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Aquatic Vegetation, Largemouth Bass and Water Quality Responses to Low-dose Fluridone Two Years Post Treatment

MARY T. BREMIGAN[1,2], S. M. HANSON[1,3], P. A. SORANNO[1], K. S. CHERUVELIL[1] AND R. D. VALLEY[1,4]

ABSTRACT

Whole-lake techniques are increasingly being used to selectively remove exotic plants, including Eurasian watermilfoil (*Myriophyllum spicatum* L.). Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone), a systemic whole-lake herbicide, is selective for Eurasian watermilfoil within a narrow low concentration range. Because fluridone applications have the potential for large effects on plant assemblages and lake food webs, they should be evaluated at the whole-lake scale. We examined effects of low-dose (5 to 8 ppb) fluridone applications by comparing submersed plant assemblages, water quality and largemouth bass (*Micropterus salmoides*) growth rates and diets between three reference lakes and three treatment lakes one- and two-years post treatment. In the treatment lakes, fluridone reduced Eurasian watermilfoil cover without reducing native plant cover, although the duration of Eurasian watermilfoil reduction varied among treatment lakes. Large pre-treatment differences among lakes in plant cover persisted, reflecting morphometric differences. We detected no treatment effects on water quality, and estimated the probability of treatment converting a lake to eutrophic conditions as being ≤0.10. Growth of largemouth bass >200 mm total length did not change in treatment lakes and we detected few treatment effects on their diet. However, in two treatment lakes, growth of smaller largemouth bass increased modestly following treatment. Overall, the extent of effects of low-dose fluridone treatment differed among lakes, apparently reflecting differences in initial conditions that, in part, were driven by lake morphometry. Therefore, future evaluations should include more lakes, chosen to represent a range of morphometry, allowing extrapolation of findings to regions containing many diverse lakes.

Key words: Sonar™, Micropterus salmoides, whole-lake experiment, macrophytes, Eurasian watermilfoil, *Myriophyllum spicatum*, fish growth.

[1]Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824-1222.
[2]bremigan@msu.edu
[3]Current Address: Professional Lake Management, P.O. Box 132, Caledonia, MI 49316.
[4]Current Address: Minnesota Department of Natural Resources, Division of Fisheries, 1200 Warner Road, St. Paul, MN 55106. Received for publication March 5, 2005 and in revised form April 28, 2005.

INTRODUCTION

Manipulation of aquatic plants commonly occurs in lakes throughout much of North America, with the potential to indirectly affect water quality, sport fisheries, and food web structure. In large part, these efforts seek to control excess exotic plant growth, especially that of hydrilla (*Hydrilla verticillata* L.f.), water-hyacinth (*Eichhornia crassipes* Mart.), and our focus, Eurasian watermilfoil (hereafter EWM). Introduced to North America by the 1940s, EWM is now widely distributed in the United States and Canada where it has displaced native plants to varying degrees (Couch and Nelson 1985, Madsen et al. 1991, Madsen 1997, Cheruvelil 2004). The potential of EWM to displace native plants results in large part from its canopied growth form (Madsen et al. 1991). Below dense EWM canopies, light, oxygen, and pH are often reduced as compared to conditions in native plant beds (Carpenter and Lodge 1986, Madsen 1997). Further, EWM can affect habitat complexity for, and biotic interactions among, zooplankton, macroinvertebrates, and fishes (Keast 1984, Lillie and Budd 1992). For example, lower macroinvertebrate densities, biomass, and species richness are associated with the interior of dense EWM beds than with their edges (Keast 1984, Sloey et al. 1997, Cheruvelil et al. 2002). Dense monospecific canopies of EWM also leave little space within them for fish to maneuver and access prey (Lillie and Budd 1992, Engel 1995, Valley and Bremigan 2002a). In contrast, native plant beds, when containing a mosaic of basal, medial, and emergent growth forms, provide a variety of interstitial spaces throughout the water column for macroinvertebrate colonization, and where fish can access prey (Downing and Anderson 1985, Valley and Bremigan 2002a). Because EWM may sometimes be integrated with native species in plant beds, we would expect its negative effect to be somewhat moderated under this condition, but to our knowledge, research has not directly addressed this topic.

Public pressure to remove EWM can be strong because its canopies not only can affect ecological interactions among lake biota, but they also can impede recreational activities (Smith and Barko 1990) and adversely affect lake aesthetics. However, uncertainty regarding the effects of EWM removal on lake ecosystems leads to controversy. For example, in Michigan, use of the herbicide fluridone to control EWM has been steadily increasing since the late 1980s (N. Rathbun, Michigan Department of Environmental Quality, pers. comm.), but opinions vary widely regarding the risks associated with its use.

Fluridone is controversial in part because it must be applied at the whole-lake scale due to the extended contact time required for its effects on plants, and in part because of concern regarding its potential direct and indirect effects. Those in favor of treatment argue that at low doses (5 to 8 ppb), fluridone removes EWM while allowing native plants to persist, and may reduce lakewide plant cover from high to moderate levels, thus potentially increasing growth rates of prominent sport fishes, such as largemouth bass. Those opposed to treatment argue that fluridone may kill some native plants, that effects of treatment on reproductive success and early life history stages of fishes and other biota are inadequately studied, that subsequent decomposition of plants after treatment may lead to algal blooms and reductions in water quality, and that removal of EWM may not be warranted if it is integrated into native plant beds rather than existing as EWM monocultures. In reality, few of these hypothesized effects have been adequately evaluated.

The lack of documented findings regarding the effects of fluridone on lake ecosystems contributes to the controversy surrounding its use. The known effects of fluridone derive from a few whole lake studies using high doses (≥10 ppb) and laboratory experiments evaluating the direct effects of both high and low doses of fluridone on EWM and other plant species. Collectively, this laboratory and field research demonstrates that at doses ≥10 ppb fluridone is non-selective, resulting in large declines in native vegetation following treatment (Netherland et al. 1997, Welling et al. 1997, Pothoven et al. 1999, Schneider 2000). However, small-scale experiments show that at low doses (5 to 8 ppb) fluridone can kill EWM without adversely affecting the four native plant species investigated (Netherland et al. 1997). Laboratory results further suggest that whole-lake treatments of 5 ppb initially, with a concentration of >2 ppb maintained for >60 days, will be most effective in controlling EWM without harming native plants (Netherland et al. 1993, Netherland and Getsinger 1995, Netherland et al. 1997).

Because knowledge is lacking regarding effects of low-dose fluridone treatment on lake ecosystems, managers are limited in their ability to consider its implications for fisheries and water quality when developing plant management policy. For lakes dominated by EWM, the negative effects of this invasive species on lake ecosystems have been relatively well investigated (Lillie and Budd 1992, Engel 1995, Sloey et al. 1997). Thus, managers must compare the potential for negative effects of EWM to the unknown effects of whole-lake herbicide treatment with low-dose fluridone when seeking to develop scientifically sound policy regarding fluridone use across many lakes. Therefore, the direct and indirect effects of low-dose fluridone treatment must be investigated and results communicated to inform management and policy decisions.

