Cladoceran grazer response to pulsed organic nutrient additions in a freshwater marsh

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Introduction

The wetland community is a complex and highly productive system through which nutrients are rapidly cycled. Abiotic factors, such as climate and hydrology affect the biota within the ecosystem. The various biotic components, adapted to the wetland environment, as well as to each other, interact as part of an intricate food web. Wetlands are unique ecosystems and should be preserved as some of their many functions include providing habitat for a diverse range of animals, including migrating waterfowl, preventing floods, stabilizing climate and acting as global carbon dioxide sinks (Mitsch and Gosselink 1993).

Pesticides, herbicides, and fertilizers from anthropogenic sources, such as from surrounding agricultural land, can potentially cause an impact on the marsh ecosystem. Researchers have taken experimental approaches to examining the effect of various stresses on wetland dynamics, including changes in foodweb interactions. Many of these have utilized an enclosure design. Hann (1991) added a pesticide which removed phytophilous cladoceran and copepod grazers and resulted in an increase in abundance and species diversity within the periphytic community. In another study, the addition of a herbicide was shown to alter the primary producer community, in which a decrease in periphyton biomass allowed a subsequent phytoplankton increase to occur (Goldsborough 1991). Inorganic nutrient enrichment has been found to increase the abundance of phytophilous cladoceran grazers (Hann 1995) and cause a shift from a periphyton-dominated to a metaphyton-dominated algal community (McDougal and Goldsborough 1995).

Interest in and appreciation of prairie wetlands is largely due to the presence of waterfowl. At Delta Marsh, waterfowl groups include dabbling ducks (*Anas* spp.) such as mallards, teals and others, as well as diving ducks (*Aythya* spp.) such as red-heads and canvasbacks (Murkin and Kadlec 1986). The birds make extensive use of this habitat for breeding, nesting and resting as well as feeding on an abundance of macrophytes and invertebrates (Murkin and Kadlec 1986; Batt *et al*. 1989). They return a proportion of this ingested material as the nutrients nitrogen and phosphorus via their feces (Bazely 1985; Manny *et al*. 1994).

A food web consists of many trophic interactions through which nutrients are continuously cycled (Carpenter 1985; Murkin 1989). Nitrogen and phosphorus that are added to the system as mineral nutrients will be incorporated by primary producers, then by different levels of consumers until these nutrients reach the top consumer in this complex interaction. In this wetland ecosystem, waterfowl are one group that holds this top status (Murkin and Kadlec 1986) and therefore, their fecal nutrient addition to the food web contributes to the cyclic nature of the web itself.

The addition of organic nutrients as feces has implications in terms of organism enhancement and/or overload to the natural system. Although some enrichment may serve to stimulate growth, addition of excess of nutrients can have negative effects, such as the death of macrophytes due to metaphyton shading (McDougal and Goldsborough 1995) and the process of eutrophication (Schindler 1971).

This study examined the effect of adding N and P, as organic nutrients in waterfowl feces, to experimental enclosures at Delta Marsh, to study the indirect effect on the microinvertebrate grazers, particularly cladocerans, many of which feed on the algal primary producers in the marsh. Cladocerans occupy two distinct habitats in the marsh: true zooplankton in the water column and phytophilous species living in association with submersed macrophytes. During the time of this study, the direct response of algae and macrophytes to waterfowl feces was examined in a study of the same enclosures by Purcell and Goldsborough (1996).

The impact of waterfowl feces on a freshwater prairie marsh has not previously been investigated. The effect of inorganic nutrients gives some indication of the potential result, but changes to the ecosystem have not yet been demonstrated using enhancement with organic nutrients.

The specific objectives of this study were: (1) to examine the indirect response of cladocerans, in terms of abundance, to the addition of high and low levels of added organic nutrients; (2) to assess the differential response of the grazer community in the open water

and that in association with macrophytes; (3) to evaluate the interaction between grazer abundance and algal biomass, which responds directly to the organic nutrient enrichment; (4) to consider the influence of other potential contributing factors, such as oxygen concentration, light and turbidity levels, on grazer dynamics.

Methods

Study Site

This study took place near the north east end of the Blind Channel at the Delta Marsh, an approximately 22,000 hectare wetland on the southern shore of Lake Manitoba, over 13 weeks from June to August 1995. The experiment was conducted in 5 x 5 metre enclosures described by Goldsborough (1993). Water impermeable woven polyethylene curtains extended approximately 50 cm into the sediments and accommodated fluctuations in water levels during the study period, with water depth ranging from 110 cm in the spring to approximately 50 cm during late summer. Any fish inside the enclosure boundaries were immediately trapped and released into the marsh. Minnow traps were retained in each enclosure for the duration of the study to catch any remaining fish or new fish fry.

The experimental design consisted of two replicates of each of two treatments (high and low organic nutrient additions) and two controls (no nutrient additions). Treatments were assigned randomly to enclosures. The spatial arrangement of the enclosures was as indicated in Purcell and Goldsborough (1996).

Macrophytes that developed in the enclosures were *Potamogeton zosteriformis, P. pectinatus, Myriophyllum spicatum* and *Ceratophyllum demersum*, submersed aquatic species which are characteristic of the Blind Channel.

Sixty-four acrylic rods, 90 cm in length and 0.64 cm in diameter, arranged in an 8x8 grid, were pushed into the sediments 30 cm, to extend vertically up into the water column. These marked the locations to be sampled within each enclosure, as selected from a random numbers table. The rod surfaces functioned as artificial substrata upon which epiphytic algae could colonize.

Nutrient Addition

Waterfowl feces for the organic nutrient addition were collected from the Delta Waterfowl Station in Delta, Manitoba. For the first addition of feces, contributing waterfowl species consisted of primarily migratory flocks of Canada Geese (*Branta canadensis)* and goslings as well as a few migratory and resident ducks and ducklings, including Mallard Ducks (*Anas platyrhychos)*, Black Ducks (*Anas rubripes*) and Wood Ducks (*Aix sponsa*). All of the faecal matter for the second addition was obtained from a captive flock of Mallard ducklings.

