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BIOMANIPULATION OF SHALLOW WETLANDS IN SOUTHERN MINNESOTA USING ROTENONE RECLAMATION AND WALLEYE FRY STOCKING: THE CONTROL OF UNDESIRABLE FISHES AND INFLUENCES ON POND ECOLOGY

by

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EXECUTIVE SUMMARY

Increasing demand for Walleye *Sander vitreus* fingerling production in Minnesota has resulted in the expansion of propagation efforts to natural shallow wetland basins (ponds) in southern Minnesota. However, the presence of non-target fishes often has resulted in reduced Walleye production. Biomanipulations have been used by the Minnesota Department of Natural Resources to mimic the effects of winterkill and enhance conditions for Walleye production with varied success. From 2002 through 2007 we studied biomanipulation using late-fall treatments with the piscicide rotenone followed by Walleye fry stocking in shallow natural ponds in southwestern Minnesota. The objectives of this study were to assess the effectiveness of rotenone treatments on improving Walleye production as well as to assess the impacts of a rotenone treatment/Walleye stocking combination on the general ecological characteristics of shallow ponds.

Based on trap-net sampling during spring following reclamation, complete fish kills occurred in only four (57%) of the seven treated ponds. These ponds remained fishless the entire first year following treatment. However, fish were encountered in all of the treated ponds by the third summer following reclamation, which indicated further treatments would be necessary to maintain 'clean' Walleye rearing ponds. Even though fish were observed in treated ponds by the third year after reclamation, the biomass of fishes was roughly 5% of that prior to treatment. The first year following reclamation provided the best Walleye production with a mean yield of 11.6 lbs/acre. Subsequently, Walleye production declined, with yields of 8.3 and 1.3 lbs/acre during the second and third year post reclamation, respectively.

Pond chemical and physical properties were relatively unchanged during this study in treatment ponds following reclamation; however, increases in water clarity and reductions in suspended solids were observed. The improved water clarity may have directly influenced aquatic macrophytes. A total of 7 of the 43 aquatic macrophyte species increased in abundance during this study in treated ponds. Submergent macrophyte species including bushy pondweed, coontail, flatstem pondweed, and sago pondweed demonstrated the largest change following reclamation. Star duckweed *Lemna trisulca* may have benefited from fish removal because it was entirely absent from transects prior to reclamation but was observed in 11% of transects by the third year after reclamation. Star duckweed has been shown to support a high ratio of aquatic organisms to plant weight and likely provided benefit to non-fish wildlife.

Zooplankton abundance crashed immediately following reclamations in treated ponds. A complete recovery of zooplankton populations was evident during the following spring, however. Zooplankton abundance in treatment ponds was not different from control ponds during the remainder of the study indicating no lasting negative effects of late-fall treatments with rotenone. Conversely, macroinvertebrate richness and abundance generally increased following reclamation in treated ponds. Although statistically insignificant, a consistently higher abundance of macroinvertebrates in treatment ponds relative to control ponds during post-reclamation time periods indicated that reclamations had positive influences on macroinvertebrates during this study.

This study demonstrated that rotenone treatments in shallow ponds in southern Minnesota obtained the primary objective of reducing undesirable fish abundance, enabled improved Walleye production, and provided ecological changes that likely benefited wildlife.

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INTRODUCTION

Many natural wetland basins (hereafter referred to as ponds) in southern Minnesota contain persistent populations of undesirable fish species such as Black Bullhead *Ameiurus melas*, Common Carp *Cyprinus carpio*, and Fathead Minnow *Pimephales promelas*. The proliferation of undesirable fish species in southern Minnesota has been attributed to artificially elevated water levels in ponds, which have been strongly influenced by agriculturally related drainage systems including buried drain tile and open drainage ditches (Blann et al. 2009). As a result, natural extirpation events (e.g., winterkill or desiccation), which had historically been relied upon by fish managers to occasionally eliminate undesirable fishes from ponds have become less frequent. In addition, the high interconnectivity among ponds has provided an avenue for migration and rapid recolonization among ponds (Norris 2007). Due to increasing social-political demand for Walleye fingerling production the Minnesota Department of Natural Resources (MNDNR) expanded the use ponds in southern Minnesota for extensive culture of Walleye *Sander vitreus*.

Given the proliferation of fishes in southern Minnesota ponds, and because Walleye culture is generally best in ponds where existing fish populations are limited (Bandow 1989; Smith and Moyle 1945; Ward et al. 2007), biomanipulation with the piscicide rotenone was conducted in an attempt to mimic natural extirpation. The use of rotenone biomanipulation in Minnesota dates back to the 1950s (Anderson 1970). Although rotenone biomanipulation has not always resulted in complete eradication of fishes it has significantly reduced fish abundance in most cases. Although not typically the primary objective, many rotenone reclamations have also resulted in a noticeable increase in water clarity and expanded submerged macrophytes. In fact, macrophyte expansion has even been documented in lakes and ponds that demonstrated an historic absence of aquatic plants (Anderson 1970). The observed benefits of Walleye production methods using rotenone biomanipulation to the improved condition of ponds has coincided with a growing pool of evidence suggesting that fish have an important influence on the ecology of ponds, mainly on the status of trophic state (Scheffer et al. 1993; Bouffard and Hanson 1997; Zimmer et al. 2000, 2001, 2003; Hanson et al. 2005). Specifically, it has been reported that planktivorous fish (e.g., Fathead Minnows) and benthivorus fish (e.g., Common Carp and Black Bullhead) may play a role in shifting ponds from a clear-water trophic state to a turbid-water trophic state (Hanson and Butler 1994; Zimmer et al. 2000, 2001, 2003; Parkos et al. 2003).

The shift in trophic state of ponds has been linked to the destruction and suppression of aquatic macrophytes, which can quickly induce the shift in trophic state and also diminish the buffering capacity toward the maintenance of a clear-water trophic state (Blindow 1992). Although aquatic macrophytes are not generally inhibited by light penetration due to the shallow nature of ponds in southern Minnesota, the prominence of a turbid trophic state in many ponds, due in part to the imposition of fishes, results in reduced abundance of aquatic macrophytes. For example, benthivorus fish tend to directly destroy aquatic macrophytes and, in doing so, allows the transfer of nutrients from the bottom sediments into the water column through disruptive substrate activities and/or excretion (Lamarra 1974; Parkos et al. 2003; Zimmer et al. 2003, 2006). The transference of nutrients within a pond by benthivorus fish can also be accomplished indirectly through the removal of wave-buffering macrophytes, which tend to limit natural re-suspension by stabilizing the bottom sediments (Moss 1990; Blindow 1992). Ultimately, the excretion and suspension of nutrients, particularly phosphorus, mitigates algal blooms (Zimmer et al. 2006). Further complicating water clarity in ponds are planktivorus fish, which have been shown to selectively feed on zooplankton grazers resulting in increased phytoplankton population abundance enabled through the lack of top-down grazing (Sarnelle 1992; Hanson and Butler 1994; Parkos et al. 2003; Zimmer et al. 2000, 2001, 2003, 2006). Regardless of the mechanisms, the destruction and suppression of aquatic macrophytes directly or indirectly by fishes often results in a multi-faceted cascading change in pond ecosystems culminating in a turbid, algae-dominated trophic state which imposes constraints on pond suitability. The notable improvements to water clarity following rotenone reclamations as part of Walleye production methods provided evidence that changes in the ecological conditions of ponds through the removal of fishes favored wildlife, thus serving a dual purpose.

Generally, where wildlife management has been the principal objective, biomanipulation using rotenone often has pursued entirely fish-free ponds. However, biomanipulations with the stocking of predators to induce top-down control on undesirable fishes has also demonstrated similar habitat improvements. Herwig et al. (2004) reported that stocking of Walleye fry was found to temporarily control Fathead Minnows. Potthoff et al. (2008) also reported that the stocking of Walleye fry into ponds quickly induced major changes in food webs and other ecological characteristics of ponds, which included increased abundance of large-bodied zooplankton, increased abundance of some macroinvertebrate species, decreased phytoplankton, and expanded macrophyte coverage. Although relying on a single method to induce biomanipulation has been successful in some cases, the common proliferation of undesirable fishes in southern Minnesota has presented a problem. Specifically, without top-down control of such fishes (e.g., Walleye foraging on Fathead Minnow) the conditions of ponds often revert following the initial biomanipulation as fish re-populate. For example, removal of Fathead Minnow resulted in improved water clarity in Prairie Pothole Region ponds, but ponds often reverted to pre-biomanipulation conditions after fishes returned (Zimmer et al 2001). Similarly, Herwig et al. (2004) found that repeated stocking with Walleye fry was necessary to maintain a clear water state. Hansel-Welch et al. (2003) also reported that the aquatic plant community improved following removal of fishes, but the macrophyte composition in ponds changed over time during the subsequent decade. A review of fish control projects by Meronek et al. (1996) reported that a combination of physical (rotenone reclamation) and biological (fish stocking) measures to control undesirable fishes could improve overall success.

Ultimately, ponds used by both fish managers for Walleye production and wildlife managers for wildlife habitat are often most suitable following natural extirpation events such as desiccation or winterkill that eradicates all or most fishes, or the use of biomanipulation which mimics those effects. To improve Walleye production potential, the MNDNR used rotenone biomanipulation to eradicate existing undesirable fish populations followed by Walleye fry stocking. This study examines the combined biomanipulation technique was investigated to determine the benefit to Walleye production and to assess the influence of treatments on pond ecology.

STUDY DESIGN AND DATA ANALYSIS

This study evaluated the use of rotenone in southwestern Minnesota ponds. Multiple treatments were used during this study and included combined effects of rotenone reclamation and three consecutive years of Walleye fry stocking. Primarily, this study was designed to determine if the rotenone treatment and fry stocking combination aided in controlling undesirable fish presence in ponds. This study also investigated the effects of rotenone and Walleye fry stocking on Walleye rearing ponds in a broader ecological perspective to determine the usefulness of the methodology in improving wildlife habitat. Therefore, this study investigated the responses of species composition and abundance of aquatic fauna, including fish, zooplankton, phytoplankton, macroinvertebrates, and macrophytes. Our goal was to first assess whether reclamation with rotenone accompanied with Walleye fry stocking was successful in eradicating and suppressing undesirable fish populations, thus facilitating 1) improved Walleye fingerling production, 2) increased aquatic vegetation occurrence, 3) reduced phytoplankton biomass measured as chlorophyll-a, 4) improved water transparency, 5) increases in abundance of large bodied daphnia *Daphnia* spp., and 6) increased macroinvertebrate abundance. The null hypothesis tested during this study was of that there was no significant difference between control and treatment ponds in various response variables. A rejection of the null hypothesis indicated that reclamation of ponds with rotenone combined with fry stocking of Walleye did have an impact on response variables that contributed to improved wildlife habitat or Walleye production in ponds.

A total of 16 ponds that had similar physical and biological parameters were selected for inclusion in this study (Table 1). Spatially, the study ponds were chosen from the Western Corn Belt Plains and Northern Glaciated Plains ecoregions of southern and southwestern Minnesota. Ponds in these regions were characterized as having relatively shallow depth (≤ 9 feet), many had been impacted by sedimentation, and most had been influenced by agricultural drainage. Landscape use in watersheds of all study ponds was agriculture in nature and included a mixture of tilled fields and grazed pasturelands. Temporally, field sampling was divided into two overlapping phases during which eight ponds were sampled during each phase. Field sampling of ponds during the initial phase of this study began in 2002 with subsequent sampling in 2003, 2004 and 2005. The second phase of the study began in fall of 2004 with field sampling conducted in 2005, 2006, and 2007. Armstrong Lake (DOW 07-0125-00), which was a scheduled treatment pond in the second phase, was removed from this study because high water levels and excessive runoff prevented reclamation during fall 2004. Thus, this study encompassed a total of 15 ponds including seven treatment ponds and eight control ponds.

Field sampling comprised collections during the fall prior to reclamations and then during summer or fall (depending on the target species) for three consecutive years following reclamations (Table 2). There were three additions to the general sampling schedule: 1) fish were sampled during the spring (May) following reclamation to determine the success of fish eradication, and 2) zooplankton were sampled two to three days post-reclamation to assess the magnitude of zooplankton population die-off due to rotenone treatment, and 3) zooplankton were sampled during May of the following spring to assess recovery during a time when Walleye fry would be stocked into ponds (Table 2).

Treatment ponds were reclaimed during the fall (i.e., October or November) using synergized powdered rotenone (*Prentox Prenfish*, Prentiss Incorporated, Floral Park, New York) administered at a target formulation concentration of 4-mg/L (0.2 mg/L actual rotenone). Rotenone was administered to ponds using a boat and a gas-powered water pump connected to a Venturi pump. Water was pumped from the intake at the back of the boat, through the Venturi pump connected to a suction tube used to draw the rotenone powder from barrels, and then the rotenone slurry was pumped out a hose to a nozzle mounted at the bow of the boat. Thus, the boat was utilized as the initial mixing device during treatment. Natural wind and wave action was relied upon for additional mixing within reclaimed ponds. Application of rotenone was completed in a zigzag pattern within each basin until the rotenone supply had been depleted.

Walleye were stocked into treatment ponds for three consecutive springs (May) following reclamation at a rate of 5,000 fry/acre. Walleye stocking rate and removal methods used during this study were similar to that used by MNDNR Fisheries to rear Walleyes for the annual stocking program. Harvest of Walleye was completed using overnight trap net sets randomly placed adjacent to shoreline in each pond. Control, or reference ponds, were not treated with rotenone and were not stocked with Walleye in order to preserve the natural pond characteristics that enabled comparison with treatment ponds to assess treatment effects.