Our collaborative study (with the US Army Engineer Research and Development Center and the Michigan Department of Environmental Quality, MDEQ) represents the first whole-lake evaluation of low-dose fluridone treatment. Our investigation is based on the experimental recommendation of 5 ppb initial fluridone concentration and 2 ppb concentration maintained for >60 days (Netherland et al. 1993, Netherland and Getsinger 1995, Netherland et al. 1997). Getsinger et al. (2002) and Madsen et al. (2002) provide detailed information on the herbicide applications and plant assemblages in our study lakes before, during, and one year after the low-dose fluridone treatment. Here, we build upon their findings by examining plant assemblages two years post-treatment, and by examining several lake response variables in addition to plants. First, we examine the response of water quality measures to fluridone treatment because of the concern that fluridone treatments may negatively affect water quality by increasing nutrients available to algae. Second, we examine the response of diet and growth of largemouth bass to fluridone treatment because bass are a socially and economically important game fish, and because largemouth bass are top predators and keystone species in lake food webs, so they are a useful indicator of changes in lake food webs. We ask two specific questions in this study:

1) *What are the effects of low-dose fluridone on aquatic plants and water quality one and two years post treatment?* We expected that the frequency of occurrence of EWM, but not of native plants, would decline following treatment and would last at least two years. We also expected an increase in pelagic algal biomass in response to reduction of EWM and subsequent release of nutrients.

2) *What are the indirect effects of low-dose fluridone treatment on largemouth bass growth rates and diet composition?* We expected that removal of EWM would improve largemouth bass growth through reductions in lake plant cover and/or conversion of any EWM canopies to structurally diverse, native plant beds (Crowder and Cooper 1979, 1982, Valley and Bremigan 2002a). Because a more structurally diverse native plant assemblage should provide better forage for largemouth bass, we expected bass diets to differ between treatment and reference lakes. In particular, we expected the amount of bluegill (*Lepomis macrochirus*) in largemouth bass diets to be greater in treatment than reference lakes, because a reduction of EWM in treatment lakes would reduce densely vegetated habitat in which bluegill could find refuge from bass predators.

MATERIALS AND METHODS

Study Design and Sample Lakes

In early 1997, a committee with representation from MDEQ, US Army Engineer Research and Development Center, and Michigan State University selected lakes for investigation of the whole-lake effects of low-dose fluridone treatment (see Getsinger et al. 2001 for full details). Initially, we selected four lakes that requested fluridone permits (treatment) and four lakes (reference) known to contain EWM and considered to be comparable to the treatment lakes in terms of location within the state, EWM presence, and lake surface area. The treatment lakes were treated with fluridone in mid-May 1997 with the goal of establishing a concentration of 5 ppb in the mixed layer (epilimnion) of the lake. A second booster treatment, aimed at restoring concentrations to 5 ppb, was applied 2 to 3 weeks later to maintain fluridone concentrations above 2 ppb for >60 days. Details of the fluridone treatments can be found in Getsinger et al. (2001 and 2002). In this manuscript, we restrict our analysis to six of the original study lakes. We dropped one treatment lake from the study because the target fluridone concentration was not reached; and we dropped one reference lake because it was treated with fluridone in 1999 (the second post-treatment year for our ongoing study).

The six study lakes are located in southeastern and southwestern Michigan. The lakes are mesotrophic and range in area from 50-200 ha (Table 1). The lakes range in mean depth from 2.2 to 7.3 m, and all stratify during the summer, but to varying degrees. Although all lakes contained EWM, they also contain a large number of other plant species (Madsen et al. 2002).

Sampling Methods

We sampled submersed aquatic vegetation in May 1997 (pre-treatment conditions) and August 1998 and 1999 (1- and 2-years post-treatment conditions), using point-based frequency of species occurrence to measure the whole-lake distribution of plants (Madsen 1999, Madsen et al. 2002). We sampled from 150 to 250 points per lake, with approximately 50 m between points except for Big Seven (40 m) and Lobdell (100 m) lakes. A grid of uniformly spaced sample points was overlain onto a lake map and located using a global positioning system. At each point, we determined species composition of plants, either visually (shallow sites) or by throwing a two-sided rake (deeper sites). We assigned each site with rooted vegetation to one of three mutually exclusive categories: only EWM present (EWM-only sites), both EWM and other, primarily native plant species present (mixed sites), and only other, primarily native plant species present (native sites). In the native category we included three exotic species (*Potamogeton crispus*, *Cabomba caroliana*, and *Najas marina*) to facilitate comparison of the effects of fluridone on EWM versus all other aquatic plants. Inclusion of these three exotic species should not alter interpretation of results because *Potamogeton crispus* was not common in our August sampling due to its proliferation during early summer, and because *Cabomba caroliana* and *Najas marina*, although exotic, typically do not form dense monospecific stands that alter littoral food webs, do not have a deleterious effect on native plant species, and were rare in our lakes (<1% of sampled points). We calculated the percent of sites lakewide that contained vegetation as the percent frequency of occurrence, which is a measure of plant percent cover. For each of the three plant categories, we also calculated the percent of vegetated sites that each comprised.

We sampled all water quality variables in the six lakes monthly during June to August 1998 and 1999 from a central sampling station located in the deepest area of each lake. Each time we measured Secchi depth (between 1000 and 1400 hours on the shady side of the boat) and we collected an integrated water sample of the epilimnion (estimated from temperature profiles) for total phosphorus (TP) and chlorophyll *a*. In the laboratory, we measured total phosphorus spectrophotometrically using the molybdate blue method (APHA 1992) and chlorophyll *a* fluorometrically after extraction in ethanol for 24 h (Yentsch and Menzel 1963, Sartory and Grobbelaar 1984). Temperature monitors placed at ~1 m depth in each lake recorded surface water temperatures from May 1 to September 31.

We collected largemouth bass in the six lakes during night electrofishing (120 volt pulsed DC) in spring 1998 to 2000. Transects averaged ten minutes in duration and were conducted parallel to shore across a variety of habitats. Each lake survey began shortly after dusk and continued until we captured approximately 50 largemouth bass. We collected 30 to 123 bass per survey. For all captured largemouth bass, we recorded length and weight, and collected approximately ten scales from below the lateral line and posterior to the pectoral fin. In the lab, we mounted largemouth bass scales for age and growth analysis and measured scale incremental growth distances using an Optimas 6.5 image analysis system and a compound microscope and camera at 20× magnification.

We back-calculated the lengths at previous ages for largemouth bass using the Fraser-Lee method (Carlander 1982). A common intercept value for the back-calculations was esti-

TABLE 1. TREATMENT HISTORY, PHYSICAL FEATURES, FLURIDONE CONCENTRATIONS FOR THIS STUDY, AND SAMPLING REGIME FOR STUDY LAKES. ALL LAKES ARE LOCATED IN SOUTHERN MICHIGAN AND ARE OF GLACIAL ORIGIN.

