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

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

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

The University of Manitoba Field Station (Delta Marsh) provided logistical support necessary for the successful completion of this project. Ken Sandilands and Kristen Elvin assisted with sample collection, April Kiers and Kristen Elvin performed analyses for water chemistry and algal biomass data, and Dr. Gordon Goldsborough provided water chemistry and algal biomass data. Dr. Norm Kenkel advised on multivariate analyses, and Thor Halldorson and Dr. Gary Stern provided invaluable assistance with analyses for chlorpyrifos. Dr. Jon Anderson (DowElanco, Winnipeg, MB) provided the Lorsban™ 4E used in this experiment.

I would like to thank my committee members, Drs. Lane Graham, Lyle Lockhart, Norm Kenkel, and Gordon Goldsborough for their support throughout my graduate studies and whose constructive comments helped improve the quality of this thesis. In particular, I would like to express my gratitude to my advisor, Dr. Brenda Hann, whose unending support and valuable guidance throughout this entire process has meant the world to me. I would also like to extend my thanks to Laurel Neufeld, Ken Sandilands, Rene Harrison, and my family, particularly my mother, Kaye Dent, for the emotional support they have given me throughout my Master's program.

This study was supported by a Natural Sciences and Engineering Research Council (NSERC) Scholarship.

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

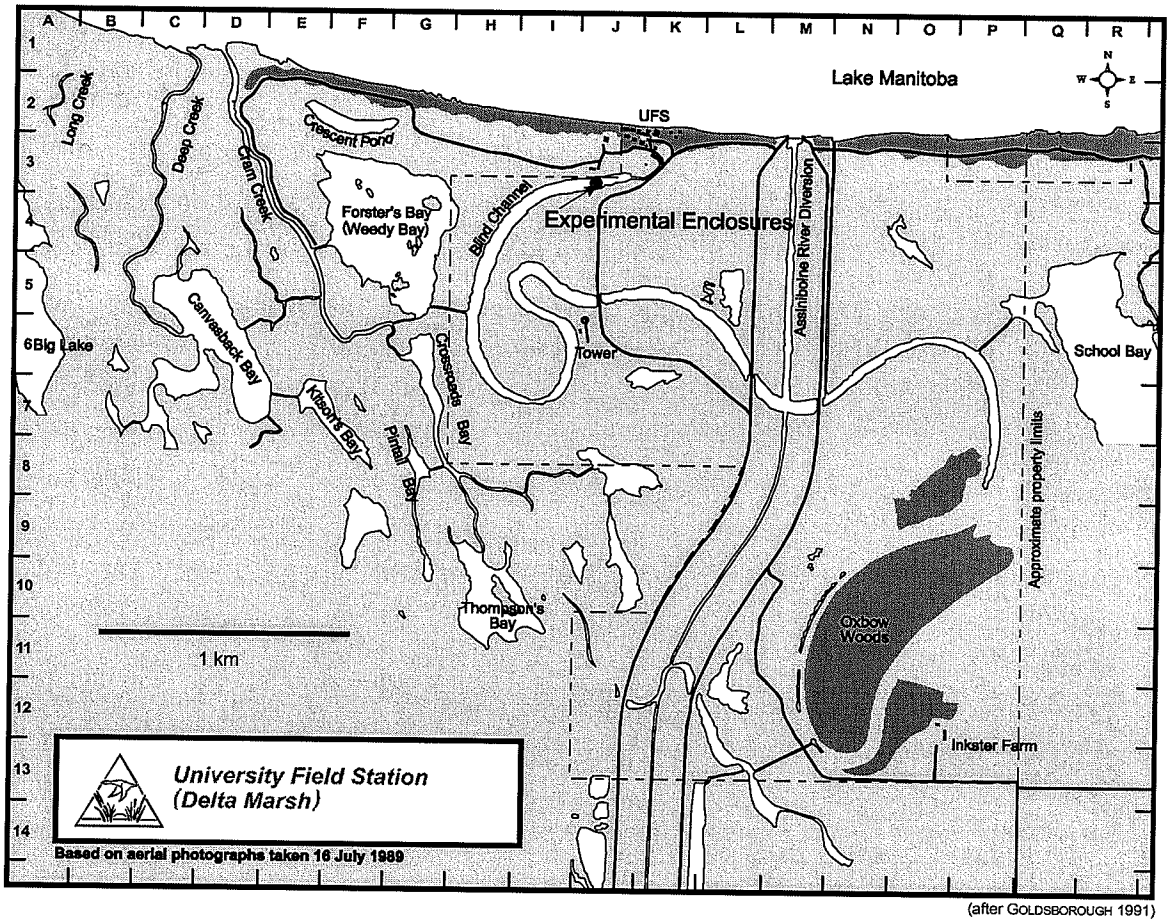


Fig. 1-1. Location of experimental enclosures in Blind Channel, Delta Marsh, 1997.

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.

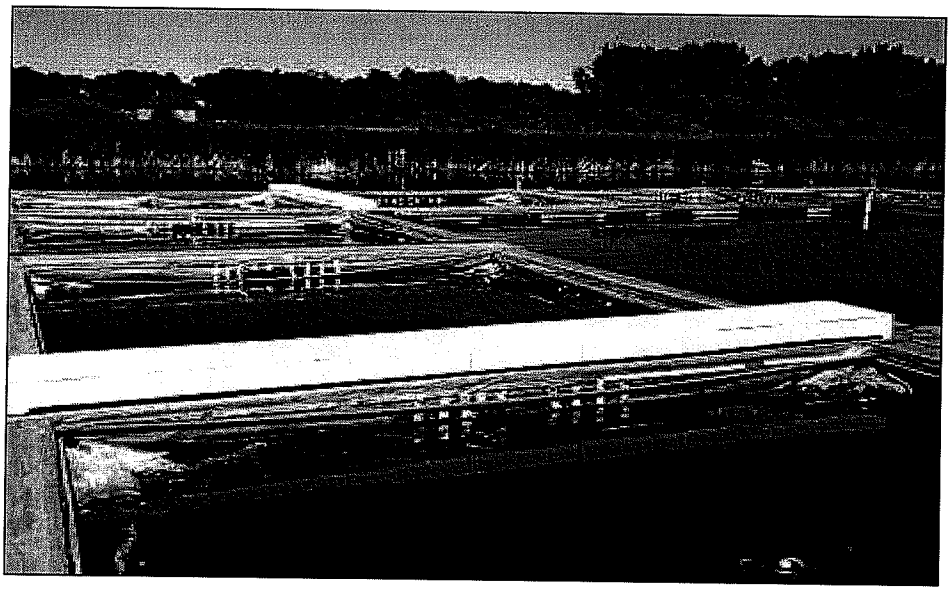
Application of chlorpyrifos and nutrients

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



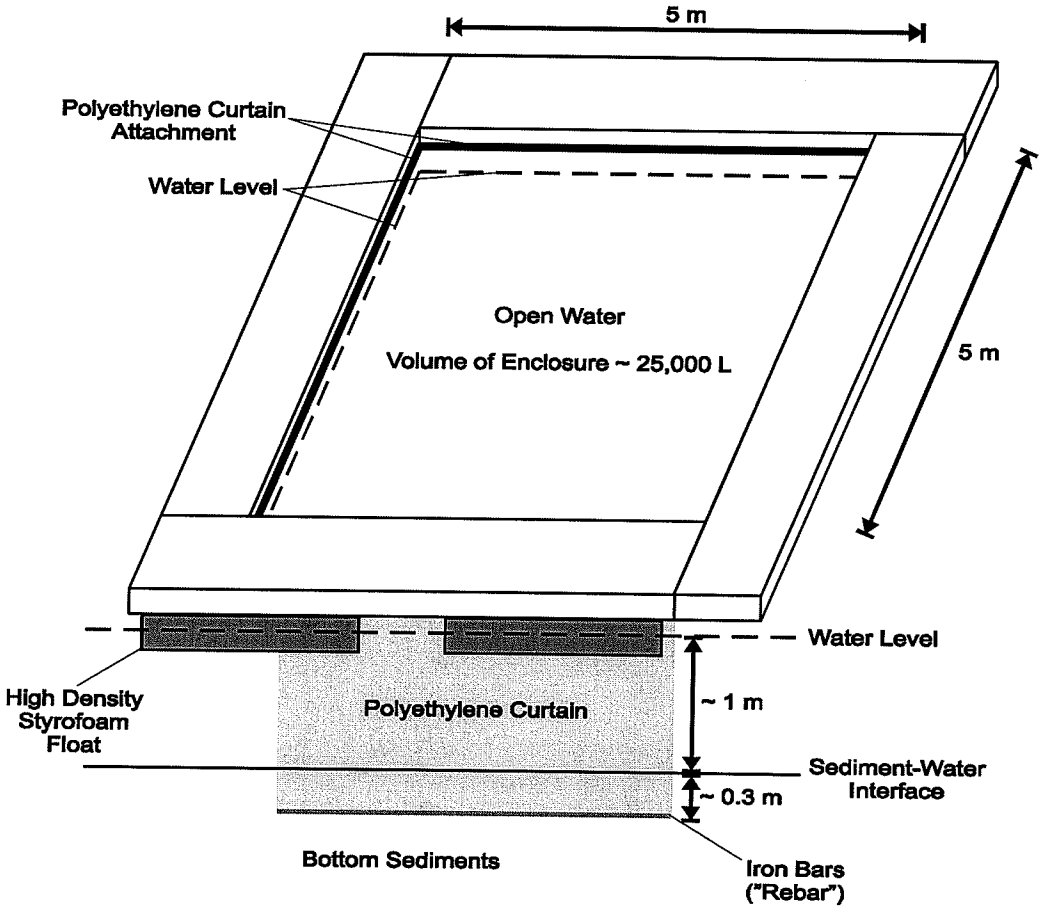


Fig. 1-2. Experimental enclosures used to model freshwater wetland communities characteristic of study site under investigation.

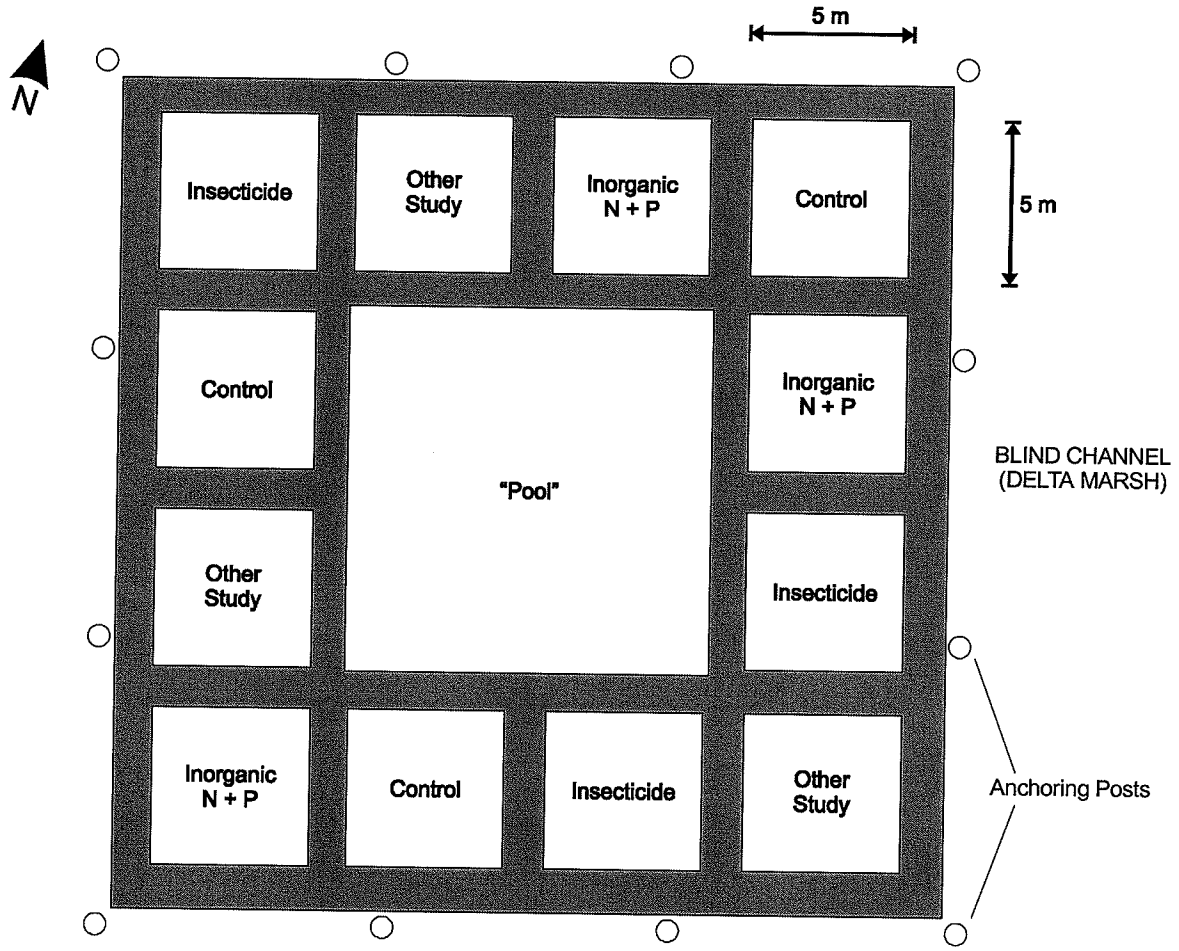


Fig. 1-3. Experimental treatments (insecticide application, inorganic nutrient enrichment, control) assigned to enclosures.

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.

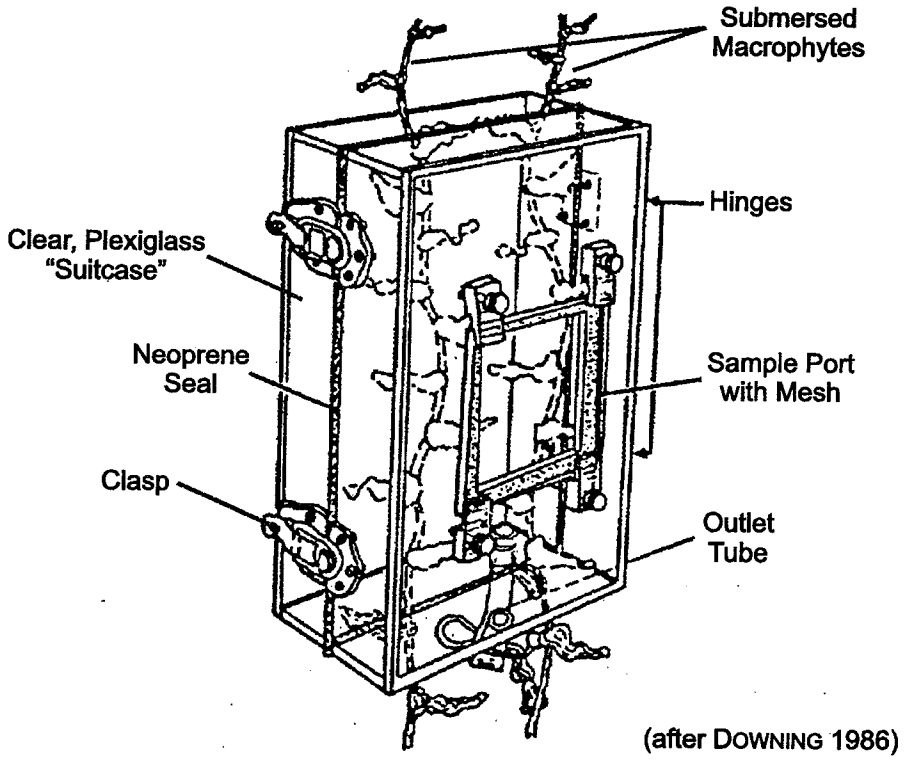


Fig. 1-4. Downing Box used to sample invertebrates and epiphyton associated with submersed aquatic macrophytes.

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 $NaH_2PO_4 \cdot 2H_20$) 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 ± 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 (≤ 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)

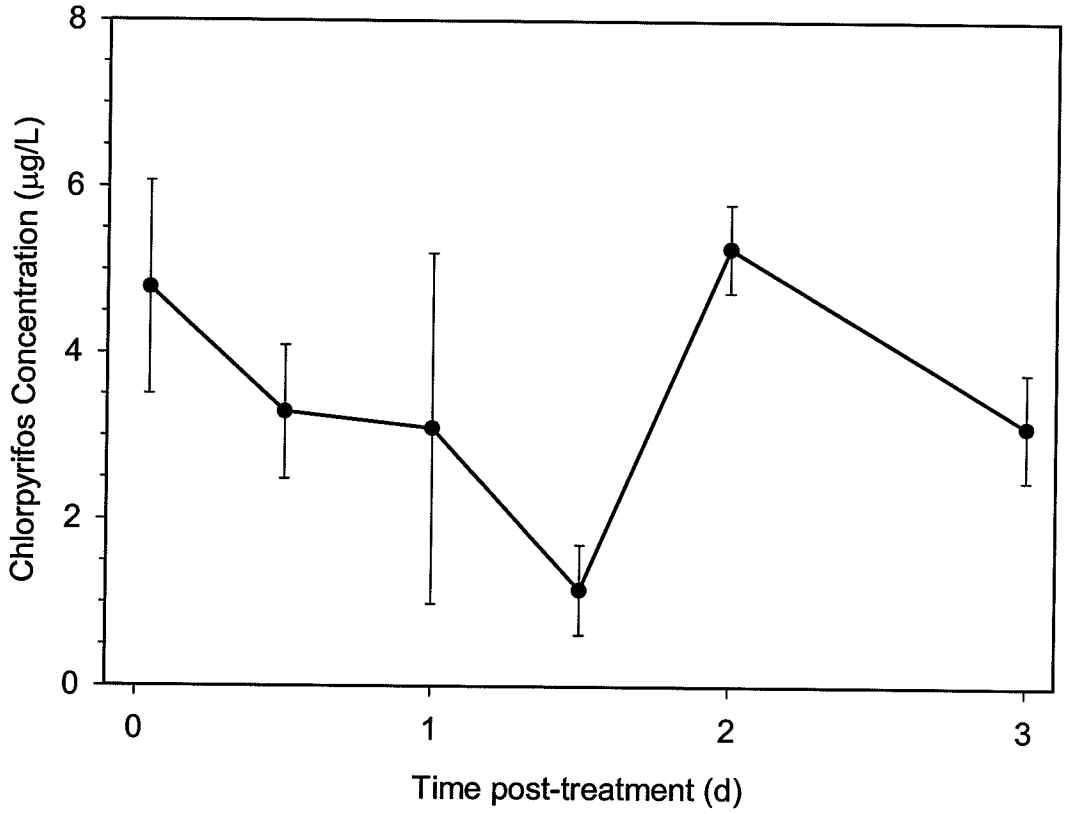


Fig. 2-1. Mean concentration of chlorpyrifos (µg/L ± SE) in the water column of enclosures treated with the insecticide chlorpyrifos after application of a nominal concentration of 10 µg/L.

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.

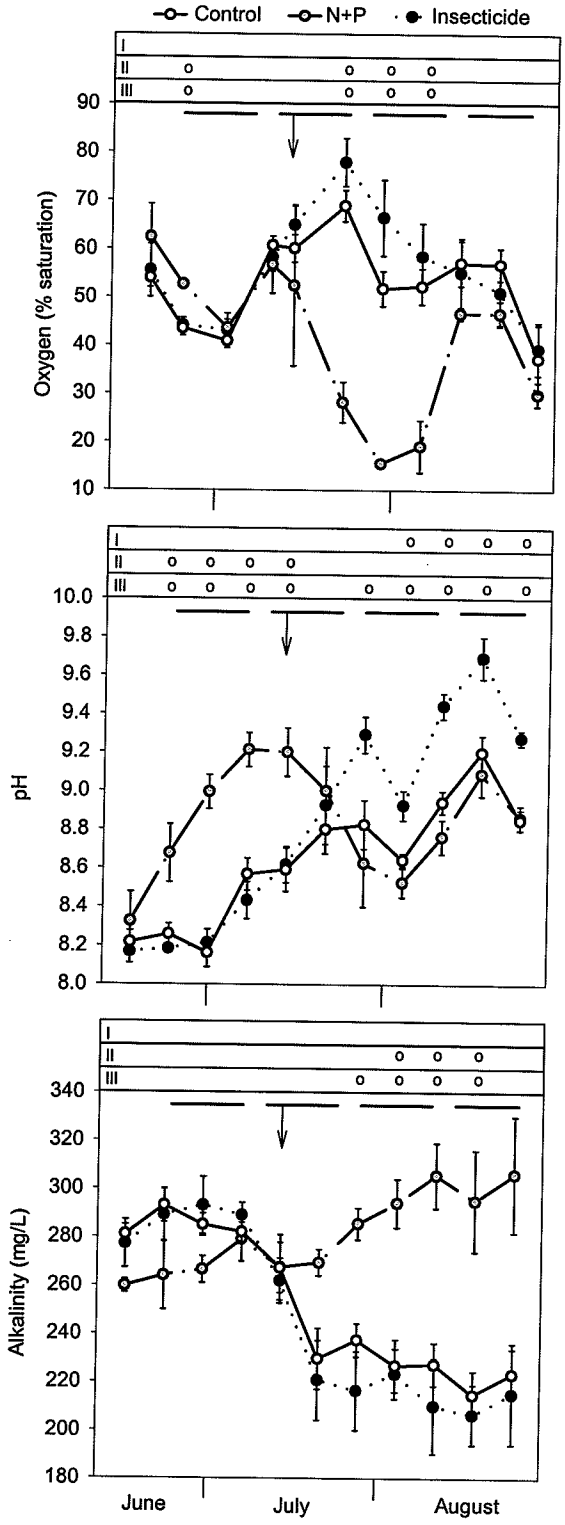
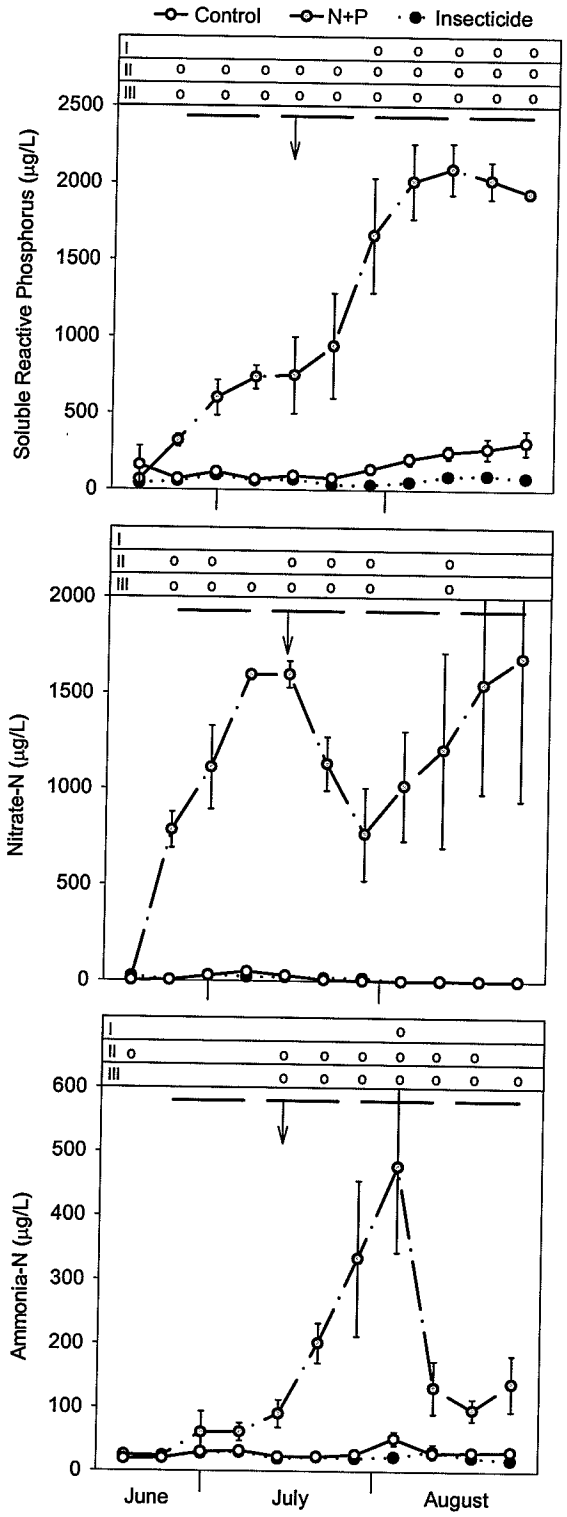

Control
N+P
Insecticide
I
II
III
Oxygen (% saturation)
90
80
70
60
50
40
30
20
10
pH
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
Alkalinity (mg/L)
340
320
300
280
260
240
220
200
180
June
July
August

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.


Control
N+P
Insecticide
Soluble Reactive Phosphorus (µg/L)
Nitrate-N (µg/L)
Ammonia-N (µg/L)
June
July
August

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.

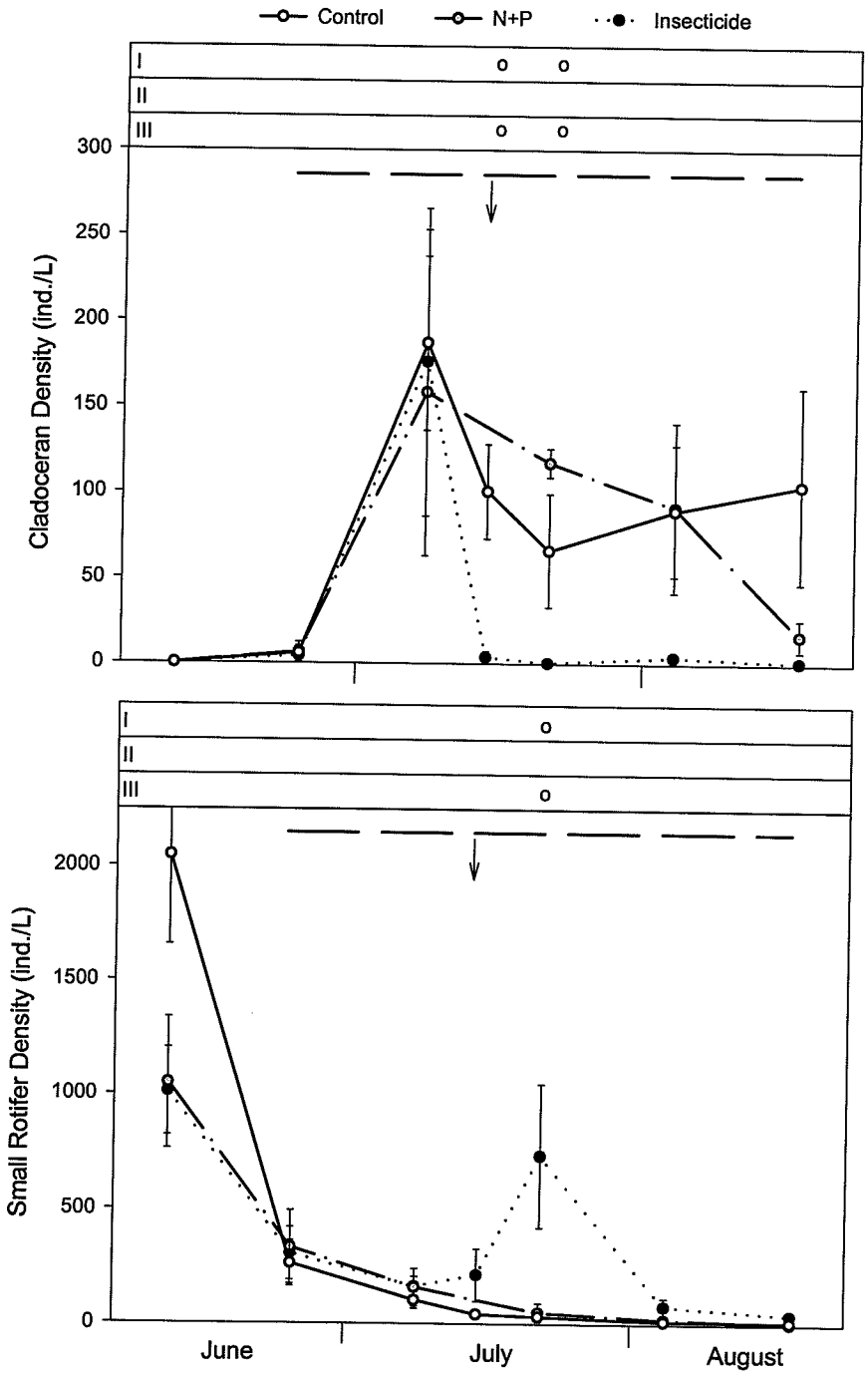

Control
N+P
Insecticide
I
II
III
Cladoceran Density (ind./L)
300
250
200
150
100
50
0
Small Rotifer Density (ind./L)
2000
1500
1000
500
0
June
July
August

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.