Fishes were sampled using trap nets during this study. The fish assemblages in each pond were evaluated by an index of species richness, as well as fish relative abundance and biomass. Six 0.25-in mesh trap nets were set randomly within each study pond overnight. Fishes sampled in the first four randomly selected "muskrat-free" trap nets were counted (six trap nets were set to enhance the odds that four would have been "muskrat-free" during the set). It was important to utilize catch from only muskrat-free nets because often muskrats chew holes in the nets, which can allow fish to escape. Captured fishes and were identified to species and enumerated. In addition, the total catch of each fish species in each net was weighed (g ± 1.0 g) and enumerated (n) to estimate biomass. All sampled fish were immediately returned to the water. The initial samples were collected in the fall (September) prior to the reclamation period (Table 2). Follow up samples were collected in early September during each subsequent year of the study. For purposes of assessing immediate effects of rotenone on fish presence, we conducted additional May sampling during the first year following treatment to assess the success of rotenone treatments on eradication of all fishes.

Thus, the immediate effects were measured by comparison of data collected in September before treatment to the collections in May following treatment. Long term comparisons in ponds were made using September sampling data from pre-treatment and each of three years post-treatment.

Zooplankton samples were collected with a 7.1 L tube sampler filled three times and then filtered through a 63-μm mesh zooplankton net and combined into a composite sample (Dettmers and Stein 1996). A total of five zooplankton samples were taken from different locations in each pond during each sample period. Samples from within each pond were then combined, as they were subsequently subsampled (5 mL) during analysis. Samples were collected two to three days prior to rotenone reclamation and then again two to three days afterward on each pond (Table 2). The following spring, on approximately May 1, samples were again collected to assess recovery. Additional samples were collected in mid-June of each year thereafter to help evaluate the presence of large zooplanktors for both Walleye and wildlife production. Zooplankton samples were preserved in alcohol, and later identified and counted in the laboratory by specialized staff from the division of Ecological Resources.

Water samples were collected using subsurface grabs in September prior to treatment (treatment ponds only) and again in June and September during three post treatment years (all ponds; Table 2). The samples were placed on ice and taken to the Minnesota Department of Agriculture (MDA) laboratory in St. Paul, where they were analyzed for the following parameters: total suspended solids (TSS), total phosphorus (PO_4), total Kjeldahl Nitrogen (TKN), nitrite (NO2) and nitrate (NO3), ammonia (NH_3), and chlorophyll-a (Chl-*a*). In addition, water temperature and secchi depth were recorded each time water samples were collected.

The relative changes in late summer macrophyte species composition and relative density, as measured by frequency of occurrence in transects, before and after the rotenone applications between the treatment and control ponds was assessed. Changes in macrophyte richness and occurrence were conducted separately for emergent, floating and submergent vegetative types. In addition, the Chl-*a* abundance was used to determine effects of macrophytes on phytoplankton production. Filamentous algae and *Chara* were also included in the analysis of macrophytes and a total of 43 taxa were assessed. Macrophyte communities were evaluated in each pond during each year of the study. Baseline reference data was collected during early summer prior to rotenone application and were re-evaluated during July of each subsequent study year (Table 2). In each pond, eight 5-m wide by 50-m long transects extending perpendicular from the shoreline were established. The locations of transects were recorded with a GPS to maintain sampling at the same location among years. Transects were sampled using a combination of surface observations and plant hook collections intended to document macrophyte species present, and frequency of occurrence. Plant hook collections were repeated until all species were identified and no new species were observed in three consecutive deployments.

Aquatic macroinvertebrates were sampled using activity traps (Murkin et al. 1983; Hanson et al. 2005). A total of eight activity traps were deployed in pairs in representative areas of the shallow near shore emergent vegetation zone, with sampling stations located between the pond edge and water depth of one meter. The bottles were filled to exclude air and placed approximately 10-cm under the surface. Pelagic species of macroinvertebrates were sampled with four vertically-oriented activity traps set for 24 hours in each study pond in August of each year, before and following, the reclamations.

Crawling and clinging macroinvertebrates were also collected in all study ponds during August with four horizontally oriented activity traps deployed for 24 hours in or near stands of vegetation. Traps were set twice during August of each study year (i.e., n = 16 per year [n = 32 in 2005]). All macroinvertebrate samples were passed through a 63 micron sieve and preserved in alcohol, and later identified and counted in the laboratory.

Table 1. Pond Type (study pond information including treatment), Administrative Area, DOW (division of water identification number), Acres (surface area), County, Years (in study), Latitude, and Longitude. Acreages are those listed by the Waters Section (1968) unless noted.

Pond Type	Administrative Area		Pond Location				Years
Pond	DOW	Acres	Area	County	Latitude	Longitude	Initial-Final
Control							
Boot	32-0015-00	89	Windom	Jackson	43.68390	-95.08258	2004-2007
Bohemian	41-0109-00	111	Ortonville	Lincoln	44.62822	-96.39459	2002-2005
Butterfield	83-0056-00	52	Windom	Watonwan	43.96032	-94.81029	2002-2005
Clam	46-0111-00	72	Windom	Martin	43.73054	-94.67474	2002-2005
Clear-Dundee	17-0041-00	222	Windom	Cottonwood	43.85187	-95.41042	2004-2007
County 13	17-0048-01	71	Windom	Cottonwood	43.95109	-95.37126	2002-2005
Oak Leaf	52-0010-00	181	Waterville	Nicollet	44.30769	-94.01545	2004-2007
South Wilson	51-0081-00	164	Windom	Murray	43.99161	-95.94045	2002-2005
Treatment							
Armstrong [1]	07-0125-00	125	Waterville	Blue Earth	44.15315	-94.34487	2004-2007
Clear	17-0008-00	76	Windom	Cottonwood	43.90051	-95.07700	2004-2007
Kinbrae	53-0018-00	38	Windom	Cottonwood	43.81923	-95.48633	2002-2005
Little Twin	46-0130-00	68	Windom	Martin	43.74256	-94.74008	2002-2005
Lower Case	83-0012-00	13	Windom	Watonwan	43.99585	-94.38505	2002-2005
Oak	41-0062-00	107	Ortonville	Lincoln	44.53671	-96.24171	2004-2007
Toners	81-0058-00	127	Waterville	Waseca	44.16520	-93.59959	2004-2007
Upper Case	83-0010-00	43	Windom	Watonwan	44.00020	-94.38750	2002-2005

[1] Removed from study due to dissimilar treatment.

Table 2. Sampling schedule for data collection from 2002 through 2007. Treatment with rotenone was conducted during October of 2002 or 2004**.**

				Long term		
Parameter	Pre-treatment	Post-Treatment	Short-term	Year 1	Year 2	Year 3
Fish	August	---	May	September	September	September
Zooplankton	2-3 days pre treat	2-3 days post treat	May	June	June	June
Phytoplankton	September	---	---	June and September	June and September	June and September
Secchi/Water Quality	September	---	---	June and September	June and September	June and September
Aquatic Vegetation	August	---	---	July	July	July
Macroinvertebrates	August	---	---	August	August	August

Statistical analysis

All parameters were analyzed with a Kruskal-Wallis test for unbalanced data sets to determine if significant differences existed before and after the rotenone treatments and/or between the control and treatment ponds. The Kruskal-Wallis test is the non-parametric equivalent to an analysis of variance (ANOVA) and determines differences in median values among sample periods (i.e., at least one periods had a different median value), and is robust to non-normality and outliers in the data. When Kruskal-Wallis tests indicated significant changes among sample periods, Wilcoxon paired comparisons were then used to determine when changes occurred and to identify differences between treatment groups.

In addition, a multivariate approach to analysis of community data was conducted using the software CANOCO (Ter Braak and Smilauer 2002). This approach was very similar to that reported by Melaas et al. (2001). In this analysis, a matched-pairs design tested for effects on the aquatic community taxa with data from the pre-treatment (before) period in each pond paired with data from the post-treatment (after) period. For short term effects, data collected in each pond prior to reclamations were paired with data collected at the earliest sampling (immediately following reclamation or during the following spring) following reclamation and the differences between sampling dates (before-after) was determined for each taxon in each pond. This approach resulted in seven replicates for treatment ponds and eight replicates for control ponds, with the number of response variables equal to the number of specific taxa of each taxa type (e.g., fishes, zooplankton, invertebrates, etc.). For long term effects, data collected prior to reclamation (before) in each pond was paired with data collected on the same general dates during each of the three years following reclamation (after), with the differences again determined for each date (before-after). This resulted in three long term sampling dates and each long term sampling date was then analyzed separately for significant change among years.

Our goal was to determine whether there was a significant effect at the community level, and to then identify specific taxa most affected by rotenone and Walleye fry stocking if a significant community-level effect was detected. We tested for significant effects at the community level using direct-gradient analysis (Ter Braak and Verdonschot 1995; Van Wijngaarden et al. 1995). Preliminary ordinations with detrended correspondence analysis showed lengths of axes in all data sets were less than 1.5 standard deviations, then we used linear model of direct-gradient analysis (redundancy analysis, RDA) instead of the unimodal model (canonical correspondence analysis). RDA has been used in several studies assessing the effects of chemical applications and environmental change on aquatic communities (Ter Braak and Wiertz 1994; Verdonschot and Ter Braak 1994; Van Wijngaarden et al. 1995; Legendre and Anderson 1999; Melaas et al. 2001). This technique is similar to MANOVA but does not restrict the number of response variables. Also, because significance is tested with Monte Carlo permutations, RDA does not require the assumption of multivariate normality (Manly 1991). RDA integrates ordination and multivariate regression, such that species are analyzed simultaneously and modeled as a function of axes that are linear combinations of environmental variables (Ter Braak 1994). In this study, there was only one qualitative environmental variable (rotenone/fry stocking or treatment group), so axis 1 is the only canonical axis. To test for a significant effect of rotenone, the variance in all taxa explained by axis 1 is determined, and the explained variance is then divided by the residual variance to produce a partial F-ratio (Ter Braak and Smilauer 2002). Significance of the observed F-ratio was determined by randomly reassigning ponds to either treatment group and determining the F-ratio of each randomization (Verdonschot and Ter Braak 1994). Numerous randomizations (n = 499) were performed, and the proportion of randomly generated F-ratios that meet or exceed the observed F-ratio represents the P value.

Species centered RDA was utilized and all ordination diagrams were reported in distance scaling with site scores as linear combinations of environmental variables to fully display effect sizes of the rotenone treatment. The use of species-centered RDA and differences of log values between the before and after sampling dates prevents abundant or rare taxa from dominating the results. As RDA was performed on the differences of log values between sampling dates (before- after), the analyses were conducted on the change in each species, not on their actual abundance. Thus, species vectors pointed in the direction of greatest decrease (or increase) in abundance over the before to after time period.

To identify rotenone effects on specific taxa, we estimated the average change in abundance between sampling dates for taxa with greater than 20% of the variance fit by the first RDA axis. For this analysis, the average difference of log values between sampling dates (before-after) and 95% confidence intervals were determined for each taxon in both the treatment and control ponds. These means and confidence intervals were then back-transformed to estimate multiplicative change in species richness, frequency of occurrence, biomass, or abundance of each taxon between the before and after sampling dates. Confidence intervals that include 1 (for decreased abundance) or –1 (for increased abundance) indicated that no significant change occurred between sampling periods.

RESULTS

Control of undesirable fishes

The mean species richness of undesirable fish (hereafter fish richness) was 2.4 for treatment and 5.8 for control ponds in the "before" samples, and ranged from 0.4 to 1.0 and 4.8 to 5.6 and treatment and control ponds, respectively (Table 3) after treatments. Undesirable fish richness indicated that four of the seven treatment ponds contained no fish during the spring following reclamation. Based on spring sampling complete fish kills occurred in only 57% of the treated ponds. Of the three ponds without complete fish kills, just two Fathead Minnows were captured in Oak Pond, 11 Fathead Minnows in Toners Pond, and two Yellow Perch *Perca flavecens* and a Pumpkinseed (Lepomis gibbosus) were captured from Lower Case Pond. On a longer-term basis, the four ponds that demonstrated complete fish eradication remained fish-free the entire year following treatment. Subsequently, only two of the treatment ponds remained fishless through the second year and 100% of the treated ponds contained undesirable fish by the third year after reclamation.

The undesirable fish richness at the initiation of the study in the treatment ponds was 2.4 (standard error = 0.5) and lower than the control pond richness of 5.8 (0.6) (Table 3). Subsequently, control ponds had significantly higher undesirable fish richness during all sample periods (P < 0.05) during this study. Among sample periods in control ponds there was no significant change throughout the study ($\chi^2 = 3.2$, $df = 4$, $P = 0.531$); however, the undesirable fish richness in treatment ponds was higher pre-treatment than post treatment sample periods ($\chi^2 = 14.8$, $df = 4$, $P = 0.005$). Generally, treatment ponds started with lower undesirable fish richness, significantly declined to zero to two species after treatment of ponds with rotenone, and then gradually increased throughout the duration of the study while control ponds remained relatively constant near five species.

The mean relative abundance (hereafter abundance) of undesirable fishes in trap nets ranged from 1,186 to 3,170 fish and 2 to 2,223 fish in control and treatment ponds, respectively (Table 3). Biomass of undesirable fishes ranged from 13,638 to 33,369 g and 58 to 16,511 g in control and treatment ponds, respectively. In control ponds no significant effect of sample period was found on undesirable fish abundance ($\chi^2 = 6.6$, $df = 4$, $P = 0.159$); however, there was a significant sample period effect on biomass ($\chi^2 = 10.5$, $df = 4$, $P = 0.033$). The significant sample period effect on undesirable fish biomass in control ponds was primarily due to the higher biomass observed during spring following reclamation than any other sample period. In treatment ponds there was a significant effect of sample period on both fish abundance ($\chi^2 = 14.8$, $df = 4$, $P = 0.005$) and fish biomass ($\chi^2 = 15.3$, $df = 4$, $P = 0.004$). The abundance and biomass of undesirable fish in treatment ponds significantly declined to near zero during the first year following treatment, but then increased to numbers that were not different from pre-treatment abundance during the remainder of the study. Intra-sample period comparisons between control and treatment ponds suggested that the biomass of undesirable fishes remained significantly higher in control ponds relative to treatment ponds ($P = 0.001$) throughout the study and indicated a significant treatment effect. In fact, the biomass of undesirable fishes in treatment ponds remained nearly 95% less the third year following treatment than prior to treatment.