Lake	Previous fluridone treatment	Ref/Trt	Surface area (ha)	Mean depth (m)	Total Phosphorus[a] (µg/L)	Fluridone concentration (ppb)[b]		Sampling schedule[e]		
						1 DAIT[c]	30 DABT[d]	1997	1998	1999
Big Seven	None	Ref	64	3.2	16.1			P	P, WQ	P, WQ, D
Clear	None	Ref	73	2.2	18.0			P	P, WQ	P, WQ
Heron	None	Ref	53	3.4	14.3			P	P, WQ	P, WQ, D
Big Crooked	None	Trt	64	4.5	21.2	3.78 (0.7)	3.47 (0.1)	P	P, WQ	P, WQ, D
Camp	None	Trt	54	7.3	24.4	4.20 (1.5)	2.86 (0.1)	P	P, WQ	P, WQ, D
Lobdell	1991, 1992	Trt	197	2.7	15.7	5.50 (2.0)	2.76 (0.2)	P	P, WQ	P, WQ

[a]Data are averages from monthly samples (June, July, August) for 1998 and 1999 combined.
[b]Estimation of the fluridone application rate is based on the assumption that the chemical will be mixed throughout the epilimnion only and will not mix into the hypolimnion. Application rates were estimated assuming a thermocline depth of 3.05 m (10 feet).
[c]DAIT = days after initial treatment. Values in parentheses are standard errors, n = 6.
[d]DABT = days after booster treatment. Values in parentheses are standard errors, n = 6. Data from Getsinger et al. 2002.
[e]P = plant sampling, WQ = water quality sampling, D = largemouth bass diet sampling. Largemouth bass were collected in all lakes during 1997, 1998, and 1999 to estimate growth rates during 1995-1999.

mated for all largemouth bass captured from all six lakes. Then, for each largemouth bass, we estimated a back-calculated length (mm) at each scale annulus and determined annual growth increments (mm/yr) by the difference in estimated largemouth bass total lengths between consecutive annuli. We tested for Lee's phenomenon, wherein younger fish from a sample would appear to be exhibiting greater growth than fish of the same age from an earlier year-class (DeVries and Frie 1996). Although no strong Lee's phenomenon was evident, we restricted growth analysis to back-calculated lengths-at-age up to four years prior to capture, and growth years from 1995 to 1999.

To determine if largemouth bass diets differed between reference and treatment lakes, we compared bass diet data between two reference lakes (Big Seven, Heron) and two treatment lakes (Big Crooked and Camp) during summer 1999 (two years post treatment). We collected largemouth bass diet samples monthly from June to September during night electrofishing using an electric water pump to flush stomach contents and preserving samples in 95% ethanol (for bass >150 mm TL) or dry ice for most bass <150 mm TL. We sampled approximately 150 to 300 bass in each lake for diet analysis.

In the lab, we measured and identified diet items to species for fish, and to order for crayfish and invertebrates. Some further distinctions in identification were made below order for some invertebrates to provide more detailed information for length-dry weight regressions. We estimated dry weight from length of diet items using length-weight regressions (G. G. Mittelbach, Michigan State University, unpubl. data, and R. D. Valley, unpublished data).

Data Analysis

Due to differences in data collection, we analyzed our four groups of variables (plants, water quality, largemouth bass growth and diet) differently, but we used SAS for all analyses (SAS Institute, Inc. 1990). We evaluated plant response to treatment using repeated measures ANOVA (von Ende 1993), comparing patterns between treatment and reference lakes immediately before (May 1997) and 1- and 2-years following treatment (August 1998 and 1999). Sampling time for plants changed from May (pre-treatment, 1997) to August (post-treatment, 1998 and 1999) due to constraints of working within a real-world management context; lakes had not been chosen by August 1996, and postponement of treatment by a year, to allow pre-treatment sampling in August 1997, was not an option. Our statistical analysis is robust to this constraint because repeated measures analysis compares patterns over time between reference and treatment lakes. In other words, a difference in the May 1997 to August 1998 comparison between reference and treatment lakes would be indicative of a treatment effect and could not be an artifact of the sampling periods.

For water quality, because there were no pre-treatment data, we compared reference and treatment lakes in post-treatment years using one-tailed t-tests on summer means 1 and 2 years after treatment to determine if TP and chlorophyll concentrations were higher, and Secchi depths lower, in treatment lakes compared to reference lakes. In addition, we interpreted our results from a Bayesian perspective (Ellison 1996) by calculating the probabilities that low-dose fluridone treatment could cause unacceptably large changes in water quality. Using a noninformative prior where all outcomes have equal probability, the posterior distribution is equivalent to the probability for a one-tailed t-test centered around the mean observed difference, in our case, the difference between treatment and reference lake means. We used these distributions to calculate the probability that an unacceptably large change in water chemistry (i.e., conversion from generally mesotrophic to generally eutrophic conditions) would occur as a result of treatment. To do so, we calculated the posterior probability, given our observed results, that treatment lakes (post-treatment) would have: TP concentrations ≥25 μg/L, chlorophyll ≥10 μg/L, or Secchi depths ≤2.5 m.

For largemouth bass growth, we compared relationships of bass annual growth increments (mm/year) as a function of back-calculated total length (mm) across levels of treatment (reference and treatment) and time (pre- and post-treatment) using a mixed ANOVA model. Fixed variables in the model were treatment and time, and the random variable was lake.

For largemouth bass diet composition, we divided the fish into four size groups, representing initial spring 1999 sizes (75 to 149 mm, 150 to 224 mm, 225 to 299 mm, and ≥300 mm TL) that each roughly corresponded to one growing season. We compared the percent composition of gut contents between reference and treatment lakes using t-tests (based on the grand mean from four monthly sampling events) for each largemouth bass size group. In addition, we used the Fish Bioenergetics 3.0 model (Hanson et al. 1997) to estimate the consumption rate (expressed as proportion of maximum possible daily consumption) that would have produced the observed annual growth increment in each lake in 1999. The bioenergetics model incorporates species-specific physiological information with observed temperature regime, diet composition, and caloric content of prey groups to estimate the consumption rate of fish, given an observed growth increment. Prey caloric content was based on values presented in Cummins and Wuycheck (1971) and Pope et al. (2001). For each of the three largest largemouth bass size class, we compared estimates of the proportion of maximum daily consumption between treatment and reference lakes using t-tests. Bioenergetics analysis requires diet information from throughout the growing season. For several lakes, bass from the smallest size class were only collected in appreciable numbers on two of the four sampling dates. Therefore, bioenergetics analysis was not conducted for the smallest bass size class. Throughout all analyses, we use 0.10, rather than 0.05, as our level of significance for Type I error to account for our small sample size and hence to increase our statistical power (Peterman 1990).

