RESPONSES OF A PRAIRIE WETLAND FOOD WEB TO ORGANOPHOSPHORUS INSECTICIDE APPLICATION AND INORGANIC NUTRIENT ENRICHMENT

LEANNE ZRUM

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

University of Manitoba

Winnipeg, Manitoba

© August, 2000

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
*****
COPYRIGHT PERMISSION PAGE

Responses of a Prairie Wetland Food Web to Organophosphorus Insecticide Application

and Inorganic Nutrient Enrichment

BY

Leanne Zrum

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University

of Manitoba in partial fulfillment of the requirements of the degree

of

Master of Science

LEANNE ZRUM © 2000

ABSTRACT

Grazer and microbial constituents of a prairie wetland food web were manipulated using mesocosms in Blind Channel, Delta Marsh, Canada. Lorsban™ 4E (active ingredient chlorpyrifos) was applied once to treatment enclosures at a concentration of 10 µg/L. Additions of inorganic nitrogen and phosphorus were made to treatment enclosures for the duration of the 10-week experimental period. Impacts of insecticide or nutrients on abundance of invertebrates (Cladocera, Cyclopoida and Calanoida Copepoda, Ostracoda, Rotifera, Insecta, Gastropoda, Amphipoda) and planktonic bacteria were limited, with relatively few significant density changes observed. In contrast, structure of invertebrate communities did change substantially in response to treatment. Differential mortality of arthropods resulted from chlorpyrifos addition; within the water column, calanoids were more tolerant than cladocerans and cyclopoids; associated with submersed macrophytes, calanoids and harpacticoid copepods were more tolerant than cladocerans, cyclopoids, and ostracods. An increase in the proportional abundance of planktonic rotifers, and macrophyte-associated rotifers and oligochaetes was observed after insecticide treatment. Nutrient enrichment did not substantially alter invertebrate community structure. Canonical correspondence analysis (CCA) was used to analyze the structure of the invertebrate communities at the species or group level. Percent cover of enclosure bottom by submersed macrophytes and alkalinity were the only significant variables in the CCA of the planktonic microinvertebrate community; 10 environmental variables in the CCA accounted for 90 % of the variance in the species data. Soluble reactive phosphorus was the only significant variable in the CCA for the macrophyte-associated microinvertebrate community; eight environmental variables in the CCA accounted for 89 % of the variance in the species data. Percent cover of enclosure bottom by submersed macrophytes and soluble reactive phosphorus were the only significant variables in the CCA of the macrophyte-associated macroinvertebrate community; eight environmental variables in the CCA accounted for 91 % of the variance in the taxa data.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: General Introduction

PROJECT BACKGROUND

Prairie lacustrine wetlands are shallow-water ecosystems typically existing for months to years in one of two states, a clear, macrophyte-dominated or a turbid, phytoplankton-dominated one. Conditions conducive to establishment and maintenance of these alternative states have been modeled (SCHEFFER et al. 1993) and investigated (SCHRIVER et al. 1995, MOSS et al. 1996, HANN & GOLDSBOROUGH 1997, McDOUGAL et al. 1997). Grazing and nutrient recycling by zooplankton and macrophyte-associated microinvertebrates are potential mechanisms for effecting control over primary producers in wetlands (VAN DONK et al. 1995, HANN & GOLDSBOROUGH 1997). Reduction of grazing pressure (e.g. insecticide application) or nutrient enrichment (e.g. fertilizers) may stimulate primary producers, thereby potentially altering the state of the shallow-water ecosystem.

Complex food web dynamics in freshwater prairie wetlands can be examined via manipulative experiments using *in situ* model ecosystems (mesocosms) that incorporate many aspects of the natural ecosystem and allow investigation of the ecological impact of contaminants potentially entering a wetland (GIDDINGS 1983, GEARING 1989). Structural (community composition and food web interactions) aspects of mesocosms exhibit both primary (direct) and secondary (indirect) effects of environmental perturbations. Survival, growth, or reproduction of aquatic organisms may exhibit primary effects due to direct, toxicological effects of a contaminant. Secondary effects follow and result from the reduction or elimination of contaminant-susceptible species (HURLBERT 1975), and are expected when direct toxicity to a contaminant results in reduction or removal of important grazers or predators that control community structure (BROCK & BUDDE 1994).

Application of insecticides and fertilizers for agricultural crop protection and enhancement results in increased pesticide contamination and nutrient

loading of wetlands adjacent to agricultural areas due to run-off, spray drift, leaching to surface and ground water, and accidental spills (NEELY & BAKER 1989, FRANK et al. 1990, RIJTEMA & KROES 1991, GOLDSBOROUGH & CRUMPTON 1998). These toxic chemicals and additional nutrients are known to affect the biotic communities of freshwater wetlands (BROCK et al. 1992a, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996, HANN & GOLDSBOROUGH 1997, MCDOUGAL et al. 1997).

A project was designed to investigate the invertebrate-algal-submersed aquatic macrophyte interactions in experimental enclosures (mesocosms) situated in a freshwater, prairie wetland. Manipulation of the primary producer-consumer interaction by differential elimination of the arthropod-grazer component through the application of an organophosphorus insecticide or by providing the primary producers with an additional source of nutrients (nitrogen and phosphorus) may provide insight into the environmental problems associated with agricultural practices in Canada. The invertebrate communities investigated occupied two different habitats: the planktonic microinvertebrate community living within the water column; and the microinvertebrate and macroinvertebrate communities living in association with submersed macrophytes. The microinvertebrates considered in this study included the following groups: (1) Cladocera, Cyclopoida and Calanoida Copepoda, and Ostracoda (arthropod filter-feeders, grazers, and predators); and (2) Rotifera and Oligochaeta (particularly, *Stylaria*) (non-arthropod grazers and detritivores). The macroinvertebrates considered included the following groups: (1) Insecta with aquatic immature life stages (arthropod grazers and predators); (2) Gastropoda (particularly, *Gyraulus* and *Physa*) (non-arthropod grazers); (3) Oligochaeta (particularly, *Chaetogaster* and *Stylaria*) (non-arthropod grazers, detritivores, and predators); and (4) Amphipoda (particularly, *Hyalella*) (arthropod grazers and detritivores). A variety of algal communities exist in a prairie wetland; the communities monitored during this study were the phytoplankton (algae entrained in the water column) and the epiphyton (algae attached to submersed

macrophytes). Submersed macrophyte community composition and biomass was evaluated throughout the course of the study. A preliminary investigation of the planktonic bacteria was also conducted.

Diverse responses by the communities described above are expected due to either organophosphorus insecticide application or inorganic nutrient enrichment. Addition of the insecticide, chlorpyrifos, results in differential mortality of the arthropod component in the invertebrate community (BROCK et al. 1992a, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996). Lorsban™ 4E (active ingredient, chlorpyrifos) is a broad spectrum organophosphorus insecticide registered in Canada for control of mosquito larvae and agricultural pests. Organophosphorus insecticides remain a popular choice because they are usually non-persistent in the environment and they do not bioaccumulate (RACKE 1993). Chlorpyrifos is known to be toxic to a range of aquatic organisms (invertebrates and vertebrates) to varying degrees (MARSHALL & ROBERTS 1978). Acute toxicity to vertebrates and invertebrates is primarily through the inhibition of the enzyme acetylcholinesterase in cholinergic synapses and neuromuscular junctions. Blocking of this enzyme results in the accumulation of the neural transmitter acetycholine, causing the disruption of normal transmission of nerve impulses, leading to death (MARSHALL & ROBERTS 1978).

Through the use of enclosures, conditions can be controlled to an extent and the consequent effects of experimental perturbations on one or more trophic levels may be investigated. However, it is critical to realize that enclosure of portions of the wetland led to physical, chemical, and biological conditions that differed from those of the unenclosed system (GOLDSBOROUGH & HANN 1996). Mesocosms are smaller than the natural system they are intended to represent, have reduced spatial and biological complexity, and contain walls that restrict exchange and provide substrata for attached organisms (e.g., algae, freshwater sponges) (PETERSEN et al. 1999). Results from manipulative enclosure experiments should only be extrapolated to the natural wetland with recognition of potential limitations due to enclosure effects (GOLDSBOROUGH & HANN 1996).

Relative to a system's natural variability, the ability to detect responses to an experimental perturbation will increase as the impact of the manipulation increases (FROST et al. 1988). However, levels of experimental perturbations are also selected in an attempt to maintain realism and sensitivity for "real world" problem solving. Experimental manipulations for this study were chosen to be representative of the level of impact that could be expected to occur under normal conditions. BROCK et al. (1992) chose a level of chlorpyrifos contamination that could be expected under a "worst case scenario" in drainage ditches adjacent to agricultural land (chlorpyrifos concentration of 35 μg/L). A level of 10 μg/L was chosen for this study as it was felt to be more representative of the degree of contamination possible under normal circumstances, but would still provide a large enough manipulation to be able to detect a response beyond the natural variability within the system (i.e., be able to detect the "signal" or response among the "noise"). Application of chlorpyrifos was made once, as would likely occur under normal agricultural practices. Nitrogen and phosphorus were added as a "press" application to the experimental system at twice the inorganic nutrient loading (HANN & GOLDSBOROUGH 1997) or equivalent nutrient loading of waterfowl feces (PETTIGREW et al. 1998) used in previous enclosure experiments in Blind Channel, Delta Marsh to produce continuous, low dose loading similar to what may be expected from overland or ground water inputs. Nutrient enrichment of the enclosures in Blind Channel at these previous levels has not produced responses detectable among the natural variability within the enclosure system.

Grazers, especially cladocerans, have been shown to be pivotal in influencing the state of shallow-water ecosystems (REYNOLDS 1994). Cladocerans and cyclopoid copepods are known to be more sensitive to chlorpyrifos than calanoid copepods (HURLBERT et al. 1970, HURLBERT et al. 1972, HURLBERT 1975, VAN DEN BRINK et al. 1995). Numbers of small rotifers tend to increase after chlorpyrifos addition (HURLBERT et al. 1972, BROCK et al. 1992a, VAN DONK et al. 1995). Differential mortality of arthropods should

increase primary producer biomass, as grazing pressure is reduced. Increases in phytoplankton and/or epiphyton in freshwater ecosystems have been observed as a result of insecticide application (HURLBERT et al. 1972, HURLBERT 1975, BROCK & BUDDE 1994).

Enrichment with inorganic nitrogen and phosphorus of indoor, freshwater microcosms (VAN DONK et al. 1995), experimental wetland enclosures (MCDOUGAL et al. 1997), and nutrient-poor (oligotrophic) wetlands (GABOR et al. 1994, MURKIN et al. 1994) enhances primary production. Total quantity of primary production, species composition, palatability, particle size, and manageability, determines the availability of resources for grazers (HANN & GOLDSBOROUGH 1997). GABOR et al. (1994) observed an increase in abundance of planktonic invertebrates in response to a single, high dose inorganic nutrient addition to an oligotrophic marsh. In contrast, MURKIN et al. (1994) did not observe any positive invertebrate response attributable to periodic, low dose inorganic nutrient additions to the same marsh. Invertebrate grazers increased in density in response to several low dose and two high dose inorganic nutrient additions in experimental wetland enclosures (HANN & GOLDSBOROUGH 1997) and indoor microcosms (VAN DONK et al. 1995). An increase in invertebrate grazers, especially cladocerans, in response to enhanced primary production may help stabilize the macrophyte-dominated, clear-water state.

Blind Channel in Delta Marsh typifies one of the two states frequently found in shallow-water ecosystems (SCHEFFER et al. 1993). It is characterized by high turbidity and phytoplankton biomass and a community proportionately dominated by copepods (particularly cyclopoids) throughout the open water season (HANN & ZRUM 1997). Experimental enclosure of sections of Blind Channel decreases turbidity in the water column by reducing resuspension of bottom sediments caused by wind and large, bottom-feeding detritivorous fish (e.g. Carp, *Cyprinus carpio*). Reduction of turbidity increases light available for submersed macrophyte growth and may permit earlier germination and

establishment of submersed macrophytes in the enclosures in comparison with Blind Channel (GOLDSBOROUGH & HANN 1996).

The overall aim of this project was to gain insight into the structure and functioning of *in situ* experimental enclosures existing in the clear water, macrophyte-dominated state by investigating their response to, and potential recovery from, controlled perturbations.

METHODS

Study site and mesocosms

The project was conducted from May to August, 1997 in Delta Marsh (MB, Canada), a 22,000 ha freshwater lacustrine wetland (98° 23' W, 50° 11' N) in south central Manitoba, bordered to the south by fertile agricultural land and aspen parkland, and separated from Lake Manitoba by a forested beach ridge (Fig. 1-1).

Experimental enclosures (mesocosms) used in this project represent the freshwater wetland communitues characteristic of the study site under investigation. Enclosures (12, 5 m x 5 m) were installed in Blind Channel on 27 May, 1997, at water depth of < 1 m. Each enclosure was constructed using impermeable woven polyethylene curtain supported on floating platforms (Fig.1-2). Curtains extended from above the water surface down to the sediments, where they were anchored with iron bars ~ 30 cm into the sediments, thereby preventing direct exchange of water between the enclosures and Blind Channel. Total volume of water per enclosure was approximately 22,000-25,000 L.

Experimental design

Experimental treatments (insecticide application, inorganic nutrient enrichment, control) were assigned to enclosures using a restricted latin square design, ensuring none of the three replicate enclosures for each treatment was adjacent
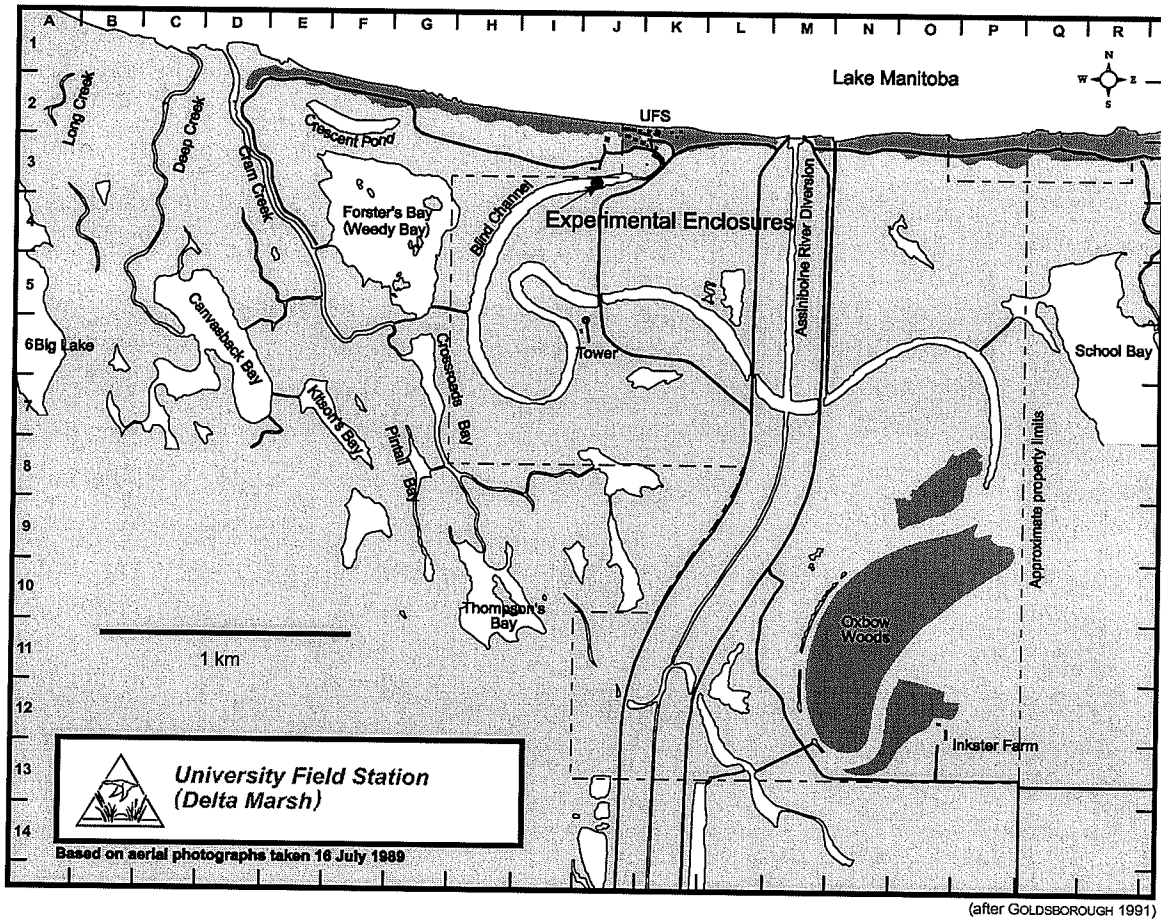
Fig. 1-1. Location of experimental enclosures in Blind Channel, Delta Marsh, 1997.

or contiguous with another (Fig. 1-3). Three additional enclosures were part of another experiment not presented with this study. Sampling of the planktonic components was initiated on 9 June, 1997, and continued weekly until 28 August, 1997. Weeks 1-2 constituted a pre-treatment period, followed by 10 weeks of treatment. Sampling of the components associated with submersed macrophytes was initiated on 9 July, 1997, the earliest date on which macrophytes could be physically sampled from the water surface, and continued weekly until 26 August, 1997.

Application of chlorpyrifos and nutrients

Insecticide applied was in the form of Lorsban™ 4E, an emulsifiable formulation with 41 % (w/w) chlorpyrifos as the active ingredient. Chlorpyrifos addition was made once on 14 July, 1997, to produce a nominal concentration of 10 μg/L in the water column. Inorganic nitrogen (as analytical grade $NaNO_3$) and phosphorus (as $NaH_2PO_4 \cdot 2H_2O$) were added to nutrient enrichment enclosures three times per week beginning on 23 June, 1997. Equal cumulative N and P loads (23.4 and 3.2 g/m^2 of wetland bottom, respectively) were added to each nutrient treatment enclosure by the end of the experiment. Water sampling for chlorpyrifos and physico-chemical analyses in the enclosures are described in CHAPTER 2 and ZRUM et al. (2000). See APPENDIX 1 for detailed chlorpyrifos sampling and analysis method.

