Planktonic microinvertebrate community structure in a prairie wetland in response to addition of inorganic nutrients and organophosphorus insecticide

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Introduction

The application of fertilizers and insecticides for agricultural crop protection results in increased nutrient loading and pesticide contamination of wetlands adjacent to agricultural areas due to run-off, spray drift, leaching to surface and ground water, and accidental spills (Wauchope 1978, Neely and Baker 1989, Frank *et al.* 1990, Rijtema and Kroes 1991). These additional nutrients and toxic chemicals are known to affect the biotic communities of freshwater wetlands (Brock *et al.* 1992a, van Donk *et al.* 1995, Hann and Goldsborough 1997, McDougal *et al.* 1997).

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 drives 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). Wetland management decisions need to be made with a more complete understanding of the ecosystem; Delta Marsh provides a unique opportunity to study a relatively unperturbed system and further our understanding of the healthy functioning of a prairie wetland, and provide insight into the restoration of negatively impacted systems.

The food web of a wetland typically comprises four trophic levels; piscivorous fish, planktivorous fish, grazing invertebrates, and primary producers (algae, submersed and emergent plants). Grazing and nutrient recycling by zooplankton and macrophyte associated microinvertebrates are potential mechanisms for effecting control over primary producers in wetlands. A combined "bottom-up" and "top-down" manipulation of the food web examining the microinvertebrate-algal interactions may provide further insight into the environmental problems associated with agricultural practices in Canada. Specifically, how does the planktonic microinvertebrate community respond to the following experimental manipulations: 1) the bottom-up changes in the community structure of phytoplankton induced by nutrient enrichment (eutrophication) via small, regular additions of inorganic nitrogen and phosphorus; and 2) the top-down changes in the community structure of the arthropod component of the planktonic microinvertebrates induced by differential mortality caused by the addition of the organophosphorus insecticide Lorsban™ 4E (active ingredient chlorpyrifos).

The microinvertebrate community consists of components occupying three different habitats: the planktonic component living in the water column; the phytophilous component living in association with submersed aquatic plants (macrophytes); and the meiofaunal component living in association with the upper layer of sediments. The planktonic microinvertebrates were the only component considered in this study and include: 1) Cladocera, Cyclopoida and Calanoida Copepoda, and Ostracoda (arthropod filter-feeders, grazers, and predators); and 2) Rotifera (non-arthropod filter-feeders, grazers, and predators). A number of algal communities exist in a prairie wetland: the phytoplankton, or algae entrained in the water column; the epiphyton, or algae attached to the submersed aquatic macrophytes; and, the epipelon, or algae inhabiting the sediments. Only the phytoplankton was considered in this study.

Diverse responses by the communities mentioned above were expected due to either nutrient or insecticide addition. The addition of inorganic nitrogen and phosphorus directly affects the primary producers in this wetland ecosystem. The phytoplankton community was expected to respond positively to nutrient addition, provided either nitrogen and/or phosphorus was limiting in the system (Hann and Goldsborough 1997, McDougal *et al.* 1997). Addition of the insecticide, chlorpyrifos, was expected to result in differential mortality of the arthropod component in the microinvertebrate community (Brock *et al.* 1992a, Brock *et al.* 1992b, Brock *et al.* 1995, van Donk *et al.* 1995, van den Brink *et al.* 1996). Lorsban™ 4E insecticide (emulsifiable concentrate; active ingredient chlorpyrifos) is a broad spectrum organophosphorus insecticide manufactured by DowElanco and registered in Canada for control of agricultural pests. Organophosphorus insecticides remain

a popular choice because they are usually non-persistent in the environment and they do not bioaccumulate (Racke 1992). The insecticide chlorpyrifos is known to be toxic to a range of aquatic organisms to varying degrees (invertebrates and vertebrates, particularly fish) (Marshall and 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 and Roberts 1978, Chambers 1992).

This paper describes the seasonal abundance patterns of the littoral microinvertebrate community in the open water and their responses to and recovery from either bottom-up (inorganic nutrient addition) manipulation of the phytoplankton community or top-down (organophosphorus insecticide) manipulation of the arthropod component of the microinvertebrate assemblage. Spatial and temporal variation in microinvertebrate species abundance and community structure were examined in experimental mesocosms in relation to some biotic environmental factors and to treatment. The goal of these experimental manipulations was to gain insight into the structure and functioning of a relatively unimpacted wetland ecosystem by studying its response to and recovery from controlled perturbations. Our ability to conserve, restore and create wetland habitat will ultimately depend on our understanding of the dynamics of a relatively healthy system.

Methods

Study site and experimental design

The study was conducted from May to August, 1997 at the Delta Marsh, a 22,000 ha freshwater lacustrine wetland (98º 23'W, 50º 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 in this project 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, MB) on 27 May at a water depth of <1 m. 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 rebar at least 30 cm 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.

