrophyte to sample both micro- and macroinvertebrates. Microinvertebrates in the sample were separated by pouring the liquid contents of the box through a 53 µm mesh net. Macrophytes were then removed from the box and shaken vigorously in a large jar with carbon-filtered water to dislodge epiphyton from their surfaces. Epiphyton was separated from macrophyte tissue and macroinvertebrates by filtering through a 1 mm mesh sieve. Macrophytes were again shaken vigorously with water to dislodge invertebrates and aid sorting in the lab. Remaining invertebrates were picked from macrophyte tissue by hand and preserved in 5% formalin. Macrophyte tissue was dried at 105° C for 24 hours, then weighed. Epiphyton biomass was determined (as Chl *a*) by methods in McDougal et al. (1997).

In samples from both water column and macrophytes, microinvertebrates were classified as organisms < 1 mm, including cladocerans and copepods; macroinvertebrates were ≥ 1 mm and not crustaceans (except amphipods). Separation based solely on size was inadequate because of overlap in size between large crustaceans and small insects. Young instars of insects (<1 mm) were grouped as macroinvertebrates because later instars were >1 mm. Large cladocerans (>1 mm) were grouped as microinvertebrates because they were in the same trophic guild and taxonomic category as smaller cladocerans. Separation into macro- and microinvertebrates was necessary so that these two groups could be analyzed independently as they play different roles in the invertebrate community.

Microinvertebrates were mostly filter-feeders whereas macroinvertebrates were scrapers, collectors, or predators.

Macroinvertebrates were counted and identified using several sources (Hilsenhoff 1995, Merritt and Cummins 1996, Wiggins 1977, Brooks and Kelton 1967), and microinvertebrates (cladocerans and copepods) were identified using Pennak (1989), Hann and Zrum (1997), and a reference collection (BJH). Insects were determined to be predators or herbivores using Merritt and Cummins (1996). Fish were monitored using two commercial minnow traps set in each enclosure. Each weekday numbers of fish per two traps were recorded then returned to their respective enclosure.

Data Analysis

In order to satisfy the assumptions of ANOVA, data were log-transformed to both normalize the data and stabilize the non-constant variance. Repeated measures (RM-ANOVA) was used to test for temporal patterns and treatment effects. Two-way RM-ANOVA was used to detect a nutrient addition effect (factor 1), a macrophyte exclusion effect (factor 2), and an interaction in the enclosures. There was no significant effect of macrophyte exclusion (two-way RM-ANOVA), thus only the effect of nutrient addition was examined in further analysis in order to increase the power of the tests. Analysis of experimental units (enclosures) grouped as controls (all enclosures without nutrient addition) and nutrient addition (all enclosures with nutrient addition) regardless of macrophyte presence was then performed. These additional analyses were performed using the SAS PROC MIXED procedure (SAS Institute Inc. 1997), which enabled use of models appropriate for the covariance structure and auto-correlation present in the data. Three models were tested for best fit, DIAG - Independence model, no correlation between repeated measurements on an experimental unit, CS - Compound Symmetric, equal correlation among measurements, and AR (1) - Auto regressive, decreasing

correlation between measurements over time (auto-correlation). The best fit model (highest Akaike's Information Criterion (AIC) value) for each variable was determined and used for analysis. The AR (1) model was used for SRP, Nitrate + Nitrite, and Ammonia. The CS model was used for all other variables. If a significant treatment x time effect was found, contrasts were performed to determine which sample dates showed a significant treatment effect.

RESULTS

Water chemistry and environmental variables

Initial mean concentrations of SRP, nitrate + nitrite, and ammonia in all enclosures were 121 µg/L, 50 µg/L, and 19.9 µg/L respectively. SRP and nitrate + nitrite increased after nutrient addition began (mean 658 µg/L and mean 1254 µg/L), whereas ammonia did not increase until August (mean 125 µg/L, Table 2-1, Appendix 1). SRP, nitrate + nitrite and ammonia were significantly higher with nutrient addition ($F_{1,7}$= 21.93 P=0.0023; $F_{1,7}$=63.61 P=0.0001; $F_{1,7}$=21.22 P=0.0025, respectively) than the control. SRP and nitrate + nitrite also showed a significant nutrient addition x time effect ($F_{17,119}$=1.80 P=0.0349; $F_{17,119}$=8.60 P=0.0001, respectively).

Turbidity in all enclosures was > 2 NTU (2.6 ± 0.56) when enclosures were installed. In control enclosures, turbidity was ≤2 NTU throughout the treatment period. Mean turbidity was significantly higher in the nutrient treatment (4.56 ± 1.87; $F_{1,7}$=5.62 P=0.0496) throughout the treatment period as a result of phytoplankton blooms (Table 2-1, Figure 2-3, Appendix 1). Temporal trends in turbidity between nutrient treatment and control were significantly different ($F_{8,56}$=6.01 P=0.0001, Appendix 1).

Table 2-1. Mean (± SE) of water chemistry, fish and algal data in control and nutrient treatment during the treatment period.

Parameter	Control	Nutrient Addition
SRP (µg/L)	72 (± 26)	658 (± 37)
Nitrate+Nitrite (µg/L)	< 50*	1254 (± 251)
Ammonia (µg/L)	22 (± 2)	125 (± 43)
Turbidity (NTU)	1.58 (± 0.13)	4.56 (± 1.87)
Oxygen (% saturation)	53 (± 2)	76 (± 9)
Fish (# enclosure/ week)	5.1 (± 3.3)	3.1 (± 1.5)
Phytoplankton Biomass (µg Chl *a*/L)	23 (± 3.9)	111 (± 15.3)
Epiphyton (µg Chl *a*/g macrophyte dry weight)	150.66 (± 77)	546.8 (± 121.6)

* Value is below the detection limit of 50 µg/L.

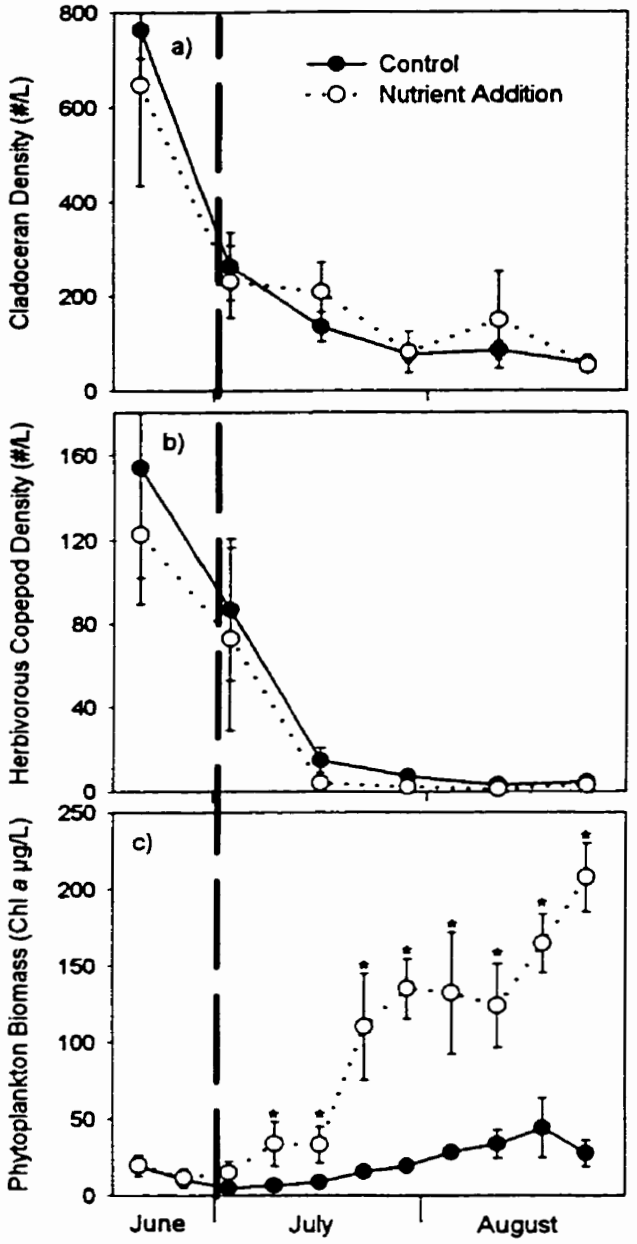

Figure 2-3. Seasonal patterns of (a) cladoceran density (mean ± SE), (b) copepod density, (c) phytoplankton biomass in the water column in experimental enclosures. The gray line indicates the start of nutrient addition. * - dates where contrasts indicate a significant treatment effect

Mean per cent oxygen saturation tended to be higher with nutrient addition (76 ± 9% saturation) than in the control (53% saturation ± 2; Table 2-1, Appendix 1). However, differences with nutrient addition were not significant ($F_{1,7}$=3.35, P=0.1022).

Treatment effects on the open water community

Cladoceran (11 species, Table 2-2) and copepod densities were highest (~120-180 ind./L, ~45-80 ind./L) in June (Figure 2-3), then declined to low density (<20 ind./L and <30 ind./L) in all enclosures for the entire treatment period. There was no significant effect of nutrient addition on densities of cladocerans or copepods ($F_{1,7}$=0.01 P=0.9240 and $F_{1,7}$=0.16 P=0.7030, respectively).

In the controls, density of planktonic rotifers was low (<1000 ind./L) throughout the study. Rotifer density increased in the nutrient treatment (to 5000 ind./L) but was not significantly different from control ($F_{1,7}$=0.57, P=0.4767).

Biomass of phytoplankton was significantly higher in the nutrient addition (100 - 200 µg Chl *a* /L; $F_{1,7}$=58.55, P=0.0001*)* than in the control (~25 µg Chl *a* /L; Figure 2-3), but the nutrient addition x time interaction was not significant ($F_{7,49}$=0.51,P=0.8283). Herbivorous macroinvertebrates were present at low numbers (<5 ind./ trap) in the water column in all enclosures. Density of herbivores did not differ with nutrient addition (F=0.05, P =0.8345). *Trichocorixa naias* (Corixidae) was the predominant predaceous invertebrate in all enclosures. Corixid density did not differ significantly with nutrient addition (F=0.45, P=0.5221).

Numbers of fish caught in each enclosure averaged 4 fish/week throughout the experiment (mostly fathead minnows). There was no significant difference in fish density with nutrient addition ($F_{1,7}$=0.31, P=0.7289). Young of the year fathead minnows were

Table 2-2. Microinvertebrate community composition.
*-Phytophilous, +- Planktonic, p-predatory, h-herbivorous

Cladocera

Alona sp.*h
Bosmina longirostris +h
Camptocercus sp.*h
Ceriodaphnia dubia *h
Chydorus sp.*h
Diaphanosoma birgei +h
Eurycercus longirostris *h
Pleuroxus denticulatus *h
Scapholeberis kingi +h
Simocephalus serrulatus *h
Simocephalus vetulus *h

Copepoda

Nauplii+
Cyclopiods +p
Calanoids +h

Rotifera

Asplanchna sp. +p
Others +h

Hydra sp. p

Ostracoda +*h

Oligochaeta

Chaetogaster sp. p
Stylaria sp. p

observed in high numbers in all enclosures but were not sampled due to the mesh size of the minnow traps.

Treatment effects associated with submersed plants

Cladoceran and copepod densities in the upper macrophyte strata (as sampled with the Downing Box) declined rapidly from 1200 cladocerans per g macrophyte dry weight and 3800 copepods per g macrophyte dry weight among macrophytes in the control, and remained at low density (Figure 2-4). Densities of cladocerans and copepods in the nutrient addition did not differ significantly from the control ($F_{1,3}$=0.00, P=0.9867, and $F_{1,3}$=0.06, P=0.8172, respectively).

Epiphyton biomass increased in the nutrient treatment from <100 µg Chl *a* per g macrophyte dry weight to >1000 µg Chl *a* per g macrophyte dry weight during the treatment period, whereas epiphyton biomass in the control peaked at ~ 250 µg Chl *a* per g macrophyte dry weight (Figure 2-4). Epiphyton biomass was not significantly higher with nutrient addition ($F_{1,3}$=4.06, P=0.1375) but the nutrient addition x time interaction was significant ($F_{8,24}$=4.72, P=0.0014).

Density of phytophilous cladocerans in the lower macrophyte strata (sampled using funnel traps) was low in June in the control (14,000/m^2 of wetland bottom), peaked in early July (42,000 ind./m^2 of wetland bottom), then declined rapidly to < 1 000 ind./m^2 by August (Figure 2-5). Density of herbivorous phytophilous copepods in the control was highest in June (71 000/m^2 of wetland bottom), then declined to < 10 000 ind./m^2 wetland bottom by late July (Figure 2-5). Densities of phytophilous cladocerans and copepods did not differ significantly with nutrient addition ($F_{1,7}$=0.16, P= 0.6984, and $F_{1,7}$=0.12, P= 0.7399 respectively).

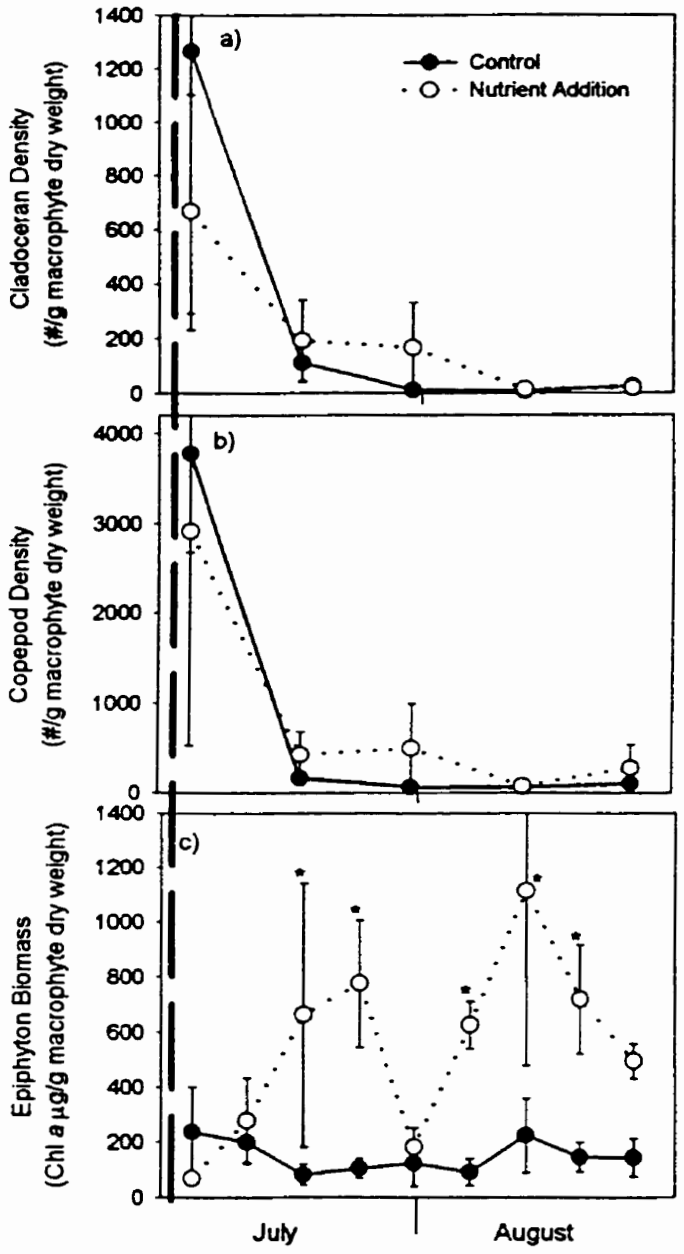

Figure 2-4. Seasonal patterns of (a) cladoceran density (mean ± SE), (b) copepod density, and (c) epiphyton biomass associated with submersed macrophytes (Downing Box). The gray line indicates that start of nutrient addition. * - dates where contrasts indicate a significant treatment effect

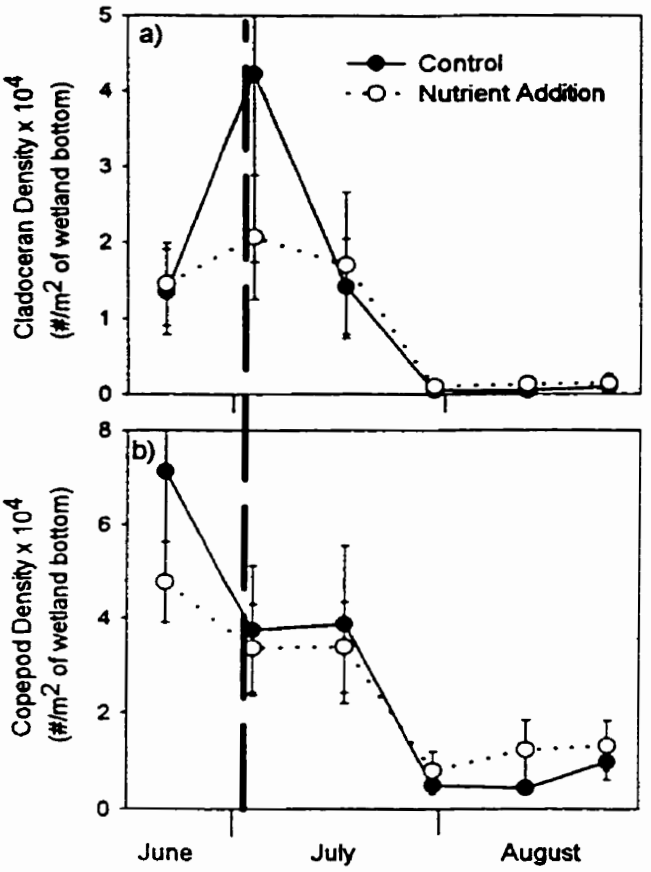

Figure 2-5. Seasonal patterns of (a) cladoceran density (mean ± SE), (b) copepod density associated with macrophytes (funnel traps). The gray line indicates the start of nutrient addition.

Mean density of herbivorous macroinvertebrates (sampled with the Downing Box) in the nutrient treatment (mean 66.6 ± 51 ind. per g macrophyte dry weight) were not significantly different from the control (mean 29.4 ± 9 ind. per g macrophyte dry weight; $F_{1,3}$=0.03, P=0.8746). However, a high density of pyralid moth larvae, *Acentria nivea* (Lepidoptera), which fed on submersed macrophytes were present in one nutrient addition enclosure.

