

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

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A Thesis

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in Partial Fulfillment of the Requirements
for the Degree of**

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**Department of Zoology
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**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

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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.

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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, MCDUGAL 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, MCDUGAL 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, *Hyaella*) (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 acetylcholine, 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 $\mu\text{g/L}$). A level of 10 $\mu\text{g/L}$ 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 (MCDUGAL 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 communities 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