Overall, the richness, abundance, and biomass of undesirable fishes were lower in treated ponds relative to control ponds based on differences in comparisons within each pond type. The ubiquitously distributed Black Bullhead was a species strongly impacted by rotenone reclamations. During this study Black Bullhead biomass (χ^2 = 15.36, *df* = 9, *P* = 0.0814) and abundance (χ^2 = 16.17, *df* = 9, *P* = 0.0633) did not change in control ponds; however, a significant change was observed for Black Bullhead in each biomass (χ^2 = 21.19, *df* = 9, *P* = 0.0119) and abundance (χ^2 = 20.66, *df* = 9, *P* = 0.0143) in treated ponds. No other undesirable fish species demonstrated a significant change in abundance during this study, which may have been attributed to the lack of universal distribution among ponds.

RDA was performed to further assess the undesirable fish assemblage response to treatments and to determine taxa specific responses (Table 4). A total of 20.8 and 29.2% of the variation in change in undesirable able fish abundance were explained by the first (among treatment group) and second (among ponds) ordination axis, respectively. Black Bullhead demonstrated the largest change in abundance during this study from pre-treatment to spring post-treatment with over 47% of the variation explained by the treatment group axis. On average Black Bullhead were roughly 60 times higher in abundance during the pre-treatment sample period than in the spring immediately following treatment. In addition, Black Bullhead abundance remained nearly 30% for all three years of sampling post-treatment, which suggests a long term reduction in Black Bullhead abundance. No other undesirable fish species demonstrated significant change in abundance during this study based on RDA, which was likely attributed to differential pre-treatment abundance among ponds.

Table 3. Trap net catches of undesirable fish species (all fish except walleye) in treated and control ponds over time. Composition is based on richness (number of species encountered), relative abundance (total number captured) and biomass (Biomass; kg) of fish captured in trap nets. Sampling was conducted using four trap nets during each sampling period: pre-treatment (September), post-treatment (May of following spring), and subsequently during September of the following three years (Year 1, Year 2, and Year 3). Values are means (and SE). Wilcoxon test statistics with $P < 0.05$ indicate significant differences between treatment and control groups. Kruskal-Wallis Statistics with $P < 0.05$ indicates the mean of at least one sample time was different for any given comparison.

		Short-term	Long term			KW-Statistics	
	Pre-Treat	Spring Year 1	Year 1	Year 2	Year 3	χ^2	*P*
			Fish (excluding walleye)				
Species Richness							
Treatment	2.4 (0.5)	0.6 (0.3)	0.4 (0.2)	1.0 (0.3)	1.0 (0.2)	**14.839**	**0.005**
Control	5.8 (0.6)	4.9 (0.6)	5.5 (0.7)	5.6 (0.6)	4.8 (0.9)	3.162	0.531
P	**0.003**	**0.001**	**< 0.001**	**0.001**	**0.001**	**---**	**---**
Biomass							
Treatment	16,510.9 (8,742.1)	57.6 (53.8)	66.4 (37.9)	3,460.7 (2,233.4)	1,007.1 (477.2)	**15.335**	**0.004**
Control	33,368.6 (8,206.8)	69,727.9 (19,341.3)	25,721.7 (9,171.6)	19,054.0 (6,591.3)	13,637.6 (2,984.5)	**10.456**	**0.033**
P	0.064	**0.001**	**0.001**	**0.028**	**0.002**	**---**	**---**
Relative Abundance							
Treatment	690.3 (317.1)	2.2 (1.5)	14.4 (10.8)	2,222.9 (2,183.8)	467.2 (262.9)	**13.069**	**0.011**
Control	1,993.5 (523.8)	1,595.9 (304.9)	1,247.7 (467.1)	3,169.9 (2,069.6)	1,186.3 (700.2)	6.598	0.159
P	**0.037**	**0.001**	**0.001**	0.621	0.247	**---**	**---**

Table 4. Results of RDA performed on undesirable fish relative abundance (total number captured). The analysis was performed on the changes in relative abundance of each species in each pond relative to pre-treatment observations. Cumulative percentage of total variance is the proportion of total variance in species data explained by each ordination axis (axis 1, treatment type; axis 2, among pond) and is a sum of Eigenvalues measuring the importance of each axis. Results of Monte Carlo permutations (n=499) were obtained to determine the significance of the treatment type on species variation (F-ratio). The total variation in species data explained by the treatment type axis (axis 1 variation %) is a sum of Eigen-values measuring axis importance. Multiplicative change represents how many fold greater was the abundance in the before period relative to the after period. Negative values represent an increase in abundance from the before to after sample period. Numbers in parenthesis represent 95% confidence intervals and confidence intervals that do not include negative one (increased occurrence) or one (decreased occurrence) indicates a significant change between periods and is denoted by an asterisk. Multiplicative change only was shown for taxa with at least 20% of variation fit by axis 1(only Bullhead in this study). An *a priori* probably level of 0.05 was used for all comparisons.

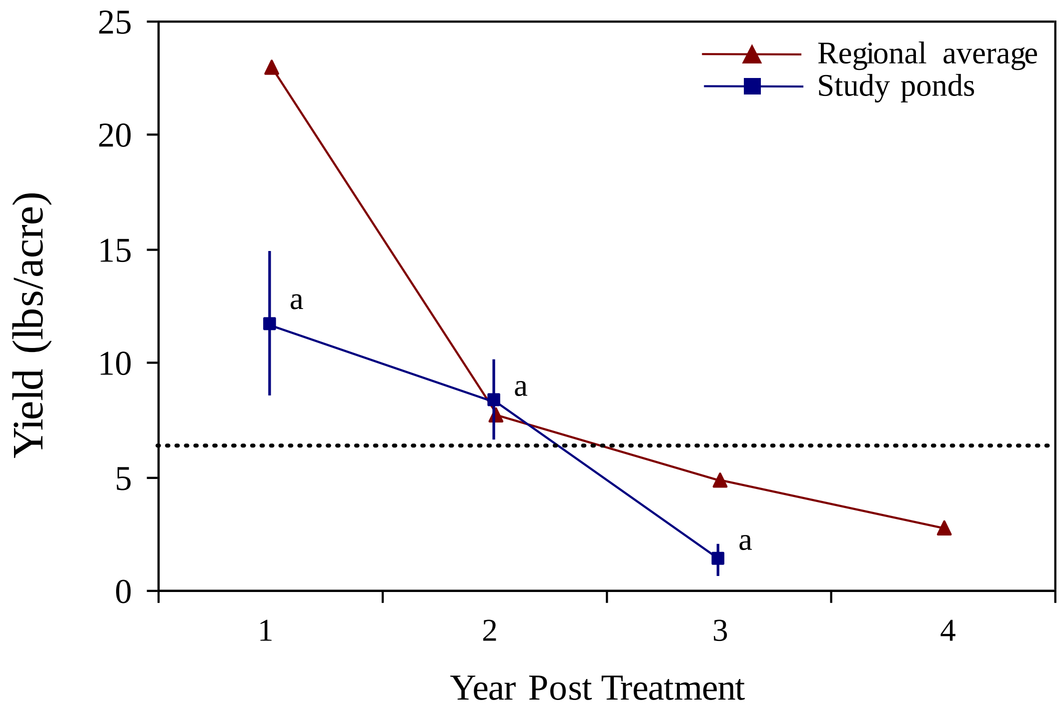
	Spring Year 1	Year 1	Year 2	Year 3
General Statistics				
Axis 1	20.8	10.7	1.5	5.2
Axis 2	29.2	37.4	35.3	32.8
Cumulative Axis Total	50.1	48.1	36.8	38.0
F-ratio	3.415	1.562	0.198	0.715
P	**0.010**	0.162	1.000	0.638
Axis 1 variation (%)[1]				
Bigmouth Buffalo	12.3	0.3	1.5	0.3
Bluegill	10.5	6.3	6.3	13.4
Bullhead	**47.3**	18.0	1.1	8.7
Brook Stickleback	6.3	---	---	---
Common Carp	0.8	0.2	< 0.1	1.3
Crappie	0.0	1.6	12.6	14.8
Common Shiner	6.3	6.3	6.3	6.3
Fathead Minnow	0.1	13.2	0.4	1.0
Golden Shiner	6.3	6.3	6.3	6.3
Green Sunfish	0.3	5.9	0.3	8.7
Iowa Darter	13.4	13.5	8.2	8.2
Johnny Darter	6.3	6.3	6.3	6.3
Northern Pike	2.1	0.1	3.8	7.6
Orangespotted Sunfish	4.4	1.5	1.1	0.5
Pumpkinseed	8.2	---	---	---
Tadpole Madtom	6.3	6.3	6.3	6.3
White Sucker	3.9	1.8	2.3	1.5
Yellow Perch	4.3	0.2	0.1	0.0
Multiplicative change (*n*)[2]				
Treatment	60.0 (3.1 – 895.0)*	29.3 (-0.3 – 1,196.2)	27.6 (-1.1 – 1,737.4)	27.8 (-0.6 – 1,288.0)
Control	-0.1 (-4.4 – 3.1)	1.2 (-0.9 – 8.4)	13.9 (2.4 – 64.2)	3.9 (0.1 – 20.0)

[1] Axis 1 variation (%) considered significant when ≥ 20.
[2] Multiplicative change shown for Bullhead species only.

Walleye production in treatment ponds

The first year following reclamation of ponds with rotenone provided the highest production of Walleye with yields ranging from 0.0 to 41.5 lbs/acre and with a mean yield of 11.6 (5.9) lbs/acre (Figure 1). Two (28.6%) of the seven treated ponds produced zero Walleye during the first year following treatment. During the second and third year following treatment production of Walleye declined each year with yields of 8.3 (3.4) lbs/acre and 1.3 (1.1) lbs/acre. There was no significant change (χ^2 = 3.4, *df* = 2, *P* = 0.183) in Walleye production yield among sample periods during this study. Walleye production was somewhat variable among ponds. Again, Walleye production was minimal in two of the ponds and indicated that those ponds were likely not good production pond candidates. Walleye production during this study compared favorably to regional mean estimates of Walleye production (Jacquelyn Bacigalupi, unpublished data) following rotenone reclamations (Figure 1).

Figure 1. Walleye production (yield, lbs/acre) in treated ponds over time post-treatment, and the regional mean response. Values for study ponds represent mean yield over all replicate ponds and vertical bars represent standard error. Regional values represent the mean yield from all ponds treated in the southern region by the Minnesota Department of Natural Resources (Jacquelyn Bacigalupi, personal communication). Means denoted with similar letters are not significantly different (Kruskal-Wallis chi-square; $P \leq 0.05$). A success threshold of 5.6 lbs/acre (horizontal line) is needed to maintain production quotas for Walleye.



Macrophytes

The mean macrophyte richness ranged from 7.3 to 9.3 and 8.4 to 12.0 for control and treatment ponds, respectively (Table 5). During this study, there was no significant sample period effect for combined (e.g., emergent, floating, and submergent) that the combined macrophyte taxa richness was significantly higher during the third year ($P \leq 0.01$) in treatment ponds but not during any other sample period. Although the between treatment group comparisons were not statistically significant, they suggest that ecologically-meaningful differences in overall macrophyte richness have been achieved among treatment groups. Especially considering that overall macrophyte richness in treatment ponds increased during each year of the study while richness in control ponds remained stable during the same period.

The increase in the combined taxa macrophyte richness in treatment ponds was strongly influenced by the positive response of submergent macrophytes (Table 5). Specifically, within macrophyte taxa types there was no difference in richness of emergent or floating macrophytes found among sample periods in either control ponds ($P > 0.60$) or treatment ponds ($P > 0.44$). In addition, there was no difference in macrophyte richness between treatment groups during any sample period ($P > 0.08$) for either emergent or floating macrophyte types. Conversely, submergent macrophyte richness did increase over time in treatment ponds ($\chi^2 = 8.0$, $df = 3$, $P = 0.05$). On average the richness of submergent macrophytes in treatment ponds increased by 2.3, 3.6 and 3.2 species during the first, second and third year relative to pre-treatment, respectively. The increase in macrophyte richness indicated an average net gain in species composition of 27 to 43% in treated ponds. The species composition of submergent macrophytes alone nearly doubled in the first year following treatment and continued to increase throughout the duration of the study. Conversely, control ponds indicated a stable or declining macrophyte richness throughout the study.

Occurrence of aquatic macrophytes for all taxa combined in transects generally followed a similar response pattern as richness (Table 5). Macrophytes for combined taxa were observed in 53% to 57% of transects in control ponds compared to 52% to 73% of transects in treatment ponds. The occurrence of macrophytes in control ponds did not change throughout the duration of the study ($\chi^2 = 0.44$, $df = 3$, $P = 0.93$); however, the occurrence of macrophytes in treatment ponds changed significantly over time ($\chi^2 = 10.5$, $df = 3$, $P = 0.02$). In addition, there was no difference in macrophyte occurrence between control and treatment ponds found during pre-treatment or the first year, but there was higher occurrence of macrophytes in treated ponds during the second ($P = 0.04$) and third year ($P = 0.05$). The occurrence of aquatic macrophytes generally increased from 50% to 70% of transects during the first year in treatment ponds, which contrasted with control ponds where macrophyte occurrence remained steady near 53% throughout the study.