The proposed experimental design examined main effects only in 1997 using three replicates of inorganic N and P addition, three replicates of insecticide addition, and three control enclosures with no manipulation. Experimental treatments were assigned to enclosures using a restricted latin square design, ensuring no replicate enclosures were adjacent or contiguous with each other. In an attempt to limit the number of experimental variables, all fish that had been trapped in the enclosures during installation were removed using commercial minnow traps and returned to Blind Channel prior to the beginning of the pre-treatment period. Enclosures were monitored daily for presence of fish with the commercial minnow traps for the duration of the experimental period.

The impact of additions of inorganic nitrogen (N) and phosphorus (P) and the insecticide Lorsban™ 4E (active ingredient chlorpyrifos) on the structure and functioning of the enclosed wetland ecosystem was investigated. 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 period. Sampling was conducted for 2 weeks to determine pre-treatment densities and community composition of planktonic microinvertebrates and phytoplankton biomass as chlorophyll a. Prior to insecticide addition, water samples were collected for analysis for the presence of chlorpyrifos. Experimental manipulation of the mesocosms and collection of biotic data was carried out for 10 consecutive weeks.

Application of nutrients and chlorpyrifos

Inorganic nitrogen (as analytical grade $NaNO_3$) and phosphorus (as $NaH_2PO_4 \cdot 2H_20$), were added to the three nutrient treatment enclosures according to the molar ratio 10N:1P. Additions were made three times a week beginning on 23 June for the 10 week treatment period. Equal cumulative N and P loads (23.4 g N m^{-2} of wetland bottom and 3.2 g P m^{-2} of wetland bottom) were added to each nutrient treatment enclosure by the end of the experiment. For each nutrient addition, pre-measured amounts of the chemicals were dissolved in 1 L of carbon-filtered water, and then sprinkled uniformly over the enclosure water surface using approximately 10 L of enclosure water.

Addition of chlorpyrifos (in Lorsban™ 4E) was made once on 14 July to produce a nominal initial concentration of 10 µg L^{-1} in the water column. Treatment was scheduled to begin in mid-July in order to give the

submersed macrophytes sufficient time to become well established in the enclosures because presence or absence of macrophytes may influence the fate and effects of chlorpyrifos (Brock *et al.* 1992a,b). For the chlorpyrifos addition a pre-determined amount of the insecticide was emulsified in 250 ml of distilled water. The distilled water containing Lorsban™ 4E was then sprinkled uniformly over the water surface of each insecticide treatment enclosure mixed in approximately 20 L of carbon-filtered water. This treatment took place in the morning on a windless day to prevent spray drift into other enclosures.

Water sampling for chlorpyrifos monitoring and analysis

Prior to insecticide addition a quantitative, depth integrated water column sample (2 L) was collected from each insecticide treatment enclosure using a transparent acrylic cylinder (50 cm x 5.5 cm) to provide measures of background concentrations of chlorpyrifos in the water column.

After insecticide addition, six sets of water samples were collected from each insecticide treatment enclosure. The first set of post-addition water samples was taken approximately one hour after addition, the second at 12 hours post-addition, the third at 24 hours post-addition, the fourth at 36 hours post-addition, the fifth at 48 hours post-addition, and the sixth at 72 hours post-addition. Each set of post-addition water samples consisted of a 2 L water sample from each insecticide treatment enclosure. Depth integrated water samples were collected, filtered through a 150 µm mesh net to remove larger planktonic microinvertebrates, and then transferred to brown glass containers.

After collection the samples were transported in coolers with ice to the Freshwater Institute (Department of Fisheries and Oceans) in Winnipeg, where they were stored in the dark at 4°C until analysis. The protocol followed for analysis of the water samples for chlorpyrifos by gas chromatography/mass spectrometry (GC/MS) using a gas chromatograph in combination with a mass selective detector (GC/MSD) is after the method of Rawn *et al.* (1998).

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 to determine the density (# L^{-1}) of planktonic microinvertebrates (see Hann and Goldsborough 1997 for method). The water column samples were filtered through a 53 µm mesh net to remove the planktonic microinvertebrates. In addition, three quantitative water column samples (1 L) were taken for the determination of biomass of phytoplankton (as chlorophyll a) (see McDougal *et al.* 1997 for method).

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. Planktonic rotifers were placed in one group, with only the predatory rotifer, *Asplanchna* sp., being counted separately.

Data analysis

For each sampling date, mean densities of planktonic microinvertebrates and fathead minnows, biomass of phytoplankton, and % cover of enclosure bottom by macrophyte were estimated. Correspondence analysis (CA) was used to examine changes in microinvertebrate assemblage structure (species composition and density) in the mesocosms in response to treatment over time. Species determined to be rare (<1% total density) 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 microinvertebrates in the plankton (# L^{-1}) were calculated for each treatment. A planktonic species x sample date (treatment) matrix was produced using ln (x+1)-transformed data to stabilize variances; the matrix included data for all enclosures as treatment means.

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 individuals with nearly identical species composition lie close together, while individuals with very different species 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.