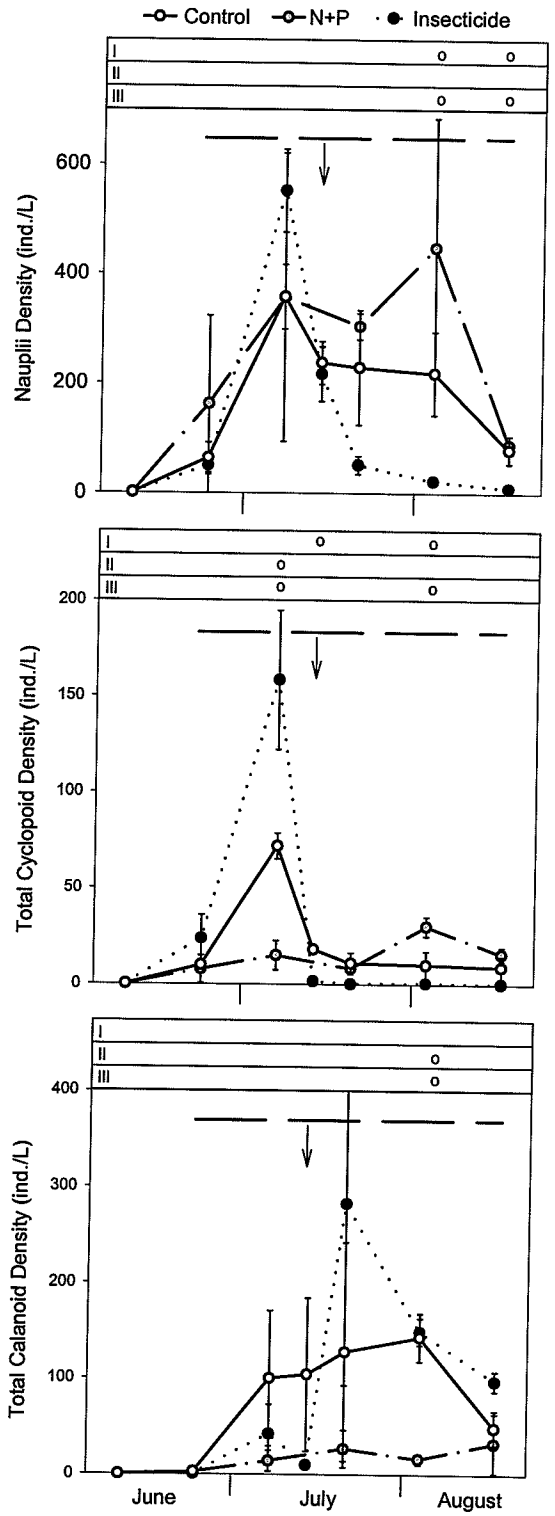
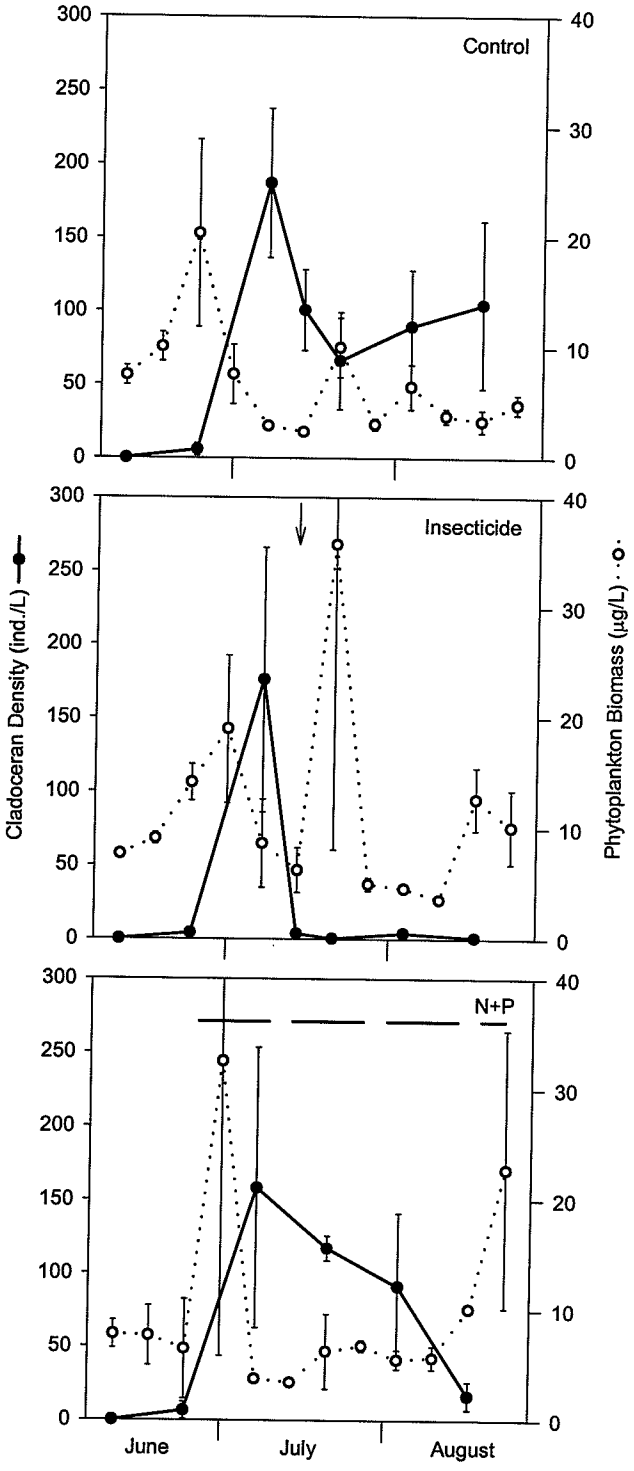

Control
N+P
Insecticide
I
II
III
Nauplii Density (ind./L)
600
400
200
0
Total Cyclopoid Density (ind./L)
200
150
100
50
0
Total Calanoid Density (ind./L)
400
300
200
100
0
June
July
August

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.


Control
Insecticide
N+P
Cladoceran Density (ind./L)
Phytoplankton Biomass (µg/L)
300
250
200
150
100
50
0
40
30
20
10
0
June
July
August

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

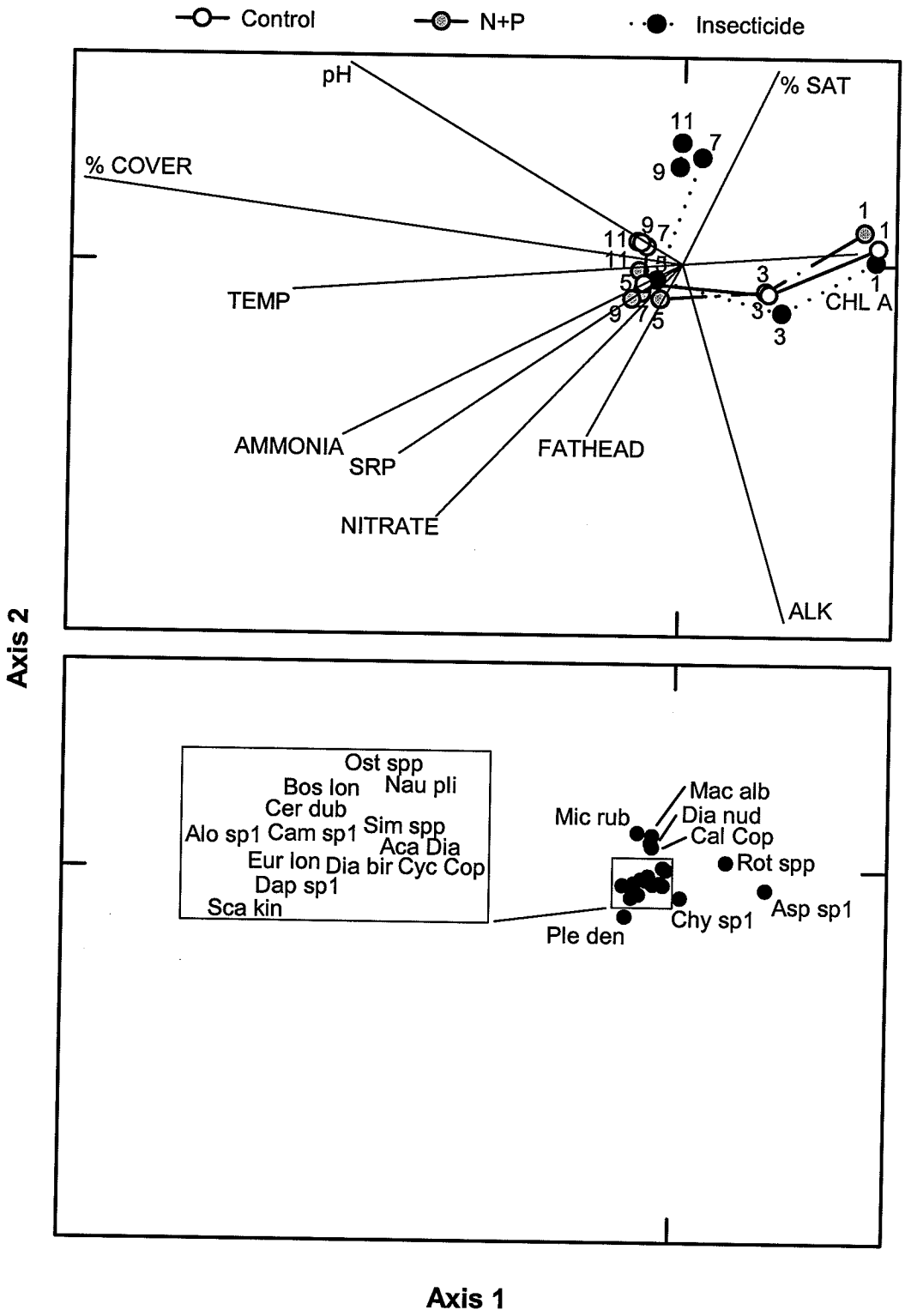
nutrient treatment had negative values on axis 2, indicating higher alkalinity and nitrate concentrations, likely related to inorganic nutrient addition. Sample dates during July and August for control and insecticide treatments (with two exceptions, (5)C and (5)I) had positive values on axis 2, indicating 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.


Control
N+P
Insecticide
pH
% SAT
% COVER
TEMP
CHL A
AMMONIA
SRP
FATHEAD
NITRATE
ALK
Ost spp
Bos lon
Nau pli
Cer dub
Alo sp1
Cam sp1
Sim spp
Aca Dia
Eur lon
Dia bir
Cyc Cop
Dap sp1
Sca kin
Mac alb
Mic rub
Dia nud
Cal Cop
Rot spp
Asp sp1
Chy sp1
Ple den
Axis 2
Axis 1

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

Sampling assistance was provided by K. SANDILANDS and K. ELVIN. The University of Manitoba Field Station (Delta Marsh) supplied logistic support essential for the completion of this project, T. HALLDORSON provided assistance with analyses for chlorpyrifos, and N. KENKEL advised on multivariate analyses. We thank Dr. J. ANDERSON (DowElanco, Winnipeg, MB) for providing the Lorsban™ 4E used in this experiment. The authors are indebted to two anonymous reviewers whose constructive comments helped improve the clarity of this manuscript. This study was supported in part by a Natural Sciences and Engineering Research Council (NSERC) Scholarship to LZ and NSERC Research Grant to LGG. This is contribution number 289 from the University of Manitoba Field Station (Delta Marsh).

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 $NaH_2PO_4 \cdot 2H_20$) 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 ($F_{2,9} = 10.49$, $p \leq 0.05$). Cladoceran density continued to decline in the insecticide treatment and was significantly lower than in the control and nutrient treatment on 6 August ($F_{2,9} = 29.84$, $p \leq 0.05$) and 20 August ($F_{2,9} = 21.28$, $p \leq 0.05$). Nutrient addition had a significant positive effect on cladoceran density ($F_{2,9} = 10.49$, $p \leq 0.05$) 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 ($F_{2,9} = 4.86$, $p \leq 0.05$) and significantly lower than in the control and insecticide treatment near the end of August ($F_{2,9} = 9.10$, $p \leq 0.05$).

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 ($F_{2,9} = 16.94$, $p \leq 0.05$) and 20 August ($F_{2,9} = 11.92$, $p \leq 0.05$). As with cladocerans, nutrient addition had a significant positive effect on ostracod density near the end of July ($F_{2,9} = 14.80$, $p \leq 0.05$).

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 ($F_{2,9} = 153.42$, $p \leq 0.05$) and 20 August ($F_{2,9} = 12.42$, $p \leq 0.05$). At the beginning of July, nauplii density was significantly lower in the nutrient treatment than in the control and insecticide treatment ($F_{2,8} = 8.42$, $p \leq 0.05$).

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 ($F_{2,9} = 15.72$, $p \leq 0.05$) and on 6 August ($F_{2,9} = 23.19$, $p \leq 0.05$) and 20 August ($F_{2,9} = 22.23$, $p \leq 0.05$). At the end of July, cyclopoid density was significantly higher in the nutrient treatment than in the control and insecticide treatment ($F_{2,9} = 15.72$, $p \leq 0.05$).

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).

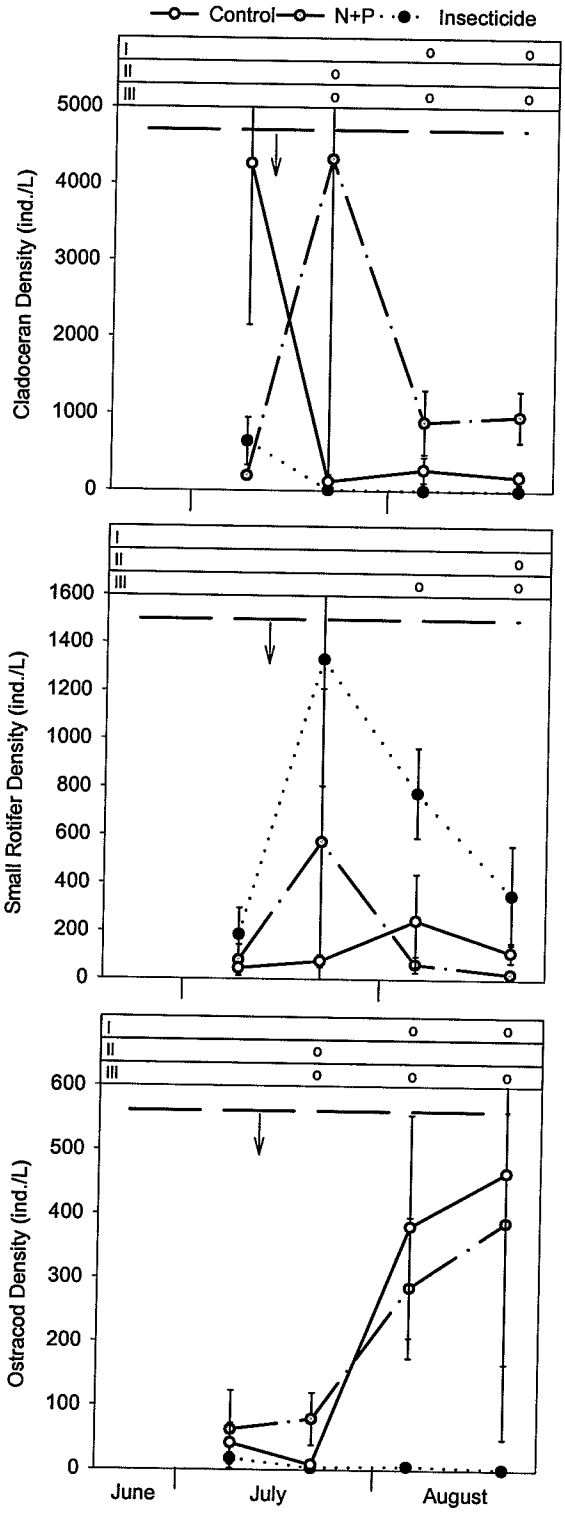

Control
N+P
Insecticide
I
II
III
Cladoceran Density (ind./L)
5000
4000
3000
2000
1000
0
Small Rotifer Density (ind./L)
1600
1400
1200
1000
800
600
400
200
0
Ostracod Density (ind./L)
600
500
400
300
200
100
0
June
July
August

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.

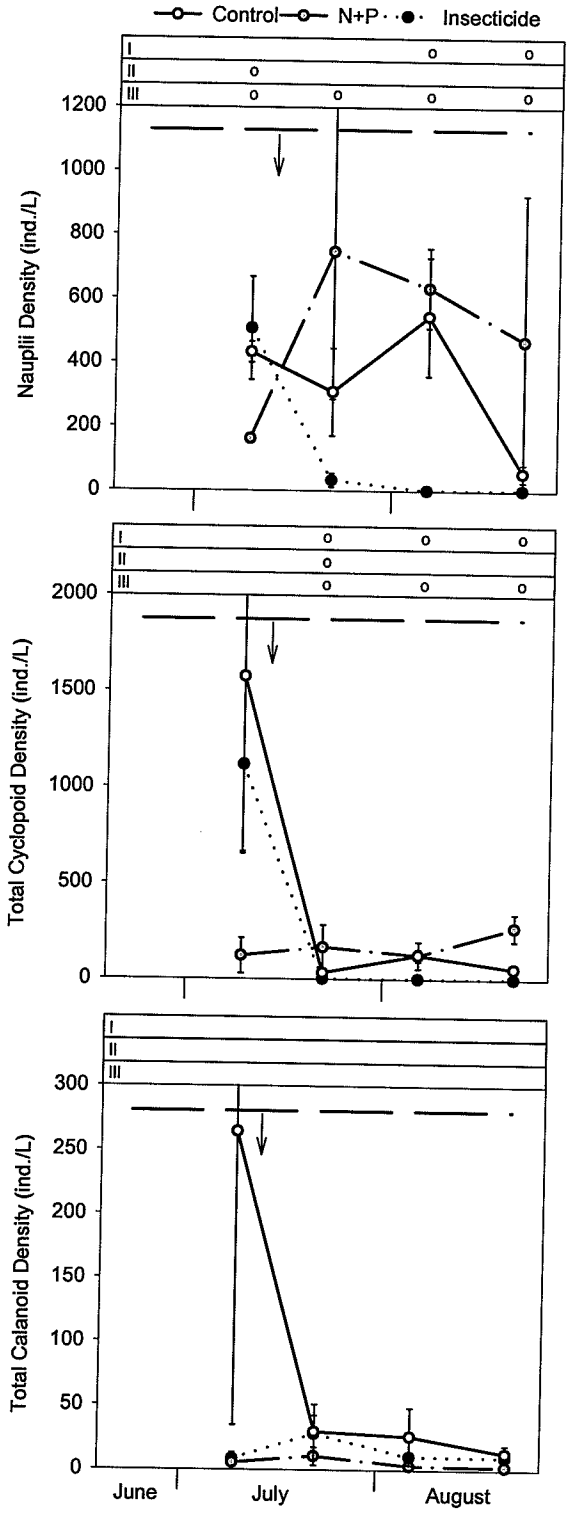
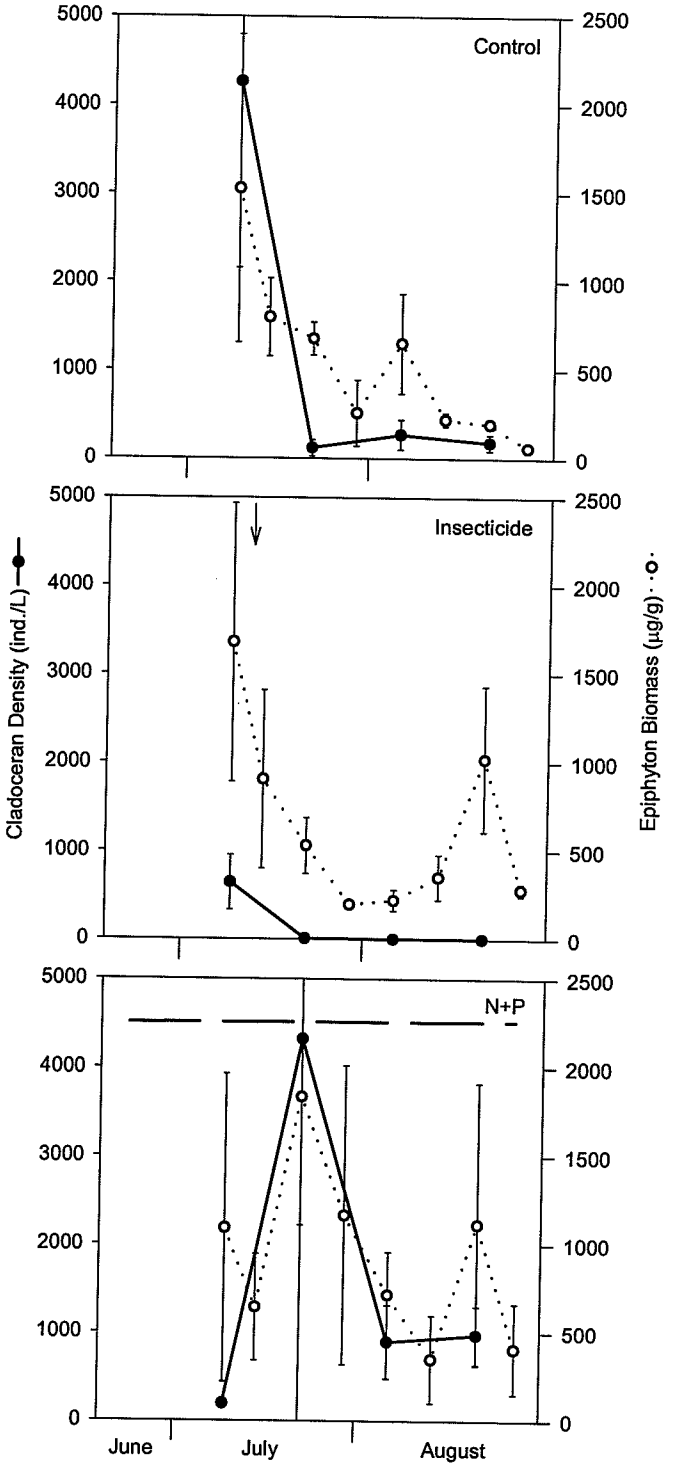

Control
N+P
Insecticide
I
II
III
Nauplii Density (ind./L)
1200
1000
800
600
400
200
0
Total Cyclopoid Density (ind./L)
2000
1500
1000
500
0
Total Calanoid Density (ind./L)
300
250
200
150
100
50
0
June
July
August

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.


Control
Insecticide
N+P
Cladoceran Density (ind./L)
Epiphyton Biomass (µg/g)
5000
4000
3000
2000
1000
0
2500
2000
1500
1000
500
0
June
July
August

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 ($F_{2,9} = 21.97$, $p \leq 0.05$). Consideration of Insecta taxa together as a group revealed no significant trends for treatments throughout the experiment.

Table 3-2. Macroinvertebrate taxa associated with submersed macrophytes occurring in experimental enclosures in Delta Marsh, Manitoba (July to August 1997).

Taxon	Control	Insecticide	N+P
Oligochaeta			
Stylaria sp.		X	
Chaetogaster sp.		X	
Amphipoda			
Hyalella sp.	X	X	X
Gastropoda			
Physa sp.	X	X	X
Gyraulus sp.	X	X	X
Hydracarina (Water Mite)	X	X	X
Insecta			
Chironomidae larvae	X	X	X
Chironomidae pupae		X	X
Acentria sp. larvae	X	X	X
Acentria sp. pupae	X		
Dytiscidae larvae	X	X	X
Ephemeroptera larvae	X		X
Trichoptera larvae	X	X	X
Zygoptera larvae	X	X	X
Corixidae nymphs	X	X	X
Corixidae adults	X		X
Notonectidae nymphs	X	X	X
Notonectidae adults	X		X
Total number of taxa	15	14	15

Microinvertebrate community structure

CCA of the microinvertebrate community in association with submersed macrophytes produced eigenvalues for the first two canonical axes of 0.177 and 0.052. CCA axis 1 was significantly related to microinvertebrate community composition ($F = 1.76$, $p = 0.05$), and the first four axes together were significant ($F = 2.13$, $p = 0.02$). The eight environmental variables included in the analysis explained 89 % of the total variance in the microinvertebrate data. When the environmental variables were forward selected, only SRP ($F = 3.92$, $p = 0.01$) was significantly related to microinvertebrate community composition. This environmental variable alone accounted for 30 % of the variance in the microinvertebrate data.

Axis 1 was most correlated with SRP and alkalinity, and axis 2 with temperature and % cover of enclosure bottom by submersed macrophytes (Table 3-3). Environmental variables were plotted in a biplot (Fig. 3-4) with site points representing each treatment on sampling dates. The temporal sequence of sample dates on axis 1 reflects primarily the influence of the abiotic parameters SRP, nitrate, and alkalinity, and to a lesser extent pH and % saturation of O_2. Sample dates for the nutrient treatment, particularly in August, had higher negative values on axis 1, indicating higher SRP, nitrate, and alkalinity concentrations, likely related to inorganic nutrient addition. Samples dates in July and August for control and insecticide treatments had more positive values on axis 1, indicating lower nutrient concentrations and increased pH and % saturation of O_2. Higher positive values on axis 1 for insecticide treatment sample dates from the middle of July through August are likely related to the application of chlorpyrifos on 14 July. Separation among sampling dates with respect to water temperature and % cover of enclosure bottom by submersed macrophytes is shown on axis 2. Sample dates in early July (week 5) for nutrient and insecticide treatment had positive values on axis 2, corresponding to warmer water temperatures and lower % cover of enclosure bottom by submersed macrophytes. All sample dates (with two exceptions, (7)N and (9)I) from the

middle of July through to the end of August for nutrient and insecticide treatment had negative values on this axis, indicating a cooling in water temperatures and higher % cover of enclosure bottom by submersed macrophytes. Therefore, on axis 2, the temporal sequence of sample dates reflects ordering of site points according to cooling of water temperature and increasing cover of enclosure bottom by submersed macrophytes. Cooling of water temperature may be indicative of the increased extent of the submersed macrophyte canopy during August and the increased reflectance of sunlight.

Microinvertebrate species were plotted on the same CCA axis 1 and 2 as for sampling dates (Fig. 3-4). Species with a positive score on axis 1 (e.g., harpacticoid copepods, *Stylaria* sp., *Microcyclops varicans rubellus*) were more abundant in waters with higher pH and % saturation of O_2. The position of species points for the above mentioned taxa, as well as for *Diaptomus nudus*, *Chaetogaster* sp., small rotifer species, and calanoid copepodites were likely also influenced by insecticide treatment, resulting in a higher positive score on axis 1. Species with a negative score on axis 1 (e.g., *Chydorus* sp., *Pseudochydorus* sp., *Scapholeberis kingi*, *Alona* sp.) occurred in waters with higher concentrations of SRP, nitrate, and alkalinity, likely related to inorganic nutrient enrichment.

