Biological Control of Eurasian watermilfoil by *Euhrychiopsis lecontei*: Assessing Efficacy and Timing of Sampling

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ABSTRACT

The milfoil weevil, *Euhrychiopsis lecontei* Dietz, is a biological control agent for Eurasian watermilfoil (EWM; *Myriophyllum spicatum* L.), a nuisance aquatic macrophyte. EnviroScience, Inc. (Stow, OH) rears and stocks *E. lecontei* for management of EWM infestations. Here, we analyze data collected by EnviroScience, Inc. from treatment (weevil-stocked) and control (unstocked) EWM beds in 30 Michigan and Wisconsin lakes over six years. Initial and final EWM and weevil densities were compared with lake-specific average and maximum lake depths, and lake surface area. The analyses showed substantive variability of weevil efficacy to control EWM. No significant associations were seen between average or maximum lake depth or lake surface area on final weevil densities or plant density changes. Only the number of days between initial and final surveys and timing of final data collection proved significant in determining final EWM densities. As more time passed between surveys, final EWM densities significantly decreased at treatment sites, possibly due to weevils, with a much smaller decrease at control sites. Also, EWM densities declined at both treatment and control sites when final survey data were collected after the start of September, a phenomenon not seen when final data were collected earlier in the year. These declines at control sites were likely due to plant senescence. Lake managers utilizing *E. lecontei* should consider the length of time between EWM surveys, as well as the timing of data collection, to avoid the confounding effect of plant senescence on data interpretation.

Key words: biological control, macrophyte management, milfoil weevil, *Myriophyllum spicatum*.

INTRODUCTION

The exotic macrophyte, Eurasian watermilfoil (*Myriophyllum spicatum* L.; hereafter EWM), has been established in the United States since at least the 1940s (Sheldon and Creed 1995). Since its introduction, EWM has become one of the most troublesome invasive aquatic macrophytes in the country (Smith and Barko 1990), establishing itself in at least 45 states and three Canadian provinces (Newman 2004).

The rapid growth rate and vegetative reproduction of EWM allows it to out-compete native macrophytes (Madsen et al. 1991), leading to ecosystem damage with striking changes in physical and chemical properties of lakes, often negatively impacting other biota (Grace and Wetzel 1978, Boylen et al. 1999). Water recreation is also hampered by dense EWM infestations, with reductions in swimming and boating, an overall decline in the fishery, and reduced aesthetic quality of the water body (Smith and Barko 1990). Coupling these negative effects with possible EWM densities of >300 stems/m^2, millions of dollars are spent annually managing and removing this plant (Sheldon and Creed 1995, Eiswerth et al. 2000).

Multiple methods have been developed to control EWM, including herbicide use (Parsons et al. 2001), mechanical treatment (Boylen et al. 1996), and biological control (Newman 2004). Here we consider the use of a native biological control agent, the milfoil weevil (*Euhrychiopsis lecontei* Dietz),

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an aquatic weevil native to northern North America that specializes on native *Myriophyllum* spp. (Newman 2004). Since the invasion by EWM, *E. lecontei* has expanded its range of host species to include EWM, preferring EWM to its native host, *M. sibiricum* (Solarz and Newman 1996, Marko et al. 2005). This preference for EWM has caused *E. lecontei* to be favorably considered as a biological control agent for EWM (Sheldon and Creed 1995). Further, these weevils show higher developmental rates on EWM than on native *Myriophyllum* spp. (Newman et al. 1997, Roley and Newman 2006). Newman (2004) provides a comprehensive review of weevil biology.

E. lecontei feeding has been associated with EWM declines in multiple studies (Sheldon and Creed 1995, Newman and Beisboer 2000, Creed and Sheldon 1995). Larval stem mining and adult consumption of leaf tissue can cause the plants to collapse and fall out of the water column (Creed et al. 1992), which reduces photosynthetic activity and plant vigor, and may promote a recovery of previously displaced and shaded-out native macrophytes. Because of these impacts, *E. lecontei* weevils have considerable potential as an EWM biological control agent, especially because they seem to have limited effects on other, native *Myriophyllum* spp. (Sheldon and Creed 2003).