Sampling and analysis of biotic communities
Communities in the water column

Three quantitative, depth integrated water column samples (4 L) were taken randomly from each enclosure weekly and filtered through a 53 μm mesh to determine densities (ind./L) of microinvertebrates (see HANN & GOLDSBOROUGH 1997 for method). A quantitative water column sample (1 L) was collected from three randomly selected positions in each enclosure weekly to provide an
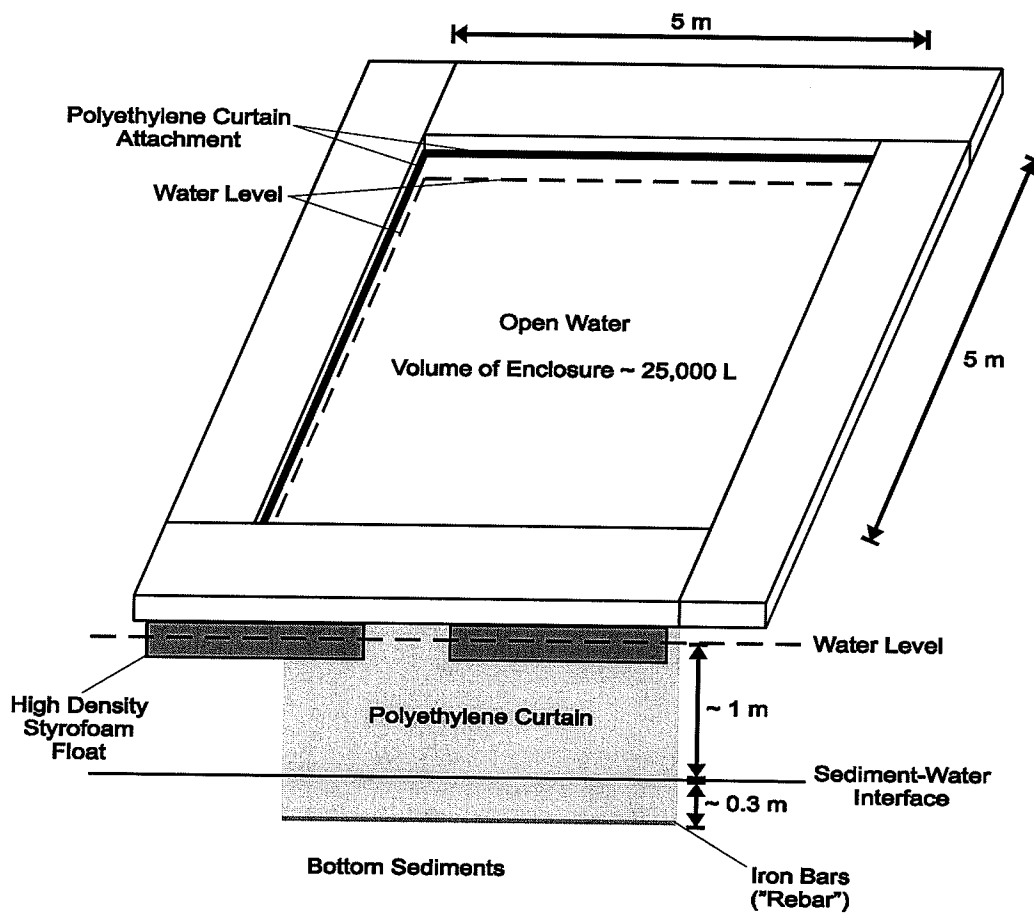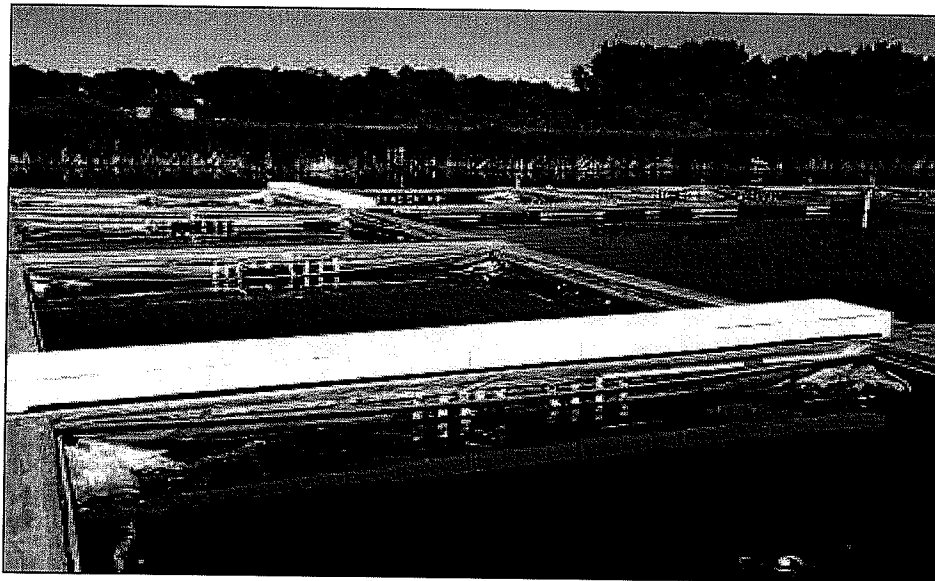
Fig. 1-2. Experimental enclosures used to model freshwater wetland communities characteristic of study site under investigation.
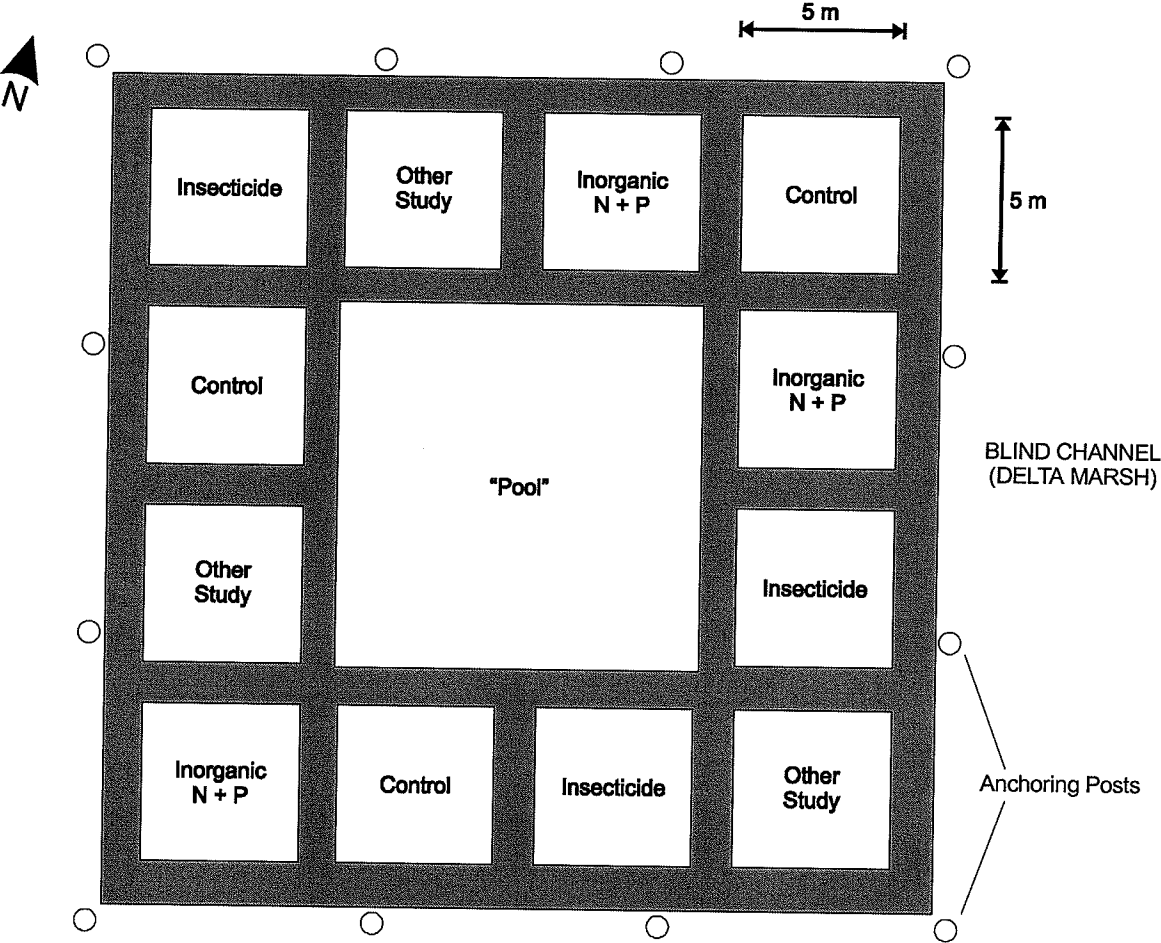
Fig. 1-3. Experimental treatments (insecticide application, inorganic nutrient enrichment, control) assigned to enclosures.

indicator of phytoplankton biomass (as chlorophyll *a*) (see MCDOUGAL et al. 1997 for method).

Microinvertebrates were identified to species using standard references, including EDMONDSON (1959), PENNAK (1978), and SMITH & FERNANDO (1978), and a reference collection (B. J. HANN). Cladocera were identified to species and enumerated. Copepoda were enumerated as nauplii, Cyclopoida and Calanoida copepodites, and Cyclopoida and Calanoida adults; only adults were identified to species. Among planktonic rotifers, only the predatory rotifer, *Asplanchna* was counted separately.

Two quantitative, depth integrated water column samples (1-1.2 L) were taken randomly from each enclosure weekly. For estimation of bacterial density (ind./mL), a 10 mL sub-sample was transferred to an acid-washed (or autoclaved) Vacutainer tube (20 mL). Within 3h of collection, 1 mL of 4 % buffered formalin was added to each sample and all were stored at 4 °C until filtration. Typically, bacteria samples were filtered within 24-72h of collection and fixation. Total bacteria were enumerated by direct count using epifluorescence microscopy (Zeiss microscope, fitted with a mercury lamp and an excitation filter set of 365 nm and 480 nm) after staining with Hoechst 33342, following the procedure of PORTER & FEIG (1980) for DAPI. Hoechst and DAPI are nucleic acid stains. When excited with the proper wavelength of light, the stain-DNA complex fluoresces bright blue, chlorophyll-bound stain fluoresces red, and unbound stain fluoresces yellow. Detailed method is presented in APPENDIX 2.

Communities associated with submersed macrophytes

Many methods have been devised for sampling invertebrates in shallow water habitats (reviewed in DOWNING 1984). A Downing Box was the optimal choice for sampling among submersed macrophytes in the shallow water of the enclosures as it permitted the simultaneous quantitative collection of phytophilous invertebrates (both microinvertebrates and macroinvertebrates), epiphyton associated with submersed macrophytes, and the macrophytes themselves.

Invertebrates and epiphyton associated with submersed aquatic macrophytes were sampled quantitatively using a Downing Box (6 L) of a design from DOWNING (1986) (Fig. 1-4). Two Downing Box samples were taken randomly from each enclosure weekly, beginning on 9 July, 1997. A Downing Box is a sampling device, resembling a "suitcase", constructed of clear plexi-glass and is used to sample invertebrates, epiphyton, and submersed macrophytes in a combined sample. Sampling is restricted to the top 0.5 m of the submersed macrophytes due to the mode of operation of the device. See APPENDIX 3 for detailed Downing Box sampling method.

Densities (ind./L) of microinvertebrates and macroinvertebrates were determined. The fresh macrophytes were sorted to species and any invertebrates still attached were removed and placed in the corresponding sample vials. Macrophyte species were dried at 106 °C for 24 h and then massed to obtain dry weight data for each species. Epiphyton was analyzed for chlorophyll *a* using methods of McDougal et al. (1997) and expressed as micrograms chlorophyll *a* per gram total dry weight of macrophytes (μg/g).

Invertebrates were identified to using standard references, including EDMONDSON (1959), PENNAK (1978), SMITH & FERNANDO (1978), and MERRITT & CUMMINS (1996), and reference collections (B. J. HANN and K. A. SANDILANDS). Cladocera were identified to species and enumerated. Copepoda were enumerated as nauplii, Cyclopoida and Calanoida copepodites, and Cyclopoida and Calanoida adults; only adults were identified to species. Among rotifers, only the predatory species, *Asplanchna* was counted separately. All macroinvertebrates were identified to order and counted, with only select taxa identified to genus.

Percent cover of enclosure bottom by submersed macrophyte species (*Potamogeton zosteriformis, P. pectinatus, Ceratophyllum demersum, Myriophyllum sibiricum*) was estimated by visual inspection each week. Submersed macrophyte biomass (as g/m^2 of wetland bottom) was measured on 16 June, 10 July, and 13 August, 1997, in each enclosure using a large plastic

cylinder (d = 0.78 m; A = 0.48 m^2). The cylinder was lowered into an enclosure and macrophytes contained within it were sheared at the sediment-water interface. Macrophyte material was sorted to species, dried at 106 °C for 24h, and then massed to obtain dry weight data for each species.

Data analysis

Specific data analysis methods (univariate and multivariate) for each component investigated are presented in the chapters to follow.

OBJECTIVES OF THE PROJECT

The experiment was designed to investigate community structure and dynamics of *in situ* mesocoms existing in the clear water, macrophyte-dominated state subject to organophosphorus insecticide application and inorganic nutrient enrichment. Specifically, the following components were examined:

1) **the response of the planktonic community to the following experimental manipulations (see CHAPTERS 2 and 5 for detailed investigation);**

   a) *direct effects* on the community structure of the arthropod component of the microinvertebrates induced by differential mortality caused by a single application of the organophosphorus insecticide chlorpyrifos, and *indirect effects* resulting from nutrient enrichment;

   b) *direct effects* on phytoplankton biomass induced by nutrient enrichment via small, periodic additions of inorganic nitrogen and phosphorus, and *indirect effects* resulting from chlorpyrifos application; and

   c) *direct effects* on bacterioplankton density induced by nutrient enrichment via small, periodic additions of inorganic nitrogen and phosphorus, and *indirect effects* resulting from chlorpyrifos application.

Submersed Macrophytes

Hinges

Clear, Plexiglass "Suitcase"

Neoprene Seal

Sample Port with Mesh

Clasp

Outlet Tube

(after DOWNING 1986)
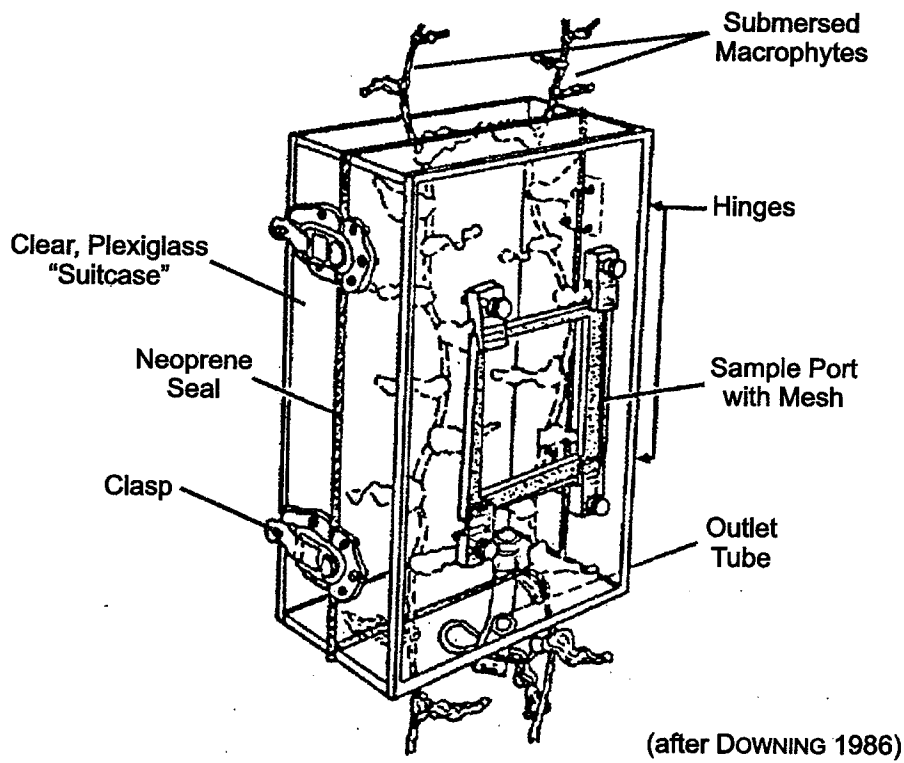
Fig. 1-4. Downing Box used to sample invertebrates and epiphyton associated with submersed aquatic macrophytes.

2) **the response of the community associated with submersed aquatic macrophytes to the following experimental manipulations (see CHAPTER 3 for detailed investigation);**

   a) *direct effects* on the community structure of the arthropod component of the microinvertebrates induced by differential mortality caused by a single application of chlorpyrifos, and *indirect effects* resulting from nutrient enrichment;

   b) *direct effects* on the community structure of the arthropod component of the macroinvertebrates induced by differential mortality caused by a single application of chlorpyrifos, and *indirect effects* resulting from nutrient enrichment; and

   c) *direct effects* on epiphyton biomass induced by nutrient enrichment via small, periodic additions of inorganic nitrogen and phosphorus, and *indirect effects* resulting from chlorpyrifos application.

HYPOTHESES

The hypotheses for the May to August, 1997, experimental period were as follows:

Organophosphorus insecticide application

Direct effects

- Planktonic arthropod microinvertebrates will decrease in density, due to mortality, in enclosures treated with insecticide in comparison to control enclosures.

- Arthropod microinvertebrates and macroinvertebrates associated with submersed macrophytes will decrease in density, due to mortality, in enclosures treated with insecticide in comparison to control enclosures.

Indirect effects

- An increase in biomass of primary producers (submersed macrophyte, epiphyton, phytoplankton) and an increase in planktonic bacterial density will be observed in treatment enclosures due to a reduction in herbivorous arthropod grazing.

- Planktonic non-arthropod microinvertebrates will increase in density and non-arthropod microinvertebrates and macroinvertebrates among submersed macrophytes will increase in density in treatment enclosures due to a reduction in competition with arthropods for food resources and arthropod predation.

- Planktonic non-arthropod microinvertebrates will increase in density in treatment enclosures if phytoplankton biomass and planktonic bacterial density increases.

- Non-arthropod microinvertebrates and macroinvertebrates associated with submersed macrophytes will increase in density in treatment enclosures if aquatic submersed macrophyte or epiphyton biomass increases.

Inorganic nutrient enrichment
Direct effects

- Nutrient addition will stimulate an increase in biomass of primary producers in the treatment enclosures in comparison to control enclosures; this may be observed as an increase in aquatic submersed macrophyte, epiphyton, or phytoplankton biomass. An increase in epiphyton biomass may be accompanied by a reduction in macrophyte biomass.

- Nutrient addition will produce an increase in the density of planktonic bacteria in the treatment enclosures in comparison to control enclosures.

Indirect effects

- Planktonic microinvertebrates will increase in density in treatment enclosures if phytoplankton biomass and/or planktonic bacterial density increases.
- Microinvertebrates and macroinvertebrates associated with submersed macrophytes will increase in density in treatment enclosures if submersed macrophyte or epiphyton biomass increases.

EXPECTED RESULTS OF EXPERIMENTAL PERTURBATIONS

The expected results from the May to August, 1997, experimental period were as follows:

All enclosures during the pre-treatment sampling period

1. The enclosures will have a sheltering effect, resulting in a decrease in turbidity relative to the Blind Channel water (GOLDSBOROUGH & HANN 1996).
2. Reduction of turbidity increases light available for submersed macrophyte growth (GOLDSBOROUGH & HANN 1996) and may permit earlier germination and establishment of submersed macrophytes in the enclosures in comparison with Blind Channel.
3. An initial peak in density of planktonic microinvertebrates will be observed in response to an exclusion of fish predators from the enclosures (HANN & GOLDSBOROUGH 1997, PETTIGREW et al. 1998).

Enclosures with organophosphorus insecticide application

1. The density of planktonic arthropod microinvertebrates and arthropod microinvertebrates and macroinvertebrates associated with submersed macrophytes will be lower in insecticide treatment enclosures than in control replicates (BROCK et al. 1992a, BROCK et al. 1992b, BROCK et al. 1995, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996); the toxic effects of the insecticide may be delayed if abundant macrophyte growth is present at the time of insecticide application due to vegetation adsorbing a large proportion

of the dose applied and hampering the mixing of the insecticide in the water (BROCK et al. 1992a).

2. The density of planktonic non-arthropod microinvertebrates and non-arthropod microinvertebrates and macroinvertebrates associated with submersed macrophytes will be higher in insecticide treatment enclosures than in control replicates (BROCK et al. 1992a, BROCK et al. 1992b, BROCK et al. 1995, VAN DEN BRINK et al. 1996).

3. The density of planktonic non-arthropod microinvertebrates will be higher than the density of planktonic arthropod microinvertebrates and the density of non-arthropod microinvertebrates and macroinvertebrates associated with submersed macrophytes will be higher than the density of arthropod microinvertebrates and macroinvertebrates associated with submersed macrophytes in insecticide treatment replicates (BROCK et al. 1992b, BROCK et al. 1995, VAN DEN BRINK et al. 1996).

4. Among arthropods, the following rank ordered response to insecticide treatment (increasing tolerance) will be observed: Cladocera < Cyclopoida Copepods < Calanoida Copepods < Predatory Macroinvertebrates < Herbivorous Macroinvertebrates. This tolerance sequence has been observed in previous field experiments using chlorpyrifos in shallow-water systems (HURLBERT et al. 1970, HURLBERT et al. 1972, HUGHES et al. 1980).

5. Recovery of arthropod microinvertebrates is expected to begin within two weeks of insecticide addition to the replicates, as was observed in another shallow-water system (HURLBERT et al. 1970).

6. Copepoda nauplii will show a more rapid decline and a somewhat more rapid recovery in density than more mature life stages (copepodites, adults) of Copepoda in the insecticide treatment replicates (BROCK et al. 1992a).

7. With macrophytes present, larger-sized Cladocera are expected to recover earlier than smaller-sized Cladocera (BROCK et al. 1992a).

8. After insecticide treatment, the biomass of primary producers (submersed macrophytes, epiphyton, phytoplankton) will increase relative to the biomass

of primary producers in control replicates (BROCK et al. 1992b, BROCK et al. 1995, VAN DONK et al. 1995); a prolific increase in epiphyton biomass in July may result in a corresponding reduction in submersed macrophyte biomass due to shading (KERSTING & VAN DEN BRINK 1997).

9. An increase in the density of planktonic bacteria will be observed in insecticide treatment replicates due to a reduction in grazing pressure (PORTER et al. 1979, SANDERS et al. 1989).

Enclosures with inorganic nutrient enrichment

If a clear water state occurs

1. The growth of submersed macrophytes up through the water column of nutrient treatment enclosures will result in a shift in biomass in the primary producers from phytoplankton to epiphyton (McDOUGAL et al. 1997).

2. The biomass of primary producers (submersed macrophytes and epiphyton) will be higher than in control replicates (BROCK et al. 1995, McDOUGAL et al. 1997).

3. The density of microinvertebrate and macroinvertebrate grazers in association with submersed macrophytes will be higher in nutrient treatment enclosures when epiphyton is the dominant primary producer (VAN DONK et al. 1995, HANN & GOLDSBOROUGH 1997).

4. The growth of sumbersed macrophytes up through the water column of nutrient treatment enclosures will result in a reduction in the density of planktonic bacteria as a shift in biomass in the primary producers from phytoplankton to epiphyton occurs (SONDERGAARD et al. 1998).

If a turbid water state occurs

1. Phytoplankton biomass will increase in nutrient treatment enclosures if submersed macrophyte biomass remains low, i.e., coverage of the bottom sediments by macrophytes is sparse.

2. The biomass of phytoplankton will be higher than in control replicates (BROCK et al. 1995, McDOUGAL et al. 1997).

3. The density of planktonic microinvertebrates will be higher in nutrient treatment enclosures when phytoplankton is the dominant primary producer (HANN & GOLDSBOROUGH 1997).