Macroinvertebrate community structure

CCA of the macroinvertebrate community produced eigenvalues for the first two canonical axes of 0.385 and 0.206. The first axis was not significantly related to macroinvertebrate community composition ($F = 1.50$, $p = 0.27$), however, the first four axes together were significant ($F = 2.40$, $p = 0.02$). The eight environmental variables included in the analysis explained 91 % of the total variance in the macroinvertebrate data. When the environmental variables were forward selected, % cover of enclosure bottom by macrophytes ($F = 4.55$, $p = 0.01$) and SRP ($F = 2.59$, $p = 0.02$) were significantly related to macroinvertebrate

community composition. Together these two variables accounted for 50 % of the total variance in the macroinvertebrate data.

Axis 1 was most strongly correlated with % cover of enclosure bottom by submersed macrophytes and alkalinity concentration, and axis 2 with SRP concentration and % saturation of O_2 (Table 3-4). Environmental variables were plotted in a biplot (Fig. 3-5) with site points representing each treatment on sampling dates. Separation among sample dates with respect to season is shown on axis 1. Sample dates in early July (week 5) for all treatments had higher negative values on axis 1, corresponding to lower % cover of enclosure bottom by submersed macrophytes. All sample dates from the middle of July through to the end of August had more positive values on this axis, indicating higher % cover of enclosure bottom by submersed macrophytes. Sample dates for the nutrient treatment had more negative values on axis 1 in comparison to the other treatments, indicating higher alkalinity, likely related to nutrient addition. The temporal sequence of sample dates on axis 2 reflects the influence primarily of the abiotic parameters SRP and % saturation of O_2, and to a lesser extent pH. Higher negative values on axis 2 for insecticide treatment sample dates from the middle of July through August are likely related to the application of chlorpyrifos on 14 July.

Macroinvertebrate taxa were plotted on the same CCA axis 1 and 2 as for sampling dates (Fig. 3-5). Macroinvertebrates with a positive score on axis 1 (e.g., *Chaetogaster* sp., *Stylaria* sp., *Acentria* sp. pupae, *Gyraulus* sp.) were more abundant in enclosures with a higher % cover of enclosure bottom by submersed macrophytes. Species with a negative score on axis 1 (e.g., Chironomidae larvae and pupae, Dytiscidae larvae) occurred in waters with a higher concentration of alkalinity, likely related to inorganic nutrient enrichment. The position of species points for *Chaetogaster* sp. and *Stylaria* sp. were likely also influenced by insecticide treatment, resulting in a higher negative score on axis 2.

Table 3-3. Weighted correlation coefficients between environmental variables and the first two CCA axes for the microinvertebrate community associated with submersed macrophytes.

Environmental variable	CCA Axis 1	CCA Axis 2
Epiphyton biomass as chlorophyll *a* (EPIPHYTON)	-0.4204	0.0764
Cover of enclosure bottom by macrophytes (% COVER)	0.4563	-0.2617
Nitrate (NITRATE)	-0.6333	0.1347
Soluble reactive phosphorus (SRP)	-0.7648	-0.2063
Alkalinity (ALK)	-0.7194	-0.0569
pH	0.4685	0.0666
% Saturation of O_2 (% SAT)	0.4895	0.1831
Water temperature (TEMP)	0.0086	0.3716

Fig. 3-4. Canonical correspondence analysis of the microinvertebrate community associated with submersed macrophytes over an 8-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 July (week 5) to end of August (week 11) (top panel). Labels for environmental variables are as in Table 3-3. 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.; Bos lon, *Bosmina longirostris*; Cam sp1, *Camptocercus* sp.; Cer dub, *Ceriodaphnia dubia*; Chy sp1, *Chydorus* sp.; Dap sp1, *Daphnia* sp.; Dia bir, *Diaphanosoma birgei*; Eur lon, *Eurycercus longirostris*; Ple den, *Pleuroxus denticulatus*; Pse sp1, *Pseudochydorus* sp.; Sca kin, *Scapholeberis kingi*; Sim spp, *Simocephalus* spp.; Nau pli, Nauplii; Cyc Cop, Cyclopoid copepodites; Aca Dia, *Acanthocyclops*/*Diacyclops* spp.; Mic rub, *Microcyclops varicans rubellus*; Cal Cop, Calanoid copepodites; Dia nud, *Diaptomus nudus*; Har Cop, Harpacticoid copepods; Rot spp, small Rotifer spp.; Asp sp1, *Asplanchna* sp.; Ost spp., Ostracod spp; Cha ter, *Chaetogaster* sp.; Sty ria, *Stylaria* sp.; Wat Mit, Water Mites.

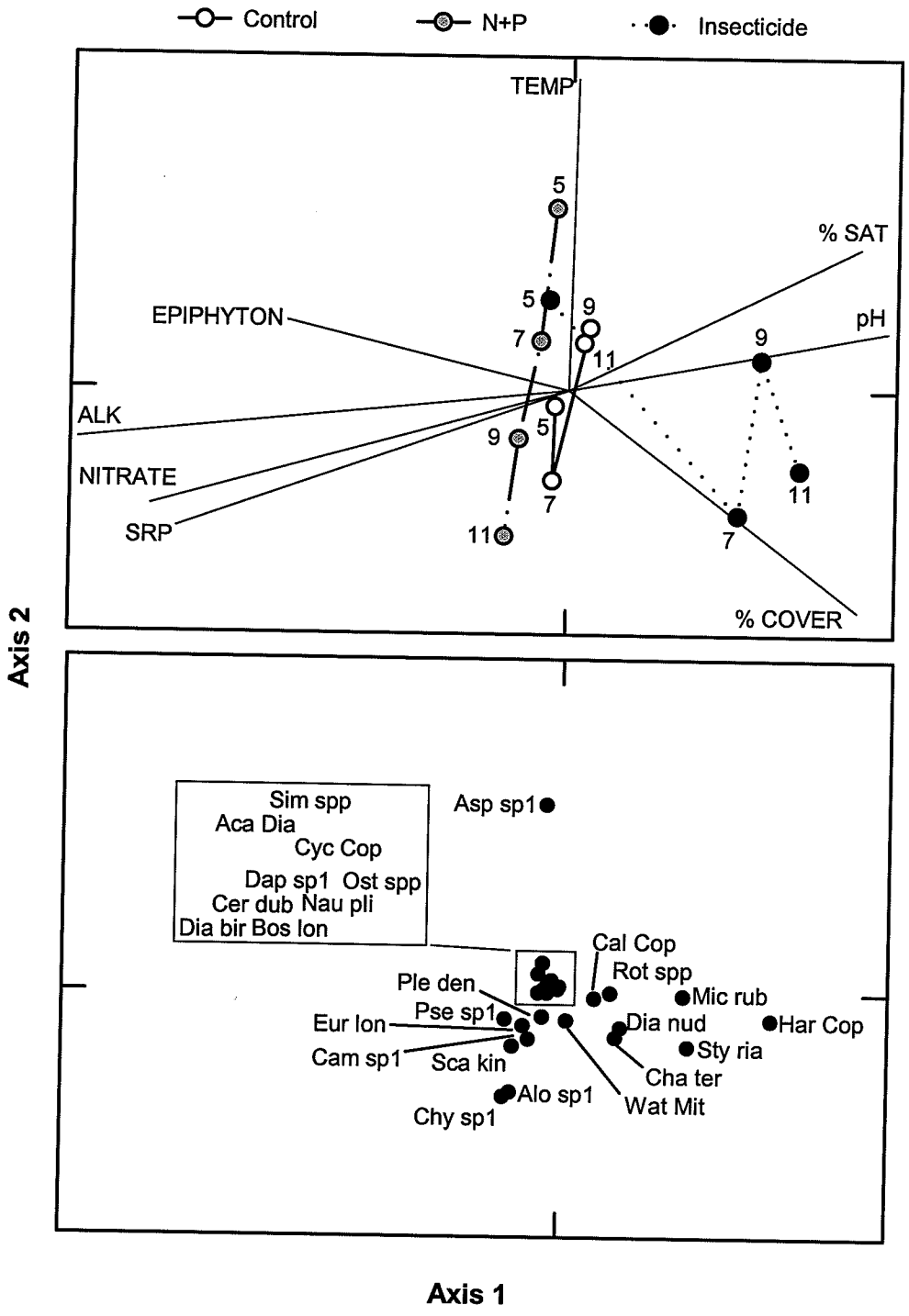
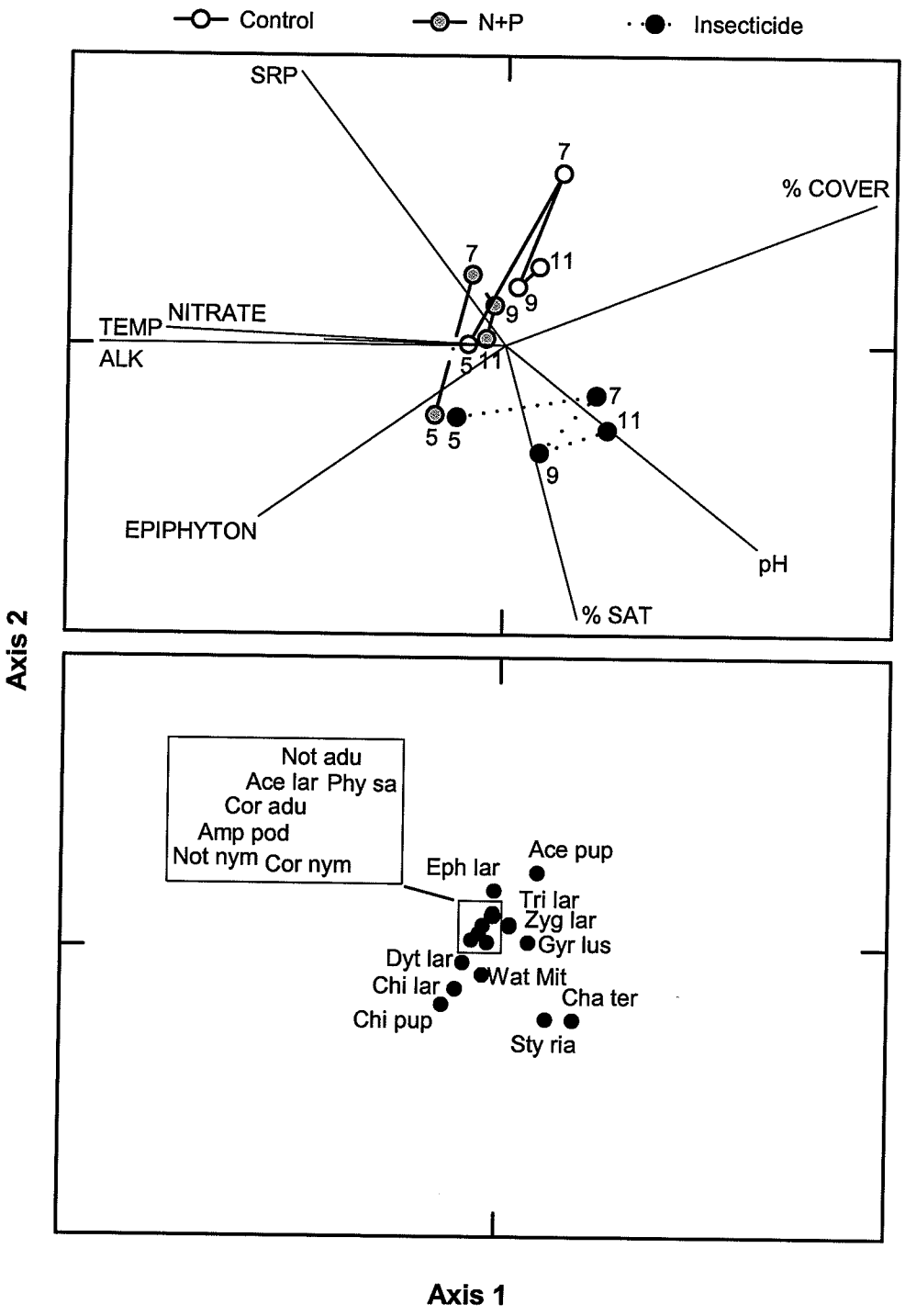

Control
N+P
Insecticide
TEMP
% SAT
EPIPHYTON
pH
ALK
NITRATE
SRP
% COVER
Axis 2
Sim spp
Aca Dia
Cyc Cop
Dap sp1 Ost spp
Cer dub Nau pli
Dia bir Bos lon
Asp sp1
Cal Cop
Rot spp
Mic rub
Ple den
Pse sp1
Eur lon
Dia nud
Har Cop
Sty ria
Cam sp1
Sca kin
Cha ter
Alo sp1
Wat Mit
Chy sp1
Axis 1

Table 3-4. Weighted correlation coefficients between environmental variables and the first two CCA axes for the macroinvertebrate community associated with submersed macrophytes.

Environmental variable	CCA Axis 1	CCA Axis 2
Epiphyton biomass as chlorophyll *a* (EPIPHYTON)	-0.5106	-0.2780
Cover of enclosure bottom by macrophytes (% COVER)	0.8332	0.2495
Nitrate (NITRATE)	-0.7030	0.0239
Soluble reactive phosphorus (SRP)	-0.5436	0.5505
Alkalinity (ALK)	-0.8175	0.0010
pH	0.5104	-0.3139
% Saturation of O_2 (% SAT)	0.1673	-0.4748
Water temperature (TEMP)	-0.3793	0.0074

Fig. 3-5. Canonical correspondence analysis of the macroinvertebrate community associated with submersed macrophytes over an 8-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 July (week 5) to end of August (week 11) (top panel). Labels for environmental variables are as in Table 3-4. Macroinvertebrates (bottom panel) are positioned on the same CCA axis 1 and 2 as for sampling dates. Taxa are coded as follows: Cha ter, *Chaetogaster* sp.; Sty ria, *Stylaria* sp.; Chi lar, Chironomidae larvae; Chi pup, Chironomidae pupae; Ace lar, *Acentria* sp. larvae; Ace pup, *Acentria* sp. pupae; Dyt lar, Dytiscidae larvae; Eph lar, Ephemeroptera larvae; Tri lar, Trichoptera larvae; Zyg lar, Zygoptera larvae; Cor nym, Corixidae nymphs; Cor adu, Corixidae adults; Not nym, Notonectidae nymphs; Not adu, Notonectidae adults; Amp pod, Amphipoda; Wat Mit, Water Mites; Phy sa, *Physa* sp.; Gyr lus, *Gyraulus* sp.


Control
N+P
Insecticide
SRP
% COVER
TEMP
NITRATE
ALK
EPIPHYTON
pH
% SAT
7
11
9
5
Not adu
Ace lar
Phy sa
Cor adu
Amp pod
Not nym
Cor nym
Eph lar
Ace pup
Tri lar
Zyg lar
Gyr lus
Dyt lar
Wat Mit
Chi lar
Cha ter
Chi pup
Sty ria
Axis 2
Axis 1

DISCUSSION

Effects of organophosphorus insecticide application

Chlorpyrifos concentrations (4.79 ± 1.28 µg/L) measured in the overlying water in the insecticide treatment have been demonstrated to be effective in reducing densities or abundance of most microinvertebrate arthropod species due to direct toxicity (MARSHALL & ROBERTS 1978, SIEFERT et al. 1989, VAN WIJNGAARDEN et al. 1993, ZRUM et al. 2000). The community composition of microinvertebrates in association with submersed macrophytes changed noticeably due to differential mortality of the arthropod component; small rotifers, calanoid copepodites, and adult *Diaptumus nudus* were more tolerant than cladocerans, ostracods, copepod nauplii, and cyclopoid copepods. Changes in planktonic microinvertebrate community composition have been shown to be similar in previous mesocosm experiments, with calanoid copepods, especially *Diaptomus nudus*, being more tolerant than cladocerans and cyclopoid copepods (HURLBERT et al. 1970, HURLBERT et al. 1972, HURLBERT 1975, VAN DEN BRINK et al. 1995, ZRUM et al. 2000).

Larger cladocerans (*Simocephalus* spp.) were most abundant in association with submersed macrophytes prior to insecticide application, but were observed in very low densities after treatment and through August. *Eurycercus longirostris*, a large cladoceran that was not observed prior to insecticide application, became the most abundant cladoceran species at the end of August. BROCK et al. (1992a) observed an earlier recovery of larger, planktonic cladocerans (*Daphnia*, *Simocephalus*) in macrophyte-dominated indoor microcosms. Smaller cladocerans (*Bosmina longirostris*, *Ceriodaphnia dubia*) appeared to be slightly more tolerant than *Simocephalus* spp., as they were observed in relatively higher densities during August. In a parallel study of the open-water planktonic community in the enclosures, ZRUM et al. (2000) found that smaller, planktonic cladocerans (*B. longirostris*, *C. dubia*, *Diaphanosoma*

birgei) were not eliminated completely by insecticide treatment and were observed in low densities for the remainder of the experiment.

Densities of small rotifers associated with submersed macrophytes were elevated in the insecticide treatment after chlorpyrifos application relative to the control and nutrient addition treatment. However, densities were not significantly higher in the insecticide treatment. In contrast, small planktonic rotifers have been shown to increase significantly in response to chlorpyrifos addition (HURLBERT et al. 1972, BROCK et al. 1992a, VAN DONK et al. 1995, ZRUM et al. 2000). ZRUM et al. (2000) suggested that reduced abundance of sensitive cladocerans and cyclopoid copepods may have indirectly contributed to these planktonic rotifer increases via reduced competition and predation (GILBERT 1988). However, in association with submersed macrophytes, these relationships may not have as strong an influence on small rotifer density, or less susceptible predators (e.g., fathead minnows, *Chaetogaster*) may have limited any increase.

Low density of ostracods in association with submersed macrophytes was found in all treatments prior to insecticide application. Ostracods declined after chlorpyrifos addition, but increased in density in the control and nutrient treatment until the end of the experiment. Ostracoda were observed to be very rare in the water column of both macrophyte-dominated and open-water indoor microcosms and were considered not to be quantitatively important (BROCK et al. 1992a). VAN DEN BRINK et al. (1996) found chlorpyrifos addition reduced the number of ostracods in combined water column samples from macrophyte-dominated and open-water locations in their outdoor experimental ditches.

Life stages of cyclopoid copepods in association with submersed macrophytes declined after insecticide application. Adults (*Acanthocyclops vernalis*, *Diacyclops thomasi*) and copepodites were nearly eliminated 1-week post-treatment and were found in very low densities through August. Nauplii were found in relatively higher densities 1-week post-treatment, but by the beginning of August densities were comparable to the other cyclopoid life stages and remained very low through to the end of the experiment. ZRUM et al. (2000)

observed a similar response in water column samples where nauplii were reduced after insecticide treatment, but not to the extent of other cyclopoid life stages. Planktonic copepod nauplii have been reported as the most susceptible life stage of copepods, declining more rapidly than more mature life stages (BROCK et al. 1992a, VAN DONK et al. 1995).

Due to low densities of all macroinvertebrate taxa associated with submersed macrophytes throughout the experiment I was unable to observe any response, either direct or indirect, by the macroinvertebrate community to chlorpyrifos application. HURLBERT et al. (1970, 1972) observed a decline in insects in the open-water after treatment of outdoor ponds with chlorpyrifos. Specifically, predaceous insect populations (e.g, Notonectidae, Dytiscidae) were more susceptible than herbivorous populations (e.g., Corixidae) and recovered more slowly (HURLBERT et al. 1972). Primary effects of chlorpyrifos have been demonstrated on macroscopic arthropod taxa (e.g., Amphipoda, Insecta, Isopoda) in macrophyte-dominated indoor microcosms using artificial substrata. BROCK et al. (1992a) concluded that amphipods (*Gammarus pulex*) and insects (*Chaoborus* sp.) were most susceptible to chlorpyrifos in macrophyte-dominated microcosms. Non-arthropod macroinvertebrates in these microcosms responded to the reduction of arthropod taxa, with decreases in turbellarian and increases in hirudinid and gastropod populations observed after insecticide treatment; oligochaetes were unaffected (BROCK et al. 1992b). In a subsequent experiment, Brock et al. (1995) found an increase in the oligochaete *Stylaria lacustris* and the gastropod *Lymnaea stagnalis*, both non-arthropod grazers on epiphytic algae, after chlorpyrifos application to nutrient enriched microcosms.

Effects of inorganic nutrient enrichment

Inorganic nutrient enrichment did not have an effect on the primary producers investigated in the mesocosms. Biomass of ephiphyton and submersed macrophytes and % cover of enclosure bottom by macrophytes in the nutrient treatment did not differ from the control. In contrast, Brock et al. (1995) and

McDOUGAL et al. (1997) observed increases in epiphyton biomass in response to nutrient loading of indoor microcosms and prairie wetland mesocosms, respectively.

The microinvertebrate community in association with aquatic macrophytes shifted in composition seasonally and periodically differed from the control. Species composition was predominantly cladocerans, with larger species (*Simocephalus* spp.) dominating in early July and smaller species (*B. longirostris*, *C. dubia*, *D. birgei*) becoming predominant by the end of July and continuing through August. The peak in cladoceran density near the end of July was significantly higher than in the control and corresponded with elevated epiphyton biomass. However, the dominant cladoceran at this time, *B. longirostris*, is a planktonic filter-feeder primarily within the water column and does not browse epiphyton. Increased abundance of smaller and more transparent species of cladocerans associated with submersed macrophytes (*B. longirostris*, *C. dubia*, *D. birgei*) may be attributed, in part, to planktivorous fish preying upon larger, more visible cladoceran species (*Simocephalus* spp.) (HESSEN 1985) in the mesocosms. Similar shifts in species composition have been observed in the presence of planktivorous fish in the water column in other mesocosm experiments (PETTIGREW et al. 1998, ZRUM et al. 2000) and in Blind Channel, Delta Marsh (HANN & ZRUM 1997). Rotifers associated with submersed macrophytes were not affected through July and August and have not been found to respond to either organic (PETTIGREW et al. 1998) or inorganic nutrient enrichment (VAN DONK et al. 1995, ZRUM et al. 2000). Densities of copepods associated with submersed macrophytes were similar to the control for the duration of the experiment, particularly through August. Copepod adults and copepodites have been found to respond positively to addition of inorganic (VAN DONK et al. 1995, HANN & GOLDSBOROUGH 1997) and organic nutrients (PETTIGREW et al. 1998).

Macroinvertebrate grazers of epiphyton did not respond to inorganic nutrient enrichment; however, I observed a significant increase in *Hyalella* sp.

near the end of August. *Hyalella* is a deposit-feeder (detritivore) and density of this amphipod has been demonstrated to be dependent on the quantity of algae associated with sediments (epipelon) and of sediment microflora (e.g., bacteria) (HARGRAVE 1970a, 1970b). Significant responses of macroinvertebrate grazers and detritivores to inorganic nutrient addition in indoor microcosms could not be demonstrated (BROCK et al. 1995, CUPPEN et al. 1995).

Community structure

Analysis of microinvertebrate community structure in association with submersed macrophytes revealed three patterns: insecticide-induced change paralleling CCA axis 1; nutrient enrichment-induced minor change paralleling CCA axis 1; and seasonal change (represented by changes in % cover by macrophytes and water temperature) correlated with CCA axis 2. The microinvertebrate community changed immediately after chlorpyrifos addition due to primary (direct) toxicological effects on cladocerans and all life stages of cyclopoid copepods (*Acanthocyclops vernalis*, *Diacyclops thomasi*). Changes continued through August as a consequence of secondary (indirect) effects on harpacticoid copepods, *Microcyclops varicans rubellus*, calanoid copepods (*Diaptomus nudus*), small rotifer species, *Stylaria*, and *Chaetogaster*. Similar response in planktonic microinvertebrate communities to chlorpyrifos application to those observed in the prairie wetland mesocosms have been demonstrated with the use of ordination (VAN DEN BRINK et al. 1995, VAN WIJNGAARDEN et al. 1995, VAN DEN BRINK et al. 1996, ZRUM et al. 2000).

Change in microinvertebrate community structure induced by nutrient loading was not as pronounced as for chlorpyrifos application. Cladoceran species dominated the community observed in the control and nutrient treatment through July, with *Scapholeberis kingi*, *Alona* sp., and *Chydorus* sp. becoming relatively more important in the nutrient enriched enclosures through August. At half the inorganic nutrient loading (HANN and GOLDSBOROUGH 1997), equivalent nutrient loading of waterfowl feces (PETTIGREW et al. 1998), or the same

inorganic nutrient loading (ZRUM et al. 2000) to that used in the present study, planktonic microinvertebrate community response was not distinguishable from the seasonal pattern in the control. PETTIGREW et al. (1998) were also unable to demonstrate discrete effects of nutrient enrichment on the community structure of microinvertebrates associated with submersed macrophytes.

Two similar patterns emerged from analyses of macroinvertebrate community structure: seasonal change (represented by changes in % cover by macrophytes) correlated with CCA axis 1; and insecticide-induced change paralleling CCA axis 2. The macroinvertebrate community changed immediately after chlorpyrifos addition due to primary (direct) toxicological effects on insects and amphipods. Changes continued through August as a consequence of secondary (indirect) effects on the oligochaetes, *Stylaria* and *Chaetogaster*. Multivariate ordinations demonstrated a similar response in macroinvertebrate communities sampled with artificial substrata to chlorpyrifos to those observed in the enclosures (VAN DEN BRINK et al. 1995, VAN WIJNGAARDEN et al. 1995, VAN DEN BRINK et al. 1996); arthropods (insects and crustaceans) were more susceptible to insecticide than oligochaetes, hirudinids, and molluscs.

Macroinvertebrate community structural response to nutrient enrichment was difficult to differentiate from the seasonal pattern observed in the control. The structure of the microinvertebrate and macroinvertebrate communities in the nutrient addition treatment was not easily distinguished from the control. Thus, nutrient enrichment appears primarily to result in increased abundance of specific invertebrate taxa, rather than any distinct change in community structure. Therefore, clear-cut treatment effects of chlorpyrifos are apparent in contrast to the muted effects of nutrient enrichment on community structure.

Dissipation of chlorpyrifos

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 h 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). 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 relatively high K_{ow} value indicates that chlorpyrifos has a strong tendency to favour the sorbed state over the dissolved state due to the nonpolar nature of the chlorpyrifos molecule. Since chlorpyrifos has a strong tendency to adsorb to surfaces, its rapid disappearance from the water column is likely attributable to adsorption of the compound on the polyethylene curtain, submersed macrophytes with attached epiphytes, and bottoms sediments (HURLBERT et al. 1970, HUGHES et al. 1980, BROCK et al. 1992a). 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.

BROCK et al. (1992a) observed considerable effects of the presence of submersed macrophytes on the distribution of chlorpyrifos in the water column of indoor microcosms; sheltered conditions and a relatively high biomass of submersed macrophytes hampered water movements and thus caused a prolonged stratification of chlorpyrifos in the water column. The sheltering effects of the enclosures and the abundance of submersed macrophytes at the time of insecticide application likely influenced the dissipation of chlorpyrifos. Micro-habitats in association with submersed macrophytes with lower insecticide concentrations may exist in enclosures treated with chlorpyrifos (BROCK et al. 1992a). Invertebrates that live in close association with submersed macrophytes and spend very little time in the open-water may be exposed to lower, less toxic concentrations of chlorpyrifos.