Given that EWM reduction by milfoil weevils may have dramatic positive effects on a given water body, an understanding of the factors correlated with *E. lecontei* effectiveness is important to aid in control efforts. Data collected by EnviroScience, Inc. (Stow, OH) regarding weevil stocking in 30 Michigan and Wisconsin lakes during six years were analyzed to provide insight into and characterize variation of *E. lecontei* efficacy as an EWM biological control agent.

METHODS

EnviroScience, Inc. (http://www.EnviroScienceinc.com) is an environmental consulting firm that rears and stocks *E. lecontei* into lakes with EWM infestations through a proprietary program termed the Middfoil® process. Weevil stocking data supplied by EnviroScience were analyzed to characterize variation in *E. lecontei* efficacy at controlling EWM beds. The dataset included initial (pre-weevil stocking) and final plant (stems/m^2) and weevil (weevils/stem) densities from 29 lakes in Michigan and one in Wisconsin (Figure 1; Table 1). These data were collected as a regular part of weevil-based EWM control efforts. Reported weevil data were a combination of all life stages (as in Jester et al. 2000) and were collected along randomly located transects running through EWM beds. Plant density data were collected from 0.3 m by 0.3 m quadrats randomly placed in EWM beds. Plants were cut near the substrate, and stems and weevils were counted on shore. Data were collected from EWM beds where weevils were stocked (treatment sites) and control EWM beds where no weevils were stocked. The number of treatment sites per lake and year varied from 1 to 10, while there were only one or two control sites per lake and year. Treatment sites were identified as large, dense beds adjacent to as much natural shoreline as possible, usually on the north side of the lake and away from boat traffic. Control sites were selected to be as similar to treatment sites as possible and were located at least several hundred meters away from treatment sites to reduce the influence of weevil dispersal among sites. The data presented here were collected during summers between 2000 and 2006. Because some lakes provided data for multiple years (n = 8 lakes), we analyzed data both within and across years for treatment and control sites because treatment and control sites remained the same from year to year.

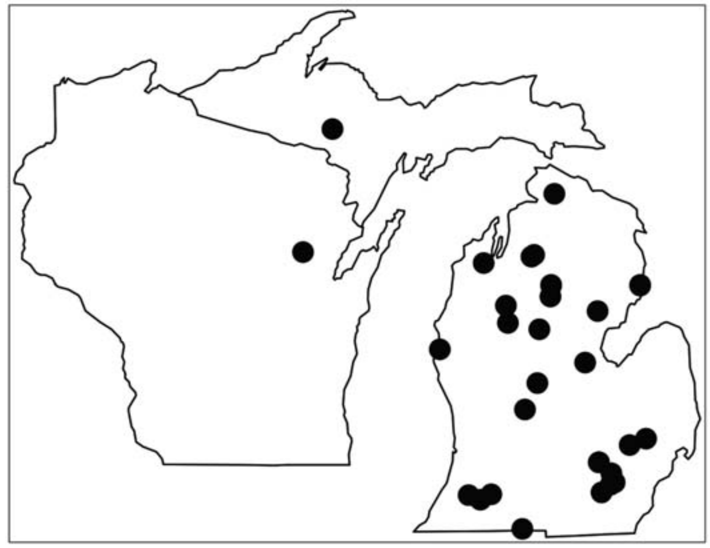

Figure 1. Geographic distribution of the Michigan and Wisconsin lakes included in this study.

For plant density analyses within years, a proportional plant density change was calculated using the equation

[(*final plant density - initial plant density*)/*initial plant density*]

to quantify intensity of plant density changes relative to initial plant density. Negative values indicate sites where EWM density decreased, possibly due to weevil control. This metric allowed comparison of treatments and control sites directly, but because sample sizes for controls are smaller (generally one per lake compared to up to 10 per lake for treatment sites), nonsignificant results for controls may have been due to smaller sample size. For this reason, in the lake characteristic analyses described below, a relative plant density difference was also calculated using the equation

$$[(C_f - T_f) - (C_i - T_i)]$$

where C_f is the final plant density at the control site, T_f is the final plant density at the treatment site, C_i is the initial plant density at the control site, and T_i is the initial plant density at the treatment site. For relative plant density difference, positive values may indicate weevil effect. This metric takes changes in control plots into account when quantifying differences in plant density.