The positive response in occurrence of macrophytes for combined taxa was largely influenced by the submergent macrophyte component in treated ponds (Table 5). For example, there was no significant change in occurrence of emergent or floating macrophytes in either control ponds ($P > 0.50$) or treatment ponds ($P > 0.51$). In addition, there was no difference in occurrence of emergent or floating macrophytes found between control and treatment ponds during any sample period ($P > 0.27$). Thus, these results revealed that emergent and floating macrophyte occurrence was similar among sample periods and between treatment groups during this study. In contrast, there was a significant sample period effect on the occurrence of submergent macrophytes in treated ponds ($\chi^2 = 17.2$, $df = 3$, $P < 0.01$), but no similar response for control ponds ($\chi^2 = 0.3$, $df = 3$, $P = 1.0$). Apparently, the occurrence of submergent macrophytes was higher during each year in treated ponds relative to pre-treatment. Further analysis indicated that the occurrence of submergent macrophytes in treated ponds was not different from control ponds during the pre-treatment sample period ($P = 0.86$) but was higher in treatment ponds during each year thereafter ($0.02 \leq P \leq 0.05$). These results provided further evidence of an immediate and long term positive response of submergent macrophytes to treatments.

RDA indicated a significant first-year effect of treatments on change in occurrence (F = 3.20, P = 0.002) of overall combined aquatic macrophytes in study ponds (Table 6). Axis 1 (representing differences among treatment groups) explained 20% and the second axis 22% of the total variation in change between sampling periods for occurrence. There was also a significant effect of treatment type on variation in species data for the second (F = 2.72, P = 0.002) and third treatment year (F = 2.55, P = 0.008). Axis 1 explained 17% and 16% of the total variation in change in occurrence from pre-treatment to the second and third year after treatment, respectively. There were no specific emergent or floating macrophyte taxa that had at least 20% of the variation in species data explained by the treatment type axis (Table 7). In most cases less than 10% of the variation in species change in occurrence among sample periods for emergent and floating macrophytes was explained by the treatment type axis. However, RDA estimated a significant effect of treatment type on change in occurrence of submergent macrophytes during each of the three years after treatment based on the high percentage of variation in species data explained by the treatment type axis (Table 8). In this case, RDA confirmed the non-parametric analysis previously reported, but further explained species that responded most positive to treatments, which included the submergent macrophyte species bushy pondweed, coontail, flatstem pondweed, and sago pondweed.

RDA revealed that bushy pondweed was the most consistently influenced taxa by treatments with a total of 42%, 45%, and 47% of the variation in bushy pondweed change in occurrence explained by the treatment type axis for the first, second, and third year, respectively (Table 8). On average bushy pondweed effect size was larger than any other taxa with multiplicative change of –10.1 (-91.2 – 0.3), -19.3 (-151.6 – -1.7), and –24.3 (-143.6 – -3.4) for Year 1, Year 2, and Year 3, respectively. Overall, multiplicative change indicated a 10 to 24-fold increase in number of transects which bushy pondweed was observed following treatment relative to pre-treatment. On average, bushy pondweed demonstrated an immediate increase in occurrence following treatment of ponds where it was observed in just 2% of transects prior to treatment and subsequently observed in 45% of transects during the July of the first year. Overall, the occurrence of bushy pondweed increased from 2% to 68% of transects from pre-treatment to the third year. Comparatively, bushy pondweed declined between 0.5 to 0.8 times in control ponds from pre-treatment to the third year.

Coontail occurrence also changed significantly in treated ponds, but not until the third year, indicating that it responded more gradually to the rotenone treatment (Table 8). A total of 25%, 21%, and 55% of the variation in coontail change in occurrence was explained by the treatment type axis for the first, second, and third years, respectively. On average coontail effect size was –4.9 (-45.9 – 0.3), -21.2 (-121.7 – -0.4), and -20.3 (-163.8 – -1.8) for Year 1, Year 2, and Year 3, respectively. Multiplicative change indicated a 5 to 22-fold increase in number of transects which coontail was observed following treatment. Similar to bushy pondweed, coontail change in occurrence declined slightly (0.1 fold) in control ponds from pre-treatment to the three years following treatment.

Flatstem pondweed had at least 20% of the variation in occurrence explained by treatment type during at least one sample time following treatment (Table 8). However, multiplicative change revealed that flatstem pondweed occurrence was not significant during this study. Multiplicative change for flatstem pondweed was -2.7 (-18.7 – 0.5) and -7.2 (-123.3 – 0.8) during the first and third year in treated ponds. The 95% confidence interval indicated high variation among ponds in change in occurrence for flatstem pondweed that likely hampered detection of significant changes. However, the occurrence of flatstem pondweed increased from just 2% of transects during pre-treatment to 29%, 18%, 46% of transects during the first, second and third years, respectively. Taken together, we suggest that there was an ecologically significant response of flatstem pondweed in many of the treatment ponds, but it was difficult to identify statistical significance because of the high variability in the response among ponds.

On average, sago pondweed occurrence increased from 30% of transects prior to treatment to 84% of transects in the third year following treatment. In contrast, the control ponds started with a higher occurrence and basically remained constant throughout the study. Sago pondweed was the most influenced taxa during the first year post-treatment with a total of 46% of the variation in sago pondweed occurrence being attributed to the treatment type axis (Table 8). Multiplicative change for sago pondweed occurrence was -6.3 (-35.7 – 0.5), which showed that sago pondweed was found in six times less transects in the pre-treatment sample period relative to the same transects the first year after treatment. However, because less than 20% of the variation in sago pondweed occurrence was explained by treatment type during the second and third year following treatment the estimation of multiplicative change was not warranted. These results suggested an immediate increase in occurrence of sago pondweed in treated ponds during the first year followed by limited change during the second and third year post-treatment. Again, due to the high variation in sago pondweed occurrence in treated ponds no significant change was detected. In contrast, sago pondweed multiplicative change was insignificant in control ponds during the same period and actually declined 0.1 (-0.1 – 0.2) times from pre-treatment to the first year.

No other macrophyte species change in occurrence from before to after treatment had more than 20% variation explained by axis 1. However, roughly 15% to 18% of the variation in occurrence from pre-treatment to the third year for common cattail, spike rush, chara, and water plantain were explained by the treatment type axis, which suggested relatively large increases in occurrence during this study (Table 8). A total of 7 of the 43 aquatic macrophyte species analyzed had roughly 18% of the variation explained by axis 1, meaning roughly 16% of the species observed during this study exhibited trends towards increased abundance following rotenone treatment in study ponds.

The expansion of submergent aquatic macrophytes following removal of fish may have had long term influence on improved water clarity through fixation of phosphorus, reduction in algae blooms, and protection of sediments from re-suspension. Algal biomass measured by chlorophyll-a concentration in study ponds demonstrated a marked decrease from pretreatment to subsequent sample periods in treatment ponds during this study (Figure 2); however, there was no statistically significant change in chlorophyll-a concentration in either control ponds ($\chi^2 = 6.0$, $df = 3$, $P = 0.111$) or treatment ponds ($\chi^2 = 2.9$, $df = 3$, $P = 0.41$). Furthermore, paired comparisons between treatment and control groups within sample periods showed no difference in chlorophyll-a concentration during pre-treatment ($P = 0.729$) or during the first ($P = 0.083$) and second year ($P = 0.165$). Conversely, the treatment group had a significantly lower chlorophyll-a concentration during the third year ($P = 0.004$). Although there was no statistically significant treatment effect on Chl-*a* found during this study some of the treatment type by time interactions were nearly significant (e.g., $P = 0.055$) and all of the estimates of the interactions showed negative effects. This means that the treatment ponds tended to be lower at each time after the overall treatment type and time effect were factored into the overall regression model. This implies that the treatment ponds showed declines in Chl-*a* following treatment that was not observed in the control ponds. Ultimately, these results provided some evidence that treatments resulted in decreased Chl-*a*, but the analyses were not clear because of high variation in the measurements within and among ponds.

An inverse relationship was found between chlorophyll-a concentration and frequency of occurrence of submergent plants in treatment ponds, but the relationship was not significant ($r^2 = 0.571$, $P = 0.245$). Most likely, the increased water clarity due to fish removal and the subsequent increased submergent plant abundance and distribution aided in reducing algal biomass. The reduction in algae blooms likely further improved water clarity prolonging the positive response of macrophytes. The aforementioned secchi depth and suspended solids results also suggested marginal improvements to water clarity in treatment ponds relative to control ponds.

Table 5. Algal abundance (chlorophyll-a ppb) and aquatic macrophyte abundance based on species composition (species richness; number of species observed) and frequency of occurrence (percentage of transects with aquatic vegetation observed) for macrophytes collected in eight 5x50 m transects from study ponds during 2002 through 2007. Sampling was conducted using standard lake survey methods during mid-summer prior to treatment and each year following treatment for three years (Year 1, Year 2, and Year 3). Values represent the mean percentage (standard error) of transects in which emergent, floating, submergent, and terrestrial aquatic vegetation were observed. Wilcoxon test statistic $P < 0.05$ indicates significant differences among treatment groups. Kruskal-Wallis Statistics with $P < 0.05$ indicates at least one sample time was different for any given comparison.

		Long term			KW-Statistics	
	Pre-Treat	Year 1	Year 2	Year 3	χ^2	*P*
Chlorophyll-a						
Control	112.9 (23.6)	128.9 (39.1)	210.3 (65.7)	132.0 (21.0)	1.6	0.658
Treatment	140.0 (54.5)	129.5 (81.4)	144.3 (75.2)	75.1 (50.6)	2.6	0.458
P	0.729	0.133	0.203	0.056	---	---
Macrophyte Species Richness						
Emergent						
Control	6.1 (1.0)	5.5 (0.8)	6.0 (0.8)	5.4 (0.6)	0.715	0.870
Treatment	5.7 (0.5)	6.0 (0.5)	6.7 (0.4)	6.4 (0.7)	2.251	0.522
P	0.859	0.479	0.549	0.305	---	---
Floating						
Control	0.5 (0.5)	0.4 (0.4)	0.6 (0.5)	0.5 (0.4)	1.875	0.599
Treatment	0.4 (0.2)	0.4 (0.2)	0.7 (0.4)	0.4 (0.3)	2.700	0.440
P	0.083	0.125	0.519	0.683	---	---
Submergent						
Control	2.6 (1.0)	2.3 (0.9)	1.9 (0.7)	1.4 (0.4)	3.852	0.278
Treatment	2.3 (0.7)	4.3 (0.5)	4.6 (0.4)	4.7 (0.6)	**8.023**	**0.046**
P	0.303	0.125	0.067	**0.004**	---	---
Overall						
Control	9.3 (2.0)	8.1 (1.6)	8.5 (1.7)	7.3 (0.8)	0.250	0.969
Treatment	8.4 (1.1)	10.7 (0.9)	12.0 (0.8)	11.6 (1.1)	7.170	0.067
P	0.597	0.079	0.080	**0.011**	---	---
Macrophyte Frequency of Occurrence						
Emergent						
Control	0.92 (0.06)	0.94 (0.06)	1.00 (0.00)	0.95 (0.03)	2.350	0.503
Treatment	0.91 (0.05)	0.95 (0.03)	0.98 (0.02)	0.95 (0.05)	2.333	0.506
P	0.580	0.296	0.285	0.741	---	---
Floating						
Control	0.13 (0.13)	0.13 (0.13)	0.14 (0.12)	0.14 (0.12)	0.603	0.896
Treatment	0.16 (0.12)	0.21 (0.14)	0.20 (0.14)	0.20 (0.14)	0.185	0.980
P	0.298	0.265	0.490	0.823	---	---
Submergent						
Control	0.55 (0.16)	0.52 (0.14)	0.50 (0.16)	0.61 (0.15)	0.317	0.957
Treatment	0.50 (0.10)	0.89 (0.07)	1.00 (0.00)	1.00 (0.00)	**17.230**	**< 0.001**
P	0.860	**0.050**	**0.016**	**0.016**	---	---
Overall						
Control	0.53 (0.08)	0.53 (0.08)	0.55 (0.08)	0.57 (0.08)	0.443	0.931
Treatment	0.52 (0.04)	0.68 (0.06)	0.73 (0.04)	0.71 (0.03)	**10.452**	**0.015**
P	0.380	0.071	**0.042**	**0.051**	---	---

Table 6. Results of RDA performed on the overall aquatic macrophyte frequency of occurrence in transects. The analysis was performed on the changes in frequency of occurrence of each species in each pond between sample times. Pre-treatment sample collections were conducted the July prior to treatment. Post-treatment samples were collected during July of the first, second, and third year following treatment. Cumulative percentage of total variance is the proportion of total variance in species data explained by each ordination axis (axis 1, treatment type; axis 2, among pond) and is a sum of Eigenvalues measuring the importance of each axis. Results of Monte Carlo permutations were obtained to determine the significance of the treatment type on species variation. An *a priori* probability level of 0.05 was used for all comparisons.