Nutrient addition occurred on two treatment dates: June 28 and July 21, 1995. On each of these dates, the feces were mixed into a liquid slurry and evenly distributed onto the surface of four treatment enclosures. Each high nutrient treatment enclosure received 7.19 kg and each low nutrient treatment enclosure received one tenth of the high treatment, 719 g wet weight of feces. The amount of each organic addition was determined on the basis of a previous experiment which involved the addition of inorganic nitrogen and phosphorus (McDougal and Goldsborough 1995). The N to P ratio used in that inorganic nutrient addition was 7:1, identical by weight to the ratio in previous research in which the N to P ratio of sediment interstitial water was determined (Kadlec 1986). As indicated by this ratio, P exists in a more limited quantity than N in the sediments, so it is this nutrient that was used to compare with the inorganic addition. The amount of P used in the inorganic addition dictated the proportion of P to use for the organic addition. The same total mass of P added in the 1994 inorganic nutrient addition experiment (Hann 1995; McDougal and Goldsborough 1995) was used in the organic nutrient addition experiment, based on chemical analysis of the feces by NorWest Lab, Winnipeg, Manitoba.

Oxygen Determination

Dissolved oxygen in the water column was measured, using a YSI Model 51B oxygen meter with attached probe, once weekly at 10 cm and 50 cm depths, in the morning and at night. These values, and water temperature at comparable depths, were used to calculate the percent oxygen saturation (Hutchinson 1957).

Water Chemistry and Chlorophyll Analysis

Surface water samples (10 cm depth) were collected from each enclosure at 3-4 day intervals. Concentrations of nutrients in the water column, including soluble reactive phosphorus and ammonia, were determined using methods of Stainton *et al.* (1977) and APHA (1985).

Phytoplankton chlorophyll *a*, as an indicator of algal biomass, and periphyton chlorophyll *a*, from the surface of the rods, were determined weekly (Purcell and Goldsborough 1996).

Sampling Methods

Three different sampling techniques were employed to determine cladoceran abundance in the different niches occupied by these organisms. Species of cladoceran zooplankton that possess a truly planktonic habit were sampled in the water column. Activity trap samples collected the various species that live amongst the submersed macrophytes. Acrylic rods, simulating macrophytes, constituted inert substrata of known surface area that allowed comparison of periphytic biomass across treatments. Rod samples were taken to capture cladoceran zooplankton inhabiting the area immediately surrounding the rods, which were colonized by algae.

Water column samples were taken using a clear acrylic cylinder, lowered vertically and stoppered at both ends below the surface of the water. A 4 L volume was poured through a 52 mm mesh sieve to concentrate the sample into a 20 mL vial. Three water column samples were taken at random locations per enclosure at weekly intervals.

Activity traps (modified from Whiteside and Williams 1975) consist of an acrylic base with 3 holes, through each of which a funnel is attached on one side to a 125 mL sample bottle on the other. These are secured via the bottle lid. The total surface area of the funnel openings is 0.02356 m^2. A flexible funnel stem addition of approximately 5-7 cm extends into the bottle to deter movement of captured organisms out of the trap. A small weight is attached to the underside to sink the trap, and a rope of sufficient length to reach the surface is tied to the upper side. A floating site marker is secured at the distal end of the rope.

Traps were set by first detaching the 3 plastic bottles, filling them with water from the sampling area and reattaching to the trap. The whole apparatus was then submerged, inverted and gently lowered to sit on the surface of submerged macrophytes. Traps were collected by slowly lifting to just below the surface, inverting, removing from the water and pouring off all excess water in the funnels. Immediately thereafter, the contents of the three bottles were emptied into a 500 mL bottle to which 10 mL of 95% ethanol, a narcotizing agent which serves to decrease the activity of any large invertebrate predators, had been added. This volume was then sieved, and the organisms concentrated into a 20 mL vial before further processing.

The use of activity traps takes advantage of the nightly vertical migration behavior of certain invertebrates (Hutchinson 1967; Whiteside and Williams 1975). These are set before dusk in the evening and retrieved in the morning, allowing the traps to remain in place for approximately 12 hours. Activity trap samples were taken once per week, two per enclosure.

Cladocera associated with epiphytic algae growing on the acrylic rods were sampled using a clear acrylic cylinder (6.4 cm in diameter), carefully lowered into the water to surround the rod. Both ends were sealed with stoppers around the rod prior to lifting the sampler and rod out of the water. This 1.6 L volume was sieved through a 52 mm mesh and concentrated in a 20 mL sample vial. Rod sampling occurred once per week, three rods per enclosure.

Sample Processing

All samples were processed as soon as possible. To each 20 mL vial, two drops of 4% formalin was added to slow the activity of the organisms and settle the contents to the bottom. Surface water was removed to 19 mL and 1 mL of 4% formalin, a preserving agent, added to achieve the desired sample volume of precisely 20 mL.

Zooplankton were identified (Pennak 1989) and counted, using a dissecting microscope, to determine abundance. Larger invertebrates, such as snails and insects, were excluded from the abundance determination. Subsample volumes used for determining density were adjusted according to the abundance of organisms present. For subsequent data analysis, the various subsamples were standardized to numbers of organisms per metre squared for the activity trap samples and numbers per litre for the water column and rod samples. The gut contents of a few selected cladoceran species were analysed, with methods as in De Infante (1978).

Results

Environmental Factors

Certain effects occurred as a result of the enclosures being put in place. These effects included changes in turbidity, timing of macrophyte development, light attenuation and dissolved oxygen concentration. Turbidity decreased rapidly within the enclosures (Goldsborough and Hann 1996; Pettigrew 1996) due to the sheltering effect from the wind provided by the curtains and the lessening of disturbance to the sediments due to the absence of fish activity. There was no discernible difference in turbidity among enclosures on any sampling date. Vertical light attenuation coefficients (K_d) were lower in all enclosures than in the Blind Channel (Goldsborough and Hann 1996; Pettigrew 1996), indicating that light was penetrating to greater depths in the enclosures. Macrophytes inside the enclosures developed earlier in the season as compared to the rest of the Blind Channel (pers. obs.), as the more transparent enclosure water allowed light

to reach the plant seedlings sooner. The oxygen content of the water was greater in the enclosures, likely due to local oxygen production by the macrophytes. Percent oxygen saturation was similar in all enclosures (Fig. 1, 2, and 3). There was a general increase to week 4 and an overall decrease thereafter. As well, measurements taken at the top of the water column in the evening were consistently higher than measurements taken in the morning or at the bottom of the water column. Fish were excluded and therefore vertebrate predation on zooplankton was greatly diminished or eliminated. The enclosure effect on zooplankton due to the elimination of fish was most easily observed during the pretreatment phase of the study (discussed later).