4. The density of planktonic bacteria will be higher than in control replicates (WAISER & ROBARTS 1997).

REFERENCES

BROCK, T. C. M., CRUM, S. J. H., VAN WIJNGAARDEN, R., BUDDE, B. J., TIJINK, J., ZUPPELLI, A. & LEEUWANGH, P. (1992a): Fate and effects of the insecticide Dursban® 4E in indoor *Elodea*-dominated and macrophyte-free freshwater model ecosystems: I. Fate and primary effects of the active ingredient chlorpyrifos. - Arch. Environ. Contam. Toxicol. **23:** 69-84.

BROCK, T. C. M., VAN DEN BOGAERT, M., BOS, A. R., VAN BREUKELEN, S. W. F., REICHE, R., TERWOERT, J., SUYKERBUYK, R. E. M. & ROIJACKERS, R. M. M. (1992b): Fate and effects of the insecticide Dursban® 4E in indoor *Elodea*-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. - Arch. Environ. Contam. Toxicol. **23:** 391-409.

BROCK, T. C. M. & BUDDE, B. J. (1994): On the choice of structural parameters and endpoints to indicate responses of freshwater ecosystems to pesticide stress. - In: HILL, I. A., HEIMBACK, F., LEEUWANGH, P. & MATTHIESEN, P. (eds.): Freshwater Field Tests For Hazard Assessment of Chemicals. - Lewis Publishers, Michigan, pp. 19-56.

BROCK, T. C. M., ROIJACKERS, R. M. M., ROLLON, R., BRANSEN, F. & VAN DER HEYDEN, L. (1995): Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms: II. Responses of macrophytes, periphyton and macroinvertebrate grazers. - Arch. Hydrobiol. **134:** 53-74.

DOWNING, J. A. (1984): Sampling the benthos of standing water. - In: DOWNING, J. A. & RIGLER, F. H. (eds.): A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters. 2nd Ed. - Blackwell Scientific Publ., London, pp. 87-130.

DOWNING, J. A. (1986): A regression technique for the estimation of epiphytic invertebrate populations. - Freshwat. Biol. **16:** 161-173.

EDMONDSON, W. T. (ed.) (1959): Freshwater Biology. 2nd Ed. - John Wiley and Sons, New York, NY.

FRANK, R., BRAUN, H. E., RIPLEY, B. D. & CLEGG, B. S.. (1990): Contamination of rural ponds with pesticide, 1971-85, Ontario, Canada. - Bull. Environ. Contam. Toxicol. **44:** 401-409.

GABOR, T. S., MURKIN, H. R., STAINTON, M. P., BOUGHEN, J. A. & TITMAN, R. D. (1994): Nutrient additions to wetlands in the Interlake region of Manitoba, Canada: effects of a single pulse addition in spring. - Hydrobiologia **279/280:** 497-510.

GEARING, J. N. (1989): The role or aquatic microcosms in ecotoxicologic research as illustrated by large marine systems. - In: LEVIN, S. A., HARWELL, M. A., KELLY, J. R. & KIMBALL, K. D. (eds.): Ecotoxicology: Problems and Approaches. - Springer-Verlag, New York, pp. 411-470.

GIDDINGS, J. M. (1983): Microcosms for assessment of chemical effects on the properties of aquatic ecosystems. - In: SAXENA, J. (ed.): Hazard Assessment of Chemicals: Current Developments. Vol. 2. - Academic Press Inc., New York, NY, pp. 45-94.

GOLDSBOROUGH, L. G. (1991): University Field Station, Delta Marsh: based on aerial photographs taken 16 July, 1989. On-line at: http://www.umanitoba.ca/faculties/ science/delta_marsh

GOLDSBOROUGH, L. G. & HANN, B. J. (1996): Enclosure affects trophic structure of a freshwater prairie wetland. - University Field Station (Delta Marsh) Annual Report **30**: 63-67. On-line at: http://www.umanitoba.ca/faculties/ science/delta_marsh

GOLDSBOROUGH, L. G. & CRUMPTON, W. G. (1998): Distribution and environmental fate of pesticides in prairie wetlands. - Great Plains Research **8**: 73-96.

HANN, B. J. & GOLDSBOROUGH, L. G. (1997): Responses of a prairie wetland to press and pulse additions of inorganic nitrogen and phosphorus: invertebrate community structure and interactions. - Arch. Hydrobiol. **140**: 169-194.

HANN, B. J. & ZRUM, L. (1997): Littoral microcrustaceans (Cladocera, Copepoda) in a prairie coastal wetland: seasonal abundance and community structure. - Hydrobiologia **357**: 37-52.

HUGHES, D. N., BOYER, M. G., PAPST, M. H., FOWLE, C. D., REES, G. A. V. & BAULU, P. (1980): Persistence of three organophosphorus insecticides in artificial ponds and some biological implications. - Arch. Environ. Contam. Toxicol. **9**: 269-279.

HURLBERT, S. H. (1975): Secondary effects of pesticides on aquatic ecosystems. - Res. Rev. **57**: 81-148.

HURLBERT, S. H., MULLA, M. S., KEITH, J. O., WESTLAKE, W. E. & DÜSCH, M. E. (1970): Biological effects and persistence of Dursban® in freshwater ponds. - J. Econ. Entomol. **63:** 43-52.

HURLBERT, S. H., MULLA, M. S. & WILSON, H. R. (1972): Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations of fresh-water ponds. - Ecol. Monogr. **42:** 269-299.

KERSTING, K. & VAN DEN BRINK, P. J. (1997): Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: responses of ecosystem metabolism. - Environ. Toxicol. Chem. **16:** 251-259.

MARSHALL, W. K. & ROBERTS, J. R. (1978): Ecotoxicology of chlorpyrifos. - Environmental Secretariat N.R.C.C. Ottawa, Canada, Na**16079**.

McDOUGAL, R. L., GOLDSBOROUGH, L. G. & HANN, B. J. (1997): Responses of a prairie wetland to press and pulse additions of inorganic nitrogen and phosphorus: production by planktonic and benthic algae. - Arch. Hydrobiol. **140:** 145-167.

MERRITT, R. W. & CUMMINS, K. W. (eds.) (1996): An Introduction to the Aquatic Insects of North America. 2nd Ed. - Kendall/Hunt Publishing Company, Dubuque, Iowa.

MOSS, B., STANSFIELD, J., IRVINE, K., PERROW, M. & PHILLIPS, G. (1996): Progressive restoration of a shallow lake: a 12-year experiment in isolation, sediment removal and biomanipulation. - J. Appl. Ecol. **33:** 71-86.

MURKIN, H. R., POLLARD, J. B., STAINTON, M. P., BOUGHEN, J. A. & TITMAN, R. D. (1994): Nutrient additions to wetlands in the interlake region of Manitoba, Canada: effects of periodic additions throughout the growing season. - Hydrobiol. **280:** 483-495.

NEELY, R. K. & BAKER, J. L. (1989): Nitrogen and phosphorus dynamics and the fate of agricultural runoff. - In: VAN DER VALK, A. (ed.): Northern Prairie Wetlands. - Iowa State Univ. Press, Ames, Iowa, pp. 92-131.

PENNAK, R. W. (1978): Freshwater Invertebrates of the United States. 2nd Ed. - John Wiley and Sons, New York, NY.

PETTIGREW, C. T., HANN, B. J. & GOLDSBOROUGH, L. G. (1998): Waterfowl feces as a source of nutrients to a prairie wetland: responses of microinvertebrates to experimental additions. - Hydrobiologia **362:** 55-66.

PORTER, K. G., PACE, M. L. & BATTEY, F. J. (1979): Ciliate protozoans as links in freshwater planktonic food chains. - Nature **277:** 563-565.

PORTER, K. G. & FEIG, Y. S. (1980): The use of DAPI for identifying and counting aquatic microflora. - Limnol. Oceanogr. **25:** 943-948.

RACKE, K. D. (1993): Environmental fate of chlorpyrifos. - Rev. Environ. Contam. Toxicol. **131:** 1-154.

REYNOLDS, C. S. (1994): The ecological basis for the successful biomanipulation of aquatic communities. - Arch. Hydrobiol. **130:** 1-33.

RIJTEMA, P. E. & KROES, J. G. (1991): Nitrogen modeling on a regional scale. - In: BOGARDI, I. & KUZELKA, R. D. (eds.): Nitrate Contamination. - NATO ASI Series, Vol. G **30**, Springer, Berlin, Heidelberg, pp. 81-95.

SANDERS, R. W., PORTER, K. G., BENNETT, S. J. & DEBIASE, A. E. (1989): Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. - Limnol. Oceanogr. **34**: 673-687.

SCHEFFER, M., HOSPER, S. H., MEIJER, M. - L., MOSS, B. & JEPPESEN, E. (1993): Alternative equilibria in shallow lakes. - Trends in Ecology and Evolution **8**: 275-279.

SCHRIVER, P., BOGESTRAND, J., JEPPESON, E. & SONDERGAARD, M. (1995): Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions: large-scale enclosure experiments in a shallow eutrophic lake. - Freshwat. Biol. **33**: 255-270.

SMITH, K. & FERNANDO, C. H. (1978): A guide to the freshwater calanoid and cyclopoid Copepod Crustacea of Ontario. - Univ. Waterloo Biol. Ser. No. **18**: 74 pp.

SONDERGAARD, M., THEIL-NIELSEN, J., CHRISTOFFERSEN, K., SCHLUTER, L., JEPPESEN, E. & SONDERGAARD, MA. (1998): Bacterioplankton and carbon turnover in a dense macrophyte canopy. - In: JEPPESEN, E., SONDERGAARD, Ma., SONDERGAARD, Mo. & CHRISTOFFERSEN, K. (eds.): The Structuring Role of Submerged Macrophytes in Lakes. - Springer-Verlag, New York, NY, pp. 250-261.

VAN DEN BRINK, P. J., VAN WIJNGAARDEN, R. P. A., LUCASSEN, W. G. H., BROCK, T. C. M. & LEEUWANGH, P. (1996): Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. - Environ. Toxicol. Chem. **15**: 1143-1153.

VAN DONK, E., PRINS, H., VOOGD, H. M., CRUM, S. J. H. & BROCK, T. C. M. (1995): Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms: I. Responses of plankton and zooplanktivorous insects. - Arch. Hydrobiol. **133**: 417-439.

WAISER, M. J. & ROBARTS, R. D. (1997): Impacts of a herbicide and fertilizers on the microbial community of a saline prairie lake. – Can. J. Fish. Aquat. Sci. **54**: 320-329.

ZRUM, L., HANN, B. J., GOLDSBOROUGH, L. G. & STERN, G. A. (2000): Effects of organophosphorus insecticide and inorganic nutrients on the planktonic microinvertebrates and algae in a prairie wetland. - Arch. Hydrobiol. **147**: 373-399.

CHAPTER 2: Effects of organophosphorus insecticide and inorganic nutrients on the planktonic microinvertebrates and algae in a prairie wetland

INTRODUCTION

Shallow-water ecosystems typically exist in one of two stable states, a clear, macrophyte-dominated or a turbid, phytoplankton-dominated one. Conditions conducive to establishment and maintenance of these alternative stable states have been modeled (SCHEFFER et al. 1993) and investigated (SCHRIVER et al. 1995, MOSS et al. 1996, HANN & GOLDSBOROUGH 1997, MCDOUGAL et al. 1997). Grazing and nutrient recycling by zooplankton and macrophyte-associated microinvertebrates are potential mechanisms for effecting control over primary producers in wetlands (VAN DONK et al. 1995, HANN & GOLDSBOROUGH 1997). Reduction of grazing pressure (e.g. insecticide application) or nutrient enrichment (e.g. fertilizers) may stimulate primary producers, thereby potentially altering the stable state of the shallow-water ecosystem.

Complex food web dynamics in freshwater prairie wetlands can be simulated via manipulative experiments using *in situ* model ecosystems (mesocosms) that incorporate many aspects of the natural ecosystem and allow examination of the ecological impact of contaminants potentially entering a wetland (GIDDINGS 1983, GEARING 1989). Structural (community composition and food web interactions) aspects of mesocosms exhibit both primary (direct) and secondary (indirect) effects of environmental perturbations. Survival, growth, or reproduction of aquatic organisms may exhibit primary effects due to direct, toxicological effects of a contaminant. Secondary effects follow and result from a reduction or elimination of contaminant-susceptible species (HURLBERT 1975) and are expected when direct toxicity to a contaminant results in reduction or removal of important grazers or predators that control community structure (BROCK & BUDDE 1994).

Application of insecticides and fertilizers for agricultural crop protection and enhancement results in increased pesticide contamination and nutrient loading of wetlands adjacent to agricultural areas; effects are due to run-off, spray drift, leaching to surface and ground water, and accidental spills (NEELY & BAKER 1989, FRANK et al. 1990, RIJTEMA & KROES 1991, GOLDSBOROUGH & CRUMPTON 1998). These toxic chemicals and additional nutrients are known to affect the biotic communities of freshwater wetlands (BROCK et al. 1992a, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996, HANN & GOLDSBOROUGH 1997, MCDOUGAL et al. 1997).

Grazers, especially cladocerans, have been shown to be pivotal in influencing the stable state of shallow-water ecosystems (REYNOLDS 1994). Addition of the insecticide, chlorpyrifos, results in differential mortality of the arthropod component in the microinvertebrate community (BROCK et al. 1992a, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996). Cladocerans and cyclopoid copepods are more sensitive than calanoid copepods (HURLBERT et al. 1970, HURLBERT et al. 1972, HURLBERT 1975, VAN DEN BRINK et al. 1995). Numbers of small rotifers tend to increase after chlorpyrifos addition (HURLBERT et al. 1972, BROCK et al. 1992a, VAN DONK et al. 1995). Differential mortality of arthropods should increase primary producer biomass, as grazing pressure is reduced. Increases in phytoplankton and/or epiphyton in freshwater ecosystems have been observed as a result of insecticide application (HURLBERT et al. 1972, HURLBERT 1975, BROCK & BUDDE 1994).

Enrichment with inorganic nitrogen and phosphorus of indoor, freshwater microcosms (VAN DONK et al. 1995), experimental wetland enclosures (MCDOUGAL et al. 1997), and nutrient-poor (oligotrophic) wetlands (GABOR et al. 1994, MURKIN et al. 1994) enhances primary production. Total quantity of primary production, species composition, palatability, particle size, and manageability, determines the availability of resources for grazers (HANN & GOLDSBOROUGH 1997). GABOR et al. (1994) observed an increase in abundance of planktonic invertebrates in response to a single, high dose inorganic nutrient

addition to an oligotrophic marsh. In contrast, MURKIN et al. (1994) did not observe any positive invertebrate response attributable to periodic, low dose inorganic nutrient additions to the same marsh. HANN & GOLDSBOROUGH (1997) and VAN DONK et al. (1995) found an increase in invertebrate grazers in response to several low dose and two high dose inorganic nutrient additions in experimental wetland enclosures and indoor microcosms, respectively. An increase in invertebrate grazers, especially cladocerans, in response to enhanced primary production may help stabilize the macrophyte-dominated, clear-water stable state.

Blind Channel in Delta Marsh typifies one of the two stable states frequently found in shallow-water ecosystems (SCHEFFER et al. 1993). It is characterized by high turbidity and phytoplankton biomass and a community proportionately dominated by copepods (particularly cyclopoids) throughout the open-water season (HANN & ZRUM 1997). Experimental enclosure of sections of Blind Channel decreases turbidity in the water column by reducing resuspension of bottom sediments caused by wind and large, bottom-feeding detritivorous fish (e.g. Carp, *Cyprinus carpio*). Reduction of turbidity increases light available for submersed macrophyte growth and may permit earlier germination and establishment of submersed macrophytes in the enclosures in comparison with Blind Channel (GOLDSBOROUGH & HANN 1996).

This paper describes results of an experiment to investigate planktonic community structure and dynamics of a wetland ecosystem subject to organophosphorus insecticide application and inorganic nutrient enrichment. Specifically, we examined responses of the planktonic microinvertebrate community to the following experimental manipulations: 1) changes in the community structure of the arthropod component of the microinvertebrates induced by differential mortality caused by a single application of the organophosphorus insecticide Lorsban™ 4E (emulsifiable formulation with 41 % (w/w) chlorpyrifos as the active ingredient); and 2) changes in phytoplankton

biomass induced by nutrient enrichment via small, periodic additions of inorganic nitrogen and phosphorus.

Our objective was to examine spatial and temporal variation in structure of the planktonic microinvertebrate community in wetland mesocosms subjected to experimental perturbations. Insecticide (chlorpyrifos) treatment is expected to result in differential mortality of the arthropod component of the microinvertebrate community; specifically, loss of efficient cladoceran grazers should increase phytoplankton biomass as grazing pressure is reduced. Inorganic nutrient enrichment is expected to alter relative abundance of primary producers, from predominantly submersed macrophytes to phytoplankton.

METHODS

Study site and experimental design

Our study was conducted from May to August, 1997 in Delta Marsh, a 22,000 ha freshwater lacustrine wetland (98° 23'W, 50° 11'N) in south-central Manitoba, bordered to the south by fertile agricultural land and aspen parkland, and separated from Lake Manitoba to the north by a forested beach ridge.

Experimental enclosures (mesocosms) model the freshwater wetland community characteristic of the study site under investigation. Enclosures (12, 5 m x 5 m) were installed in Blind Channel on 27 May at a water depth of < 1 m. Each enclosure was constructed using impermeable woven polyethylene curtain supported on floating platforms. Curtains extended from above the water surface down to the sediments, where they were anchored with iron bars at least 30 cm into the sediments, thereby preventing direct exchange of water between the enclosures and Blind Channel. Enclosures were open on top to the atmosphere. Total volume of water per enclosure was approximately 22,000 L. Fish (primarily fathead minnows, *Pimephales promelas*) trapped during installation were removed using commercial minnow traps, monitored daily for the duration of the experiment.

Experimental treatments (insecticide addition, nutrient enrichment, control) were assigned to enclosures using a restricted latin square design, ensuring none of the three replicate enclosures for each treatment was adjacent or contiguous with another. Three additional enclosures were part of another experiment not presented with our study. Sampling was initiated on 9 June and continued weekly until 28 August. Weeks 1-2 constituted a pre-treatment period, followed by 10 weeks of treatment.

Application of chlorpyrifos and nutrients

Lorsban™ 4E insecticide is a broad spectrum organophosphorus insecticide manufactured by DowElanco and registered in Canada for control of agricultural pests. The active ingredient, chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate], has an anticholinesterase mode of action and is known to be toxic to a range of aquatic organisms (invertebrates and vertebrates, particularly fish) to varying degrees (MARSHALL & ROBERTS 1978).

Chlorpyrifos addition was made once on 14 July to produce a nominal concentration of 10 µg/L in the water column. Addition was delayed until mid-July to give submersed macrophytes (primarily *Ceratophyllum* sp. and *Potamogeton* spp.) sufficient time to germinate and become established in the experimental enclosure system. Experiments performed by BROCK et al. (1992a, 1992b) demonstrated that presence of macrophytes influences the fate and effects of chlorpyrifos, with the rate of chlorpyrifos disappearance in water with macrophytes being more rapid than in open-water systems; chlorpyrifos application to agricultural crops in Manitoba also typically occurs in late July-early August (RAWN 1998). Insecticide was emulsified in 250 mL of distilled water, then sprinkled uniformly over the water surface of each insecticide treatment enclosure mixed in approximately 20 L of carbon-filtered water. Application took place in the morning on a windless day to prevent spray drift into other enclosures.

Inorganic nitrogen (as analytical grade $NaNO_3$) and phosphorus (as $NaH_2PO_4 \cdot 2H_2O$) were added three times per week beginning on 23 June for the 10 week treatment period. Equal cumulative N and P loads (23.4 g/m^2 of wetland bottom and 3.2 g/m^2 of wetland bottom, respectively) were added to each nutrient treatment enclosure by the end of the experiment. Each nutrient addition was prepared by dissolving the chemicals in 1 L of carbon-filtered water, and then sprinkled uniformly over the enclosure water surface using approximately 10 L of enclosure water.

Water sampling for chlorpyrifos and physico-chemical analysis

Prior to insecticide addition, water samples were taken to provide measures of background concentrations of chlorpyrifos in the water column. Duplicate depth-integrated water column samples were collected from each insecticide treatment enclosure using a transparent acrylic cylinder (50 cm x 5.5 cm), filtered through a 150 μm mesh net to remove larger planktonic microinvertebrates, and combined in brown glass bottles for analysis (2 L total volume). After insecticide addition, six sets of water samples were collected from each insecticide treatment enclosure following the same protocol used for pre-addition samples. Samples were collected at 1, 12, 24, 36, 48, and 72 hours post-addition and transported on ice to the Freshwater Institute (Department of Fisheries and Oceans) in Winnipeg, where they were stored in the dark at 4°C until processing.

Samples were extracted and analyzed for chlorpyrifos using the method described by RAWN (1998). Extracts were analyzed using a Hewlett Packard (HP) 5890 series II gas chromatograph with electronic pressure control coupled to an HP 5971 mass selective detector operating in the selective ion mode. Detection criteria were the correct ratios of two characteristic ions, 197 m/z (quantifier ion) and 199 m/z (qualifier ion), and a retention time of 29.00 min. Chlorpyrifos concentration was quantified using external standard solutions and corrected for volume changes. The instrument detection limit for chlorpyrifos was

5 pg/μL. See APPENDIX 1 for detailed chlorpyrifos sampling and analysis method.

