ORIGINAL ARTICLES

Effects of Fathead Minnow Colonization and Removal on a Prairie Wetland Ecosystem

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ABSTRACT

Fish communities in prairie wetlands are extremely dynamic. Due to complete winterkills and periodic colonization, individual basins alternate between supporting a fish population and being fishless. Here we assess the ecological consequences of colonization and subsequent extinction of a fathead minnow population in a prairie wetland. We used a BACI-type approach (before–after control impact) in which data from the colonized wetland were paired with data from a similar fishless site first when both wetlands were fishless (1996), then when the minnow population reached moderate densities in the colonized site (1998), and then again when the colonized site became fishless after treatment with rotenone (1999). Fish colonization resulted in significant increases in turbidity, total phosphorus, and chlorophyll *a* in the water column; it also caused significant decreases in the abundance of aquatic insects and large cladocerans. Elimination of the minnow population largely reversed the effects of minnow colonization. Our results indicate that characteristics of prairie wetlands can vary as they alternate between supporting fathead minnow populations and being fishless and that ecological characteristics may change rapidly in response to minnow colonization or elimination.

Key words: prairie wetlands; aquatic invertebrates; water quality; fathead minnows; intervention analysis; BACI; before–after control impact.

INTRODUCTION

Numerous studies have examined the effects of planktivorous fish on aquatic ecosystems, mainly by comparing ecosystems with fish to fishless sites (Hanson and Riggs 1995; Zimmer and others 2000) or by adding fish to mesocosms (McQueen and others 1992; Pérez-Fuentetaja and others 1996; Vanni and Layne 1997; Vanni and others 1997). Studies assessing the effects of the introduction of planktivorous fish to fishless ecosystems are less common (for example, Spencer and King 1984; He and others 1994), as are assessments following fish removal (for example, Reinertsen and others 1990; Hanson and Butler 1994; He and others 1994). Although specific results have varied, many studies have found that planktivorous fish are associated with an increase in the phosphorus and algae concentrations in the water column, higher turbidity, and a reduction in the abundance of some planktonic and nektonic invertebrates.

Although the composition of fish communities varies among lentic ecosystems (Tonn and Magnuson 1982; Magnuson and others 1998), the extinction of fish communities is probably a rare event. Thus, relatively few ecosystems alternate naturally between supporting a dense fish population and being virtually fishless. However, the presence or absence of planktivorous fish in individual wetlands of the Prairie Pot-

hole Region (PPR) of central North America is probably highly variable, and relatively few wetlands support permanent populations (Euliss and others 1999). This variability is due to the climate and landscape features of the PPR, as well as the physical and biological characteristics of the wetlands themselves.

Most wetlands in the PPR are very productive and relatively shallow, promoting frequent winterkills (Kantrud and others 1989). The climate of the PPR alternates between wet and dry, with each phase lasting approximately 10–20 years (Diaz 1983). The wet–dry cycle has dramatic effects on the physical, chemical, and biological properties of PPR wetlands (Euliss and others 1999), including fish populations. During the dry phase, fish populations are greatly reduced or eliminated by lower water depths that promote winterkills as well as by the actual drying of basins (Kantrud and others 1989). Additionally, the probability that fishless habitats will be colonized is likely lower due to a reduced flow of overland water between basins. In contrast, greater wetland depth and increased overland water flow during the wet cycle increase the likelihood that fish populations will persist and new habitats will be colonized.

Historically, the PPR was a mosaic of wetlands embedded within prairie uplands; most watersheds were primarily nonintegrated, limiting the dispersal of fish among the wetlands (Kantrud and others 1989; Peterka 1989; Euliss and others 1999). Since the late 20th century, the consolidation of local watersheds to drain shallower wetlands for agriculture has increased the proportion and perhaps the number of deeper basins in many areas (Euliss and others 1999). These remaining basins may serve as refuges for fish during winter or low-water periods. Moreover, the ditches, culverts, and subsurface tile used to drain wetlands probably increase dispersal rates. Thus, fish probably exist as metapopulations throughout the PPR, with local extinctions and periodic colonizations from more permanent wetland habitats.

Fathead minnows (*Pimephales promelas*) and brook sticklebacks (*Culaea inconstans*) are usually the only species of fish that occur naturally in PPR wetlands (Peterka 1989). These two species often co-occur, but wetlands with fatheads only are relatively common whereas wetlands with sticklebacks only are more rare (Held and Peterka 1974; Peterka 1989; K. D. Zimmer and others unpublished). In wetlands where they co-occur, fathead minnows are usually dominant in terms of both numbers of individuals and biomass (Zimmer and others 2000). Thus, fathead minnows are probably the native fish species with the greatest potential to influence wetland ecosystems in the PPR.

Aquatic invertebrates are an important food resource for fathead minnows (Held and Peterka 1974), and comparative studies from PPR wetlands have found that fathead minnows are associated with a reduction in the abundance and biomass of several types of aquatic invertebrates (Hanson and Riggs 1995; Zimmer and others 2000). Predation pressure from minnow populations can be high. Duffy (1998) estimated that consumption of aquatic invertebrates by fathead minnows in four South Dakota wetlands approximately equaled invertebrate production rates. However, the response of invertebrate communities to minnow colonization and elimination is still not well understood. Nor is it known how these two events influence other wetland characteristics, such as water clarity, nutrient concentrations in the water column, and phytoplankton abundance.