Nutrient Addition Effects

The soluble reactive phosphorus concentration (Fig. 4) in the control enclosures was consistently low all summer. The low nutrient addition enclosures showed small peaks immediately after each nutrient addition. In the high nutrient enclosures, sharp increases of phosphorus resulted briefly after the first and second nutrient additions and during week 13 of the study.

In the control enclosures, the ammonia concentration was close to zero for all weeks of the study (Fig. 5). There were small increases in the low nutrient enclosures and large increases in the high nutrient enclosures, corresponding to the addition of organic nutrients, that lasted for less than a week in the low enclosures and less than two weeks in the high enclosures.

Microinvertebrate Response

Of the three zooplankton groups occurring in the marsh, cladocerans, copepods and rotifers, the cladocerans appeared to display the most clear-cut enclosure and treatment effects. For this reason, a detailed comparison of cladoceran abundance in each habitat was presented, while copepods and rotifers were not addressed in this paper.

Cladocera in the Water Column

At the beginning of the study, cladoceran abundance was low in all enclosures (Fig. 6). During the pretreatment period in all enclosures, population numbers had substantially increased by week 3 and declined again by week 5. The addition of the low level of organic nutrients did not appear to elicit any response in cladoceran abundance different from the controls following either addition of organic nutrients.

No detectable response in cladoceran abundance occurred after the first high nutrient addition in week 4, as means of the replicate high treatment enclosures did

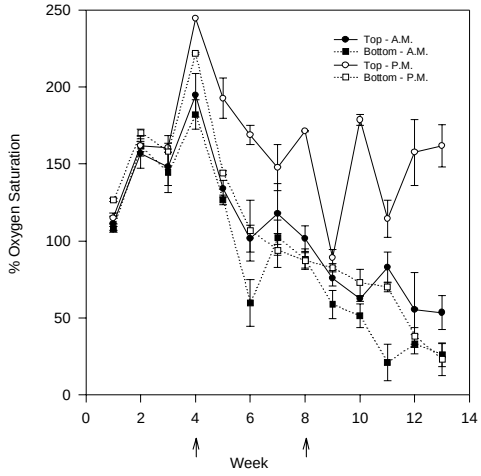

Figure 1. Oxygen saturation (% of maximum) in replicate control enclosures.

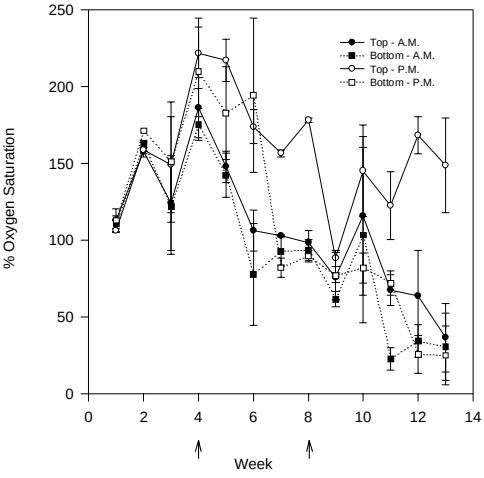

Figure 2. Oxygen saturation (% of maximum) in replicate enclosures to which a low level of waterfowl feces were added (vertical arrows).

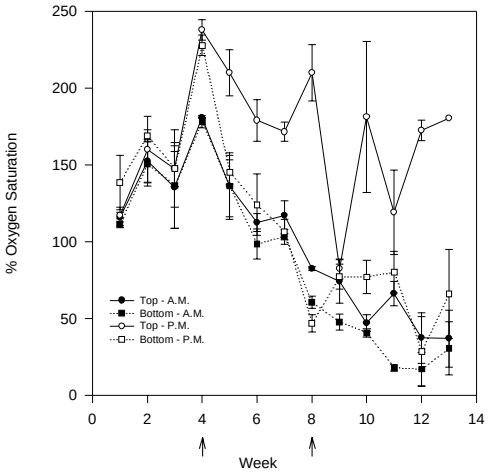

Figure 3. Oxygen saturation (% of maximum) in replicate enclosures to which a high level of waterfowl feces were added (vertical arrows).

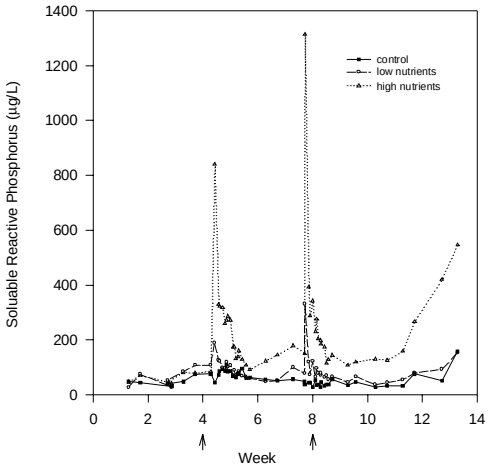

Figure 4. Soluble reactive phosphorus concentration (µg/L) in replicate control enclosures and in those to which low or high levels of waterfowl feces were added (vertical arrows).