RESULTS

Direct Effects of Low-dose Fluridone on Plants

Percent frequency of occurrence of aquatic plants ranged from 40 to 95% across lakes (Figure 1A). These differences in plant cover among lakes were mainly due to depth differ-

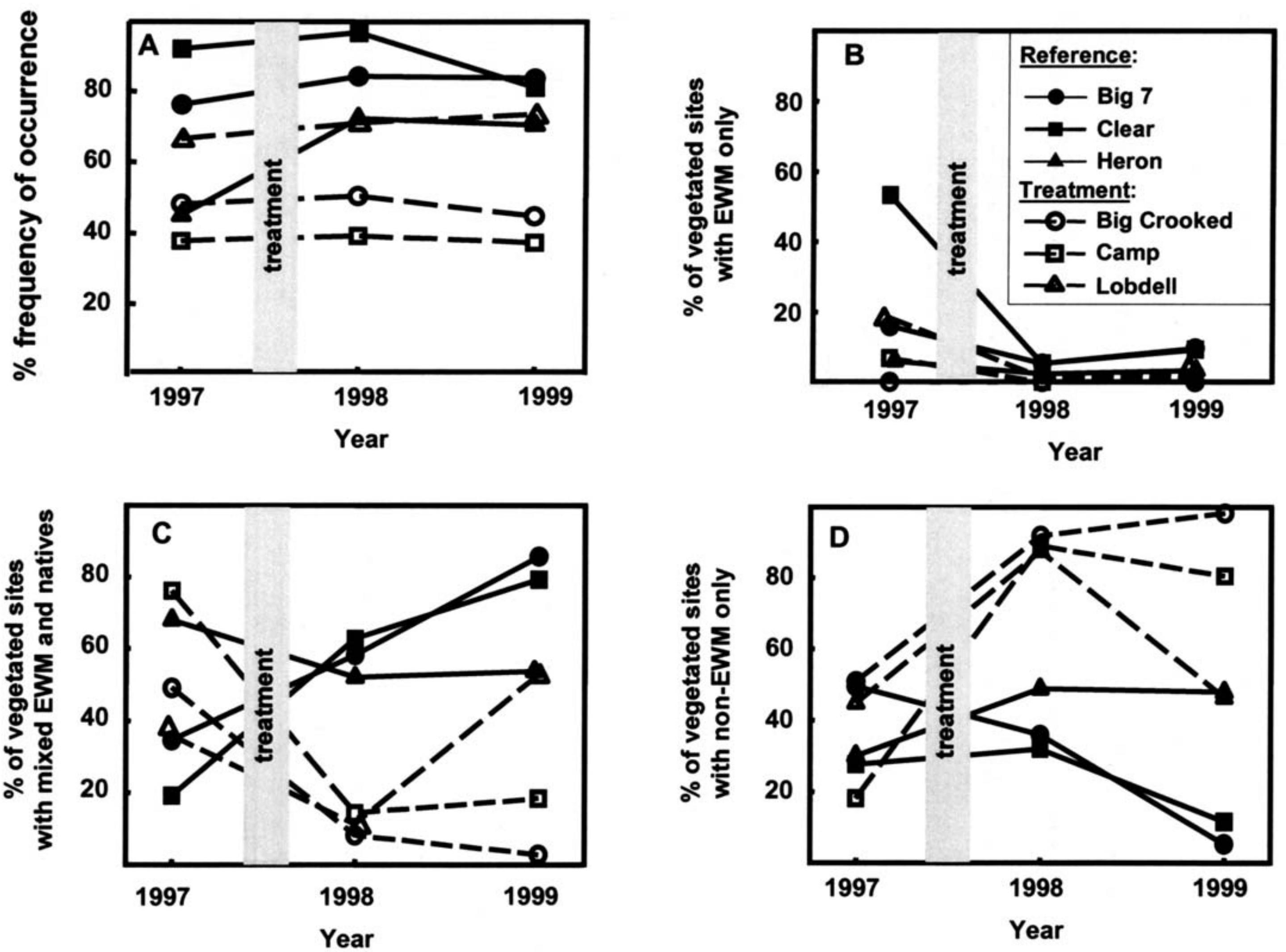

Figure 1. Direct effects of low-dose fluridone treatment on frequency of occurrence of aquatic plants. Results of repeated measures ANOVA are in Table 2. Panel A represents percent of all points (lakewide) sampled at which rooted aquatic plants were present (% frequency of occurrence). Considering only vegetated sites, we compare: (B) the % of EWM-only sites, (C) the % of mixed sites, and (D) the % of native plant sites.

ences, given that for all lakes, the majority of littoral zone sites (84-94%) were vegetated (Cheruvelil, unpubl. data). Repeated measures analysis of variance showed that low-dose fluridone application selectively reduced EWM cover in treatment lakes, without adversely affecting percent occurrence of native plants (Table 2). Prior to treatment, EWM-only plant beds were relatively uncommon (<20%) in most lakes. Rather, EWM and native plants occurred together in mixed beds. Therefore, the effect of treatment was primarily that of conversion of mixed sites to native sites, rather than a change in the overall percent frequency of plant occurrence.

Treatment lakes tended to have lower percent frequency of occurrence (all rooted plants combined) than did reference lakes both before and after treatment, although this difference was not statistically significant (Table 2, Figure 1A). Although percent frequency of plant occurrence changed over time, the treatment*time interaction was not significant, indicating that temporal patterns did not differ between reference and treatment lakes. Considering specific time intervals, the change in percent frequency of occurrence during the first time interval was significant, although the treatment*time interaction was not. No significant changes occurred between the first and second year post treatment.

The percent frequency of occurrence of EWM-only sites did not vary between treatment and reference lakes or over time (Table 2, Figure 1B). The percent frequency of occurrence of mixed sites differed between reference and treatment lakes, with a higher percent of mixed sites in reference lakes, particularly during the post-treatment years (Figure 1C; Table 2). During the first time interval, the time*treatment interaction was significant, indicating that changes in the percent of mixed sites differed between reference and treatment lakes. In the treatment lakes, percent of mixed sites fell to below 20% in 1998, the first post-treatment year, whereas values in the reference lakes remained steady or increased, ranging 50 to 70% (Figure 1C). Changes did not occur during the later time interval. The percent frequency of occurrence of native sites differed between reference and

TABLE 2. MULTIVARIATE REPEATED-MEASURES ANALYSIS OF VARIANCE FOR EACH OF THE FOUR PLANT METRICS IN THE SIX STUDY LAKES. THE TREATMENT EFFECT IDENTIFIES DIFFERENCES BETWEEN LAKES TREATED WITH FLURIDONE AND UNTREATED REFERENCE LAKES. THE TIME EFFECT IDENTIFIES CHANGES IN PLANT ASSEMBLAGES OVER THE THREE YEARS OF THE STUDY. A SIGNIFICANT INTERACTION TERM IDENTIFIES A DIFFERENT RESPONSE OF PLANT ASSEMBLAGES OVER TIME BETWEEN TREATMENT AND REFERENCE LAKES. "DIRECTION OF CHANGE" INDICATES THE DIRECTION OF CHANGE (POSITIVE OR NEGATIVE) IN THE PLANT METRIC FOR THE TREATMENT LAKES RELATIVE TO THE REFERENCE LAKES. FOR EACH METRIC WE PERFORMED AN ANALYSIS OF CONTRASTS, WHICH TESTS FOR TIME AND TIME*TREATMENT EFFECTS DURING EACH TIME INTERVAL. INTERVAL 1 COMPARES MAY 1997 (PRE-TREATMENT) TO AUGUST 1998 (ONE YEAR AFTER TREATMENT). INTERVAL 2 COMPARES AUGUST 1998 TO AUGUST 1999 (TWO YEARS AFTER TREATMENT). TO CONTROL FOR THE MULTIPLE COMPARISONS BEING DRAWN (I.E., TWO TIME INTERVALS WITHIN EACH ANALYSIS), WE DIVIDED 0.10/2 TO ACHIEVE A SIGNIFICANCE LEVEL OF 0.05 FOR THE ANALYSIS OF CONTRASTS. SIGNIFICANT EFFECTS ARE IN BOLD.