Trichocorixa naias was the most abundant predaceous macroinvertebrate among the macrophytes in all enclosures. Density did not differ significantly with nutrient addition during the treatment period ($F_{1,3}$=0.67, P=0.4407).

DISCUSSION

It was predicted that nutrient addition alone would not produce turbid conditions in the enclosures because of mechanisms maintaining clear-water conditions, mainly the presence of submersed macrophytes and associated epiphytes and the refugium they provide for zooplankton grazers. However, in my experiment phytoplankton blooms (>80-100 µg Chl *a*/L), and thus turbid conditions, were produced with nutrient addition alone.

The level of nutrients used in this study may have been too high for maintenance of clear water conditions regardless of macrophyte/epiphyte and grazer density. Jeppesen et al. (1991) suggested that clear water conditions in shallow lakes can occur over a range of 50-125 µg P/L. Mean concentration of SRP in the nutrient treatment was 658 µg P/L, well above the range suggested by Jeppesen et al. (1991). Nutrient loading was much higher (2,288 µg N/L/week, 300 µg P/L/week) than in other enclosure studies, e.g. 326.9 µg N/L/week, 70 µg P/L/week (Drenner et al. 1990), 112 µg N/L/week^{-1}, 8.6 µg P/L/ week (Proulx et al. 1996) where phytoplankton blooms occurred at much lower

concentrations of P in the water column. It may be that nutrients made available in the water column were in excess of what could be used by macrophytes and associated epiphytes or absorbed into the sediments. However, phytoplankton blooms did not occur in a previous enclosure experiment in Delta Marsh where nutrients were added at half the levels of this study, where SRP was 330 µg/L (McDougal et al. 1997).

Low density of filter-feeding zooplankton also contributed to phytoplankton dominance (>80-100 µg Chl *a*/L) with nutrient addition. When normal densities of zooplankton are present, populations in the open water and associated with submersed macrophytes can effectively graze and control phytoplankton (Schriver et al. 1995, Timms and Moss 1984), thereby maintaining clear-water conditions. Due to low zooplankton densities, phytoplankton blooms and thus turbidity were not controlled in my study. There was no response of the microinvertebrate community to nutrient addition despite the strong response of phytoplankton and epiphyton in those enclosures. Phytoplankton biomass was almost an order of magnitude higher in the nutrient addition than in the control, yet no complementary response was seen in zooplankton (see Figure 2-1 for trophic relationship).

Scheffer (1991) predicted that nutrient enrichment, when a high density of fish are present, will decrease zooplankton density and increase algal density. In my experiment, the low density of zooplankton in the open water was probably the result of predation from planktivorous fathead minnows that feed extensively on zooplankton and other invertebrates (Held and Peterka 1974). Most cladocerans that did survive in the open water were *Diaphanosoma birgei*, which are transparent and less susceptible to predation.

It is suspected that the density of fathead minnows was unusually high since they were confined to the enclosures. Adult fish present in the enclosures were unable to migrate to the lake in July, as occurred in the surrounding marsh (Kiers and Hann 1996).

High densities can occur in wetlands in the absence of predators (Carlson and Berry 1990). This was the case as piscivorous fish were excluded from the enclosures. Fathead minnows are fractional spawners (Gale and Buynak 1982), meaning eggs are laid multiple times throughout the season, producing numerous cohorts during the summer. Young fish can grow rapidly reaching adult size in 90 days (Held and Peterka 1974). Large numbers of young of the year (YOY) minnows were present in all enclosures, and increased in size throughout the study. Predation pressure on zooplankton would have increased proportionately throughout the study as the large cohort of YOY fish grew throughout the season. This intense control of zooplankton by minnows may have contributed to the shift to phytoplankton dominance.

Schriver et al. (1995) observed that phytoplankton was present only at low densities of macrophytes. Low density of macrophytes in the nutrient enclosures (macrophytes sparse in the nutrient addition only enclosures, and absent in the macrophyte exclusion x nutrient addition enclosures) would also contribute to algal blooms and turbid conditions.

The complex structure of macrophytes typically acts as a habitat for zooplankton and refuge from planktivorous fish. Zooplankton, protected in macrophyte beds during the day, migrate horizontally at night to open water to graze phytoplankton (Timms and Moss 1984). This strategy offers protection from visually feeding planktivorous fish during the day as well as at night. Zooplankton among the macrophytes graze phytoplankton that enters the macrophyte bed via water currents, either by filter feeding directly from the water column, or by scraping phytoplankton that adheres to epiphyton (Irvine et al. 1989), allowing the macrophyte bed to act as a filter for phytoplankton. However, in my experiment, phytophilous zooplankton were present in very low densities throughout the season. Stephen et al. (1998) also found that macrophytes did not provide a refuge for zooplankton from sticklebacks, a minnow similar in size to fathead

minnows. As a consequence of artificially elevated density of planktivorous fish, and low density of macrophytes, a refuge was not provided for zooplankton grazers.

Macrophyte exclusion alone was expected to produce turbid conditions as there would be no refuge for zooplankton from predation by fish (Stansfield et al. 1997, Beklioglu and Moss 1996a,b, Moss 1995), and more nutrients available to phytoplankton. Phytoplankton blooms have occurred after the loss of submersed macrophytes (Timms and Moss 1984). Schriver et al. (1995) observed an increase in biovolume of phytoplankton in response to macrophyte exclusion using a black weed barrier on the sediments. However, in my study, no phytoplankton response or turbid conditions (>80-100 µg Chl *a*/L) were produced even though macrophytes and associated epiphytes were effectively excluded and densities of zooplankton were very low throughout the study. This suggests that phytoplankton in this treatment may have been nutrient limited. The level of nutrients in this treatment did not differ from the control, thus availability of nutrients in the water column was probably low.

Herbivorous macroinvertebrates that feed on epiphyton (Figure 2-1) did not show any response to nutrient addition or macrophyte exclusion despite increased epiphyton biomass in the nutrient addition treatment. The availability of epiphyton may have been less since submersed macrophytes were sparse in the nutrient treatment, resulting in less surface area to feed upon.

Lack of response by predaceous macroinvertebrates (corixids) may be due to the low density of prey. These predators would be expected to prey upon a lower trophic level, namely zooplankton (Figure 2-1), which were present at low densities. The indirect response of corixids to nutrient addition would be expected to be small as response to biomanipulation weakens with each level in the food web (McQueen et al. 1986). Manipulations such as nutrient addition and macrophyte exclusion would not be expected to affect the upper levels of the food web.

Further Study

Observations of the enclosures throughout the study suggest that macrophytes may play a role in maintaining clear-water conditions, especially when nutrients are added. In studies where macrophytes were present (Campeau et al. 1994, Gabor et al. 1994, Van Donk et al. 1995, McDougal et al. 1997), only moderately elevated phytoplankton biomass (<30 µg/L Chl a) occurred in response to nutrient addition. However, in other studies where macrophytes were absent, phytoplankton blooms of 600-700 µg/L Chl *a* (Proulx et al. 1996), and 100, and 230 µg/L Chl *a* (Drenner et al. 1990) occurred with nutrient addition. Previous enclosure experiments at Delta Marsh (in which macrophytes were present) showed no phytoplankton response to nutrient addition (Hann and Goldsborough 1997, McDougal et al. 1997, McDougal and Goldsborough 1996). Schriver et al. (1995) observed that phytoplankton was present only at low densities of macrophytes. In my study, phytoplankton blooms occurred in response to nutrient addition only where macrophytes were successfully excluded or were sparse. Macrophytes may be able to maintain clear water conditions only if they are established before nutrient loading occurs and are not subject to intense herbivory. The role of macrophytes needs further study in order to determine if nutrient addition at different stages of macrophyte development results in phytoplankton blooms and thus turbid conditions.

It is not clear in this study if phytoplankton became dominant because of high nutrient loading or lack of control of phytoplankton by grazers. In order to protect wetlands from shifts to phytoplankton dominance in the future, the minimum level of nutrient loading needed to cause such a shift to turbid conditions, and other factors involved in such a shift, need to be determined. Once these factors are determined, manipulations can then be considered to maintain clear water conditions. Manipulations

to maintain clear water may be necessary as nutrient loading is likely to increase in the future due to increased fertilizer use and run-off caused by draining of wetlands (prairie potholes) in agricultural land.

CHAPTER 3

THE IMPACT OF FATHEAD MINNOWS AND NUTRIENTS ON INVERTEBRATES IN A PRAIRIE WETLAND, DELTA MARSH, MANITOBA.

INTRODUCTION

Top-down effects of predation from fish and bottom-up effects of nutrients have been shown to be important in determining phytoplankton abundance and thus water quality (Moss 1990). Shallow freshwater ecosystems often exhibit either clear conditions with low phytoplankton abundance and submersed macrophytes, or turbid conditions where submersed macrophytes are sparse and phytoplankton dominates (Scheffer et al. 1993, Moss 1990). If nutrient levels are moderate, the presence of large cladoceran zooplankton is frequently the key to clear-water conditions (Timms and Moss 1984, Stansfield et al. 1997) because of their efficient filter-feeding (Brooks and Dodson 1965).

The abundance of cladoceran zooplankton is highly dependent on the intensity of predation from planktivorous fish, which in turn is affected by the density of planktivorous fish and density of macrophytes. Macrophytes may provide a refuge for zooplankton against predation from fish (Timms and Moss 1984) by providing low light conditions (Timms and Moss 1984) and structural complexity (Crowder and Cooper 1979, Persson and Crowder 1998), making feeding difficult for visually feeding planktivorous fish. Since presence of planktivorous fish directly affects zooplankton densities, I decided to study this interaction in the presence of macrophytes, a potential refuge for zooplankton. Planktivorous fish indirectly led to increased phytoplankton biomass via trophic cascades in other studies (Zimmer personal comm.) in which trophic cascades were documented (Carpenter et al. 1985, Romare et al. 1999, Moss 1990, Schriver et al. 1995). Clear water conditions are dependent on densities of zooplankton, densities of planktivores, densities of submersed macrophytes, and level of nutrient loading.

The objectives of the study were: 1) to determine if addition of fathead minnows could produce turbid conditions via loss of top-down grazing control on phytoplankton; 2) to determine if submersed macrophytes provide a refuge for zooplankton from predation by fathead minnows; and 3) to determine if experimental nutrient additions were sufficient to produce turbid conditions via phytoplankton blooms.

I predicted that fathead minnow addition would result in turbid conditions because fathead minnow predation should deplete zooplankton grazer populations, allowing phytoplankton to flourish. Fathead minnows will have the most impact in open water where zooplankton have no protection from predation, and will have the least impact on grazers among macrophytes because of the refuge effect.

I predicted that nutrient addition would not produce turbid conditions due to the presence of submersed macrophytes and the habitat they provide for zooplankton grazers.

METHODS AND MATERIALS

Experimental Design

A description of the experimental enclosures and study site is found in Chapter 2. The enclosures were installed on May 29, 1997. The fathead minnow treatment was conducted from June 18 to Aug. 29 (10 weeks), and the nutrient addition treatment from June 23 to Aug. 29 (9 weeks).

The experiment was designed to examine two main factors, fathead minnow addition and inorganic nutrient addition. Three replicates of each experimental treatment was assigned randomly to the enclosures using a latin square design (Figure 3-1). Treatment combinations included, 1) fathead minnows added, no nutrient addition, and

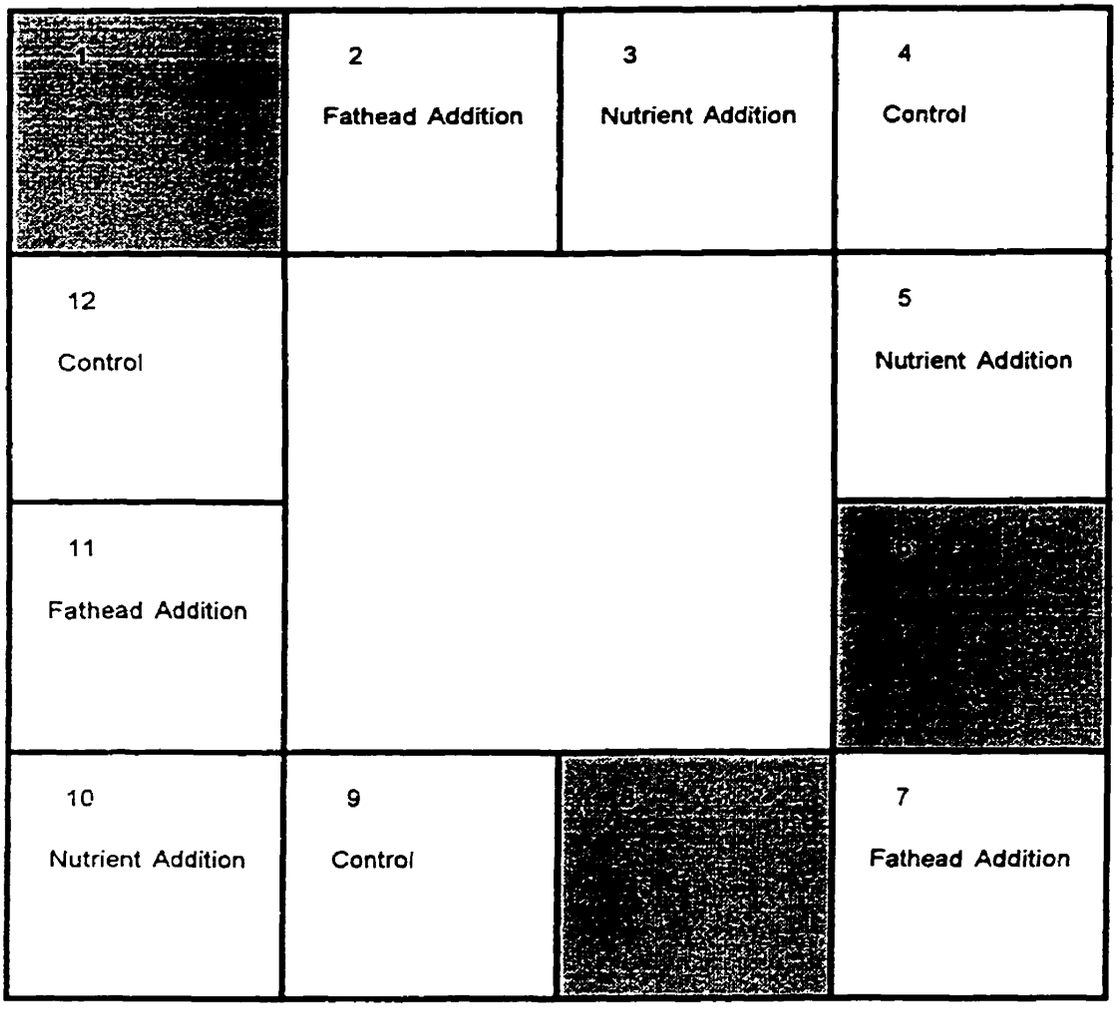

Figure 3-1. Schematic diagram of setup of experimental encloures in 1997. Gray enclosures not used in this experiment.

2) no fathead minnows, nutrients added. Control enclosures (3 replicates) were not manipulated and had no fathead minnows and no nutrient addition.

Fish were initially removed from all enclosures until fathead minnow addition began. Minnow traps were set in each enclosure to trap any remaining fish before treatment began. Traps were kept in control and nutrient enclosures for the rest of the summer.

On June 18, 125 adult fathead minnows (mean weight 1.72 g) were added to each fathead treatment enclosure. The density of 5 fathead minnows/ m^2 wetland was estimated by Spencer and King (1984) to be a realistic natural density in shallow water. For the first two weeks, any minnows that were found dead were replaced with live minnows in order to maintain 125 fish per enclosure. On July 2-3, a storm raised the water level in the marsh, perhaps briefly pulling the curtains out of the sediment, letting fish into non-fish treatment enclosures and possibly out of the treatment enclosures. Most fish were trapped out of control and nutrient enclosures soon after. Since it was impossible to tell whether there were still 125 minnows in treatment enclosures, one minnow trap was placed in each fathead treatment and checked daily (with replacement of trapped minnows) to monitor minnow density through the rest of the study. Two traps were also checked daily and any fish removed from all non-fathead enclosures.

Inorganic nutrients, nitrogen (N) as $NaNO_3$, and phosphorus (P) as $NaH_2PO_4 \cdot 2H_2O$, were added three times a week (28 additions) during the nutrient addition treatment (June 23 - Aug 29). This produced a cumulative loading of 115.4 g/m^2 for N and 15.1 g/m^2 for P. Nutrients were dissolved in 1L of carbon filtered water, mixed with water from the appropriate enclosure, and sprinkled evenly over it.

Water Chemistry

Water samples were collected twice weekly at ~30 cm depth from each enclosure throughout the study period. Alkalinity, pH, soluble reactive phosphorus (SRP), nitrate + nitrite ($NO_3 + NO_2$), and ammonia (NH_3) were determined using methods described in Stainton et al. (1977) and APHA (1992). Oxygen concentration and water temperature were determined weekly mid-morning at 10 cm depth in each enclosure with a YSI oxygen meter (Model # 51).

Biota Sampling

All groups of invertebrates present in the water column and associated with submersed macrophytes were sampled, processed, identified, and counted as in 1996 (see Chapter 2). Phytoplankton and epiphyton biomass were determined as in Chapter 2 following the methods in McDougal et al. (1997). Macrophyte biomass was estimated in each enclosure each month by lowering a large plastic tube to the sediments and harvesting all submersed macrophytes for dry weight determination (G. Goldsborough, pers. comm.). Additional invertebrate sampling included an estimation of the biomass of micro and macroinvertebrates associated with submersed plants. Funnel traps were set twice weekly (see Chapter 2) to obtain an estimate of the number of individuals (one set of samples) and an estimate of the biomass (second set of samples). Biomass samples were sorted as microinvertebrates or macroinvertebrates, frozen, and stored for later dry weight determination. Samples were weighed immediately after being dried for 24 hours at 60°C.

Data Analysis

Only two replicates of the nutrient treatment were used in analyses. One replicate was excluded due to a phytoplankton bloom which caused turbid conditions, and few submersed macrophytes were present at the start of treatment. In contrast, the

other two replicates (included in analyses) had submersed macrophytes present before treatment began, and clear conditions.