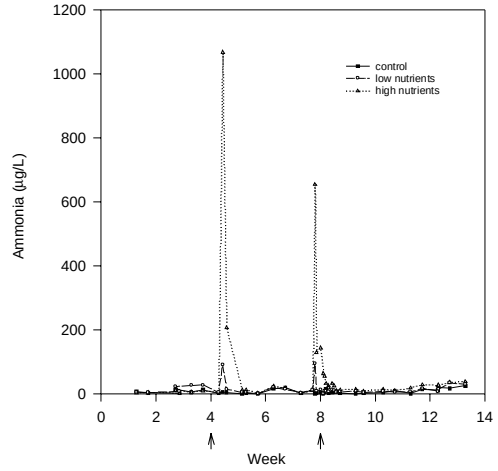

Figure 5. Ammonium-N concentration (µg/L) in replicate control enclosures and in those to which low or high levels of waterfowl feces were added (vertical arrows).

not differ from the control mean abundance. After the second addition of organic nutrients to the high treatment enclosures in week 8 (Fig. 6), mean cladoceran abundance showed a marked increase. However, the two replicate enclosures responded quite differently. Enclosure 1 (Fig. 7), showed a dramatic increase in cladoceran abundance after the second nutrient addition, while enclosure 8 (Fig. 8) showed no immediate response.

Cladoceran - Phytoplankton Interaction

The species of cladocerans sampled from the water column feed primarily on phytoplanktonic algae. Gut content analyses were conducted to confirm algae as a food source for the cladoceran species involved in this study. Specimens of *Ceriodaphnia dubia*, the most abundant cladoceran, *Bosmina longirostris* and *Scapholeberis kingi* were examined from early and late summer. Diatoms, blue-green algae and green algae were found in their guts.

In order to examine the numbers of cladocerans relative to their food source, cladoceran abundance and phytoplankton biomass were simultaneously plotted over time. During the pretreatment period in both high addition enclosures (Figs. 7 and 8), phytoplankton decreased while the cladocerans increased from week 1 to week 3, as the cladocerans grazed their primary food source. After the first nutrient addition the high algal response occurred in only enclosure 1. Grazer abundance was higher in this than in the other enclosure, and likely contributed to the abrupt decrease in algal biomass (Fig. 7). In the other high nutrient enclosure, enclosure 8, the trends in cladoceran abundance paralleled phytoplankton abundance (Fig. 8).

After the second high nutrient addition, there was an increase in phosphorus and ammonia and a brief increase in algal abundance. This increase was more substantial in enclosure 1 than in enclosure 8 and had declined in both enclosures by week 9. In enclosure 1 (Fig. 7), algal biomass remained low and cladoceran abundance increased substantially. In enclosure 8, cladocerans showed no response while algal biomass increased beyond week 11.

Cladocerans Associated with Macrophytes

Mean cladoceran abundance in the low nutrient addition enclosures remained very similar to the control enclosures following the addition of the two pulses of the

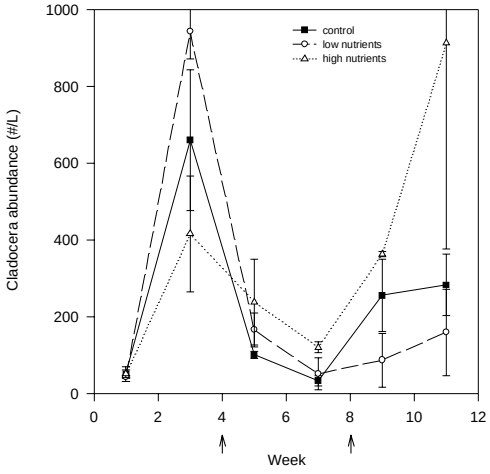

Figure 6. Cladoceran abundance (#/L) in the water column on replicate control enclosures and in those to which low or high levels of waterfowl feces were added (vertical arrows).

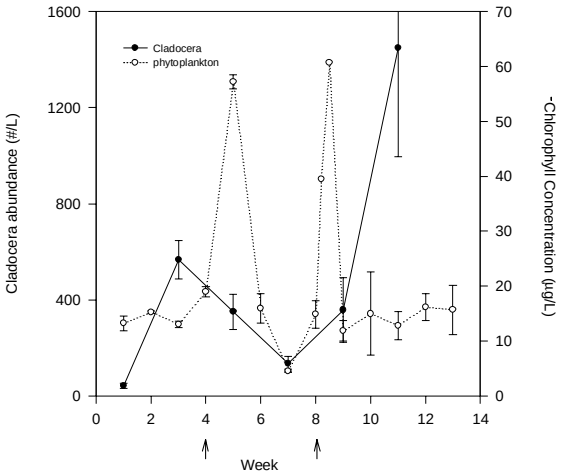

Figure 7. Cladoceran abundance (#/L) versus phytoplankton biomass (µg/L) in an enclosure (#1) to which a high level of waterfowl feces was added (vertical arrows).

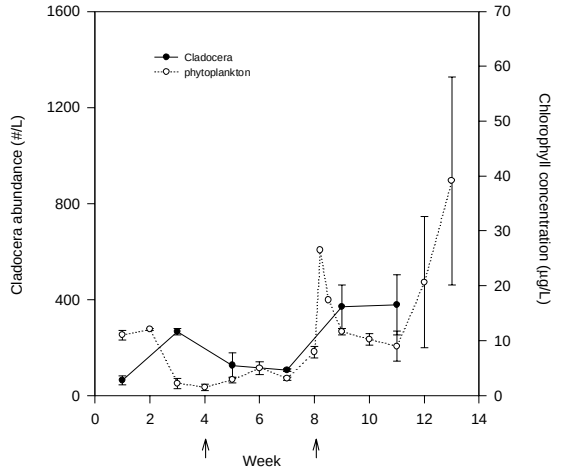

Figure 8. Cladoceran abundance (#/L) versus phytoplankton biomass (µg/L) in an enclosure (#8) to which a high level of waterfowl feces was added (vertical arrows).

low concentration of organic nutrients (Fig. 9). In contrast, mean cladoceran abundance in the high nutrient enclosures increased following both nutrient additions. However, as in the water column, the high nutrient response differed between replicate enclosures, with a strong response apparent in only one enclosure. Cladoceran abundance began increasing by week 7 in enclosure 8, and continued to increase dramatically after the second addition of nutrients. In enclosure 1, cladoceran abundance increased by week 9, but subsequently declined by week 11.