Average and maximum lake depths and lake surface area data, provided by lake managers or collected by EnviroScience, Inc. employees, were analyzed for correlations with plant density metrics and final weevil densities for lakes where these data were available (Table 1). No individual bed-level characteristics were collected for either treatment or control sites. The statistical test used to analyze these lake characteristic data was the Two-Dimensional Kolmogorov-

TABLE 1. LAKES USED IN THIS ANALYSIS, THEIR LOCATIONS (COUNTY, STATE), SURFACE AREA, AVERAGE AND MAXIMUM DEPTHS, AND MEAN/RANGE OF FINAL WEEVIL DENSITIES (# / STEM) AT TREATMENT SITES. NR = DATA NOT REPORTED.

Lake	Location (County, State)	Surface area (ha)	Avg. depth (m)	Max. depth (m)	Avg. final weevil density #/stem (Mean; Range)
Bankson Lake	Van Buren, MI	87.82	NR	NR	0.07; 0.07-0.07
Barton Pond	Washtenaw, MI	140.83	3.05	6.10	0.01; 0.00-0.03
Bass Lake	Montcalm, MI	41.28	2.29	3.96	0.32; 0.00-0.77
Big Lake	Oakland, MI	97.12	1.83	NR	0.01; 0.00-0.03
Burt Lake	Cheboygan, MI	NR	NR	NR	0.56; 0.56-0.56
Lake Cadillac	Wexford, MI	465.39	2.13	9.14	0.00; 0.00-0.00
Eagle Lake	Van Burren, MI	77.70	7.47	18.29	0.02; 0.00-0.07
Gilead Lake	Branch, MI	NR	2.44	13.72	0.38; 0.33-0.43
Higgins Lake	Roscommon, MI	NR	2.44	50.29	0.08; 0.06-0.10
Hougton Lake	Roscommon, MI	8111.52	1.52	5.79	0.48; 0.48-0.48
Hunter Lake	Midland, MI	56.66	2.59	13.72	0.16; 0.07-0.33
Independence Lake	Washtenaw, MI	80.94	2.74	8.53	3.30; 2.30-4.30
Indian Wood Lake	Oakland, MI	5.67	1.83	NR	0.10; 0.10-0.10
Lake Diane	Washtenaw, MI	161.87	3.05	15.24	0.24; 0.00-0.47
Lily Lake	Clare, MI	80.94	1.83	4.27	0.18; 0.13-0.23
Long Lake	Grand Traverse, MI	1205.96	7.56	27.43	0.34; 0.00-0.90
Long Lake	Ionia, MI	141.64	NR	NR	0.15; 0.00-0.33
Manistee Lake	Kalkaska, MI	348.03	2.26	4.27	0.10; 0.00-0.23
Mills Lake	Ogemaw, MI	10.52	3.66	NR	0.20; 0.20-0.20
North Lake	Livingston, MI	91.86	1.52	15.24	0.10; 0.00-0.20
Parkview Hills	Kalamazoo, MI	NR	1.52	NR	0.48; 0.36-0.60
Pentwater Lake	Oceana, MI	176.44	NR	NR	0.00; 0.00-0.00
Pickerel Lake	Kalkaska, MI	28.33	19.81	27.43	0.44; 0.17-0.71
Pleasant Lake	Washtenaw, MI	81.75	4.57	10.06	0.72; 0.30-1.40
Rose Lake	Osceola, MI	150.95	4.88	9.45	0.40; 0.40-0.40
Sawyer Lake	Dickinson, MI	80.94	3.96	6.10	0.16; 0.00-0.66
Lake Shangrila	Livingston, MI	NR	NR	NR	0.40; 0.37-0.43
Silver Lake	Livingston, MI	61.51	5.49	13.72	0.20; 0.20-0.20
Van Etten Lake	Iosco, MI	570.20	4.27	80.77	0.45; 0.00-1.25
Loon Lake	Shawano, WI	123.43	2.59	6.71	0.37; 0.00-0.97