Surface water samples (1 L) were collected from each enclosure twice weekly for the determination of chemical parameters. Samples were analyzed for pH, alkalinity (acid titration; APHA 1992), soluble reactive phosphorus (SRP) (acid molybdate method; STAINTON et al. 1977), ammonium-N (NH_3-N) (hypochlorite method; STAINTON et al. 1977), and nitrate+nitrite-N (NO_3-N) (UV absorption method; APHA 1992). Dissolved oxygen and water temperature (at 10 and 50 cm depths) were measured weekly, in the morning, using a YSI Model 51B meter. Turbidity (at 30 cm depth) in each enclosure was determined weekly using a Hach Model 2100B turbidimeter.

Sampling and analysis of planktonic microinvertebrates and phytoplankton
Three quantitative, depth integrated water column samples (4 L) were taken randomly from each enclosure weekly and filtered through a 53 μm mesh to determine densities (ind./L) of microinvertebrates (see HANN & GOLDSBOROUGH 1997 for method). A quantitative water column sample (1 L) was collected from three randomly selected positions in each enclosure weekly to estimate biomass of phytoplankton (as chlorophyll a) (see MCDOUGAL et al. 1997 for method).

Percent cover of enclosure bottom by submersed macrophyte species (*Potamogeton zosteriformis, P. pectinatus, Ceratophyllum demersum,* and *Myriophyllum sibiricum*) was estimated by visual inspection weekly. Mesocosm size made it difficult to take representative samples of the macrophyte community at weekly intervals without disturbing the enclosures. Macrophyte biomass (g/m^2 of wetland bottom) was measured on 16 June, 10 July, and 13 August in each enclosure using a plastic cylinder (d = 0.78 m; A = 0.48 m^2).The cylinder was lowered into an enclosure and macrophytes contained within it were sheared at the sediment-water interface. Macrophyte material was dried at 106°C for 24 h and then weighed to obtain dry mass data.

Microinvertebrates were identified to species using standard references, including PENNAK (1978), EDMONDSON (1959), and SMITH & FERNANDO (1978), and a reference collection (BJH). Cladocera were identified to species and counted. Copepoda were counted as nauplii, Cyclopoida and Calanoida copepodites, and Cyclopoida and Calanoida adults; only adults were identified to species. Among planktonic rotifers, only the predatory rotifer, *Asplanchna* sp., was counted separately.

Data analysis

Univariate analysis

Insecticide addition and control treatments both contained three submersed macrophyte-dominated replicates for the duration of the experiment. Inorganic nutrient addition treatment was reduced to two replicates due to a persistent phytoplankton bloom and late development of submersed macrophytes in one enclosure. Replicates included in data analysis were characterized by clear water (low phytoplankton biomass) and similar development in areal proportion and density of submersed macrophytes. We felt it was important for replicates to resemble each other with respect to macrophyte development as the fate and effects of chlorpyrifos differ between water with macrophytes and open-water systems (BROCK et al. 1992a, 1992b). For each sampling date, mean densities of planktonic microinvertebrates (ind./L) and fathead minnows (ind./week), mean phytoplankton biomass as chlorophyll *a* (μg/L), and mean values for all physico-chemical parameters were estimated for all replicates; differences between treated and control enclosures were statistically compared. Data were tested for normal distribution and homogeneity of variance, and, if necessary, ln (x+1) - transformed prior to analysis using a one-way ANOVA for each sampling date. If differences in the mean values among treatment groups were greater than would be expected by chance, pairwise multiple comparisons among treatments were carried out using the Student-Newman-Keuls method. Treatment effects were considered statistically significant at p values < 0.05. Our ability to detect

differences among treatments was limited due to lack of statistical power (small number of replicates). All estimates of treatment and control means are presented as mean $\pm$ SE.

Multivariate analysis

For each sampling date, mean densities of planktonic microinvertebrate species and fathead minnows, mean biomass of phytoplankton, % cover of enclosure bottom by macrophytes, mean concentrations of ammonia, nitrate, soluble reactive phosphorus, and alkalinity, pH, % saturation of oxygen, and water temperature were estimated for all treated and control enclosures.

Relationships between microinvertebrate species and environmental data were examined using canonical correspondence analysis (CCA). Species determined to be rare ($\leq$ 20 % of frequency of most common species) were downweighted in importance during analysis. Ordinations were performed using the program CANOCO (version 3.10, TER BRAAK 1988). For each sampling date, mean densities of microinvertebrate species in the plankton (ind./L) were calculated for each treatment. A species x sample date (for each treatment) matrix was produced using ln (x+1)-transformed data to stabilize variances. Biotic environmental parameters, fathead minnow abundance (FATHEAD), biomass of phytoplankton (CHL A), and % cover of enclosure bottom by submersed macrophytes (% COVER), and abiotic parameters, mean concentration of ammonia (AMMONIA), nitrate (NITRATE), soluble reactive phosphorus (SRP), and alkalinity (ALK), pH (pH), % saturation of oxygen (% SAT), and water temperature (TEMP) were included in an environmental variable x sample date (for each treatment) matrix. If necessary, environmental data were ln (x+1)-transformed to stabilize variances. The statistical significance of the relationship between species composition and canonical axes (constrained by set of environmental variables) was tested using a Monte Carlo permutation test; the statistical importance of specific environmental variables was determined

through forward selection of environmental variables and subsequent testing with a Monte Carlo permutation test (TER BRAAK 1988).

CCA assumes that species abundances are unimodal functions along environmental gradients. Axes are constrained to the fraction of total variance in the data that is explained by the environmental variables measured; a set of species is related directly to a set of environmental variables and an ordination diagram is produced by detecting patterns of variation in species community composition that can be best accounted for by the environmental variables quantified (TER BRAAK 1986). The diagram shows the pattern of variation in species composition as accounted for by the environmental variables measured and the distributions of species along environmental gradients. Species and sites are represented as points and environmental variables as lines (or vectors). Longer environmental vectors are more highly correlated with the ordination axes and the corresponding environmental variable has a greater influence on the pattern of species community variation (TER BRAAK 1988). Site points lie at the centroid of the species points that occur in them; a site that lies close to a species likely has a high density of that species. Sites that are similar in species composition and relative density will lie close together on the diagram, while sites that differ in relative density of a similar set of species or in their species composition will lie further apart.

RESULTS

Fathead minnow density

Seasonal mean fathead density (ind./week) in control, insecticide treatment, and nutrient treatment enclosures declined during the experiment as there was mortality without recruitment of adults into the enclosures from Blind Channel, and did not vary significantly among treatments for the entire experiment (ANOVA, $p > 0.05$).

Chlorpyrifos in the water column

Chlorpyrifos concentrations of 0.35 to 1.11 µg/L (0.83 ± 0.24 µg/L) were measured in the water column of the insecticide treatment enclosures prior to addition. One hour after application, chlorpyrifos concentrations of 2.96 to 7.26 µg/L (4.79 ± 1.28 µg/L) were detected in the insecticide enclosures (Table 2-1) (Fig. 2-1). After 12 h, 61 - 82 %, and after 24 h, 18 - 100 % of the measured dose could be detected in the water column. Chlorpyrifos concentrations in the water column declined until 1.5 days after application. On day 2 after application, the concentration of chlorpyrifos in the insecticide enclosures increased to 5.26 ± 0.53 µg/L; by day 3 after application, chlorpyrifos concentrations had declined to levels similar to 1 day post-treatment.

Environmental variables

Seasonal mean water temperature increased during the experiment as daytime air temperature rose, but did not vary among control, insecticide treatment, and nutrient treatment (Table 2-1). Turbidity declined at the beginning of the experiment as enclosure curtains and developing macrophytes reduced sediment resuspension by wind and did not vary among treatments (Table 2-1).

Percent oxygen saturation was significantly higher in the nutrient treatment than in the control or insecticide treatment at the beginning of nutrient addition in the middle of June (week 3) (ANOVA, $p < 0.05$) (Fig. 2-2). However, oxygen was significantly lower in the nutrient treatment than in the control or insecticide treatment from the middle of July (week 7) until the beginning of August (week 9) (ANOVA, $p < 0.05$). Changes in oxygen saturation were similar in all treatments for the remainder of August. Overall, seasonal mean % saturation of oxygen was lower in the nutrient treatment than in the control and insecticide treatment (Table 2-1).

From mid-June (week 3) to mid-July (week 6), pH was significantly higher

Table 2-1. Mean (± SE) of biotic and abiotic environmental parameters in experimental enclosures in Delta Marsh, Manitoba (June to August 1997).

Environmental Parameter	Control	Insecticide	N+P
Fathead minnow density (ind./week)	3 (± 1)	7 (± 4)	2 (± 1)
Biomass of phytoplankton as chlorophyll a (µg/L)	6.9 (± 1.5)	11.4 (± 2.6)	9.9 (± 2.5)
Cover of enclosure bottom by submersed macrophytes (% cover estimated)	52 (± 11)	55 (± 12)	47 (± 7)
Biomass of submersed macrophytes (g/m^2 enclosure bottom)	90 (± 31)	111 (± 33)	186 (± 59)
Initial chlorpyrifos concentration (µg/L)	—	4.79 (± 1.28)	—
Turbidity (NTU)	1.0 (± 0.1)	1.1 (± 0.1)	1.1 (± 0.1)
Water temperature (° C)	20.1 (± 1.0)	20.1 (± 1.0)	20.1 (± 1.0)
Oxygen (% saturation)	56.0 (± 3.5)	57.6 (± 4.6)	46.2 (± 6.6)
pH	8.6 (± 0.1)	8.7 (± 0.2)	8.7 (± 0.1)
Alkalinity (mg/L)	255 (± 14)	251 (± 16)	278 (± 5)
Ammonia (µg/L)	29 (± 5)	23 (± 1)	170 (± 65)
Nitrate (µg/L)	< 50 (below detection limit)	< 50 (below detection limit)	883 (± 212)
SRP (µg/L)	140 (± 35)	55 (± 9)	1080 (± 387)
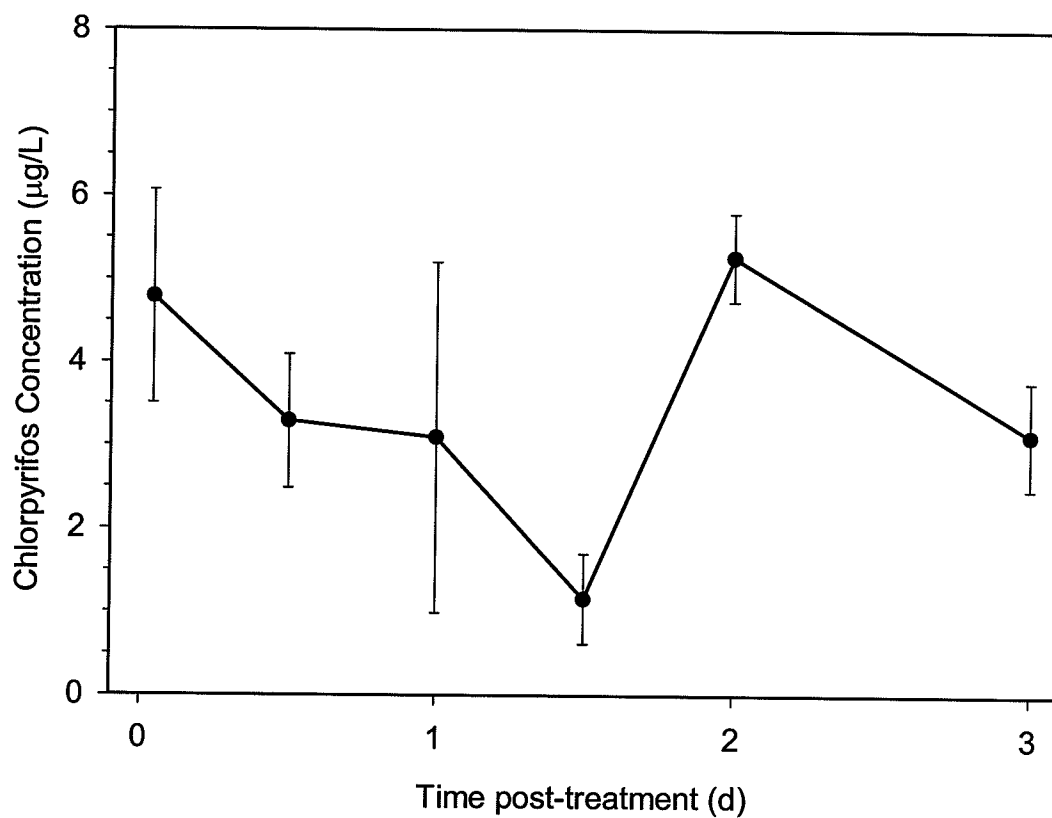
Fig. 2-1. Mean concentration of chlorpyrifos (µg/L ± SE) in the water column of enclosures treated with the insecticide chlorpyrifos after application of a nominal concentration of 10 µg/L.

in the nutrient treatment (ANOVA, $p < 0.05$) with values increasing from ~8.3 to ~9.2 (Fig. 2-2). There was no difference between the control and insecticide treatment during this time period, although values increased from ~8.2 in early June to ~ 8.6. After week 6, pH declined in the nutrient treatment to approach values in the control, and increased in the insecticide treatment. At the end of July, pH was significantly higher in the insecticide treatment than in the nutrient treatment (ANOVA, $p < 0.05$). In August (weeks 9-12), pH was significantly higher in the insecticide treatment than in the control and nutrient treatment (ANOVA, $p < 0.05$), with values increasing from ~9.1 to ~9.6. There was no difference between control and nutrient treatment during this time period, although values increased from ~8.6 to > 9.0.

Alkalinity in the water column of all treatments was similar from the beginning of June to mid-July (Fig. 2-2). Control and insecticide treatment diverged from nutrient treatment by the end of July, when alkalinity decreased (~225 mg/L) in control and insecticide treatment and remained high (~300 mg/L) in nutrient treatment. Alkalinity was significantly higher in the nutrient treatment for most of August (ANOVA, $p < 0.05$). Overall, seasonal mean alkalinity was higher in the nutrient treatment than in control and insecticide treatment (Table 2-1).

Levels of inorganic N and P in the water column of control and insecticide treatment were low (SRP ~55-140 µg/L, NO_3-N <50 µg/L or below detection limit, NH_3-N ~23-29 µg/L) and remained nearly constant over the experiment (Table 2-1, Fig. 2-3); SRP increased slightly in the control from the end of July through August (weeks 8-12). Ambient N and P levels in the nutrient treatment (SRP ~1080 µg/L, NO_3-N ~883 µg/L, NH_3-N ~170 µg/L) were significantly higher than in control and insecticide treatment for most of the experiment (ANOVA, $p < 0.05$) (Table 2-1, Fig. 2-3). SRP in the nutrient treatment increased gradually, reaching a maximum of ~2.1 mg/L by mid-August (week 10) (Fig. 2-3). Nitrate-N levels in the nutrient treatment increased after the first addition in June (week 3)

and reached a maximum of 1.6 mg/L by mid-July (week 6) (Fig. 2-3). Ammonia-N in the nutrient treatment increased throughout most of the treatment period, particularly in mid-July, reaching a maximum of 477 µg/L at the beginning of August (week 9) (Fig. 2-3).

Microinvertebrate abundance

The microcrustacean community included 12 species of cladocerans, 4 species of cyclopoid copepods, and the calanoid copepod *Diaptomus nudus* throughout the experiment (Table 2-2). Only 10 microcrustacean species were found in the insecticide treatment, compared with 15 species in the nutrient treatment and 13 in the control.

Maximum density of cladocerans (158-187 ind./L), consisting primarily of *Bosmina longirostris*, *Diaphanosoma birgei*, and *Ceriodaphnia dubia*, occurred at the beginning of July, two weeks after nutrient addition treatment had begun and 1 week prior to insecticide addition (Fig. 2-4). After insecticide application on 14 July (week 6), cladoceran density declined to < 4 ind./L and was significantly lower than in the control and nutrient treatment for 1-week post-treatment (ANOVA, $p < 0.05$). Nutrient addition had no significant effect on cladoceran density (ANOVA, $p > 0.05$).

Small planktonic rotifers showed pre-treatment density peaks (1012-2049 ind./L) in all treatments, but decreased throughout the experiment (Fig. 2-4). Insecticide addition resulted in a significant increase in small rotifer density to ~734 ind./L by 1-week post-treatment (ANOVA, $p < 0.05$); density declined and was similar to control and nutrient treatment during August. Nutrient addition had no significant effect on rotifer density (ANOVA, $p > 0.05$).

Copepod nauplii increased to high density (357-552 ind./L) in all treatments at the beginning of July (week 5) (Fig. 2-5). Insecticide addition reduced nauplii density to ~51 ind./L; density continued to decline through the end of July and August. Nauplii density was significantly lower in the insecticide

Fig. 2-2. Changes in % saturation of oxygen (± SE), pH (± SE), and alkalinity (mg/L ± SE) in the water column over a 12-week period in control enclosures (Control), enclosures loaded with inorganic nutrients (N+P), and in enclosures treated with the insecticide chlorpyrifos (Insecticide). The horizontal dotted line denotes thrice weekly additions of inorganic nutrients from 23 June to 27 August; the arrow denotes the moment of insecticide application. Significant differences (**O** = 1-way ANOVA, $p <$ 0.05) between treatments are presented in the horizontal bars at the top of the graph: I = Control versus Insecticide enclosures; II = Control versus N+P enclosures; III = Insecticide versus N+P enclosures.
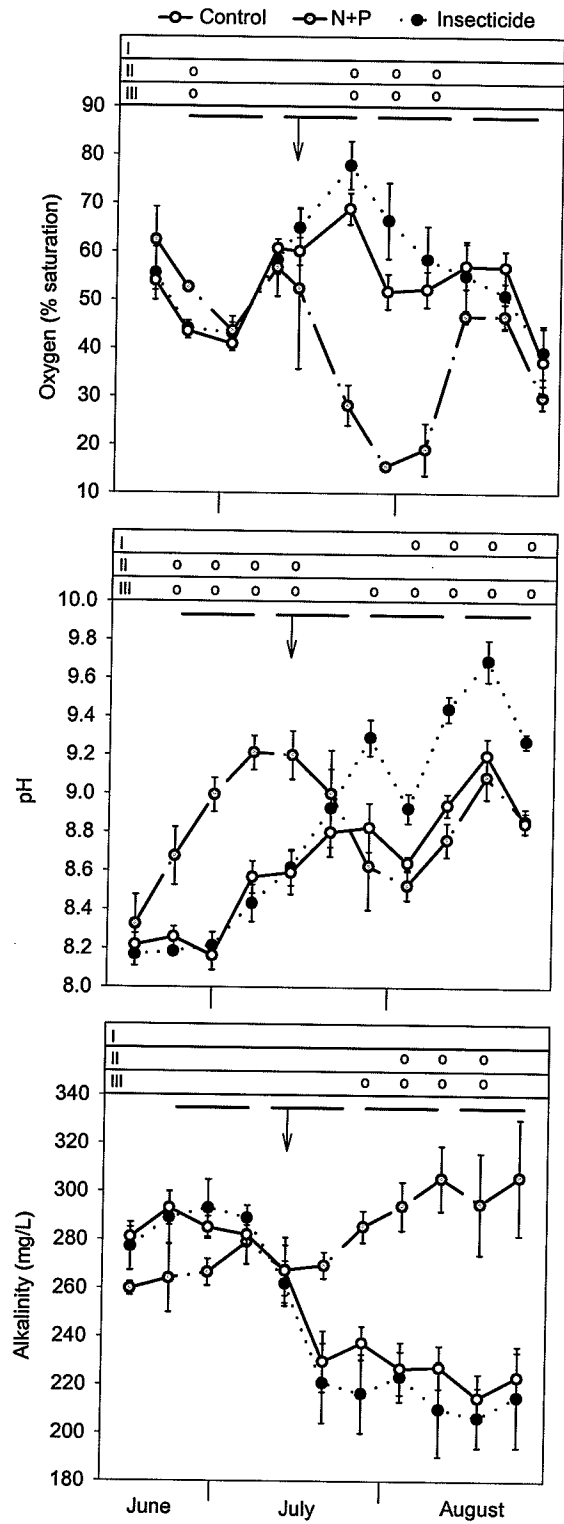
Fig. 2-3. Changes in soluble reactive phosphorus (μg/L ± SE), nitrate-N (μg/L ± SE), and ammonia-N (μg/L ± SE) in the water column over a 12-week period in control enclosures (Control), enclosures loaded with inorganic nutrients (N+P), and in enclosures treated with the insecticide chlorpyrifos (Insecticide). Symbols are identified as in Fig. 2-2.
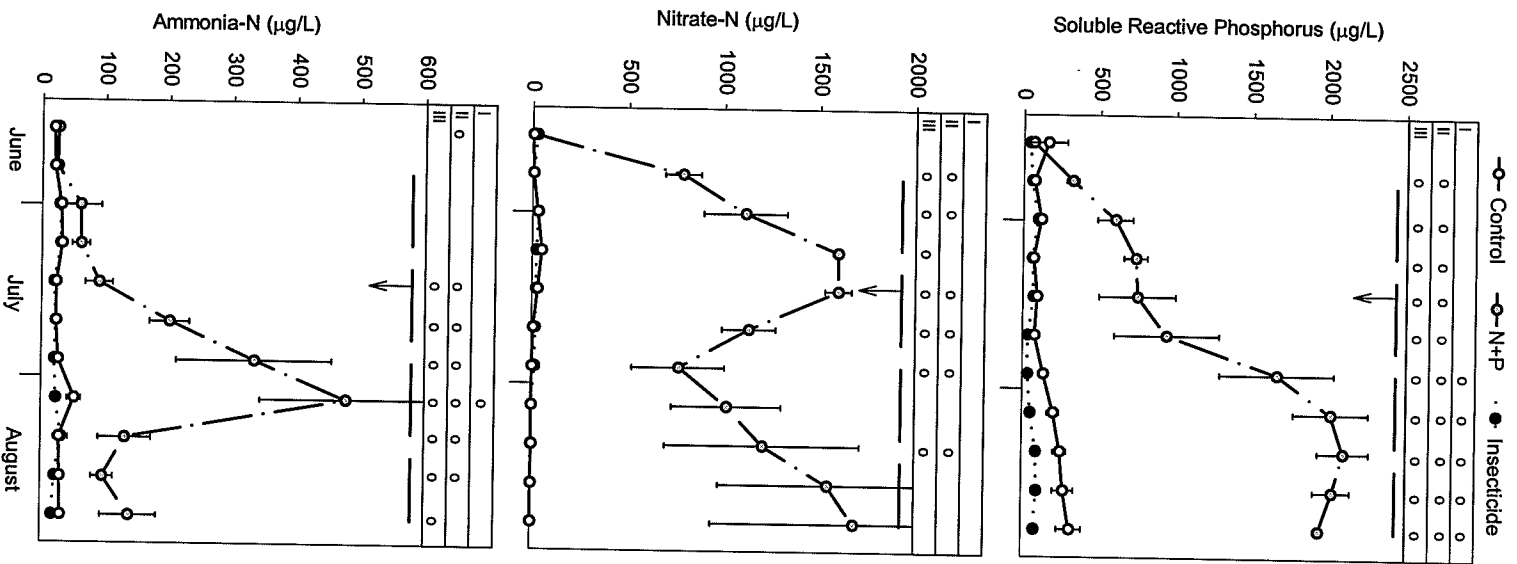
treatment than in the control and nutrient treatment through August (ANOVA, $p < 0.05$). Nutrient addition had no significant effect on nauplii density (ANOVA, $p > 0.05$).