Cladocera Associated with Acrylic Rods

Cladoceran abundance (Fig. 10) was low in all enclosures in week 1 of the study, followed by a

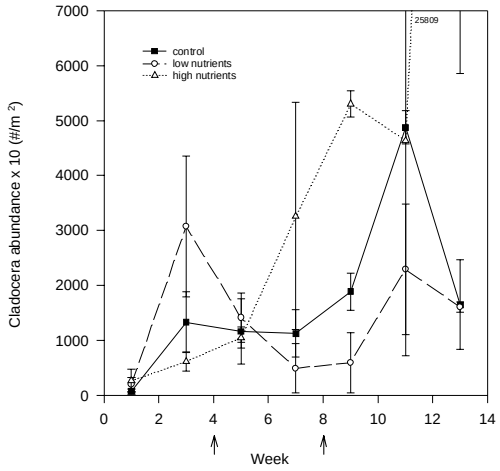

Figure 9. Cladoceran abundance (#/L) associated with submersed macrophytes in replicate control enclosures and in those to which low or high levels of waterfowl feces were added (vertical arrows).

pretreatment peak in week 3. By week 5, this peak declined dramatically in the control enclosures. Cladocerans in the high nutrient enclosures showed a slight increase on this week, following the addition of nutrients in week 4. A decrease to a similar level as in the control enclosures occurred by week 7 in the high nutrient enclosures. Treatment responses did not appear to occur in the cladocerans following the addition of the low amount of organic loading.

By week 9, the second addition of nutrients had been added and there was an increase in cladoceran abundance in all enclosures. Cladocerans in the high nutrient enclosures (Fig. 10) appeared to show a response to the nutrient loading in weeks 9 and 11, as cladoceran abundance far exceeded that in the control situation. By the last week of sampling, the abundance in all enclosures had decreased to approximately the same low numbers. As the response that occurred in the two high nutrient enclosures appeared variable, it was necessary to examine these individually (Fig. 11 and 12). Although the magnitude of changes were considerably more dramatic in enclosure 1, the overall trends were identical.

Cladoceran - Periphyton Interaction

The cladocerans sampled in association with the acrylic rods were likely feeding largely on the periphytic algae attached to the rods. For this reason, cladoceran abundance was plotted along with periphyton biomass through time to provide potential reasons for the trends in abundance that occurred.

In the high nutrient enclosures, there was a high level of phosphorus and ammonia in the water after the first

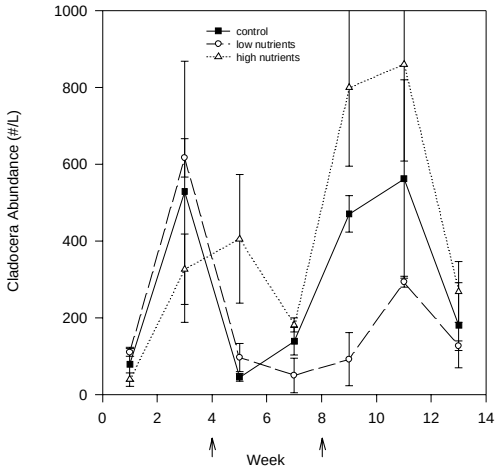

Figure 10. Cladoceran abundance (#/L) associated with artificial substrata in replicate control enclosures and in those to which low or high levels of waterfowl feces were added (vertical arrows).

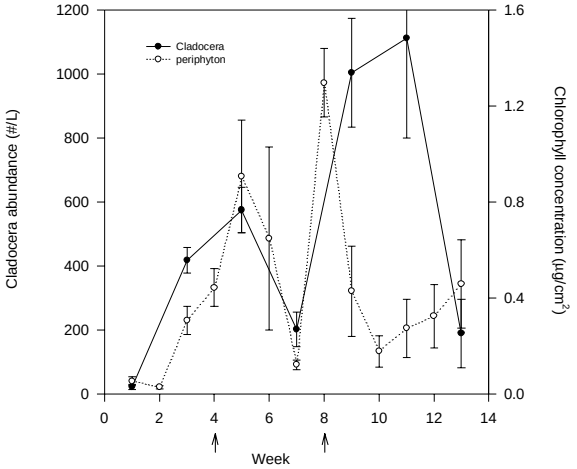

Figure 11. Cladoceran abundance (#/L) versus periphyton biomass (µg/cm^2) in an enclosure (#1) to which a high level of feces was added (vertical arrows).

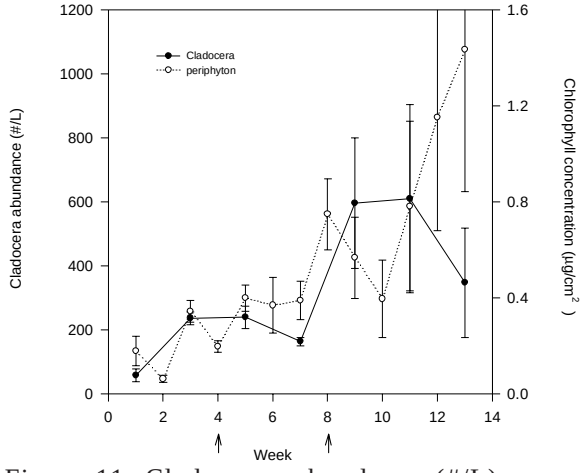

Figure 11. Cladoceran abundance (#/L) versus periphyton biomass (µg/cm^2) in an enclosure (#1) to which a high level of feces was added (vertical arrows).

and second additions of nutrients. There were corresponding algal increases after the additions (Figs. 11 and 12). The cladocerans did not appear to respond to the first addition, but there was a response to the second nutrient addition. The stronger cladoceran response in enclosure 1 (Fig. 11) corresponded to a larger increase in periphyton in this same enclosure, as compared to the response of both groups in enclosure 8 (Fig. 12).