Smirnov (2DKS) test, a nonparametric analysis for nonrandom distributions in bivariate data with nonlinear relationships (test statistic is "*D*"; Garvey et al. 1998). The 2DKS tests for significant threshold points on the x-axis where the mean and variance of the y-axis data significantly change. This is accomplished by comparing the proportion of points in each of four quadrants around each point to a null expectation of equal distribution among these four quadrants. The 2DKS test provides x,y coordinates (D_{BKS}) where the maximum difference between observed and expected point distribution exists. One can use this point to identify the value on the x-axis where the mean and variance of points along the y-axis changes (see Garvey et al. 1998). This test does not indicate direction of association, which must be inferred from the shape of the point distribution within the plot. The available lake characteristic data were non-normally distributed and heteroscedastic, violating the assumptions required by linear regression models, so this nonparametric test was chosen to analyze these data. Because no data were available for EWM bed-specific characteristics and the plant and weevil density data were collected on a finer scale than the lake depths, lake-wide averages by year of plant density metrics and final weevil density were used for 2DKS analyses.

In addition to the 2DKS tests, linear regression was used to analyze the effect of final weevil density on proportional plant density change, as well as the effect of days passed between surveys on final plant density. To analyze the effect of weevil treatment on final plant density for the lakes with multiple years reported ($n = 8$), we also performed linear regressions on the plots of final plant density by year for each lake. This approach was taken because seven of the eight lakes with multiple years reported had only two years of data. Repeated measures ANOVAs were inappropriate given that missing data were correlated with duration of the study. Treatment and control sites were analyzed separately using initial plant density as a covariate in the analysis to remove the effects of differences in initial plant density between lakes and years. A line was fitted to these final plant densities across years, and the slopes of these lines (partial regression coefficient) were used to indicate how final plant density changed over years. To analyze the effect of weevil treatment across years relative to controls, a one-tailed, one-sample *t*-test was performed on the differences of the slopes (treatment sites slope - control sites slope) from the above regressions. Prior to any analyses, a Kruskal-Wallis test was performed on initial plant densities at control ($n = 41$) versus treatment ($n = 167$) sites to ensure the initial densities were not significantly different between control and treatment beds ($H = 0.3996$; $P = 0.5272$).

To test if time of year of data collection affected plant density outcomes, we calculated the day of year when final surveys were conducted (range: day 219-261). We then plotted lake-specific averages for proportional plant density change at control sites versus proportional plant density change at respective treatment sites. The points in this plot were split into two groups by the range of day of year of final collection (days 219 to 240 and days 241 to 261). A Chi-Square test with Monte Carlo Simulation was performed on each date range, treating each graph quadrant as a cell in the analysis.

Averages by lake and year for treatment and control sites were used in all the analyses described above. Using these averages produced nonsignificant results, so in some cases, less conservative tests were performed using each treatment and control site separately for each lake to increase the number of data points and tease out effects that were not present in the original analyses. The lake characteristic 2DKS analyses and the Chi-Square analysis of the final survey date ranges are reported here using lake and year averages. However, the analyses regarding days passed between surveys within years, and across-year effects on final plant density, along with the linear regression of final weevil density versus proportional plant density change, are reported using individual treatment and control sites.

RESULTS AND DISCUSSION

Proportional plant density change was not related to final weevil density at treatment sites (linear regression $F = 0.0006$; $P = 0.9796$; $n = 115$) or controls ($F = 0.0918$ $P = 0.76363$; $n = 38$). Also, substantial variation was seen in the effectiveness of *E. lecontei* at controlling EWM (Figure 2). In some cases, plant densities declined at treatment sites and increased at control sites (quadrant IV), while in others, plant densities decreased at control sites and increased at treatment sites (quadrant II). Congruently, plant densities dropped at both control and treatment sites in some instances (quadrant III), while they increased at both in others (quadrant I). This broad range of variation suggested the role of other factors affecting EWM abundance and warranted examination of the lake characteristics included in the data set.