Relationships between microinvertebrate species and environmental data were examined using canonical correspondence analysis (CCA). Species determined to be rare (<1% total density) 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 microinvertebrates in the plankton (# L^{-1}) were calculated for each treatment. A planktonic species x sample date (for each treatment) matrix was produced using ln (x+1)-transformed data to stabilize variances. One environmental variable x sample date (for each treatment) matrix was produced; the matrix included untransformed values for chlorophyll a (CHLOROPA), fathead minnow density (FATHEAD), and % cover of enclosure bottom by submersed macrophyte (MACROPH). Environmental variables, particularly % cover of enclosure bottom by submersed macrophytes, play an important role in structuring the microinvertebrate community.

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 (individuals) represented as points and environmental variables as arrows (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, we 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

Chlorpyrifos monitoring in the water column

The GC/MSD method used to determine the concentration of chlorpyrifos in the water column provided a 33.0 % recovery for the chlorpyrifos ion. Contamination of the equipment by samples resulted in a background concentration of 2.87 µg of chlorpyrifos L^{-1} HPLC-grade water (Fig. 1A), subtracted from the concentration determined for each water sample collected from the enclosures (Fig. 1B). At day 1 (14 July, 9 am) after application, when the insecticide had mixed homogeneously in the water column, chlorpyrifos concentrations in the range of 1.15 to 10.33 µg L^{-1} were measured in the insecticide enclosures (Fig. 1C). In all the enclosures treated with chlorpyrifos the rate of disappearance from the water column was high. After 12 hours (14 July, 9 pm) between 65 and 10 %, and after 24 hours (15 July) between 51 and 0% of the dose, could be detected in the water column of the three chlorpyrifos enclosures. At day four (17 July) after application, the concentration of chlorpyrifos in one of the insecticide enclosures increased to 1.82 µg L^{-1} from 0 µg L^{-1}. The rapid initial disappearance of the insecticide from the water column is primarily attributable to adsorption of the compound on the polyethylene curtain and on submersed macrophytes with attached epiphyton; desorption from these surfaces may have contributed to the residual concentration ("reflux") measured in the water column of one enclosure (Hughes *et al.* 1980, Siefert *et al.* 1989).

Correspondence analysis

The microinvertebrate community in the water column was very similar in ordination space for all treatments until insecticide application in the middle of July (Fig. 2). The first two ordination axes cumulatively explained 70.4% of the total variance in the species data. CA axis 1 appears to separate the communities by sampling date (or season), showing the change in the