Discussion

Nutrient Loading

Many studies have involved the addition of inorganic nutrients and found responses in the zooplankton community (Campeau *et al.* 1994; Van Donk *et al.* 1995). The high amount of nutrients added in this experiment was equivalent, in terms of P, to the amount of inorganic nutrients added to enclosures in a previous study (Hann 1995; McDougal and Goldsborough 1995). The consistency in nutrient loading rates in this study with the previous experiment allowed for a comparison between the response of zooplankton to a similar increase of inorganic versus organic nutrients.

The type of organic nutrients chosen for the nutrient additions in this study was waterfowl feces. As waterfowl inhabit the marsh, this was a realistic source of organic loading. The combined duck and goose populations in the Delta Marsh area in the last few years have been estimated to be approximately 60,000 at the peak of migration (Bob Jones, Manitoba Natural Resources, Winnipeg, Manitoba, pers. comm.). Manny *et al.* (1994) made conservative estimates of Canada goose defecation rates during the day and night equal to 1.96 and 0.37 droppings per goose per hour, respectively. The dry weight of each dropping was indicated to be 1.17 grams. The nitrogen and phosphorus content of waterfowl feces from the Delta Marsh were found to be 52.3 and 17.4 mg/g dry weight, respectively (Norwest Lab, Winnipeg, Manitoba). Taking into consideration the values above and the area of the marsh, the amount of organic loading that the natural density of waterfowl would have contributed to the marsh would be 7.51 g N/ ha/ day and 2.5 g P/ ha/ day. This level of organic nutrient loading to an enclosure was calculated to be approximately 1.88 x 10^{-2} g of nitrogen and 0.63 x 10^{-2} g of P/enclosure/day, both substantially smaller loading rates than those used in this experiment.

General Pattern of Response

The addition of organic nutrients produced immediate increases of phosphorus (SRP) and nitrogen

(ammonia) in the water column. This effect was slight in the low nutrient addition enclosures and dramatic in the high nutrient addition enclosures. These increases were short-lived, as has been observed with the addition of inorganic N and P in other enclosure experiments (Goldsborough 1993; McDougal and Goldsborough 1995; Van Donk *et al*. 1995). Nutrients in the water column can be depleted by uptake by macrophytes (Brock *et al*. 1995), algae, and sediments and by the process of denitrification (Van Donk *et al*. 1995). Although algal consumption of nutrients in the water column may be limited as macrophytes are likely superior competitors in terms of storage and uptake (Brock *et al*. 1995), phytoplanktonic and periphytic algae have demonstrated a short-term direct response to a nutrient addition (Gabor *et al*. 1994; Hann 1995).

The bottom-up trophic effect of nutrients on primary producers is counteracted by the top-down effect of invertebrate grazers (Carpenter *et al*. 1985). The effects of low and high nutrient pulsed additions on the cladoceran grazer community in the water column were examined in this study. No detectable responses occurred following the addition of the low level of nutrients, whereas in the high nutrient addition enclosures, a dramatic response occurred only following the second addition of organic nutrients.

Cladocerans were the only zooplankton group examined in this study to be greatly affected by the addition of nutrients. Nutrient concentrations can indirectly influence cladoceran abundance through direct effects on algae. The prediction is that chlorophyll *a* values, which are indicative of algal biomass, will decrease as grazer density increases (Carpenter *et al.* 1985). Similarly, it is an increase in algal abundance that can cause a corresponding increase in grazer abundance (Gabor *et al.* 1994). During many weeks of this study, cladoceran abundance appeared to closely track algal abundance, with one group increasing while the other decreased, and vice versa. The trend was that algae would increase, leading to a corresponding increase in the cladoceran grazers until they depleted this food source, thereby decreasing the algae, leading to a subsequent decrease in the grazers. This response occurred because many cladocerans are highly effective herbivorous grazers of phytoplankton and periphyton (Porter 1977; Hann 1991). The dominant species involved in this study were filter-feeders with appendages that can filter algae at rapid rates (Hutchinson 1967). As well, cladocerans reproduce asexually (Wetzel 1983), and therefore have the ability to respond quickly to changing amounts of food by increasing population size.

During the last few weeks of the study, there were high concentrations of phosphorus in the water column in the high nutrient addition enclosures. This corresponded to the time of macrophyte senescence in these enclosures, and the breakdown of macrophyte tissue would have leaked nutrients into the water (Landers 1982). These nutrients would have been available for uptake by phytoplankton and periphyton. It can be speculated that the macrophytes in enclosure 8 senesced earlier than in enclosure 1, as indicated by the substantial phosphorus increase in late August in the former enclosure. Algal biomass increases were found to occur in the high nutrient enclosure 8 during this time of macrophyte senescence. In the other high nutrient enclosure, enclosure 1, algal biomass remained low likely due to a combination of lower nutrients and high cladoceran densities during this time.

Comparison of Response in Different Subhabitats

Cladocerans in the water column, associated with macrophytes, and with artificial substrata displayed obvious responses to the pulsed nutrient addition. The response in the cladoceran community to the second high nutrient pulse was markedly different in the two replicate enclosures. In enclosure 1, the planktonic Cladocera increased in abundance, heavily grazing the phytoplankton, but there was little response in the phytophilous Cladocera. The opposite pattern was observed in enclosure 8 in which the phytophilous cladocerans increased. Phytoplankton was high in this enclosure, while the zooplankton community showed a muted response.

The differential responses among the communities occupying different niches (and sampled via different methods) could have been due to the three-dimensional partitioning of the habitat. The water column and rod samples were taken high in the water, while activity traps were lowered to the surface of the macrophytes, nearer to the sediments. Therefore, the first method sampled planktonic cladocerans in the water column, while the second should have sampled individuals directly associated with periphytic algae on the rods as well as some zooplankton from the surrounding water column. An overlap of communities can occur, so it is predictable that the response should be similar in the grazers inhabiting these two niches. In contrast, the activity traps were placed to recover primarily phytophilous species, grazing the epiphytic algae on the macrophyte surface. As this is mainly a different community from that described above, it is understandable that a different response occurred from that in the water column and rod samples.