Here we assess the influence of fathead minnow colonization and their subsequent removal on the invertebrate community and water-quality characteristics of a PPR wetland. We tested for significant changes in the perturbed wetland using a modified BACI approach (before–after control impact) (Stewart-Oaten and others 1986), a technique commonly applied in other unreplicated impact studies (Carpenter and others 1989; Schroeter and others 1993; Smith and others 1993; Stout and Rondinelli 1995). We provide supporting evidence to show that the minnow population was the cause of the observed changes by relating the results of this study to those of a larger replicated study that assessed the effects of fathead minnows by comparing multiple wetlands with minnow populations to fishless sites (Zimmer and others 2000; K. D. Zimmer and others unpublished).

METHODS

Field Sampling

In the BACI approach, data from the perturbed site are paired with data collected simultaneously at a reference site to determine if significant changes occur in the perturbed site (Stewart-Oaten and others 1986). Both the perturbed and reference wetlands used here were included in the larger study (Zimmer and others 2000; K. D. Zimmer and others unpublished). We define the wetland that was colonized by fathead minnows and subsequently treated with rotenone as the treatment site. This wetland was fishless throughout 1996. Fathead minnows were first observed here in spring of 1997, and only fathead min-
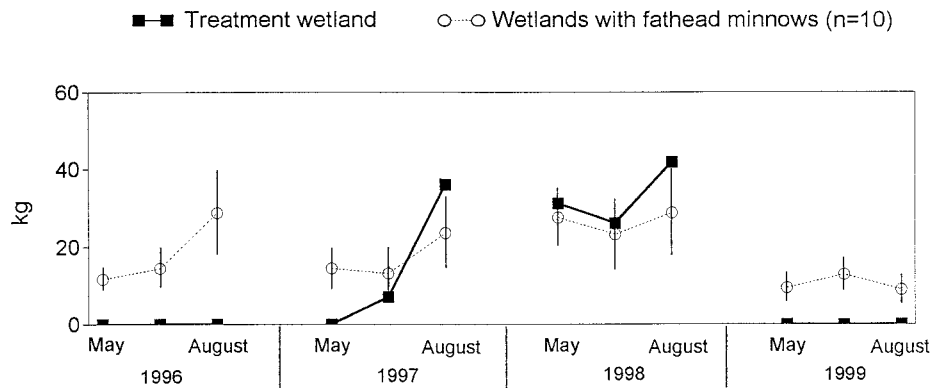
Figure 1. Relative abundance of fathead minnows in the treatment wetland and the 10 wetlands used in our larger study (Zimmer and others unpublished) in 1996 through 1999. Graphs show the total biomass of fathead minnows captured in five traps in the treatment site and the average total biomass ($\pm$ 1 standard error) captured in five traps in the 10 wetlands on each date.

nows colonized this site. The mechanism of the colonization is unknown, but it likely resulted from diversion of overland water flow.

Minnow abundance was low in spring of 1997, but it increased exponentially as the summer progressed (Figure 1). Thus, 1997 was a transitional year in terms of minnow abundance, and 1998 was the first full year that the treatment site supported a minnow population comparable in density to those in wetlands used in our larger study (K. D. Zimmer and others unpublished) (Figure 1). The treatment wetland was then treated with rotenone in the fall of 1998, so that it was again fishless throughout 1999. Minnow carcasses were left in the treated wetland following the rotenone application. The reference site used here was a fishless wetland (also included in our larger study), and it remained fishless during all study years. We selected this reference site because its depth was similar to that of the treatment site, and depth has a significant influence on aquatic invertebrate communities in prairie wetlands (Zimmer and others 2000). To eliminate potential bias associated with the chemical treatment, the reference wetland was also treated with rotenone at the same time as the treatment site.

Both wetlands are located on US Fish and Wildlife Service property (Waterfowl Production Areas) in west-central Minnesota and are approximately 80 km apart. The wetlands have a semipermanent hydroperiod (following the classification of Stewart and Kantrud 1971) and occasionally dry. The watershed of both wetlands is largely grasses. The reference site had a surface area of 8.1 ha and a maximum depth (Z_{max}) of 1.9 m; the treatment site was 4.1 ha with a Z_{max} of 2.1 m. Sampling was conducted in each wetland from May through August in 1996 through 1999. Five sampling transects were established in each wetland every year by randomly selecting five compass bearings (0–360° from north). A compass was used to establish the

five transects that radiated from the wetland center to the edge. Two sampling stations were established along each transect, one at the interface of emergent vegetation and open water (interface station) and the other one-half the distance from the interface station to the center of the wetland (open-water station). All aquatic invertebrate and fish sampling was done along these transects. Both wetlands were sampled within 4 days of each other during each sampling rotation.

Funnel-type minnow traps were used to sample fish in early May, mid-July, and late August of each year in both wetlands. One trap was set at each interface station and collected after 24 h. Results for each date are expressed as the total biomass of fathead minnows captured in the five traps.

Aquatic invertebrates were sampled with integrated column samples (Swanson 1978) and activity traps (AT) following the design of Murkin and others (1983). Samples were collected every 3rd week from May through August, for a total of six sampling dates in each year; AT samples were collected concurrently with column samples. Column samples were taken at the five open-water stations of each wetland on each date and concentrated by filtration through a 68-μm mesh funnel. Ten activity traps were deployed in each wetland on each date, one at each interface and open-water station. Traps were suspended horizontally 30 cm below the water surface and retrieved after 24 h. AT contents were concentrated by passing samples through a 140-μm mesh funnel. Both column and AT samples were preserved with 70% ethanol.