In order to satisfy the assumptions of ANOVA, data were log-transformed to normalize data and to stabilize non-constant variance. Repeated measures analysis of variance (RM-ANOVA) was used to test for temporal patterns and treatment effects (nutrient or fathead addition). SAS PROC MIXED procedure (SAS Institute Inc.1997) enabled use of models appropriate for the covariance structure and auto-correlation present in the data. Three models were tested for best fit: DIAG - Independence model which assumes no correlation between repeated measurements on an experimental unit, CS - Compound Symmetric which assumes equal correlation among measurements, and AR (1) - Auto regressive which assumes decreasing correlation between measurements over time (auto-correlation). The best-fit model as determined from the highest Akaike's Information Criterion (AIC) value for each variable was used for analysis. The AR (1) model was used for SRP, Nitrate + Nitrite, Ammonia, % Oxygen saturation, and density of predators in the Downing Box. The CS model was used for all other variables.

RESULTS

Fathead Addition

Density of fathead minnows and total fish caught in enclosures was high (~55 trapped/day) at the start of the fathead treatment, whereas fish density was low in the controls (<4 fish trapped/day; Figure 3-2). Density of adult fatheads in the fathead treatment declined to an average of 35 (± 10) fatheads trapped/day through July and August. Small numbers of fatheads were present in some control enclosures for the first two weeks of July until they were trapped and removed. In late July and August, one control enclosure had bullheads that were trapped and removed (mean 17.5 trapped/

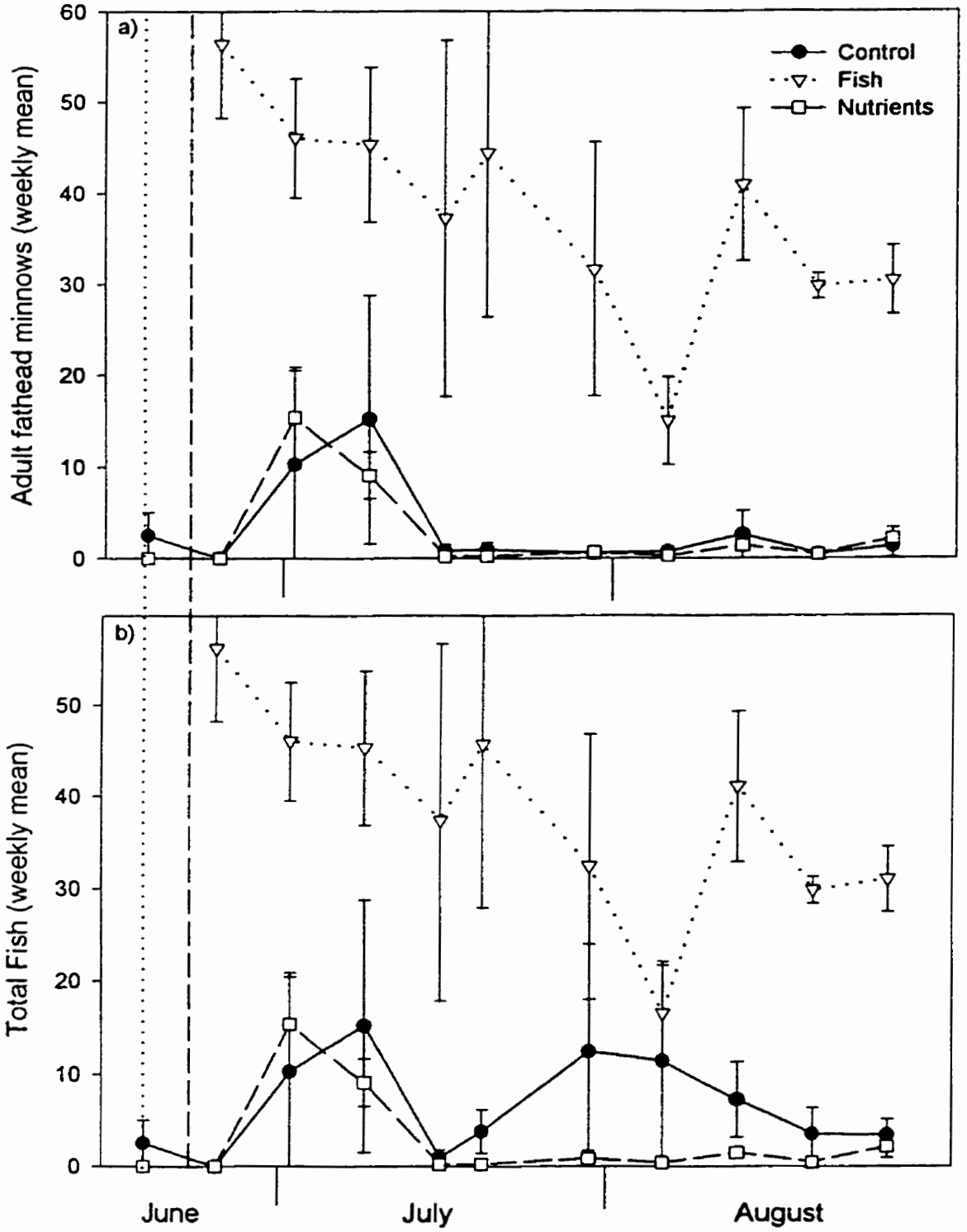

Figure 3-2. Seasonal patterns of (a) adult fathead minnows trapped (mean ± SE), and (b) total fish trapped in each treatment. The dotted line indicates the start of fathead treatment, the dashed line indicates the start of nutrient treatment.

day). The number of fatheads was significantly higher in the fathead treatment due to treatment alone ($F_{1,5}$ = 36.54, P = 0.0018) but not with treatment x time ($F_{9,45}$ = 1.48, P = 0.1830). The number of total fish (including bullheads) was still significantly higher with treatment ($F_{1,5}$ = 16.04, P = 0.0103) and treatment x time ($F_{9,45}$ = 3.99, P = 0.0009). Many small young of the year (YOY) fatheads, which increased in size throughout the study, were present in the fathead treatment, but were not sampled due to the mesh size of the traps. Few YOY fatheads were observed in control enclosures.

Water chemistry

Concentrations of SRP, nitrate and ammonia in the water column were low in the fathead treatment throughout the study (Figure 3-3). Concentrations of SRP, nitrate, and ammonia were not significantly different with fathead treatment ($F_{1,5}$ = 0.14, P = 0.7226, $F_{1,5}$ = 4.14, P = 0.0975, and $F_{1,5}$ = 0.58, P = 0.4818, respectively. Only nitrate concentration was higher with treatment x time ($F_{19,95}$ = 2.67, P = 0.0009), but most values were zero and 3 values were very low.

Per cent oxygen saturation in the fathead treatment was very similar to the control (Figure 3-3d). There was no significant effect of treatment on percent oxygen saturation ($F_{1,5}$ = 1.49, P = 0.2769) or treatment x time ($F_{9,45}$=0.86, P= 0.5762).

Biomass of macrophytes increased to a maximum much earlier with nutrient addition than in controls (Table 3-1), although the area that macrophytes occupied in the enclosures decreased as caterpillars broke up the macrophyte structure, causing them to collapse.

Treatment effects on the open water community

The seasonal pattern of cladoceran density was similar in the fathead treatment to the control (Figure 3-4a). The mean density of cladocerans in the control was ~80

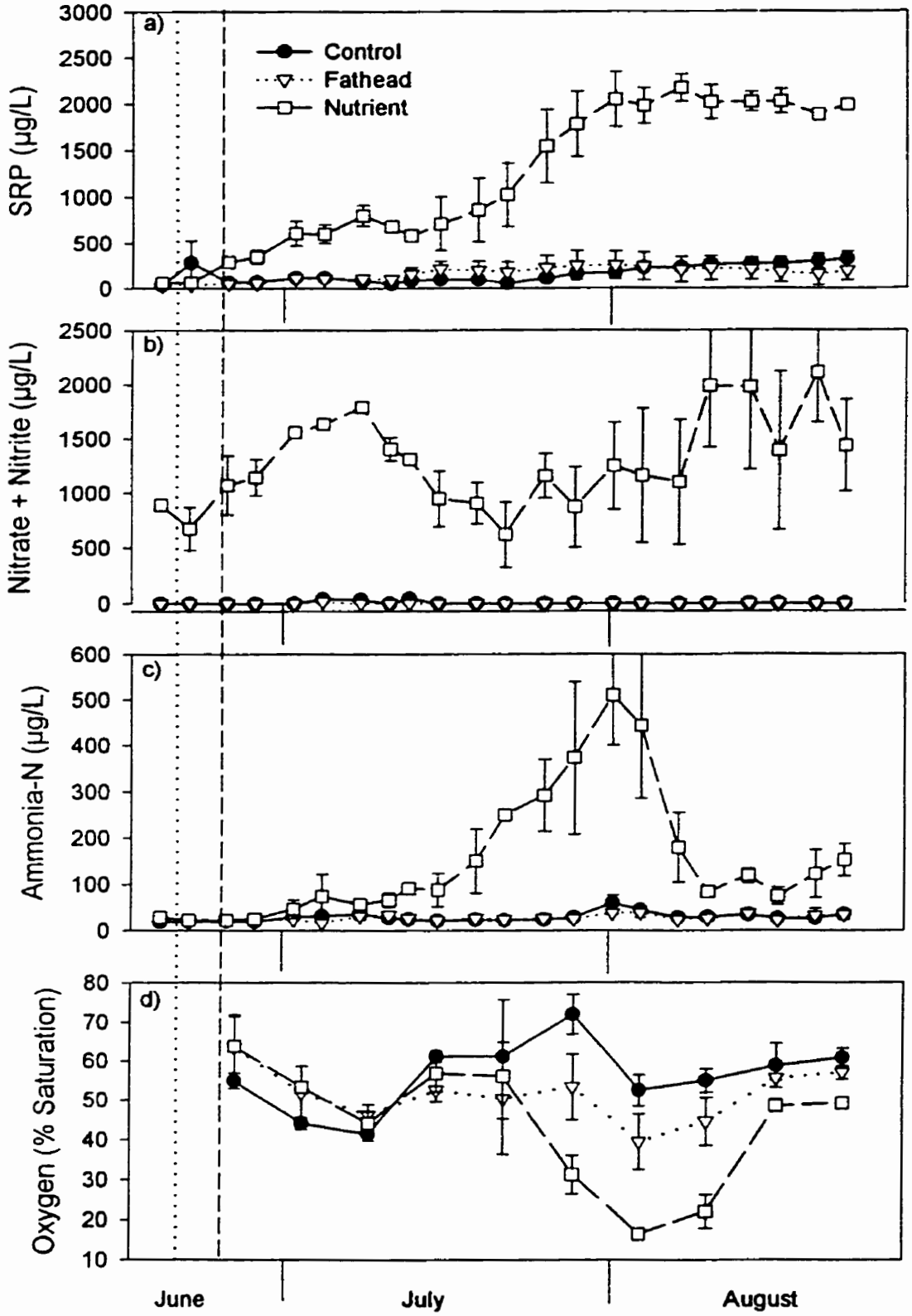

Figure 3-3. Seasonal patterns of (a) soluble reactive phosphorous (SRP; mean ± SE) (b) nitrate + nitrite, (c) ammonia, and (d) % oxygen saturation at 10cm in AM in the water column. The dotted line indicates start of fathead treatment, the dashed line indicates start of nutrient treatment.

Table 3-1. Macrophyte biomass in each treatment (g/m^2 wetland bottom in 1997.

	June 16	July 10	Aug. 13
Control	3 (± 0.6)	76 (± 31)	191 (± 51)
Fatheads	36 (± 32)	223 (± 63)	210 (± 67)
Nutrients	181 (± 144)	306 (± 99)	277 (± 165)

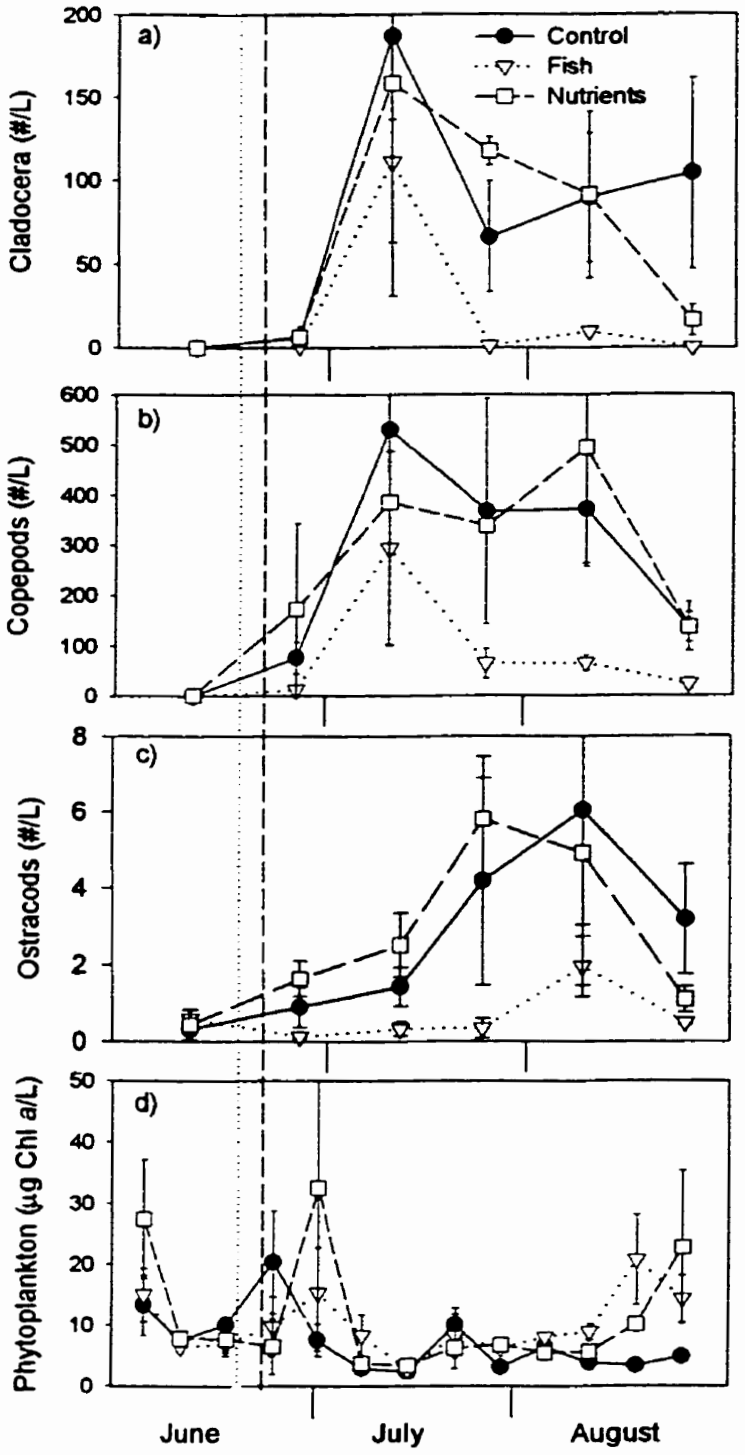

Figure 3-4. Seasonal patterns of (a) cladoceran density (mean ± SE), (b) copepod density, (c) ostracod density, and (d) phytoplankton biomass in the water column in experimental enclosures. The dotted line indicates the start of fathead treatment, the dashed line indicates the start of nutrient treatment

ind./L over the last six weeks of the study, whereas in the fathead treatment the mean density was <15 ind./L. The density of cladocerans was significantly lower with fathead treatment ($F_{1,4}$ = 15.14, P = 0.0115) than in the controls.

The seasonal pattern of copepod density was also similar to the control (Figure 3-4b). Mean density of copepods in the last six weeks in the control was 292.7 ind./L, whereas in the fathead treatment mean density was 50.9 ind./L. The density of copepods was significantly lower with fathead treatment ($F_{1,4}$ = 9.29, P = 0.0285) than in the controls in the water column.

Ostracod density tended to be lower in the fathead treatment (<2 ind./L, Figure 3-4c) than the control (6 ind./L) although this difference was not statistically significant ($F_{1,5}$ = 5.53, P= 0.0654).

Phytoplankton biomass in the fathead treatment reached its maximum near the end of August (~20 µg/L), during which time phytoplankton biomass in the control was low (<5 µg/L; Figure 3-4d). Biomass of phytoplankton was significantly higher with fathead treatment ($F_{1,5}$ = 9.83, P = 0.0258) and treatment x time ($F_{9,45}$ = 2.36, P = 0.0280); however, phytoplankton biomass differed at the end of the experiment only and the difference from the control was small.

Density of macroinvertebrate predators and herbivores did not respond to fathead minnow addition (Figure 3-5). Densities of predators and herbivores were not significantly different with treatment ($F_{1,5}$ = 0.52, P = 0.5020, $F_{1,5}$ = 0.01, P = 0.9342, respectively) from the control.

Treatment effects associated with submersed macrophytes

Upper Macrophyte Strata

Cladoceran and copepod densities were high in the control (~24,000 and ~11,000 ind./m^2 wetland bottom, respectively; Figure 3-6) when sampling began.