Final conclusions

Specific treatment effects of chlorpyrifos were apparent in contrast to muted effects of nutrient enrichment on microinvertebrate community structure. The microinvertebrate community changed immediately after chlorpyrifos addition due to primary (direct) toxicological effects on cladocerans and all life stages of cyclopoid copepods, resulting in a decline in their abundance. Changes continued through August as a consequence of secondary (indirect) effects on small rotifers and calanoid copepods in the water column and on harpacticoid copepods, *Microcyclops varicans rubellus*, calanoid copepods, small rotifers, *Stylaria*, and *Chaetogaster* in association with submersed macrophytes, resulting in an increase in the relative abundance of these taxa. After insecticide addition, insects and amphipods declined and *Stylaria* and *Chaetogaster* became relatively more important components of the macroinvertebrate community in association with submersed macrophytes.

Discrete treatment effects of inorganic nutrient enrichment on the primary producers in the mesocosms were not found. Biomass of algae (phytoplankton, epiphyton) and submersed macrophytes and % cover of enclosure bottom by macrophytes were not markedly different from the control. The planktonic microinvertebrate community and in association with aquatic macrophytes shifted in composition seasonally, but did not differ substantially from the control. Macroinvertebrate grazers of epiphyton did not respond to inorganic nutrient enrichment.

ACKNOWLEDGEMENTS

The University of Manitoba Field Station (Delta Marsh) provided logistical support necessary for the successful completion of this project. K. SANDILANDS and K. ELVIN assisted with sample collection and analyses, N. KENKEL advised on multivariate analyses, and T. HALLDORSON provided assistance with analyses for chlorpyrifos. Dr. J. ANDERSON (DowElanco, Winnipeg, MB) provided the Lorsban™ 4E used in this experiment. This study was supported in part by a Natural Sciences and Engineering Research Council (NSERC) Scholarship to LZ and NSERC Research Grants to BJH and LGG.

REFERENCES

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., 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.

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.

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.

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.

HARGRAVE, B. T. (1970a): The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). - J. Anim. Ecol. **39:** 427-437.

HARGRAVE, B. T. (1970b): Distribution, growth, and seasonal abundance of *Hyalella azteca* (Amphipoda) in relation to sediment microflora. - J. Fish. Res. Bd. Can. **27:** 685-699.

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.

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.

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

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.

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.

SANDILANDS, K. A., HANN, B. J., & GOLDSBOROUGH, L. G. (2000): The impact of nutrients and submersed macrophytes on invertebrates in a prairie wetland, Delta Marsh, Manitoba. - Arch. Hydrobiol. **148:** 441-459.

SCHEFFER, M. (1998): Ecology of Shallow Lakes. 1st Ed. - Chapman and Hall, London.

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.

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.

SPSS for Windows. Version 10.0, SPSS Inc. ©1989-1999.

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.

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:** (submitted). On-line at: http://www.umanitoba.ca/faculties/science/delta_marsh

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 4: Ordination methods for investigating responses of invertebrate communities to experimental manipulation

INTRODUCTION

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. 1992, VAN DONK et al. 1995, VAN DEN BRINK et al. 1996, HANN & GOLDSBOROUGH 1997, MCDOUGAL et al. 1997, ZRUM et al. 2000). Freshwater wetlands are complex ecosystems and invertebrate communities are influenced not only by the addition of toxic chemicals and nutrients, but also by the biotic and abiotic composition of their environment. The organisms inhabiting Delta Marsh (MB, Canada) and the surrounding area, including Lake Manitoba, are linked in a complex food web. In order to further our understanding of what organizes the food web in prairie wetlands, manipulative experiments using mesocosms are a valuable approach because they incorporate many aspects of the natural ecosystem and allow insight into the ecological impact of contaminants potentially entering a wetland (GIDDINGS 1983, GEARING 1989).

The complexity of the wetland ecosystem and the numerous interacting environmental factors (including pesticide contamination and nutrient enrichment) make it difficult to determine the effects of a specific contaminant on the structure of invertebrate communities. Multivariate methods are capable of describing patterns in the species density data and sites (samples). The procedure of arranging samples on the basis of species composition data is termed ordination (TER BRAAK 1987). Although ordination techniques have the ability to provide a visualization of a community's response to environmental

parameters or an experimental manipulation, these methods have been infrequently employed in experiments using micro- and mesocosms (experimental enclosures) (VAN WIJNGAARDEN et al. 1995).

Application of ordination in prairie wetland mesocosm experiments is advantageous for several reasons: 1) ordination allows the investigator to identify environmental variables (season, treatment) influencing the invertebrate composition in samples collected over time; 2) analysis of effects on separate groups or species is often difficult due to the low densities of some invertebrates and the inherent variability among replicates; 3) patterns of coincidence of several invertebrate taxa are often of greater use in detecting relationships among species and their environment; 4) ordination allows the investigator to visualize invertebrate taxa in relation to samples over time in a diagram (e.g., samples with similar invertebrate composition will lie close together in an ordination diagram and samples with dissimilar composition will lie further away from each other); and 5) some ordination techniques provide the investigator with statistical analyses capable of determining the significance of specific environmental factors or treatments on the invertebrate community structure.

Correspondence analysis (CA) is an ordination method in which it is assumed that the abundances of species increase and decrease within a limited range of values of an environmental variable (unimodal response) (TER BRAAK 1985, VAN WIJNGAARDEN et al. 1995). Use of CA to examine the structure of the invertebrate communities in prairie wetland mesocosm experiments conducted during the open-water season is appropriate as most species demonstrate a unimodal response to the changing environment. CA is referred to as indirect gradient analysis, as species occurrences are not related directly to environmental variables (TER BRAAK 1985). As in CA, canonical correspondence analysis (CCA) assumes that species increase and decrease along an environmental gradient. CCA is referred to as direct gradient analysis, as species occurrences are related directly to environmental variables quantified by the investigator (TER BRAAK 1986). The use of CA in combination with CCA extends

the analytical power of ordination by allowing the comparison of results from indirect and direct gradient analysis (TER BRAAK 1986). By using the two methods in combination, shortcomings with respect to one's ability to quantify adequately the many environmental variables influencing the dynamics of invertebrate communities in prairie wetland mescosm experiments may be identified. Use of the ordination techniques principal component analysis (PCA) and redundancy analysis (RDA) in combination has been considered a valuable tool for evaluating the invertebrate community responses in indoor microcosms to experimental manipulation (VAN WIJNGAARDEN et al. 1995).

This paper describes the usefulness of the unimodal response ordination techniques CA and CCA when used in combination for examining the responses of the planktonic microinvertebrate community and the invertebrate communities associated with submersed macrophytes to changing environmental variables ("season") and to experimental perturbations (insecticide application and inorganic nutrient enrichment) in prairie wetland mesocosms.

METHODS

Enclosure experiment

The 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. Experimental enclosures (mesocosms) used in this study model the freshwater wetland community characteristic of the study site under investigation. An experimental system consisting of 12, 5 m by 5 m mesocosms was installed at a site in Blind Channel (Delta Marsh) on 27 May at a water depth of < 1 m. The experimental design has been described in detail by ZRUM et al. (2000). Each enclosure consisted of a floating wooden platform with an impermeable woven polyethylene curtain attached. The curtains extended from above the water surface down to the sediments, where they were anchored with iron bars at least 0.30 m into the sediments, thereby preventing direct exchange of water between

the enclosures and Blind Channel. The 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 for the duration of the experiment. Three enclosures were treated with a single addition of Lorsban™ 4E (active ingredient chlorpyrifos) on 14 July to produce a nominal concentration of 10 µg/L in the water column, three enclosures received inorganic nitrogen (as analytical grade $NaNO_3$) and phosphorus (as $NaH_2PO_4 \cdot 2H_20$) thrice weekly beginning on 23 June for a 10 week treatment period, and three served as controls.

Surface water samples were collected from each enclosure twice weekly for the determination of chemical parameters (see ZRUM et al. 2000 and CHAPTER 3 for methods). Samples were analyzed for pH, alkalinity, soluble reactive phosphorus (SRP), ammonium-N (NH_3-N), and nitrate+nitrite-N (NO_3-N). Dissolved oxygen and water temperature (at 10 and 50 cm depths) were measured weekly, in the morning, and turbidity (at 30 cm depth) in each enclosure was determined weekly. Planktonic microinvertebrate (ind./L) and phytoplankton (biomass as chlorophyll *a*) 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 nutrient enrichment. Sampling of invertebrates (ind./L) and epiphyton (biomass as chlorophyll *a*) associated with submersed macrophytes using a Downing Box (DOWNING 1986) was initiated on 9 July and continued weekly until 28 August. Percent cover of enclosure bottom by submersed macrophytes was estimated visually each week and submersed macrophyte biomass (g/m^2 of wetland bottom) was determined once in June, July, and August.

Dissipation of chlorpyrifos, nutrient enrichment dynamics, and treatment effects on invertebrate populations and primary producers in the enclosures have been described by ZRUM et al. (2000) and in CHAPTER 3.

Multivariate data analysis

Correspondence analysis

Correspondence analysis (CA) was used to examine changes in invertebrate community structure (species composition and density) in the mesocosms in response to treatment over time. Ordinations were performed using the program CANOCO (version 3.10, TER BRAAK 1988). For each sampling date, mean densities (ind./L) of microinvertebrates in the plankton, mean densities (ind./L) of microinvertebrates associated with submersed macrophytes, and mean densities (ind./L) of macroinvertebrates associated with submersed macrophytes were calculated for each treatment. Invertebrate taxa x sample date (for each treatment) matrices were produced using ln (x+1)-transformed data to stabilize variances.

Correspondence analysis (CA) may be recognized as a special case of principal component analysis (PCA), in which a double standardization (i.e., by both the columns and rows of the raw data) is performed prior to eigenanalysis being carried out. Since the data are in the form of a contingency table, CA is able to examine the relationships among individuals simultaneously classified into two categorical variables. CA is an ordination technique based on a unimodal response model, or a model in which it is assumed the abundance of species increase and decrease within a limited range of values of an environmental variable (TER BRAAK 1985, VAN WIJNGAARDEN et al. 1995). The ordination axes extracted in CA can be thought of as corresponding to environmental gradients; because species occurrences are not related directly to environmental variables, though, CA is referred to as indirect gradient analysis (TER BRAAK 1985). CA produces an ordination diagram in which sites (sample dates for each treatment) with nearly identical invertebrate taxa composition lie close together, whereas sites with very different invertebrate taxa composition lie far apart. If the environmental variables measured relate to the first few ordination axes and appear to be able to predict the species composition, then they likely account for a large amount of the variation observed in the species

data. Likewise, if the environmental variables do not relate to the first few ordination axes, they do not account for the variation in the main trends of the species data, but may account for a portion of the residual variation.

Canonical correspondence analysis

Relationships between invertebrate 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 (ind./L) of microinvertebrates in the plankton, mean densities (ind./L) of microinvertebrates associated with submersed macrophytes, and mean densities (ind./L) of macroinvertebrates associated with submersed macrophytes, mean biomass of phytoplankton and epiphyton, % cover of enclosure bottom by macrophytes, mean total dry weight of macrophytes in Downing Box samples, 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. Invertebrate taxa x sample date (for each treatment) matrices were produced using ln (x+1)-transformed data to stabilize variances.

For the planktonic microinvertebrate data set, biotic environmental parameters, fathead minnow density (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.

For the invertebrates associated with submersed macrophytes, 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. 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 invertebrate taxa composition and canonical axes (constrained by the set of environmental variables) was tested for the planktonic microinvertebrates, the microinvertebrates associated with submersed macrophytes, and the macroinvertebrates associated with submersed macrophytes 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).

As in CA, the underlying model in CCA assumes that species abundances are unimodal functions along environmental gradients. In CA the ordination axes extracted take into account all the variance of the data set, whereas in CCA the axes are constrained to the fraction of the total variance in the data that is explained by the environmental variables measured. In CCA 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). As in CA, the resulting ordination diagram shows the pattern of variation in species composition, but CCA also shows the main relationships between the species and environmental variables. The ordination diagram shows species and sites (sample dates for each treatment) 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 ordination diagram, while sites that differ in the relative density of a similar set of species or in their species composition will lie further apart.

The use of CA in combination with CCA extends the analytical power of ordination by allowing the comparison of results from indirect and direct gradient analysis (TER BRAAK 1986). When the resulting ordination diagrams do not differ substantially, one can infer that the environmental variables quantified may account for the main variation in the species data (TER BRAAK 1986). If the diagrams do differ, the environmental variables either account for residual variation in the species data (high correlations between species and environment axes) or they cannot account for any of the variation (low correlations) (TER BRAAK 1986).

RESULTS

Planktonic microinvertebrates

Correspondence analysis

The eigenvalues of the first two CA ordination axes were 0.303 and 0.142, respectively, cumulatively explaining 76 % of the total variance in the species data. Initially, site points of the treatment enclosures lie close to the site points for the control in the ordination diagram, indicating the microinvertebrate community in the water column was similar in composition for all treatments until insecticide application in the middle of July (week 6) (Fig. 4-1). CA axis 1 appears to separate the communities by sampling date (or season), showing the

change in the community from the beginning of June to the end of August. CA axis 2 appears to represent insecticide treatment effect, showing the change in the community structure from pre-treatment (week 1 to week 5) to post-treatment (week 7 to week 11), with the insecticide treatment being separated from control and nutrient treatment after insecticide application in the middle of July. Pre-treatment samples were separated, indicating differences in the microinvertebrate community composition among sample dates, but not among treatments.

Canonical correspondence analysis

Eigenvalues of the first and second CCA axes constrained to the environmental variables were 0.295 and 0.122, respectively. 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; these two environmental variables accounted for 60 % of the total variance in the species data. CCA axis 1 was most strongly correlated with % cover of enclosure bottom by submersed macrophyte and water temperature, and CCA axis 2 with alkalinity and nitrate concentrations (Table 4-1).

A separation is shown among sample dates with respect to season on axis 1 in the ordination diagram (Fig. 4-2). Those species with a high positive score on axis 1 (e.g., small rotifer species, *Asplanchna* sp.) were relatively more abundant in the samples taken in June, and those with a negative score on axis 1 (e.g., *Diaphanosoma birgei*, *Bosmina longirostris*, Ostracod spp., *Diaptomus nudus*, *Microcyclops varicans rubellus*) were relatively more abundant in July and

August. A separation is shown among sample dates with respect to insecticide treatment on axis 2 (Fig. 4-2). After chlorpyrifos application in the middle of July (week 6), calanoid copepod copepodites, adult *Diaptomus nudus*, and *Microcyclops varicans rubellus* were relatively more abundant (higher positive score on axis 2) in the insecticide treatment.

Eigenvalues of the first two axes in CA and CCA were very similar (0.303 and 0.142, and 0.295 and 0.122, respectively). Since the first two axes in CCA were constrained to the variation explained by the environmental variables, the change in the planktonic microinvertebrate community observed with respect to the first two CA axes may also be explained by season (sampling date) and insecticide treatment. A separation is shown among sample dates with respect to season on axis 1 and with respect to insecticide treatment on axis 2 in both ordination diagrams. Positioning of species points in both ordinations with respect to season (sampling date) and insecticide application were practically identical.

Microinvertebrates associated with submersed macrophytes

Correspondence analysis

The eigenvalues of the first two CA ordination axes were 0.18 and 0.11, respectively, cumulatively explaining 65 % of the total variance in the species data. Initially, site points of the treatment enclosures lie close to the site point for the control in the ordination diagram, indicating the microinvertebrate community in association with submersed macrophytes was similar in composition for all treatments until insecticide application in the middle of July (week 6) (Fig. 4-3). CA axis 1 appears to represent insecticide treatment effect, showing the change in the community structure from pre-treatment (week 5) to post-treatment (week 7 to week 11), with the insecticide treatment being separated from the control and nutrient treatment after insecticide addition in the middle of July. CA axis 2 appears to separate the communities by sampling date (or season), showing the change in the community from the beginning of July to the end of August.

Fig. 4-1. 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). Microinvertebrate species (bottom panel) are positioned on the same CA axis 1 and 2 as for sampling dates. Species are identified 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.

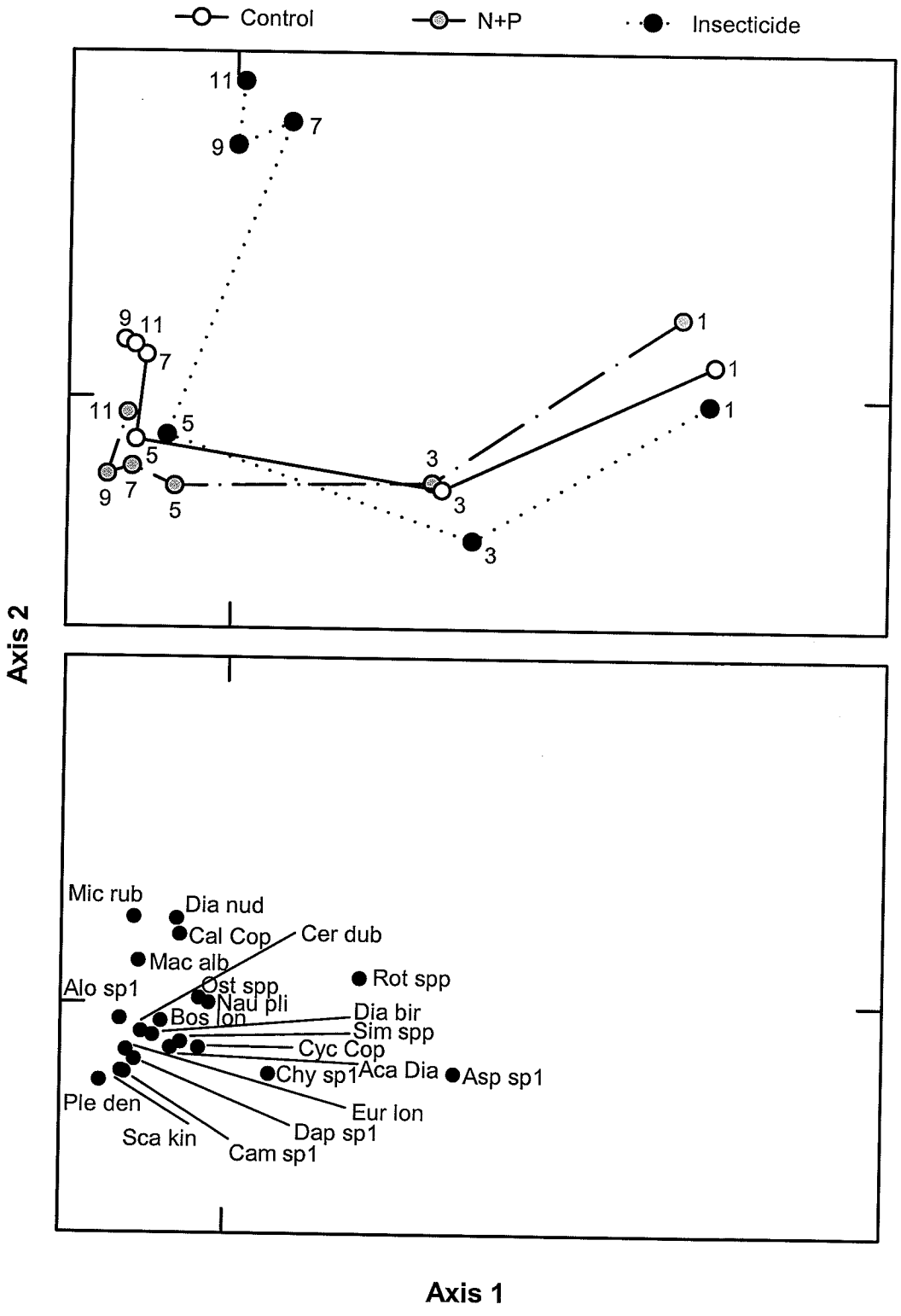
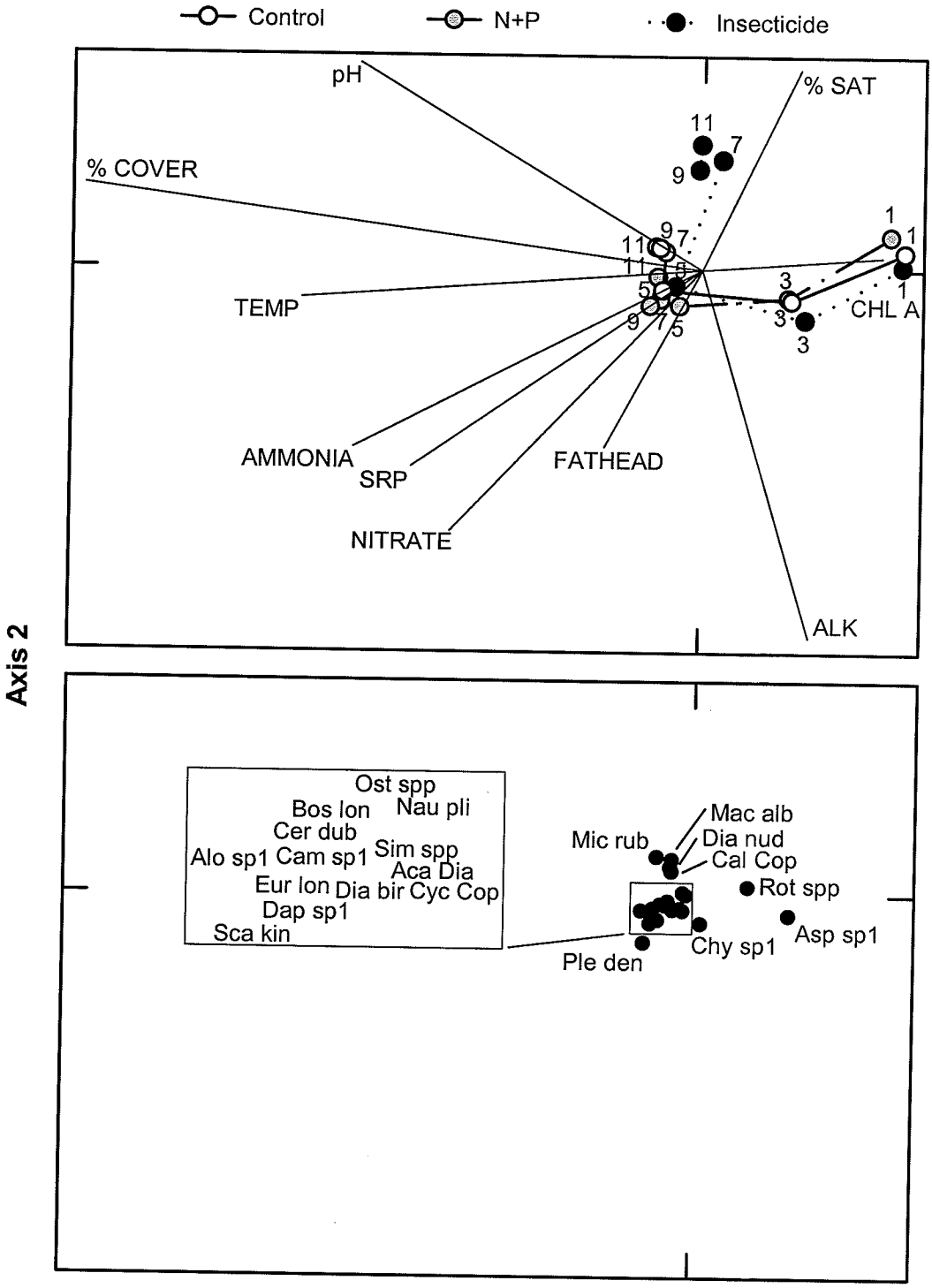

Control
N+P
Insecticide
11
7
9
9
11
7
11
5
5
9
7
5
3
3
3
1
1
1
Axis 2
Mic rub
Dia nud
Cal Cop
Cer dub
Mac alb
Alo sp1
Ost spp
Nau pli
Rot spp
Bos lon
Dia bir
Sim spp
Cyc Cop
Chy sp1
Aca Dia
Asp sp1
Ple den
Eur lon
Dap sp1
Sca kin
Cam sp1
Axis 1

Table 4-1. 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. 4-2. 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 4-1. Microinvertebrate species (bottom panel) are positioned on the same CCA axis 1 and 2 as for sampling dates. Species are identified as in Fig. 4-1.


Control
N+P
Insecticide
pH
% SAT
% COVER
TEMP
CHL A
AMMONIA
SRP
FATHEAD
NITRATE
ALK
Ost spp
Bos lon
Nau pli
Cer dub
Alo sp1
Cam sp1
Sim spp
Aca Dia
Eur lon
Dia bir
Cyc Cop
Dap sp1
Sca kin
Mac alb
Mic rub
Dia nud
Cal Cop
Rot spp
Asp sp1
Chy sp1
Ple den
Axis 2
Axis 1

Canonical correspondence analysis

Eigenvalues of the first and second CCA axes constrained to the environmental variables were 0.177 and 0.052, respectively. CCA axis 1 was significantly related to microinvertebrate community composition (Monte Carlo permutation test, $F = 1.76$, $p = 0.05$) and the first four axes together were significant ($F = 2.13$, $p = 0.02$). The eight environmental variables included in the analysis explained 89 % of the total variance in the species data. When the environmental variables were forward selected, only SRP (Monte Carlo permutation test, $F = 3.92$, $p = 0.01$) was significantly related to microinvertebrate community composition; this environmental variable alone accounted for 30 % of the total variance in the species data. CCA axis 1 was most strongly correlated with SRP and alkalinity, and CCA axis 2 with water temperature and % cover of enclosure bottom by submersed macrophytes (Table 4-2).

A separation is shown among sample dates with respect to insecticide treatment on axis 1 (Fig. 4-4). After chlorpyrifos application in the middle of July (week 6), harpacticoid copepods, *Stylaria* sp., *Microcyclops varicans rubellus*, *Diaptomus nudus*, *Chaetogaster* sp., small rotifer species, and calanoid copepod copepodites and were relatively more abundant (higher positive score on axis 1) in the insecticide treatment. A separation is shown among sample dates with respect to season on axis 2 in the ordination diagram (Fig. 4-4). Those species with a more negative score on axis 2 (e.g., *Asplanchna* sp., *Simocephalus* spp.) were relatively more abundant in the samples taken in early July, prior to insecticide application, and those with a higher positive score on axis 2 (e.g., *Chydorus* sp., *Alona* sp.) were relatively more abundant during August, particularly in the nutrient treatment.

Eigenvalues of the first axis in CA and CCA were very similar (0.184 and 0.177, respectively); however, eigenvalues for the second axis differed (0.110 and 0.052, respectively). Since the first two axes in CCA were constrained to the variation explained by the environmental variables, the change in the

microinvertebrate community associated with submersed macrophytes observed with respect to the first two CA axes may also be explained by insecticide treatment and season (sampling date), but due to the dissimilarity in comparison to the CCA diagram there is likely some unmeasured factor(s) contributing to the changes in the community structure. A separation is shown among sample dates with respect to insecticide treatment on axis 1 and with respect to season on axis 2 in both ordination diagrams. Positioning of sample dates in both ordinations with respect to insecticide application were very similar, reflecting the similarity in eigenvalues of the first axes. However, the positioning of sample dates with respect to season differed noticeably between the two diagrams, particularly for the control. Positioning of species points in both ordinations with respect to insecticide application were practically identical. Differences among positions of species points with respect to season likely reflected the dissimilar positions of sample dates, primarily for the control.

Macroinvertebrates associated with submersed macrophytes

Correspondence analysis

The eigenvalues of the first two CA ordination axes were 0.53 and 0.32, respectively, cumulatively explaining 74 % of the total variance in the species data. Initially, site points of the treatment enclosures lie close to the site point for the control in the ordination diagram, indicating the macroinvertebrate community in association with submersed macrophytes was similar in composition for all treatments until insecticide application in the middle of July (week 6) (Fig. 4-5). CA axis 1 appears to separate the communities by sampling date (or season), showing the change in the community from the beginning of July to the end of August. CA axis 2 appears to represent insecticide treatment effect, showing the change in the community structure from pre-treatment (week 5) to post-treatment (week 7 to week 11), with the insecticide treatment being separated from the control and nutrient treatment after insecticide addition in the middle of July.