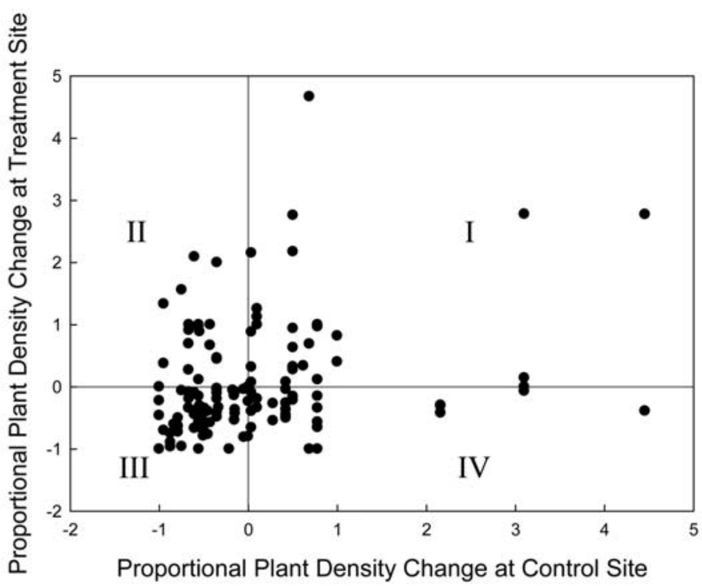

Figure 2. Changes in proportional plant density [*(final plant density - initial plant density)/initial plant density*] at lake-specific control sites vs. treatment sites. Points in quadrant I represent an increase in EWM density at both control and treatment sites, implying no weevil effect. Points in quadrant II represent EWM density increases at treatment sites and EWM density declines at control sites, implying no weevil effect. Points in quadrant III represent EWM density declines at both control and treatment sites—weevil effect unknown. Points in quadrant IV represent EWM density declines at treatment sites and EWM density increases at control sites, implying a weevil effect on EWM density.

No significant associations were found between any of the available lake characteristics and the lake and year averages of proportional plant density change, relative plant density difference, or final weevil density (2DKS all P-values > 0.05). These results are similar to Jester et al. (2000), who found that no lake-wide characteristics (including those examined here) influenced weevil abundance, which can in turn be directly related to efficacy of plant control (Newman 2004). Jester et al. (2000) did find, however, that weevil abundance negatively correlated with several bed-level characteristics, including plant bed depth and percent sandy shoreline adjacent to the plant bed. They also found that weevil abundance was positively correlated with number of *M. spicatum* apical tips per plant, distance from shore to milfoil bed, and percent natural shoreline in the Wisconsin lakes they studied. Bed-level characteristics such as these should be measured during future weevil stocking efforts or subsequent monitoring of those sites. See Newman (2004) for a review of factors known to influence weevil density.

Weevil densities should clearly be related to reduction of EWM densities. Newman (2004) noted that weevil densities >1 weevil/stem can consistently control EWM, while densities <0.1 weevils/stem may not be sufficient for plant control. Twenty-three of the 30 lakes reported here had average final weevil densities >0.1 weevils/stem (Table 1) for weevil stocking sites. However, we saw no relationship between final weevil density and proportional plant density change. Because of this, further research is warranted to better link when and how high weevil densities influence efficacy of EWM control.

Another important consideration for using weevils in EWM control is the timing of data collection. Clearly, maximizing the time between stocking and end of season sampling is desirable to best measure weevil effects. The timing of data collection proved significant in determining final plant densities at both treatment, and to a lesser extent, control sites. Final EWM densities at individual sites were negatively correlated with the number of days that elapsed between initial and final plant surveys (range 35 to 106 days). This relationship held true for treatment sites (linear regression $F = 30.8046$; $P < 0.0001$; $n = 109$), although the relationship was marginally nonsignificant for the control sites ($F = 4.0884$; $P = 0.0516$; $n = 34$; Figure 3). This stronger relationship in treatment than control sites is consistent with the hypothesis that the weevils are having a negative impact on EWM because the plants are showing stronger declines with time at weevil stocking sites. However, the lack of a significant relationship at control sites may also be due to reduced statistical power resulting from a much smaller sample size. Analyses to examine the effect of weevils on final plant density while controlling for multiple treatment sites and multiple years with lake and year-specific averages of final plant densities for both control and treatment sites were not significant (all P-values > 0.05).