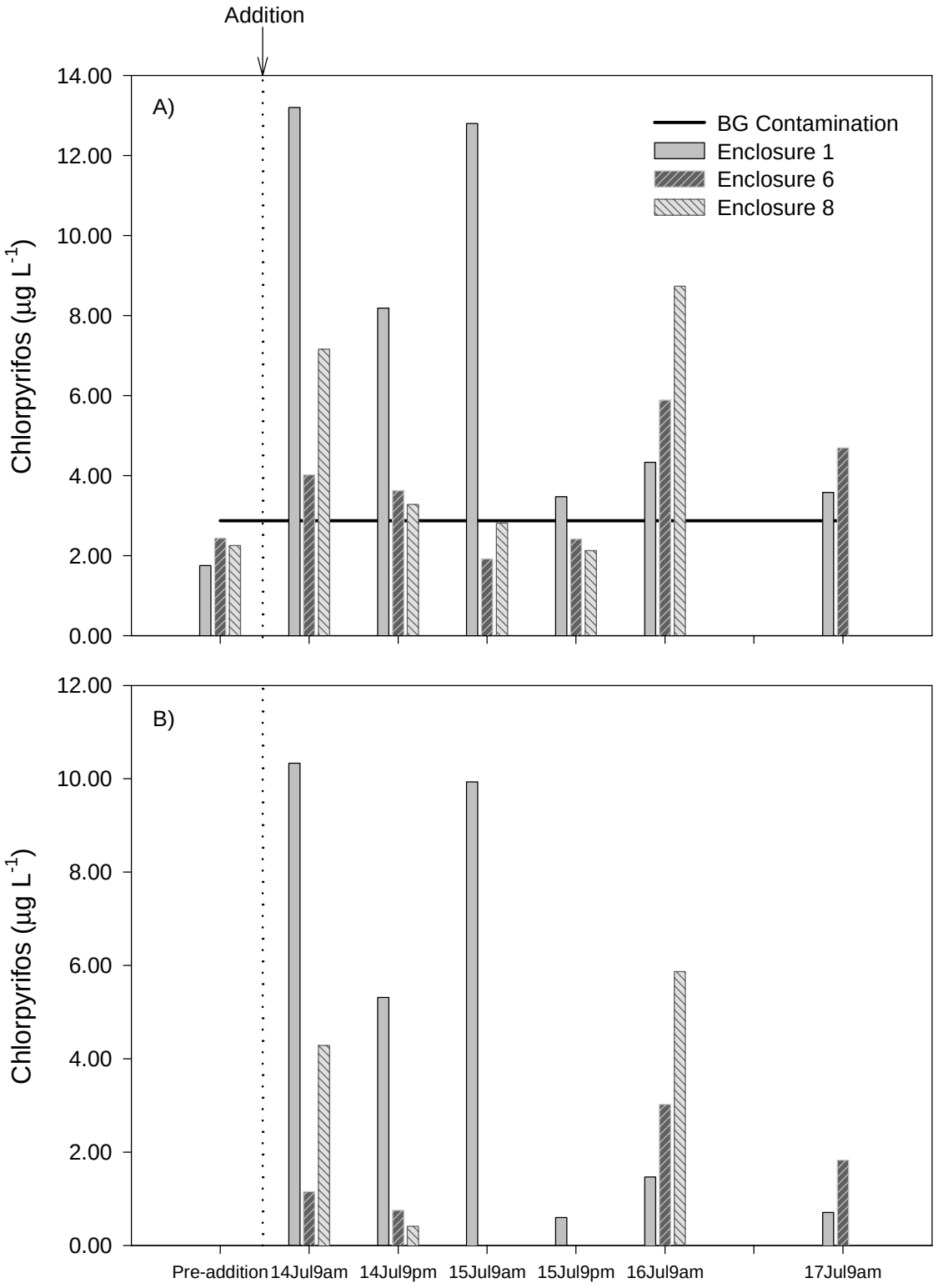

Figure 1. A. Chlorpyrifos concentrations (µg L^{-1}) in depth-integrated water column samples after application of a nominal concentration of 10 µg L^{-1}, with background contamination. B. Chlorpyrifos concentrations (µg L^{-1}) in depth-integrated water column samples after application of a nominal concentration of 10 µg L^{-1}, with background contamination removed.

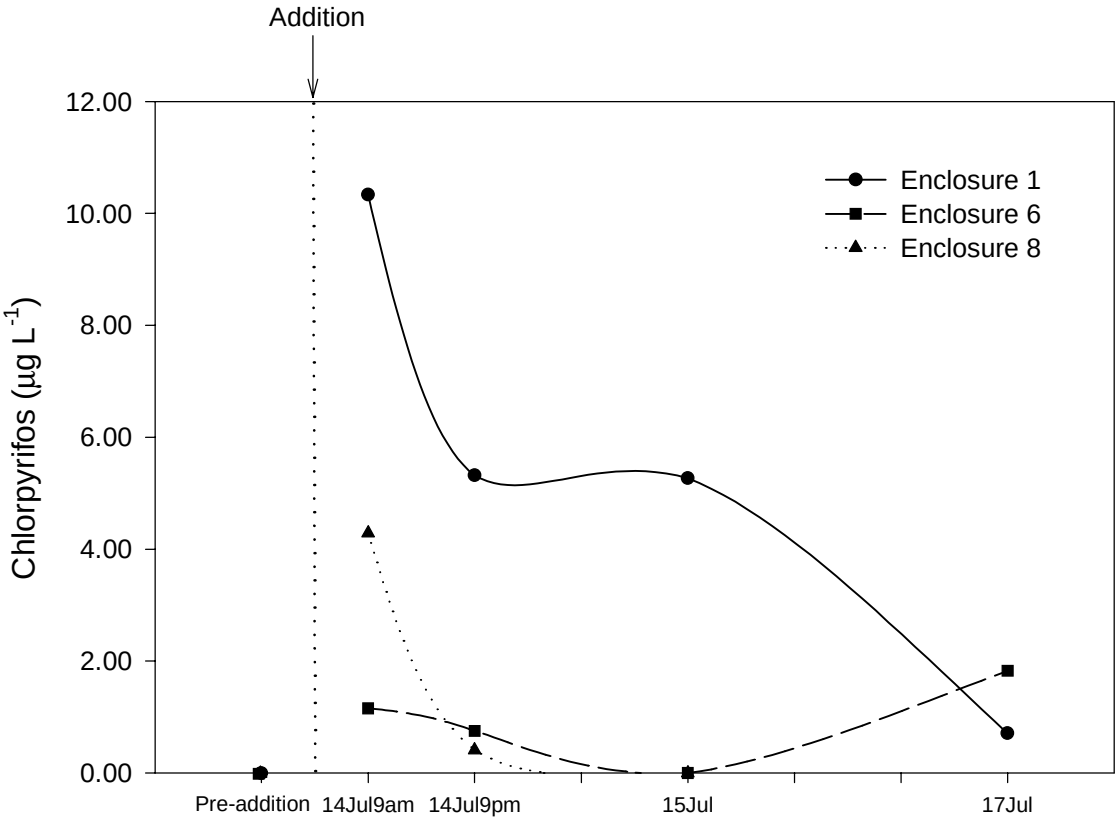

Figure 1. C. Dissipation of mean chlorpyrifos concentrations (µg L^{-1}) in depth-integrated water column samples after application of a nominal concentration of 10 µg L^{-1}, with background contamination removed.