Fig. 4-3. Correspondence analysis of the microinvertebrate community associated with submersed macrophytes over an 8-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 July (week 5) to end of August (week 11) (top panel). Microinvertebrate species (bottom panel) are positioned on the same CA axis 1 and 2 as for sampling dates. Species are coded as follows: Alo sp1, *Alona* sp.; Bos lon, *Bosmina longirostris*; Cam sp1, *Camptocercus* sp.; Cer dub, *Ceriodaphnia dubia*; Chy sp1, *Chydorus* sp.; Dap sp1, *Daphnia* sp.; Dia bir, *Diaphanosoma birgei*; Eur lon, *Eurycercus longirostris*; Ple den, *Pleuroxus denticulatus*; Pse sp1, *Pseudochydorus* sp.; Sca kin, *Scapholeberis kingi*; Sim spp, *Simocephalus* spp.; Nau pli, Nauplii; Cyc Cop, Cyclopoid copepodites; Aca Dia, *Acanthocyclops*/*Diacyclops* spp.; Mic rub, *Microcyclops varicans rubellus*; Cal Cop, Calanoid copepodites; Dia nud, *Diaptomus nudus*; Har Cop, Harpacticoid copepods; Rot spp, small Rotifer spp.; Asp sp1, *Asplanchna* sp.; Ost spp., Ostracod spp; Cha ter, *Chaetogaster* sp.; Sty ria, *Stylaria* sp.; Wat Mit, Water Mites.

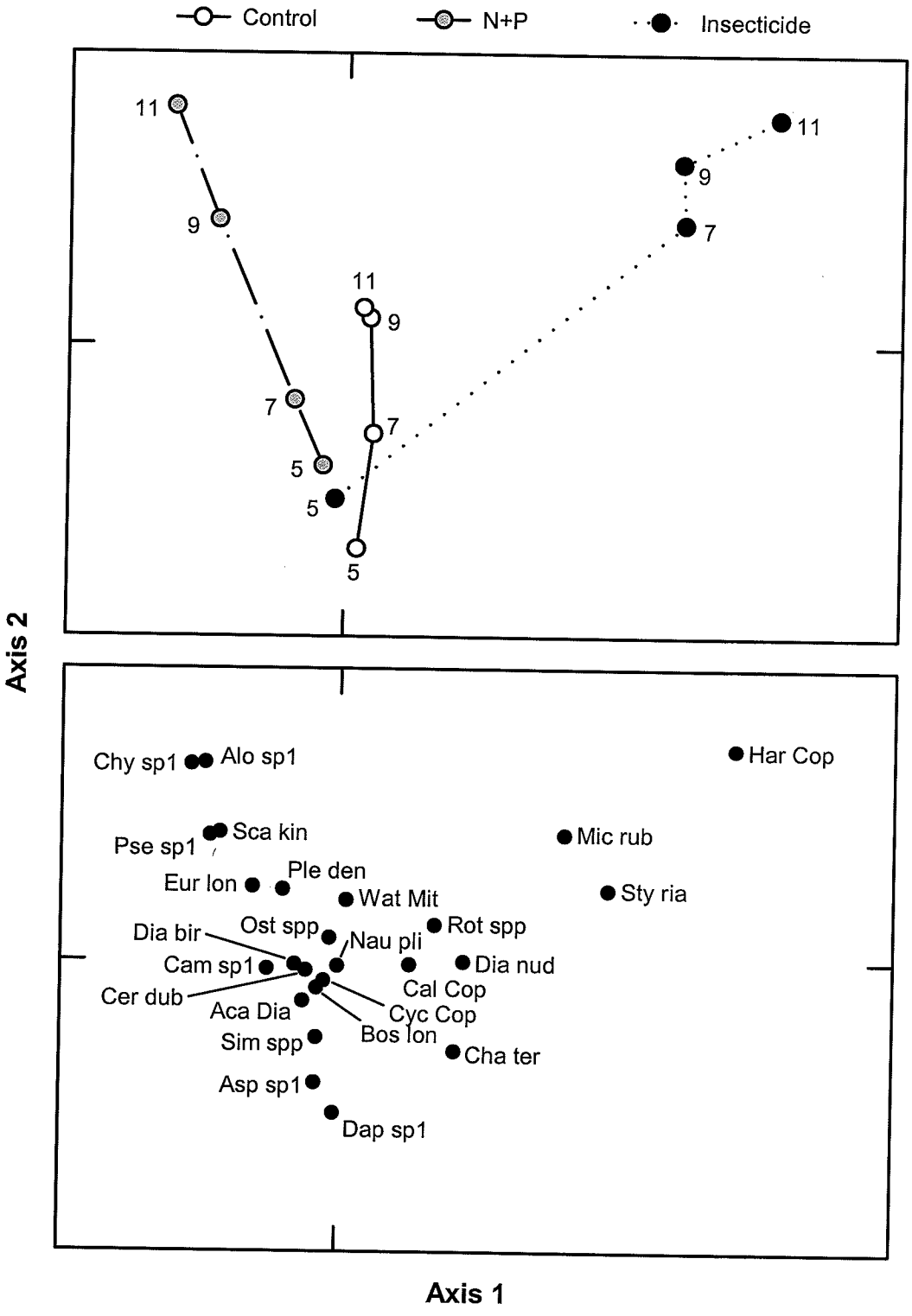
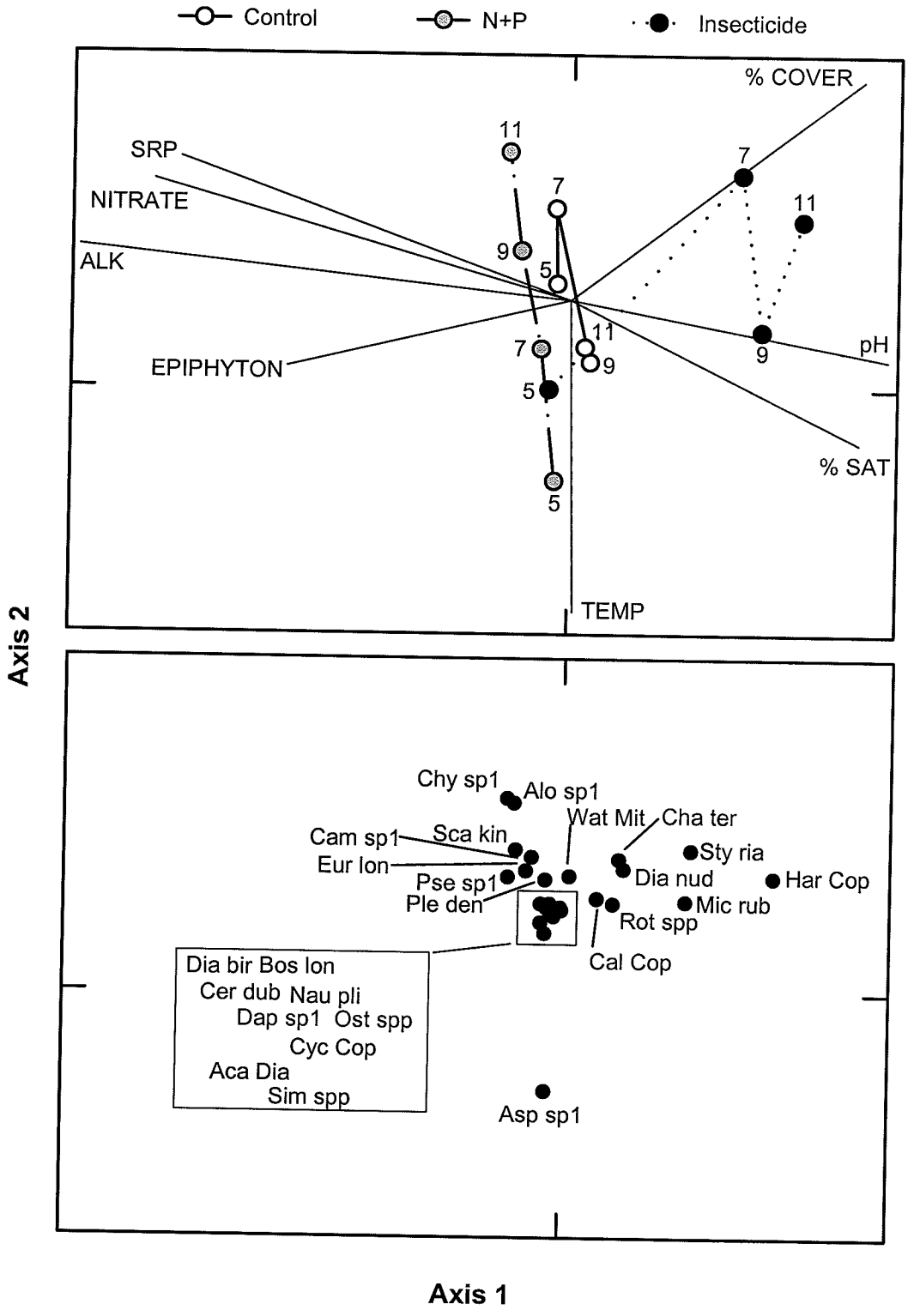

Control
N+P
Insecticide
11
9
7
5
11
9
7
5
11
9
7
Axis 2
Chy sp1
Alo sp1
Har Cop
Pse sp1
Sca kin
Mic rub
Eur lon
Ple den
Wat Mit
Sty ria
Dia bir
Ost spp
Nau pli
Rot spp
Cam sp1
Dia nud
Cer dub
Cal Cop
Aca Dia
Cyc Cop
Sim spp
Bos lon
Cha ter
Asp sp1
Dap sp1
Axis 1

Table 4-2. Weighted correlation coefficients between environmental variables and the first two CCA axes for the microinvertebrate community associated with submersed macrophytes.

Environmental variable	CCA Axis 1	CCA Axis 2
Epiphyton biomass as chlorophyll *a* (EPIPHYTON)	-0.4204	0.0764
Cover of enclosure bottom by macrophytes (% COVER)	0.4563	-0.2617
Nitrate (NITRATE)	-0.6333	0.1347
Soluble reactive phosphorus (SRP)	-0.7648	-0.2063
Alkalinity (ALK)	-0.7194	-0.0569
pH	0.4685	0.0666
% Saturation of O_2 (% SAT)	0.4895	0.1831
Water temperature (TEMP)	0.0086	0.3716

Fig. 4-4. Canonical correspondence analysis of the microinvertebrate community associated with submersed macrophytes over an 8-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 July (week 5) to end of August (week 11) (top panel). Labels for environmental variables are as in Table 4-2. Microinvertebrate species (bottom panel) are positioned on the same CCA axis 1 and 2 as for sampling dates. Species are identified as in Fig. 4-3.


Control
N+P
Insecticide
% COVER
SRP
NITRATE
ALK
EPIPHYTON
pH
% SAT
TEMP
11
7
9
5
Axis 2
Chy sp1
Alo sp1
Wat Mit
Cha ter
Cam sp1
Sca kin
Sty ria
Eur lon
Dia nud
Har Cop
Pse sp1
Ple den
Mic rub
Rot spp
Cal Cop
Dia bir Bos lon
Cer dub Nau pli
Dap sp1 Ost spp
Cyc Cop
Aca Dia
Sim spp
Asp sp1
Axis 1

Canonical correspondence analysis

Eigenvalues of the first and second CCA axes constrained to the environmental variables were 0.385 and 0.206, respectively. CCA axis 1 was not significantly related to macroinvertebrate community composition (Monte Carlo permutation test, $F = 1.50$, $p = 0.27$), however, the first four axes together were significant ($F = 2.40$, $p = 0.02$). The eight environmental variables included in the analysis explained 91 % of the total variance in the species data. When the environmental variables were forward selected, % cover of enclosure bottom by submersed macrophytes (Monte Carlo permutation test, $F = 4.55$, $p = 0.01$) and SRP ($F = 2.59$, $p = 0.02$) were significantly related to macroinvertebrate community composition; these environmental variables accounted for 50 % of the total variance in the species data. CCA axis 1 was most strongly correlated with % cover of enclosure bottom by submersed macrophytes and alkalinity concentration, and CCA axis 2 with SRP concentration and % saturation of O_2 (Table 4-3).

A separation is shown among sample dates with respect to season on axis 1 in the ordination diagram (Fig. 4-6). Those taxa with a more positive score on axis 1 (e.g., Chironomidae pupae and larvae, Dytiscidae larvae) were relatively more abundant in the samples taken in early July, prior to insecticide application, and those with a higher negative score on axis 1 (e.g., Corixidae nymphs, water mites, Notonectidae nymphs) were relatively more abundant during August. A separation is shown among sample dates with respect to insecticide treatment on axis 2 (Fig. 4-6). After chlorpyrifos application in the middle of July (week 6), *Stylaria* sp. and *Chaetogaster* sp. were relatively more abundant (higher positive score on axis 2) in the insecticide treatment.

Eigenvalues of the first two axes in CA and CCA were relatively dissimilar (0.527 and 0.385, and 0.318 and 0.206, respectively). The first two axes in CCA were constrained to the variation explained by the environmental variables. Change in the macroinvertebrate community observed with respect to the first two CA axes is partially explained by season (sampling date) and insecticide

treatment, but due to the dissimilarity in comparison to the CCA ordination there is likely some unmeasured factor(s) contributing to the structure of the community. A separation is shown among sample dates with respect to season on axis 1 and with respect to insecticide treatment on axis 2 in both ordination diagrams. Positioning of sample dates differed noticeably between the two ordinations, particularly with respect to season. Differences among positions of species points with respect to season likely reflected the dissimilar positions of sample dates. Positioning of species points in both diagrams with respect to sample dates for the insecticide treatment were very similar.

DISCUSSION

Interpretation of ordinations

Effects of organophosphorus insecticide application

Application of insecticide resulted in a pronounced change in the composition of the invertebrate communities due to the differential mortality among arthropods. After chlorpyrifos addition, calanoid copepodites and adult *Diaptomus nudus* dominated the planktonic community and, along with harpacticoid copepods, *Stylaria* sp., *Microcyclops varicans rubellus*, *Chaetogaster* sp., and small rotifer species, also the microinvertebrate community associated with submersed macrophytes. In general, the findings of the ordinations were in agreement with the literature. Calanoid copepods have been observed as being less susceptible to chlorpyrifos than cladocerans and cyclopoid copepods (HURLBERT et al. 1970, HURLBERT et al. 1972, VAN DEN BRINK et al. 1995). Within the macroinvertebrate community in association with submersed macrophytes, *Stylaria* sp. and *Chaetogaster* sp. (oligochaetes) were relatively more abundant after insecticide application. A similar response in macroinvertebrate communities to chlorpyrifos to those observed in the enclosures has been reported in the literature; arthropods (insects and crustaceans) were more susceptible to insecticide than

Fig. 4-5. Correspondence analysis of the macroinvertebrate community associated with submersed macrophytes over an 8-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 July (week 5) to end of August (week 11) (top panel). Macroinvertebrates (bottom panel) are positioned on the same CA axis 1 and 2 as for sampling dates. Taxa are coded as follows: Cha ter, *Chaetogaster* sp.; Sty ria, *Stylaria* sp.; Chi lar, Chironomidae larvae; Chi pup, Chironomidae pupae; Ace lar, *Acentria* sp. larvae; Ace pup, *Acentria* sp. pupae; Dyt lar, Dytiscidae larvae; Eph lar, Ephemeroptera larvae; Tri lar, Trichoptera larvae; Zyg lar, Zygoptera larvae; Cor nym, Corixidae nymphs; Cor adu, Corixidae adults; Not nym, Notonectidae nymphs; Not adu, Notonectidae adults; Amp pod, Amphipoda; Wat Mit, Water Mites; Phy sa, *Physa* sp.; Gyr lus, *Gyraulus* sp.

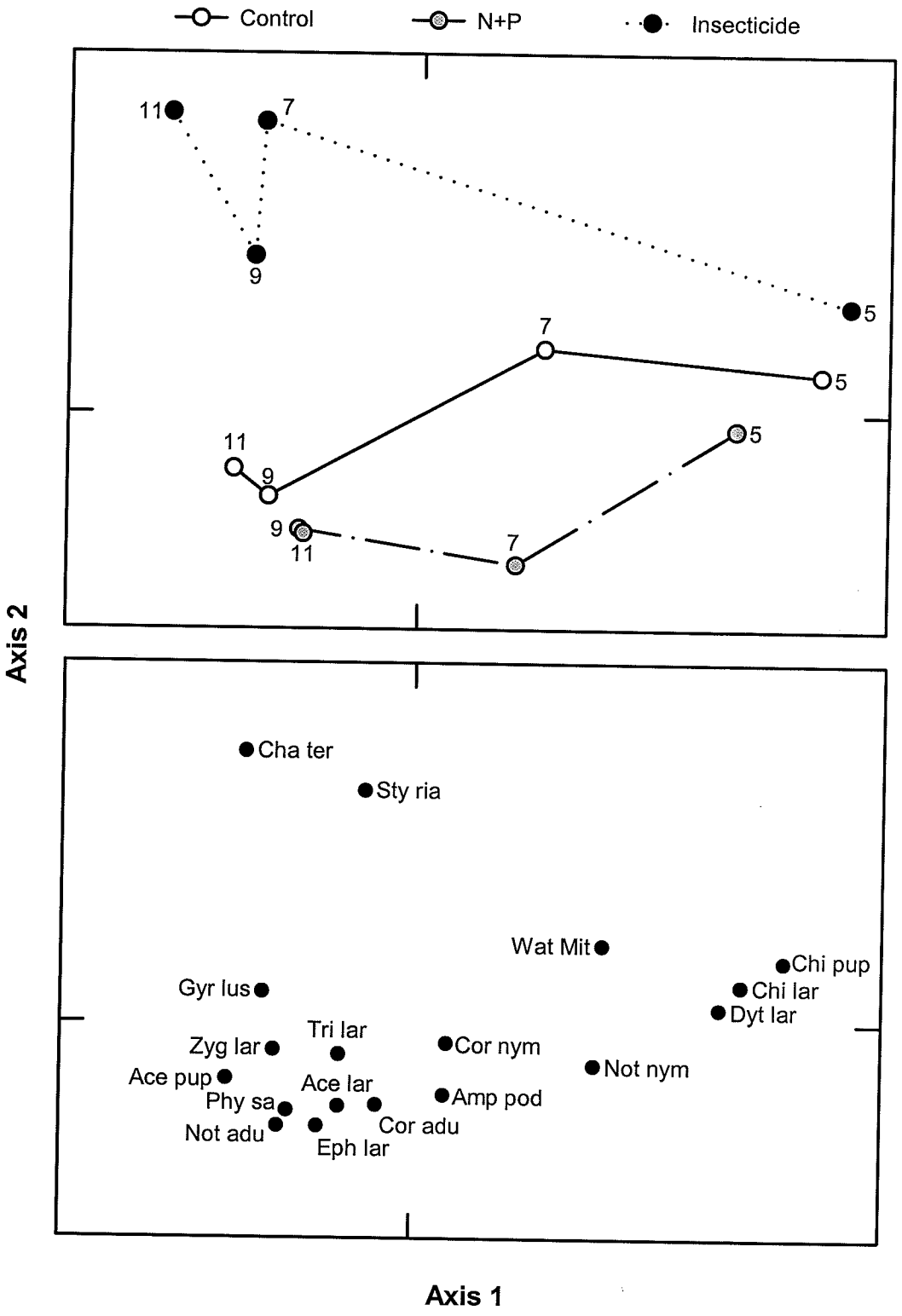
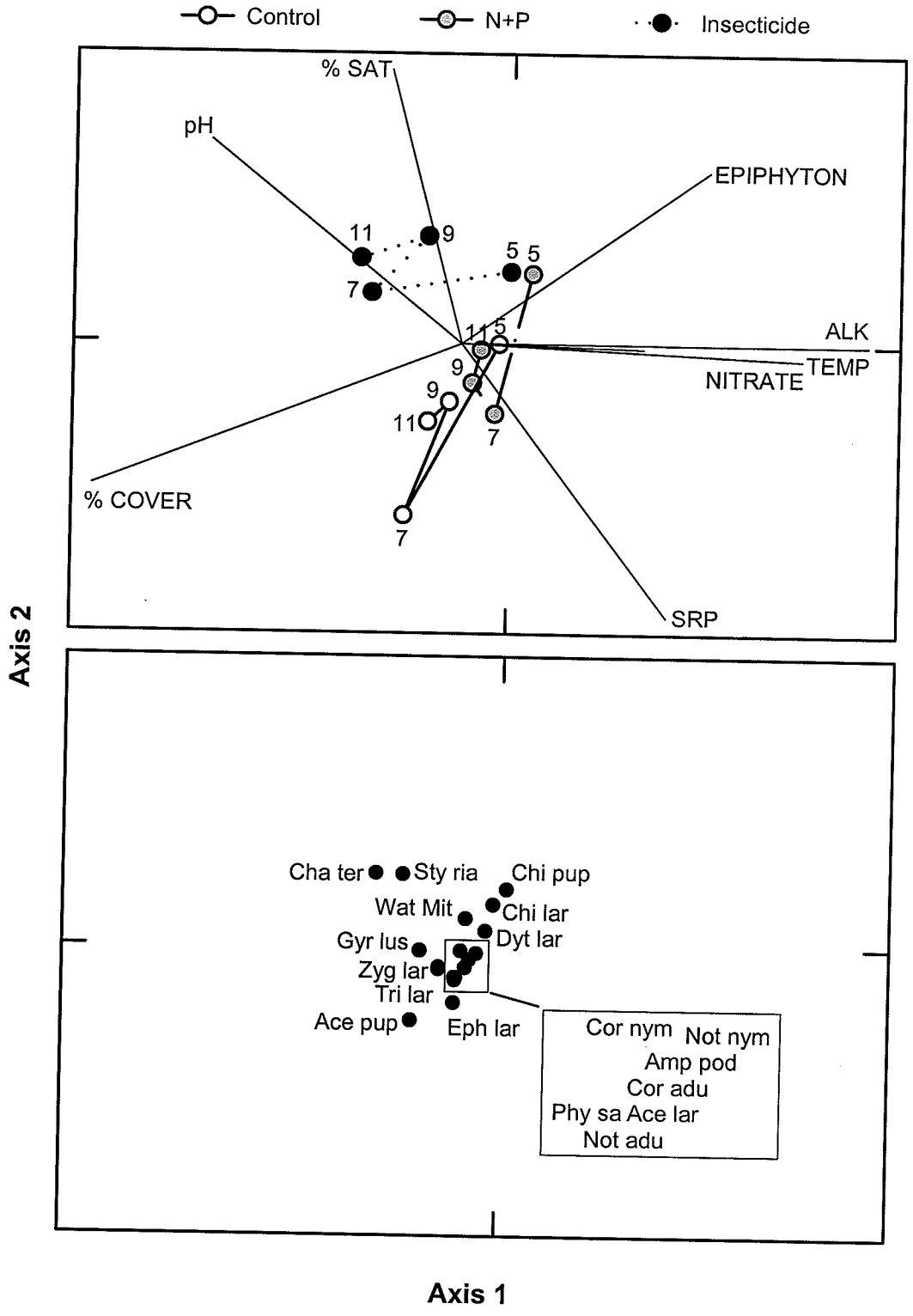

Control
N+P
Insecticide
11
7
9
5
7
5
11
9
5
9
11
7
Axis 2
Cha ter
Sty ria
Wat Mit
Chi pup
Gyr lus
Chi lar
Dyt lar
Tri lar
Zyg lar
Cor nym
Not nym
Ace pup
Ace lar
Amp pod
Phy sa
Cor adu
Not adu
Eph lar
Axis 1

Table 4-3. Weighted correlation coefficients between environmental variables and the first two CCA axes for the macroinvertebrate community associated with submersed macrophytes.

Environmental variable	CCA Axis 1	CCA Axis 2
Epiphyton biomass as chlorophyll *a* (EPIPHYTON)	-0.5106	-0.2780
Cover of enclosure bottom by macrophytes (% COVER)	0.8332	0.2495
Nitrate (NITRATE)	-0.7030	0.0239
Soluble reactive phosphorus (SRP)	-0.5436	0.5505
Alkalinity (ALK)	-0.8175	0.0010
pH	0.5104	-0.3139
% Saturation of O_2 (% SAT)	0.1673	-0.4748
Water temperature (TEMP)	-0.3793	0.0074

Fig. 4-6. Canonical correspondence analysis of the macroinvertebrate community associated with submersed macrophytes over an 8-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 July (week 5) to end of August (week 11) (top panel). Labels for environmental variables are as in Table 4-3. Macroinvertebrates (bottom panel) are positioned on the same CCA axis 1 and 2 as for sampling dates. Taxa are coded as in Fig. 4-5.


Control
N+P
Insecticide
% SAT
pH
EPIPHYTON
ALK
TEMP
NITRATE
% COVER
SRP
11
9
5
5
7
11
5
9
9
11
7
7
Axis 2
Cha ter
Sty ria
Chi pup
Wat Mit
Chi lar
Gyr lus
Dyt lar
Zyg lar
Tri lar
Ace pup
Eph lar
Cor nym
Not nym
Amp pod
Cor adu
Phy sa
Ace lar
Not adu
Axis 1

oligochaetes, hirudinids, and molluscs (VAN DEN BRINK et al. 1995, VAN WIJNGAARDEN et al. 1995, VAN DEN BRINK et al. 1996).

Effects of inorganic nutrient loading

Invertebrate community composition in the inorganic nutrient addition treatment changed seasonally in response to a number of environmental parameters. It is difficult to ascertain treatment effects on the composition of the planktonic community or the community in association with submersed macrophytes, as the enclosures receiving nutrients did not differ substantially from the control.

Correspondence and canonical correspondence analyses

A better separation of the seasonal effects and the insecticide treatment occurred in the ordination diagrams produced using CA for the three invertebrate communities investigated, particularly for the communities associated with submersed macrophytes. In CA, environmental variables are not included directly in the ordination of the species data, i.e., the resulting pattern of site and invertebrate taxa points is not constrained by environmental variables and the ordination axes extracted take into account all the variance of the species data set. In comparison, in CCA, environmental variables are included directly in the ordination of the invertebrate taxa data, such that the resulting ordination diagram is constrained by the quantified environmental variables and the ordination axes extracted only account for the total variance in the species data that is explained by the environmental variables included in the analysis.

The resulting ordination diagrams using CA and CCA were most similar for the planktonic microinvertebrate community, less similar for the microinvertebrate community associated with submersed macrophytes, and least similar for the macroinvertebrate community associated with submersed macrophytes. The ordinations produced using the two methods were very similar for the planktonic microinvertebrate community and the correlations between the most important environmental variables and the first two canonical axes in CCA

were relatively high. From this one is able to infer that the environmental variables quantified accounted for a portion of the main variation in the species data (TER BRAAK 1986). The open-water portions of the enclosures likely were relatively homogeneous with respect to nutrient concentrations and other abiotic and biotic environmental parameters. As a result, the environmental parameters measured in the open-water described adequately the open-water habitat and the structure of the planktonic microinvertebrate community corresponded to this homogeneous environment.