Invertebrates were identified to the lowest feasible taxonomic group and counted. Invertebrates were pooled into the following nine aggregate taxa for statistical analysis: aquatic insects (excluding Corixidae), Corixidae, *Hyalella azteca, Gammarus lacustris*, ostracods, large-bodied cladocera (body length >1 mm, mainly *Daphnia* and *Simocephalus*),

small-bodied cladocera (body length <1 mm, mainly Chydoridae and Bosminidae), calanoid copepods, and cyclopoid copepods. The most abundant taxa in the group "aquatic insects" were Chironomidae, Chaoboridae, Ephemeroptera, and Zygoptera, which represented 41%, 18%, 11%, and 7% of total individuals, respectively. We analyzed Corixidae separately from other aquatic insects because our previous work has shown a positive relationship between corixid and fathead minnow abundances but a negative relationship for most other types of insects (Zimmer and others 2000). Similarly, we observed (Zimmer and others 2000) a negative relationship between abundance of *H. azteca* and fathead minnows but not for *G. lacustris*. Therefore, we analyzed these taxa separately. Contents of the five column samples and 10 AT samples on each date were pooled for each invertebrate group to produce a total count for each wetland. This resulted in six estimates of abundance for each of the nine invertebrate groups in each wetland in each year.

We collected surface-water samples from the center of each wetland on the same dates we sampled aquatic invertebrates, and water samples were analyzed for total phosphorus, total nitrogen, and chlorophyll *a* (APHA 1992). Turbidity was measured in the field on each sampling date with a portable nephelometer. As with invertebrates, this resulted in six estimates per year for each water-quality variable in each wetland.

Statistical Analysis

The traditional application of the BACI approach involves the assessment of a single perturbation and utilizes data collected before and after the event (Stewart-Oaten and others 1986). Such an analysis uses two data sets, collected before and after the event, and tests for significant change may involve a randomization test (Carpenter and others 1989), *t* test (Stewart-Oaten and others 1986), or a nonparametric equivalent (Smith and others 1993). However, we assessed two perturbations (minnow colonization and removal), resulting in three data sets (before minnow colonization, after colonization, and after minnow removal). Thus, we used one-way analysis of variance (ANOVA) to test for significant changes in response to both minnow colonization and removal. We did not include data from 1997 in our analysis because this year was transitional in terms of minnow abundance in the treatment site, with the minnow population increasing from very low numbers in May to moderate numbers by August. Thus, we used data from 1996 as the before-colonization period (Before),

1998 data to assess the effects of minnow colonization (Colonization), and 1999 data to assess effects of minnow removal (Removal).

In a BACI design, data from the treatment site are matched with data from the reference site collected on the same date; the difference between the two (delta) is determined for each sampling date (Stewart-Oaten and others 1986). This was done for data in the Before (B_Δ), Colonization (C_Δ), and Removal (R_Δ) periods. This resulted in six deltas for each of the three time periods for each response variable. Our null hypothesis was H_o: $\bar{B}_\Delta = \bar{C}_\Delta = \bar{R}_\Delta$, with a significant difference among deltas indicating that a significant change occurred in the treatment wetland.

The validity of the BACI approach is dependent on four assumptions: normal distributions and equal variances, additivity, independence of the deltas, and lack of a trend in deltas in the Before period (Stewart-Oaten and others 1992; Smith and others 1993). We transformed our data in an attempt to increase additivity and normality and to equalize variance (Stewart-Oaten and others 1986). Our data sets for small cladocerans, corixids, *H. azteca*, and *G. lacustris* contained zeros, and so were $\log_{10} (n+1)$ transformed; other variables were $\log_{10}$ transformed without the constant. We tested all transformed data for additivity with Tukey's one-degree-of-freedom test (Tukey 1949) and for trends in the B_Δ by determining the correlation between the B_Δ and their respective sampling dates (Smith and others 1993). Variables that failed either the additivity or trend tests were not subjected to tests for significant change. Independence of the deltas within each time period was assessed using the Durbin-Watson test for first-order autocorrelation with the SAS AUTOREG procedure (SAS 1988). We tested for positive autocorrelation only, as it inflates the type I error rate and is of more concern than negative autocorrelation (Carpenter and others 1989; Bence 1995). Previous studies have assessed autocorrelation in the Before period only, but we took a more conservative approach and assessed autocorrelation in all three time periods. It is difficult to assess autocorrelation with sample sizes as small as ours (Bence 1995); thus, results of significance testing should be scrutinized in light of the estimated autocorrelation of each variable.

A one-way ANOVA was used to test for significant differences among deltas in the three time periods using the SAS GLM procedure (SAS 1990). To maintain an overall error rate of *P* less than 0.05 across all ANOVAs, the *P* values of water quality and invertebrate variables from ANOVA were each adjusted using a sequential-Bonferroni correction

(Rice 1990). Thus, all reported P values less than 0.05 represent the value after correction. When the ANOVA yielded significant results, the Ryan-Einot-Gabriel-Welsch multiple-comparison test (SAS 1990) was used to determine which time periods differed significantly from others. We interpreted significant differences between B_Δ and C_Δ as significant effects of minnow colonization, and significant differences between C_Δ and R_Δ as significant effects of minnow removal.