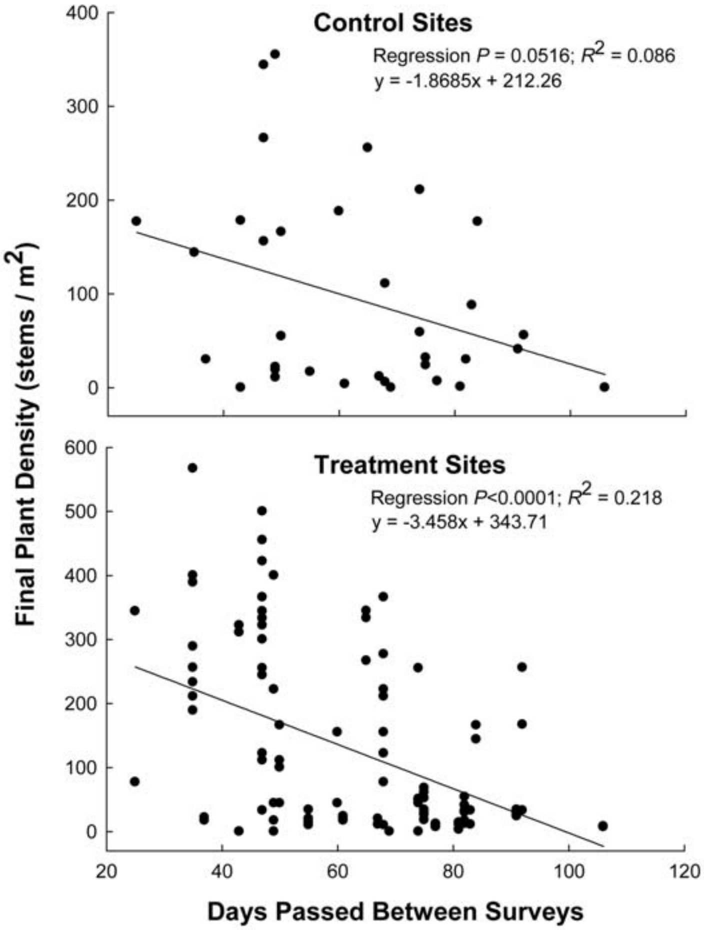

Figure 3. The relationship between final EWM densities (stems/m^2) and the number of days passed between initial and final surveys for control and treatment sites.

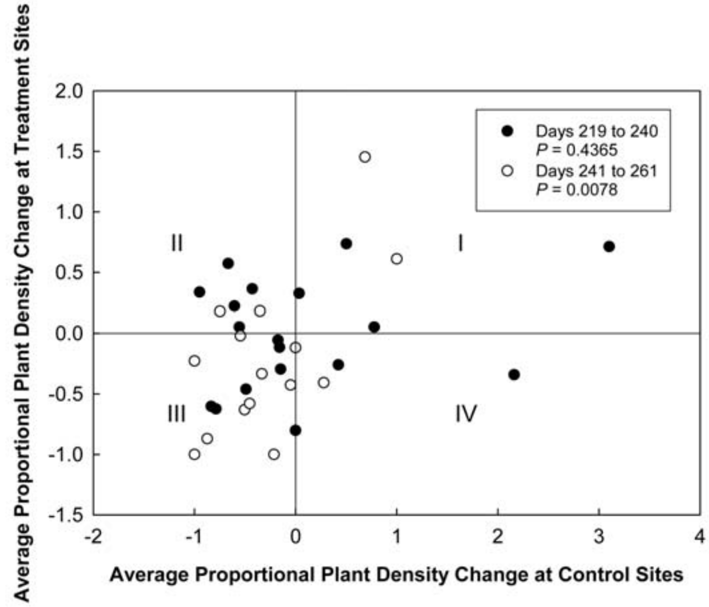

Figure 4. Average proportional EWM density change by lake and year at control vs. treatment sites. Filled circles represent lakes where the final survey was conducted between days 219 and 240 (~7 August to end of August); open circles represent lakes where the final survey was conducted after day 240 (very late August/early September). *P*-values are results from Chi-Square test (with Monte Carlo Simulation) of distribution of points among the four graph quadrants.