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 (beginning of June to beginning of July) to post-treatment (end of July to end of August), 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 between sample dates, but not between treatments. Species composition and relative abundance of planktonic microinvertebrates in all treatments was dominated in June by small rotifers and the predatory rotifer *Asplanchna* sp. By the beginning of July the trajectories for control and nutrient addition treatment have coalesced showing substantially less community change between sample dates for the month of July. Several planktonic species of Cladocera (*Bosmina longirostris*, *Daphnia* sp., *Diaphanosoma birgei*), cyclopoid copepod copepodites (immature life stage) and adult cyclopoid copepods, *Acanthocyclops/Diacyclops* spp. were represented in the community of the nutrient addition treatment through July and August. The control diverges slightly from the trajectory of the nutrient addition treatment at the end of July, with the planktonic community being dominated by the cladoceran *Bosmina longirostris*, nauplii (immature copepod life stage), and calanoid copepod copepodites (immature life stage). After insecticide application in the middle of July the trajectory for insecticide treatment diverged from control and nutrient addition treatment and coalesced showing much less community change between sample dates for the month of August. The insecticide treatment community structure from the end of July to the end of August was dominated by small rotifers, calanoid copepod copepodites (immature life stage), and the adult calanoid copepod *Diaptomus nudus*.

Canonical correspondence analysis

For the open water community in the CCA using species and environmental data sets, the eigenvalues for the first two canonical axes were 0.256 and 0.048. The variance accounted for in the species data by the first two canonical axes is 98.1% when the environmental data is included. The environmental variables included in the analysis explained 45.4% of the total variance in the species data. Axis 1 was strongly correlated with