Estimating the degree of change in response to a perturbation is as important as determining whether a significant change has occurred (Stewart-Oaten and others 1992; Schroeter and others 1993). For each variable, we estimated the magnitude of change in the treatment wetland in response to both minnow colonization and removal and compared this change to that observed in the reference wetland over the same time period. This was done by pairing the log-value of each response variable on each sampling date within one wetland to the log-value on the same date in the same wetland in a different year and determining the difference. For each response variable, this resulted in six estimates of change between 1996 and 1998 and between 1998 and 1999 within each wetland. We then determined the mean difference and confidence interval between both time periods and back-transformed these values. The back-transformed mean is equivalent to the average ratio of the geometric means of the untransformed data and provides an estimate of the multiplicative change between time periods. Paired differences between 1996 and 1998 in the treatment wetland represented the change in response to minnow colonization, and paired differences between 1998 and 1999 represented the change in response to minnow removal. Estimates of change between the same time periods were also determined in the same manner for the reference site, providing an estimate of natural variability in the absence of the perturbations.

Estimating the degree of change from time-series data that are positively autocorrelated can result in biased estimates and an underestimation of confidence intervals (Bence 1995). Thus, we tested for positive, first-order autocorrelation in the data used to estimate change between time periods with the SAS AUTOREG procedure (SAS 1988). When results indicated modest autocorrelation (P less than 0.50), maximum likelihood methods were used to estimate the average change and confidence interval (Bence 1995). Estimates of change in data sets with nonsignificant autocorrelation were generated with ordinary least-squares. Detecting autocorrela-

tion is difficult with sample sizes as small as ours, and estimates may still be biased after correction with maximum likelihood methods (Bence 1995). Thus, all estimates of change and the associated confidence intervals are considered approximate.

RESULTS

Ostracods had significant additivity and trend tests ($P = 0.047$, $P = 0.022$, respectively) and calanoid copepods had a significant additivity test ($P = 0.017$), so these two invertebrate groups were not tested for significant change. All other variables had nonsignificant tests for additivity and trend (all P greater than 0.05). Several variables exhibited significant first-order autocorrelation, including turbidity ($P = 0.019$), total nitrogen ($P = 0.028$), aquatic insects ($P = 0.042$), corixids ($P = 0.001$), cyclopoid copepods ($P = 0.012$), and $G.$ $lacustris$ ($P = 0.016$), but all were significant in only 1 of the 3 years (Table 1). No significant autocorrelation was detected in any other variables in any year (all P greater than 0.05) (Table 1).

Among the water-quality variables, significant changes in the treatment wetland were detected in turbidity (P less than 0.0001), chlorophyll a ($P = 0.0216$), and total phosphorus ($P = 0.0048$), but no changes were detected in total nitrogen ($P = 0.0865$) (Figure 2). However, total nitrogen concentrations increased sharply on the last 3 dates of 1998. Multiple-comparison tests conducted on each significant variable showed complementary patterns of response to minnow colonization and removal. For each significant variable, 1998 was significantly different from 1996 and 1999, but no difference was detected between 1996 and 1999. Colonization by minnows resulted in increased turbidity and higher concentrations of chlorophyll a and total phosphorus in the water column, whereas removal of the minnow population had the opposite effect. The lack of significant differences between 1996 and 1999 indicates that removal of the minnow population largely reversed the effects of colonization.

Significant effects were detected on the abundance of large cladocerans (P less than 0.0001), aquatic insects ($P = 0.0002$), and $H.$ $azteca$ ($P = 0.0001$) (Figure 3). Similar to the significant water-quality variables, multiple comparison tests for large cladocerans and aquatic insects indicated that 1998 was significantly different from 1996 and 1999, with no difference detected between 1996 and 1999. The abundance of both taxonomic groups decreased sharply after colonization, then increased following minnow removal. This again

Table 1. Results of Testing for First-Order Autocorrelation in B_{deltas} (1996), C_{deltas} (1998), and R_{deltas} (1999)

Variable	B_Δ *d (P)*	C_Δ *d (P)*	R_Δ *d (P)*
Turbidity	2.49 (0.747)	0.67 (0.019)[a]	1.17 (0.123)
Chlorophyll *a*	1.87 (0.429)	1.74 (0.361)	3.17 (0.961)
Total phosphorus	1.23 (0.144)	1.71 (0.349)	2.07 (0.538)
Total nitrogen	1.52 (0.258)	0.75 (0.028)[a]	3.52 (0.995)
Aquatic insects	0.98 (0.068)	0.84 (0.042)[a]	2.92 (0.906)
Corixidae	2.64 (0.807)	0.36 (0.001)[a]	1.87 (0.431)
Hyalella azteca	1.90 (0.444)	1.13 (0.110)	1.25 (0.153)
Gammarus lacustris	1.96 (0.481)	2.02 (0.513)	0.64 (0.016)[a]
Large cladocerans	1.74 (0.360)	3.27 (0.974)	1.39 (0.204)
Small cladocerans	1.25 (0.153)	2.90 (0.900)	1.87 (0.431)
Cyclopoid copepods	1.28 (0.162)	0.58 (0.012)[a]	2.19 (0.601)