	Axis 1 %		
Taxon	Year 1	Year 2	Year 3
Cumulative percentage of variance			
Axis 1 (treatment type)	19.6	17.3	16.4
Axis 2 (among pond)	22.2	14.8	21.8
Total	41.8	32.1	38.2
Monte Carlo Test			
F-ratio	3.160	2.718	2.545
P	0.002	0.002	0.008

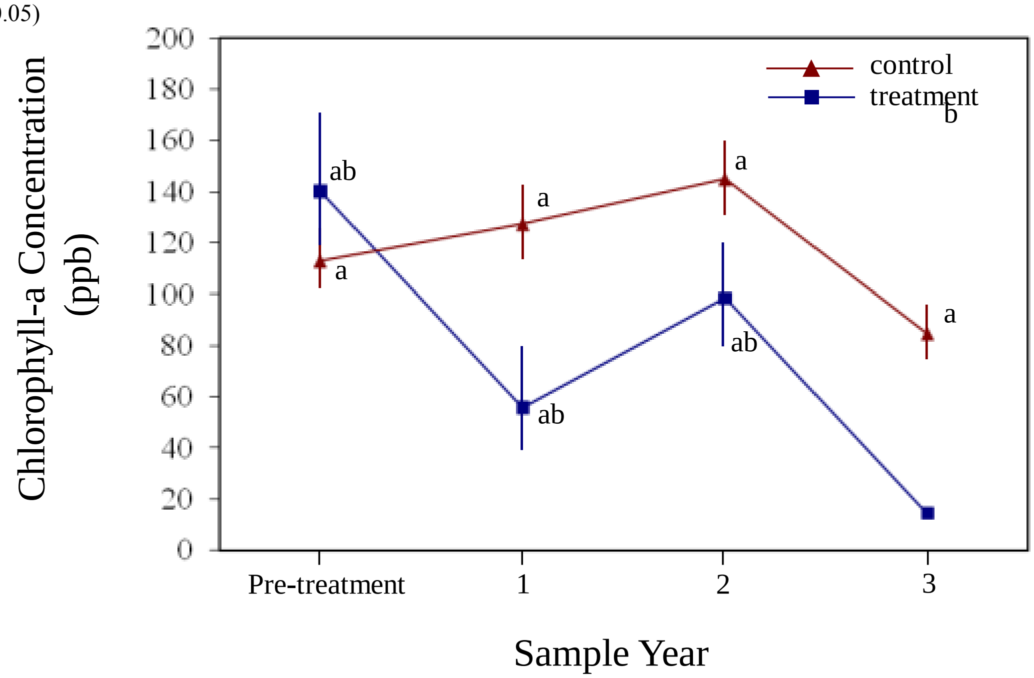
Table 7. Results of RDA performed on the emergent and floating aquatic macrophyte frequency of occurrence in transects. The analysis was performed on the changes in frequency of occurrence of each species in each pond between sample times. Pre-treatment sample collections were conducted the July prior to treatment. Post-treatment samples were collected during July of the first, second, and third year following treatment. Values represent the cumulative fit per species as a fraction of the total variation explained for each species by the first ordination axis (axis 1 %).

	Axis 1 %		
Taxon	Year 1	Year 2	Year 3
Emergent			
Arrowhead	9.4	18.4	6.7
Arum-leaved arrowhead	8.2	8.2	8.2
Bog sedge	---	6.3	6.3
Chufa	13.4	5.2	5.2
Common cattail	14.5	9.7	18.0
Comosa sedge	0.0	---	6.3
Giant burreed	0.1	8.2	0.8
Hardstem bulrush	1.3	2.6	15.5
Narrowleaf cattail	7.3	11.5	2.7
Needles rush	11.9	14.1	3.2
Needle spike rush	6.3	6.3	---
Pusillis	8.2	8.2	8.2
Reed canary grass	9.1	5.7	< 0.1
River bulrush	1.1	6.8	5.5
Marsh skullcap	0.0	6.3	---
Softstem bulrush	0.1	0.4	3.7
Spatterdock	6.3	6.3	6.3
Spike rush	---	17.6	17.6
3-square bulrush	6.3	15.1	3.8
Wapato	8.2	---	---
Water stargrass	8.2	8.2	---
White water buttercup	6.3	---	---
Floating			
Floatingleaf pondweed	6.3	10.3	5.6
Greater duckweed	6.3	6.3	6.3
Lesser duckweed	8.2	8.2	0.4
Star duckweed	0.0	8.2	8.2
White waterlilly	8.2	13.4	13.4
Yellow waterlilly	6.3	6.3	6.3

Table 8. Results of RDA performed on the submergent aquatic macrophyte frequency of occurrence in transects. The analysis was performed on the changes in frequency of occurrence of each species in each pond between sample times. Pre-treatment sample collections were conducted the July prior to treatment. Post-treatment samples were collected during July of the first, second, and third year following treatment. Values represent the cumulative fit per species as a fraction of the total variation explained for each species by the first ordination axis (axis 1 %) and the multiplicative change observed (95% confidence interval) in the abundance of taxa in the treatment and control ponds. Multiplicative change represents how many fold greater was the abundance of each taxon in the before period relative to the after period. Negative values represent an increase in abundance from the before to after sample period. Multiplicative change was only shown for taxa with ≥ 20% of variation fit by axis 1. Confidence intervals that do not include negative one (increased occurrence), or one (decreased occurrence), indicate a significant change between periods and are indicated by an asterisk.

		Multiplicative change	
Taxon	Axis-1 %	treatment	control
Pre-treatment to Year 1			
Bushy pondweed	41.6	-10.1 (-91.2 – 0.3)	0.7 (-0.9 – 4.1)
Chara	22.0	-1.0 (-6.7 – 0.9)	0.6 (-0.3 – 2.5)
Claspingleaf pondweed	2.8	---	---
Coontail	25.0	-4.9 (-45.9 – 0.3)	-0.1 (-0.4 – 0.1)
Curly leaf pondweed	0.4	---	---
Filamentous algae	28.1	-4.0 (-30.7 – 0.3)	<-0.1 (-0.1 – <0.1)
Flatstem pondweed	25.1	-2.7 (-18.7 – 0.5)	<0.1 (-0.1 – 0.1)
Frie’s pondweed	6.3	---	---
Northern water milfoil	11.4	---	---
Plantain	17.8	---	---
Sago pondweed	46.1	-6.3 (-35.7 – 0.5)	0.1 (-0.1 – 0.2)
Watercress	6.3	---	---
Pre-treatment to Year 2			
Bushy pondweed	44.9	-19.3 (-151.6 – -1.7)*	0.8 (-2.2 – 9.4)
Chara	49.1	-0.8 (-3.4 – 0.4)	6.7 (0.8 – 31.5)
Claspingleaf pondweed	0.2	---	---
Coontail	21.2	-12.2 (-121.7 – -0.4)	-0.9 (6.1 – 1.1)
Curly leaf pondweed	2.9	---	---
Filamentous algae	20.3	-10.4 (-94.5 – 2.1)*	-0.8 (-5.8 – 1.0)
Flatstem pondweed	13.3	---	---
Frie’s pondweed	6.3	---	---
Northern water milfoil	7.2	---	---
Plantain	16.7	---	---
Sago pondweed	18.9	---	---
Watercress	6.3	---	---
Pre-treatment to Year 3			
Bushy pondweed	46.8	-24.3 (-143.6 – -3.4)*	0.5 (-3.3 – 8.9)
Chara	15.9	---	---
Claspingleaf pondweed	6.7	---	---
Coontail	55.3	-20.3 (-163.8 – -1.8)*	0.1 (-0.1 – 0.3)
Curly leaf pondweed	7.7	---	---
Filamentous algae	4.6	---	---
Flatstem pondweed	27.9	-7.2 (-123.3 – 0.8)	0.6 (-1.0 – 4.2)
Frie’s pondweed	6.3	---	---
Northern water milfoil	5.8	---	---
Plantain	17.3	---	---
Sago pondweed	14.2	---	---
Watercress	6.3	---	---

Figure 2. Algae abundance (mean chlorophyll-a concentration ±1 SE; ppb) and submergent aquatic macrophyte frequency of occurrence (% of transects). Means denoted with similar letters are not significantly different based on Kruskal-Wallis chi-square or Wilcoxon test scores ($P \leq 0.05$)



Pond chemical and physical properties

Nutrient dynamics: Ammonia levels evidenced a significant time effect in both control ponds (χ^2 = 9.2, df = 3, P = 0.026) and treatment ponds (χ^2 = 13.3, df = 3, P = 0.004) (Table 9). Generally, ammonia was similar in control and treatment ponds during all but the third year during which ammonia labels were significantly higher in treatment than control ponds *(P* = 0.001). No differences in Kjeldahl nitrogen, Nitrite/Nitrate, or total nitrogen were detected between control and treatment ponds or among sample periods within treatment groups during this study.

No significant differences were found for total phosphorus during this study between control and treatment ponds or within treatment groups among sample periods (Table 9). However, the release of phosphorus from the fish biomass following reclamation was apparent by the nearly doubling of total phosphorus from pre-treatment to the first year following treatment, which was followed by a gradual decline through the remainder of the study. Control ponds remained constant throughout the study, indicating a possible biological treatment effect.

Water clarity: There was no significant time effect found in either control ponds (χ^2 = 1.3, df = 3, P = 0.529) or treatment ponds (χ^2 = 2.2, df = 3, P = 0.327) for secchi depth (Table 9). The secchi depth ranged from 9.7 to 16.8 inches and 28.7 to 52.0 inches in control and treatment ponds, respectively. Secchi depth was lower in control than treatment ponds at the initiation of the study. However, comparisons of secchi depth between control and treatment ponds indicated that treatment ponds had higher secchi depth than control ponds during Year 1 (P = 0.048) and Year 3 (P = 0.004), but not during pre-treatment (P = 0.208). Unfortunately, secchi depth was not recorded during the second year following treatment during this study. Although there was no significant time effect on secchi depth in treatment ponds the average secchi depth increased substantially from 28.7 (15.7) inches to 52.0 (8.0) inches in the first year post-treatment. Again, as with phosphorus results no similar pattern was observed in control ponds suggesting improved water clarity.

There was no significant time effect on suspended solids in control ponds (χ^2 = 1.8, df = 3, P = 0.614) or in treated ponds (χ^2 = 6.0, df = 3, P = 0.113) (Table 9). Furthermore, paired comparisons between control and treatment ponds within sample periods indicated similar suspended solids during all but Year 3 (P = 0.018). Although the analysis resulted in no statistically significant decline in suspended solids following rotenone application in treated ponds, there was a moderate reduction in suspended solids in treated ponds indicating improved water clarity, which corroborated total phosphorus and secchi depth results.

Table 9. Pond chemical and physical properties estimated from water samples collected during September during pre-treatment and three years following treatment from study ponds from 2002–2007. All measurements were reported in ppm unless noted. Values represent treatment means (standard error) for each parameter for control and treatment ponds. Wilcoxon test statistics with $P < 0.05$ indicate significant differences among treatment groups. Kruskal-Wallis Statistics with $P < 0.05$ indicates at least one sample time was different for any given comparison.

Pond Parameter	Pre-treatment	Year 1	Year 2	Year 3	KW-Statistics	
Ammonia (NH_3)						
Control	0.03 (0.00)	0.13 (0.05)	0.13 (0.08)	0.03 (0.00)	9.2	**0.026**
Treatment	0.03 (0.00)	0.13 (0.05)	0.06 (0.01)	0.25 (0.09)	13.3	**0.004**
P	0.771	0.451	0.164	**0.001**	---	---
Total N ($NO_2 + NO_3 + NH_3$)						
Control	0.06 (0.01)	0.14 (0.05)	0.15 (0.09)	0.23 (0.07)	4.0	0.257
Treatment	0.05 (0.01)	0.16 (0.07)	0.09 (0.02)	0.43 (0.13)	9.0	0.029
P	0.861	0.451	0.354	0.072	---	---
Phosphorus (PO_4)						
Control	0.172 (0.025)	0.149 (0.017)	0.178 (0.034)	0.167 (0.017)	0.7	0.881
Treatment	0.318 (0.156)	0.543 (0.293)	0.399 (0.190)	0.269 (0.107)	0.6	0.908
P	0.729	0.247	0.203	0.563	---	---
N:P Ratio [1]						
Control	0.47 (0.18)	1.33 (0.53)	1.76 (1.38)	1.81 (0.70)	1.4	0.711
Treatment	0.45 (0.20)	1.43 (1.08)	0.90 (0.51)	2.76 (1.23)	7.3	0.064
P	0.643	0.729	0.908	0.488	---	---
Secchi Depth (inches)						
Control	12.0 (1.4)	16.8 (7.0)	---	9.7 (1.7)	1.3	0.529
Treatment	28.7 (15.7)	52.0 (8.0)	---	42.0 (8.5)	2.2	0.327
P	0.208	**0.048**	---	**0.004**	---	---
Total Suspended Solids						
Control	58.9 (11.3)	50.1 (12.5)	49.5 (10.2)	41.8 (10.5)	1.4	0.711
Treatment	82.1 (32.1)	60.5 (49.5)	37.0 (17.6)	12.2 (4.2)	6.0	0.113
P	0.729	0.083	0.165	**0.018**	---	---

[1] Total nitrogen ($NO_2 + NO_3 + NH_3$) to phosphorus (PO_4) ratio.

Zooplankton

Zooplankton was not identified to the species level during this study so determining exact zooplankton species composition was not possible. However, the effects of treatment on 12 aggregate taxa groups indicated no change in richness of taxa groups among sample periods in control ponds (χ^2=3.354, df = 5, P = 0.37) or treatment ponds (χ^2=7.0, df = 5, P = 0.22) (Table 10). There were no significant differences in the richness of zooplankton taxa groups between treatment and control ponds during any specific sample period using paired comparisons (P > 0.05), except for higher zooplankton richness in control ponds during June of the second (P = 0.039) year. Overall, zooplankton richness ranged from 5.4 (0.3) to 6.1 (0.3) and 3.7 (0.7) to 5.7 (0.3) in control and treatment ponds, respectively.

There was a significant effect of sample period on the overall zooplankton abundance during this study in treatment ponds (χ^2=20.49, df = 5, P = 0.001) but not in control ponds (χ^2=5.22, df = 5, P = 0.39) (Table 10). A similar response was observed for zooplankton biomass in treatment ponds (χ^2=16.81, df = 5, P = 0.005) and control ponds (χ^2=6.09, df = 5, P = 0.298). The significant sample period effect reflected the drastic initial reduction in zooplankton populations observed after reclamation of ponds with rotenone followed by a rapid recovery during the following spring. During this study copepod and cladocera zooplankton generally responded similarly among sample periods and between control and treatment groups (Table 10). There was an immediately significant decline in copepoda abundance (P = 0.038) and biomass (P = 0.005) in treatment ponds, but no significant decline in control ponds (P > 0.05). On average the abundance of copepods declined from 94 (25) copepods/L to 1 (1) copepod/L in treatment ponds. During the post-treatment sample there was also a significant difference in abundance (P < 0.001) and biomass (P < 0.001) between control and treatment ponds indicating that the response of copepods in treatment ponds differed from the response in control ponds. In this case, copepod abundance and biomass approached zero in treatment ponds but remained higher in control ponds. Furthermore, during the recovery period copepod populations rebounded to pretreatment levels in the treated ponds (P > 0.05) and there was no apparent long term effect of rotenone treatments on copepod abundance or biomass during the three years of this study.