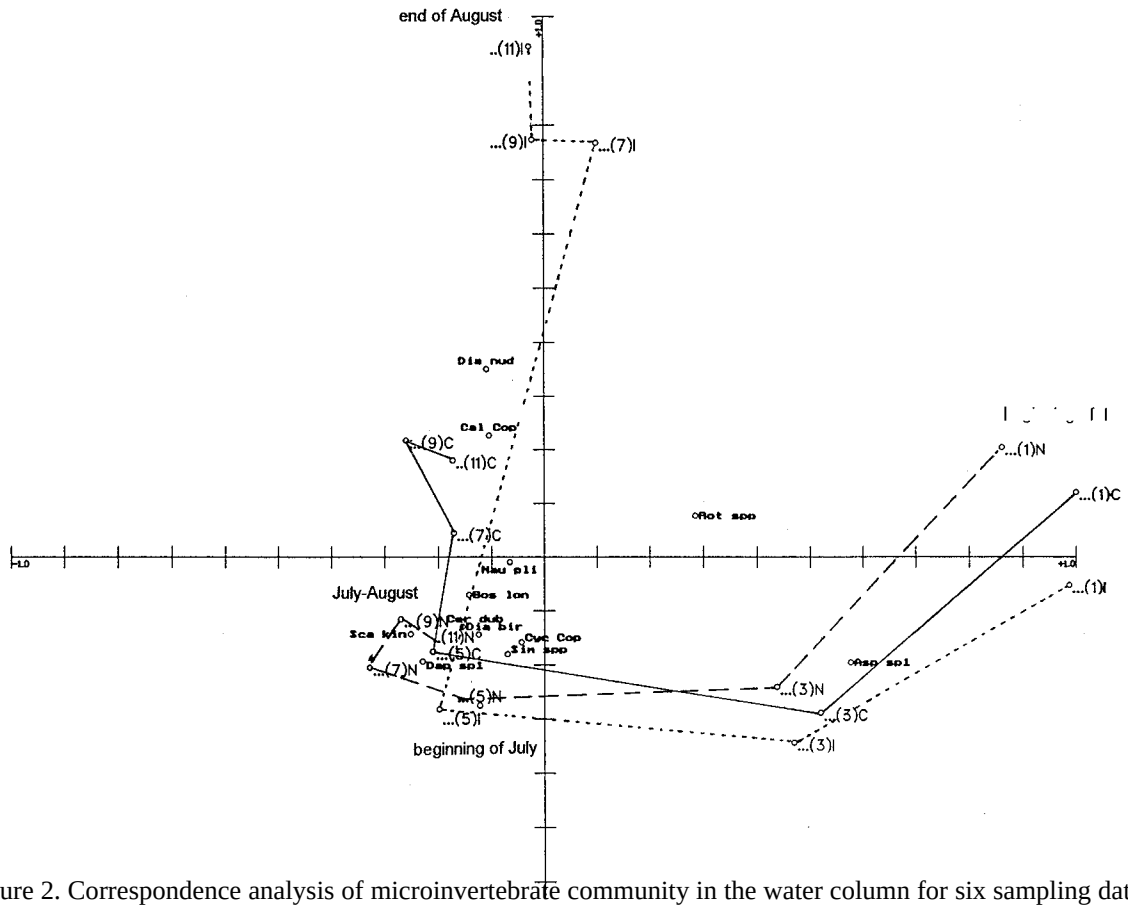

Figure 2. Correspondence analysis of microinvertebrate community in the water column for six sampling dates in controls (C), inorganic nutrient addition (N) treatment, and insecticide application (I) treatment. Changes in community structure are shown connected through points for each sampling date from June to August and in species space. Microinvertebrate 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 Copepod Copepodites; Aca Dia, *Acanthocyclops*/*Diacyclops* spp.; Mac alb, *Macrocyclops albidus*; Cyc rub, *Cyclops varicans rubellus*; Cal Cop, Calanoid Copepod Copepodites; Dia nud, *Diaptomus nudus*; Rot spp, small Rotifer spp.; Asp sp1, *Asplanchna* sp.1; Ost spp, Ostracod spp.

only one environmental variable, % cover of enclosure bottom by submersed macrophyte, and axis 2 with fathead minnow density (Table 1).

Site points, representing sample dates, and microinvertebrate species points were plotted with environmental variables in a triplot (Fig. 3). A separation is shown between sample dates with respect to season (sampling date) on axis 1 in the ordination diagram. All sample dates in June for all treatments had positive values on axis 1, corresponding to lower % cover of enclosure bottom by submersed macrophyte and higher chlorophyll a values, whereas all (with two exceptions, (5)C and (7)N) sample dates in July and August for all treatments had negative values on that axis, indicating higher %

Table 1. Weighted correlation coefficients between environmental variables and the first two CCA axes for the microinvertebrate community in the water column; CCA using species and environmental data sets.

Environmental variable	Axis 1	Axis 2
Fathead minnow density (FATHEAD)	0.0381	0.5438
Chlorophyll a(CHLOROPA)	0.1003	0.0711
% Cover of enclosure bottom by submersed macrophyte (MACROPH)	- 0.8655	- 0.1779

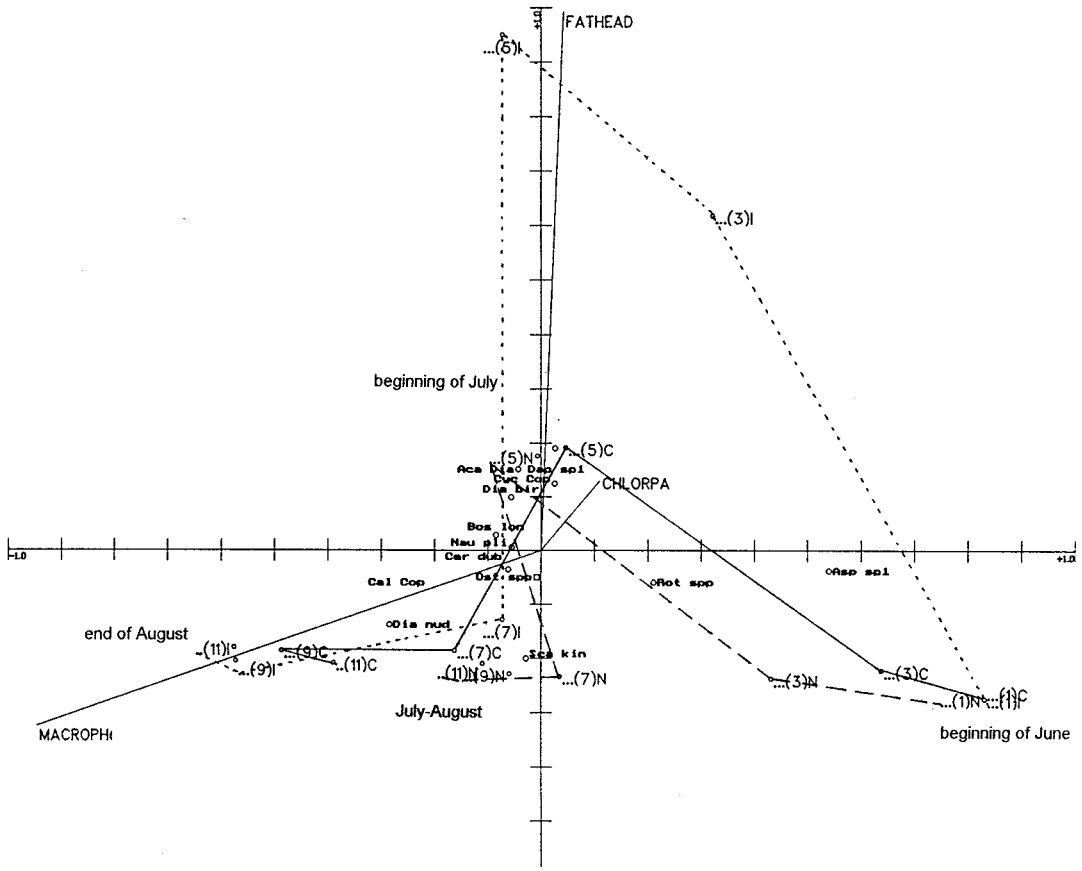

Figure 3. Canonical correspondence analysis triplot for microinvertebrate species, environmental variables, and sampling dates in controls (C), inorganic nutrient addition (N) treatment, and insecticide application (I) treatment over week 1 (beginning of June) - week 11 (end of August). Labels for environmental variables are as in Table 1. Site points, representing sampling dates, are shown as circles and designated by week number and treatment. Site points are connected by lines showing the temporal sequence of samples from week 1-11. Site points of samples with high similarity in species composition lie close together, while site points with dissimilar species composition lie far apart. Microinvertebrate species are identified as in Fig. 2.