	All data		Direction of change	Specific time intervals	
	F statistic	p-value		Interval 1 (1997-1998) p-value	Interval 2 (1998-1999) p-value
% frequency of occurrence			none		
Treatment	4.37	0.105			
Time	7.27	**0.071**		**0.037**	0.260
Treatment*Time	3.58	0.161		0.097	0.407
% EWM only			none		
Treatment	3.95	0.118			
Time	4.25	0.133		0.057	**0.032**
Treatment*Time	0.09	0.917		0.653	0.770
% natives and EWM			–		
Treatment	19.11	**0.012**			
Time	3.98	0.143		0.219	0.193
Treatment*Time	5.14	0.108		**0.035**	0.860
% natives			+		
Treatment	15.12	**0.018**			
Time	13.13	**0.033**		**0.012**	0.160
Treatment*Time	6.47	**0.082**		**0.017**	0.720

treatment lakes as well (Figure 1D; Table 2), with generally higher values in treatment lakes, particularly in 1998. Values also varied over time. In particular, during the first time interval, both the time and time*treatment interaction effects were significant (Table 2). The frequency of native sites increased to >80% in the treatment lakes between 1997 and 1998, whereas it remained steady at 30 to 50% in the reference lakes. This difference between reference and treatment lakes in 1998 persisted through 1999. However, the duration of fluridone effects on plants varied among the three treatment lakes. In two of the treatment lakes (Big Crooked and Camp), EWM appeared to be suppressed for two full years following the year of treatment, whereas in the third treatment lake (Lobdell), the plant assemblage appeared to return to pre-treatment conditions by the second year following treatment (Figure 1C, D).

Indirect Effects of Low-dose Fluridone on Water Quality

We did not detect statistically significant differences in water quality between reference and treatment lakes during the two years following treatment as measured by TP, Secchi depth and chlorophyll *a* (Figure 2). Although visual inspection suggests a pattern of higher total phosphorus (TP) and chlorophyll *a* values in two of the treatment lakes (Big Crooked and Camp) relative to the reference lakes, these patterns were not statistically significant. To examine these results in more detail, we calculated the probability that the treatment lakes would switch from mesotrophic to eutrophic conditions following treatment for our three measures of water quality. We found that the probability of a lake switching from mesotrophic to eutrophic conditions was generally low for all measures. The probability that TP would increase above 25 μg/L was 0.10, that chlorophyll would increase above 10 μg/L was 0.09 and that Secchi depth would decrease below 2.5 m was 0.02.

Indirect Effects of Low-dose Fluridone on Largemouth Bass

We found that largemouth bass growth increments significantly increased in the treatment lakes during post-treatment years, but only for relatively small bass (<200 mm TL; Figure 3). Specifically, the mixed ANOVA model documented a significant three way interaction effect of back-calculated total length*time*treatment ($p < 0.001$) on annual growth increment, confirming that bass growth increments in reference and treatment lakes differed across time periods, with the magnitude of the effect dependent on fish length. Overall, we detected a moderate increase in growth of small bass in treatment lakes. For example, the predicted annual growth increment for a largemouth bass starting at 100 mm TL in spring was similar (55 mm) between reference and treatment lakes prior to treatment. After treatment, the predicted annual growth increment in treatment lakes increased to 61 mm, representing a 12% increase from pre-treatment conditions.

Diet composition varied among largemouth bass sizes classes and study lakes, but we found no consistent differences between reference and treatment lakes (Figure 4; based on 20 individual t-tests). Zooplankton comprised only a minor component of the diet, never exceeding 10%. The percent of crayfish was variable, but generally low, never exceeding 20%.