Cladoceran zooplankton responded similarly to copepods during this study, although the initial decline in cladocera abundance and biomass from the pre-treatment to the post-treatment period was not significant (Table 10). On average, cladocera abundance declined from 183 (153) zooplankton/L to less than 1 (1) zooplankton/L. This was followed by a significant increase in cladocera abundance (P < 0.001) and biomass (P < 0.001) from post-treatment to the recovery period. Cladocera abundance and biomass followed a similar trajectory in treated and control ponds between these periods, and were not significantly different during the recovery period (P > 0.05). The long term cladocera populations in treated ponds were not different from control ponds indicating complete recovery of cladocera following rotenone treatment.

Short term RDA indicated a significant immediate effect of rotenone on zooplankton abundance (F = 0.313, P = 0.002), with axis 1 (representing differences between treatment groups) explaining 31% and axis 2 33% of the total variance in community change between sampling dates. The high amount of variation explained by the second axis indicated that abundance of zooplankton taxa was variable among study ponds, especially treatment ponds. Based on short term RDA virtually all zooplankton taxa were significantly affected by rotenone treatments. Calanoid copepods were the most influenced by rotenone treatments with a total of 46% of the variation in calanoid copepods change in abundance being attributed to the treatment type axis (Table 11). Calanoid copepod abundance was roughly 28 times (95% confidence interval; 4.7 – 141.7) higher during the pre-treatment sample relative to the post-treatment sample. Cyclopoida, daphniidae and bosminidae had 29% to 37% of the total variation in abundance change explained by the treatment group axis. Effect size for these zooplankton taxa ranged from 6 to 16 times higher abundance pre-treatment relative to post-treatment. Nauplii, cyclopoida, and daphniidae demonstrated much less variation among treatment ponds; however, these groups displayed the largest change in abundance (multiplicative change of 28 and 16, respectively) from pre-treatment to post-treatment, owing to higher initial abundance. Except for sididae, zooplankton taxa experienced a greater reduction in abundance in treatment than in control ponds. Although sididae abundance change was significant, with 25% of the variation explained by the treatment group axis, the abundance of sididae actually declined most in control ponds (Table 11).

The multiplicative change in abundance of sididae was just -0.1 (-0.1 – 0.1) and 0.5 (-0.1 – 1.3) for treatment and control ponds, respectively. Overall, the abundance of sididae was relatively low throughout the duration of this study, as was the abundance of harpacticoida and chydoridae, which translated into minimal change among sample periods.

Following the significant decline in the overall zooplankton population abundance following rotenone treatments, RDA indicated a significant increase (F = 0.182, P = 0.028) in overall zooplankton abundance during the recovery period (Table 6) relative to pre-treatment. Axis 1 explained 18% and the second axis 41% of the total variance in change between sampling dates for zooplankton abundance. In this case, the pre-treatment zooplankton abundance (before) was paired with the recovery period (after). Four of twelve zooplankton taxa experienced a significant change in abundance from pre-treatment to the recovery period. Sididae, cyclopoida, and nauplii abundance increased the most in treatment ponds with a total of 34%, 32%, and 24% of the variation explained by the treatment type axis (Table 11). Daphiidae, bosminidae and harpacticoida all indicated greater increases in abundance in treatment ponds; however, the percentage of the total variation explained by the treatment type axis was not greater than 20% for these zooplankton taxa (Table 11) and changes in abundance were largely influenced by among pond variation. Overall, the abundance of all zooplankton taxa, besides calanoida, increased more in treatment than in control ponds during the recovery period. Effect sizes revealed that abundance of most zooplankton taxa in treatment ponds were from 0.1 to 9.2 times lower during the pre-treatment sample than recovery period sample, indicating that zooplankton recovered from the rotenone treatment. A single exception were calanoid copepods, which were 7.9 (0.8 – 44.2) times lower in abundance during the recovery sample relative to the pre-treatment sample, indicating a delayed recovery relative to other zooplankton taxa. By the following June, calanoids had rebounded to pre-treatment levels. In control ponds most zooplankton taxa yielded higher abundance pre-treatment relative to the recovery period. Overall, the direction of species vectors and estimated multiplicative change between pre-treatment and recovery time periods indicated that zooplankton populations increased most in treatment ponds (Table 11).

During this study, there was a significant increase in the overall zooplankton population abundance from post-treatment to the recovery period (F = 0.406, P = 0.002). A total of 41% of the variation in zooplankton population abundance was explained by axis 1 while axis 2 contributed 21% of the total variation. This finding indicated that following the significant reduction of zooplankton abundance that the zooplankton populations as a whole had recovered. In fact, cyclopoida (51%), daphniidae (47%), nauplii (47%), and bosminidae (38%) each had nearly 50% of the total variation in abundance explained by the treatment type axis (Table 11) indicating a strong treatment effect. From post-treatment to the recovery period the overall zooplankton population abundance in treated ponds increased from 1 (1) to 371 (84) zooplankton/L, which marked a 370% increase (Table 10). Conversely, in control ponds the zooplankton abundance increased from 150 (34) to 218 (78) zooplankton/L, which marked only a 45% increase, significantly less than in treated ponds. Multiplicative change suggested that zooplankton in treatment ponds were from 7 to 68 times higher during the recovery period relative to the post-treatment period (Table 17). Similarly, zooplankton abundance in control ponds also increased during the recovery period relative to the post-treatment period, but the magnitude of change never exceeded three times (only daphniidae were significant), much less change than observed in treatment ponds. Again, this indicated that zooplankton populations significantly declined in treatment ponds, but increased at a faster rate than control ponds during the recovery period.

Following the significant decline in zooplankton population abundance following rotenone treatments and subsequent recovery during the following spring RDA indicated no significant change from pre-treatment to the summer following treatment (F = 0.023, P = 0.964) (Table 10). Only 2% of the variation in change in zooplankton population abundance from pre-treatment to the first summer following treatment was explained by the treatment type axis while nearly 50% was explained by the among pond variation. None of the zooplankton taxa demonstrated a significant change in abundance from pre-treatment to the following summer period. In fact, for most zooplankton taxa less than 10% of the variation was explained by the treatment type axis and most variance was a function of among pond variation. Based on the average number of zooplankton/L among ponds within each treatment group the treatment ponds increased from 278 (157) to 284 (148) and the control group from 444 (217) to 503 (172) during the pre-treatment and summer following treatment, respectively. Overall, the change in zooplankton population abundance was equal from pre-treatment to the first year following treatment and subsequent summer samples indicating complete and total recovery of the zooplankton populations with no long term significant effects from treatments, so no further analysis was attempted.

Table 10. Zooplankton composition based on species richness (number of species observed), relative abundance (number/L) and biomass (µg/L) for zooplankton collected from study ponds during 2002 through 2007. Samples were collected from five locations in each pond and pooled. Three replicate samples were analyzed in the lab. A replicate average was then calculated to represent the relative abundance and biomass for each sample time. Samples were collected prior to treatment (Pre-Treat), the week after treatment (Post-Treat), during May of the following spring (Recovery), and again during mid -June of each year following treatment for three years (Year 1, Year 2, and Year 3). Overall values represent the average (standard error) over ponds within treatment groups. Wilcoxon test statistic (*P* < 0.050 indicate significant differences among treatment groups. Kruskal-Wallis Statistics with *P* < 0.05 indicates at least one sample time was different for any given comparison.

	Short term			Long term			KW-Statistics	
	Pre-Treat	Post-Treat	Recovery	Year 1	Year 2	Year 3	χ^2	*P*
Species Richness								
Treatment	4.9 (0.4)	3.7 (0.7)	5.0 (0.2)	5.7 (0.3)	5.0 (0.2)	5.3 (0.3)	6.992	0.221
Control	5.8 (0.4)	5.5 (0.3)	5.4 (0.3)	5.8 (0.3)	6.1 (0.4)	6.1 (0.3)	5.354	0.374
P	0.161	0.057	0.248	0.675	**0.039**	0.076	---	---
Relative Abundance								
Copepoda								
Treatment	94 (25)	1 (1)	278 (96)	144 (73)	98 (69)	119 (41)	20.130	**0.001**
Control	129 (29)	69 (14)	84 (18)	183 (55)	184 (99)	219 (71)	5.449	0.364
P	0.418	**0.001**	**0.049**	0.699	0.908	0.247	---	---
Cladocera								
Treatment	183 (153)	< 1 (< 1)	93 (20)	140 (75)	68 (22)	65 (25)	17.490	**0.004**
Control	315 (214)	81 (25)	134 (66)	320 (128)	172 (88)	250 (74)	5.405	0.369
P	0.105	**0.001**	0.488	0.197	0.908	0.064	---	---
Overall								
Treatment	278 (157)	1 (1)	371 (84)	284 (148)	165 (68)	185 (43)	20.490	**0.001**
Control	444 (217)	150 (34)	218 (78)	503 (172)	356 (165)	469 (121)	5.217	0.390
P	0.247	**0.001**	0.083	0.245	1.000	0.133	---	---
Biomass								
Copepoda								
Treatment	592 (142)	4 (4)	535 (161)	476 (220)	356 (268)	369 (181)	18.290	**0.003**
Control	557 (192)	215 (76)	293 (100)	317 (91)	275 (136)	667 (182)	7.616	0.179
P	0.643	**0.001**	0.247	0.897	1.000	0.247		
Cladocera								
Treatment	589 (339)	1 (< 1)	469 (178)	588 (145)	519 (317)	527 (229)	17.413	**0.004**
Control	488 (335)	147 (59)	346 (113)	448 (143)	171 (163)	617 (184)	4.115	0.533
P	0.643	**0.001**	0.817	0.302	0.488	0.643		
Overall								
Treatment	1,181 (385)	5 (4)	1,004 (197)	1,065 (296)	875 (382)	896 (303)	16.811	**0.005**
Control	1,045 (369)	362 (106)	639 (188)	764 (210)	607 (255)	1,285 (339)	6.089	0.298
P	0.817	**0.001**	0.105	0.302	0.643	0.418	---	---

Table 11. Results of short term and long term RDA performed on the zooplankton relative abundance (#/L). The analysis was performed on the changes in relative abundance of each taxon group in each pond between sample times for immediate short term (pre-treatment to post-treatment the week following treatment), short term recovery (pre-treatment to the spring following treatment), post-treatment recovery (post-treatment to spring following treatment), and long term (pre-treatment to following June). Pre-treatment sample collections were conducted the week prior to reclamation. Post-treatment samples were collected the week following treatment, recovery samples were collected during early May of the year following reclamation, and Year 1 samples were collected the June following reclamation. Multiplicative change represents how many fold greater was the abundance of each taxon in the before period relative to the after period. Conversely, negative numbers represent how many fold greater was the abundance of each taxon in the after period relative to the before period. Multiplicative change was only shown for taxa with ≥ 20% of variation fit by axis 1 (treatment type axis). Confidence intervals that do not include one indicate a significant change between periods and are indicated by bold type.

		Multiplicative change			Multiplicative change	
Taxon	Axis-1 % variation	treatment	control	Axis-1 % variation	treatment	control
	Pre-treatment to Post-treatment			**Pre-treatment to Recovery**		
Nauplii	**23.6**	3.5 (0.4 – 13.8)	0.2 (-1.6 – 2.6)	**23.9**	**-4.2 (-20.9 – -0.2)**	<-0.1 (-2.8 – 2.5)
Harpacticoida	6.3	---	---	12.2	---	---
Calanoida	**45.9**	**27.5 (4.7 – 141.7)**	1.7 (0.2 – 5.0)	**29.1**	**7.9 (0.8 – 44.2)**	0.9 (<0.1 – 2.5)
Cyclopoida	**36.5**	**5.7 (1.6 – 16.6)**	1.0 (0.1 – 2.5)	**31.9**	**-9.2 (-43.3 – -1.4)**	0.1 (-4.3 – 4.9)
Daphniidae	**31.5**	**6.3 (1.1 – 24.5)**	0.8 (-0.1 – 2.6)	11.9	---	---
Chydoridae	< 0.1	---	---	< 0.1	---	---
Bosminidae	**28.8**	**16.2 (1.3 – 129.3)**	1.4 (<0.1 – 4.6)	6.0	---	---
Sididae	**25.0**	**<-0.1 (<-0.1 – <0.1)**	0.5 (<-0.1 – 1.3)	**34.2**	**-0.1 (-0.5 – 0.2)**	0.7 (<0.1 – 1.6)
Macrothricidae	---	---	---	6.3	---	---
	Post-treatment to Recovery			**Pre-treatment to following June**		
Nauplii	**47.2**	**-22.7 (-134.0 – -3.2)**	-0.2 (-3.2 – 1.9)	1.0	---	---
Harpacticoida	8.2	---	---	5.8	---	---
Calanoida	12.6	---	---	10.1	---	---
Cyclopoida	**50.5**	**-67.7 (-443.3 – -9.6)**	-0.9 (-7.5 – 1.5)	4.6	---	---
Daphniidae	**46.6**	**-44.0 (-137.5 – -13.6)**	**-2.9 (-13.2 – -0.1)**	1.8	---	---
Chydoridae	0.1	---	---	1.6	---	---
Bosminidae	**38.1**	**-6.7 (-41.5 – -0.4)**	2.2 (-1.4 – 24.2)	0.0	---	---
Sididae	15.4	---	---	0.7	---	---
Macrothricidae	6.3	---	---	---	---	---

Macroinvertebrates

Our results indicated that treatment possibly resulted in increased species richness of aquatic macroinvertebrates (Table 12). Generally, richness increased in treatment ponds while richness declined or remained steady in control ponds. However, there was no significant effect of sample period on the overall richness of aquatic macroinvertebrates found during this study in either control ponds (χ^2=6.691, df=3, P=0.082) or treatment ponds (χ^2=2.884, df=3, P=0.410). Unfortunately, ponds that entered the study in 2003 were not sampled for aquatic macroinvertebrates prior to treatment in August 2002. Therefore, a total of four control ponds and three treatment ponds that entered the study in 2005 were sampled prior to treatment in August 2004 and had data for before-after comparison. Paired comparisons between treatment groups during each sample time indicated no significant difference during pre-treatment (P = 1.000); however, there was a significant difference in species richness between control and treatment ponds during each year following treatment ($P <$ 0.020). Based on the results of this study it is likely that aquatic macroinvertebrate species richness was higher overall in treatment ponds relative to control ponds throughout the duration of this study, but without pre-treatment data available for all ponds it is impossible to determine whether rotenone treatments had a significant impact on aquatic macroinvertebrate species richness.