Shown is the Durbin-Watson statistic (d), where d = 0 indicates positive autocorrelation, d = 2.0 no autocorrelation, and d = 4.0 negative autocorrelation. P represents the probability of significant positive autocorrelation.
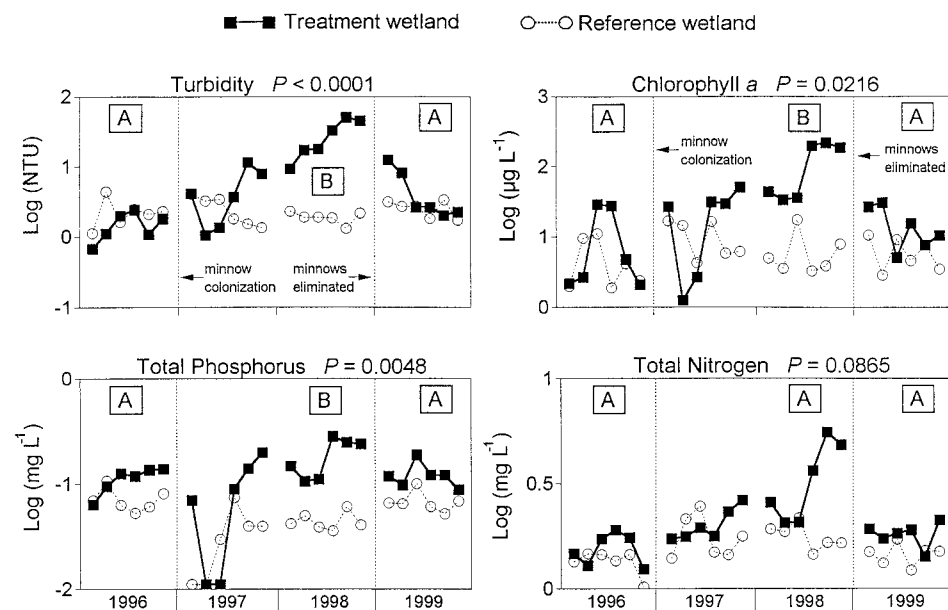[a]*Significant results.*

Figure 2. Values of turbidity, chlorophyll *a*, total phosphorus, and total nitrogen in the treatment and reference wetlands on six sampling dates in 1996 through 1999. The dashed vertical lines indicate the times of minnow colonization and removal in the treatment site. The *P* values indicate results of the BACI ANOVA tests for significant differences among the deltas of 1996, 1998, and 1999; years with common letters are not significantly different.

indicates an effect of both minnow colonization and removal and that removal of the minnow population reversed the effects of colonization. For *H. azteca*, all 3 years were significantly different. Abundance of *H. azteca* was higher in the treatment wetland in 1996, higher in the reference site in 1998, but similar between the two sites in 1999. No significant effects were detected on the abundance of small cladocerans ($P = 0.0768$), Corixidae ($P = 0.0948$), *G. lacustris* ($P = 0.7170$), or cyclopoid copepods ($P = 0.2929$) (Figure 3), indicating that minnow colonization and removal did not affect the abundance of these invertebrates.

Estimates of the degree of change in the response variables in the treatment wetland corroborated the results of significance testing (Table 2). Variables for which we detected significant changes with ANOVA exhibited much greater change between time periods relative to variables with nonsignificant results. Additionally, changes observed in a number of variables in the treatment wetland were dramatic relative to changes in the reference wetland. The largest changes observed in invertebrate abundance in the treatment wetland were for large cladocerans and aquatic insects; large cladocerans were 206 times and aquatic insects 36 times more
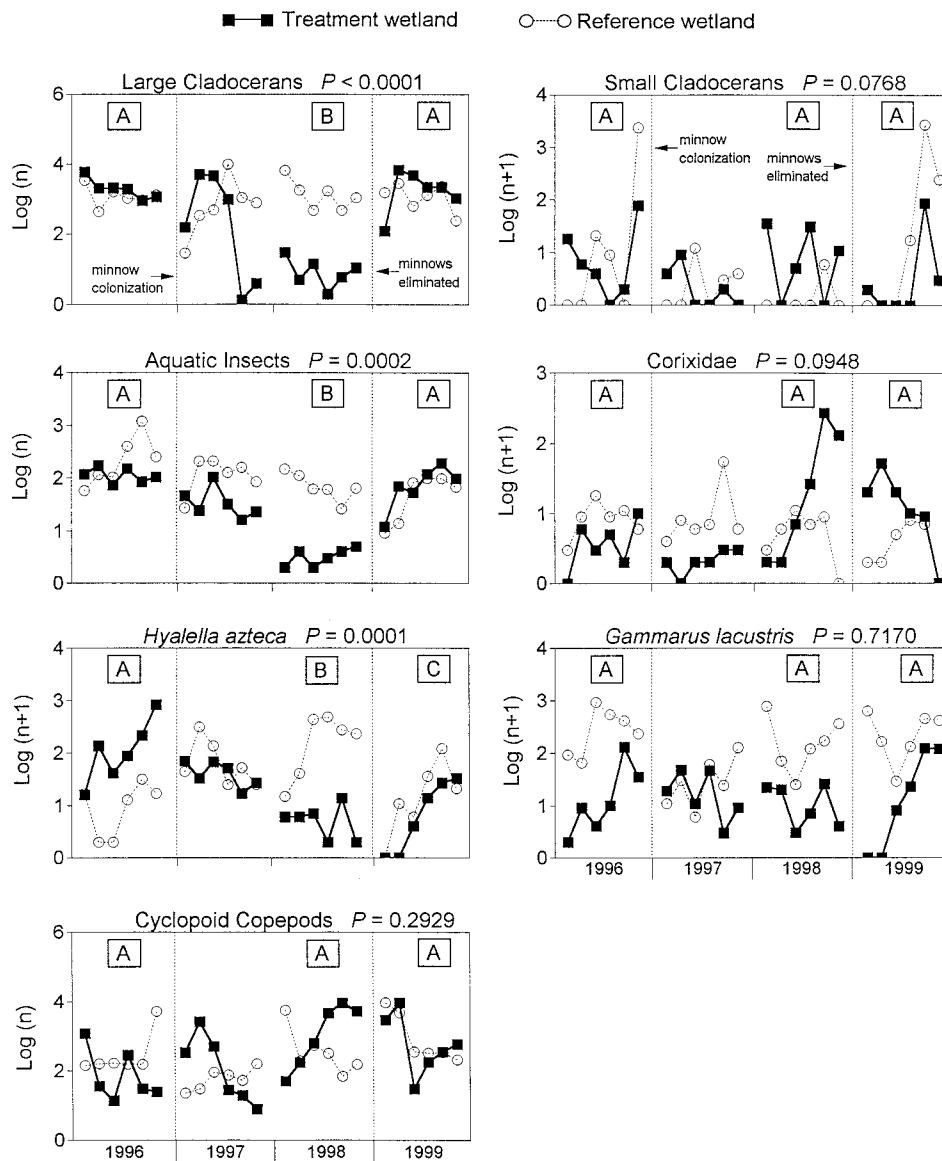
Figure 3. Abundance of large cladocerans, small cladocerans, aquatic insects, Corixidae, *Hyalella azteca, Gammarus lacustris*, and cyclopoid copepods in the treatment and reference wetlands on six sampling dates in 1996 through 1999. The dashed vertical lines indicate the times of minnow colonization and removal in the treatment site. The *P* values indicate results of the BACI ANOVA tests for significant differences among the deltas of 1996, 1998, and 1999; years with common letters are not significantly different.