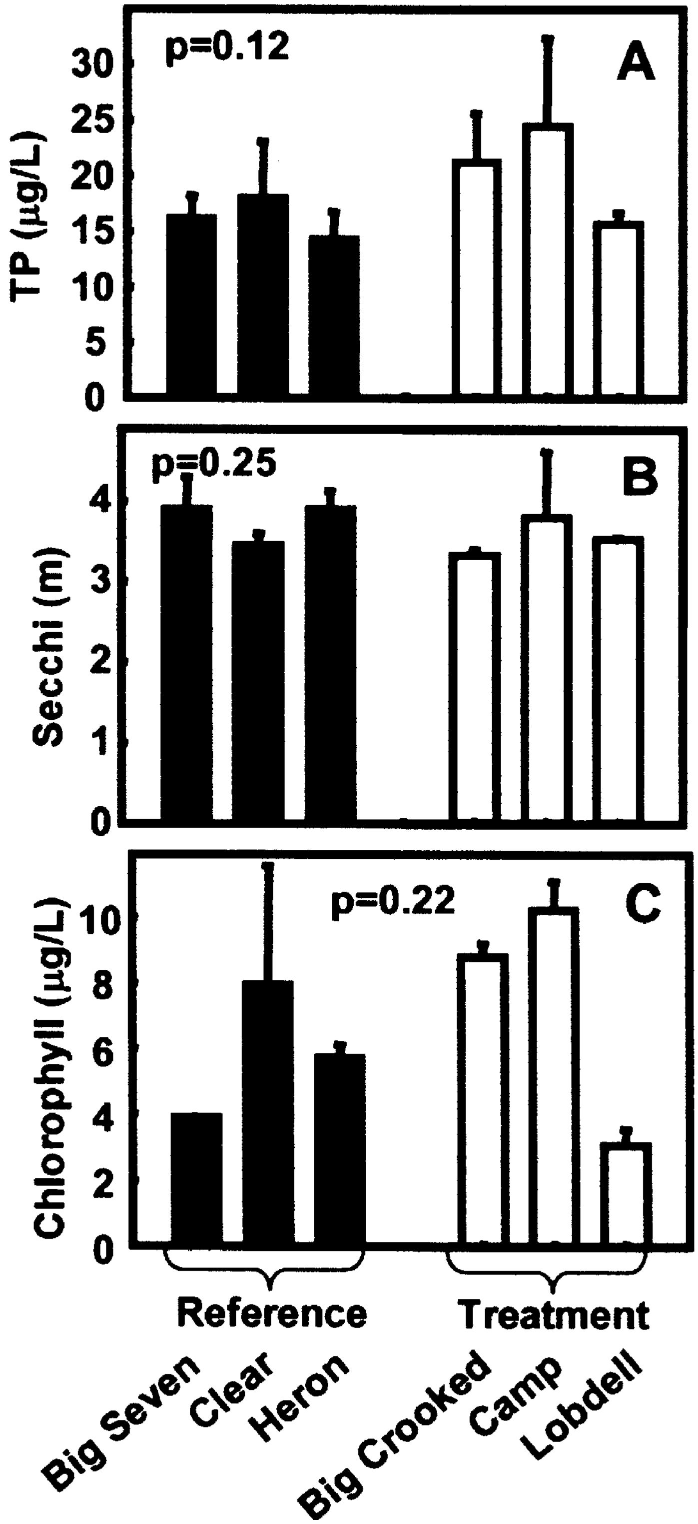

Figure 2. Water quality characteristics of sampled lakes (A-C). One tailed t-tests were used to compare mean annual values between treatment and reference lakes during the two years following low-dose fluridone treatment.

Macroinvertebrates (primarily dipterans and ephemeropterans) typically comprised the largest percentage of the diet, ranging 25 to73%. Fish (bluegill, cyprinids, yellow perch (*Perca flavescens*), and largemouth bass) comprised approximately 40% of diets, with the relative amounts of bluegill versus other fishes being quite variable. T-tests comparing diet proportions between treatment and reference lakes generally showed that diet composition did not differ between treatment and reference lakes (p-values typically >0.35). Only two t-tests yielded statistically significant differences, both for the 300 mm TL size group. For this size group, bluegill comprised a larger proportion of the diet in reference lakes than treatment lakes ($p < 0.07$), contrary to expectations. In addition, other fish comprised a larger proportion of the diet in treatment than in reference lakes ($p < 0.047$). Bioenergetics estimates of consumption rate (expressed as proportion of maximum possible daily consumption) did not vary between treatment and reference lakes. Proportion of maximum consumption ranged 0.41 to 0.47 for the 150 mm TL size group, 0.37 to 0.41 for the 225 mm TL size group, and 0.34 to 0.37 for the 300 mm TL size group, but did not vary predictably between reference and treatment lakes (t-test p-values >0.30).

DISCUSSION

We evaluated whole-lake low-dose fluridone treatments for reducing EWM by quantifying the direct effects of treatment on plant assemblages and the indirect effects on water quality and largemouth bass diet and growth. We found that the direct effects of low-dose fluridone on plant assemblages matched predictions from previous small-scale experimental work (Netherland et al. 1997). Eurasian watermilfoil frequency of occurrence was reduced for at least 1 to 2 years in treatment lakes, without a decline in the frequency of occurrence of native plants. Overall, indirect effects of low-dose fluridone treatment were of relatively limited magnitude. There was no statistically significant effect on water quality parameters, although a non-significant trend toward higher phosphorus and chlorophyll levels in treatment lakes was noted. Growth rates of largemouth bass <200 mm TL increased about 12%, but few differences between reference and treatment lakes in diets of bass >75 mm TL were detected. Taken together, our results indicate that in conditions similar to those of our study, low-dose fluridone treatment may be an effective management tool for short-term (one to two year) reduction of EWM prevalence without negatively affecting native plants, water quality, or largemouth bass growth. Interpretation of our results to inform lake management policy for a wider variety of lakes and circumstances must thoroughly consider the context within which our study was conducted (and hence within which its results can be extrapolated), and must identify the critical uncertainties that remain. We highlight these below.

Direct Effects of Low-dose Fluridone on Plants

Overall, the magnitude and duration of effects of whole-lake fluridone treatment are likely to depend on a combination of factors, including fluridone concentration and initial lake conditions, such as plant species composition, percent plant cover, lake depth and trophic status. Our study sought to determine whether reductions in EWM persisted for two years following treatment because the MDEQ plans a three-year cycle for fluridone treatments in Michigan. Our results indicate