Many factors were considered to explain potentially the differing responses of the algae and grazers in enclosures 1 and 8. It was found that percent oxygen

saturation (Fig. 1, 2 and 3), turbidity and light extinction did not differ between any enclosures, including between the replicate high nutrient addition enclosures (Goldsborough and Hann 1996; Pettigrew 1996). Neither enclosure contained planktivorous fish. Observations of macrophyte density suggested that biomass was similar in enclosure 1 and 8. It is unlikely that any of these factors affected the communities differently in the two enclosures.

The differences in grazer abundance in enclosures 1 and 8 may have been due to stronger invertebrate predation in enclosure 8. Odonate larvae in the activity trap samples consisted of more larger individuals in enclosure 8 versus enclosure 1. These species are predators of certain cladocerans (Johnson 1973; Johnson *et al.* 1985), and therefore could have kept cladoceran abundance low. Phytoplankton and periphyton were high in this enclosure, likely a function of decreased cladoceran grazing pressure. The odonate larvae present in enclosure 1 were considerably smaller and less abundant than the individuals in enclosure 8. As a result, cladoceran abundance after the second nutrient addition would not have been restrained by heavy predation, and therefore would have been able to demonstrate a clear response to the nutrient addition.

Phytophilous cladocerans, sampled by the activity traps, demonstrated a dramatic response to the second nutrient addition in enclosure 8 and a limited response in enclosure 1. After week 11, phosphorus concentrations began to increase in the water column in enclosure 1, suggesting that macrophyte senescence was occurring more quickly in this enclosure than in enclosure 8, and subsequently leaking nutrients into the water. With the senescence of the macrophytes in this enclosure, the structure of the macrophyte bed would have begun to break down, including the substrata for epiphytic algal attachment (Burkholder and Wetzel 1989). The decline in food quality of the epiphytes in enclosure 1 could account for the weak response in the phytophilous grazers after the second nutrient addition. In enclosure 8, slight nutrient leakage into the water column began to occur by week 13. As the senescence of the macrophytes in this enclosure was delayed, good macrophyte and epiphyte structure would have still been present after the second nutrient addition, resulting in a plentiful food source and allowing the phytophilous cladocerans to dramatically increase in abundance. The dominant phytophilous species present in this enclosure were *Chydorus* spp. As these species have a compact body form, they would not have been as vulnerable to invertebrate predation as were the planktonic species in this enclosure.

Differences in species composition can also be accounted for by the different niches the cladocerans occupy. Samples taken high in the water column, including the rod samples, contained planktonic species, dominated by *Ceriodaphnia dubia*. Samples taken among the macrophytes also collected *Chydorus* spp., epiphyton grazers that were not primarily located in the water column. The rod samples also contained some of these species as this subhabitat included individuals grazing the periphytic algae on the surface of the rods (*Chydorus* spp.) as well as planktonic species (*C. dubia*) from the surrounding water column.

Response to Organic Nutrient Addition

Inorganic nutrient additions in enclosure situations have been found to directly affect phytoplankton and periphyton biomass, thereby indirectly influencing the grazer community (Fairchild *et al.* 1989; Van Donk *et al.* 1995). As well, nutrient enrichment of wetlands, using inorganic nutrients, have led to increased algal biomass (Rader and Richardson 1992; Gabor *et al*. 1994). This study examined the response of the zooplankton to the addition of organic nutrients. Unlike inorganic nutrients, which can be added in a form desirable for uptake by primary producers, organic nutrients might be largely inaccessible, as these nutrients may be bound in the form of complex organic particles. This likely contributed to the results in this experiment not being as clear-cut as in studies with inorganic additions.

The absence of response in the low nutrient addition enclosures suggests that this amount of organic addition had no detectable effect on the abundance of these invertebrates. In a nutrient addition experiment in an oligotrophic wetland, Murkin *et al*. (1994) also found that the nutrient amount used was insufficient to elicit a response in the invertebrate groups studied. More consistent (in the high nutrient enclosures) and pronounced responses might have occurred if a higher level of nutrients were added. Unlike with the algae, the response of the grazers is indirect, and small increases at one trophic level may not be substantial enough to transfer an adequate amount of energy to the next trophic level to produce an increase there as well.

The graphical interpretation of these data suggest that a response, in cladoceran abundance, occurred in the high nutrient enclosures. However, this was substantial in only one enclosure, and marginal in the other. Biological systems are inherently variable and when dealing with enclosures in the field, the community in each enclosed space will be different to some degree. Although precautions are taken to achieve as much homogeneity between enclosures as possible, each enclosure will contain those species, and descendants of those species, sectioned off in the water

column, as well as the unique seed and egg banks that exist in the sediments. As these community interaction are complex, added nutrients that affected the community of one enclosure may not have the same effect on another because of confounding factors. However, the cladoceran grazer abundance was more explainable upon examination of the corresponding algal assemblage present. But this does not account for bottom-up differences in terms of the nutrient addition. Although not quantified, perhaps the macrophyte densities may have been different in the two enclosures, giving the macrophytes in one enclosure a stronger competitive advantage over the algae in the uptake of the added nutrients. In a nutrient addition experiment, Brock *et al*. (1995) found that the majority of added nutrients was taken up by the macrophytes present.

Conclusions

The levels of organic loading in this experiment far exceeded the natural levels of waterfowl feces that are normally added to the marsh. Even at these high nutrient addition levels, responses in the community were short-lived and the system quickly returned to its previous condition. The aquatic community at the Delta Marsh proved resilient to extremely large organic nutrient additions. This suggests that the birds occupying the marsh area each year do not negatively impact the system and that even unrealistically high densities of waterfowl over a short period of time would potentially produce only temporary responses by the resident plankton.

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References

American Public Health Association (APHA). 1985. Standard methods for the examination of water and wastewater. 16th ed. Washington, DC.

Batt, B.D.J., Anderson, M.G., Anderson, C.D. and Caswell, F.D. 1989. The use of prairie potholes by North American ducks. In Northern prairie wetlands. *Edited by* A. Van der Valk. Iowa State University Press, Ames. pp. 204-227

Bazely, D.R. and Jefferies, R.L. 1985. Goose feces: A source of nitrogen for plant growth in a grazed salt marsh. J. Appl. Ecol. **22**: 693-703.