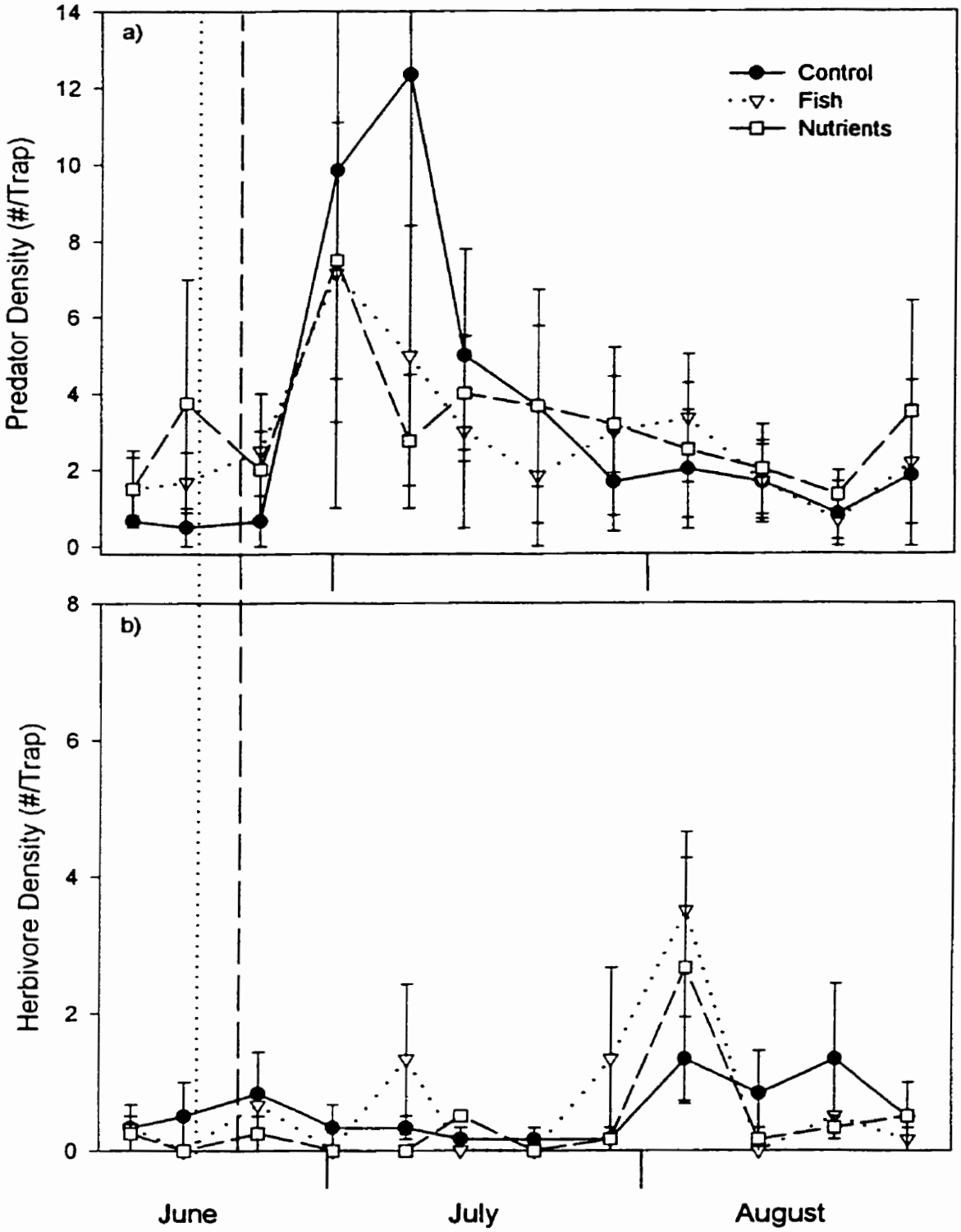

Figure 3-5. Density of (a) macroinvertebrate predators (mean ± SE) and (b) macroinvertebrate herbivores in the water column throughout the study (activity traps). The dotted line indicates the start of fathead treatment, the dashed line indicates the start of nutrient treatment.

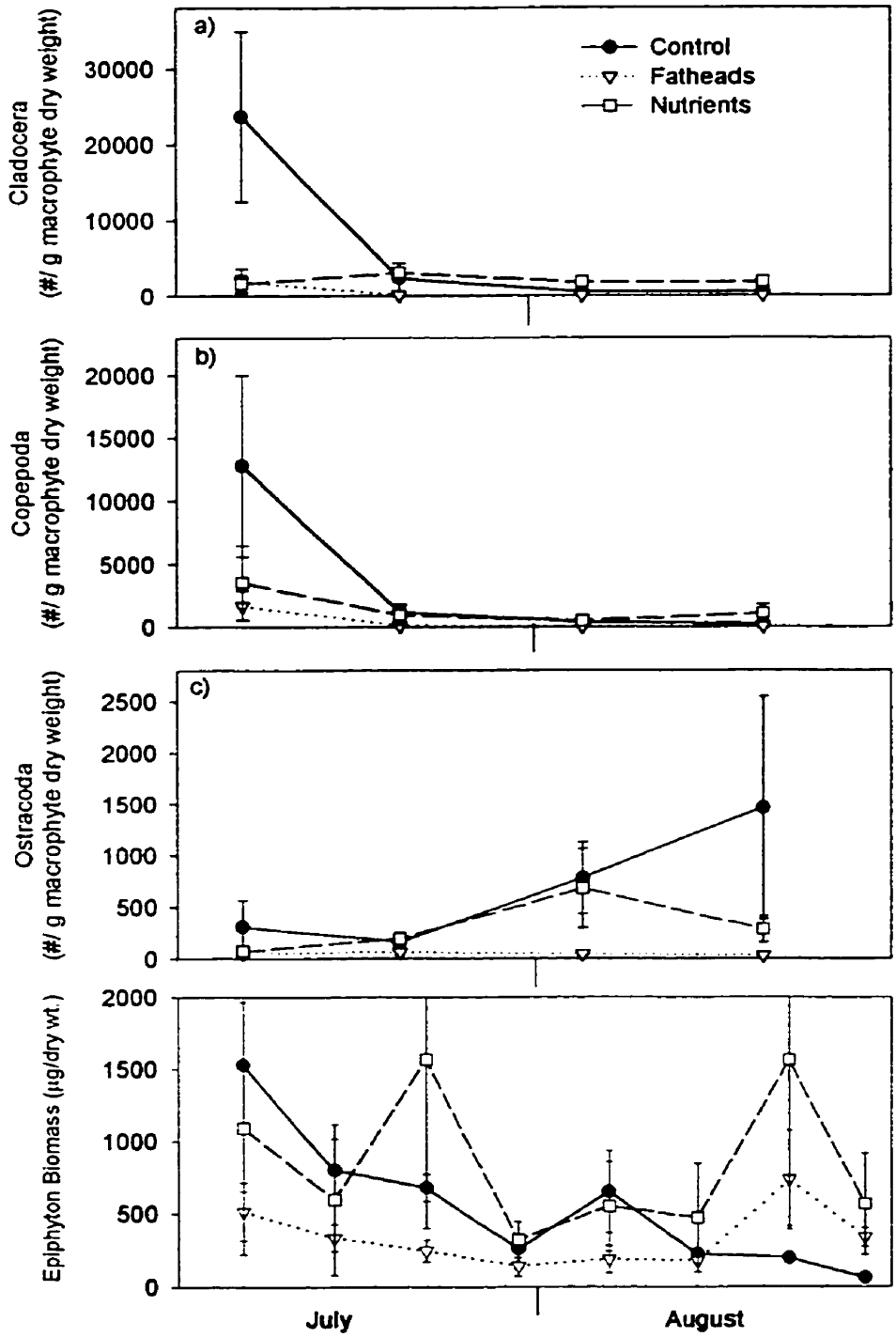

Figure 3-6. Seasonal patterns of (a) cladoceran density (mean ± SE), (b) copepod density, (c) ostracod density, and (d) epiphyton biomass associated with submersed macrophytes (upper strata; Downing Box).

Densities were low throughout the study in the fathead treatment (< 2000 ind./ m^2 wetland bottom). There was a significant effect of fatheads on cladoceran and copepod density ($F_{1,5}$ = 21.08, P = 0.0059; $F_{1,5}$ = 14.62, P = 0.0123) but no significant effect of treatment x time ($F_{3,15}$ = 0.08, P = 0.9699; F= 0.01, P = 0.9285) was detected.

Ostracod density in the upper macrophyte strata was very low (< 70 ind./ m^2 wetland bottom) throughout the study compared to the control which reached a mean density of ~180,000 ind./g macrophyte dry weight at the end of the study (Figure 3-6). There was no significant effect on ostracod density of treatment alone ($F_{1,5}$ = 5.01, P = 0.0754), however treatment x time was significant ($F_{3,15}$ = 3.52, P = 0.0414).

Epiphyton biomass tended to be lower than control in the fathead treatment until mid-August when biomass reached a peak of ~700 µg/g macrophyte dry weight (Figure 3-6d). Epiphyton biomass was not significantly affected by treatment alone ($F_{1,5}$ = 0.18, P = 0.6900) but was with treatment x time ($F_{7,35}$ = 4.21, P = 0.0018).

Predaceous macroinvertebrate density in the upper macrophyte strata was lower in the fathead treatment reaching a peak of 6 ind./g macrophyte dry weight. The control peaked at 12 ind./g macrophyte dry weight (Figure 3-7a). There was no significant effect of fathead treatment on the density of predaceous macroinvertebrates ($F_{1,5}$ = 0.72, P = 0.4355) and no significant effect of fatheads x time ($F_{6,30}$ = 0.30, P = 0.9313). Density of herbivorous macroinvertebrates (dominated by snails) in the fathead treatment was higher than in the control in July and tended to be lower than in the control in August (Figure 3-7b). There was no significant effect of fathead treatment alone ($F_{1,5}$ = 0.43, P = 0.5390) but there was a significant treatment x time effect ($F_{6,30}$ = 3.36, P = 0.0118).

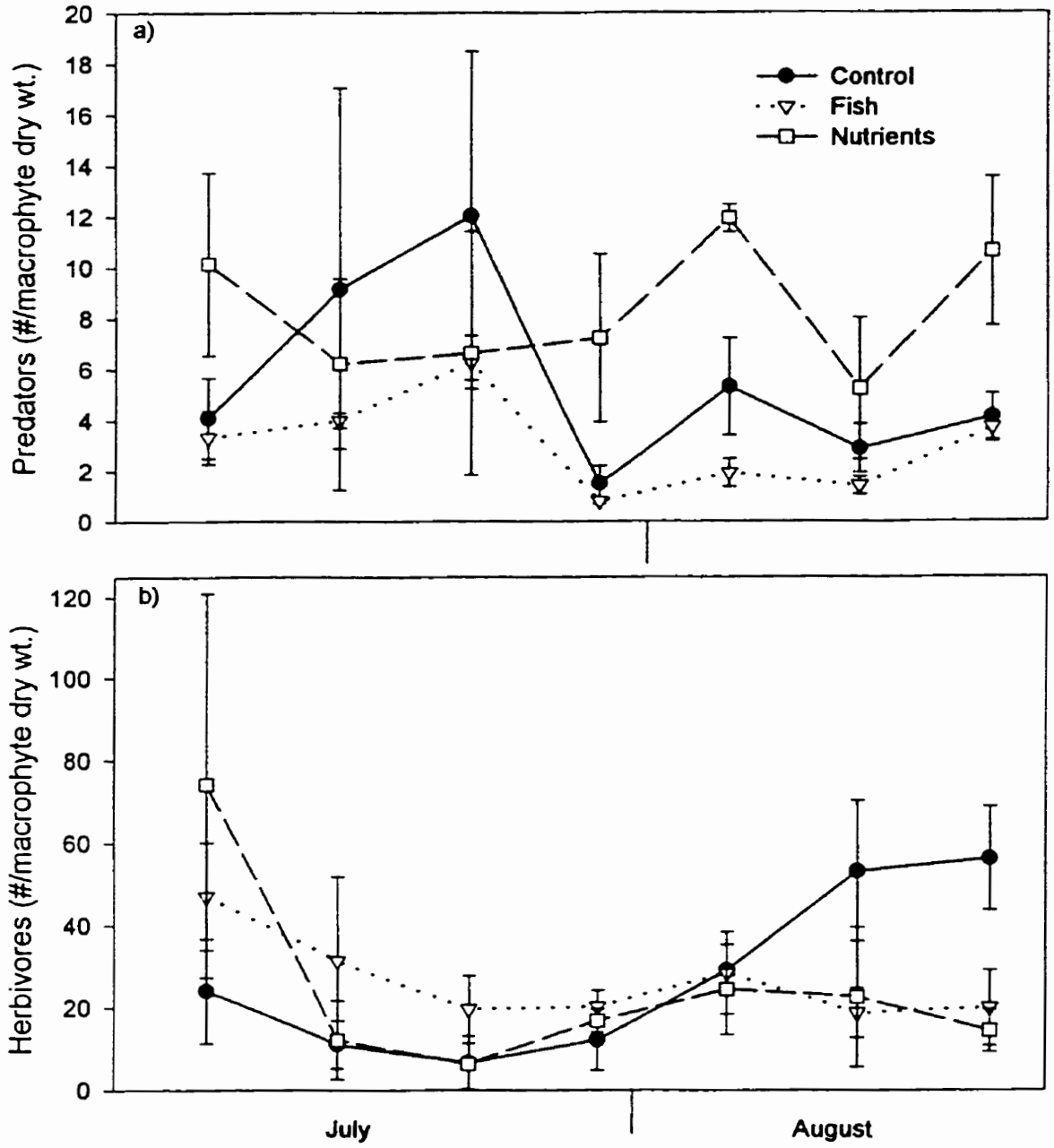

Figure 3-7. Seasonal pattern of (a) predaceous macroinvertebrates (mean ± SE), and (b) herbivorous macroinvertebrates associated with the upper macrophyte strata (Downing Box).

Lower Macrophyte Strata

Maximum mean densities of cladocerans and copepods in the fathead treatment were ~11,000 ind./m^2 wetland bottom and ~30,000 ind./m^2 wetland bottom, respectively (Figure 3-8). These maxima were lower than in the control (~32,000 and ~48,000 ind./m^2 wetland bottom); however, there was no significant treatment effect ($F = 4.94$, $P = 0.0770$, $F = 2.27$, $P = 0.1921$).

Ostracod density in the fathead treatment was low (<40 000 ind./m^2 wetland bottom) throughout the study (Figure 3-8c). In the control, mean ostracod density was an order of magnitude higher in mid-August (~400,000 ind./m^2 wetland bottom). This difference was detected on only one sampling date with the funnel traps; however this large population of ostracods was also detected in the water column and upper macrophyte strata. Ostracod density was significantly lower with fathead treatment ($F_{1,4} = 7.88$, $P = 0.0377$) and treatment x time ($F_{4,16} = 3.51$, $P = 0.0250$).

Mean density of small non-predaceous rotifers was higher after fatheads were added (~14,000 ind./m^2 wetland bottom) and on one sampling date mid-August (~11,000 ind./m^2 wetland bottom) in the fathead treatment. Rotifer density in the control was low (<2,000 ind./L) on both these dates (Figure 3-8d). Rotifer density was not significantly higher with fathead treatment ($F_{1,5} = 3.78$, $P = 0.1094$) but was with treatment x time ($F_{4,20} = 5.04$, $P = 0.0056$).

Densities of predaceous macroinvertebrates were higher at the start of the fathead treatment (~2100 ind./m^2 wetland bottom, dominated by the oligochaete *Stylaria* spp.) than in the control (~300 ind./m^2 wetland bottom; Figure 3-9a) and similar for the rest of the experiment. Densities of herbivorous macroinvertebrates were higher at the end of the experiment in the fathead treatment (~10,000ind./m^2 wetland bottom; Figure 3-9b, dominated by amphipods and snails) than the control (~3,500 ind./m^2 wetland

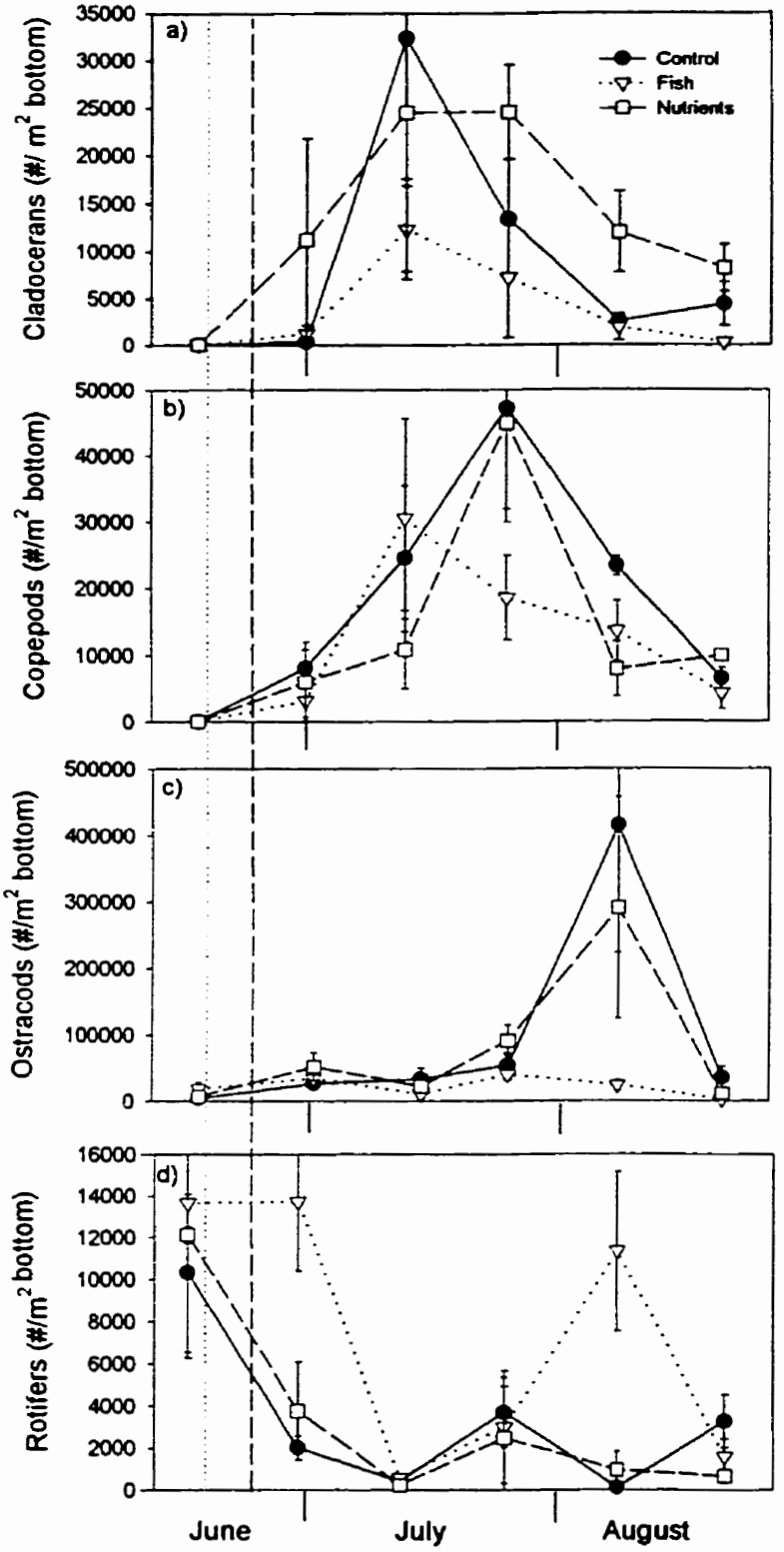

Figure 3-8. Seasonal patterns of (a) cladoceran density (mean ± SE), (b) copepod density, (c) ostracod density, and rotifer density (excluding *Asplanchna*) associated with the lower macrophyte strata (funnel traps). The dotted line indicates the start of fathead treatment, the dashed line indicates the start of nutrient treatment.