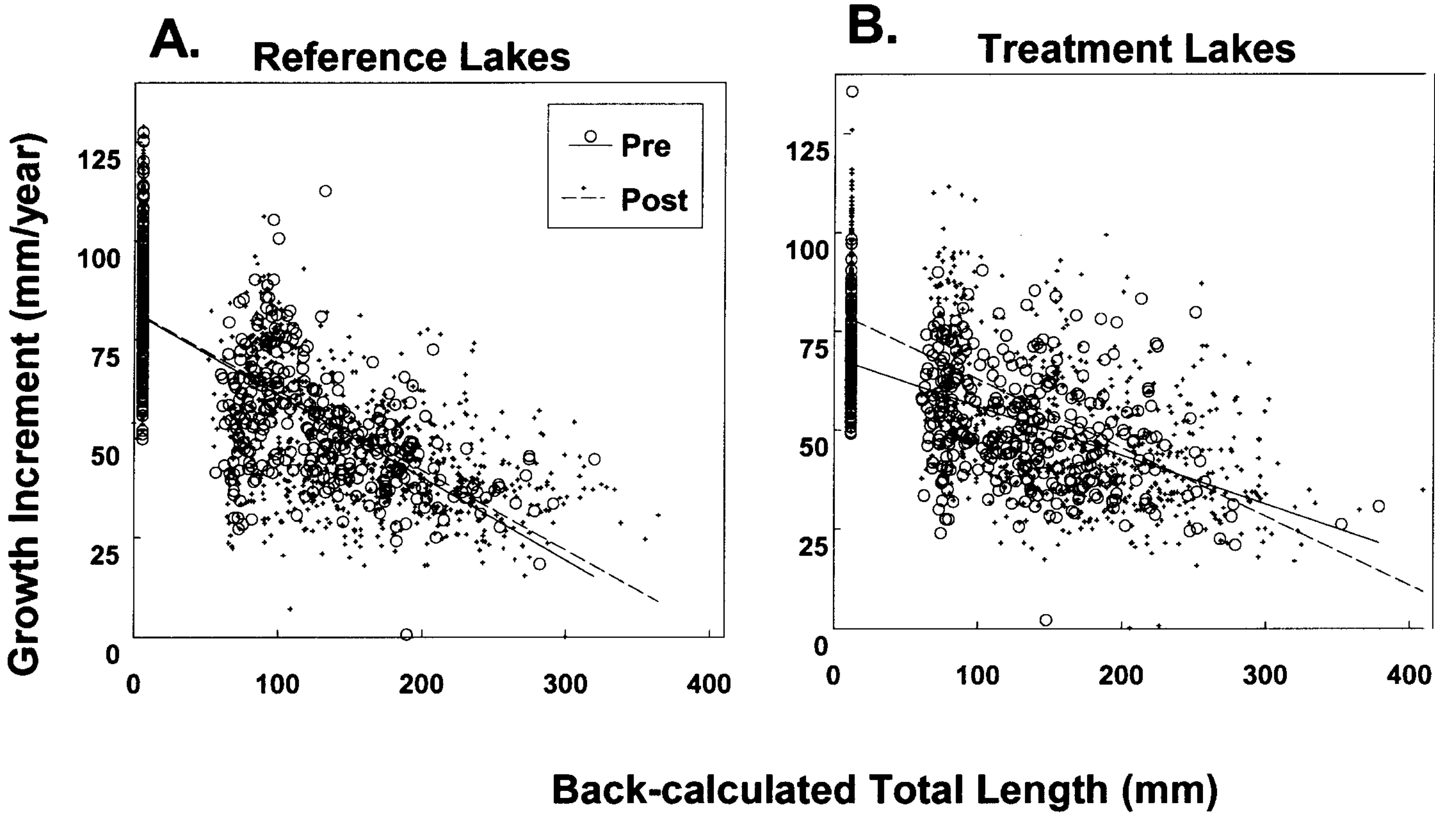

Figure 3. Estimated relationships of bass growth increment as a function of back-calculated total length for (A) reference and (B) treatment lakes during pre- and post-treatment periods. Separate regressions were calculated for pre-treatment (1995-1996) and post-treatment (1997-1999) years.

that the duration of effects varies by lake. In two treatment lakes (Big Crooked and Camp), control appeared to be effective for two complete summers following the year of treatment, but in one treatment lake (Lobdell), EWM frequency of occurrence in the second year post treatment was comparable to its pre-treatment level. One key difference among the treatment lakes is that the frequency of occurrence of all plants prior to treatment was higher in Lobdell Lake (the lake that had short-lived control) than in the two other treatment lakes. These results suggest that EWM reduction for two years following treatment may be more difficult to achieve in lakes where plant cover (especially EWM) is relatively high. Another key difference among the lakes is that Lobdell Lake received fluridone treatment in earlier years (1991 and 1992). Although our data are insufficient to determine whether cumulative effects of repeated fluridone treatment (such as increased resistance to fluridone within the EWM population) are likely to occur, it is a potential concern that should be addressed.

Effects of fluridone on species richness and plant cover likely increase with fluridone concentration and decrease with initial plant species richness. For example, in our study, plant assemblages were similar across all lakes prior to treatment with native plants and EWM occurring in mixed-species plant beds. The primary response of plants to treatment was that of conversion from mixed sites to native sites. Accordingly, plant species richness did not differ between treatment and reference lakes (Madsen et al. 2002). Madsen et al. (2002) noted an increase in the exotic *Potamogeton crispus*, although this species increased in the reference lakes as well, to a somewhat lesser degree, suggesting regional climatic influences on all lakes. Madsen et al. (2002) also show that there was no evidence of negative effects of fluridone on individual species of native plants. Similarly, plant cover showed little response to fluridone treatment. This result is in contrast to a recent whole-lake study in two Minnesota lakes that was conducted using higher concentrations of fluridone (23 ppb). This treatment concentration resulted in severe reduction of plant cover in the two lakes (from 100% to 33% and 63%) and at least short-term reductions in plant species richness (Pothoven et al. 1999). Differences between our study and the Pothoven et al. (1999) study in initial plant conditions (in addition to the difference in fluridone concentration) may have influenced the response of the plant assemblages to treatment. In all treatment lakes in our study, native plants were prevalent at the time of treatment. However, one of the two treatment lakes in the Pothoven et al. (1999) study contained a very depauperate plant community at the time of treatment (Pothoven et al. 1999). For lakes in which EWM dominates the plant community in extensive monospecific plant beds, selective removal likely will greatly reduce plant cover, at least initially. As a result, indirect effects on water quality and fish also may be very different from those that occur when EWM is present with native plants. Ultimately, scientifically-sound policy regarding the use of whole-lake fluridone should build upon knowledge of effects of repeated treatments across a gradient of lakes that range from extreme