cover of enclosure bottom by submersed macrophyte and lower chlorophyll a values. On axis 1, the temporal sequence of sample dates reflects ordering of site points according to increasing values over the summer season of % cover of enclosure bottom by submersed macrophyte and to decreasing values over the summer season of chlorophyll a values. The additional separation of insecticide site points (particularly (9)I and (11)I) on axis 1 are likely the result of insecticide treatment effect on their microinvertebrate species composition. The temporal sequence of sample dates on axis 2 reflects the influence of primarily fathead minnow density, and to a lesser extent chlorophyll a values, on species composition from the beginning of June to the end of August. Those species with a high positive score on axis 1 (e.g., small rotifer species, Asplanchna sp.) were 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*) were more abundant in July and August; the position of the species points for calanoid copepod copepodites and *Diaptomus nudus* are likely also influenced by the effects of the insecticide treatment. Similarly, those species with a positive score on axis 2 occurred at higher fathead minnow densities.

Discussion

Correspondence and canonical correspondence analyses

The resulting ordination diagrams using CA (microinvertebrate species data set) (Fig. 2) and CCA (microinvertebrate species with environmental variables data sets) (Fig. 3) differed slightly, with a better separation of the insecticide treatment occurring in the ordination diagram produced using CA. In CA, environmental variables are not included directly in the

ordination of the species data, i.e., the resulting pattern of site (sampling date) and species 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 species 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.

Although the ordinations produced by CA (Fig. 2) and CCA (Fig. 3) differed, the correlations between environmental variables and the first two canonical axes were high (MACROPH with CCA axis 1 = -0.8655; FATHEAD with CCA axis 2 = 0.5438), indicating that the environmental variables quantified accounted for residual variation in the species data, but not for the main variation (Ter Braak 1986). Due to the limited number of environmental variables included in the analyses, it is not surprising that a large portion of the variance in the species data set is unaccounted for. The complex nature of a wetland ecosystem limits our ability to adequately quantify the environmental parameters influencing the dynamics of the planktonic microinvertebrate community under investigation.

Dissipation of chlorpyrifos

The single addition of Lorsban™ 4E (active ingredient chlorpyrifos) was made on 14 July 1997 in order to give the submersed macrophytes (*Ceratophyllum* sp., *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 disappearance of chlorpyrifos in water with macrophytes being more rapid than in open-water systems.

The rate of disappearance of chlorpyrifos in the treated enclosures was high, with between 51 and 0% of the original dose being detected in the treated enclosures only 24 hours after addition (Fig. 1C). Other studies have reported the 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 and Kline 1990).

The initial rapid loss of chlorpyrifos after application may be partially attributable to volatilization from the surface water (Racke 1993). Chlorpyrifos is very insoluble in water and has a very high soil-water partition coefficient (K_d), ranging from 13.4 to close to 2,000 (Racke 1993). A high K_d value indicates that chlorpyrifos has a strong tendency to favour the sorbed state over the dissolved state and is a result of the nonpolar nature of the chlorpyrifos molecule. Due to the nature 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 and on submersed macrophytes with attached epiphytes (Hurlbert *et al.* 1970, Hughes *et al.* 1980, Brock *et al.* 1992a). Sorption of chlorpyrifos by enclosure curtains, macrophytes, and epiphyton 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 (1.15 to 10.33 µg L^{-1}) measured at day 1 after addition (Fig. 1C). Sorption-desorption processes are a major factor in determining the distribution and persistence of available chlorpyrifos in the water column (Marshall and Roberts 1978). The "reflux" of chlorpyrifos in the water column seen at day 4 after addition demonstrates the potential of the molecule to become "secondarily" available for absorptive uptake by susceptible aquatic organisms upon desorption from binding surfaces.