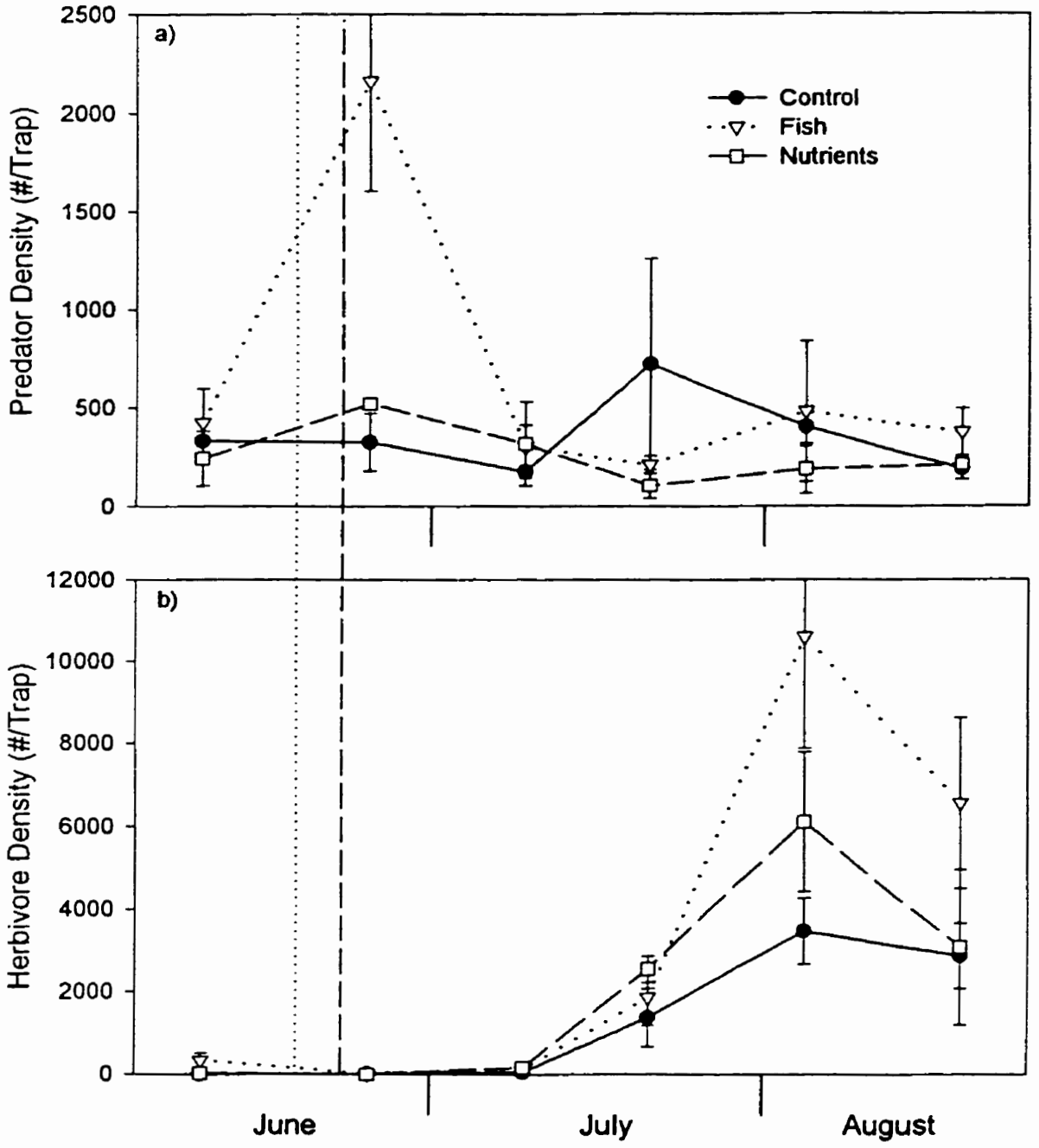

Figure 3-9. Seasonal density of (a) predaceous macroinvertebrates (mean ± SE), and (b) herbivorous macroinvertebrates associated with submersed macrophytes throughout the study. (funnel traps). The dotted line indicates the start of fathead treatment, the dashed line indicates the start of nutrient treatment.

bottom). Densities of predators or herbivores were not significantly different with treatment ($F_{1,4} = 4.61$, $P = 0.0845$ and $F_{4,20} = 4.43$, $P = 0.0892$).

Biomass of microinvertebrates in the funnel traps tended to be lower in the fathead treatment than in the control from mid-July to the end of the experiment (Figure 3-10a). This difference was not significant with treatment alone ($F_{1,5} = 3.64$, $P = 0.1145$). Biomass of macroinvertebrates in these samples tended to be slightly higher (mean 0.086 g/trap) than in the control (mean 0.052 g/trap) in August (Figure 3-10b). However, this difference was not significant with treatment ($F_{1,5} = 2.87$, $P = 0.1508$).

Nutrient Addition

Water Chemistry

Soluble reactive phosphorus (SRP) increased in the nutrient treatment throughout July and leveled off at ~2000 µg/L in August (Figure 3-3a). SRP increased very slightly in the control reaching a maximum of ~300 µg/L at the end of the experiment. Nitrate + nitrite increased rapidly in the nutrient treatment in the first 3 weeks of treatment, then declined and generally increased again in August (Figure 3-3b). Ammonia increased later in July and reached a peak (~500 µg/L) in early August then decreased rapidly again to ~120 µg/L for the last two weeks (Figure 3-3c). Nitrate + nitrite and ammonia were present at low levels in the control throughout the experiment. Levels of SRP, nitrate + nitrite and ammonia were significantly higher with nutrient addition ($F_{1,5} = 30.93$, $P = 0.0026$, $F_{1,5} = 1258.88$, $P = 0.0001$, and $F_{1,5} = 129.7$, $P = 0.0001$, respectively) and nutrient addition x time ($F_{19,94} = 0.52$, $P = 0.9452$, $F_{19,94} = 2.51$, $P = 0.0018$, and $F_{19,94} = 3.14$, $P = 0.0001$, respectively).

Per cent oxygen saturation was similar in the nutrient addition and control until the end of July, when the level of oxygen in the nutrient enclosures declined for 3 weeks and then returned to similar levels at the end of the experiment (Figure 3-3d). Per cent

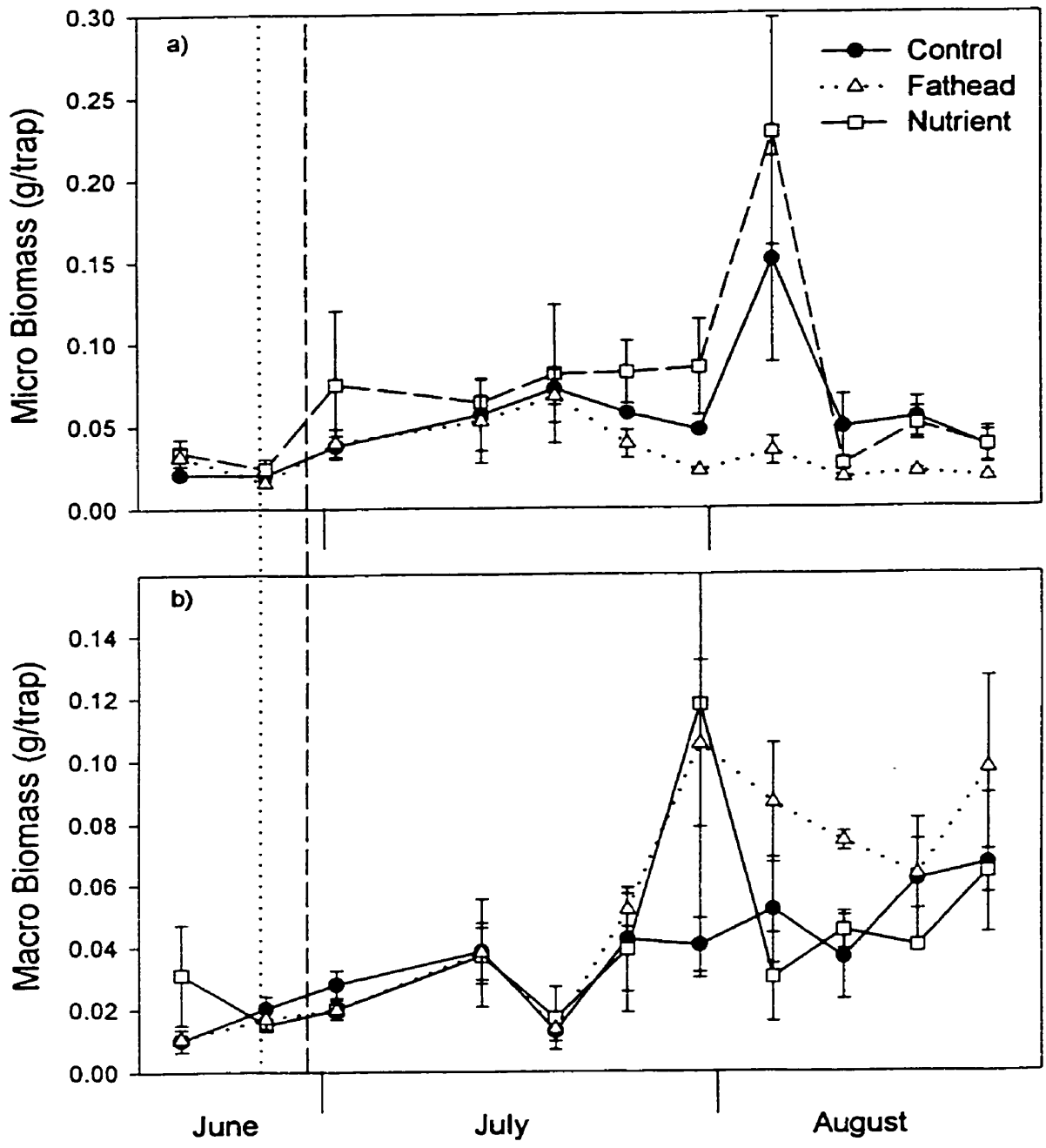

Figure 3-10. Seasonal pattern of (a) microinvertebrate biomass (mean ± SE) and, (b) macroinvertebrate biomass associated with the lower macrophyte strata (funnel traps). The dotted line indicates the start of fathead treatment, the dashed line indicates the start of nutrient treatment.

oxygen saturation was significantly lower with nutrient treatment ($F_{1,5}$ = 12.27, P = 0.0172) and nutrient treatment x time ($F_{10,50}$ = 5.21, P = 0.0001).

Treatment effects on the open water community

Cladoceran densities were low at the start of the experiment and increased rapidly by mid-July reaching ~180 ind./L in the control and ~160 ind./L in the nutrient treatment (3-4a). Density of cladocerans in the nutrient treatment declined slowly reaching low density by the end of the experiment. Populations of cladocerans in both the control and nutrient addition were dominated by *Bosmina longirostris*. Few large Cladocera (*Daphnia* spp., *Diaphanosoma*, and *Simocephalus* spp.) were present during peak density in mid-July. Copepod density was low initially and increased to reach a peak in early July (~500 ind./L) in the control: however, in the nutrient treatment, copepods peaked in mid-August (~500 ind./L, Figure 3-4b). There was no significant effect of the nutrient treatment on densities of cladocerans or copepods ($F_{1,5}$ = 0.02, P = 0.8956, $F_{1,5}$ = 0.11, P = 0.7555). Ostracod density peaked early to mid-August in both the control and nutrient treatment (~6 ind./L; Figure 3-4 c). There was no significant effect of nutrient treatment on ostracod density ($F_{1,5}$ = 0.16, P = 0.7097).

Phytoplankton reached its highest biomass (~20 µg/L) in the control at the beginning of the treatment period then declined and averaged ~5 µg/L for the rest of the study (Figure 3-4d). Phytoplankton biomass in the nutrient treatment peaked a week later at ~33 µg/L, and also declined rapidly to levels similar to the control, and then peaked again at the end of the experiment (~22 µg/L). Phytoplankton biomass did not differ significantly with nutrient treatment alone ($F_{1,5}$ = 3.69, P = 0.1128) but did differ significantly with nutrient treatment x time ($F_{9,45}$ = 2.51, P = 0.0201). However, when contrasts were performed, only the last 2 weeks showed significantly higher phytoplankton biomass.

Densities of macroinvertebrate herbivores and predators showed no response to nutrient addition in the water column (Figure 3-5). Differences were not significant with nutrient treatment ($F_{1,5} = 0.09$, $P = 0.7779$, and $F_{1,5} = 0.20$, $P = 0.6728$, respectively).

Treatment effects associated with submersed macrophytes

Upper macrophyte strata

Cladoceran and copepod density in the nutrient treatment was low throughout July and August (<4000 ind./m^2 wetland bottom) compared to the control which had densities of ~24,000 cladocerans and ~11,000 copepods/ m^2 wetland bottom in early July (Figure 3-6 a,b). From mid-July, densities of cladocerans and copepods were low in both nutrient treatment and control. Cladoceran and copepod densities were not significantly affected by nutrient treatment ($F_{1,5} = 0.54$, $P = 0.4959$, $F_{1,5} = 0.01$, $P = 0.9285$ respectively), however the treatment x time effect was significant for cladocerans ($F_{3,12} = 3.96$, $P = 0.0291$). Ostracod density with nutrient treatment was not significantly different from control density ($F_{1,5} = 0.02$, $P = 0.8856$), except for the last sampling date when density with nutrient treatment was ~350 ind./g macrophyte dry weight, and density in the control was ~13,000 ind./g macrophyte dry weight (Figure 3-6c).

Epiphyton biomass generally declined throughout the study in the control, but peaked twice with nutrient addition in mid-July and mid-August at ~1600 µg/g dry weight of macrophyte (Figure 3-6d). Epiphyton density with nutrient addition alone was not significantly different from controls ($F_{1,5} = 0.7$, $P = 0.4410$) but was with nutrient x time ($F_{7,35} = 3.04$, $P = 0.0133$). Metaphyton and/or a green algal film were observed on nutrient addition enclosures throughout the nutrient treatment. A layer of metaphyton developed in one of the enclosures from a high epiphyton load, covering as much as one quarter of the surface area over a ~10 day period in late July.

Predaceous macroinvertebrate density tended to be higher in August with nutrient treatment (dominated by zygopteran larvae) than the control (Figure 3-7a), but the difference was not significant with treatment ($F_{1,5} = 3.16$, $P = 0.1358$) or treatment x time ($F_{6,30} = 1.4$, $P = 0.2468$). Herbivorous macroinvertebrate density (predominantly snails) was lower with nutrient addition than in the controls in August (Figure 3-7b), but this difference was not significant with treatment ($F_{1,5} = 0.01$, $P = 0.9416$) or treatment x time ($F_{6,30} = 2.23$, $P = 0.0675$).

Lower macrophyte strata

Cladoceran density peaked in the nutrient treatment mid-July at ~25 000 ind./m^2 wetland bottom, and in the control at ~32 000 ind./m^2 wetland bottom (Figure 3-8a). High density in the nutrient treatment persisted longer than in the control, and did not decline to as low numbers in August. Density of cladocerans was marginally higher with nutrient treatment ($F_{1,5} = 5.72$, $P = 0.0623$) but not with nutrient treatment x time ($F_{3,12} = 0.81$, $P = 0.5309$).

Copepod density peaked at ~48 000 ind./m^2 wetland bottom in both the control and nutrient treatment mid-July then decreased rapidly in August (Figure 3-8b). Copepod (both calanoid and cyclopoid) density did not differ with nutrient addition ($F_{1,5} = 1.67$, $P = 0.2627$).

Ostracod density peaked in mid-August at ~ 40,000 ind./m^2 and 30,000 ind./m^2 wetland bottom in the control and nutrient treatment, respectively (Figure 3-8c). There was no significant difference with nutrient treatment on ostracod density ($F_{1,5} = 0.00$, $P = 0.9617$).

Densities of macroinvertebrate predators and herbivores in the nutrient treatment did not differ significantly from the control ($F_{1,5}=1.31$, $P=0.3043$; $F_{1,5}=1.25$, $P=0.3147$).

Microinvertebrate biomass peaked early August in both the control and nutrient treatment (Figure 3-10a) and was not affected by nutrient treatment ($F_{1,5} = 1.25$, $P =$

0.3138). Macroinvertebrate biomass increased gradually in the control and in the nutrient treatment throughout the study (Figure 3-10b). A large peak in macroinvertebrate biomass was observed in early August (~0.12 g/trap). Macroinvertebrate biomass was not affected significantly by nutrient treatment ($F_{1,5}$ = 0.06, P = 0.8116).

DISCUSSION

Fathead Minnow Addition

I predicted that fathead minnow addition would cause turbid conditions because their top-down predation of zooplankton would result in an increase in phytoplankton biomass. Addition of fathead minnows exerted a top-down control on zooplankton in the open water and the upper macrophyte strata. However, turbid conditions did not occur with fathead minnow addition, despite the significant increase in phytoplankton. The trophic cascade resulting from predation by fathead minnows did not occur to the extent predicted. Fathead minnows can have a large effect on zooplankton communities as well as other invertebrates (Zimmer et al. 2000, Held and Peterka 1974, Hambright and Hall 1992, Price et al. 1991, Hanson and Riggs 1995) even when fatheads are present at low densities (Zimmer et al. 2000). Zimmer found that predation from fathead minnows introduced to a wetland resulted in large phytoplankton blooms (Zimmer personal communication), and thus turbid conditions, via the trophic cascade (Carpenter et al. 1985).

Strong top-down control on phytoplankton via the trophic cascade may not have occurred in this study because of the complexity of the food web. Strong (1992) suggests that true trophic 'cascades' may not occur, instead trophic 'tangles' or 'trickles'.

Shallow complex systems may resemble 'trophic webs' rather than 'trophic ladders' typical of simpler less diverse pelagic systems (Strong 1992).