The ordinations produced using CA and CCA were less similar for the microinvertebrate community associated with the submersed macrophytes in comparison to the planktonic microinvertebrate community. Although the ordinations produced using the two methods differed slightly, the correlations between the most important environmental variables and the first two canonical axes in CCA were still relatively high, indicating that the environmental variables quantified accounted for residual variation in the species data, but not for a portion of the main variation (TER BRAAK 1986). The portions of the enclosures with submersed macrophytes likely were heterogeneous with respect to nutrient concentrations and other abiotic and biotic environmental parameters. The presence of submersed macrophytes increases the physical complexity of the aquatic environment and inhibits the homogenization of the water column, thereby potentially altering the dynamics of nutrient concentrations and other abiotic and biotic environmental parameters (e.g., contaminant and nutrient gradients) (SCHEFFER 1998). As a result, the environmental parameters measured in the open-water did not describe the more complex habitat in association with the submersed macrophytes as adequately as they did the open-water and my ability to explain the main variance observed in the microinvertebrate community was hampered.

The ordinations produced using the two techniques were the least similar for the macroinvertebrate community associated with the submersed macrophytes in comparison to both the planktonic microinvertebrate community

and the microinvertebrate community associated with submersed macrophytes. Although the ordinations produced using the two methods differed more than for the other two communities investigated, the correlations between the most important environmental variables and the first two canonical axes in CCA were still relatively high, indicating that the environmental variables quantified still accounted for residual variation in the invertebrate taxa data (TER BRAAK 1986). Since the environmental parameters measured in the open-water poorly described the more complex habitat in association with the submersed macrophytes, particularly for the macroinvertebrate community, my ability to explain the main variance in the macroinvertebrate community constrained by the quantified environmental variables is even more limited. Additionally, the sampling method may not have been optimal for accurately estimating the abundances of many macroinvertebrate taxa. Macroinvertebrates are larger organisms, tend to be spatially heterogeneous in distribution, and tend to exist in relatively lower abundances than smaller organisms. As a consequence, some larger taxa were likely underrepresented or not represented in the macroinvertebrate community.

My ability to explain the variation in the invertebrate communities associated with submersed macrophytes would potentially have been improved if abiotic and biotic environmental parameters were measured in a fashion and at a scale more representative of the complex habitat in association with submersed macrophytes had been measured. Quantifying environmental variables in closer association with the invertebrates amongst the macrophytes would likely improve the interpretation of the environmental factors influencing the dynamics of the invertebrate communities under investigation and increase the overall understanding of the complex interactions occurring within the mesocosms.

Advantages of multivariate ordination

Information gathered from the enclosure experiment conducted in Delta Marsh yielded very large data sets, characterized by variability in the densities of

invertebrate taxa collected (see ZRUM et al. 2000, CHAPTER 3). Multivariate ordination techniques were invaluable in condensing these data and providing an effective tool for looking at large amounts of data in a concise approach.

CA produces ordination diagrams useful for visualizing differences in invertebrate community structure among sites (sample dates for each treatment) and revealing the specific invertebrate taxa associated with different sites. Indirect inferences may be made with respect to the influence of environmental parameters (i.e., seasonal changes, treatment effects) on the community structure. CA is useful as it provides an overview and quantification of the explained variation in samples. However, species occurrences are not related directly to environmental variables, thus, one is not able to specify the contribution or importance of any one environmental variable to the structure of the invertebrate community.

Further analysis of environmental parameters and their contributions to the explained variation in samples may be achieved with CCA. CCA produces ordination diagrams useful for visualizing differences in invertebrate community structure among sites constrained to environmental variables quantified by the investigator. Although neither chlorpyrifos concentration nor sampling date (i.e., "time") was included among the environmental variables, I believe that CCA produces an ordination diagram interpretable with respect to treatment effects. As this study was conducted in outdoor enclosures in a natural setting, I felt it was critical to consider seasonal aspects, such as natural species succession, in addition to treatment effects. By analyzing the data in this manner, I was able to visualize the invertebrate communities with respect to environmental factors (i.e., "seasonality") that determine their structure throughout the experiment. In addition, any change in community structure due to treatment, not seasonality, was apparent as treatment trajectories diverged. This pattern emerged in the CCA as insecticide treatment diverged from control and nutrient treatment immediately after chlorpyrifos application.

Statistical evaluation of CCA results may be done with Monte Carlo permutation testing. For the analysis of experiments, CCA is similar to a MANOVA followed by the extraction of canonical variates [Canonical Variate (Multiple Discriminant) Analysis]. Two important differences exist, however: 1) CCA is able to analyze any number of species, whereas both MANOVA and CVA impose an upper limit determined by the number of samples, which is a problem in experiments where the number of samples is less than the number of species; and 2) the statistical tests carried out in MANOVA require the assumption that the data are multivariate normal, whereas in CCA the standard test uses Monte Carlo permutation which does not require multivariate normality (VERDONSCHOT & TER BRAAK 1994). Within CCA, covariables may also be introduced in an attempt to compensate or account for systematic differences among samples or enclosures.

This experiment demonstrated the ability of CA used in combination with CCA to provide a clear overview of seasonal and treatment (particularly insecticide) effects on the planktonic invertebrate community structure and the invertebrates in association with submersed macrophytes of outdoor mesocosms in Delta Marsh and identify shortcomings with respect to our ability to quantify adequately the many environmental variables influencing the dynamics of the invertebrate communities investigated.

ACKNOWLEDGMENTS

Sampling assistance was provided by K. SANDILANDS, K. ELVIN, and L. G. GOLDSBOROUGH. The University of Manitoba Field Station (Delta Marsh) supplied logistic support essential for the completion of this project, K. ELVIN and L. G. GOLDSBOROUGH provided data for algal biomass as chlorophyll *a* and water chemistry, G. STERN and T. HALLDORSON provided assistance with analyses for chlorpyrifos, and N. KENKEL advised on multivariate analyses. Dr. J. ANDERSON (DowElanco, Winnipeg, MB) provided the Lorsban™ 4E used in this experiment. This study was supported by a Natural Sciences and Engineering Research Council (NSERC) Scholarship to LZ and NSERC Research Grants to BJH and LGG.

REFERENCES

BROCK, T. C. M., CRUM, S. J. H., VAN WIJNGAARDEN, R., BUDDE, B. J., TIJINK, J., ZUPPELLI, A. & LEEUWANGH, P. (1992): 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.

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

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.

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.

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.

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.

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.

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.

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.

SCHEFFER, M. (1998): Ecology of Shallow Lakes. 1st Ed. - Chapman and Hall, London.

TER BRAAK, C. J. F. (1985): Correspondence analysis of incidence and abundance data: properties in terms of a unimodal response model. - Biometrics **41:** 859-873.

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. (1987): Ordination. - In: JONGMAN, R. G. H., TER BRAAK, C. J. F. & VAN TONGEREN, O. F. R. (eds.): Data Analysis in Community and Landscape Ecology. - Wageningen, Pudoc, pp. 91-173.

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.

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.

VERDONSCHOT, P. & TER BRAAK, C. J. F. (1994): An experimental manipulation of oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions: multivariate analysis with Monte Carlo permutation tests. - Hydrobiologia **278:** 251-266.

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 5: Effects of organophosphorus insecticide and inorganic nutrients on the planktonic bacteria in a prairie wetland

INTRODUCTION

The specific roles microorganisms perform in freshwater ecosystems are still relatively unclear (PORTER et al. 1988), particularly in shallow-water ecosystems such as prairie wetlands. In the context of a limnetic food web model, PORTER (1996) emphasized the importance of considering the microbial web (bacteria, flagellates, ciliates) as an integral component of the traditional, algal-based food web (algae, invertebrates, fish), interacting with the algal-based food web at all trophic levels (Fig. 5-1). Direct and indirect consumers of bacterial production exist at all trophic levels. Omnivory is prevalent among many groups of invertebrates, i.e., many consumers feed at more than one trophic level. For example, rotifers and cladocerans are known to feed not only on phytoplankton, but also on bacteria and predators on bacteria (flagellates, ciliates) (SANDERS et al. 1989). Bacterivorous organisms (such as flagellates, rotifers, cladocerans) may decrease bacteria by direct grazing, or alternatively, cladocerans may graze flagellates, thereby releasing bacteria from grazing pressure.

The responses of planktonic microinvertebrate and phytoplankton communities to manipulations of higher trophic levels (e.g., planktivorous fish) have been reported (CARPENTER et al. 1987). However, the responses of planktonic bacteria to these manipulations remain virtually undocumented (VAQUE & PACE 1992). Manipulation of higher trophic levels provides an opportunity to investigate trophic cascades through the two integrally linked food webs. Direct or indirect manipulation of the grazer community will likely result in changes in the abundance of planktonic bacteria.

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 invertebrate and algal 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, ZRUM et al. 2000). However, very little information is available pertaining to either the direct or the indirect response of planktonic bacteria to contaminants and nutrient loading in freshwater prairie wetlands.

Relatively fewer investigations of the microbial web in shallow water ecosystems have been conducted in comparison to larger lakes. One of the longer term studies of an unmanipulated shallow-water system was conducted by JEPPESEN et al. (1997); an eight-year study of Lake Sobygard in Denmark examined the seasonal variation in bacterioplankton abundance, biomass, and production. Experimental manipulation of components of the microbial web have primarily focused on the effects of additional nutrients (nitrogen and phosphorus) directly on bacteria by increasing resources and indirectly through increased phytoplankton (COTTINGHAM et al. 1997), with little being known with respect to the effects of toxic stresses (e.g., herbicides, insecticides) on components of the microbial food web (WAISER & ROBARTS 1997).

This paper describes results of a study to investigate the dynamics of the planktonic bacteria in a prairie wetland ecosystem subject to controlled organophosphorus insecticide application and inorganic nutrient enrichment. Specifically, I examined the indirect responses of the bacteria in the water column to the following experimental manipulations: 1) alterations in the community structure of the microinvertebrates (specifically, Cladocera, 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); and 2) changes in phytoplankton 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 planktonic microinvertebrates and phytoplankton (ZRUM et al. 2000).

Organophosphorus insecticide treatment was expected to result in differential mortality of the arthropod component of the microinvertebrate community. Specifically, a reduction in arthropod grazer control of phytoplankton would lead to an increase in phytoplankton biomass relative to the control, provided resources were not limiting and non-arthropod herbivores did not increase in abundance. Insecticide treatment was expected also to result in an increase in bacterial density in the water column, provided non-arthropod bacterivores (specifically, flagellates and ciliates) did not increase in response to reduced competition and predation by arthropod microinvertebrates. Inorganic nutrient enrichment was expected to result in an increase in phytoplankton biomass and bacterial density in the water column relative to the control.

The first objective in this present paper was to examine temporal variation in density of bacteria in the water column of prairie wetland mesocosms subjected to experimental perturbations (treatments). Secondly, interactions in patterns of abundance within the microbial web, among bacteria, microinvertebrates (specifically, planktonic cladocerans and small rotifers), and phytoplankton were examined.

METHODS

Study site and experimental design

This 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.

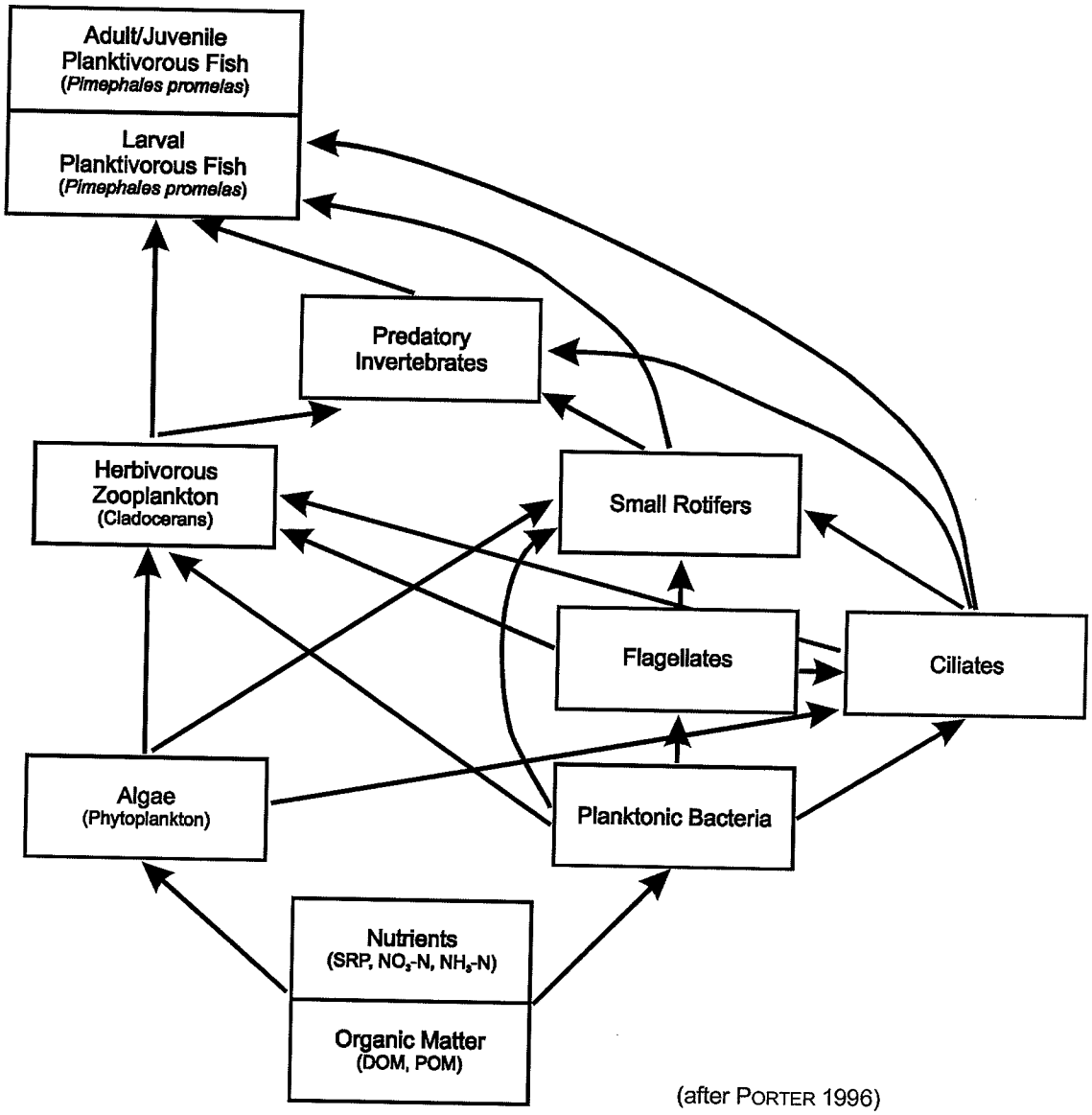


Fig. 5-1. A generalized planktonic food web integrating the microbial loop with the traditional, algal-based food chain.

Experimental enclosures (mesocosms) used represent 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 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.

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 $NaH_2PO_4 \cdot 2H_20$) 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). Water sampling for chlopryrifos and physico-chemical analyses in the enclosures have been described in ZRUM et al. (2000).

Sampling and analysis of planktonic bacteria and phytoplankton

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, bacterial samples were filtered within 24-72h of collection and fixation. The volume of water filtered varied among enclosures and was determined by the density of bacteria in the previous week's samples. 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 a bright blue, chlorophyll-bound stain fluoresces red, and unbound stain fluoresces yellow. Detailed method is presented in APPENDIX 2.

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 planktonic 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).

Cladocera were identified to species using standard references, including EDMONDSON (1959) and PENNAK (1978), and a reference collection (BJH) and enumerated. Among planktonic rotifers, only the predatory rotifer, *Asplanchna* sp., was identified and counted separately.

Data analysis

Insecticide and control treatments both contained three submersed macrophyte-dominated replicates for the duration of the experiment, however inorganic nutrient addition treatment was reduced to two replicates due to a persistent phytoplankton bloom and hence, 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. I 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 bacteria (ind./mL) and planktonic microinvertebrates (ind./L) and mean phytoplankton biomass as chlorophyll-*a* (µg/L) were estimated for all replicates; differences between treated and control enclosures 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 groups were greater than would be expected by chance, pairwise multiple comparisons among treatments were carried out using the Student-Newman-Keuls (SNK) method. Treatment effects were considered statistically significant at p values ≤ 0.05. My ability to detect differences among treatments was limited due to lack of statistical power (small number of replicates). For all sample dates for all treatments the number of degrees of freedom (df) was 8 due to the reduction of the nutrient addition treatment to two replicates. All estimates of treatment and control means are presented as mean $\pm$ SE.

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.

Nutrient dynamics

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.

Density of planktonic bacteria

All bacteria enumerated consisted of single cells; no colonies or filaments were observed. Density of planktonic bacteria (0.4-2.1 ind./mL x 10^5) was relatively low in all treatments at the beginning of June (Fig. 5-2). However, density in the control was significantly lower than in the enclosures designated as treatment ($F_{2,8} = 8.53$, $p \leq 0.05$). Maximum density (7.5 ind./mL x 10^5) occurred in the nutrient treatment near the end of June, three days after nutrient enrichment had

began. Planktonic bacterial density peaked in the control (10.4 ind./mL x 10^5) two weeks later. Density in the insecticide treatment (10.8 ± 3.6 ind./mL x 10^5) did not peak until the middle of August, concurrent with a second peak in the control (10.6 ± 5.8 ind./mL x 10^5). Insecticide application and nutrient enrichment had no significant effect on planktonic bacterial density.

Interactions among bacteria, microinvertebrates, and phytoplankton

Microinvertebrate densities and phytoplankton biomass have been described in detail for the control and treatments by ZRUM et al. (2000).

In the control, small planktonic rotifers showed a pre-treatment density peak (~2049 ind./L), declined rapidly by the end of June (~300 ind./L) and continued to decline throughout the experiment (< 100 ind./L) (Fig. 5-3). Phytoplankton biomass peaked near the end of June (~21 μg/L), concurrent with the rapid decline in small rotifer density (Fig. 5-3). Near the beginning of July, planktonic cladocerans peaked at a density of ~187 ind./L and bacteria peaked at a density of ~10.4 ind./mL x 10^5 (Fig. 5-3). Phytoplankton biomass declined to ~ 2.5 μg/L as cladoceran and bacterial densities increased and remained low (< 10 μg/L) throughout the remainder of the experiment. Cladoceran density declined to ~70 ind./L by the end of July, but remained relatively constant through August (~90 ind./L). Bacterial density declined to ~3.5 ind./mL x 10^5 by the beginning of August, however a second peak in density (~10.6 ind./mL x 10^5) was observed around the middle of the month.

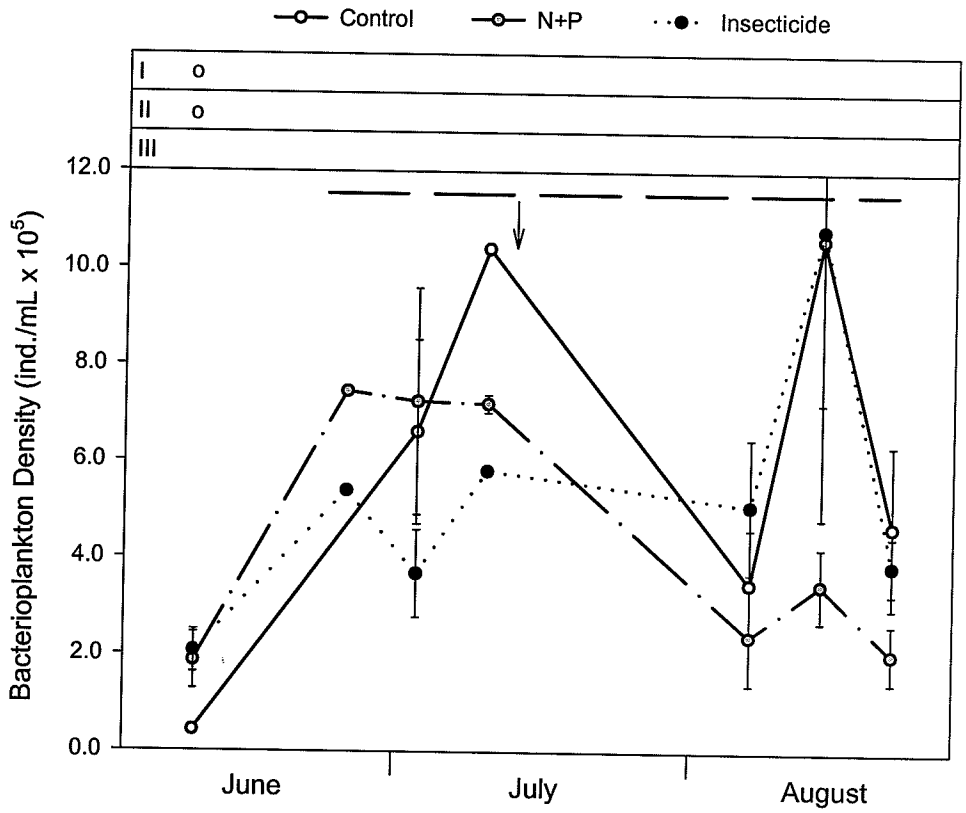
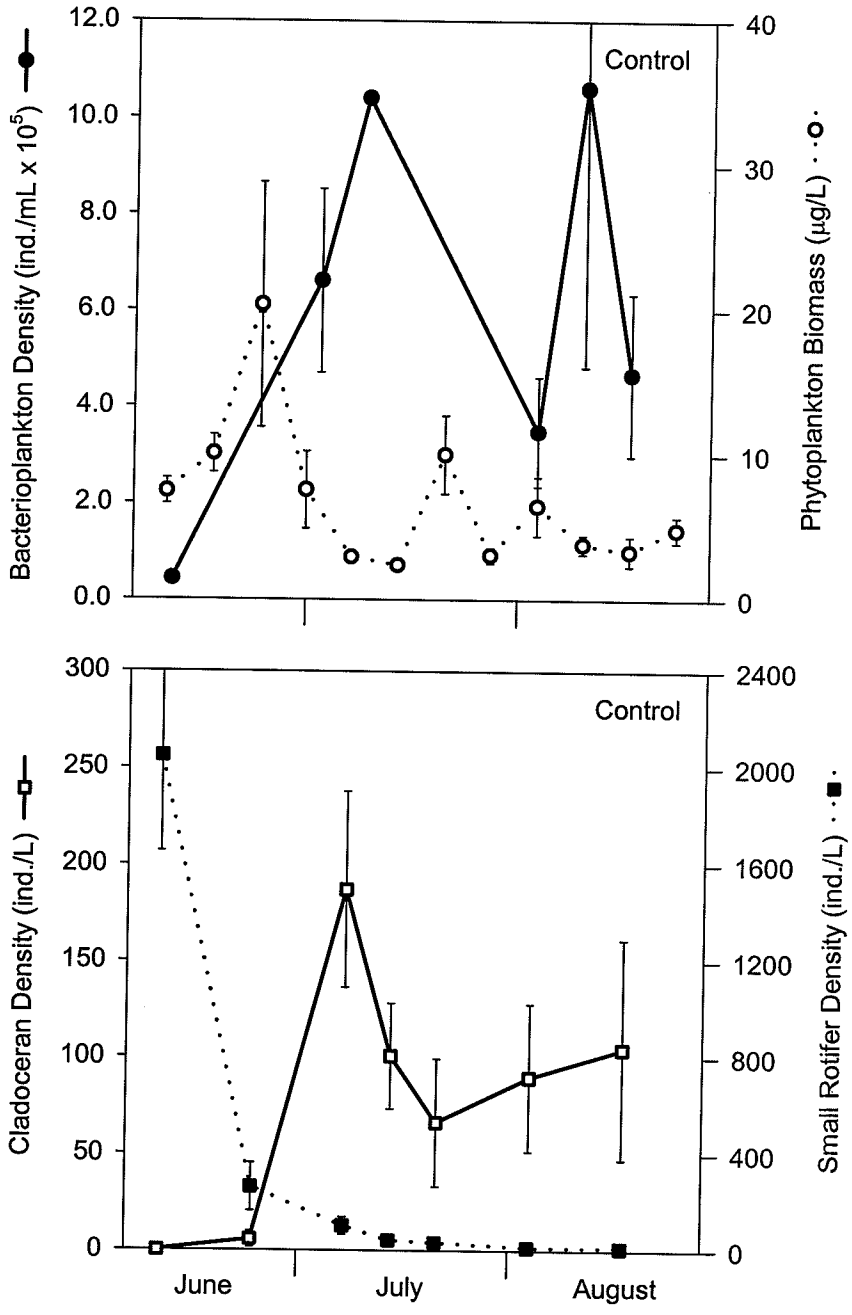


Fig. 5-2. Changes in bacterial density (ind./mL ± SE) in the water column over a 12-week period in control enclosures (Control), enclosures loaded with inorganic nutrients (N+P), and in enclosures treated with the insecticide chlorpyrifos (Insecticide). The horizontal dotted line denotes thrice weekly additions of inorganic nutrients from 23 June to 27 August; the arrow denotes the moment of insecticide application. Significant differences ($p < 0.05$) 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. 5-3. Changes in bacterial density (ind./mL ± SE), phytoplankton biomass (μg/L ± SE), cladoceran density (ind./L ± SE), and small rotifer density (ind./L ± SE) in the water column over a 12-week period in control enclosures (Control).


Control
Bacterioplankton Density (ind./mL x 10^5)
Phytoplankton Biomass (µg/L)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
40
30
20
10
0
Control
Cladoceran Density (ind./L)
Small Rotifer Density (ind./L)
300
250
200
150
100
50
0
2400
2000
1600
1200
800
400
0
June
July
August

In the insecticide treatment, small planktonic rotifers showed a pre-treatment density peak (~1012 ind./L) and declined rapidly by the end of June (~300 ind./L) (Fig. 5-4). Phytoplankton biomass peaked at the end of June (~19 μg/L), concurrent with the rapid decline in small rotifer density (Fig. 5-4). Near the beginning of July, planktonic cladocerans peaked at a density of ~175 ind./L and bacteria peaked at a density of ~5.8 ind./mL x 10^5 (Fig. 5-4). Phytoplankton biomass declined to ~7 μg/L as cladoceran and bacterial densities increased. Immediately after chlorpyrifos application, cladoceran density rapidly declined to < 4 ind./L. Small planktonic rotifers increased in density 1-week post-treatment to ~734 ind./L, concurrent with a peak in phytoplankton biomass (~36 μg/L). Small planktonic rotifer density and phytoplankton biomass declined to pre-treatment values by the beginning of August. Subsequently, bacterial density increased, peaking at ~10.8 ind./mL x 10^5 by the middle of August.

In the nutrient addition treatment, small planktonic rotifers showed a pre-treatment density peak (~1050 ind./L), declined rapidly by the end of June (~250 ind./L) and continued to decline throughout the experiment (< 100 ind./L) (Fig. 5-5). Phytoplankton biomass peaked 1-week after nutrient addition had begun (~33 μg/L), concurrent with the rapid decline in small rotifer density (Fig. 5-5). Near the beginning of July, planktonic cladocerans peaked at a density of ~158 ind./L and from the end of June to near the middle of July, bacteria exhibited an extended peak at a density of ~7.2-7.5 ind./mL x 10^5 (Fig. 5-5). Phytoplankton biomass declined to ~ 3.5 μg/L as cladoceran and bacterial densities increased and remained low (< 10 μg/L) until the end of August. Cladoceran density gradually declined to ~90 ind./L by the beginning of August, but declined sharply by the end of the month (~15 ind./L). Phytoplankton biomass increased to ~22.5 μg/L shortly after this rapid decline in cladoceran density. Bacterial density declined to ~3.5 ind./mL x 10^5 by the beginning of August and remained relatively constant through the remainder of the month.

Fig. 5-4. Changes in bacterial density (ind./mL ± SE), phytoplankton biomass (μg/L ± SE), cladoceran density (ind./L ± SE), and small rotifer density (ind./L ± SE) in the water column over a 12-week period in enclosures treated with the insecticide chlorpyrifos (Insecticide). Symbols identified as in Fig. 5-2.