The relative abundance of aquatic macroinvertebrates indicated a similar response as found for species richness (Table 12). Again, no significant effect of sample period was found on macroinvertebrate relative abundance in control ponds (χ^2=3.078, df=3, P=0.380) or treatment ponds (χ^2=0.724, df=3, P=0.868). In addition, a paired comparison between control and treatment ponds revealed no difference in macroinvertebrate abundance during pre-treatment (P = 0.724). However, the abundance of macroinvertebrates in treatment ponds was significantly higher than control ponds during Year 1 (P = 0.002), Year 2 (P = 0.021) and Year 3 (P = 0.011). Inspection of macroinvertebrate abundance among sample periods indicated that macroinvertebrate abundance in treatment ponds increased during each year following treatment relative to control ponds, which remained stable or declined subtly. The increase in macroinvertebrate abundance over time in treated ponds accompanied by the significantly higher abundance of macroinvertebrates in treated ponds relative to control ponds following rotenone treatments suggested a significant increase of aquatic macroinvertebrate abundance during this study. Both vertical and horizontal macroinvertebrate trap types followed the same pattern during this study with generally increasing abundance in treatment ponds and steady or declining abundance in control ponds. Thus, during this study there was no apparent difference in abundance of macroinvertebrates in pelagic water column traps relative to near-shore traps. Both trap types indicated no community level effect of rotenone treatments on macroinvertebrate relative abundance (P > 0.05).

Because the various trap types (i.e., vertical and horizontal) indicated similar responses of macroinvertebrates to rotenone treatments during this study the data were combined for RDA. RDA on the overall activity trap data indicated that the application of rotenone did not have a significant first-year effect on the overall invertebrate communities (F =1.234; P = 0.294) (Table 12). This first axis explained roughly 20% of the total variation and the second axis, representing differences among ponds, explained 49% of the total variation in change between sampling dates for relative abundance. The high variation explained by the second axis indicated that there was a large variation in macroinvertebrate abundance among study ponds.

Although there was no significant impact of rotenone treatments on the overall macroinvertebrate population abundance there were specific species that experienced significant changes in abundance. Based on the short term RDA odonata and amphipoda were the most influenced by rotenone treatments with over 40% of the variation in relative abundance being attributed to the treatment type axis multiplicative change for odonata abundance was -0.7 (-3.3 – 0.5), which indicated that the abundance of odonata roughly doubled on average during the first year following treatment. In addition, based on multiplicative change the relative abundances of belostomidae, curculionidae, dyticidae, halipidae, nepidae, and simulidae each increased during the first year in treatment ponds. The abundance of these species increased from 0.1 to 0.7 times during the first year following rotenone treatments. However, the variation among treated ponds was quite high for these species relative to odonata. Axis 2 was largely a gradient of variability in change in abundances of corixidae, poduridae, and hydrophilidae which were not affected by treatment but were highly variable among both treated and control ponds.

RDA on the overall activity trap data indicated that the application of rotenone also did not have a significant long term (August prior to treatment compared to August three years following treatment) effect on the overall invertebrate communities ($F = 0.125$; $P = 0.6220$) (Table 12). Axis 1 explained only 13% of the total variation and the second axis 63% of the total variation in change between sampling dates for relative abundance. Based on RDA a total of eight taxa had at least 20% of the variation explained by the treatment type axis (Table 13). However, only belostomatidae, curculionidae, dytiscidae, and hirunidae indicated a significant change in abundance from prior to treatment to the third year after treatment as indicated by 95% confidence intervals. Generally, the abundance of aquatic macroinvertebrate taxa increased by only 0.1 to 1.0 times on average from pre-treatment to the third year following treatment. During the same time period the abundance of macroinvertebrates in control ponds generally declined by 0.1 times.

Table 12. Macroinvertebrate composition based on species richness (number of species observed) and relative abundance (number/net) for macroinvertebrates collected from study ponds during 2002 through 2007. Samples were collected from eight locations in each pond including four offshore sets, in which traps were suspended vertically in the water column and four littoral sets, in which traps were suspended horizontally near aquatic vegetation nearer shore. Sampling was conducted twice during August of each sample year. Total numbers of macroinvertebrates were summed over all traps to estimate a number/trap for each sampling period. Samples were collected prior to treatment (Pre), and following treatment for three consecutive years (Year 1, Year 2, and Year 3). Overall values represent the average (standard error) over ponds within treatment groups. Wilcoxon test statistic P < 0.05 indicates significant differences among treatment groups. Kruskal-Wallis Statistics with P < 0.05 indicates at least one sample time was different for any given comparison.

	Short term	Long term			KW-Statistics	
	Pre-Treat	Year 1	Year 2	Year 3	χ^2	*P*
Species Richness						
Overall						
Treatment	10.3 (1.9)	11.0 (0.8)	12.6 (0.6)	11.6 (0.4)	2.884	0.410
Control	9.8 (0.9)	8.4 (0.5)	8.3 (0.5)	7.3 (0.5)	6.691	0.082
P	1.000	**0.022**	**0.002**	**0.001**	---	---
Relative Abundance						
Vertical						
Treatment	21.2 (11.3)	23.0 (3.9)	25.9 (8.9)	112.9 (93.0)	4.944	0.176
Control	31.5 (25.6)	3.7 (0.8)	11.3 (3.3)	6.8 (2.1)	2.800	0.424
P	0.724	**0.002**	0.118	**0.015**	---	---
Horizontal						
Treatment	24.5 (11.5)	19.7 (3.5)	34.0 (8.2)	27.5 (8.8)	0.529	0.913
Control	20.9 (13.7)	5.4 (1.5)	10.9 (2.8)	7.8 (2.1)	1.158	0.763
P	0.724	**0.003**	**0.011**	**0.028**	---	---
Overall						
Treatment	22.8 (11.4)	21.3 (3.5)	30.0 (7.2)	70.2 (50.3)	0.724	0.868
Control	26.2 (19.6)	4.6 (1.1)	11.1 (2.8)	7.4 (2.0)	3.078	0.380
P	0.724	**0.002**	**0.021**	**0.011**	---	---

Table 13. Results of RDA performed on the aquatic macroinvertebrate relative abundance (#/net). The analysis was performed on the changes in relative abundance of each taxon group in each pond between sample times for 1-year (pre-treatment to August following treatment) and long term (first year following treatment to third year following treatment). Pre-treatment sample collections were conducted the August prior to reclamation. Post-treatment samples were collected during August of the first year and third year following reclamation. Values represent the cumulative fit per species as a fraction of the total variance explained for each species by the first ordination axis (%) and the multiplicative change observed (95% confidence interval) in the abundance of taxa in the treatment and control ponds based on combined vertical and horizontal trap data. Multiplicative change represents how many fold greater was the abundance of each taxon in the after period relative to the before period. Multiplicative change was only shown for taxa with ≥ 20% of variation fit by axis 1. Confidence intervals that do not include one indicate a significant change between periods and are indicated by bold type.

	Year 1			Year 3		
		Multiplicative Change			Multiplicative Change	
Taxon	Axis 1% variation	Treatment	Control	Axis 1% variation	Treatment	Control
Amphipoda	43.0	-1.2 (-36.5 – 6.4)	1.6 (-1.7 – 17.0)	10.1	---	---
Belostomatidae	22.2	**<-0.1 (-0.1 – 0.1)**	0.0 (0.0 – 0.0)	33.4	**-0.1 (-0.6 – 0.3)**	0.0 (0.0 – 0.0)
Chaoboridae	0.2	---	---	8.3	---	---
Chironomidae	2.3	---	---	13.7	---	---
Corixidae	15.3	---	---	27.5	-1.0 (-18.2 – 3.9)	1.4 (-5.7 – 36.8)
Curculionidae	22.2	**0.1 (-0.3 – 0.5)**	0.0 (0.0 – 0.0)	22.2	**0.1 (-0.4 – 0.7)**	0.0 (0.0 – 0.0)
Dytiscidae	37.0	**-0.3 (-0.7 – <0.1)**	<-0.1 (-0.5 – 0.5)	93.7	**-0.4 (-0.7 – 0.2)**	<-0.1 (-0.1 – 0.1)
Ephemeroptera	0.1	---	---	6.4	---	---
Gyrinidae	1.2	---	---	1.2	---	---
Haliplidae	30.2	**-0.7 (-4.4 – 0.9)**	-0.1 (-0.8 – 0.6)	5.5	---	---
Hirunidae	0.8	---	---	46.6	**-0.1 (-0.7 – 0.3)**	0.1 (<-0.1 – 0.1)
Hydracarina	3.1	---	---	5.5	---	---
Hydrophilidae	17.0	---	---	17.0	---	---
Nepidae	22.2	**<-0.1 (0.1 – 0.1)**	0.0 (0.0 – 0.0)	---	---	---
Notonectidae	34.3	-0.7 (-8.6 – 2.4)	<0.1 (-0.1 – 0.2)	34.5	-0.4 (-4.0 – 1.6)	0.2 (-0.3 – 0.8)
Odonata	46.5	**-0.7 (-3.3 – 0.5)**	-0.1 (-0.4 – 0.1)	5.4	---	---
Odonata	46.5	**-0.7 (-3.3 – 0.5)**	-0.1 (-0.4 – 0.1)	5.4	---	---
Poduridae	17.9	---	---	12.5	---	---
Simulidae	22.2	**<0.1 (-0.1 – 0.1)**	0.0 (0.0 – 0.0)	4.1	---	---
Trichoptera	12.4	---	---	1.7	---	---

DISCUSSION

Control of undesirable fishes

As expected, rotenone reclamations during this study had an immediate population reducing effect on undesirable fishes in treated ponds. Following reclamation the richness, abundance, and biomass of undesirable fishes in treated ponds approached zero. In fact, the reduction in abundance of undesirable fishes from pre-treatment to the first year was 97.9%; biomass was reduced by 99.6%. However, as these percentages illustrate the reclamation of ponds with rotenone failed to completely eradicate undesirable fishes from all ponds. Undesirable fishes were captured in low numbers from 43% of treated ponds during the spring following reclamation.

The failure of rotenone reclamation efforts to achieve complete fish eradication is not unique to this study. Turner (1959) reported complete kills had occurred in just 72% of 32 Kentucky ponds treated during fall, which compared to 97% of 24 ponds having complete kills when treated during summer. The lower success rate encountered between summer and fall treatments was attributed to the availability of deep-water refuge during fall, which was considered unavailable during summer due to stagnation. Apparently, variation in environmental conditions at the time of rotenone application can have tremendous influence on treatment effectiveness. Physical pond characteristics such as incomplete coverage or inadequate dispersion of toxicants in shallow and deep water or in pond borders, water temperatures being too high or too low, the presence of a strong thermocline, high turbidity, the dilution of toxicants by untimely rains, and/or failure to treat isolated waters have all been attributed to reduced rotenone effectiveness (Post 1958). Also, soft or mucky bottoms that permit fish to burrow in to escape the toxicant can result in incomplete fish eradication. In the latter case, fish tend to seek refuge in dense beds of submersed aquatic vegetation, in burrows made by a variety of aquatic animals, or in springs, seeps or inflowing streams (Lennon et al. 1971). Although physical pond characteristics could not all be completely controlled during this study the treatment of ponds during fall overturn and after plant growth had subsided was expected to alleviate some of the physical factors that would limit reclamation success. Furthermore, the application of rotenone during late fall after water temperatures had cooled also enabled an extended period of maintaining appropriate rotenone concentrations because degradation rates were slower in cooler water (Gilderhus et al. 1988).

Biological factors could also have contributed to reduced rotenone reclamation success and may have been an issue during this study. Biological factors included resistance of some species or individuals even to strong doses of toxicants, re-invasion of fish through overflows or from other bodies of water in the watershed, and illegal or unauthorized activities (Lennon et al. 1971). During this study, undesirable fish species captured in spring following reclamation included Fathead Minnow, Yellow Perch, and Pumpkinseed. Consequently, Fathead Minnow have been reported to demonstrate strong tolerance to rotenone and the fishes presence was not unlikely; however, Yellow Perch, and Pumpkinseed were considered only moderately tolerant to rotenone (Jenkins 1956). Surprisingly, Black Bullheads, which are considered a species most tolerant to rotenone were not observed in any treatment pond during spring following reclamation. Comparatively, Black Bullheads were encountered in each of the eight control ponds during spring post-treatment sampling. The absence of highly tolerant fish species and the presence of less tolerant fish species during spring following treatment in three of the seven ponds may have indicated natural or unauthorized stocking between reclamation and spring sampling.