abundant prior to minnow colonization compared to afterward. Similarly, large cladocerans were 177 times and aquatic insects 18 times more abundant after minnow removal than they were when fish were present. In contrast, the greatest change in the abundance of these taxa in the reference site was a three-fold decrease in aquatic insects between 1996 and 1998. The abundance of *H. azteca* in the treatment wetland dropped sharply following colonization, but the abundance was also highly variable in the reference site. Cyclopoid copepods were the only other group in the treatment wetland that fluctuated between 1996 and 1998, but the abundance of this taxon was highly variable. Overall, *H. azteca* and small cladocerans showed the greatest changes between time periods in the reference site

(Table 2). However, in both cases the relative abundance between years was highly variable.

Most water-quality variables with significant BACI results also changed substantially between time periods in the treatment site (Table 2). Turbidity was 18 times and chlorophyll *a* 17 times higher following minnow colonization. Turbidity was five times and chlorophyll *a* six times higher when the fish were present relative to the period following minnow removal. Changes observed in total phosphorus concentration were considerably less dramatic; it was 1.6 times higher after minnow colonization and 1.5 times higher prior to minnow removal relative to after. The BACI ANOVA did not indicate any significant changes in total nitrogen concentrations, but the changes in the treatment

Table 2. Multiplicative Change Observed (95% Confidence Interval) in the Treatment and Reference Wetlands between 1996 and 1998 and between 1998 and 1999

	Multiplicative Change Observed			
	Treatment Wetland		Reference Wetland	
Variable	1996–98	1998–99	1996–98	1998–99
Large cladocerans	– 206.1 (35.8, 1185.8)	+176.8 (33.1, 943.4)[a]	+ 1.1 (0.5, 2.4)	– 1.2 (0.4, 3.1)
Aquatic insects	– 35.5 (19.8, 63.7)[a]	+ 17.8 (5.9, 53.6)[a]	– 2.5 (0.3, 12.3)[a]	– 2.2 (0.2, 19.0)[a]
Hyalella azteca	– 20.9 (6.6, 66.1)[a]	+ 1.7 (0.1, 19.5)[a]	+16.6 (1.0, 138.0)[a]	– 7.8 (0.7, 89.1)[a]
Corixidae	+ 4.9 (0.4, 56.4)[a]	– 4.1 (0.2, 935.5)[a]	– 1.6 (1.0, 2.6)[a]	– 1.5 (0.9, 2.4)[a]
Cyclopoid copepods	+ 8.2 (0.02, 328.1)[a]	– 1.2 (0.01, 237.1)[a]	+ 1.2 (0.02, 99.2)[a]	+2.4 (0.8, 7.0)
Gammarus lacustris	1.0 (0.01, 241.0)	+ 2.2 (0.02, 257.0)[a]	– 1.5 (0.2, 12.9)[a]	+1.4 (1.0, 2.0)
Small cladocerans	1.0 (0.2, 5.1)	– 2.2 (0.2, 22.4)	– 6.5 (0.4, 82.1)	+13.0 (0.1, 554.7)[a]
Turbidity	+ 17.7 (9.4, 33.5)[a]	– 4.7 (1.4, 15.9)[a]	– 1.1 (0.7, 1.8)	+1.3 (1.0, 1.9)
Chlorophyll *a*	+ 17.1 (2.4, 119.4)[a]	– 6.1 (1.1, 33.8)[a]	+ 1.4 (0.7, 2.7)	– 1.1 (0.7, 1.6)
Total phosphorus	+ 1.6 (1.2, 2.2)	– 1.5 (0.8, 2.8)[a]	– 1.1 (0.8, 1.3)	+1.1 (0.7, 1.7)
Total nitrogen	+ 2.4 (1.0, 5.3)[a]	– 2.0 (1.0, 3.3)[a]	+ 1.3 (1.2, 1.5)	– 1.2 (1.0, 1.4)[a]

+, indicates variable increased between time periods; –, variable decreased between time periods. Change observed is the ratio of geometric means in each wetland between time periods, with 1996–98 representing the response to minnow colonization and 1998–99 the response to minnow removal in the treatment wetland.
Underlined change indicates variables that changed significantly between time periods as determined by the BACI ANOVA.
[a]*variables with significant-first-order-autocorrelation (P <0.50) where estimates were adjusted with maximum likelihood methods; all others are least-squares estimates.*

wetland were actually larger than those observed for total phosphorus. As with aquatic invertebrates, the water-quality variables changed far less between time periods in the reference site relative to the treatment site.