Total cyclopoid copepod density peaked at the beginning of July in the control and insecticide treatment (72-158 ind./L) and was significantly higher than in the nutrient treatment (ANOVA, $p < 0.05$) (Fig. 2-5). Insecticide addition reduced cyclopoid density to < 2 ind./L. Cyclopoid density was significantly lower than in the control immediately after insecticide application, and in the control and nutrient treatment at the beginning of August (ANOVA, $p < 0.05$).

Calanoid copepods were rare (0-2 ind./L) in the water column in all treatments during June (Fig. 2-5). Calanoid density in the control and insecticide treatment was significantly higher than in the nutrient treatment at the beginning of August (ANOVA, $p < 0.05$).

Phytoplankton biomass was elevated in all treatments at the end of June - early July, after nutrient addition had begun, and at the end of August in the insecticide and nutrient treatments (Fig. 2-6). Peaks in phytoplankton biomass at the end of June - early July (~19-33 µg/L) were followed 1-2 weeks later by high densities of cladocerans in all treatments (Fig. 2-6). Insecticide addition resulted in a brief phytoplankton bloom (~36 µg/L) 1-week post-treatment, but it was not significant (ANOVA, $p > 0.05$); biomass decreased and was similar to controls and nutrient treatment during August. The phytoplankton bloom occurred simultaneously with an increase in small rotifer density (~734 ind./L) (Figs. 2-4 & 2-6).

Mean percent cover of enclosure bottom by submersed macrophytes and macrophyte biomass did not differ, suggesting there was no differential response to treatments (Table 2-1).

Table 2-2. Microcrustacean species occurring in experimental enclosures in Delta Marsh, Manitoba (June to August 1997).

Taxon	Control	Insecticide	N+P
Cladocera			
Diaphanosoma birgei KORINEK 1981	X	X	X
Bosmina longirostris (O.F. MÜLLER) 1785	X	X	X
Ceriodaphnia dubia RICHARD 1894	X	X	X
Daphnia sp.	X	X	X
Scapholeberis kingi SARS 1903		X	X
Simocephalus serrulatus (KOCH) 1841	X	X	X
Simocephalus vetulus SCHÖDLER 1858	X	X	X
Alona sp.	X		X
Camptocercus sp.			X
Chydorus sp.			X
Eurycercus longirostris HANN 1982	X		X
Pleuroxus denticulatus BIRGE 1878			X
Copepoda (Cyclopoida)			
Microcyclops varicans rubellus (LILLJEBORG) 1901	X		
Macrocyclops albidus (JURINE) 1820	X		
Acanthocyclops vernalis (FISCHER) 1853	X	X	X
Diacyclops thomasi (S.A. FORBES) 1882	X	X	X
Copepoda (Calanoida)			
Diaptomus nudus MARSH 1904	X	X	X
Total number of species	13	10	15

Fig. 2-4. Changes in cladoceran and small rotifer density (ind./L ± SE) in the water column over a 12-week period in control enclosures (Control), enclosures loaded with inorganic nutrients (N+P), and in enclosures treated with the insecticide chlorpyrifos (Insecticide). Symbols are identified as in Fig. 2-2.
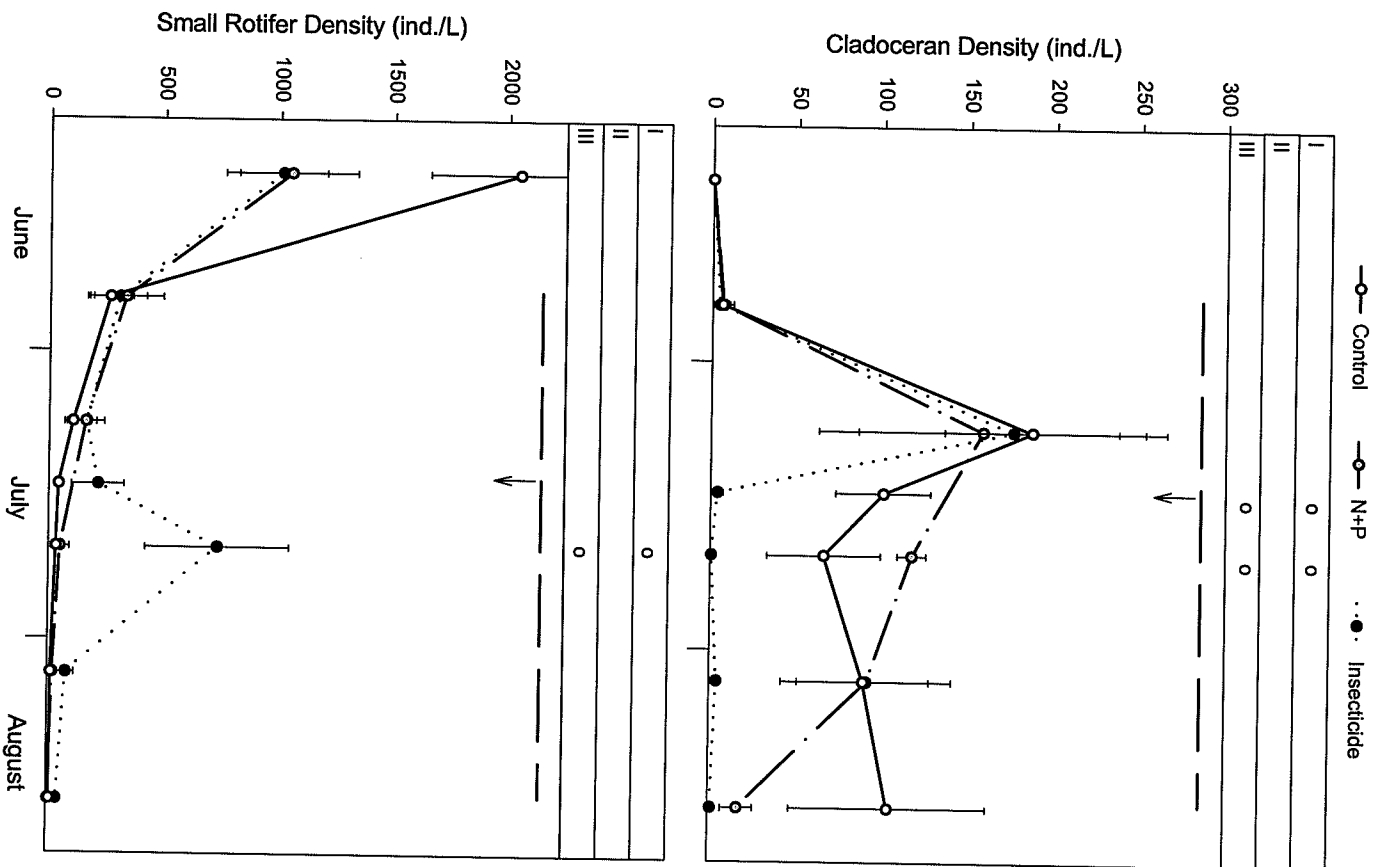
Fig. 2-5. Changes in copepod nauplii, total (copepodites and adults) cyclopoid copepod, and total (copepodites and adults) calanoid copepod density (ind./L ± SE) in the water column over a 12-week period in control enclosures (Control), enclosures loaded with inorganic nutrients (N+P), and in enclosures treated with the insecticide chlorpyrifos (Insecticide). Symbols are identified as in Fig. 2-2.
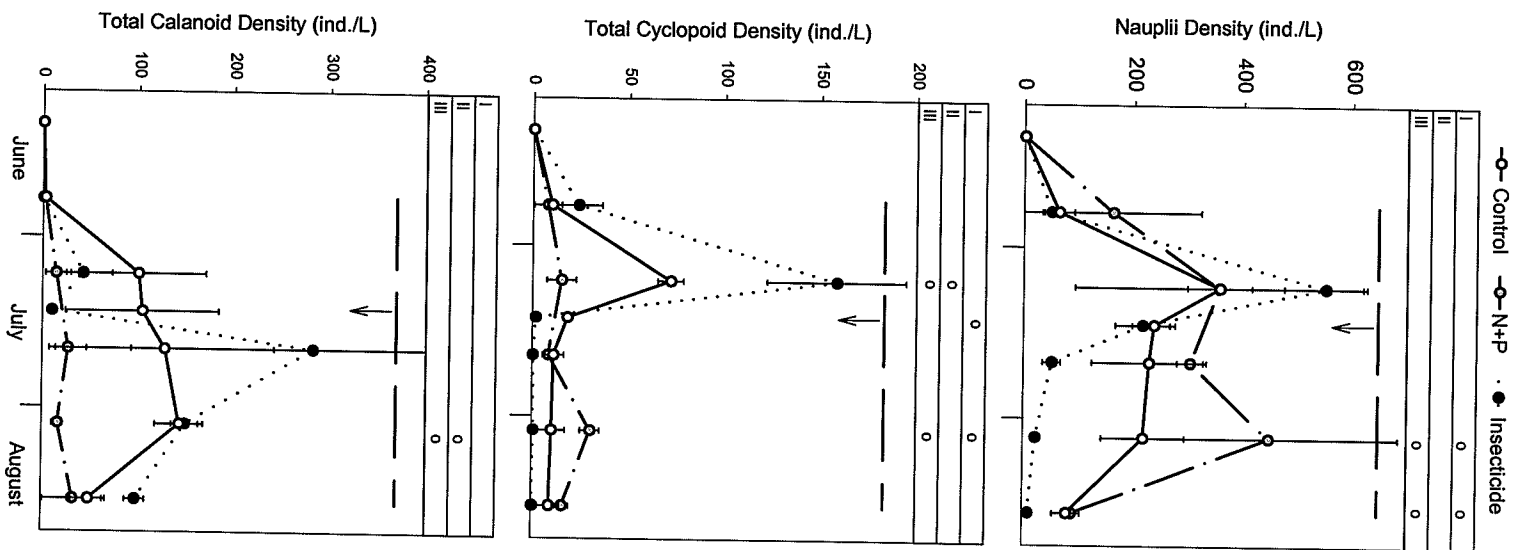
Fig. 2-6. Changes in cladoceran density (ind./L ± SE) in the water column and phytoplankton biomass as chlorophyll *a* (µg/L ± SE) over a 12-week period in control enclosures (Control), enclosures treated with the insecticide chlorpyrifos (Insecticide), and enclosures loaded with inorganic nutrients (N+P). The horizontal dotted line denotes thrice weekly additions of inorganic nutrients from 23 June to 27 August; the arrow denotes the moment of insecticide application.
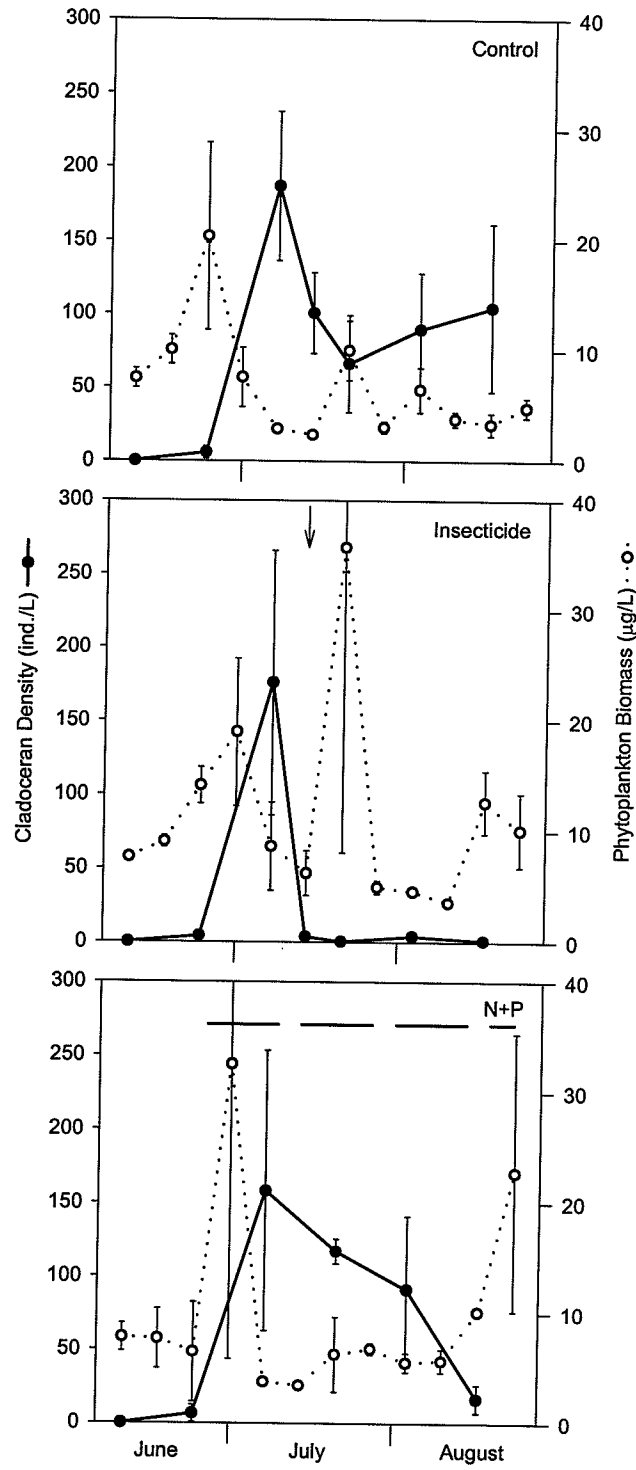
Microinvertebrate community structure

Canonical correspondence analysis

CCA of the open water community produced eigenvalues for the first two canonical axes of 0.295 and 0.122. CCA axis 1 was significantly related to microinvertebrate community composition (Monte Carlo permutation test, F = 7.14, p = 0.01), and the first four axes together were significant (F = 6.36, p = 0.01). The 10 environmental variables included in the analysis explained 90 % of the total variance in the species data. When the environmental variables were forward selected, only % cover of enclosure bottom by macrophytes (Monte Carlo permutation test, F = 12.15, p = 0.01) and alkalinity (F = 6.70, p = 0.01) were significantly related to microinvertebrate community composition; they accounted for 60 % of the total variance in the species data.

Axis 1 was most strongly correlated with % cover of enclosure bottom by submersed macrophytes and water temperature, and axis 2 with alkalinity and nitrate concentrations (Table 2-3). Site points, representing sample dates, were plotted with environmental variables in a biplot (Fig. 2-7). Separation between sample dates with respect to season (sampling date) is shown on axis 1. Sample dates in June (weeks 1-3) for all treatments had positive values on axis 1, corresponding to lower % cover of enclosure bottom by submersed macrophytes, higher phytoplankton biomass (as chlorophyll a), and cooler water temperatures. All sample dates (with two exceptions, (7)I and (11)I) in July and August (weeks 5-11) for all treatments had negative values on that axis, indicating higher % cover of enclosure bottom by submersed macrophytes, reduced phytoplankton biomass, and warmer water temperatures. On axis 1, therefore, the temporal sequence of sample dates reflects ordering of site points according to warming of water temperature and a shift in primary producers from phytoplankton to increasing cover of enclosure bottom by submersed macrophytes. The temporal sequence of sample dates on axis 2 reflects the influence primarily of the abiotic parameters alkalinity and nitrate, and to a lesser extent pH and % saturation of O_2. Sample dates in July and August for the

nutrient treatment had negative values on axis 2, indicating higher alkalinity and nitrate concentrations, likely related to inorganic nutrient addition. Sample dates during July and August for control and insecticide treatments (with two exceptions, (5)C and (5)I) had positive values on axis 2, indicating lower alkalinity and nitrate concentrations and increased pH and % saturation of O_2. Higher positive values on axis 2 of sample dates from the middle of July through August for insecticide treatment is likely related to the application of chlorpyrifos on 14 July.

Microinvertebrate species were plotted on the same CCA axis 1 and 2 as for sampling dates (Fig. 2-7). Species with a high positive score on axis 1 (e.g. small rotifer species, *Asplanchna* sp.) were more abundant in June, and those with a negative score on axis 1 (e.g. *Diaphanosoma birgei*, *Bosmina longirostris*, Ostracod spp., *Diaptomus nudus*) were more abundant in July and August. Species with a negative score on axis 1 occurred in warmer waters with a higher % cover of enclosure bottom with submersed macrophytes. The position of species points for small rotifer species, calanoid copepodites, and *Diaptomus nudus* were likely also influenced by insecticide treatment, resulting in a higher positive score on axis 2.

Table 2-3. Weighted correlation coefficients between environmental variables and the first two CCA axes for the microinvertebrate community in the water column.