Brock, T.C.M., Roijackers, R.M.M., Rolton, R., Bransen, F. and 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.

Burkholder, J.M. and Wetzel, R.G. 1989. Epiphytic microalgae on natural substrata in a hardwater lake: seasonal dynamics of community structure, biomass and ATP content. Arch. Hydrobiol. **83**: 1-56.

Campeau, S., Murkin, H.R., and Titman, R.D. 1994. Relative importance of algae and emergent plant litter to freshwater marsh invertebrates. Can. J. Fish. Aquat. Sci. **51**: 681-692.

Carpenter, S.R., Kitchell, J.F. and Hodgson, J.R. 1985. Cascading trophic interactions and lake productivity. BioScience **35**: 634-639.

De Infante, A. 1978. A method for the study of foods of herbivorous zooplankton. Trans. Amer. Microscop. Soc. **97**: 256-258.

Fairchild, G.W., Campbell, J.M. and Lowe, R.L. 1989. Numerical response of chydorids (Cladocera) and chironomids (Diptera) to nutrient-enhanced periphyton growth. Arch. Hydrobiol. **114**: 369-382.

Gabor, T.S., Murkin, H.R., Stainton, M.P., Boughen, J.A. and 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.

Goldsborough, L.G. 1991. Effects of hexazinone on water chemistry and periphyton biomass and productivity in large littoral enclosures. University Field Station (Delta Marsh) Annual Report **26**: 50-62.

Goldsborough, L.G. 1993. Responses of marsh algal communities to controlled nitrogen and phosphorus enrichment. University Field Station (Delta Marsh) Annual Report **28**: 35-40.

Goldsborough, L.G. and Hann, B.J. 1996. Enclosure affects trophic structure of a freshwater prairie wetland. University Field Station (Delta Marsh) Annual Report **30**: 63-67.

Hann, B.J. 1991. Invertebrate grazer-periphyton interactions in a eutrophic marsh pond. Freshwater Biology **26**: 87-96.

Hann, B.J. 1995. Responses of aquatic invertebrates to experimental nutrient enrichment of a wetland. University Field Station (Delta Marsh) Annual Report **29**: 83-87.

Hutchinson, G.E. 1957. A Treatise on Limnology. Vol.

I. Geography, physics, and chemistry. John Wiley & Sons, Inc., New York.

Hutchinson, G.E. 1967. A Treatise on Limnology. Volume II. Introduction to Lake Biology and the Limnoplankton. John Wiley & Sons, Inc., New York.

Johnson, D.M. 1973. Predation by damselfly naiads on cladoceran populations: fluctuating intensity. Ecology **54**: 251-268.

Johnson, D.M., Crowley, P.H., Bohanan, R.E., Watson, C.N., and Martin, T.H. 1985. Competition among larval dragonflies: a field enclosure experiment. Ecology **66**: 119-128.

Kadlec, J.A. 1986. Effects of flooding on dissolved and suspended nutrients in small diked marshes. Can. J. Fish. Aquat. Sci. **43**: 1999-2008.

Landers, D.H. 1982. Effects of naturally senescing aquatic macrophytes on nutrient chemistry and chlorophyll *a* of surrounding waters. Limnol. Oceanogr. **27**: 428-439.

Manny, B.A., Johnson, W.C. and Wetzel, R.G. 1994. Nutrient additions by waterfowl to lakes and reservoirs: predicting their effects on productivity and water quality. Hydrobiologia **279/280**: 121-132.

McDougal, R.L. and Goldsborough, L.G. 1995. Responses of wetland algae and macrophytes to press and pulse additions of inorganic nitrogen and phosphorus. University Field Station (Delta Marsh) Annual Report **29**: 117-126.

Mitsch, W.J. and Gosselink, J.G. 1993. Wetlands, 2nd edition. Van Nostrand Reinhold, New York.

Murkin, H.R. 1989. The basis for food chains in prairie wetlands. In Northern prairie wetlands. *Edited by* A. G. Van der Valk. Iowa State University Press, Ames, IA. pp. 316-338.

Murkin, H.R. and Kadlec, J.A. 1986. Relationships between waterfowl and macroinvertebrate densities in a northern prairie marsh. J. Wildl. Manage. **50**: 212-217.

Murkin, H.R., Pollard, J.B., Stainton, M.P., Boughen, J.A. and Titman, R.D. 1994. Nutrient additions to wetlands in the Interlake region of Manitoba, Canada: effects of periodic additions throughout the growing season. Hydrobiologia **279/280**: 483-495.

Pennak, R.W. 1989. Fresh-water Invertebrates of the United States. 3rd ed. The Ronald Press, New York.

Pettigrew, C.T. 1996. Response of microinvertebrates to organic nutrient additions in a prairie wetland. Honors Thesis, Dept. of Zoology, University of Manitoba. 87 pp.

Porter, K.G. 1977. The plant-animal interface in freshwater ecosystems. American Scientist **65**: 159-170.

Purcell S. and Goldsborough, L.G. 1996. The significance of waterfowl feces as a source of nutrients to algae in a prairie wetland. University Field Station (Delta Marsh) Annual Report **30**: 43-51.

Rader, R.B., and Richardson, C.J. 1992. The effects of nutrient enrichment on algae and macroinvertebrates in the Everglades: a review. Wetlands **12**: 121-135.

Schindler, D.W., Armstrong, F.A.J., Holmgren, S.K., and Brunskill, G.J. 1971. Eutrophication of Lake 227, Experimental Lakes Area, Northwestern Ontario, by addition of phosphate and nitrate. J. Fish. Res. Bd Can. **28**: 1762-1783.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of fresh water. 2nd ed. Fish Mar. Serv. Misc. Spec. Publ. 25.

Van Donk, E., Prins, H., Voogd, H.M., Crum, S.J.H. and 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.

Wetzel, R.G. 1983. Limnology. 2nd. ed. Saunders College Publishing, Philadelphia.

Whiteside, M.C. and Williams, J.B. 1975. A new sampling technique for aquatic ecologists. Verh. Internat. Verein. Limnol. **19**:1534-1539.