Although reclamations during this study did not establish complete fish eradication, the complementary stocking of Walleye fry into treated ponds did likely contribute to undesirable fish control. After three years of Walleye fry stocking the biomass of fishes in treatment ponds remained more than 90% reduced on average relative to pre-treatment. Similarly, Meronek et al. (1996) reported that nearly two-thirds of biomanipulations that relied on a combination of rotenone treatments and predator stocking were considered successful compared to only 48% and 24% of biomanipulations using just rotenone or fish stocking, respectively.

Walleye production

Walleye production was highest during the first year following reclamation in treated ponds. Based on the total production quota for Walleye by the MNDNR during 2008 (126,715 lbs) and the total surface area utilized for Walleye production (22,668 acres) a yield threshold of 5.6 lbs/acre would need to be averaged on a statewide basis to have achieved production quota goals. On average a yield of 5.6 lbs/acre was considered successful production. Walleye production in rotenone treated ponds during this study were considered successful during the first and second year following treatment with yields exceeding 5.6 lbs/acre; however, Walleye production declined below the 'success threshold' during the third year following treatment when only 1.3 lbs/acre were produced. Since most treated ponds had re-established populations of undesirable fishes and contained carry over Walleye by the third year following treatment, Walleye production was likely negatively influenced. Thus, this study suggested that rotenone reclamation efforts were needed every two to three years to maintain successful Walleye production ponds.

Pond ecology

The reclamation of ponds and stocking of Walleye fry during this study, although resulting in the reduction of undesirable fishes by over 90% after three years, did not provoke a wide range of statistically significant ecological changes in ponds. These results contradicted many previous studies which had indicated that biomanipulation to remove undesirable fishes often resulted in a cascading changes to the entire pond ecosystem (Zimmer et al. 2001; Welch et al. 2003; Herwig et al. 2004; Potthoff et al. 2008). Typically, populations of phytoplankton and submergent aquatic macrophytes characterize the productivity of ponds. Some ponds are dominated by submergent aquatic macrophytes and often demonstrate high water clarity. Conversely, other ponds have been dominated by phytoplankton, maintained with limited submergent aquatic macrophytes, and demonstrate turbid conditions. The existence of aquatic ecosystems having multiple states of equilibrium has been coined "alternative stable states." Most research on the existence of alternate stable states of pond communities has indicated that aquatic macrophytes are important to the maintenance of the clear-water state (Scheffer et al. 1993; Weisner et al. 1997; McGowan et al. 2005). Apparently, the most important mechanism that determines which state (clear or turbid) a pond exhibits has been the topic of much research (e.g., Jeppesen et al. 1990; Scheffer et al. 1993), but robust and stable submerged plant communities are generally agreed upon as important for maintaining long term persistence of clear water conditions (Bayley and Prather 2003). Most importantly, submergent plants maintain a clear water condition through reduced sediment suspension and competition for nutrients with phytoplankton (van Donk and van de Bund 2002). Therefore, any improvements to the abundance of aquatic macrophytes during this study would have been expected to influence the trophic state of ponds.

Submergent macrophytes in treated ponds during this study actually did demonstrate a remarkable increase in richness and occurrence. In fact, the overall average frequency of occurrence in treated ponds increased from roughly 50% of transects prior to treatment to 100% of transects after three years - indicating complete coverage of submergent macrophytes in ponds. Specifically, aquatic macrophyte species such as bushy pondweed, coontail, flatstem pondweed, and sago pondweed exhibited greatest improvement. Interestingly, each submergent macrophtye species demonstrated various response timing. For example, sago pondweed immediately expanded during the first year following reclamation while coontail had a delayed response with higher occurrence each year post-reclamation. This indicated that as time elapses following biomanipulations that the response of aquatic macrophyte communities changed. Similarly, King and Hunt (1967) reported that Common Carp selectively effected chara and leafy pondweeds and that certain macrophyte species increased in dispersion within two months of Common Carp removal, which indicated species-specific responses. Hansel-Welch et al. (2003) also reported that the macrophyte composition in ponds changed over time following biomanipulation in prairie ponds.

Although only over a period of three years this study likely also captured some response changes to the aquatic macrophyte community over time following rotenone reclamations.

In many cases, fish introduce a disruptive role in the maintenance of sound aquatic macrophyte communities in ponds, which apparently influences the trophic state of ponds and induces higher abundance of phytoplankton. Although fish biomass was reduced and aquatic macrophtye abundance significantly increased during this study there was no corresponding significant reduction in phytoplankton abundance based on Chl-*a* in treated ponds. With that under consideration, the algal abundance marginally declined each year following reclamation in ponds and was roughly 50% lower in the third year relative to pre-treatment. Hypothetically, the algal populations in treatment ponds may have shifted from being dominated by smaller green algae to larger blue-green algae. A shift in composition of various algae types could result in similar Chl-*a* concentrations, but water clarity would likely improve due to higher light penetration allowed by the larger-particle blue-green algae dominance. Unfortunately, algae were not identified during this study.

The chemical properties of ponds also did not differ substantially between control and treatment ponds. Total phosphorus concentrations in the water column increased insignificantly in treated ponds during the first year, but then declined throughout the remainder of the study. Some studies have reported a decrease in total phosphorus in the water column of ponds following the removal of Fathead Minnow, which has been attributed to the rapid cycling of phosphorus from various source pools (e.g., Zimmer et al. 2001). However, Potthoff et al. (2008) reported similar results as this study for total phosphorus in a biomanipulation study. In Potthoff's study Walleye were stocked into ponds with existing populations of Fathead Minnow. Although no changes were detected for total phosphorus there were higher concentrations of total dissolved phosphorus in ponds stocked with Walleye fry, which was attributed to phytoplankton shifting from a nutrient to a grazer limitation due to increased zooplankton abundance. Apparently, the phosphorus was re-apportioned to various forms due to changes in the food web of the ponds, which would not have been inferred from estimates of total phosphorus solely (Elser et al. 2000). Therefore, there may have been phosphorus re-appropriation dynamics during this study that confounded the analysis of total phosphorus. At any rate, the immediate increase in total phosphorus during the first year after reclamation of this study was followed by a gradual decline to pre-treatment levels. The total phosphorus dynamics reported here may have resulted from a release of phosphorus of fish biomass following rotenone reclamation and then gradual fixation by increasingly abundant aquatic plants.

Although total phosphorus did not demonstrate significant reductions, the water clarity marginally improved following rotenone treatments during this study. Secchi depth increased an average of 13 inches in treated ponds while the total suspended solids declined roughly 85% from pre-treatment to the third year. Although not statistically significant, the marginal increases in water clarity during this study may have been due to reductions in turbidity from re-suspension of solids caused from benthivorus fish activities (e.g., Black Bullhead). Scheffer et al. (2003) reported that sediment re-suspension from wind was augmented when coupled with decreased stability of sediments due to feeding activities of benthivorous fish, or the lack of aquatic macrophytes. Furthermore, Zimmer et al. (2006) reported that detritus was a major diet component of Fathead Minnows in shallow wetlands and that subsequent excretion represented a major nutrient flux from wetland sediments to the water column. Therefore, the removal of fish likely had direct water clarity improving impact that was expected to sustain higher aquatic macrophyte abundance due to improved light penetration in ponds.

Given the improved conditions of ponds including improved water clarity and expanded macrophytes it could be assumed that aquatic macroinvertebrates would have increased in abundance due to the improved habitat conditions. However, during this study the richness and abundance of aquatic macroinvertebrates did not differ significantly among sample periods in either treatment, but differences were observed between treatment groups during post-treatment periods. Melaas et al. (2001) reported similar results for prairie wetlands treated with rotenone. In that study the overall results indicated no significant effect of rotenone on the long term abundance of aquatic invertebrates. Furthermore, a short term reduction in invertebrate abundance was followed by a rapid repopulation and recovery within the first year after treatment. During this study there was no sampling of aquatic invertebrates immediately after treatment, which made the detection of short term changes in invertebrate abundance impossible to ascertain. In addition, the lack of sampling in all ponds prior to treatment resulted in the inability to determine if the higher species composition and abundance in treated ponds relative to control ponds was a result of rotenone treatments or natural variation among ponds. In addition, most evidence suggested that rotenone treatments did not have a statistical significant impact on aquatic macroinvertebrate abundance. However, the results of this study suggested that treatment of ponds with rotenone had a positive effect on aquatic macroinvertebrate species richness and abundance because both increased in treated ponds relative to control ponds following rotenone treatments, although insignificantly.

Aquatic macroinvertebrates are also an important link between primary producers and vertebrate consumers in ponds in the prairie pothole region of southwestern Minnesota (Euliss et al. 1999). Reductions of herbivorous zooplankton through top down predation by planktivorous fish have been shown to negatively impact water transparency (Scheffer et al. 1993; Scheffer 1998), which can subsequently reduce macrophyte growth and aquatic invertebrate abundance. Studies of the rotenone influence on macroinvertebrates have indicated that populations can be substantially reduced (e.g., Burress 1982; Mangum and Madrigal 1999); however, Engstrom-Heg et al. (1978) and Dudgeon (1990) both indicated that macroinvertebrate sensitivity to rotenone was highly variable by species and depended heavily on their metabolic oxygen demand. Research has also demonstrated minimal impacts from rotenone applications on benthic invertebrates that live primarily in the sediments (e.g., Melaas et al. 2000). Mischke et al. (2001) suggested that the sediments, particularly those high in organic content, buffered benthic invertebrates from rotenone. Understanding the effects of rotenone on aquatic macroinvertebrates was important because they provide sustenance for pond dwelling organisms and migrating birds. Furthermore, aquatic macroinvertebrates are an important food resource for Walleye at certain times; thus play a role in the production of Walleye in natural rearing ponds (Herwig et al. 2004).

For many years, rotenone was not believed to have any substantial impact on invertebrate populations (Oglesby 1964); however, numerous studies have demonstrated the adverse acute impacts of rotenone introduction on zooplankton (e.g., Neves 1975; Serns 1979; Melass et al. 2001). During this study the treatment of ponds with rotenone significantly affected zooplankton population abundance and biomass. Overall, there was a decline in zooplankton abundance and biomass in all ponds from pre-treatment to post-treatment, which was likely due to short term toxicity of rotenone known to influence zooplankton populations, coupled with the natural seasonal chronology (decrease) in zooplankton as water temperatures and food resources declined. The latter mechanism also seemed to play a role as evidenced by the large decrease in control ponds as well. However, significantly lower abundance and biomass in treated ponds indicated this pattern was strongly influenced by rotenone application. Most importantly, the zooplankton abundance and biomass had achieved pre-treatment levels during the recovery period and there were apparently no long term effects of the rotenone application on zooplankton populations observed based on the similarities between control and treatment ponds in subsequent June samples. This was particularly important because a rebound in zooplankton populations was necessary to provide food for Walleye fry and waterfowl during the spring following reclamation.

Given the differences in life history between copepoda and cladocera it was also important to determine if there were different responses to rotenone treatments in ponds by the various zooplankton groups. Apparently, the timing of rotenone applications can be critical. MNDNR fisheries staff had noted the quick and complete recovery of zooplankton in ponds where the reclamations were completed during the late fall (J. Gorton, unpublished report; S. Fisher, unpublished data). In other studies (e.g., Kiser et al. 1963; Bandow 1980) and through MNDNR observations of spring, summer, and early fall reclamations, zooplankton recovery, particularly the copepoda appeared to be quite slow—taking years to achieve zooplankton recovery similar to that seen in late fall-reclaimed waters. Furthermore, zooplankton recovery does not occur after all fall reclamations. Late-fall egg deposition is a common trait of northern latitude zooplankton (Allan 1976). It has been suggested by DNR fisheries staff that the success of zooplankton recovery is related to the proportion of females that drop their egg cases prior to the fall reclamation. The presence of these eggs, presumably not affected by the rotenone treatment, may be critical to rejuvenating the decimated immediate post-reclamation zooplankton community. However, both copepoda and cladocera zooplankton responded similarly during this study indicating that fall treatments were not inhibiting to zooplankton populations.

Although no long term significant effect of rotenone treatments on zooplankton and macroinvertebrates was found during this study the increased occurrence of aquatic macrophytes in treated ponds during this study likely had a an important effect on other aquatic organisms including aquatic macroinvertebrates, amphibians, and waterfowl through improved habitat availability (Krull 1970). For example, star duckweed and coontail ranked first and third in importance to waterfowl as a food plant (Krull 1970). Given the significant increase in dispersion of coontail in treated ponds during this study there was likely a benefit to waterfowl following rotenone treatments. Subsequently, star duckweed may have benefited from fish removal in treated ponds because it was entirely absent from transects prior to reclamation but was observed in 11% of transects in the third year. Star duckweed has been shown to support a high ratio of aquatic organisms to plant weight that likely provided benefit to non-fish wildlife in treated ponds during this study even though the increase in occurrence was not considered statistically significant (Krull 1970).

This study demonstrated that rotenone treatments in shallow ponds in southern Minnesota obtained the primary objective of reducing undesirable fishes that may limit Walleye production and deteriorate overall pond condition. Subsequently, Walleye production was considered acceptable the first and second year following reclamations. Furthermore, the removal of fish biomass from ponds had a cascading effect that resulted in—at minimum—marginal reductions in phytoplankton abundance and improved water clarity, which enabled increased aquatic macrophyte abundance and dispersion. The zooplankton populations experienced an initial decline to near zero abundance, which was followed by a rapid recovery to pre-treatment abundance levels by the following spring. Macroinvertebrate abundance demonstrated increases from the beginning to the end of the study in treatment ponds that was not replicated in control ponds, which was most likely attributed to improved habitat availability. Thus, the rotenone application and fry stocking combination may have provided the means to alter the ecology of ponds to enable higher Walleye production and increased benefit to wildlife. Ultimately, the re-population of ponds with undesirable fish indicated that additional rotenone treatments would be necessary every two to three years to maintain a complete eradication of undesirable fishes in ponds, but that with the combined used of rotenone reclamation and Walleye fry stocking the influence of fishes on ponds could be minimized.

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