Environmental variable	CCA Axis 1	CCA Axis 2
Fathead minnow (FATHEAD)	-0.1381	-0.3865
Chlorophyll a (CHL A)	0.2609	0.0283
Cover of enclosure bottom by macrophytes (% COVER)	-0.8949	0.1789
Ammonia (AMMONIA)	-0.5020	-0.3893
Nitrate (NITRATE)	-0.3606	-0.5715
Soluble reactive phosphorus (SRP)	-0.4178	-0.4311
Alkalinity (ALK)	0.1598	-0.8006
pH	-0.4982	0.4456
% Saturation of O_2 (% SAT)	0.1388	0.4358
Water temperature (TEMP)	-0.5796	-0.0653

Fig. 2-7. Canonical correspondence analysis of the microinvertebrate community in the water column over an 11-week period in control enclosures (Control), enclosures loaded with inorganic nutrients (N+P), and in enclosures treated with the insecticide chlorpyrifos (Insecticide). Changes in community structure are shown connected through points for each week from beginning of June (week 1) to end of August (week 11) (top panel). Labels for environmental variables are as in Table 2-3. Microinvertebrate species (bottom panel) are positioned on the same CCA axis 1 and 2 as for sampling dates. Species are coded as follows: Alo sp1, *Alona* sp.1; Bos lon, *Bosmina longirostris*; Cam sp1, *Camptocercus* sp.1; Cer dub, *Ceriodaphnia dubia*; Chy sp1, *Chydorus* sp.1; Dap sp1, *Daphnia* sp.1; Dia bir, *Diaphanosoma birgei*; Eur lon, *Eurycercus longirostris*; Ple den, *Pleuroxus denticulatus*; Sca kin, *Scapholeberis kingi*; Sim spp, *Simocephalus* spp.; Nau pli, Nauplii; Cyc Cop, Cyclopoid copepodites; Aca Dia, *Acanthocyclops/ Diacyclops* spp.; Mac alb, *Macrocyclops albidus*; Mic rub, *Microcyclops varicans rubellus*; Cal Cop, Calanoid copepodites; Dia nud, *Diaptomus nudus*; Rot spp, small Rotifer spp.; Asp sp1, *Asplanchna* sp.1; Ost spp., Ostracod spp.
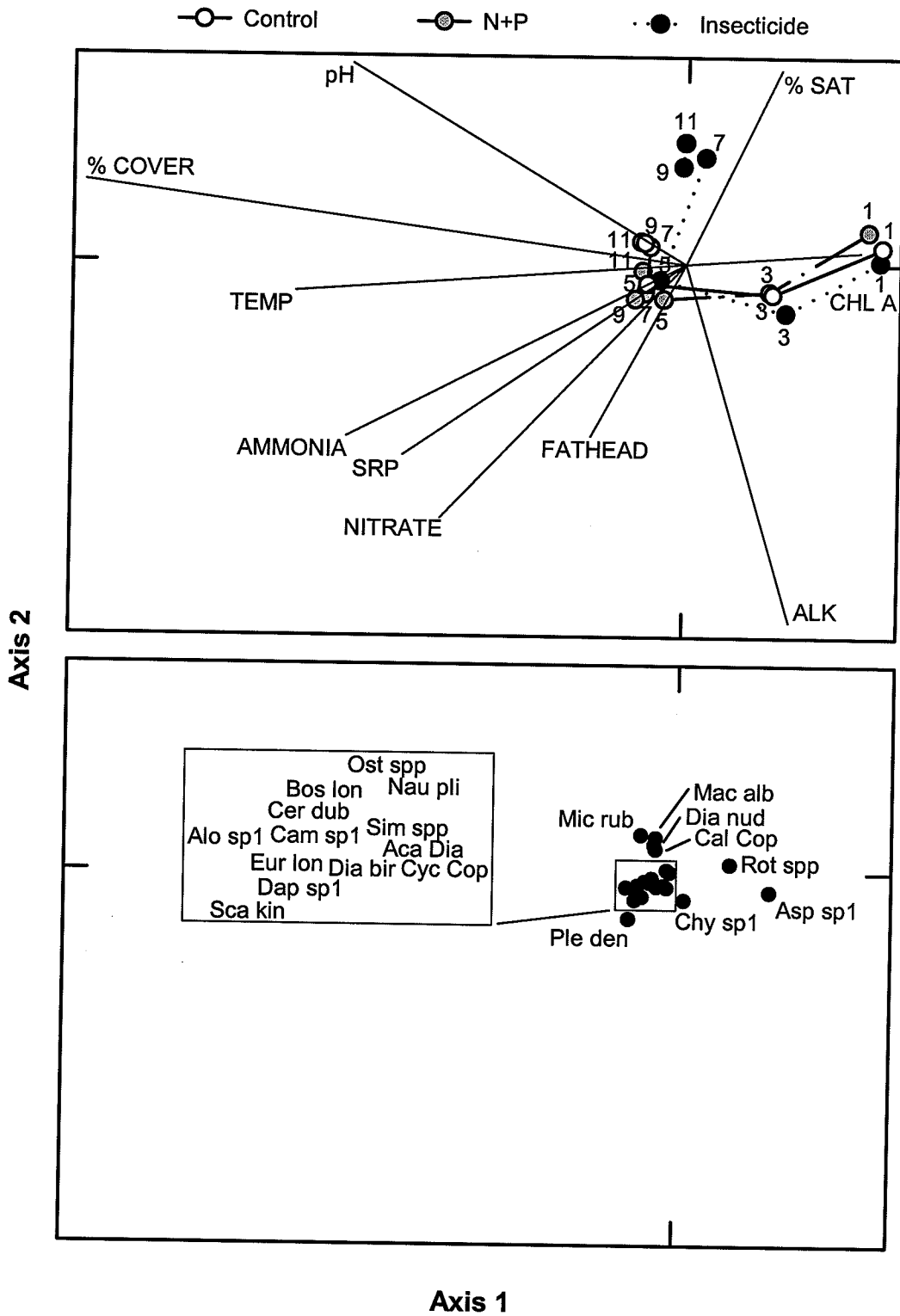
DISCUSSION

Effects of organophosphorus insecticide application

Initial chlorpyrifos concentrations measured in the water column of our insecticide treatment enclosures (4.79 ± 1.28 µg/L) have been shown to be effective in reducing most microinvertebrate arthropod species due to direct toxicity (MARSHALL & ROBERTS 1978, SIEFERT et al. 1989, VAN WIJNGAARDEN et al. 1993). Planktonic microinvertebrate community composition changed markedly due to differential mortality of arthropod microinvertebrates, with calanoid copepodites and adult *Diaptomus nudus* being more tolerant than Cladocera and cyclopoid copepods (HURLBERT et al. 1970, HURLBERT et al. 1972, HURLBERT 1975, VAN DEN BRINK et al. 1995).

Larger cladocerans were eliminated completely by the insecticide treatment and did not reappear for the remainder of the experiment. Smaller cladocerans (*Bosmina longirostris, Ceriodaphnia dubia, Diaphanosoma birgei*) were slightly more tolerant than larger forms (*Daphnia* sp., *Simocephalus serrulatus, Simocephalus vetulus*), showing some recovery during August. In contrast, BROCK et al. (1992a) found that larger, planktonic cladocerans (*Daphnia, Simocephalus*) showed an earlier recovery than smaller species (*Alona, Bosmina*) in macrophyte-dominated indoor microcosms.

Life stages of cyclopoid copepods responded differentially to insecticide application. Adults (*Acanthocyclops vernalis, Diacyclops thomasi*) and copepodites were nearly eliminated and showed no recovery during August. Nauplii were reduced immediately after insecticide application, but were not as devastated as copepodites and adults. They continued to decline in abundance throughout August, attributable to either maturation of a cohort, or delayed insecticide toxicity. In other studies, copepod nauplii were the most susceptible life stage of copepods, showing a more rapid decline and recovery than copepodites and adults (BROCK et al. 1992a, VAN DONK et al. 1995).

Increases in small planktonic rotifers in the insecticide enclosures after treatment have also been observed by HURLBERT et al. (1972), BROCK et al. (1992a), and VAN DONK et al. (1995). These increases may be indirect consequences of reduced abundance of sensitive Cladocera and cyclopoid copepods. Cladocerans are able to suppress rotifers both by competition for the shared phytoplankton resource and by mechanical interference (GILBERT 1988), and the cyclopoid copepods *Acanthocyclops vernalis* and *Diacyclops thomasi* present prior to treatment are known to prey on small rotifers (FRYER 1957). With fewer competitors and predators, small rotifers increased in density through rapid asexual (parthenogenetic) reproduction. A short-lived increase in phytoplankton biomass was observed the week after insecticide addition, concurrent with the increase in small rotifers. HURLBERT et al. (1972) and BROCK et al. (1992b) found that a decline in cladoceran abundance due to direct insecticide toxicity led to an increase in rotifers, which then fed on phytoplankton; a subsequent increase in *Asplanchna* preyed on the small rotifers, thereby reducing their population. *Asplanchna* densities did not increase in response to the abundance of small rotifers in our enclosures after insecticide treatment. Perhaps our experiment was not of sufficient length for secondary, indirect effects resulting from insecticide addition to be fully observed.

Effects of inorganic nutrient enrichment

We did not observe specific treatment effects of nutrient enrichment on the primary producers investigated. Biomass of both phytoplankton and submersed macrophytes and % cover of enclosure bottom by macrophytes was not markedly different from controls. However, primary producers in a wetland ecosystem (e.g. phytoplankton, epiphyton, submersed macrophytes) have been shown to respond positively, but differentially, to nutrient enrichment (MURKIN et al. 1994, MCDOUGAL et al. 1997). In previous experiments (using half the nutrient loading concentrations) in Blind Channel neither phytoplankton nor macrophyte biomass were affected by periodic nutrient addition; biomass of metaphyton

(detached epiphyton mats) and, to a lesser extent, epiphyton showed greatest increases in response to nutrient enrichment (MCDOUGAL et al. 1997).

The nutrient enriched planktonic microinvertebrate community changed seasonally, but in a pattern that did not differ substantially from the controls. Species composition in the nutrient treatment was predominantly cladocerans through July and August. Relatively larger cladocerans (*Daphnia* sp.) are more effective at filtering phytoplankton from the water column than smaller cladoceran species or copepods (KNOECHEL & HOLTBY 1986, VANNI 1987), and are frequently cited as keystone species in stabilizing the macrophyte-dominated clear-water state (MOSS et al. 1996, SARNELLE 1992). Presence of *Daphnia* sp. may have limited the positive response of phytoplankton biomass to nutrient addition, particularly at the beginning of August when its population peaked. Large *Daphnia* are relatively rare in Blind Channel, Delta Marsh, typically occurring in early spring, but not persisting later in the season (HANN & ZRUM 1997). Even in previous enclosure experiments, *Daphnia* sp. were abundant in June, but were replaced by the smaller *Ceriodaphnia dubia* through July and August (HANN & GOLDSBOROUGH 1997).

Predominance of smaller and more transparent species of cladocerans (*Bosmina longirostris, Ceriodaphnia dubia, Diaphanosoma birgei*) than *Daphnia* sp. may have been due, in part, to the size- and visibility-selectivity of planktivorous fish in the experimental enclosures (HESSEN 1985). In earlier nutrient addition enclosure experiments, PETTIGREW et al. (1998) observed a shift from the relatively large planktonic species *Ceriodaphnia dubia*, with a highly visible black eye, to smaller *Chydorus* spp., with an inconspicuous eye and a more phytophilous lifestyle, sheltered from fish predation amongst the submersed macrophytes. Cyclopoid copepods and small, transparent species of cladocerans were found to predominate the microinvertebrate community in Blind Channel, with planktivorous fish present (HANN & ZRUM 1997).

Community structure

The multivariate ordination method (CCA) used in our study emphasized changes in the structure (species composition) of the microinvertebrate community, or changes in the proportional (relative) abundances of species. Similarity of ordination diagrams using correspondence analysis (CA) and CCA techniques reinforced our confidence that the environmental variables included in CCA adequately explained the patterns of change observed in the community (ZRUM & HANN 1998). Furthermore, the high percentage of variance (90 %) in the species data explained by the environmental variables suggests that they accounted for the main variation in species data with respect to treatment over the sampling season. By comparison, values of 30 to 40 % for the fraction of total variance in a species data set explained by a suite of environmental variables is common in CCA in ecological studies (TER BRAAK 1988).

Two patterns emerge from our analyses of community structure: seasonal change (represented by increasing water temperature and % macrophyte cover), correlated with axis 1 of CCA; and insecticide-induced change, paralleling axis 2 of CCA. Effects of chlorpyrifos on invertebrate community structure have been evaluated in indoor experimental freshwater microcosms intended to mimic drainage ditches in and around agricultural areas (BROCK et al. 1992a, BROCK et al. 1992b, BROCK et al. 1995, CUPPEN et al. 1995, VAN DEN BRINK et al. 1995, VAN DONK et al. 1995, VAN WIJNGAARDEN et al. 1995) and in outdoor experimental ditches, which more closely resemble a natural system (VAN DEN BRINK et al. 1996). Ordinations demonstrated similar response in zooplankton communities to chlorpyrifos to those observed in our outdoor wetland enclosures (VAN DEN BRINK et al. 1995, VAN WIJNGAARDEN et al. 1995, VAN DEN BRINK et al. 1996). Communities changed immediately after insecticide application due to primary (direct) toxicological effects on cladocerans and cyclopoid copepods, and continued to change over time as a consequence of secondary (indirect) effects on rotifers and calanoid copepods (VAN DEN BRINK et al. 1995, VAN DEN BRINK et al. 1996).

Community structural response to our experimental nutrient enrichment was not discrete or separable from the prevailing seasonal pattern in the unmanipulated microinvertebrate community. Similarly, at half the inorganic nutrient loading (HANN & GOLDSBOROUGH 1997), or equivalent nutrient loading of waterfowl feces (PETTIGREW et al. 1998) to that used in our study, changes in community structure paralleled those occurring in controls. Thus, nutrient enrichment appears primarily to result in increased abundance of microinvertebrates, rather than any change in community structure. Therefore, specific treatment effects of chlorpyrifos are apparent in contrast to the muted effects of nutrient enrichment on community structure.

Dissipation of chlorpyrifos

Background (pre-treatment) chlorpyrifos levels (0.83 ± 0.24 µg/L) measured in the water column of insecticide enclosures prior to addition in mid-July were higher than previously reported values for surface water in Lake Manitoba (CURRIE & WILLIAMSON 1995) and the Red River and its tributaries (RAWN 1998). The proximity of the enclosures to agricultural land may have increased the potential for contamination due to pesticide aerial drift and resulted in higher chlorpyrifos concentrations being detected. Detrimental effects of chronic low levels of chlorpyrifos (0.1 µg/L) on invertebrate community structure in indoor freshwater microcosms have been reported (VAN DEN BRINK et al. 1995).

Rate of disappearance of chlorpyrifos in the treated enclosures was high, but variable, with between 18 and 100 % of the original dose being detected 24 hours after addition. Other studies have reported rapid disappearance of chlorpyrifos during the first few days after application, with initial half-lives ranging from a few hours to 1-3 days (MACEK et al. 1972, HUGHES et al. 1980, BRAZNER & KLINE 1990). Initial rapid loss of chlorpyrifos after application may be partially attributable to volatilization from the surface water (RACKE 1993). Chlorpyrifos has a low water solubility and a high octanol-water partition coefficient (K_{ow}) (log K_{ow} of 4.7-5.3; MCDONALD et al. 1985, DE BRUIJN et al.

1989). A high K_{ow} value indicates that chlorpyrifos has a strong tendency to favour the sorbed state over the dissolved state as a result of the nonpolar nature of the chlorpyrifos molecule. Due to the tendency of chlorpyrifos to adsorb to surfaces, its rapid disappearance from the water column is likely also attributable to adsorption of the compound on the polyethylene curtain, submersed macrophytes with attached epiphytes, and sediments (HURLBERT et al. 1970, HUGHES et al. 1980, BROCK et al. 1992a). Sorption of chlorpyrifos by enclosure curtains, macrophytes, epiphyton, and sediments would have limited its availability for absorption by the microinvertebrates (RACKE 1993); differences between insecticide treatment enclosures with respect to biomass of submersed macrophytes and epiphyton may have contributed to the range of chlorpyrifos concentrations (2.96 to 7.26 µg/L) measured at 1 hour after addition. Sorption-desorption processes are a major factor in determining the distribution and persistence of available chlorpyrifos in the water column (MARSHALL & ROBERTS 1978). The "reflux" of chlorpyrifos in the water column seen at day 2 after addition (5.27 ± 0.53 µg/L), likely indicates the potential of the molecule to become "secondarily" available for absorptive uptake by susceptible aquatic organisms upon desorption from binding surfaces.

ACKNOWLEDGMENTS

REFERENCES

American Public Health Association (APHA) (1992): Standard Methods for the Examination of Water and Wastewater. 18th Ed. - GREENBERG, A. E., CLESCERI, L. S. & EATON, A. D. (eds.). Washington, D.C.

BRAZNER, J. C. & KLINE, E. R. (1990): Effects of chlorpyrifos on the diet and growth of larval fathead minnows, *Pimephales promelas*, in littoral enclosures. - Can. J. Fish. Aquat. Sci. **47:** 1157-1165.

BROCK, T. C. M., CRUM, S. J. H., VAN WIJNGAARDEN, R., BUDDE, B. J., TIJINK, J., ZUPPELLI, A. & LEEUWANGH, P. (1992a): Fate and effects of the insecticide Dursban® 4E in indoor *Elodea*-dominated and macrophyte-free freshwater model ecosystems: I. Fate and primary effects of the active ingredient chlorpyrifos. - Arch. Environ. Contam. Toxicol. **23:** 69-84.

BROCK, T. C. M., VAN DEN BOGAERT, M., BOS, A. R., VAN BREUKELEN, S. W. F., REICHE, R., TERWOERT, J., SUYKERBUYK, R. E. M. & ROIJACKERS, R. M. M. (1992b): Fate and effects of the insecticide Dursban® 4E in indoor *Elodea*-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. - Arch. Environ. Contam. Toxicol. **23:** 391-409.

BROCK, T. C. M. & BUDDE, B. J. (1994): On the choice of structural parameters and endpoints to indicate responses of freshwater ecosystems to pesticide stress. - In: HILL, I. A., HEIMBACK, F., LEEUWANGH, P. & MATTHIESEN, P. (eds.): Freshwater Field Tests For Hazard Assessment of Chemicals. - Lewis Publishers, Michigan, pp. 19-56.

BROCK, T. C. M., ROIJACKERS, R. M. M., ROLLON, R., BRANSEN, F. & VAN DER HEYDEN, L. (1995): Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms: II. Responses of macrophytes, periphyton and macroinvertebrate grazers. - Arch. Hydrobiol. **134:** 53-74.

CUPPEN, J. G. M., GYLSTRA, R., VAN BEUSEKOM, S., BUDDE, B. J. & BROCK, T. C. M. (1995): Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms: III. Responses of macroinvertebrate detritivores, breakdown of plant litter, and final conclusions. - Arch. Hydrobiol. **134:** 157-177.

CURRIE, R. S. & WILLIAMSON, D. A. (1995): An assessment of pesticide residues in surface waters of Manitoba, Canada. - Manitoba Environment Report No. **95-08**.

DE BRUIJN, J., BUSSER, F., SEINEN, W. & HERMENS, J. (1989): Determinations of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. - Environ. Toxicol. Chem. **8:** 499-512.

EDMONDSON, W. T. (ed.) (1959): Freshwater Biology. 2nd Ed. - John Wiley and Sons, New York, NY.

FRANK, R., BRAUN, H. E., RIPLEY, B. D. & CLEGG, B. S. (1990): Contamination of rural ponds with pesticide, 1971-85, Ontario, Canada. - Bull. Environ. Contam. Toxicol. **44:** 401-409.

FRYER, G. (1957): The food of some freshwater cyclopoid copepods and its ecological significance. - J. Anim. Ecol. **26:** 263-286.

GABOR, T. S., MURKIN, H. R., STAINTON, M. P., BOUGHEN, J. A. & TITMAN, R. D. (1994): Nutrient additions to wetlands in the Interlake region of Manitoba, Canada: effects of a single pulse addition in spring. - Hydrobiologia **279/280**: 497-510.

GEARING, J. N. (1989): The role or aquatic microcosms in ecotoxicologic research as illustrated by large marine systems. - In: LEVIN, S. A., HARWELL, M. A., KELLY, J. R. & KIMBALL, K. D. (eds.): Ecotoxicology: Problems and Approaches. - Springer-Verlag, New York, pp. 411-470.

GIDDINGS, J. M. (1983): Microcosms for assessment of chemical effects on the properties of aquatic ecosystems. - In: SAXENA, J. (ed.): Hazard Assessment of Chemicals: Current Developments. Vol. 2. - Academic Press Inc., New York, pp. 45-94.

GILBERT, J. J. (1988): Suppression of rotifer populations by *Daphnia*: A review of the evidence, the mechanisms, and the effects on zooplankton community structure. - Limnol. Oceanogr. **33**: 1286-1303.

GOLDSBOROUGH, L. G. & HANN, B. J. (1996): Enclosure affects trophic structure of a freshwater prairie wetland. - University Field Station (Delta Marsh) Annual Report **30**: 63-67. On-line at: http://www.umanitoba.ca/faculties/ science/delta_marsh

GOLDSBOROUGH, L. G. & CRUMPTON, W. G. (1998): Distribution and environmental fate of pesticides in prairie wetlands. - Great Plains Research **8**: 73-96.

HANN, B. J. & GOLDSBOROUGH, L. G. (1997): Responses of a prairie wetland to press and pulse additions of inorganic nitrogen and phosphorus: invertebrate community structure and interactions. - Arch. Hydrobiol. **140:** 169-194.

HANN, B. J. & ZRUM, L. (1997): Littoral microcrustaceans (Cladocera, Copepoda) in a prairie coastal wetland: seasonal abundance and community structure. - Hydrobiologia **357:** 37-52.

HESSEN, D. O. (1985): Selective zooplankton predation by pre-adult roach (*Rutilus rutilus*): The size-selective hypothesis versus the visibility-selective hypothesis. - Hydrobiologia **124:** 73-79.

HUGHES, D. N., BOYER, M. G., PAPST, M. H., FOWLE, C. D., REES, G. A. V. & BAULU, P. (1980): Persistence of three organophosphorus insecticides in artificial ponds and some biological implications. - Arch. Environ. Contam. Toxicol. **9:** 269-279.

HURLBERT, S. H. (1975): Secondary effects of pesticides on aquatic ecosystems. - Res. Rev. **57:** 81-148.

HURLBERT, S. H., MULLA, M. S., KEITH, J. O., WESTLAKE, W. E. & DÜSCH, M. E. (1970): Biological effects and persistence of Dursban® in freshwater ponds. - J. Econ. Entomol. **63:** 43-52.

HURLBERT, S. H., MULLA, M. S. & WILSON, H. R. (1972): Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations of fresh-water ponds. - Ecol. Monogr. **42:** 269-299.

KNOECHEL, R. & HOLTBY, L. B. (1986): Construction and validation of a body-length model for the prediction of cladoceran community filtering rates. - Limnol. Oceanogr. **31:** 1-16.

MACEK, K. J., WALSH, D. F., HOGAN, J. W. & HOLZ, D. D. (1972): Toxicity of the insecticide Dursban to fish and aquatic invertebrates in ponds. - Trans. Am. Fish. Soc. **3:** 420-427.