DISCUSSION

Our results indicate that both minnow colonization and removal resulted in changes in the invertebrate community and water-quality characteristics of this prairie wetland. We observed significant changes in the abundance of large-bodied cladocerans, aquatic insects, and *H. azteca*, as well as in turbidity and concentrations of total phosphorus and chlorophyll *a* in the water column. Additionally, the changes we observed in response to both events were much larger than those in the reference site. However, because of the lack of replication, the BACI approach only indicates whether a nonrandom change has occurred. Additional evidence must be used to support the claim that the minnow population caused the changes (Stewart-Oaten and others 1986; Carpenter 1990). Several lines of additional evidence support this hypothesis.

First, for all variables for which the ANOVA results were significant, changes were detected in response to both minnow colonization and minnow removal. The likelihood of observing significant but opposite changes in association with the two events is low if these changes were due to natural variability or other influences. A second line of supporting evidence is the consistency of these results with those we obtained in our larger, replicated study comparing wetlands with fathead minnows to fishless sites (Zimmer and others 2000; K. D. Zimmer and others unpublished). The larger study showed that wetlands with fathead minnows are characterized by lower abundances of large and small-bodied cladocerans, *H. azteca*, and aquatic insects, as well as higher turbidity, chlorophyll *a*, and total phosphorus concentrations relative to fishless sites. Furthermore, no differences in abundance of cyclopoid copepods, *G. lacustris*, or concentrations of total nitrogen are associated with fish in the replicated study. Thus, with the exception of small-bodied cladocerans, the findings from our larger study conform to the results observed here. Additionally, the direction of change associated with both minnow colonization and removal was consistent with the results from the larger study. For example, the larger study showed that turbidity was significantly higher in wetlands with minnows; colonization resulted in a significant increase in turbidity in the treatment site, whereas removal resulted in a significant decrease. Finally, our results are consistent with the predictions of Carpenter and others (1985) and similar to the effects of changes in minnow

abundance observed by Spencer and King (1984) and Carpenter and Kitchell (1988).

These lines of evidence strongly support the hypothesis that the changes we detected were due to the fish population. Although replication is obviously desirable in any study, it is often impossible in unplanned ecosystem-scale studies such as this (Carpenter 1990). When properly analyzed, unreplicated ecosystem studies are powerful approaches that can provide insight not apparent in smaller-scale, replicated studies (Carpenter 1989, 1990; Schindler 1998).

H. azteca was the only variable with equivocal results. The BACI ANOVA indicated significant differences among all 3 years, suggesting a significant response to both minnow colonization and removal. However, the abundance of this taxon was also highly variable in the reference wetland. In particular, it appears the significant difference between 1998 and 1999 was driven by reduced abundance in the reference site in 1999, not by changes in the treatment site. Thus, the effect of minnow colonization and removal on the abundance of this taxon, relative to natural variability in its abundance, is unclear.

The degree to which minnow colonization and removal influences wetland ecosystems is likely to vary. The abundance of aquatic macrophytes in the treatment wetland was low relative to other fishless wetlands (K. D. Zimmer and others unpublished), and it is possible that aquatic macrophytes may buffer some of the effects of minnow colonization in other sites. Aquatic macrophytes provide aquatic invertebrates with a refuge from fish predation (Crowder and Cooper 1982; Schriver and others 1995; Perrow and others 1999), and a scarcity of macrophytes at our treatment site may exacerbate minnow effects. Additionally, the water-quality characteristics of shallow aquatic ecosystems are strongly influenced by aquatic macrophytes, with high plant abundance facilitating high water clarity, low phytoplankton abundance, and low concentrations of nutrients in the water column (reviewed by Scheffer 1998). Thus, changes in the water-quality characteristics in our treatment site may be more pronounced than changes observed in other colonized wetlands with better-developed plant communities. In general, there is a negative relationship between abundance of aquatic macrophytes and depth in prairie wetlands (Zimmer and others 2000); thus, the effects of minnow colonization seem likely to be most pronounced in deeper prairie wetlands. Increased turbidity associated with minnows may also cause a reduction in macrophyte abundance (Spencer and King 1984), and this effect

is also likely to be most pronounced in deeper basins.

The response of a wetland to minnow removal is also likely to vary both in terms of magnitude of change and speed of response. Fathead minnows were only present in our treatment site for 2 years, and this short time period may have facilitated the rapid recovery of the impacted invertebrates once the minnow population was removed. However, species in our taxonomic group "large cladocerans" are capable of explosive population growth from initially low densities (Carpenter and others 1987). Large-bodied cladocerans such as *Daphnia* are thought to be the most effective grazers on phytoplankton (reviewed by Sterner 1989), and their rapid population growth may facilitate equally rapid changes in phytoplankton abundance and turbidity in response to minnow removal.