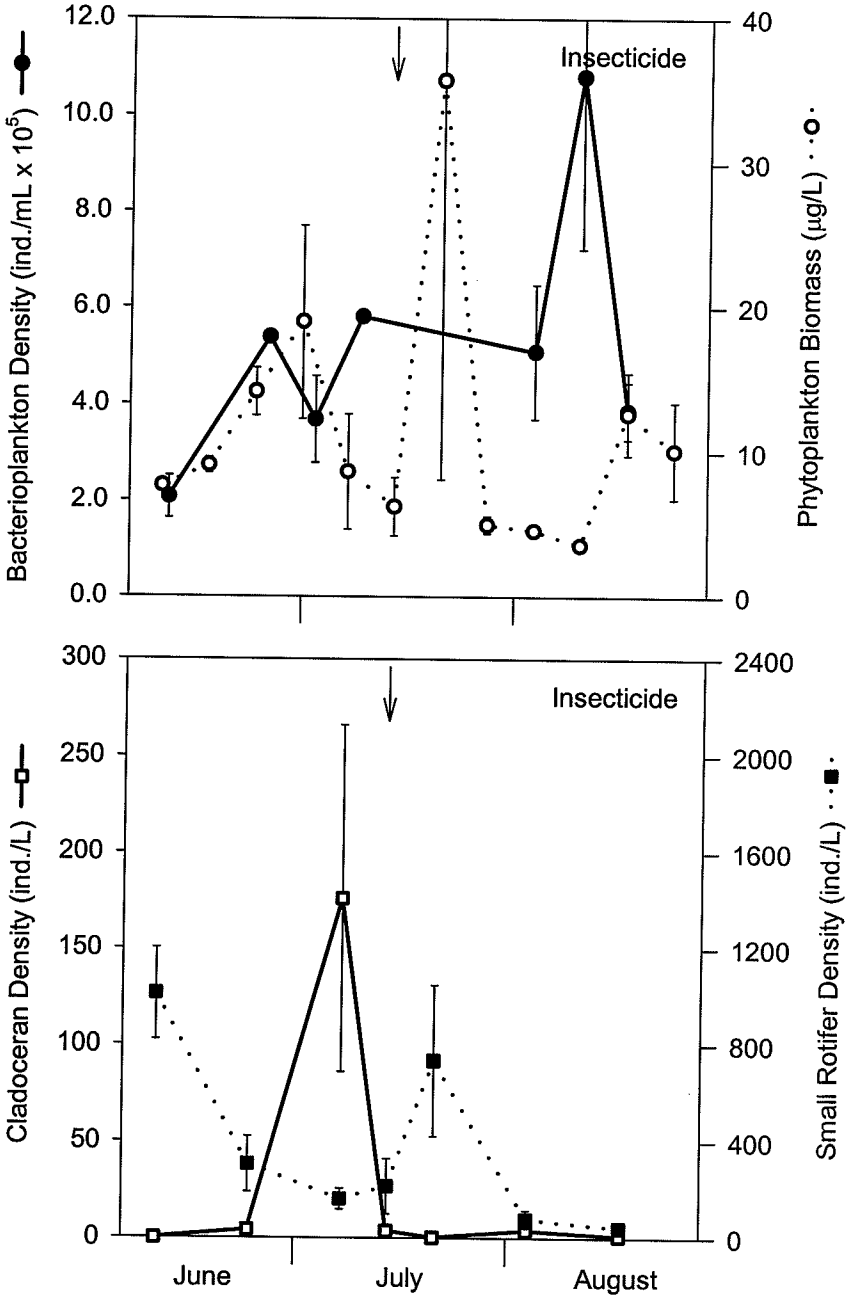
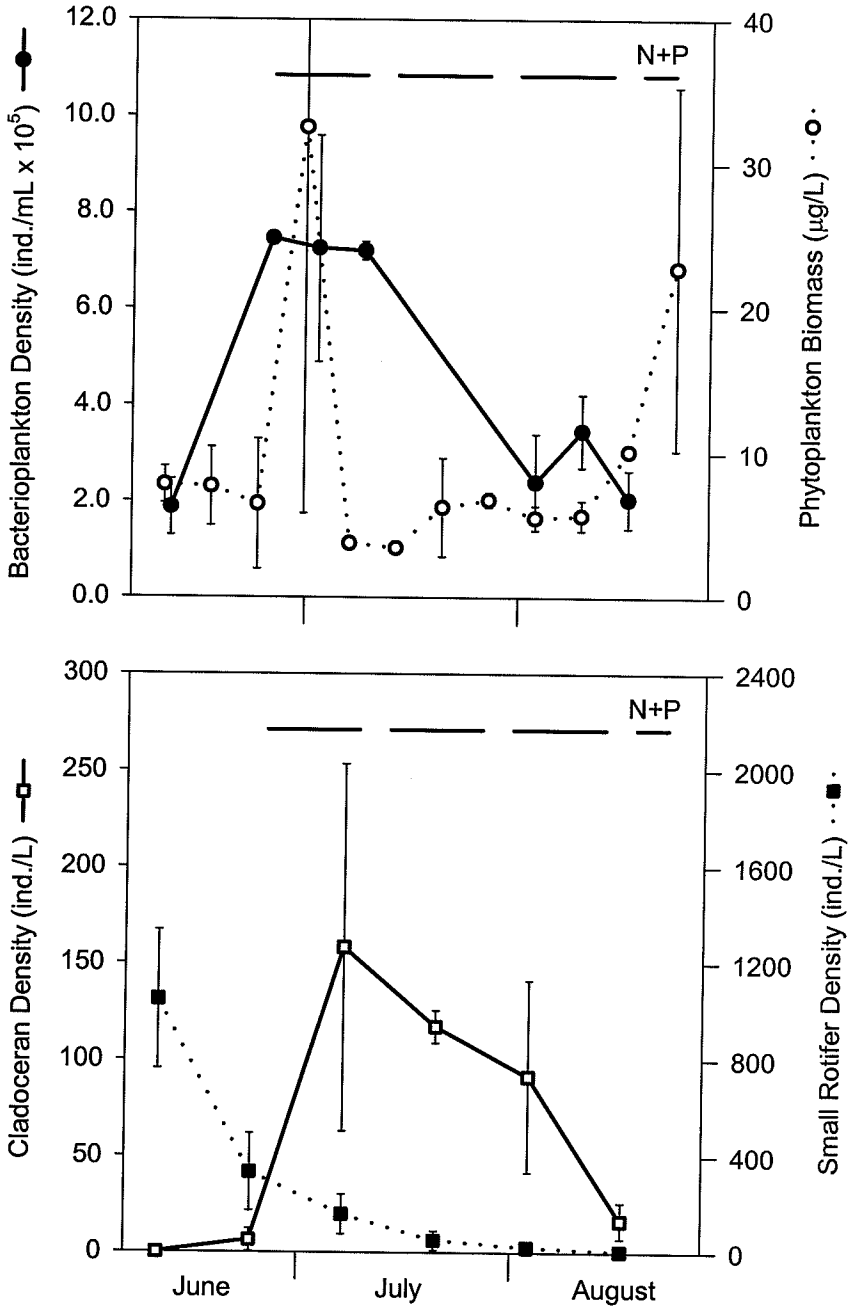

Bacterioplankton Density (ind./mL x 10^5)
Phytoplankton Biomass (µg/L)
Insecticide
12.0
10.0
8.0
6.0
4.0
2.0
0.0
40
30
20
10
0
Cladoceran Density (ind./L)
Small Rotifer Density (ind./L)
Insecticide
300
250
200
150
100
50
0
2400
2000
1600
1200
800
400
0
June
July
August

Fig. 5-5. Changes in bacterial density (ind./mL ± SE), phytoplankton biomass (μg/L ± SE), cladoceran density (ind./L ± SE), and small rotifer density (ind./L ± SE) in the water column over a 12-week period in enclosures loaded with inorganic nutrients (N+P). Symbols identified as in Fig. 5-2.


Bacterioplankton Density (ind./mL x 10^5)
Phytoplankton Biomass (µg/L)
N+P
12.0
10.0
8.0
6.0
4.0
2.0
0.0
40
30
20
10
0
Cladoceran Density (ind./L)
Small Rotifer Density (ind./L)
N+P
300
250
200
150
100
50
0
2400
2000
1600
1200
800
400
0
June
July
August

DISCUSSION

Density of bacterioplankton was initially relatively low in all enclosures near the beginning of June. Densities increased as the open-water season progressed, typically peaking in July and declining into August; secondary peaks were observed around the middle of August for all treatments. Bacterioplankton abundance in shallow, more eutrophic systems, is typically characterized by lower abundances through winter, with the highest bacterial densities occurring in early summer and undergoing fluctuations throughout the open-water season (JEPPESEN et al. 1997, KIRSCHNER & VELIMIROV 1997).

Bacterial density in the water column was not significantly different in response to either insecticide application or inorganic nutrient enrichment. Interactions among bacteria, microinvertebrates (specifically, planktonic cladocerans and small rotifers), and phytoplankton, revealed some evidence of trophic cascades, via the microbial food web, operating in similar fashions in all enclosures (Fig. 5-1). Small planktonic rotifers showed early June (pre-treatment) density peaks in all treatments, but declined rapidly by the end of June and continued to decline throughout the experiment, except in the insecticide treatment. The early summer peak in small rotifer density may have resulted from rapid asexual (parthenogenetic) reproduction in response to favorable environmental conditions (e.g., water temperature, food availability and quality). The subsequent seasonal decline in small rotifer density may have been a consequence of a reduction in food quality or crowding.

Upon release from small rotifer consumption, a short-lived peak in phytoplankton biomass was observed in all treatments at the end of June. Subsequently, phytoplankton biomass declined as planktonic cladoceran and bacterial densities increased. Grazing of phytoplankton by planktonic cladocerans leading to a period of clear water has been reported in the literature (LAMPERT et al. 1986, SOMMER et al. 1986). An increase in small rotifers in response to the increase in phytoplankton biomass was not observed likely due to the suppression of rotifers by cladocerans both by competition for the shared

algal resource and by mechanical interference (GILBERT 1988). Small rotifers have been observed to be relatively more important than cladocerans as consumers of planktonic bacteria (SANDERS et al. 1989). Additionally, cladocerans are important grazers of flagellates and will also consume the larger ciliates, both of which are voracious feeders on bacteria (PORTER et al. 1979, SANDERS et al. 1989). The observed increase in planktonic bacterial density was likely the result of the following series of events: low densities of small rotifers released bacteria from predation; inefficient grazing of bacteria by cladocerans allowed an increase in bacterial density; and, consumption of bacterivorous flagellates and ciliates by cladocerans released bacteria from predation.

Experimental perturbations of the integrated food web as a consequence of insecticide and nutrient treatment resembled changes observed in other ecosystems. The increase in small planktonic rotifers in the insecticide enclosures after chlorpyrifos application has also been observed by HURLBERT et al. (1972), BROCK et al. (1992a), and VAN DONK et al. (1995). A short-lived increase in phytoplankton biomass was also observed the week after insecticide addition, concurrent with the increase in small rotifers. These increases may have been an indirect consequence of reduced abundance of insecticide-sensitive cladocerans which were able to suppress the rotifers prior to chlorpyrifos application by competition for phytoplankton and by mechanical interference. With fewer competitors, small rotifers increased in density through rapid asexual reproduction. HURLBERT et al. (1972) and BROCK et al. (1992b) found that a reduction in cladoceran density due to direct insecticide toxicity led to an increase in rotifers, which then fed upon the phytoplankton. Small planktonic rotifer density and phytoplankton biomass declined to pre-treatment values by the beginning of August. Subsequently, bacterial density increased, likely due to a release from predation. In microcosm studies conducted by WAISER & ROBARTS (1997), addition of a herbicide was observed to indirectly stimulate planktonic bacterial numbers, but only in combination with nitrogen and phosphorus enrichment.

When the interactions among bacteria, planktonic cladocerans and small rotifers, and phytoplankton in the nutrient enrichment treatment were examined, the pattern did not differ substantially from the one observed in the control for the entire experimental period, except during the second half of August. Cladoceran density remained relatively high in the nutrient treatment through August. A sharp decline in cladoceran density in the nutrient treatment was observed near the end of August and an increase in phytoplankton biomass occurred approximately 1-week later. The increase in phytoplankton biomass was likely due to the combination of reduced consumption by cladocerans and increased availability of nutrients from inorganic nitrogen and phosphorus addition. Increases in bacterioplankton density in response to increased nutrient loading could not be demonstrated. In contrast, addition of nitrogen and phosphorus to an oligotrophic prairie lake was shown to stimulate phytoplankton biomass and planktonic bacterial density (WAISER & ROBARTS 1997).

Although this study was preliminary in nature, some valuable insights have been gained with respect to increasing our understanding of the importance and regulation of microbial processes in freshwater, prairie wetland ecosystems. The seasonal pattern of bacterioplankton density appears to resemble that of other comparable systems. When interactions among bacteria, planktonic cladocerans and small rotifers, and phytoplankton were considered, they revealed some evidence of trophic cascades functioning in the enclosures (e.g., inferred predation by some planktonic microinvertebrates on bacteria in the water column and competition for a shared algal food source among planktonic invertebrates). The need exists to explore the dynamics of other important bacterivorous organisms, namely the flagellates and ciliates, and their effects on the microbial components of the food web. Additionally, very little is known about the strength of interactions among members of the food web existing in the experimental enclosures and how these interactions are influenced by perturbation (e.g., insecticide application, inorganic nutrient enrichment) or season.

ACKNOWLEDGEMENTS

The University of Manitoba Field Station (Delta Marsh) provided logistical support necessary for the successful completion of this project. K. SANDILANDS and K. ELVIN assisted with sample collection, L. G. GOLDSBOROUGH provided chlorophyll *a* data, and T. HALLDORSON provided assistance with analyses for chlorpyrifos. Thank you to E. HUEBNER for the use of his epifluorescence microscope. Dr. J. ANDERSON (DowElanco, Winnipeg, MB) provided the Lorsban™ 4E used in this experiment. This study was supported in part by a Natural Sciences and Engineering Research Council (NSERC) Scholarship to LZ and NSERC Research Grants to BJH and LGG.

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.

CARPENTER, S. R., KITCHELL, J. F., HODGSON, J. R., COCHRAN, P. A., ELSER, J. J., ELSER, M. M., LODGE, D. M., KRETCHMER, D., HE, X. & VON ENDE, C. N. (1987): Regulation of lake primary productivity by food web structure. - Ecology **68:** 1863-1876.

COTTINGHAM, K. L., KNIGHT, S. E., CARPENTER, S. R., COLE, J. J., PACE, M. L. & WAGNER, A. E. (1997): Response of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment. - J. Plankton Res. **19:** 995-1010.

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.

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. & 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.

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.

JEPPESEN, E., ERLANDSEN, M. & SONDERGAARD, M. (1997): Can simple empirical equations describe the seasonal dynamics of bacterioplankton in lakes: an eight-year study in shallow hypertrophic and biologically highly dynamic Lake Sobygard, Denmark. - Microb. Ecol. **34:** 11-26.

KIRSCHNER, A. K. T. & VELIMIROV, B. (1997): A seasonal study of bacterial community succession in a temperate backwater system, indicated by variation in morphotype numbers, biomass, and secondary production. - Microb. Ecol. **34:** 27-38.

LAMPERT, W., FLECKNER, W., RAI, H. & TAYLOR, B. E. (1986): Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. - Limnol. Oceanogr. **31:** 478-490.

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.

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.

PORTER, K. G. (1996): Integrating the microbial loop and the classic food chain into a realistic planktonic food web. - In: POLIS, G. A. & WINEMILLER, K. O. (eds.): Food Webs: Integration of Patterns and Dynamics. - Chapman and Hall, New York, NY, pp. 51-59.

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.

PORTER, K. G., PAERL, H., HODSON, R., PACE, M., PRISCU, J., RIEMANN, B., SCAVIA, D. & STOCKNER, J. (1988): Microbial interactions in lake food webs. - In: CARPENTER, S. R. (ed.): Complex Interactions in Lake Communities. - Springer-Verlag, New York, NY, pp. 209-227.

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.

SOMMER, U., GLIWICZ, Z. M., LAMPERT, W. & DUNCAN, A. (1986): The PEG-model of seasonal succession of planktonic events in fresh waters. - Arch. Hydrobiol. **106:** 433-471.

SPSS for Windows. Version 10.0, SPSS Inc. ©1989-1999.

VAQUE, D. & PACE, M. L. (1992): Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure. - J. Plankton Res. **14:** 307-321.

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 6: Synopsis and Major Conclusions

PERTURBATION OF SHALLOW-WATER ECOSYSTEMS

Grazer and microbial constituents of a prairie wetland food web were examined using *in situ* mesocosms in Blind Channel, Delta Marsh, during the open-water season in 1997. Direct and indirect effects of a "top-down" perturbation (application of the arthropod-specific insecticide, chlorpyrifos) and a "bottom-up" manipulation (inorganic N and P enrichment) were monitored over a 10-week experimental period. The detailed consequences, both acute and prolonged, have been presented in CHAPTERS 2 through 5, isolating those effects observed on the communities in the water column of the experimental enclosures from those documented for the communities associated with submersed macrophytes.

A synopsis of food web effects is presented here beginning with a picture of the unmanipulated food web in the mesocosms (Fig. 6-1), derived primarily from previous experiments with the enclosure system (SANDILANDS et al. 2000), and the potential linkages among trophic groups. The unmanipulated food web illustrates the traditional grazer food web as it interfaces with the microbial food web. More components of the overall web were examined in the present study than have been attempted previously, allowing a relatively comprehensive study of trophic cascades through the food web existing in the mesocosms.

SPATIAL HETEROGENEITY AMONG EXPERIMENTAL ENCLOSURES

Our understanding of complex interactions and community structure has come primarily from studies of planktonic communities in lakes (LODGE et al. 1988). LODGE et al. (1988) attribute the success of these studies to the relative simplicity of the planktonic communities and the relative ease of efficiently sampling and manipulating these communities. Shallow-water ecosystems (e.g., wetlands) are typically considered to be more complex than large, deep lakes; interactions occurring within these shallow-water systems are inherently more difficult to

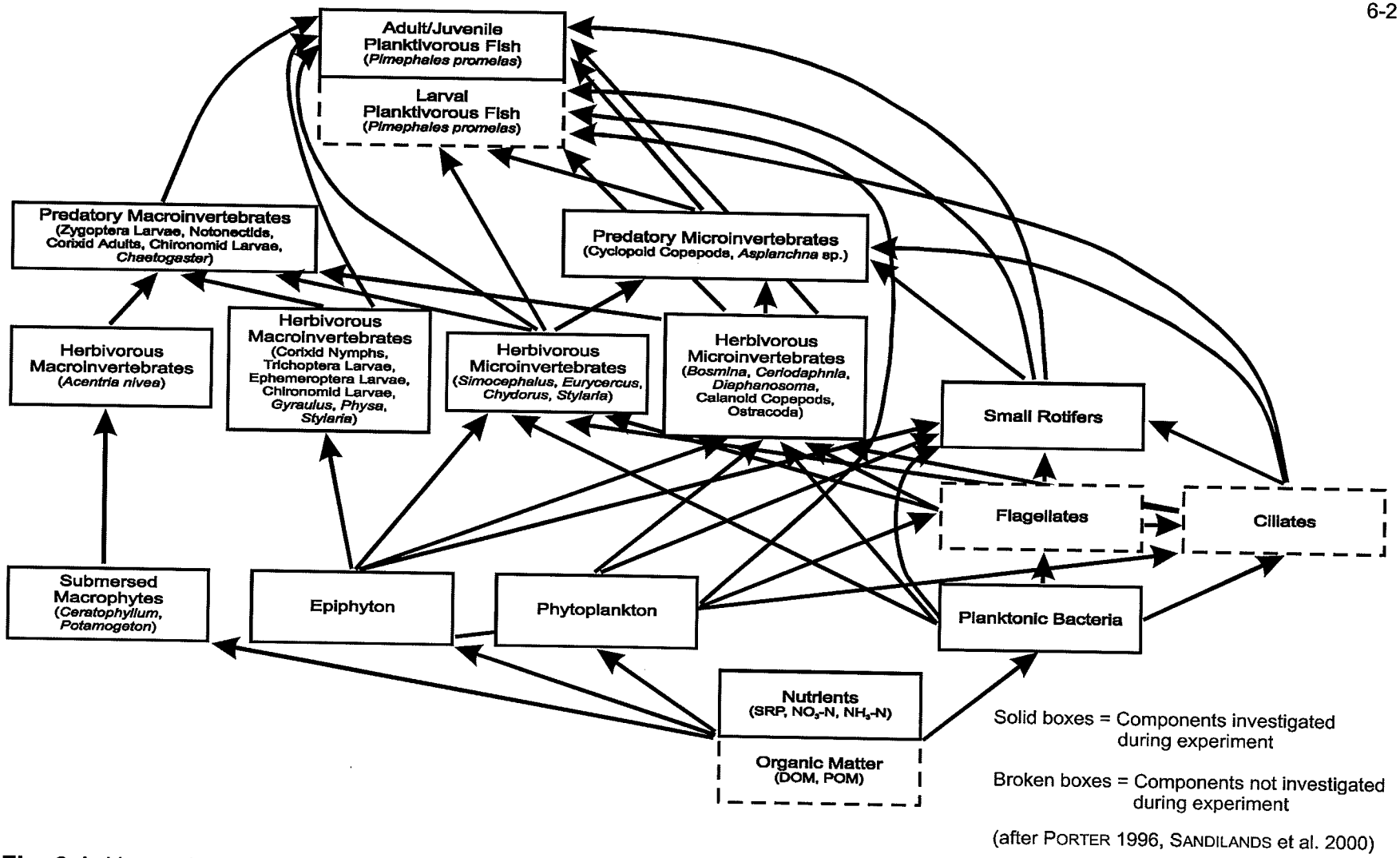


Fig. 6-1. Unmanipulated food web in experimental enclosures in Blind Channel, Delta Marsh.

understand. The increase in complexity is largely due to the intense sediment-water interaction and the potentially large impact of submersed aquatic macrophtyes (SCHEFFER 1998).

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). Additionally, spatial variation in distribution of organisms is likely greater both horizontally and vertically (i.e., water depth) in beds of submersed macrophytes than in the open-water. The "patchiness" of invertebrates was reflected in the variability within and among treatment replicates for the experimental enclosures of Blind Channel, Delta Marsh.

The difference required to detect a response in the invertebrate taxa measured relates to the power of the statistical test. The variability among treatment replicates (visualized by large standard error bars) results in low power for most comparisons among treatments on sampling dates; however, power increased as variability among treatment replicates declined. Therefore, depending on the degree of variability among treatment replicates, power of the statistical test was higher or lower. In order to increase consistently the power of statistical tests, a greater number of treatment replicates would be required to reduce the degree of variability observed for a treatment effect. Logistical limitations, however, prevented having more than three replicates for each treatment and the control.

EXPERIMENTAL PERTURBATIONS

Organophosphorus insecticide application

Manipulation of the primary producer-consumer interaction by differential elimination of the arthropod-grazer component through the application of the organophosphorus insecticide, chlorpyrifos, was conducted. Addition of chlorpyrifos resulted in the differential mortality of the planktonic arthropod microinvertebrate community and the macrophyte-associated microinvertebrate and macroinvertebrate communities (direct effects) (Fig. 6-2). Differential mortality of arthropod grazers should increase primary producer biomass (phytoplankton, epiphyton, submersed macrophytes), as grazing pressure is reduced (indirect effects) (Fig. 6-2), but was not observed. Knowledge of edibility of primary producers (especially phytoplankton and epiphyton) would be required to address this issue.

Direct (primary) effects

- Differential mortality of planktonic arthropod microinvertebrates resulted from chlorpyrifos application; calanoid copepods (*Diaptomus nudus*) were observed to be more tolerant than cladocerans, copepod nauplii, and cyclopoid copepod adults (*Acanthocyclops vernalis*, *Diacyclops thomasi*) and copepodites.
- Differential mortality of macrophyte-associated microinvertebrates resulted from chlorpyrifos application; calanoid copepods (*Diaptomus nudus*), harpacticoid copepods, and *Microcyclops varicans rubellus* (cyclopoid copepod) were observed to be more tolerant than cladocerans, copepod nauplii, cyclopoid copepod adults (*Acanthocyclops vernalis*, *Diacyclops thomasi*) and copepodites, and ostracods.

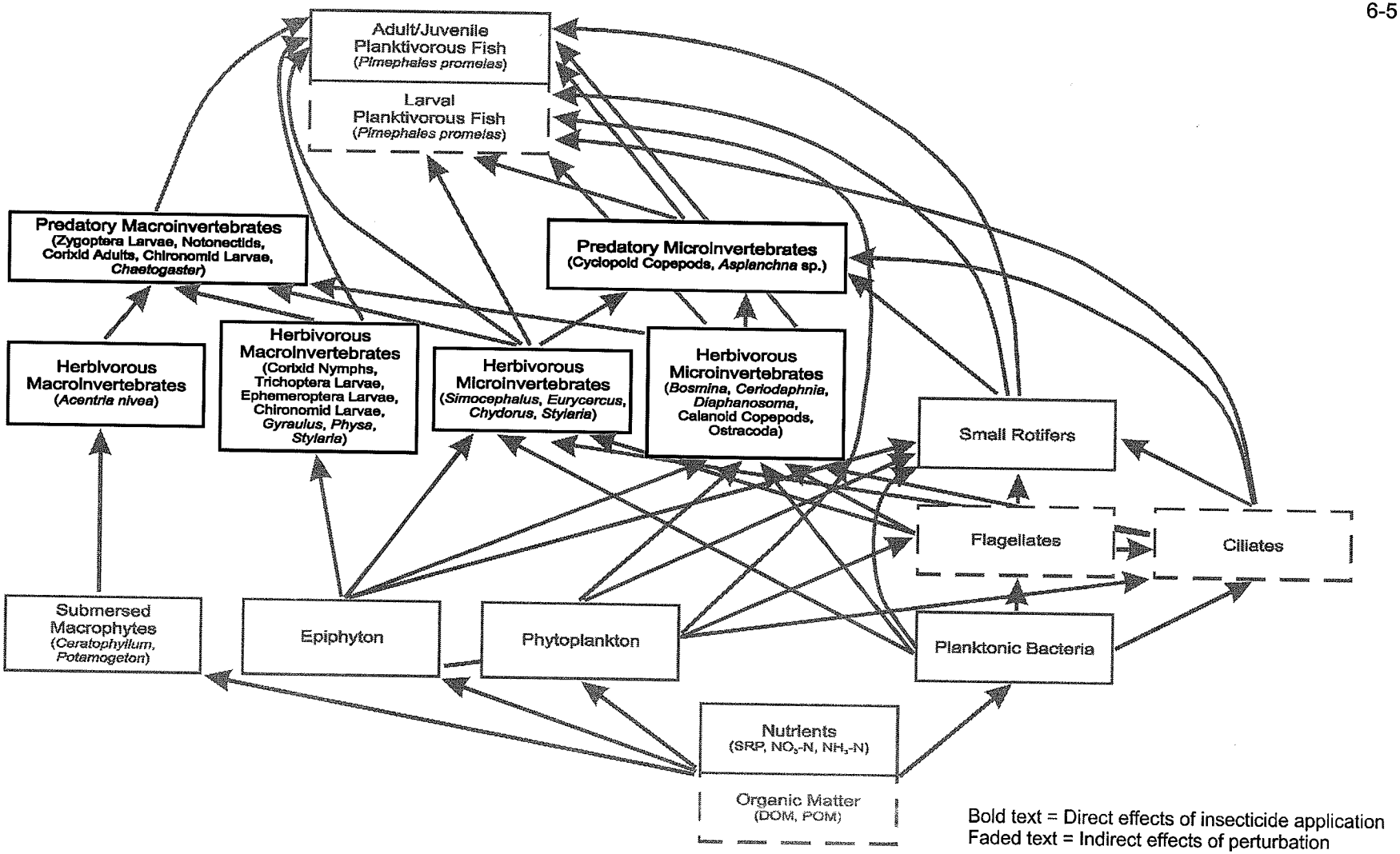


Fig. 6-2. Manipulated food web in experimental enclosures in Blind Channel, Delta Marsh, through the application of the insecticide, chlorpyrifos.