MARSHALL, W. K. & ROBERTS, J. R. (1978): Ecotoxicology of chlorpyrifos. - Environmental Secretariat N.R.C.C. Ottawa, Canada, Na **16079**.

MCDONALD, I. A., HOWES, D. A. & GILLIS, N. A. (1985): The determination of the physico-chemical parameters of chlorpyrifos. Rep. GH-C **1393**. DowElanco, Indianapolis, IN.

MCDOUGAL, R. L., GOLDSBOROUGH, L. G. & HANN, B. J. (1997): Responses of a prairie wetland to press and pulse additions of inorganic nitrogen and phosphorus: production by planktonic and benthic algae. - Arch. Hydrobiol. **140:** 145-167.

MOSS, B., STANSFIELD, J., IRVINE, K., PERROW, M. & PHILLIPS, G. (1996): Progressive restoration of a shallow lake: a 12-year experiment in isolation, sediment removal and biomanipulation. - J. Appl. Ecol. **33:** 71-86.

MURKIN, H. R., POLLARD, J. B., STAINTON, M. P., BOUGHEN, J. A. & TITMAN, R. D. (1994): Nutrient additions to wetlands in the interlake region of Manitoba, Canada: effects of periodic additions throughout the growing season. - Hydrobiol. **280:** 483-495.

NEELY, R. K. & BAKER, J. L. (1989): Nitrogen and phosphorus dynamics and the fate of agricultural runoff. - In: VAN DER VALK, A. (ed.): Northern Prairie Wetlands. - Iowa State Univ. Press, Ames, Iowa, pp. 92-131.

PENNAK, R. W. (1978): Freshwater Invertebrates of the United States. 2nd Ed. - John Wiley and Sons, New York, NY.

PETTIGREW, C. T., HANN, B. J. & GOLDSBOROUGH, L. G. (1998): Waterfowl feces as a source of nutrients to a prairie wetland: responses of microinvertebrates to experimental additions. - Hydrobiologia **362:** 55-66.

RACKE, K. D. (1993): Environmental fate of chlorpyrifos. - Rev. Environ. Contam. Toxicol. **131:** 1-154.

RAWN, D. F. K. (1998): The transport and deposition of current use pesticides and PCBs to surface waters in the Red River drainage basin. - Ph. D. Thesis. University of Manitoba. 393 pp.

REYNOLDS, C. S. (1994): The ecological basis for the successful biomanipulation of aquatic communities. - Arch. Hydrobiol. **130:** 1-33.

RIJTEMA, P. E. & KROES, J. G. (1991): Nitrogen modeling on a regional scale. - In: BOGARDI, I. & KUZELKA, R. D. (eds.): Nitrate Contamination. - NATO ASI Series, Vol. G **30**, Springer, Berlin, Heidelberg, pp. 81-95.

SARNELLE, O. (1992): Nutrient enrichment and grazer effects on phytoplankton in lakes. - Ecology **73:** 551-560.

SCHEFFER, M., HOSPER, S. H., MEIJER, M. - L., MOSS, B. & JEPPESEN, E. (1993): Alternative equilibria in shallow lakes. - Trends in Ecology and Evolution **8:** 275-279.

SCHRIVER, P., BOGESTRAND, J., JEPPESON, E. & SONDERGAARD, M. (1995): Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions: large-scale enclosure experiments in a shallow eutrophic lake. - Freshwat. Biol. **33:** 255-270.

SIEFERT, R. E., LOZANO, S. J., BRAZNER, J. C. & KNUTH, M. L. (1989): Littoral enclosures for aquatic field testing of pesticides: effects of chlorpyrifos on a natural system. - Miscellaneous Publication Series Entomological Society of America **75:** 57-73.

SMITH, K. & FERNANDO, C. H. (1978): A guide to the freshwater calanoid and cyclopoid Copepod Crustacea of Ontario. - Univ. Waterloo Biol. Ser. No. **18:** 74 pp.

STAINTON, M. P., CAPEL, M. J. & ARMSTRONG, F. A. J. (1977): The chemical analysis of freshwater. 2nd Ed. - Fisheries and Environment Canada. Misc. Spec. Publ. **25**.

TER BRAAK, C. J. F. (1986): Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. - Ecology **67:** 1167-1179.

TER BRAAK, C. J. F. (1988): CANOCO - a FORTRAN program for canonical community ordination. - Microcomputer Power, Ithaca, NY.

VAN DEN BRINK, P. J., VAN DONK, E., GYLSTRA, R., CRUM, S. J. H. & BROCK, T. C. M. (1995): Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. - Chemosphere **31**: 3181-3200.

VAN DEN BRINK, P. J., VAN WIJNGAARDEN, R. P. A., LUCASSEN, W. G. H., BROCK, T. C. M. & LEEUWANGH, P. (1996): Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. - Environ. Toxicol. Chem. **15**: 1143-1153.

VAN DONK, E., PRINS, H., VOOGD, H. M., CRUM, S. J. H. & BROCK, T. C. M. (1995): Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms: I. Responses of plankton and zooplanktivorous insects. - Arch. Hydrobiol. **133**: 417-439.

VANNI, M. J. (1987): Effects of nutrients and zooplankton size on the structure of a phytoplankton community. - Ecology **68**: 624-635.

VAN WIJNGAARDEN, R., LEEUWANGH, P., LUCASSEN, W. G. H., ROMIJN, K., RONDAY, R., VAN DER VELDE, R. & WILLIGENBURG, W. (1993): Acute toxicity of chlorpyrifos to fish, a newt, and aquatic invertebrates. - Bull. Environ. Contam. Toxicol. **51**: 716-723.

VAN WIJNGAARDEN, R. P. A., VAN DEN BRINK, P. J., VOSHAAR, J. H. O. & LEEUWANGH, P. (1995): Ordination techniques for analysing response of biological communities to toxic stress in experimental ecosystems. - Ecotoxicology **4**: 61-77.

ZRUM, L. & HANN, B. J. (1998): Responses of a prairie wetland to press additions
of inorganic nitrogen and phosphorus and to the addition of the insecticide
Lorsban™ 4E (active ingredient chlorpyrifos): Planktonic microinvertebrate
community structure. - University Field Station (Delta Marsh) Annual
Report **32:** 56-67. On-line at: http://www.umanitoba.ca/faculties/
science/delta_marsh

CHAPTER 3: Effects of organophosphorus insecticide and inorganic nutrients on the invertebrate community and algae associated with submersed macrophytes in a prairie wetland

INTRODUCTION

Prairie wetlands are shallow-water ecosystems typically existing in one of two states, a clear-water, macrophyte-dominated or a turbid, phytoplankton-dominated one. The ambient factors facilitating the establishment and maintenance of these alternative states have been modeled by SCHEFFER et al. (1993), extensively discussed in SCHEFFER (1998), and investigated in a prairie wetland ecosystem using experimental enclosures (HANN & GOLDSBOROUGH 1997, MCDOUGAL et al. 1997, SANDILANDS et al. 2000, ZRUM et al. 2000). Grazing and nutrient recycling by planktonic microinvertebrates and macrophyte-associated invertebrates are potential mechanisms for effecting control over primary producers in shallow-water ecosystems (BROCK et al. 1995, VAN DONK et al. 1995, HANN & GOLDSBOROUGH 1997). Primary producers may be stimulated through a reduction in grazing pressure (direct toxicity of insecticide to arthropods) or nutrient enrichment (increased nutrients available for reproduction and growth), thereby potentially altering the stable state of the prairie wetland.

Blind Channel in Delta Marsh (MB, Canada) typifies one of the two states frequently observed in shallow-water ecosystems (SCHEFFER et al. 1993). It is characterized by relatively high turbidity and phytoplankton biomass and an invertebrate community proportionately dominated by copepods (particularly cyclopoids) throughout the open-water season (HANN & ZRUM 1997). Experimental enclosure of sections of Blind Channel leads to a decrease in turbidity in the water column by limiting resuspension of bottom sediments through wind action and the feeding activity of large, detritivorous fish (e.g., Carp, *Cyprinus carpio*). A reduction of turbidity increases the light available for submersed macrophyte growth and may permit earlier germination and establishment of submersed macrophytes in the enclosures relative to Blind

Channel (GOLDSBOROUGH & HANN 1996). Once established, the enclosures are typified by clear-water. Over the open-water season, the relative proportion of open water and submersed macrophytes changes. Early in the season the water in the enclosures clears and submersed macrophytes are sparse; as the season progresses, open water becomes less apparent as submersed macrophytes grow and occupy a greater proportion of space within the enclosures. The experimental enclosures develop over time to exist in the clear-water, macrophyte-dominated state.

Many of the freshwater wetlands in North America are infringed upon by agricultural land. Common use of pesticides (herbicides and insecticides) and fertilizers by agriculture for commercial crop protection and improved production has resulted in increased pesticide contamination and nutrient loading of adjacent wetlands via run-off, spray drift, leaching to surface and ground water, and accidental spills (NEELY & BAKER 1989, FRANK et al. 1990, RIJTEMA & KROES 1991, GOLDSBOROUGH & CRUMPTON 1998). These toxic chemicals and additional nutrients are known to affect the biotic communities of freshwater wetlands (BROCK et al. 1992a, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996, HANN & GOLDSBOROUGH 1997, MCDOUGAL et al. 1997, SANDILANDS et al. 2000, ZRUM et al. 2000). However, little information is available pertaining to either the direct or the indirect response of the invertebrate community associated with submersed macrophytes to contaminants and nutrient loading in freshwater prairie wetlands.

The presence of abundant submersed macrophytes alters the functioning of shallow-water systems in a number of ways, including the following: 1) they provide a refuge for smaller invertebrates from predation by planktivorous fish (e.g., fathead minnows, *Pimephales promelas*) and invertebrate predators (e.g., insect larvae, *Hydra*, flatworms); 2) they potentially alter the chemical dynamics of the system by inhibiting the homogenization of water (e.g., contaminant and nutrient gradients); 3) they stabilize the bottom sediments, thereby limiting the resuspension of bottom sediments; and 4) they provide an immense surface for

the growth of attached algae (epiphyton) and biofilms (complex communities of algae, bacteria, and small animals), thereby providing an abundant food source for larger organisms (SCHEFFER 1998).

This paper describes results of a study to investigate the invertebrate dynamics and community structure of prairie wetland enclosures in the clear-water, macrophyte-dominated state subject to controlled organophosphorus insecticide application and inorganic nutrient enrichment. Specifically, responses of the microinvertebrate and macroinvertebrate communities in association with submersed macrophytes to the following experimental manipulations were examined: 1) alterations in the community structure of the microinvertebrates (e.g., Cladocera, Copepoda, Ostracoda, Rotifera) induced by differential mortality caused by a single application of the organophosphorus insecticide Lorsban™ 4E (emulsifiable formulation with 41 % (w/w) chlorpyrifos as the active ingredient); 2) changes in the community structure of the macroinvertebrates (e.g., Insecta, Oligochaeta, Amphipoda, Gastropoda) induced by differential mortality caused by a single application of chlorpyrifos; and 3) changes in epiphytic algal biomass induced by inorganic nutrient enrichment via small additions of nitrogen and phosphorus at regular, frequent intervals. Within the scope of the present study a previous paper presented results for the microinvertebrates and algae in the open water of the same experimental system (ZRUM et al. 2000).

Organophosphorus insecticide treatment was expected to result in differential mortality of the arthropod component of the microinvertebrate and macroinvertebrate communities. Specifically, a reduction in arthropod grazer control of epiphytic algae was expected to lead to an increase in epiphyton biomass relative to the control, provided resources (e.g., nutrients) were not limiting and non-arthropod herbivores (e.g., gastropods, *Stylaria*) did not increase in abundance. Inorganic nutrient enrichment was expected to result in an increase in epiphyton biomass relative to the control.

The first objective in this paper was to examine temporal variation in structure of the microinvertebrate community in association with submersed macrophytes in prairie wetland mesocosms subjected to experimental perturbations (treatments). Secondly, variation in the structure of the macroinvertebrate community in association with submersed macrophytes subjected to the same treatments was examined. The final objective was to present a concise synthesis of the impacts of insecticide application and nutrient enrichment on the dynamics and community structure of both the open-water planktonic community and the complex community associated with submersed macrophytes in the prairie wetland mesocosms.

METHODS

Study site and experimental design

The experiment was conducted in Blind Channel in Delta Marsh, Manitoba, a 22,000 ha freshwater lacustrine wetland (98° 23'W, 50° 11'N) in south-central Manitoba, bordered to the south by fertile agricultural land and aspen parkland, and separated from Lake Manitoba to the north by a forested beach ridge.

Experimental enclosures (mesocosms) used model the freshwater wetland community characteristic of the study site under investigation. Enclosures (12, 5 m x 5 m) were installed in Blind Channel on 27 May at a water depth of < 1 m. Each enclosure was constructed using impermeable woven polyethylene curtain supported on floating platforms. Curtains extended from above the water surface down to the sediments, where they were anchored with iron bars at least 30 cm into the sediments, thereby preventing direct exchange of water between the enclosures and Blind Channel. Enclosures were open on top to the atmosphere. Total volume of water per enclosure was approximately 22,000 L. Fish (primarily fathead minnows, *Pimephales promelas*) trapped during installation were removed using commercial minnow traps, monitored daily for the duration of the experiment.

Experimental treatments (insecticide addition, nutrient enrichment, control) were assigned to enclosures using a restricted latin square design, ensuring none of the three replicate enclosures for each treatment was adjacent or contiguous with another. An additional three enclosures were part of another experiment not presented with this study. Sampling for this component of the study was initiated on 9 July (week 5) and continued weekly until 26 August (week 12), 1997. Insecticide applied was in the form of Lorsban™ 4E, an emulsifiable formulation with chlorpyrifos as the active ingredient. Chlorpyrifos addition was made once on 14 July to produce a nominal concentration of 10 µg/L in the water column. Inorganic nitrogen (as analytical grade $NaNO_3$) and phosphorus (as $NaH_2PO_4 \cdot 2H_2O$) were added to nutrient enrichment enclosures three times per week beginning on 23 June. Equal cumulative N and P loads (23.4 and 3.2 g/m^2 of wetland bottom, respectively) were added to each nutrient treatment enclosure by the end of the experiment. Water sampling for chlorpyrifos and physico-chemical analyses in the enclosures have been described by ZRUM et al. (2000). See APPENDIX 1 for detailed chlorpyrifos sampling and analysis method.

Sampling and analysis of invertebrates and algae

Invertebrates and epiphyton associated with submersed aquatic macrophytes were sampled quantitatively using a Downing Box of a design from DOWNING (1986) (see APPENDIX 3 for detailed method). Two Downing Box samples (6 L) were taken randomly from each enclosure weekly, beginning on 9 July. Water from the sample collected was filtered through a 53 µm mesh and densities (ind./L) of microinvertebrates collected were determined (see HANN & GOLDSBOROUGH 1997 for method). Macroinvertebrates were removed from the fresh macrophytes, collected separately, and density (ind./L) was determined. The fresh macrophytes were sorted to species, dried at 106 °C for 24 h, and then weighed to obtain dry weight data for each species. Epiphyton on fresh macrophytes was sampled, analyzed for chlorophyll *a* using methods of

MCDOUGAL et al. (1997), and expressed as micrograms chlorophyll *a* per gram total dry weight of macrophytes (μg/g).

Percent cover of enclosure bottom by submersed macrophyte species (*Potamogeton zosteriformis*, *P. pectinatus*, *Ceratophyllum demersum*, and *Myriophyllum sibiricum*) was estimated by visual inspection each week. Macrophyte biomass (g/m^2 of wetland bottom) was measured on 16 June, 10 July, and 13 August in each enclosure using a large plastic cylinder (d = 0.78 m; A = 0.48 m^2) (see ZRUM et al. 2000 for method). The size of the mesocosms made it difficult to obtain representative samples of the macrophyte community more frequently. The alteration of the macrophyte canopy caused by sampling could change substantially the chemical and biological environment for subsequent samples.

The Downing Box samples habitat comprising both water and submersed macrophytes in varying proportions. Therefore, samples obtained may be treated in two ways: 1) density of invertebrates in the sample calculated as the number of individuals per unit volume; or 2) density of invertebrates in the sample calculated as the number of individuals per unit dry weight of macrophyte as used in DOWNING (1986). Dependent on the proportion of macrophytes relative to water in a sample, numbers of invertebrates will be estimated more accurately as either individuals per unit volume of water or individuals per unit dry weight of macrophyte. Due to the facultative nature of most invertebrates, i.e., they occur everywhere in shallow-water ecosystems, but some species are more frequently encountered in the water column and others in association with macrophytes, it is difficult to estimate accurately the densities of individuals represented in Downing Box samples. In order to compare results from this component of the study with those presented in ZRUM et al. (2000), all invertebrate densities are expressed as individuals per unit volume.

Invertebrates were identified using standard references, including EDMONDSON (1959), PENNAK (1978), SMITH & FERNANDO (1978), and MERRITT & CUMMINS (1996), and reference collections (BJH and KAS). Cladocera were

identified to species and enumerated. Copepoda were counted as nauplii and Cyclopoida and Calanoida copepodites and adults; only adults were identified to species. Among rotifers, only the predatory species, *Asplanchna* sp., was enumerated separately. All macroinvertebrates were identified to order and counted, with only select taxa identified to genus.

Data analysis

Univariate analysis

Insecticide addition, nutrient enrichment, and control treatments all contained three, submersed macrophyte-dominated replicates. Replicates included in data analysis were characterized by clear water (low phytoplankton biomass) and submersed macrophytes with similar areal proportions and density for the duration of the study. BROCK et al. (1992a, 1992b) observed differences in the fate and effects of chlorpyrifos between water with macrophytes and open-water systems, so the inter-replicate similarity with respect to development of submersed macrophytes was an important aspect of the experiment. For each sampling date, mean densities of microinvertebrates (ind./L) and macroinvertebrates (ind./L), mean epiphyton biomass as chlorophyll *a* (μg/g dry weight of macrophytes), and mean values for all physico-chemical parameters were estimated for all enclosures. Any differences between treated and control replicates were compared statistically using the program SPSS (version 10.0, SPSS Inc.). Data were tested for normal distribution and homogeneity of variance, and, if necessary, ln (x+1)-transformed prior to analysis using one-way ANOVA for each sampling date. If differences in the mean values among treatment replicates were greater than would be expected by chance alone, pair-wise multiple comparisons among treatments were carried out using the Student-Newman-Keuls (SNK) method. Treatment effects were considered statistically significant at $p \leq 0.05$. The ability to detect significant differences among treatments was limited due to a lack of statistical power in most instances as a result of the small number of replicates. For the first sample date (week 5) for all

treatments the number of degrees of freedom (df) was 8 due to the reduction of the nutrient addition treatment to two replicates as submersed macrophytes had not sufficiently developed in one replicate to allow for efficient sampling with the Downing Box. For all subsequent dates and treatments, df = 9 as all treatments contained three replicates. All estimates of treatment and control means are presented as mean ± SE.

Multivariate analysis

Relationships between microinvertebrate species and environmental data and between macroinvertebrate taxa and environmental data were examined using canonical correspondence analysis (CCA). Ordinations were performed using the program CANOCO (version 3.10, TER BRAAK 1988). For each sampling date, mean densities of microinvertebrate species and macroinvertebrate taxa, mean biomass of epiphyton, % cover of enclosure bottom by macrophytes, mean total dry weight of macrophytes in Downing Box samples, mean concentrations of nitrate, soluble reactive phosphorus, and alkalinity, pH, % saturation of oxygen, and water temperature were estimated for all enclosures.

For the microinvertebrates, mean densities of species (ind./L) were calculated for each sampling date for each treatment. A species x sample date (for each treatment) matrix was produced using ln (x+1)-transformed data to stabilize variances. Biotic environmental parameters, biomass of epiphyton (EPIPHYTON) and % cover of enclosure bottom by submersed macrophytes (% COVER), and abiotic parameters, mean concentration of nitrate (NITRATE), soluble reactive phosphorus (SRP), and alkalinity (ALK), pH (pH), % saturation of oxygen (% SAT), and water temperature (TEMP) were included in an environmental variable x sample date (for each treatment) matrix. If necessary, environmental data were ln (x+1)-transformed to stabilize variances. The statistical significance of the relationship between species composition and canonical axes (constrained by the set of environmental variables) was tested using a Monte Carlo permutation test (n=999); the statistical importance of

specific environmental variables was determined through forward selection of environmental variables and subsequent testing with a Monte Carlo permutation test (n=999) (TER BRAAK 1988).

For the macroinvertebrates, mean densities of taxa (ind./L) were calculated for each sampling date for each treatment. A taxa x sample date (for each treatment) matrix was produced using ln (x+1)-transformed data. The environmental variable x sample matrix produced for the microinvertebrate data was used. Total dry weight of macrophytes in Downing Box samples was included in a covariable x sample data (for each treatment) matrix in an attempt to account for differences in the relative amount of total macrophytes obtained in a sample; covariable data were ln (x+1)-transformed. The statistical significance of the relationship between taxa composition and canonical axes (constrained by the set of environmental variables) was tested using a Monte Carlo permutation test. The statistical importance of specific environmental variables was again determined through forward selection of environmental variables and subsequent testing with a Monte Carlo permutation test (TER BRAAK 1988).