The effect of planktivores on zooplankton communities depends on which planktivores are present and their feeding behaviour. Hall et al. (1976) predicted that size-selective predation by planktivorous fish would result in selective predation on the larger cladocerans, resulting in a shift in the community to smaller cladocerans. There was no evidence of size-selective feeding by fathead minnows in my experiment as large cladocerans were sparse before treatment began, and the density of small zooplankton species likely declined as a result of fathead minnow predation. Fathead minnows have been shown to affect the whole zooplankton community (Zimmer et al. 2000; Hambright and Hall 1992), likely the result of fathead minnows being filter-feeders as well as particulate feeders (Hambright and Hall 1992). Particulate feeding is used to feed on large prey, and filter-feeding is used to feed on smaller prey (Hambright and Hall 1992).

Fathead minnows are fractional spawners, producing several cohorts throughout the summer (Gale and Buynak 1982). Therefore, fathead minnow populations consist predominantly of YOY as the summer progresses (Zimmer personal communication), representing the majority of predation pressure on zooplankton (Price et al. 1991, Zimmer et al. 2000), and to a lesser extent on ostracods (Price et al. 1991). In the enclosures in my study, YOY fathead minnows became predominant as the summer progressed, increasing in size and density (Sandilands, personal observation).

I expected that macrophytes would provide a refuge for zooplankton from predation from fatheads. Submersed macrophytes provide a refuge for zooplankton from predation by planktivorous fish (Timms and Moss 1984, Schriver et al. 1995, Stansfield, et al. 1997, Perrow et al. 1999). Despite growing evidence that macrophytes provide a refuge effect, I did not detect such an effect in this study, since cladoceran

density in macrophytes (especially upper macrophytes) was lower in the fathead treatment than in the control.

Perhaps macrophytes did not provide a refuge for zooplankton because of the high density of enclosed fatheads present. Fatheads may have been forced to feed among macrophytes after preferred food in the open water column was consumed, and as macrophytes occupied more of the enclosure volume throughout the season. The refuge was probably ineffective against the large population of YOY fatheads whose small size allows them to penetrate the complex structure of the macrophytes more effectively than larger fish (Persson and Crowder 1998). Furthermore, YOY use mainly particulate feeding (Hambright and Hall, 1992), which would allow fatheads to prey upon phytophilous zooplankton grazing epiphyton on macrophyte surfaces.

Schriver et al. (1995) suggested that the refuge effect for large cladocerans disappears over the threshold level of ~2 fish /m^2 and that the refuge for smaller cladocerans disappears at a threshold of ~4 fish/m^2 (also Jeppesen et al. 1998). The density of adult fatheads in the enclosures was 5/m^2, not including the many YOY present. Thus the density of fish in this experiment was likely well above the threshold of 4/m^2 (Schriver et al. 1995). A refuge effect was not detected in enclosure experiments where the density of stickleback planktivores (a fish similar in size to fathead minnows) was 11-18/m^2 (Stephen et al. 1998).

The effect of fatheads has been examined in few studies (or other planktivorous fish) on the densities of macroinvertebrates (Zimmer et al. 2000, Mallory et al. 1994). Mallory et al. (1994) found that wetlands with planktivorous fish had fewer macroinvertebrates than fishless wetlands. In wetlands with fathead minnows present, densities of many macroinvertebrates declined, e.g. ephemeropterans, trichopterans, amphipods, and notonectids (Zimmer et al. 2000). I found that fathead minnows did not have an effect on macroinvertebrates despite the findings of Zimmer et al. (2000) and

Mallory et al. (1994). Since my enclosures were dominated by young of the year, an effect on macroinvertebrates would be less likely due to the smaller gape size of juvenile fatheads. Smaller fatheads feed less on amphipods than large fathead minnows (Held and Peterka 1974). Zimmer et al. (2000) and Hann (1999) found that corixids became abundant and were the dominant macroinvertebrate when fathead minnows were present. However, corixids were not the dominant macroinvertebrates in the fathead treatment, although they had been in 1996 (at similar densities to Hann 1999; Sandilands, unpublished data) when fish were also present. A lack of corixid response in 1997 may be because adult corixids were not present at the time of enclosure installation. Hann (1996) found that adult Corixids first appeared in the open water in early June. Thus, installation of enclosures in 1997 (May 29) may have been before corixids were present, whereas in 1996, enclosures were installed later (June 11) after the appearance of adult corixids in the open water.

Nutrient Addition

Addition of inorganic N and P did not cause turbid conditions as predicted. Even though there was a significant response of phytoplankton to nutrient addition over time (~33 µg Chl *a*/L), the response was small and did not produce turbid conditions (defined as >80 - 100 µg/L Chl *a*, based on a review of the literature). Levels of nutrients(SRP) in the water column were similar to or higher than previous nutrient additions of the same loading that produced turbid conditions (100 - 200 µg Chl *a*/L; Chapter 2). However, densities of submersed macrophytes were much higher in this study (see Table 3.1 and Table A-1 in Appendix 2), and may have contributed to maintenance of clear water conditions. Macrophyte biomass was higher in the nutrient treatment than the controls before treatment began, and macrophyte biomass peaked with nutrient addition earlier than controls, perhaps due to increased nutrient availability.

The presence of a high biomass of macrophytes in the nutrient treatment relative to controls and to macrophyte biomass in 1996 likely explains the lack of phytoplankton response in 1997, and the strong response of phytoplankton in 1996. Biomass of macrophytes affects the level of nutrients available to phytoplankton in the water column. Macrophytes may potentially compete with phytoplankton for nutrients, as Scheffer (1998) has found that macrophyte beds can result in a decrease of dissolved nutrients in the water column. *Ceratophyllum demersum*, which was abundant in the enclosures, can inhibit phytoplankton development (Mjelde and Faafeng 1997). Irvine et al. (1989) suggested that macrophytes maintain clear water conditions by luxury uptake of nutrients, inhibition of algae by allelopathy (also see Wium-Andersen et al. 1982, Wium-Andersen et al. 1983), shedding of leaves with high epiphyton, and providing habitat and refuge for zooplankton grazers (discussed later). Macrophytes may act as a sink for nitrogen and phosphorus (van Donk et al. 1993, Scheffer 1998). I also noted that short stands of the macroalga *Chara* on the sediments in 1997, but not in 1996. However, biomass of *Chara* could not be quantified as it was difficult to sample the short *Chara* stands growing among taller, dense stands of macrophytes. The presence of *Chara* in 1997 and not in 1996 may further explain the lack of phytoplankton response in 1997 due to its allelopathic effect on phytoplankton (Wium-Andersen et al. 1982).

Increased biomass of macrophytes over the control would also provide more habitat for zooplankton and surfaces for epiphytes to colonize. In fact, there was more epiphyton per m^2 wetland bottom in the nutrient addition. Mats of metaphyton or thin films of green algae also occurred in the nutrient addition.

Oxygen saturation in the water column was significantly lower with nutrient addition. Dense macrophyte beds can cause low levels of oxygen at night due to respiration (Scheffer 1998). Since biomass of both macrophytes and epiphytes was higher with nutrient addition, oxygen concentration should have been lowest in the

morning due to respiration. The higher the primary productivity, the higher the respiration and the more depleted oxygen concentrations are in the morning. Chow-Fraser (1998) found that high algal biomass resulted in low oxygen concentrations near macrophyte beds, with a large diurnal fluctuation.

The increase of phytoplankton in response to nutrient addition did not result in an increase in planktonic zooplankton, contrary to other studies (Gabor et al. 1994, Van Donk et al. 1995, Campeau et al. 1994). Zooplankton may not have had a chance to respond to the increased availability of food since the increase in phytoplankton was of short duration and at the end of the experiment.

Cladocerans associated with macrophytes increased in abundance in response to nutrient addition. Phytophilous cladocerans increased in abundance in response to nutrient addition and subsequent increase in availability of food for these phytophilous grazers (Hann and Goldsborough 1997, Pettigrew et al. 1998) in previous enclosure experiments at Delta Marsh.

Phytoplankton blooms occurred in the enclosure experiments when either inorganic nutrients (Chapter 2) or organic nutrients were added (although the response was brief, Pettigrew et al. 1998). Phytoplankton blooms did not occur where other primary producers (macrophytes and associated epiphyton) were eliminated and top-down control was weak (Chapter 2). Therefore, phytoplankton may be nutrient limited in Delta Marsh, or at least sufficient nutrients are not available to produce large phytoplankton blooms and thus turbid conditions.

CHAPTER 4

FACTORS AFFECTING SPECIES COMPOSITION OF THE INVERTEBRATE COMMUNITY ASSOCIATED WITH SUBMERSED MACROPHYTES IN EXPERIMENTAL ENCLOSURES TREATED WITH NUTRIENT AND FATHEAD MINNOW ADDITION.

INTRODUCTION

Submersed macrophyte beds and associated zooplankton may be thought of as a phytoplankton filter. Macrophytes provide habitat and refuge (when planktivorous fish are present) for zooplankton filter-feeders which are important grazers of phytoplankton (Irvine et al. 1989, Timms and Moss 1984), and thus maintain the clear water state (Scheffer et al. 1993, Moss, 1990; see Chapter 2). When planktivorous fish are present, zooplankton in the macrophyte beds ultimately determine the stable state of the system. Therefore, it is necessary to understand how planktivorous fish, nutrients and macrophyte density affect zooplankton density and community structure among the macrophytes. In this study, two important factors in the stable state model, nutrients (nitrogen and phosphorus) and planktivorous fish (fathead minnows) were added in separate enclosures to examine their effects on the invertebrate community.

The microcrustacean zooplankton community associated with submersed macrophytes consists of two components: planktonic filter-feeders and phytophilous scrapers. Planktonic members, present in the water column around macrophytes, include species such as *Bosmina longirostris*, *Diaphanosoma birgei*, *Ceriodaphnia dubia*, and also calanoid and cyclopoid copepods. Phytophilous scrapers, present on the surfaces of macrophytes, include chydorid cladocerans such as *Eurycercus longirostris*, *Pleuroxus denticulatus*, and *Chydorus* spp. Zooplankton associated with submersed macrophytes can feed by one of two methods; thus they can control

phytoplankton in two ways. First, filter-feeders can feed on phytoplankton that is carried into macrophyte beds by water currents, or filter feeders can migrate horizontally from macrophyte beds into the open water column to feed at night (Timms and Moss 1984), when predation pressure from visually feeding fish is low. Second, and probably less effective, scrapers can feed on phytoplankton that is carried into macrophyte beds and is trapped on the epiphyton on macrophyte surfaces (Irvine et al. 1989).

Two important factors that affect zooplankton densities among macrophytes are density of macrophytes and density of predators. Dense macrophyte beds provide habitat for phytophilous zooplankton, and surfaces for epiphytes to colonize (especially if epiphyton growth is stimulated with high nutrient loading), and thus provide food for phytophilous grazers. Dense macrophytes may also provide a refuge from predation by planktivorous fish. Sparse macrophytes may not provide an effective refuge as fish may be able to forage more freely (Irvine et al. 1989, Lougheed and Chow-Fraser 1998).

Vertebrate predators (planktivorous fish) can also affect zooplankton density directly. Planktivorous fish feed mainly on zooplankton, and can have a large impact on zooplankton in the open water, and to a lesser extent on zooplankton associated with macrophytes.

Macrophyte and planktivore density can also indirectly affect phytoplankton biomass. Since both macrophytes and planktivores affect zooplankton directly, and zooplankton directly affects phytoplankton, macrophytes and planktivores indirectly affect phytoplankton via this trophic cascade (Carpenter et al. 1985).

Macroinvertebrates may also affect zooplankton density and biomass of primary producers indirectly. Predators in macrophytes may prey upon zooplankton, indirectly reducing grazing pressure on phytoplankton and epiphyton. Macroinvertebrate herbivores (snails, amphipods, *Acentria* larvae) can graze on epiphyton (Brönmark 1989, Pennak 1989) and macrophytes themselves (*Acentria*; Merrit and Cummins 1996),

thereby affecting available food and habitat for phytophilous grazers. Epiphyton grazers may improve growth and survival of macrophytes by reducing epiphyton that shades the surfaces of macrophytes upon which they grow (Brönmark 1985,1989, Underwood et al. 1992, Thomas 1987).

The objectives of the study were: 1) to examine changes in species composition of the microinvertebrate and macroinvertebrate community throughout the season, 2) to determine the effects of nutrient (nitrogen and phosphorus) addition and fathead minnow addition on the zooplankton species composition and macroinvertebrate community associated with the macrophytes, and 3) to determine the effect of environmental variables on species composition.

METHODS

A description of the study site and the experimental design can be found in Chapters 2 and 3, respectively. This chapter deals only with Downing Box data collected from July 9 to August 20, 1997 in all 3 treatments (control, nutrient addition, and fathead minnow addition). Sampling started 3 weeks after treatment began because macrophytes were sparse until this time. Therefore, the examination of seasonal changes in the invertebrate community in this chapter does not represent the entire open water season.

Biotic Sampling

The zooplankton community associated with submersed aquatic plants was sampled using the Downing box sampler (Downing 1986), an 'acrylic suitcase' (4 L), lowered into the water and closed around approximately the top 30 cm of macrophyte (Figure 4-1). The liquid contents of the box were then poured through a 53 μm mesh net

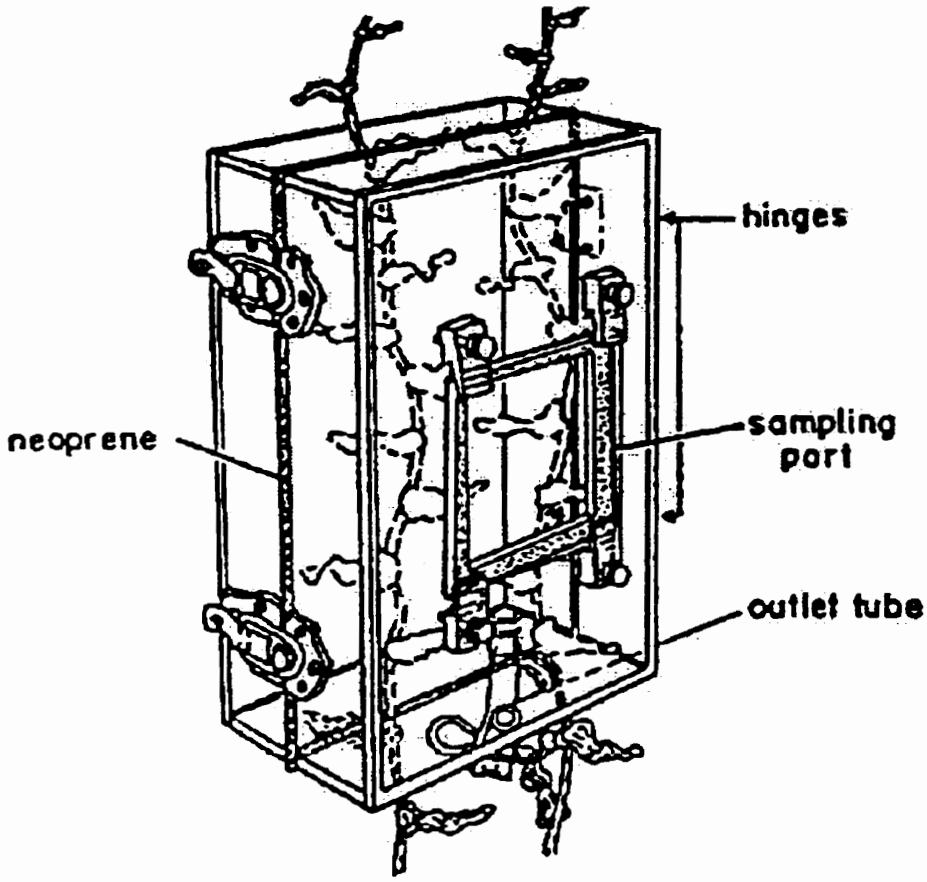

Figure 4-1. The Downing Box consists of clear acrylic (which is gently closed around a submersed macrophyte) enclosing a macrophyte and associated fauna and flora (Downing, 1986)

to collect microinvertebrates. The plant tissue was shaken vigorously with carbon filtered water to remove macroinvertebrates and epiphyton. Biomass of both epiphyton and macrophytes was determined (methods in Chapter 2). Samples often contained 2 species of macrophyte which were later separated for dry weight determination; however, invertebrates and epiphyton could not be separated according to species of macrophyte. Phytoplankton biomass was determined from 3 samples per enclosure, collected in mid water column as described in Chapter 2.

Zooplankton were identified using Pennak (1989) and a reference collection (BJH). 'Species data' used in analyses included: cladocerans (*Bosmina longirostris*, *Ceriodaphnia dubia*, *Diaphanosoma birgei*, *Simocephalus* sp., *Eurycercus longirostris*, *Chydorus* spp., *Pleuroxus denticulatus*, *Daphnia* spp.), copepods (nauplii, cyclopoid copepodites, cyclopoid adults, calanoid copepodites, calanoid adults), small non-predaceous rotifers, and ostracods.

Fish were monitored daily using two minnow traps in each enclosure. Fish caught in the fathead minnow treatment enclosures were counted and returned to the enclosure. Fish caught in all other enclosures were counted and removed. Mean daily fish catch was then calculated for each enclosure for each week. Only adult fish, most of which were fathead minnows, were large enough to be caught in the traps. In most enclosures (especially fathead treatment) there were young of the year (YOY) fatheads which could not be sampled quantitatively, but relative abundance was noted if they were present.

Data Analysis

Correspondence Analysis (CA) was performed on LOG (x+1) treatment mean data standardized per gram of macrophyte biomass sampled on each date in order to

explore the species composition of zooplankton among treatments. Data were standardized per unit macrophyte dry weight so that samples with a very small or large biomass of macrophytes would not result in an under or over estimate of the proportion of phytophilous species. Changes in species composition throughout the season were examined by connecting successive points on the biplots. CA is an indirect gradient analysis much like principal component analysis with double standardization of the data before eigenanalysis (ter Braak 1985). CA groups samples with similar "species" composition close together on an ordination diagram, while placing samples with different species composition far apart.

The effect of environmental variables on microinvertebrate and macroinvertebrate species composition was also examined with the use of Canonical Correspondence Analysis (CCA). CCA is a direct gradient analysis method which incorporates the response of species to environmental variables used in the method. As with CA, the CCA ordination diagram places samples with similar species composition and relative abundance close together, and samples with different species compositions and relative abundance far apart. Environmental variables are represented on the CCA triplots as vectors which increase in value along the vector from the origin. The importance of the factor is also proportional to the length of the vector (ter Braak, 1986).