- Smaller species of planktonic and macrophyte-associated Cladocera (*Bosmina longirostris*, *Ceriodaphnia dubia*, *Diaphanosoma birgei*) appeared to be more tolerant than larger species of planktonic and macrophyte-associated cladocerans (*Daphnia*, *Simocephalus*).
- Planktonic and macrophtye-associated nauplii were reduced after chlorpyrifos application, but not to the extent of other cyclopoid life stages.
- The macrophyte-associated macroinvertebrate community changed after chlorpyrifos addition due to direct toxicity on the arthropod groups Insecta and Amphipoda (*Hyalella*).

Indirect (secondary) effects

- Small planktonic rotifers increased in response to the differential mortality of planktonic arthropod microinvertebrates.
- The relative importance of small rotifers and oligochaetes (*Chaetogaster*, *Stylaria*) in association with submersed macrophytes increased in response to the differential mortality of macrophyte-associated arthropod microinvertebrates.
- The relative importance of *Chaetogaster* and *Stylaria* in association with submersed macrophytes increased in response to the differential mortality of macrophyte-associated arthropod macroinvertebrates.
- A definitive positive response by the primary producers to the differential mortality of arthropod invertebrates could not be demonstrated.
- Density of planktonic bacteria did not positively respond to the differential mortality of planktonic arthropod microinvertebrates.

Inorganic nutrient enrichment

Manipulation of the primary producer-consumer interaction by providing the primary producers with an additional source of nutrients (inorganic nitrogen and phosphorus) was conducted. Addition of inorganic nitrogen and phosphorus

should increase primary producer biomass (phytoplankton, epiphyton, submersed macrophytes) and density of plankonic bacteria (direct effects) (Fig. 6-3). Increased primary producer biomass should result in increased densities of microinvertebrate and macroinvertebrate grazers (indirect effects) (Fig. 6-3), but neither was observed.

Direct (primary) effects

- A definitive response by the primary producers to increased availability of inorganic nitrogen and phosphorus could not be demonstrated.
- Density of planktonic bacteria did not positively respond to inorganic nutrient enrichment.

Indirect (secondary) effects

- A definitive response by the planktonic microinvertebrate or the submersed macrophyte associated microinvertebrate and macroinvertebrate communities to potentially increased primary producer biomass could not be demonstrated.

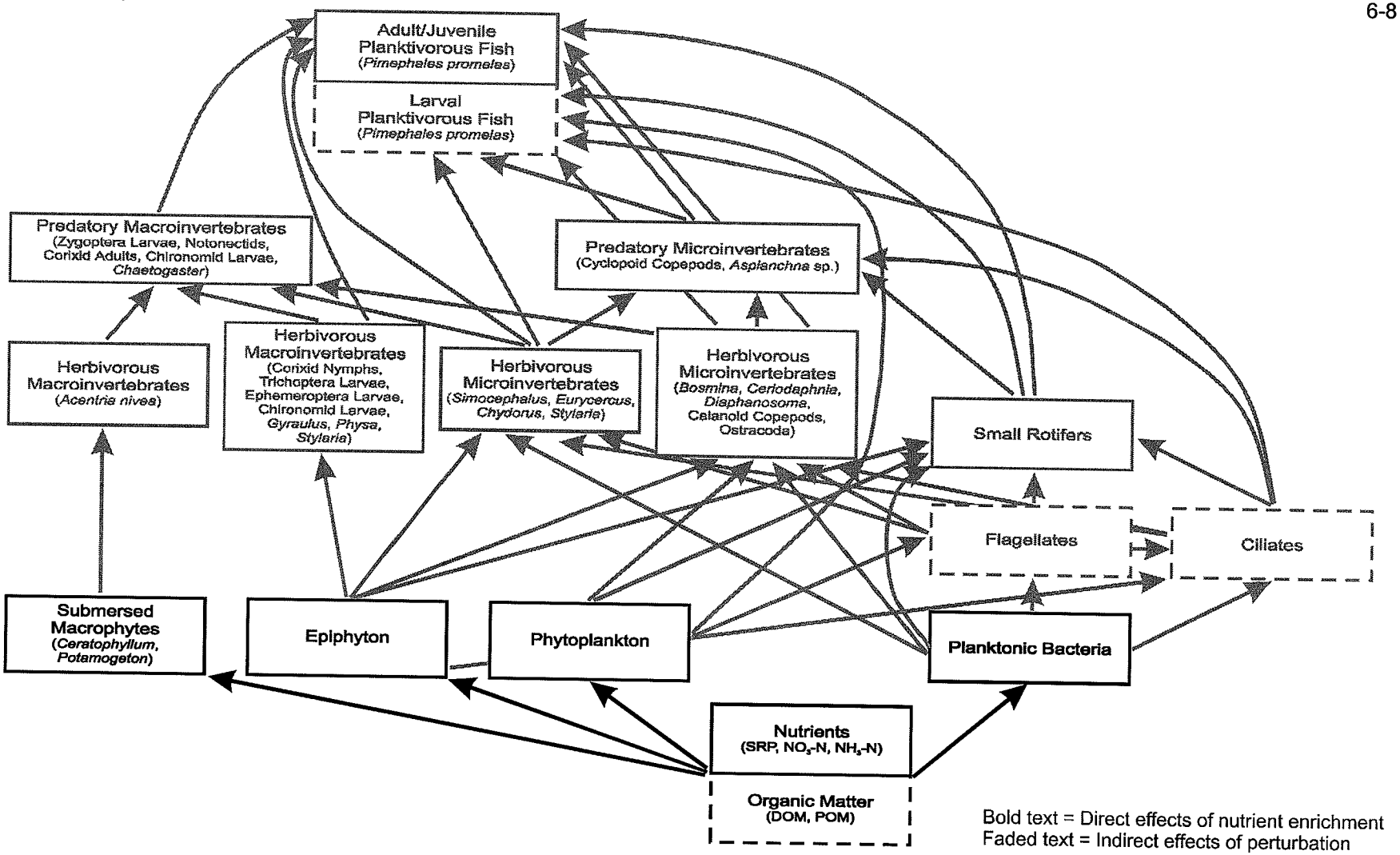


Fig. 6-3. Manipulated food web in experimental enclosures in Blind Channel, Delta Marsh, through inorganic nutrient enrichment.

REFERENCES

BROCK, T. C. M., CRUM, S. J. H., VAN WIJNGAARDEN, R., BUDDE, B. J., TIJINK, J., ZUPPELLI, A. & LEEUWANGH, P. (1992): 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.

FROST, T. M., DEANGELIS, D. L., BARTELL, S. M., HALL, D. J. & HURLBERT, S. H. (1988): Scale in the design and interpretation of aquatic community research. - In: Carpenter, S. R. (ed.): Complex Interactions in Lake Communities. - Springer-Verlag, New York, NY, pp. 229-258.

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

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.

LODGE, D. M., BARKO, J. W., STRAYER, D., MELACK, J. M., MITTELBACH, G. G., HOWARTH, R. W., MENGE, B. & TITUS, J. E. (1988): Spatial heterogeneity and habitat interactions in lake communities. - In: Carpenter, S. R. (ed.): Complex Interactions in Lake Communities. - Springer-Verlag, New York, NY, pp. 181-208.

PETERSEN, J. E., CORNWELL, J. C. & KEMP, W. M. (1999): Implicit scaling in the design of experimental aquatic ecosystems. - Oikos **85:** 3-18.

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. (1996): Integrating the microbial loop and the classic food chain into a realistic planktonic food web. - In: POLIS, G. A. & WINEMILLER, K. O. (eds.): Food Webs: Integration of Patterns and Dynamics. - Chapman and Hall, New York, NY, pp. 51-59.

SANDILANDS, K. A., HANN, B. J., & GOLDSBOROUGH, L. G. (2000): The impact of nutrients and submersed macrophytes on invertebrates in a prairie wetland, Delta Marsh, Manitoba. - Arch. Hydrobiol. **148:** 441-459.

SCHEFFER, M. (1998): Ecology of Shallow Lakes. 1st Ed. - Chapman and Hall, London.

APPENDIX 1: Detailed method for insecticide application and analysis of chlorpyrifos in water

Water samples were extracted and analyzed for chlorpyrifos using the method described by RAWN (1998). This method originally involved HPLC analysis (BURKHARDT et al. 1994) and was modified significantly by RAWN (1998) to include clean-up of water sample extracts and use of a gas chromatograph in combination with a mass selective detector as the detection instrument.

DILUTION OF CHLORPYRIFOS

- Lorsban™4E: emulsifiable formulation with 41 % (w/w) chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] as the active ingredient
- Lorsban™4E has a guarantee of 480 g chlorpyrifos per L, or 480,000 µg/mL
- dilution of 1mL of the emulsifiable concentrate with 1mL of distilled water produced a concentration of 240,000 µg/mL
- for each insecticide treatment enclosure, 1mL of the 240,000 µg/mL dilution was pipetted into a 250 mL brown glass bottle, which was then filled with distilled water
- the bottles were stored at 4°C in the dark until the time of addition
- all dilution steps took place in a fumehood.

APPLICATION OF CHLORPYRIFOS

- the approximate volumes of insecticide treatment enclosures 1, 6, and 8 were 21,500 L, 22,250 L, and 22,500 L, respectively
- to each enclosure 240 000 µg of chlorpyrifos was added to produce a nominal concentration of approximately 10 µg/L
- addition of chlorpyrifos occurred on Monday, 14 July, at 9:00 AM
- weather conditions were partly cloudy with a light breeze and an air temperature of 15-20°C

- for each insecticide treatment enclosure the following addition protocol was used:
    1) a 10 L watering can was used;
    2) the watering can was rinsed with enclosure water prior to addition;
    3) the watering can was filled with 5 L of carbon-filtered water and then half of the 250 mL bottle was added to it; the contents of the can were swirled and then sprinkled over the entire enclosure using the catwalk;
    4) step 3) was repeated with the remainder of the 250 mL bottle and the enclosure was sprinkled in the opposite direction;
    5) the 250 mL bottle was rinsed three times with carbon-filtered water into the watering can and 5 L of carbon-filtered water was added; the contents were then swirled and the enclosure sprinkled; and
    6) an additional 5 L of carbon-filtered water was used to rinse the watering can and sprinkle the enclosure for a fourth time.

WATER SAMPLING FOR CHLORPYRIFOS ANALYSIS

- the following sampling schedule was used:
    1) 97-07-14 8:30 AM - pre-addition water samples taken;
    2) 97-07-14 9:00 AM - chlorpyrifos addition;
    3) 97-07-14 9:30 AM - first set of post-addition water samples taken (1 h post-addition);
    4) 97-07-14 9:00 PM - second set of post-addition water samples taken (12 h post-addition);
    5) 97-07-15 9:00 AM - third set of post-addition water samples taken (24 h post-addition);
    6) 97-07-15 9:00 PM - fourth set of post-addition water samples taken (36 h post-addition);
    7) 97-07-16 9:00 AM - fifth set of post-addition water samples taken (48 h post-addition); and

8) 97-07-17 9:00 AM - sixth set of post-addition water samples taken (72 h post-addition).

- prior to chlorpyrifos addition water samples were collected from each insecticide treatment enclosure at 8:30 AM to provide background levels of chlorpyrifos in the water column
- all water samples (pre- and post-addition) were collected using the following protocol:
    1) prior to water collection, the brown glass collection containers and collection equipment was rinsed thoroughly with enclosure water, pouring the water back into the enclosure;
    2) duplicate depth-integrated water column samples (from the center and the northeast corner of the enclosure) were collected from each insecticide treatment enclosure using a transparent acrylic cylinder (50 cm x 5.5 cm)
    3) total volume of each sample was 2 L;
    4) a glass funnel with 150 µm mesh net covering its opening was used to transfer the sample into a pre-labeled brown glass collection container and remove larger planktonic microinvertebrates;
    5) after collection, the samples were transported to the Freshwater Institute (Department of Fisheries and Oceans) in Winnipeg packed in coolers with ice; and
    6) samples were stored at 4 °C in the dark until further processing and analysis.

MATERIALS FOR PROCESSING AND ANALYSIS

detection instrument

- a Hewlett Packard (HP) 5890 series II gas chromatograph (GC) with electronic pressure control coupled to an HP 5971 mass selective detector (MSD) operating in the selective ion mode

water pumps

- one Fluid Metering Inc. (FMI) lab pump, model QD ("Q" Pump) with stainless steel AXYS filter holder and teflon tubing (referred to as "filtration unit")
- three FMI lab pumps, model RHSY with teflon tubing (referred to as "extraction units")

filters

- GelmanSciences type A/E glass, 142 mm
- GelmanSciences ACRODISC CR PTFE 1.0 µm filter pack

cartridges

- disposable solid phase extraction (SPE) tubes containing adsorbents [Supelco Inc., Custom, Supelclean™ Envi-Carb, 6ml (0.5g), teflon frits]

analytical evaporator

- N-evap™ by Organomation

syringe

- Hamilton 5.0ml (5000µl) luer-lock syringe

WATER SAMPLE PROCESSING

The following protocol was used to prepare the water samples for analysis:

- **filtering of solid phase extraction (SPE) water samples**
    1) wash the AXYS filtration unit sequentially with 100 mL distilled in glass (DIG) grade methanol and 100 mL high performance liquid chromatography (HPLC) grade water prior to filtration;
    2) disassemble the AXYS filtration unit and wipe the inside with kimwipes;

3) handling filters only with acetone cleaned forceps, place one baked (@ 275 °C for 8 h) type A/E glass filter with rough side facing down on bottom half of filtration unit, replace screen, place second baked type A/E glass filter with rough side facing up on top half of filtration unit and reassemble the AXYS filtration unit;
4) clean outside of teflon tubing with hexane
5) filter 2 L water sample into baked brown glass collection container;
6) after sample is filtered, disassemble AXYS filtration unit, remove type A/E glass filters with acetone cleaned forceps, place on hexane cleaned tinfoil, wrap, place in brown glass jars, and store at 4 °C in the dark;
7) analysis of the particles collected on the type A/E glass filters may be analyzed at a later date for chlorpyrifos; and
8) reassemble AXYS filtration unit and repeat steps 1) through 7) for additional water samples.

- **extraction of SPE water samples**

1) weigh three SPE cartridges prior to cartridge conditioning and sample extraction;
2) condition the three SPE cartridges sequentially with 10 mL 80% dichloromethane (DCM)/20% methanol (MeOH) solution, 5 mL DIG grade MeOH, and 15 mL of a 10 g/L solution of ascorbic acid;
3) the cartridges must be used within 8 hours of conditioning, or reconditioned;

NOTE: ascorbic acid solution is prepared in HPLC grade water and has a shelf-life of one month only; this solution must be stored in a brown glass or foil wrapped container at 4 °C.

4) to each filtered 2 L water sample add 20 g of baked (@ 600 °C for 8 h) NaCl and 20 µL of the internal standard deuterated (d_3) 2,4-D (concentration of 50 µg per mL methanol);

NOTE: the syringe used to add (d_3) 2,4-D must be cleaned with acetone and then pumped with the internal standard to ensure no bubbles are present.

5) clean each extraction unit sequentially with 30 mL DIG grade methanol and 30 mL HPLC grade water;
6) pump the 2 L water sample through a SPE cartridge at a flow rate of 20-25 mL/min. into a receiving beaker;
7) record the sample volume collected using a 1000 mL graduated cylinder;
8) dry the SPE cartridge over positive pressure nitrogen gas at 140 psi for 18 minutes; and
9) weigh the cartridge to ensure it is dry.

- **elution procedure for SPE cartridges**

A. base fraction

1) elute the cartridge sequentially with 1 mL DIG grade methanol and 6 mL 80%DCM/20%MeOH at a flow rate of 1 drop per second; and
2) collect eluant in a baked (@ 275 °C for 8h) 15 mL graduated test tube.

B. acid fraction

1) elute the cartridge with 8 mL of 0.2% trifluoracetic acid (TFA) in 80%DCM/20%MeOH at a flow rate of 1 drop per second; and
2) collect both the base and acid fractions in the same 15mL graduated test tube.

NOTE: at this point, the graduated test tubes can be wrapped in foil and left until there are enough samples filtered, extracted, and eluted to make further processing efficient.

NOTE: acid and base fractions were collected for potential future analysis of major metabolites of chlorpyrifos [TCP (3,5,6-trichloro-2-pyridinol) and TMP (3,5,6-trichloro-2-methoxypyridine)].

- **further sample processing**

The remaining steps are carried out most efficiently when there are several eluted samples ready for further processing:

1) N-evap™ each sample to 500 µL;
2) be careful samples do not bubble over;
3) add 500 µL or 1 mL of diazomethane (derivatizing agent) to each sample, vortex each sample, open and close the lid of each to relieve the pressure, and let each sit for 1h;

NOTE: derivatization was carried out for potential future analysis of major metabolites of chlorpyrifos (TCP and TMP).

4) bring the volume of each sample to 5 mL with hexane and vortex;
5) N-evap™ each sample to 1 mL;
6) bring the volume of each sample to 5 mL with hexane and vortex
7) at this point check for a layer of water in each sample, dry over anhydrous sodium sulfate (Na_2SO_4) if necessary and vortex;
8) filter sample using GelmanSciences filter pack and Hamilton 5.0 mL luer-lock syringe into a new baked 15 mL graduated test tube;
9) syringe is cleaned sequentially with DIG grade methanol and hexane prior to filtration;
10) filter pack is rinsed with hexane prior to filtration;
11) after sample has been put through the filter pack, rinse the original test tube with 2 mL of hexane twice and put through filter pack and rinse syringe with 2 mL of hexane;

NOTE: it is important to unscrew the GelmanSciences filter pack from the syringe each time before the plunger is removed; if it is not removed, the filter pack will be damaged.

12) N-evap™ each sample to 1 mL;
13) spike each sample with 10 µL of the volume corrector $13C_{12}$ PCB 52 (2,2',5,5'-tetrachlorbiphenyl);

NOTE: the 10 µL syringe used to add the volume corrector must be cleaned sequentially with acetone and hexane; the syringe must be pumped with the volume corrector to ensure no bubbles are present.

14) vortex each sample and transfer into small brown vials (2 mL) with lids;
15) mark the meniscus of each so that the samples may be analyzed by the GC/MSD more than once; and
16) the samples should be stored at 4 °C until they are to be analyzed by the instrument.

WATER SAMPLE ANALYSIS

Sample extracts were analyzed using a 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.

REFERENCES

BURKHARDT, M. R. & WERNER, S. W. (1994): Methods of analysis by the National Water Quality Laboratory: determination of pesticides in water by solid-phase extraction and high performance liquid chromatography. - Schedule **2050/2051**.

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.

APPENDIX 2: Detailed method for the investigation of bacteria in the water column

Water samples were collected to determine the density of bacteria in the water column of each enclosure. Total bacteria were enumerated by epifluorescence microscopy after staining with Hoechst 33342, following the procedure of PORTER & FEIG (1980) for DAPI (4'6-diamidino-2-phenylindole). Hoechst 33342 and DAPI are fluorescing DNA-specific stains and when excited with the proper wavelength of light, the stain-DNA complex fluoresces a bright blue.

SAMPLING OF BACTERIA IN THE WATER COLUMN

- two quantitative, depth integrated water column samples (1-1.2 L) were taken randomly from each enclosure weekly using a transparent acrylic cylinder (50 cm x 5.5 cm)
- for estimation of bacterial density, a 10 mL subsample was transferred to an acid-washed (or autoclaved) Vacutainer tube (20 mL) that had been rinsed 2X with the sample water
- within 3h of collection, 1 mL of 4 % buffered formalin was added to each sample and all were stored at 4 °C until filtration

MATERIALS FOR FILTRATION, SLIDE PREPARATION, AND ENUMERATION

sample membrane (filter)

- black, 25 mm diameter, 0.2 µm porosity Isopore™ polycarbonate membrane with low fluorescent background

backing filter

- 1.0 µm porosity GF/A glass microfibre filter

sample stain

- Hoechst 33342
- stain is specific for DNA
- unbound stain fluoresces yellow, DNA-bound stain fluoresces blue, and chlorophyll-bound stain fluoresces red against the black polycarbonate filter background

glass microanalysis filter holder assembly (Fisher Scientific)

- glass filtration tower assembly has a volume of 15 mL
- the frit glass support is 25 mm in diameter and has a filtration area of 2.1cm^2

epifluorescent microscope

- a filter set of 365 nm and 480 nm was used for Hoechst stain

PREPARATION OF HOECHST STAIN

- the concentration of the Hoechst stock solution used was 500 µg/mL
- dilution of 100 µl of the stock solution 5X produced 500 µL of a 100 µg/mL Hoechst solution used for staining of microbial samples
- for staining, 2 µL of the 5X diluted Hoechst solution was used per mL of water sample filtered; this produced a final concentration of 0.2 µg Hoechst/mL sample filtered

WATER SAMPLE FILTRATION AND SLIDE PREPARATION

Typically, bacterioplankton samples were filtered within 24-72 h of collection and fixation. The volume of water filtered varied among enclosures and was determined by the density of bacteria in the previous week's samples.

- **filtration procedure**
    1) prepare the glass filtration tower;

2) filter approximately 100 mL of distilled water through a 1.0 µm porosity GF/A glass microfibre filter;
3) remove the filter holder (do not disassemble) and rinse the filtrate collection vacuum flask and a 1 L flask thoroughly with this filtrate
4) discard the filtrate;
5) replace the filter holder and continue to filter approximately 1000 mL of distilled water;
6) the filtrate in the 1 L flask is to be used for dilutions and rinsing as necessary;
7) remove and discard the cellulose acetate filter, reassemble the filtration tower, and rinse well with filtered distilled water;
8) transfer 100 mL of filtered distilled water to a 250 mL beaker to be used for wetting the black, polycarbonate filters prior to placing them on the filtration tower base;
9) place a wetted glass microfibre filter on the tower base and a wetted, black, polycarbonate filter on the backing filter and assemble the filtration tower;
10) the backing filter will help distribute the sample more evenly;
11) the 5X diluted Hoechst solution is for use with sample volumes of 2 mL;
12) if 2 mL of sample is not used, add filtered distilled water to the tower in whatever volume is necessary so that the total volume in the tower prior to addition of stain equals 2 mL;
13) the volume of sample filtered is adjusted depending on the bacterial density in the previous week's sample to give a countable and statistically significant number of bacterial cells per field of view;
14) add 4.0 µL of stain and carefully remove the entire filtration tower and mix the contents by swirling for 1 minute;

15) let the sample sit for another 3 minutes, then add 3 mL of filtered distilled water, swirl the contents for 30 seconds, and filter using a low vacuum (< 15 cm Hg);
16) rinse the filter once with 2 mL of filtered distilled water;
17) filter the sample not quite to dryness;
18) remove the filter while still under vacuum to minimize water retention by the filter and prevent cells from floating off the filter surface;
19) the filtration tower funnel should be thoroughly rinsed with filtered distilled water between samples; and
20) for each week, a blank slide should be prepared using the same procedure as for the water column samples to obtain a background count of bacteria in the filtered distilled water.

- **slide preparation procedure**

Plain glass microscope slides used for mounting of the polycarbonate filters were pre-labeled with the sample date, enclosure number, and replicate letter.

1) place a small drop of non-fluorescent immersion oil on a cleaned plain glass slide and spread into a thin film using another glass slide;
2) place the polycarbonate filter onto the area of the slide covered with oil and then place another small drop of oil on the centre of the filter (excess oil will result in "floating" of the cells off the filter);
3) place a cover slip onto the filter; gently press the cover slip down with a pencil eraser until the oil moves out to the edge of the cover slip and forms a seal;
4) pressure applied must ensure no lateral movement of the cover slip as this can result in unequal distribution of cells on the filter;
5) at this stage the mounted polycarbonate filter can be frozen at -20 °C (or lower) for up to 70 d without any loss in bacterial cells (TURLEY 1993); and

6) sealing the edges of the cover slip with melted paraffin or clear fingernail polish is recommended for long-term storage to prevent movement of the cover slips and changes in the moisture content of the preparations.

- **sample enumeration**

Total bacteria were enumerated by epifluorescence microscopy (Zeiss, fitted with a mercury lamp and an excitation filter set of 365 nm and 480 nm) after staining with Hoechst, following the procedure of PORTER and FEIG (1980).

1) estimates of the number of bacteria contained in a sample are made by enumerating cells on some fraction of the filter;
2) the portion of the filter examined corresponds to a number of fields of view from various segments of the filter;
3) bacteria may not be distributed evenly so it is important to include fields of view from near the center and the edges of the filter;
4) slides were thawed and bacteria were enumerated at a magnification of 1000X;
5) up to 40 fields of view were examined and a minimum of 600 individuals were counted for each sample, giving a precision of approximately ± 10 % at the 95 % confidence interval (TURLEY 1993);
6) the density of bacteria in the original sample is determined from knowing the sample volume filtered, the average number of bacteria per field of view counted, the area of the filter covered by sample (determined by the filter funnel diameter), the area of the filter examined per field of view, and the dilution of the sample due to the addition of preservative (a constant factor of 1.0 if a 1:10 preservative:sample ratio is used);
7) if these features are constant, then they can be combined into a single conversion factor; and
8) the following equation was used to calculate bacterial density (TURLEY 1993):

$$\text{Density (ind./mL)} = \frac{[(SC-BC)xCFxF]}{V}$$

where:

SC = mean of sample counts per field of view

BC = mean of background counts per field of view

CF = effective filter area/field of view area

F = (volume fixative/volume sample fixed) + 1

V = volume of fixed sample filtered (mL)

REFERENCES

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

TURLEY, C. M. (1993): Direct estimates of bacterial numbers in seawater samples without incurring cell loss due to sample storage. - In: KEMP, P. F., SHERR, B. F., SHERR, E. B. & COLE, J. J. (ed.): Handbook of Methods in Aquatic Microbial Ecology. - Lewis Publishers, Boca Raton, Florida, pp. 143-147.

APPENDIX 3: Detailed method for obtaining a Downing Box sample

A Downing Box sample is obtained using the following procedure:

1) the Downing Box is slowly and carefully lowered in the open position into the enclosure where a sample is to be taken, thereby minimizing disturbance;
2) once the device is positioned where the sample is to be taken, the investigator slowly and carefully closes the Downing Box, sampling a water volume of 6 L together with zooplankton, phytophilous invertebrates, and submersed macrophytes with associated epiphyton;
3) submersed macrophytes are trimmed around the outside edges of the Downing Box and the sample is slowly lifted to the enclosure deck;
4) once on the enclosure deck, the outlet tube is opened and the water contained in the Downing Box is filtered through a 53 μm mesh and the retained microinvertebrates are collected in a sample vial (20 mL);
5) remaining macrophytes are placed into a 2 L jar with a tight-fitting lid containing a pre-measured volume of carbon filtered water;
6) the jar with macrophytes and carbon filtered water is shaken for approximately 1 minute to remove macroinvertebrates and attached epiphyton from the macrophytes;
7) the macrophytes are removed from the jar and placed into labeled bags and stored in a cooler for transport back to the Field Station laboratory;
8) the carbon filtered water is filtered through a 1 mm mesh to remove the macroinvertebrates and the water containing the remaining epiphyton is collected in a 1 L plastic bottle for chlorophyll *a* analysis; and
9) the macroinvertebrates are washed from the 1 mm mesh and collected in a sample vial (20 mL).