While the effect of weevils within years is one indication of success in controlling EWM, it is also important to look at effects over several years. No significant across year effects were seen; the differences of the slopes of the lines of the final plant densities in successive years at treatment versus respective control sites were not significantly different than zero (one-tailed, one sample *t*-test $t = -1.0569$; $P = 0.1628$; $n = 8$). In examining these slope differences, however, five of the eight lakes showed negative values, implying that plant densities were dropping more or increasing less at the treatment versus respective control sites in these lakes. The overall average of these slope differences between treatment and control sites was -71.5135, indicating that a weevil effect across years may be seen with more than eight lakes. If more data can be collected, this analysis should be performed again.

Extended periods of time between initial and final surveys may give *E. lecontei* more time to reproduce and damage plant tissue; however, plant senescence may also contribute to reduced EWM densities observed during late summer. To examine a possible effect of plant senescence on final EWM densities, we characterized average lake and year specific proportional plant density changes at control and treatment sites by the day of year of the final survey (Figure 4). Across lakes and years, the day of the year of the final EWM survey ranged from day 219 (around 10 August) through day 261 (mid-September). We split this range in half and graphed points in two groups: those whose final survey was conducted between days 219 to 240 and those surveyed between days 241 to 261. Day 241 provided a biologically relevant reference point of around the start of September. In examining EWM senescence in an Indiana reservoir, Landers (1982) noted that senescence started in August and became "advanced" by the start of September (see Figure 5 in Landers 1982). Analysis of the distribution of the points (Figure 4) showed that the data points from the first date range were randomly distributed among the four graph quadrants (Chi-Square $\chi^2 = 2.79$; $P = 0.4365$; $n = 19$). However, during the second half of the date range, data points were significantly clustered in Quadrant III, where plant densities declined at both control and treatment sites ($\chi^2 = 11.80$; $P = 0.0078$; $n = 15$), indicating that plant senescence may be skewing the data and our understanding of the role of *E. lecontei* in reducing EWM density. Weevil densities also start to decline in September as weevils leave lakes to overwinter on shore (Newman et al. 2001), making careful choice of final sampling date even more important.

Investigators examining the effectiveness of *E. lecontei* as a biological control agent for EWM using population augmentation should allow similar periods of time to go by between initial and final surveys both within and across years from lake to lake, while making sure to have all data collected before plant senescence becomes a factor. It remains desirable, however, to give the weevils as much time as possible to damage EWM before assessing success of the control effort. Because senescence seems to start in late August (though this may vary based on latitude and weather), we propose that final surveys of *E. lecontei* and EWM density should be collected by mid-August to reduce the risk of plant senescence or departing weevils skewing data interpretation.

More research is warranted to better understand when and where the weevils will be effective at controlling EWM. We propose that future research be conducted in lakes with an equal number of similar treatment and control EWM beds, paired *a priori*, while keeping in mind the data collection timing issues noted above. Also, lakes should be surveyed for multiple years after a stocking event, both with and without yearly stocking, to gain a better understanding of how effective the weevils are over a period of years. The dataset considered here involved evaluation of patterns over a few months to three years, and large-scale changes in such complicated systems could reasonably be expected to require more time to develop. Further, more specific experiments utilizing collection of EWM bed-specific measures would help gain a deeper understanding of the variation of final weevil densities (Table 1). Data such as those collected in Jester et al. (2000) on *M. spicatum* bed-specific characteristics (and any number of novel variables) should be collected when possible. Taking these factors into account would provide a much greater understanding of what affects weevil density (and therefore efficacy) and may eventually lead to a suite of characteristics that will predict weevil success.

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