CCA was performed on LOG (x+1) (for microinvertebrates) and LOG (x+10) (for macroinvertebrates) transformed treatment mean data standardized to volume on each date. Macroinvertebrate density was LOG (x+10) transformed because of the low abundance of many species which were present but not sampled. If a taxa was not sampled, the LOG (x+10) transformation results in a value of 1. I believe this is valid since only taxa which did occur were included in analyses. This problem is a result of sampling organisms which are commonly present, but are not always effectively sampled. The CCA on LOG (x+10) more accurately reflected true dominance of species

in the ordination. The quantity of macrophytes sampled varied between samples and between treatments, so data were standardized to volume so that treatment effects could be examined. Also, environmental data such as species of macrophyte can be included in a triplot. Separate CCAs were performed on microinvertebrates and macroinvertebrates because microinvertebrates are much more abundant than macroinvertebrates, and therefore would represent an overwhelmingly large proportion of the community if both groups were treated together, even though macroinvertebrates may have a greater biomass than microinvertebrates. Factors which affect zooplankton directly were used in the CCA. 'Environmental' variables were: young of the year fish density (YOY), epiphyton biomass (µg Chl a/sample), phytoplankton biomass (µg Chl a/L), macrophyte biomass (dry weight/sample) and species of macrophyte (dry weight/sample for each species). Weighted average (WA) scores were used in the triplots. CA and CCA were performed using CANOCO version 3.10 (ter Braak 1988).

Young of the year fish (YOY) grew throughout the season and exerted increasing predation pressure on the zooplankton, but their numbers were not quantified due to sampling difficulties. YOY can be included as an environmental variable since the YOY fish feed differently than adults, i.e. as particulate feeders rather than filter feeders, (Hambright and Hall 1992). Some YOY were present in most enclosures, not just the fathead treatment, so a simulated environmental variable was constructed to represent predation pressure from YOY fatheads, estimated to increase exponentially through August. This increase in predation pressure is exponential because of the rapid growth of YOY (Held and Peterka 1974), and low mortality in the population due to enclosure (discussed in Chapter 3). Data were estimated for each enclosure based on the relative number of YOY observed in each enclosure. Data constructed for each treatment on each date were LOG (x+1) transformed and used in the CCA. YOY was added as a

separate environmental variable, with a unimodal distribution, satisfying the assumptions of the method (ter Braak 1986).

RESULTS

Species Composition

The first CA axis accounted for 47.5% of the variance in the data (Figure 4-2). When the points were connected between successive sampling dates, the pattern for all treatments paralleled the first axis (Figure 4-2) from left to right, thus representing the seasonal development of the zooplankton community. In early July, the community was composed mostly of filter-feeding species (*Bosmina longirostris*, *Simocephalus* sp. and calanoid copepods). By the end of the experiment, the zooplankton community was composed of phytophilous chydorid scrapers (*Pleuroxus denticulatus*, *Chydorus* spp., *Eurycercus longirostris*) and ostracods.

The second CA axis accounted for 19.6% of the variation in the data, and is important in separating the effects of experimental treatment on the species composition of the community (Figure 4-2). The species compositions in the two treatments and the control were similar in early July, then diverged from one another throughout the season (Figure 4-2). The control was located between the two treatments on the ordination, with *Diaphonosoma* more abundant than in the treatments. The fathead treatment was most separated and there was a change in species composition to ostracods and rotifers by the end of the experiment. In the nutrient treatment, there was a shift to phytophilous species (*Pleuroxus denticulatus* and *Chydorus* spp.) that scrape epiphyton from macrophyte surfaces.

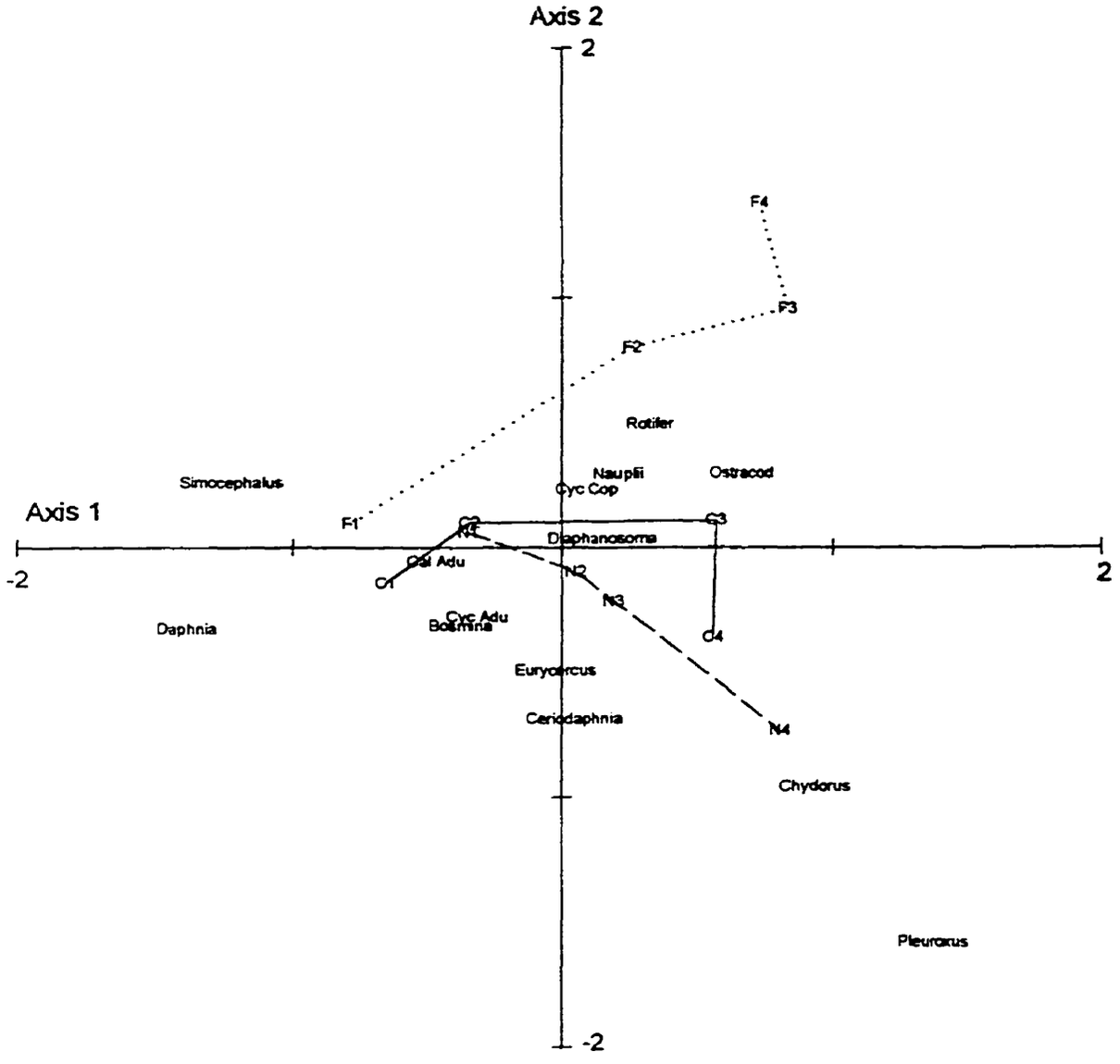

Figure 4-2. Correspondence Analysis biplot of numbers of individuals per unit macrophyte dry weight sampled with the Downing Box. Labels for treatment mean sites (connected points) are coded C - control, F - fathead minnow treatment, N - nutrient treatment, 1- July 9, 2 - July 23, 3 - Aug. 6, and 4 - Aug. 20, 1997. Species are identified as follows: Bosmina, *Bosmina longirostris*; Ceriodaphnia, *Ceriodaphnia dubia*; Chydorus, *Chydorus* sp.; Diaphanosoma, *Diaphanosoma birgei*; Eurycercus, *Eurycercus longirostris*; Pleuroxus, *Pleuroxus denticulatus*; Simocephalus, *Simocephalus* spp.; Daphnia, *Daphnia* sp. Rotifer, small rotifers; Nauplii, copepod nauplii, Cyc Cop, cyclopoid copepodites; Cyc Adu, cyclopoid adults; Cal Cop,calanoid copepodites, Cal Adu, calanoid adults; and Ostracod, Ostracod spp.

Effect of environmental variables

Environmental variables explained 59.0% (sum of unconstrained eigenvalues/ sum of constrained eigenvalues x 100) of the variance in the species composition (Figure 4-3) in the CCA. YOY fathead minnows and macrophyte biomass were highly correlated with the first axis (R= 0.9016 and R= 0.7778 respectively, Table 4-1). YOY predation pressure contributed to the shift to phytophilous species throughout the season. Macrophyte biomass also increased throughout the season (Figure 4-3), with a concurrent shift to phytophilous species.

Phytoplankton and epiphyton biomass were correlated with the second axis of the ordination (R= 0.4522, and R= -0.5738 respectively, Table 4-1), separating the treatments along the second CCA axis (Figure 4-3). Phytoplankton biomass was highest in the fish treatments, and epiphyton biomass was highest in the nutrient treatment (confirmed in Chapter 3). There was a higher proportion of phytophilous cladocerans (*Chydorus* sp. *Pleuroxus*, and *Eurycercus*) present in the nutrient treatment where epiphyton biomass was highest.

Dry weights of individual macrophyte species were also used as environmental variables to examine effects of macrophyte species on zooplankton species composition. *Ceratophyllum demersum* and *Potamogeton zosteriformis* were both present later in the season when there was a higher proportion of phytophilous species (Figure 4-4). Planktonic species of zooplankton were in higher abundance where *P. pectinatus* biomass was higher earlier in the season.

When a CCA was performed on macroinvertebrate abundance data, 65.2% of the variation in the species data was explained by environmental variables (Figure 4-5). Again, the first CCA axis accounted for the seasonal trend in the macroinvertebrate

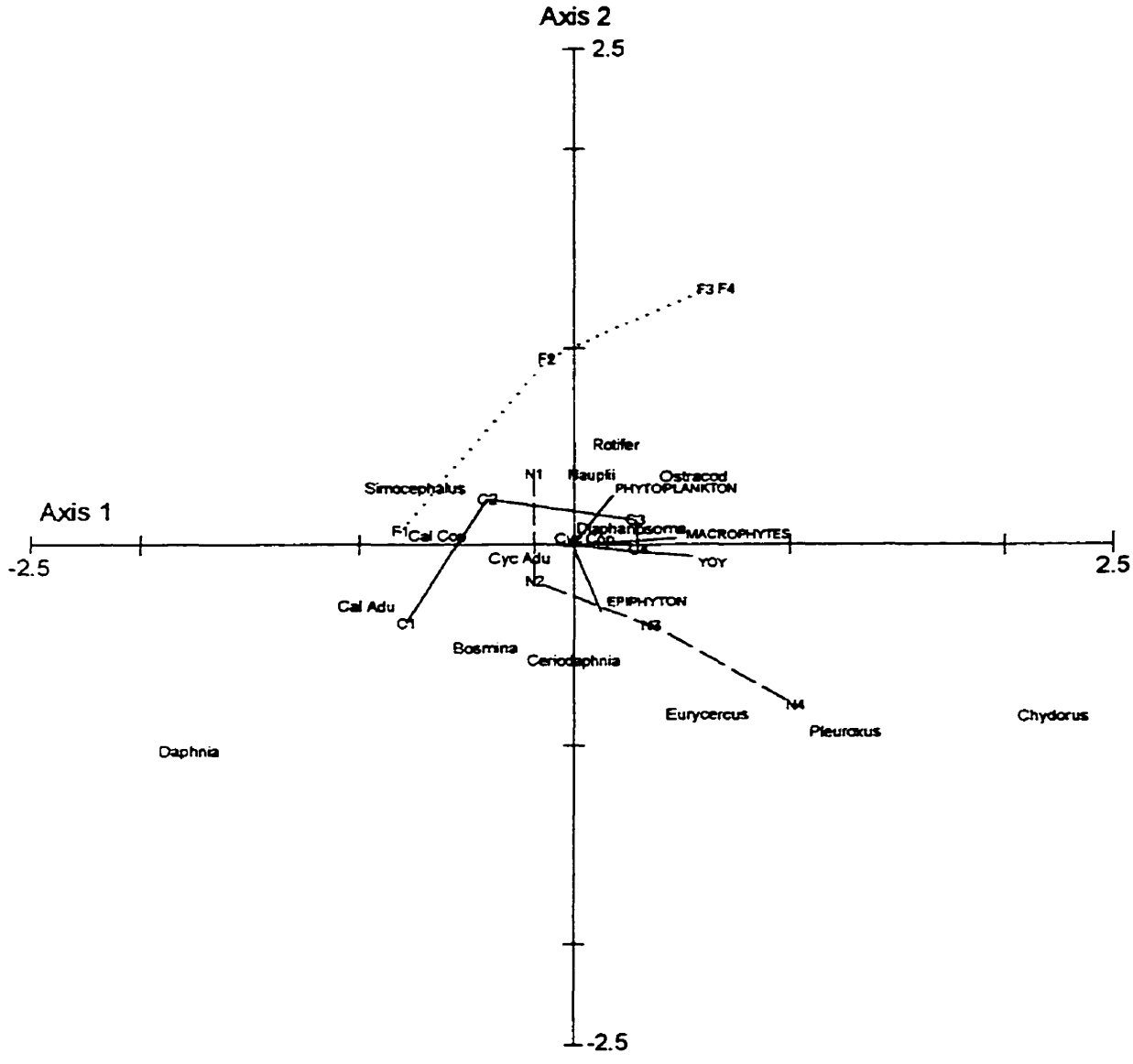

Figure 4-3. Canonical Correspondence Analysis triplot for numbers of individuals per Downing Box sample. Labels for treatment mean sites and zooplankton species are as in Figure 2. Environmental variables are labeled as follows: EPIPHYTON, mean epiphyton Chl a per downing box sample per treatment; and PHYTOPLANKTON, mean phytoplankton Chl a per treatment; MACROPHYTE, mean total macrophyte biomass (dry weight) per treatment; and YOY, estimated predation pressure from young of the year fathead minnows for each treatment.

Table 4-1. Weighted correlation coefficients between environmental variables and the first two CCA axes for the zooplankton community in submersed macrophytes (Figure 4-3).

Environmental Variable	Axis 1	Axis 2
Macrophyte Biomass	0.778	0.086
YOY Fish	0.902	-0.080
Epiphyton Biomass	0.217	-0.574
Phytoplankton Biomass	0.314	0.452

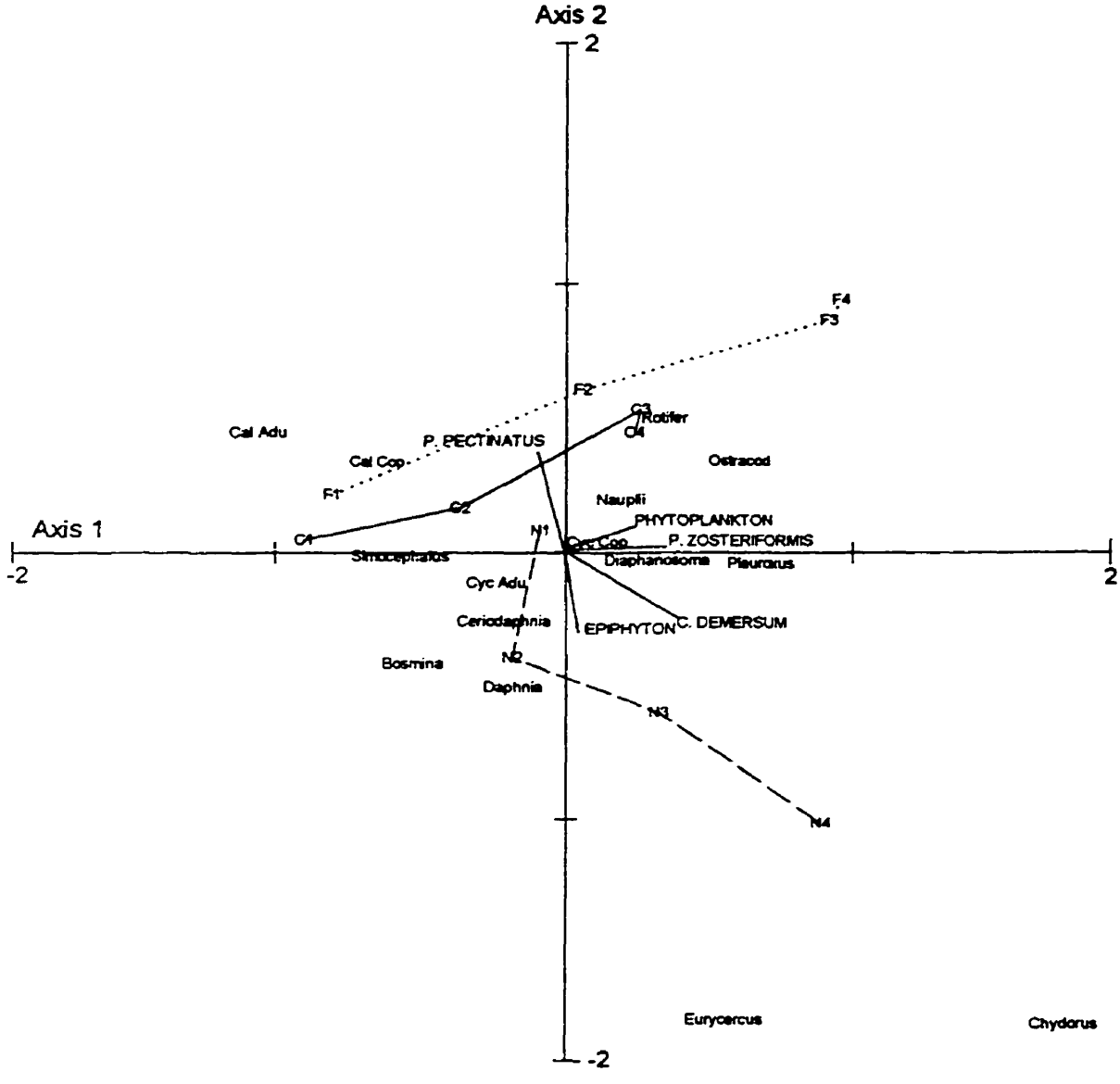

Figure 4-4. Canonical Correspondence Analysis triplot for numbers of individuals per downing box sample. Labels for treatment mean sites and zooplankton species are as in Figure 4-2. Environment variables are labeled as follows: EPIPHYTON, mean epiphyton Chl a per downing box sample per treatment; and PHYTOPLANKTON, mean phytoplankton Chl a per treatment; P.PECTUNATUS, mean dry weight of *Potamogeton pectinatus* per treatment; P.ZOSTERIFORMIS, mean dry weight of *Potamogeton zosteriformis* per treatment; and C.DEMERSUM, mean dry weight of *Ceratophyllum demersum* per treatment.