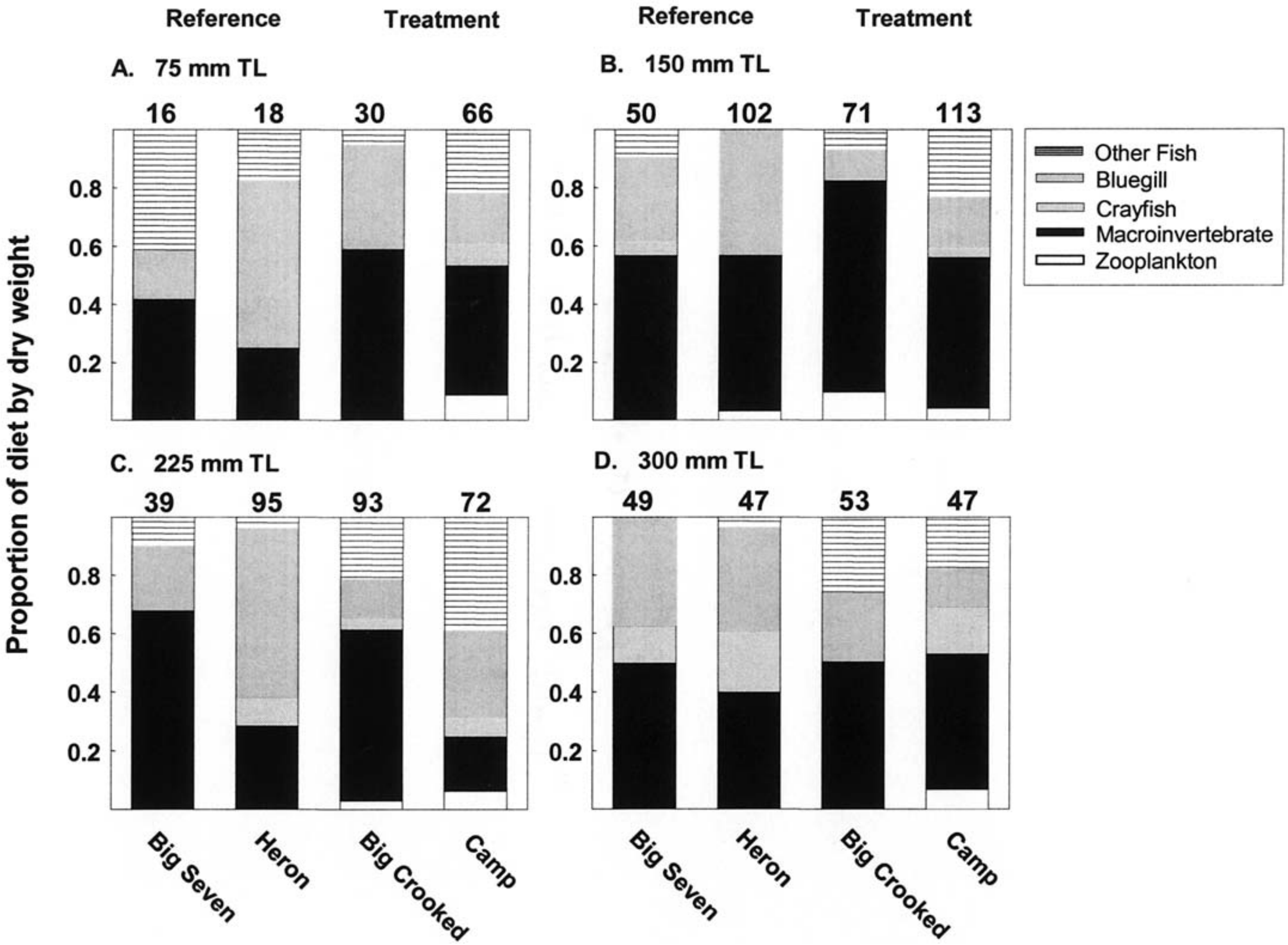

Figure 4. Diet composition of largemouth bass in two reference and two treatment lakes during summer 1999, the second year following low-dose fluridone treatment. Diet composition represents the proportion of diet contents by estimated dry weight. T-tests were used to compare proportions between reference and treatment lakes for each diet item and bass size group (see text for results). Size groups represent bass with an initial size of (A) 75 mm TL, (B) 150 mm TL, (C) 225 mm TL, and (D) 300 mm TL in spring 1999. Sample sizes are shown for each size group and lake combination.

EWM dominance to low to moderate levels of EWM integrated as one component of a diverse plant assemblage.

Indirect Effects of Low-dose Fluridone on Water Quality and Largemouth Bass Diet and Growth

We found no statistical differences in water quality between our treatment and reference lakes. The calculated probability of our study lakes switching from mesotrophic to eutrophic conditions was generally low and ranged from 0.02 to 0.10. Overall, we can conclude that dramatic reductions in water quality did not occur in the study lakes, and may not be expected to occur in fluridone-treated lakes with EWM mixed within a relatively diverse plant community. The effects on water quality in fluridone-treated lakes where EWM is the dominant plant species, however, remain to be seen.

Similarly, the effects of low-dose fluridone treatment on largemouth bass diet and growth were generally low, and only detected for small bass, which is supported by other whole-lake macrophyte manipulation studies. For example, Carpenter et al. (1995) found that the power to detect changes in growth increments in younger fish (bluegill in this case) was greater than the power to detect changes in growth of older fish. In addition, a previous comparison of age-0 largemouth bass growth in our study lakes only during post-treatment years, showed that growth rates were greater in two of the treatment lakes (Big Crooked and Camp) than in two of the reference lakes (Big Seven and Heron) or the other treatment lake (Lobdell; Valley and Bremigan 2002b). The higher growth of age-0 largemouth bass in two treatment lakes was likely due to greater availability of macroinvertebrates (Cheruvelil et al. 2002) and age-0 bluegill (Valley and Bremigan 2002b). In this manuscript's growth analysis, we were able to compare bass growth rates before and after treatment, and documented that growth rates of age-0 bass (and other bass <200 mm TL) in two treatment lakes significantly increased following treatment. Similarly, Maceina and Slipke (2004) documented neutral or small positive effects on age-0 bass due to *Hydrilla* removal using fluridone and Aquathol. Herein, we found that growth rates of largemouth bass >200 mm TL did not change following treatment, nor did estimates of the proportion of maximum consumption differ between treatment and reference lakes. Our expectation that the amount of bluegill in largemouth bass diets would be greater in treatment than reference lakes was not supported by our data. In their evaluation of the indirect effects of a much higher dose (23 ppb) of fluridone, Pothoven et al. (1999) documented enhanced piscivory by largemouth bass in just one of two treatment lakes and only during the year of treatment. Perhaps if enhanced piscivory by largemouth bass does occur, it is short-term in nature and limited to situations with severe reductions in plant cover, likely associated with high fluridone concentration and/or low prevalence of native plant species prior to treatment.

Management Implications

As for most whole lake studies, our study lakes were not perfect replicates of each other. To account for this potential problem, we carefully chose our statistics (focusing on changes through time) and were cautious in our interpretation of our data. Clearly more whole-lake evaluations are needed of such important management techniques as the use of whole-lake fluridone. Our results should help guide future evaluations. For example, the differences in response among our three treatment lakes reflect the challenges in conducting whole-lake studies of management techniques that can be extrapolated to a much larger (and diverse) population of lakes, which is needed to make policy decisions. Two of the treatment lakes (Big Crooked and Camp) demonstrate similar patterns: EWM reduction through two years following treatment, relatively high TP and chlorophyll concentrations, and faster growth of young bass. In contrast, our third treatment lake (Lobdell) experienced EWM reduction only through one year following treatment, had relatively low TP and chlorophyll concentrations, and relatively slow young bass growth. Thus, lakes will not necessarily respond similarly to the same management technique, in large part, presumably, due to natural differences among lakes. Big Crooked and Camp lakes (treatment lakes with similar responses) were the two deepest lakes in our study, with mean depths of 4.5 and 7.3 m respectively, in contrast to Lobdell's 2.7 m mean depth. Lakes with mean depth <3 m are typically considered "shallow", with a high proportion of the lake bottom in the littoral zone and with the possibility of destratification in summer. Initial plant conditions appeared to vary among lakes according to lake depth as well. Specifically, the frequency of occurrence of plants decreased with increasing mean depth (correlation coefficient, $r = -0.88$, $p = 0.02$). Because the frequency of occurrence of plants was not affected by EWM removal, this mean depth "signature" was not removed following treatment.

Our results suggest that controlling for lake depth will likely improve the statistical power of low-dose fluridone evaluations, given that variation among lakes in our study appears to correspond to mean depth. But given that fluridone is likely to be used across an even wider lake depth gradient than the one in our study, what depths should be selected for future study? We recommend future evaluations of fluridone be conducted in treatment-reference lake pairs as recommended by Carpenter (1989) who argued that the goal of lake choice should be to reduce unexplained variability among lakes. However, we argue that the paired lakes should be chosen along a lake mean depth gradient to reduce a potentially large amount of the unexplained variation while representing a broad range of lake conditions. Conducting management evaluations along such environmental gradients will increase the workload demands in the short term, but should lead more quickly to robust policy development. In addition, this approach may serve as a useful educational tool, by helping lake property owners and others managing lake plants to recognize the broad diversity of lakes. The recognition of lake types along environmental gradients should encourage development of reasonable expectations for lakes that address recreational and aesthetic demands within the natural constraints or predisposition of individual lakes.

Controversy regarding the use of whole-lake herbicides, such as fluridone, is likely to continue, unless uncertainty regarding their effects is reduced. This goal can be best achieved through conducting treatments within an experimental context or study design that allows robust extrapolation of findings. Our study indicates that single, low-dose, whole-lake fluridone treatments are unlikely to cause large reductions in water quality, and may produce a minor increase in growth of young bass. These findings should be incorporated into a broader body of studies investigating short- and long-term responses of a variety of lake types to repeated fluridone treatments (i.e., once every 3 years). Such an endeavor requires coordination among policy makers, researchers, applicators, and stakeholders.

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