Effects of inorganic nutrient loading

The planktonic microinvertebrate 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 species composition of the community, as the enclosures receiving nutrients did not differ substantially from the control. The species composition of the community in the nutrient treatment differed slightly from the control during the month of August, with the community being predominantly cladocerans in contrast to one with more copepods represented (Figs. 2, 3). Primary producers in a wetland ecosystem (e.g., phytoplankton, epiphyton, epipelon, macrophytes) have been shown to respond positively to nutrient enrichment (Murkin *et al.* 1994, McDougal *et al*. 1997). An explanation for the differences between controls and nutrient enrichment enclosures may be that the primary producer communities responded differentially to the nutrient loading. The % cover of enclosure bottom by submersed macrophyte was lower in the inorganic nutrient treatment than in the controls (Fig. 3), indicating the additional nutrients were likely assimilated by an algal community rather than by the macrophytes (McDougal *et al.* 1997). Dominance of the microinvertebrate community in the nutrient addition treatment by both planktonic and phytophilous cladocerans reflects the availability of

suitable food for these organisms in the form of either phytoplankton or epiphytic algae attached to the macrophytes. It is important to keep in mind that the ordination methods used are for the analysis of changes in the structure (species composition) of the microinvertebrate community, or changes in the proportional (relative) abundances of species, not differences in absolute abundances of species. Nutrient enrichment did not substantially affect microinvertebrate community structure, but there may have been a positive numerical response to the increased availability of edible algae either as phytoplankton or epiphyton.

Effects of organophosphorus insecticide application

The initial chlorpyrifos concentrations measured in the water column of the insecticide treatment enclosures (Fig. 1C) are effective in reducing most microinvertebrate arthropod species due to direct toxicity (Marshall and Roberts 1978, Siefert *et al.* 1989, van Wijngaarden *et al.* 1993). Application of insecticide resulted in a pronounced change in the community composition of the planktonic microinvertebrates (Figs. 2, 3). Calanoid copepod copepodites and adult calanoid copepods dominated the community after insecticide addition. Differential mortality of arthropod microinvertebrates resulted from chlorpyrifos application, with calanoid copepods being more tolerant than the Cladocera and cyclopoid copepods (Hurlbert *et al.* 1970, Hurlbert *et al.* 1972, van den Brink *et al.* 1995). Calanoid copepods are able to feed by grazing epiphyton attached to macrophytes and by filtering phytoplankton from the water column (Pennak 1978). It is possible that calanoid copepods are not as efficient at filtering as the cladocerans and are subsequently outcompeted for this food resource in the controls and insecticide enclosures prior to chlorpyrifos addition. If the calanoids exist among the macrophytes, grazing on epiphyton in the insecticide enclosures, they will be able to respond to the elimination of the cladocerans and move into the water column to take advantage of an unoccupied niche with little competition.

The relative increase of small planktonic rotifers in the insecticide enclosures after addition (Figs. 2, 3) might be explained by the reduction in 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 species of cyclopoid copepods present are known to prey on small rotifers (Fryer 1957). Once free of competition and with reduced predation pressure, the small rotifers were able to increase in population size through rapid asexual (parthenogenetic) reproduction to take advantage of available resources.

Implications of research for management decisions

Delta Marsh is relatively uncontaminated lacustrine wetland, bordered to the south by agricultural land, where pesticides and fertilizers are in use, and Lake Manitoba to the north. The wetland acts as a buffer zone between the agricultural land and Lake Manitoba, filtering out nutrients from fertilizer and pesticide residues before they can enter the lake. Continued pressures from agricultural practices may be detrimental to the overall health and functioning of Delta Marsh, thereby negatively impacting Lake Manitoba and the species that depend on it.

The microinvertebrate community plays a large role in the wetland food web in regulating both the biomass of phytoplankton in the water column and epiphyton on submersed aquatic macrophytes through grazing, and as food items for other organisms, particularly planktivorous fish. Microinvertebrates are a crucial component in a complex food web affecting the following interacting organisms, in particular:

1. Forage fish species (e.g., fathead minnows, spottail shiners) use the wetland extensively for spawning and nursery ground for young fish, migrating seasonally in and out of Delta Marsh (Whiteside 1988, Whiteside *et al.* 1985). The wetland is ideal for the growth of planktivorous young-of-the-year and juvenile fish because microinvertebrates as food are plentiful, and emergent and submergent macrophytes provide a refuge from predators.
2. Success of the forage fish species is critical for the maintenance of piscivorous fish species (e.g., walleye, sauger, northern pike), many of which are important to the commercial and sport fisheries existing on Lake Manitoba.
3. Ducks and geese also depend upon wetland microinvertebrates, particularly during the spring months, to provide a protein-, lipid-, and calcium-rich food source necessary for breeding, egg-laying, and brood-rearing (Krull 1970).
4. Potential effects with respect to competition between planktivorous fish species and breeding waterfowl for this shared valuable microinvertebrate food source have not been thoroughly evaluated in prairie wetlands; the consideration of microinvertebrate community dynamics is important in contributing to our understanding of this interaction for a shared food resource.

The organisms inhabiting Delta Marsh and the surrounding area, including Lake Manitoba, are all linked in a complex food web. In order to further our understanding of what drives the food web in prairie wetlands, manipulative experiments using large

mesocosms are a good approach because they incorporate many aspects of the natural ecosystem. Wetland management decisions need to be made with a more complete understanding of the ecosystem; Delta Marsh provides a unique opportunity to study a relatively unperturbed system and furthering our understanding of the healthy functioning of a prairie wetland and provide insight into the restoration of negatively impacted systems.

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