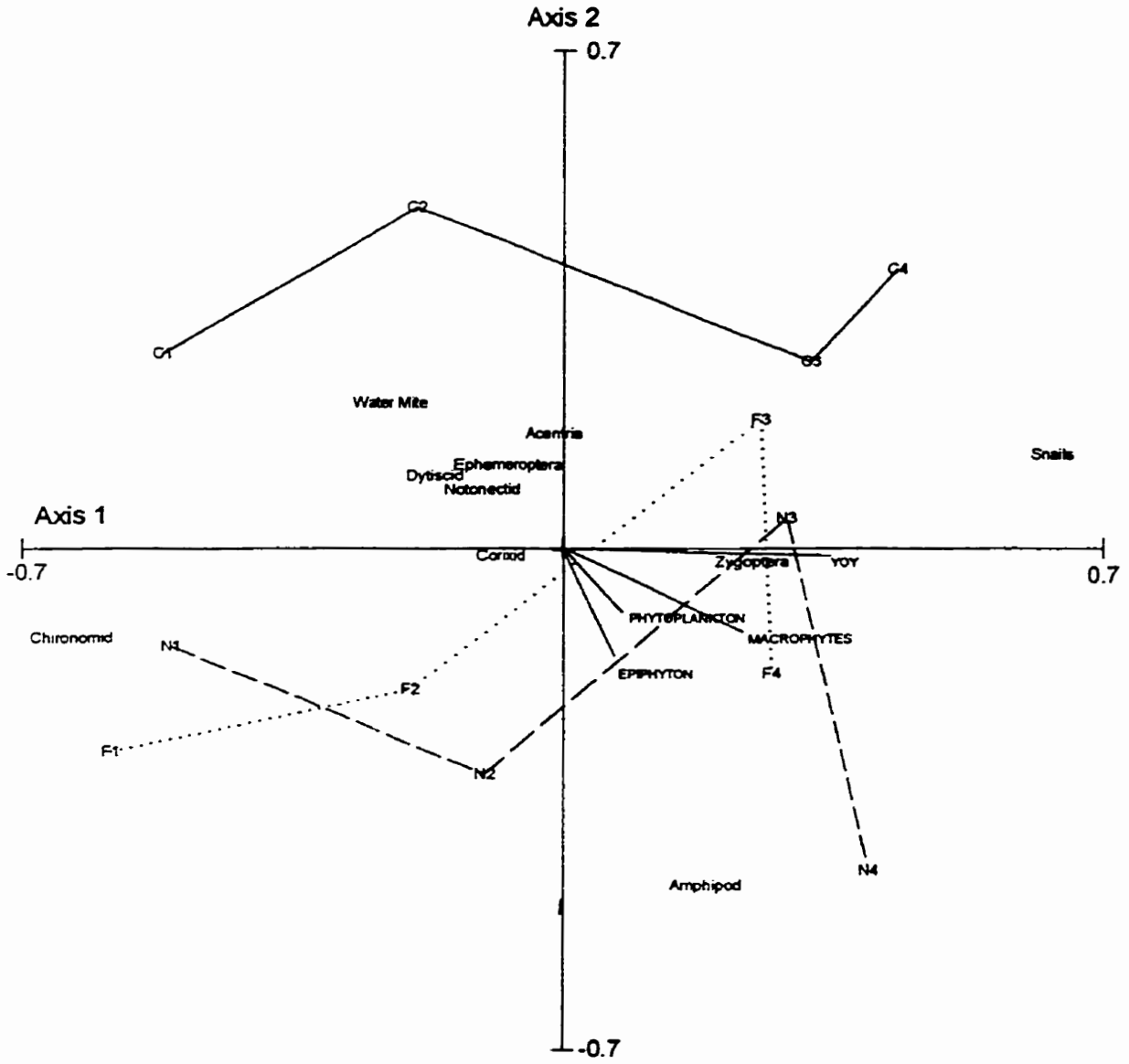

Figure 4-5. Canonical Correspondence Analysis triplot for numbers of macroinvertebrates (log x+10) per Downing Box sample. Labels for treatment mean sites (connected points) are coded as in Figure 2. Macroinvertebrates are identified as follows: Water Mite, water mites; Acentria, *Acentria nivea* larvae; Dytiscid, dytiscid larvae; Ephemeroptera, ephemerotera larvae; Notonectid, notonectid nymphs and adults; Corixid, corixid nymphs and adults; Chiromomid, chironomid larvae; Zygoptera, zygoptera larvae; Snails, *Gyraulus* sp. and *Physa* sp.; and Amphipod, amphipods.

community as each treatment paralleled the first axis throughout the season. Early in July, the community was dominated by dytiscid larvae, water mites, and chironomid larvae. At the end of August, the community was dominated by snails, *Acentria* larvae, zygopteran larvae, and amphipods. The control was widely separated from the two treatments on the second axis throughout the season. In August, the control was dominated by snails and *Acentria* larvae, whereas the fathead and nutrient treatments were dominated by zygopteran larvae and amphipods.

DISCUSSION

Seasonal change in species composition

The most dramatic result found in this study was the change in species composition of the zooplankton community throughout the season. Pettigrew et al. (1998) and Zrum et al. (2000) also found that this seasonal change in species composition was stronger than treatment effects themselves. The proportion of phytophilous grazers (scrapers) in the community increased as the biomass of macrophytes increased throughout the season. There was more habitat available for these phytophilous species which graze epiphyton from macrophyte surfaces. Lougheed and Chow-Fraser (1998) also found that macrophyte cover was important in determining the composition of the zooplankton community in a hypereutrophic Great Lakes wetland. This shift from planktonic species (*Daphnia* sp. and *Bosmina longirostris*) to phytophilous species (*Eurycercus longirostris*, *Ceriodaphnia dubia*, *Chydorus* sp. and *Pleuroxus denticulatus*) also occurred in previous enclosure experiments (Hann and Goldsborough 1997, Pettigrew et al. 1998, and Zrum et al. 2000) and in the Blind Channel (Hann and Zrum 1997). However in this study, *Simocephalus*

spp. were important in early July, whereas *Simocephalus* spp. became important later in previous experiments (Hann and Goldsborough 1997, Pettigrew et al. 1998).

YOY fathead minnows also had a substantial effect on the seasonal change in the community. Predation by YOY fish led to a decline in proportion of planktonic filter-feeders throughout the season. Filter-feeding zooplankton, e.g. *Simocephalus* and *Daphnia*, were depredated first, as they were in the open water, and were easier prey than scrapers which were closely associated with macrophytes. The disappearance of *Simocephalus* early is contrary to Hann and Goldsborough(1997) and Pettigrew et al. (1998) who found that *Simocephalus* became important later in the season. The decline of *Daphnia* followed by *Simocephalus* was probably the consequence of fish selectively feeding on larger cladocerans, in accordance with the size efficiency hypothesis (Brooks and Dodson 1965) and size selective predation (Hall et al. 1976). Since *Daphnia* are found in the open water column, and thus the easiest prey for fatheads, they would be depredated first. As *Daphnia* became scarce in the water column, fatheads probably started foraging in macrophytes while they were still sparse early in the season and would prey upon *Simocephalus*, the largest prey item present. Fish were not present in previous enclosure experiments (Hann and Goldsborough 1997, Pettigrew et al. 1998) thus *Simocephalus* would have been exposed to less predation pressure than in this study. Cyclopoid copepods declined later since they exhibit evasive or escape behaviour in the presence of fish (Drenner et al. 1978).

The macroinvertebrate community also changed throughout the season. The community was dominated by chironomid and dytiscid larvae in early July, and shifted to dominance of amphipods and snails by the end of August. It is expected that as macrophyte biomass increases throughout the season, snails and amphipods would also respond to the increased habitat available. It is also possible that certain groups were not sampled effectively early in the season, and became large enough to be caught in

the sieve (>1mm) later in the season. However, survival of both snails and amphipods would benefit from increased availability of epiphyton as food on macrophyte surfaces. As 'sit and wait' predators, zygopterans may also have increased in abundance as more habitat became available.

Effect of treatment

Fathead Minnow Addition

Treatments were separated on the second CCA ordination axis as the season progressed. Early in the experiment in the fathead treatment, there was a higher proportion of cladocerans which later declined, as they were preyed upon selectively by fatheads. At the end of the season, the zooplankton community was dominated by rotifers and ostracods in the fathead treatment. Others have documented a shift in the zooplankton community to rotifers, when other filter feeders have been eliminated (Drenner et al. 1990). The fathead treatment also had a relatively low proportion of phytophilous cladocerans in August. This may have occurred from YOY predation as YOY fatheads may prey upon smaller phytophilous species since YOY are smaller and can potentially penetrate macrophyte beds better than adult fish. Phytoplankton was the dominant algal component and thus would be unfavourable for phytophilous species. Hann and Zrum (1997) also found that fewer phytophilous species were common in an area of the marsh where fish were present (*Bosmina longirostris*, *Simocephalus serrulatus*) than in an area where fish were absent (*Alonella excisa*, *Kurzia latissima*, *Pleuroxus procurvus*, *Chydorus* sp. and *Microcyclops rubellus*). Ostracod density in the fathead treatment was significantly lower than control (see Chapter 3); however ostracods were still dominant. Ostracods may be the least preferred food item, and only became dominant after more suitable prey had been depleted. Held and Peterka (1974) found that fathead minnows eat fewer ostracods than they do cladocerans and

copepods, suggesting that cladocerans and copepods are preferred over ostracods (assuming that all are equally available).

In the absence of efficient filter feeders (cladocerans), phytoplankton biomass increased with fathead treatment (significantly higher than control, see Chapter 3). Ostracods do not feed effectively on phytoplankton, thus phytoplankton was not controlled in the fish treatment near the end of the season, despite the abundance of ostracods. Thus fish had a top-down effect on the food web, decreasing cladoceran zooplankton abundance, reducing grazing pressure on phytoplankton, and therefore increasing phytoplankton biomass via the trophic cascade (Carpenter et al. 1985).

Fish treatment resulted in the macroinvertebrate community being dominated by amphipods and snails. Snails and amphipods may not have been preyed upon by the young of the year fish due to gape limitation (Held and Peterka 1974). Amphipods were abundant among macrophytes (especially *C. demersum*, personal observation), probably because they feed on epiphyton and other organic matter on plant surfaces (Pennak 1989).

In previous studies, corixids became the dominant macroinvertebrate when fish were present (Zimmer et al. 2000, Hann 1999). Indeed, corixid density was high in the 1996 enclosure experiment (Sandilands, unpublished) with fish present and densities comparable to unenclosed areas of Delta marsh with fish (Hann 1999). However, corixid density in the 1997 experiment was comparatively low in the fathead treatment. Hann (1996) found that adult corixids first appeared in the open water in early June. As enclosures were installed earlier in 1997 (May 29) than in 1996 (June 11), it is possible that corixid adults were excluded in the 1997 experiment, resulting in anomolously low densities throughout the season (see Chapter 3).

Nutrient Treatment

The nutrient addition treatment was also separated from the controls on the second CCA ordination axis over time. The cladoceran community composition changed to a higher proportion of phytophilous species (*Eurycercus longirostris*, *Pleuroxus denticulatus*, and *Chydorus* sp.) than in the control. Biomass of epiphyton increased throughout the season (see Chapter 3) as the epiphyton vector increased with nutrient addition throughout the season. Therefore, it seems the cladoceran community shifted to more phytophilous species as epiphyton biomass increased. This shift in the community is expected as phytophilous grazers, which scrape epiphyton from macrophyte surfaces, would increase in response to greater availability of epiphyton in the nutrient treatment.

At the end of the study, the nutrient addition treatment had a high proportion of amphipods relative to other macroinvertebrates. Amphipods could have responded to the increase in habitat provided by macrophytes, and associated increase in epiphyton available as food (Pennak 1989).

The proportion of *Potamogeton pectinatus* was higher early in the season, and then decreased as *C. demersum* and *P. zosteriformis* became dominant. Filter-feeders were present where there was a higher biomass of *P. pectinatus*. This is expected as *P. pectinatus* has a simple structure and less surface area, and therefore would offer less habitat for phytophilous species of zooplankton. *Potamogeton pectinatus* often occurred alone, and due to lower habitat complexity, samples with this macrophyte would contain a higher proportion of planktonic species as more of the sample would represent water column. In comparison, *P. zosteriformis*, and especially *C. demersum*, have much greater structural complexity and surface areas which favoured phytophilous species over planktonic species by offering more surface area for feeding. Similarly fewer planktonic species were found among dense macrophyte beds in Cresent Pond, Delta

Marsh (Hann 1999). The vectors for *C. demersum* and *P. zosteriformis* were similar, indicating that they had similar invertebrate communities. Hann (1999) also found that these species of submersed macrophyte supported similar compositions of microinvertebrates.

In summary, macrophyte biomass, predation from fish, and their interaction were important in determining the species composition of the zooplankton community throughout the season. Phytoplankton and epiphyton biomass were important in determining differences in species composition between treatments of fish and nutrient addition.

CHAPTER 5 - CONCLUSIONS

1) In 1996, nutrient addition to enclosures resulted in phytoplankton blooms, and thus turbid conditions. No response of invertebrates was detected to increased food availability (phytoplankton and epiphyton) most likely because their populations were decimated by planktivorous fish.

2) In 1997, nutrient addition did not result in turbid conditions via phytoplankton blooms, probably due to the higher biomass of submersed macrophytes present.

3) Exclusion of macrophytes alone did not affect phytoplankton biomass or invertebrate communities in the water column.

4) Fathead minnow addition resulted in decreased densities of microinvertebrates but not macroinvertebrates, and a small increase in phytoplankton due to decreased top-down control via the trophic cascade; however, phytoplankton response was too small to produce turbid conditions.

5) Submersed macrophytes did not provide an effective refuge for zooplankton from predation by fathead minnows. The lack of refuge may be a consequence of the high density of YOY fathead minnows in the enclosures or YOY can penetrate macrophyte beds.

6) Zooplankton community composition among the macrophytes was affected more by seasonal change in composition than by treatment effects, with planktonic species dominant early in the season and phytophilous species dominant later in the season.

These enclosure experiments are probably most applicable to sheltered areas of the marsh where wind and wave action are reduced. The sheltering effect of the enclosures caused decreased turbidity from lack of wind and wave action. The turbid

water state is more likely to occur in the marsh if nutrient loading increases due to the increased turbidity from wind action.

The results of this experiment may have been greatly affected by enclosure, thereby reducing the applicability of this study on the rest of the marsh. The enclosure experiment was designed to include as many components of the marsh ecosystem as possible to increase realism, and was much more realistic than laboratory experiments. To understand fully the effect of the treatments used on the marsh, large portions of the marsh would have to be closed off or isolated ponds used for experimentation, as was attempted in the Marsh Ecology Research Program (MERP) (Murkin et al. 2000). However, this introduces other logistic difficulties for sampling and monitoring of habitat quality.

One component of the two stable state model is the presence of a refuge for zooplankton among the macrophytes as a stabilizer of the clear water state. However, in this study, macrophytes did not provide a refuge for zooplankton against predation by planktivorous fish, most likely due to the presence of an anomolously high density of YOY fathead minnows. Macrophytes may not have had as strong a stabilizing effect when YOY planktivorous fish were present, due to the possible ineffectiveness of the refuge under these conditions. The effectiveness of the refuge is probably dependent on the density, species, feeding behaviour, and size of the YOY planktivores present.

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APPENDIX 1

Table A-1. Mean water temperature (C° ± SE) at 10 cm depth throughout the treatment period in control and nutrient addition in 1996.

Date	Control	Nutrient addition
July 11	19 ± 0.0	19.1 ± 0.1
July 18	22.5 ± 0.0	22.5 ± 0.0
July 25	19.7 ± 0.1	19.8 ± 0.1
August 1	20.1 ± 0.1	20 ± 0.0
August 8	18 ± 0.0	17.9 ± 0.1
August 15	19.1 ± 0.1	19.1 ± 0.1
August 22	17.5 ± 0.0	17.6 ± 0.1

APPENDIX 2

Table A-2. Macrophyte biomass in control and nutrient treatment in 1996. (g/m^2 wetland bottom ± SE).

	June 17	July 15	Aug. 12	Aug. 27
Control	40 (± 30)	70 (± 36)	59 (± 9)	55 (± 11)
Nutrients	35 (± 34)	61 (± 43)	35 (± 10)	65 (± 0.2)

Table A-3. Mean water temperature (C° ± SE) at 10 cm depth throughout the treatment period in control, fathead and nutrient treatment in 1997.

Date	Control	Fathead Treatment	Nutrient Treatment
June 19	18.0 ± 0.0	18.0 ± 0.0	18.0 ± 0.0
June 25	19.5 ± 0.0	19.3 ± 0.2	19.5 ± 0.0
July 3	14.0 ± 0.0	14.2 ± 0.2	14.0 ± 0.0
July 11	22.5 ± 0.0	22.5 ± 0.0	22.5 ± 0.0
July 15	24.5 ± 0.0	24.3 ± 0.2	24.3 ± 0.3
July 24	23.7 ± 0.2	23.5 ± 0.0	23.5 ± 0.0
July 31	22.0 ± 0.0	22.0 ± 0.0	22.0 ± 0.0
August 7	23.0 ± 0.0	23.0 ± 0.0	23.0 ± 0.0
August 14	17.0 ± 0.0	17.0 ± 0.0	17.0 ± 0.0
August 21	18.5 ± 0.0	18.3 ± 0.2	18.3 ± 0.3
August 28	19.3 ± 0.2	19.5 ± 0.0	19.8 ± 0.3