Differences between the treatment site and the reference site during 1998 (when minnows were present in the treatment site) were greater than the differences observed between wetlands with and without minnow populations in our larger study (Zimmer and others unpublished). For example, in 1998, turbidity was 13 times higher in the treatment site relative to the reference site, while turbidity was approximately four times higher in wetlands with minnows than in the fishless sites in our larger study. This indicates that the short-term effects of minnow colonization can be dramatic, but over time the ecosystem may display resilience that mutes differences between sites with and without minnows. This resilience may arise from compensatory changes in the food web (Frost and others 1995), such as an increased abundance of small-bodied zooplankton compensating for the decreased abundance of large-bodied zooplankton.

Changes in turbidity in the treatment site were largely driven by changes in the abundance of phytoplankton (chlorophyll *a*). A regression of turbidity on chlorophyll *a* in the treatment site on the 18 sampling dates revealed a strong relationship (adjusted $R^2 = 0.78$), but the relative importance of mechanisms driving the changes in phytoplankton abundance is less clear. Changes in total phosphorus concentrations mirrored changes in phytoplankton abundance, whereas phytoplankton and large cladocerans exhibited opposing patterns of change. Thus, changes in phytoplankton abundance could be due to reduced grazing pressure from cladocerans (top-down effects), changes in the availability of phosphorus (bottom-up effects), or some combination of both (Vanni and Layne 1997; Vanni and others 1997). The bottom-up mechanism could involve higher availability of phospho-

rus due to excretion by the minnows, resulting in higher concentrations of phytoplankton (McQueen and others 1992; Schindler 1992; Schindler and others 1993). Alternatively, higher phosphorus concentrations could simply be due to indirect, top-down effects, where low herbivory results in higher phytoplankton abundance, resulting in increased concentrations of phosphorus in the water column (Vanni and others 1997). Relatively little work has been done on these questions in prairie wetlands, and the relative importance of top-down and bottom-up influences on phytoplankton abundance has not been established.

Fathead minnows probably have great potential to influence phosphorus cycling in wetlands due to their life-history characteristics and feeding habits. Fathead minnows are fractional spawners, resulting in high fecundity and production of young-of-the-year throughout the summer (Peterka 1989). High recruitment and growth rates, coupled with the absence of piscivorous fish in most wetlands, can lead to dense populations and high production rates in fathead minnow populations. For example, in a study of fathead minnow populations in four South Dakota wetlands, Duffy (1998) estimated that adult standing-stock biomass in June ranged from 144 to 482 kg ha^{-1} and exhibited daily net-production rates of 3–14 kg ha^{-1} d^{-1}. Fathead minnows are also short-lived; their natural mortality rates are high (Duffy 1998), and most adults die after spawning in their 2nd year (Peterka 1989). High production rates, coupled with high mortality rates, result in high consumption of phosphorus from various pools and rapid turnover of this nutrient through the minnow population. Additionally, fathead minnows are omnivorous (Litvak and Hansell 1990), and benthic feeding and subsequent translocation of phosphorus to the water column may enhance phytoplankton growth (Schaus and Vanni 2000). However, we still do not understand the effects of fathead minnows on phosphorus cycling or its relative importance in structuring algal communities in prairie wetlands.

Most lakes do not alternate naturally between supporting planktivorous fish populations and being fishless over short time scales, but the presence or absence of fish populations in the wetlands of the PPR is highly variable (Kantrud and others 1989; Peterka 1989), making our results applicable to a large number of ecosystems. However, we assessed the effects of complete removal of a minnow population—an event that is likely to occur less often than extensive but incomplete kills. It is unknown whether strong kills will result in changes similar to those observed here, or if complete kills are needed to induce such dramatic changes. Although our results indicate that the presence or absence of fathead minnows has considerable influence on the ecological characteristics of prairie wetlands, several important questions remain: (a) How variable is the response to minnow colonization and removal? (b) Does the abundance of aquatic macrophytes buffer the effects of colonization, and does the length of time minnows were present influence the recovery time? (c) Will the effects of minnow colonization dampen through time—that is, will the ecosystem show any degree of resilience?

The variability in the presence of minnows in wetlands may also influence other wetland-dependent species, including birds, amphibians, and hydrophytes (reviewed by Bouffard and Hanson 1997). Variability in the physical and chemical characteristics of prairie wetlands, as well as variability in climate and the surrounding landscape, are all important for maintaining regional biotic diversity in these ecosystems (Kantrud and others 1989; Euliss and other 1999). Similarly, variability in the presence of minnows in prairie wetlands may result in different assemblages of wetland biota, with fishless basins favoring certain assemblages of organisms and basins with minnows favoring others. As with other biotic and abiotic variables, interbasin and interannual variability in the presence of minnows may be important for maintaining diverse assemblages of species at the landscape level. In turn, the presence or absence of minnows is itself influenced by variability in a number of biotic and abiotic factors. These interactions result in dynamic ecosystems, where biota respond to both spatial and temporal variability in biotic and biotic influences.

Anthropogenic disturbances of the watersheds of wetlands in the PPR and the basins themselves also affect the ecological characteristics of these ecosystems (Kantrud and others 1989; Euliss and others 1999). Landscape alterations, such as the digging of ditches, road construction, and the installation of culverts may alter the number of wetlands supporting minnow populations (Zimmer and others 2000). This study and several others have shown that minnows can influence the ecological characteristics of prairie wetlands (Hanson and Riggs 1995; Cox and others 1998; Duffy 1998; Zimmer and others, forthcoming). Thus, landscape manipulations that alter the proportion of basins supporting fathead minnow populations may also influence the ecological characteristics of prairie wetlands at the regional scale.

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