Invertebrate densities are assumed to be unimodal functions along environmental gradients in CCA. Axes are constrained by the fraction of total variance in the invertebrate data that is explained by the environmental variables measured; an ordination diagram is produced by detecting patterns of variation in invertebrate community composition that can be best accounted for by the environmental variables quantified (TER BRAAK 1986). The diagram shows the pattern of variation in invertebrate composition as accounted for by the environmental variables measured and the distributions of invertebrates along environmental gradients. The influence of an environmental variable on the distribution of invertebrates may be limited by designating it as a covariable. Use of covariables allows one potentially to account for systematic differences among samples taken. Invertebrate taxa and sites (each treatment over time) are represented as points and environmental variables as lines (or vectors). Site points lie at the centroid of the invertebrate taxa points that occur in them; a site

point that lies close to an invertebrate point likely has a high density of that particular invertebrate taxon. Sites that are similar in invertebrate composition and relative density will lie close together on the diagram, while sites that differ in relative density of a similar set of invertebrates or in their invertebrate composition will lie further apart. Longer environmental vectors are more highly correlated with the ordination axes shown and the corresponding environmental variable has a greater influence on the pattern of invertebrate community variation (TER BRAAK 1988).

RESULTS

Chlorpyrifos in the water column

Chlorpyrifos concentrations in the overlying water in the insecticide treatment enclosures following addition on 14 July are summarized by ZRUM et al. (2000). The chlorpyrifos concentration in the insecticide treatment one hour after application was 4.79 ± 1.28 µg/L. After 12 hours, 61 - 82 %, and after 24 hours, 18 - 100 % of the measured dose could be detected in the water column. Chlorpyrifos concentrations in the water column declined until 1.5 days after application. On day 2 after application, the concentration of chlorpyrifos in the insecticide enclosures increased to 5.26 ± 0.53 µg/L; by day 3 after application, chlorpyrifos concentrations had declined to levels similar to 1 day post-treatment.

Environmental variables

Water temperature, percent oxygen saturation, pH, and selected water chemistry parameters are summarized in ZRUM et al. (2000). Mean water temperature increased during June and fluctuated among treatments during July and August. Dissolved oxygen measurements at 10 and 50 cm depths did not differ significantly in any enclosure for the entire experimental period (ANOVA, p > 0.05), therefore an average value was used for each sampling date. Percent

oxygen saturation was significantly lower in the nutrient addition treatment than in the control or insecticide treatment from the middle of July through to the beginning of August (ZRUM et al. 2000). From the middle of June through to the middle of July, pH was significantly higher in the nutrient treatment. In August, pH was significantly higher in the insecticide treatment than in the control and nutrient treatment (ZRUM et al. 2000).

The N and P concentrations in the water column of control and insecticide treatment were low (SRP ~55-140 µg/L ; NO_3-N <50 µg/L, or below detection limit; NH_3-N ~23-29 µg/L) and the nutrient treatment was observed consistently to exceed control and insecticide treatment concentrations (ZRUM et al. 2000). Levels of inorganic N and P in the nutrient treatment (SRP ~1080 µg/L ; NO_3-N ~883 µg/L; NH_3-N ~170 µg/L) were significantly higher than in control and insecticide treatment for most of the experiment.

Submersed macrophytes were not affected by treatments as mean percent cover of enclosure bottom by submersed macrophytes and macrophyte biomass in treatments and control replicate enclosures were not different statistically (ZRUM et al. 2000).

Microinvertebrate abundance

The microcrustacean community associated with submersed macrophytes included 12 species of cladocerans, 3 species of cyclopoid copepods, the calanoid copepod *Diaptomus nudus*, and a harpacticoid copepod (Table 3-1). There were 14 microcrustacean species found in the insecticide treatment, compared with 15 species in each of the control and nutrient treatment; 12 species were common to all treatments.

Maximum density of cladocerans (641-4264 ind./L), consisting primarily of *Bosmina longirostris, Ceriodaphnia dubia*, and *Simocephalus* spp., occurred in the control and insecticide treatment near the beginning of July, one week prior to insecticide application (Fig. 3-1). Cladoceran density peaked (4321 ind./L) in

the nutrient treatment one week later and consisted primarily of the same species. Following insecticide addition on 14 July, cladoceran density in the insecticide treatment declined to 6 ind./L, but was significantly lower than in the nutrient treatment for 1-week post-treatment only ($F_{2,9}$ = 10.49, p ≤ 0.05). Cladoceran density continued to decline in the insecticide treatment and was significantly lower than in the control and nutrient treatment on 6 August ($F_{2,9}$ = 29.84, p ≤ 0.05) and 20 August ($F_{2,9}$ = 21.28, p ≤ 0.05). Nutrient addition had a significant positive effect on cladoceran density ($F_{2,9}$ = 10.49, p ≤ 0.05) near the end of July only, approximately one month after nutrient addition treatment had begun.

Small rotifers peaked in density (572-1332 ind./L) in the insecticide and nutrient treatments near the end of July, but did not peak in the control until the beginning of August (245 ind./L) (Fig. 3-1). Density of small rotifers declined in all treatments throughout August. Insecticide addition had no significant effect on small rotifer density on 23 July ($F_{2,9}$ = 4.15, p > 0.05), 6 August ($F_{2,9}$ = 4.86, p > 0.05), or 20 August ($F_{2,9}$ = 9.10, p > 0.05). The density of small rotifers in the nutrient treatment was significantly lower than in the insecticide treatment at the beginning of August ($F_{2,9}$ = 4.86, p ≤ 0.05) and significantly lower than in the control and insecticide treatment near the end of August ($F_{2,9}$ = 9.10, p ≤ 0.05).

Density of ostracods was low (17-62 ind./L) in all treatments near the beginning of July (Fig. 3-1). After insecticide application, ostracod density declined to 3 ind./L, but was not significantly lower than in the control ($F_{2,9}$ = 14.80, p > 0.05). Ostracods increased in density in the control and nutrient treatment through August and were significantly higher than in the insecticide treatment on 6 August ($F_{2,9}$ = 16.94, p ≤ 0.05) and 20 August ($F_{2,9}$ = 11.92, p ≤ 0.05). As with cladocerans, nutrient addition had a significant positive effect on ostracod density near the end of July ($F_{2,9}$ = 14.80, p ≤ 0.05).

Copepod nauplii density was highest (506 ind./L) in the insecticide treatment at the beginning of July (Fig. 3-2). However, nauplii density did not

peak in the nutrient treatment (746 ind./L) until the end of July and the control (543 ind./L) until the beginning of August. Insecticide application reduced nauplii density to 33 ind./L; density continued to decline through the end of August. Nauplii density was significantly lower in the insecticide treatment than in the control and nutrient treatment on 6 August ($F_{2,9}$ = 153.42, p ≤ 0.05) and 20 August ($F_{2,9}$ = 12.42, p ≤ 0.05). At the beginning of July, nauplii density was significantly lower in the nutrient treatment than in the control and insecticide treatment ($F_{2,8}$ = 8.42, p ≤ 0.05).

Total cyclopoid copepod density peaked at the beginning of July in the control and insecticide treatment (1116-1574 ind./L), but was not significantly higher than in the nutrient treatment ($F_{2,8}$ = 4.39, p > 0.05) (Fig. 3-2). Addition of chlorpyrifos reduced cyclopoid density to < 1 ind./L. Cyclopoid density was significantly lower in the insecticide treatment than in the control and nutrient treatment 1-week after chlorpyrifos addition ($F_{2,9}$ = 15.72, p ≤ 0.05) and on 6 August ($F_{2,9}$ = 23.19, p ≤ 0.05) and 20 August ($F_{2,9}$ = 22.23, p ≤ 0.05). At the end of July, cyclopoid density was significantly higher in the nutrient treatment than in the control and insecticide treatment ($F_{2,9}$ = 15.72, p ≤ 0.05).

Calanoid copepods were rare in association with submersed macrophytes in all treatments throughout the experiment (3-29 ind./L), except in the control near the beginning of July (264 ind./L) (Fig. 3-2). Calanoid density did not differ significantly among treatments near the beginning of July ($F_{2,8}$ = 2.38, p > 0.05), 1-week after chlorpyrifos application ($F_{2,9}$ = 0.03, p > 0.05), on 6 August ($F_{2,9}$ = 0.19, p > 0.05), or 20 August ($F_{2,9}$ = 1.09, p > 0.05).

Epiphyton biomass was elevated in the control and insecticide treatment near the beginning of July and in the nutrient treatment at the end of July, approximately one month after nutrient addition was initiated (Fig. 3-3). Peaks in epiphyton biomass at the beginning of July in the control and insecticide treatment (1526-1678 µg/g) and at the end of July in the nutrient treatment (1835 µg/g) corresponded to higher densities of cladocerans (Fig. 3-3).

Table 3-1. Microcrustacean species associated with submersed macrophytes occurring in experimental enclosures in Delta Marsh, Manitoba (July to August 1997).

| Species	Control	Insecticide	N+P
Cladocera			
Pseudochydorus globosus (Baird) 1850			X
Diaphanosoma birgei KORINEK 1981	X	X	X
Bosmina longirostris (O.F. MÜLLER) 1785	X	X	X
Ceriodaphnia dubia RICHARD 1894	X	X	X
Daphnia sp.	X	X	X
Scapholeberis kingi SARS 1903	X	X	X
Simocephalus spp.	X	X	X
Alona sp.	X	X	X
Camptocercus sp.	X		X
Chydorus sp.	X		X
Eurycercus longirostris HANN 1982	X	X	X
Pleuroxus denticulatus BIRGE 1878	X	X	X
Copepoda (Cyclopoida)			
Microcyclops varicans rubellus (LILLJEBORG) 1901	X	X	
Acanthocyclops vernalis (FISCHER) 1853	X	X	X
Diacyclops thomasi (S.A. FORBES) 1882	X	X	X
Copepoda (Calanoida)			
Diaptomus nudus MARSH 1904	X	X	X
Copepoda (Harpacticoida)		X	
Total number of species	15	14	15

Fig. 3-1. Changes in cladoceran, small rotifer, and ostracod density (ind./L ± SE) in association with submersed macrophytes over an 8-week period in enclosures treated with the insecticide chlorpyrifos (Insecticide), enclosures loaded with inorganic nutrients (N+P), and in control enclosures (Control). The horizontal dotted line denotes thrice weekly press additions of inorganic nutrients from 23 June to 27 August; the arrow denotes the moment of insecticide application. Significant differences ($p < 0.05$) between treatments are presented in the horizontal bars at the top of the graph: I = Control versus Insecticide enclosures; II = Control versus N+P enclosures; III = Insecticide versus N+P enclosures. **O** = 1-way ANOVA significant, followed by post-hoc multiple comparisons test (SNK).
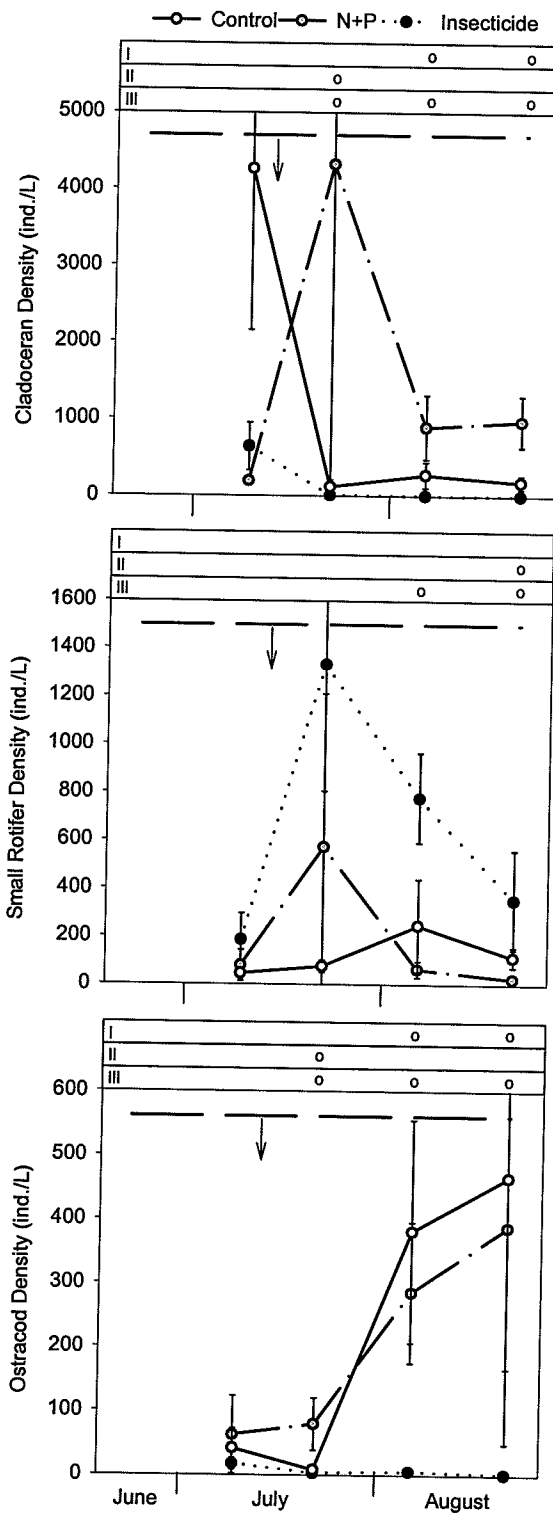
Fig. 3-2. Changes in copepod nauplii, total (copepodites and adults) cyclopoid copepod, and total (copepodites and adults) calanoid copepod density (ind./L ± SE) in association with submersed macrophytes over an 8-week period in enclosures treated with the insecticide chlorpyrifos (Insecticide), enclosures loaded with inorganic nutrients (N+P), and in control enclosures (Control). Symbols are identified as in Fig. 3-1.
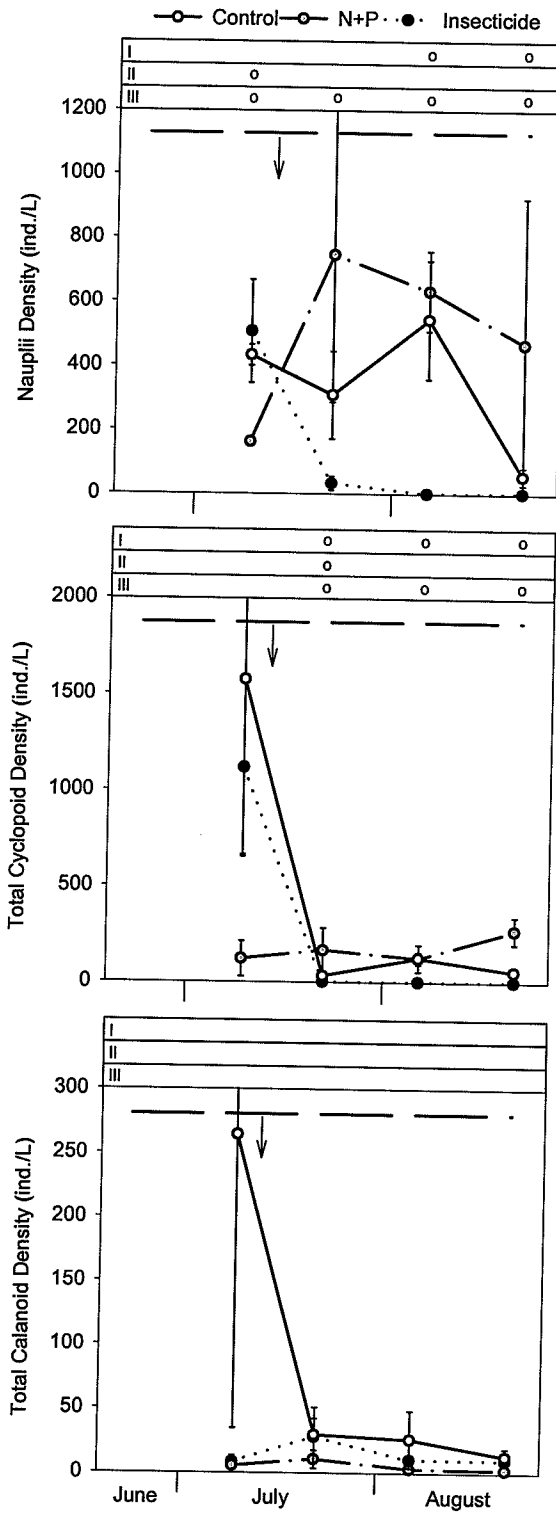
Fig. 3-3. Changes in cladoceran density (ind./L ± SE) in association with submersed macrophytes and epiphyton biomass as chlorophyll *a* (µg/g ± SE) over an 8-week period in control enclosures (Control), enclosures treated with the insecticide chlorpyrifos (Insecticide), and enclosures loaded with inorganic nutrients (N+P). The horizontal dotted line denotes thrice weekly press additions of inorganic nutrients from 23 June to 27 August; the arrow denotes the moment of insecticide application.
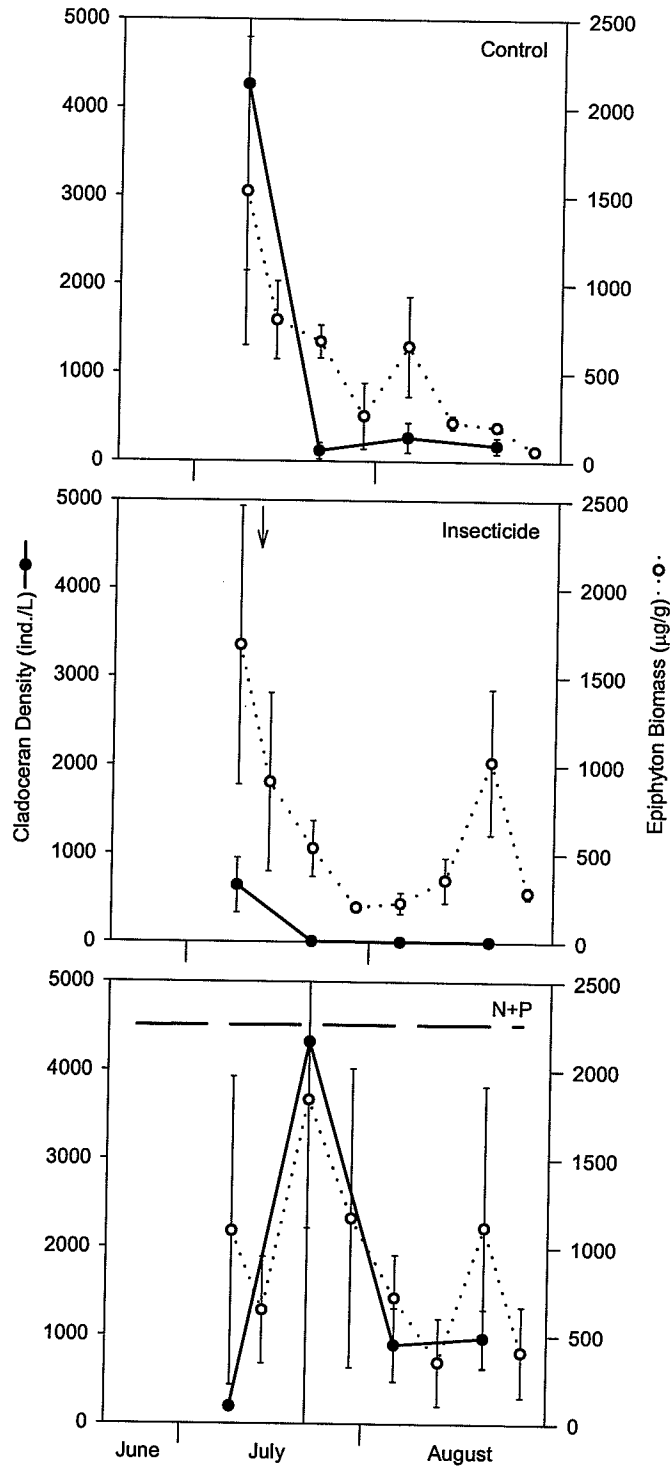
Epiphyton biomass did not differ significantly among treatments throughout the experiment (p > 0.05).

Macroinvertebrate abundance

The macroinvertebrate community included 2 species of oligochaetes, the amphipod species *Hyalella* sp., 2 gastropod species, several unidentified species of water mites, and 12 taxa of insects throughout the experiment (Table 3-2). There were 14 macroinvertebrate taxa observed in the insecticide treatment, compared with 15 taxa in each of the control and nutrient treatment; 11 taxa were common to all treatments.

Macroinvertebrate taxa were rare (< 1-24 ind./L) in comparison to most microinvertebrates observed in association with submersed macrophytes. Three taxa that occurred in higher densities were zygopteran larvae, *Hyalella* sp., and *Gyraulus* sp. Densities of zygopteran larvae and *Gyraulus* sp. did not differ significantly among treatments for the duration of the experiment (p > 0.05). Density of *Hyalella* sp. was significantly higher in the nutrient treatment than in the control and insecticide treatment at the end of August ($F_{2,9}$ = 21.97, p ≤ 0.05). Consideration of Insecta taxa together as a group revealed no significant trends for treatments throughout the experiment.