Overwintering habitat requirements of the milfoil weevil, *Euhrychiopsis lecontei*, in two central Wisconsin lakes

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ABSTRACT

The native milfoil weevil, Euhrychiopsis lecontei (Dietz), shows potential to be an effective biological control for Eurasian watermilfoil, Myriophyllum spicatum L. To better define shoreland habitat requirements for overwintering success, univariate and multivariate (discriminate analysis) statistical methods were used to identify the habitat variables that best define weevil overwintering habitat at two lakes in Portage County, Wisconsin: Thomas Lake, a glacial seepage lake, and Springville Pond, an impoundment of the Little Plover River. Weevil presence and abundance along the shore were evaluated in relation to the presence of milfoil fragments along shore, distance from shoreline, height above water, habitat type, soil texture, soil and duff moisture, soil and duff organic matter, duff depth, and duff composition. The results suggest that higher elevation sites closer to shore, with more duff material, are associated with weevil presence, and that management activities that remove duff material from the shoreland, such as mowing and raking, may be disadvantatgeous to weevil populations. It was inconclusive whether duff composition was truly correlated with weevil abundance, suggesting that lake residents and lake managers may not need to be concerned about planting specific plant species for weevil habitat.

Key words: Eurasian watermilfoil, Myriophyllum spicatum, biological control, shoreland habitat, hibernation habitat.

INTRODUCTION

Declines in Eurasian watermilfoil (*Myriophyllum spicatum*) have been associated with several herbivorous invertebrates, but primarily the native milfoil weevil, *Euhrychiopsis lecontei* (Dietz), that feeds exclusively on milfoil species (Sheldon and Creed 1995, Newman et al. 1996, Buckingham 1998, Newman 2004, Newman et al. 2006). Research suggests the milfoil weevil has the potential to be a biological control

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agent on Eurasian watermilfoil when their population densities are high, but more study on factors limiting populations adequate for control is needed (Creed and Sheldon 1995, Sheldon and Creed 1995, Creed 2000, Jester et al. 2000, Madsen et al. 2000, Newman 2004, Cuda et al. 2008).

Shoreland habitat for overwintering may be one important factor in sustaining high milfoil weevil populations. In fall (September through November), weevils move to shore where they overwinter at the soil-leaf litter interface (Newman et al. 2001). Many anthropogenic impacts may disturb soil or remove the leaf litter, but the minimum leaf litter requirements are unknown. Newman et al. (2001) found that populations were most commonly found at two to six meters from the shoreline, and were significantly lower in sites with soil moisture > 15%. In spring, between ice-out and mid-May, they return to the lake, where they live on milfoil (Newman et al. 2001).

Several questions remain about factors important to overwintering habitat requirements. It is currently unknown how they move to shore in fall. They have been documented to fly in spring, but this has not been documented in fall (Newman et al. 2001). It is unknown whether they are strong enough fliers to select habitat, their direction is controlled by wind speed and direction, or they may simply raft to shore in fall on milfoil fragments. Jester et al. (2000) found milfoil weevil population density correlated negatively with bare, sand shorelands, and positively with natural shoreland vegetation (Jester et al. 2000), but "natural" vegetation can vary widely. Newman et al. (2001) documented weevils can be successful on natural grass riparian areas (i.e., prairie sites), but correlations with other vegetation types is unknown. Other studies (Jester et al. 2000, Newman and Inglis 2009) have related in-lake density to shoreland habitat, but relatively little is known about the relationship between shoreland habitat and overwinter density of weevils. More whole-lake studies are needed to better understand overwintering habitat requirements (Jester et al. 2000, Newman et al. 2001, Newman 2004).

The objectve of this study was to evaluate, in two lakes, shoreland habitat characteristics that help discriminate shoreland characteristics where weevils overwinter versus site characteristics where they are absent. Defining shoreland characteristics that correlate with weevils may provide guidance for shoreland management on lakes where biological control is a desired management tool.

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MATERIALS AND METHODS

Study area

Our study area included two lakes in Portage County, Wisconsin: 1) Thomas Lake (2 November to 7 November 2009) and 2) the eastern third of Springville Pond (13 November to 21 November 2009). Both study sites consisted primarily of natural (undisturbed) shoreland.

Thomas Lake $(44^{\circ}28'24.50''N 89^{\circ}23'24.22''W)$ is a 13hectare hard-water seepage lake, with a maximum depth of 9 meters. In 2009, frequency of occurrence in vegetated sites (n = 51) was 57% for *M. spicatum*, and *M. sibiricum* was only visually observed twice (unpublished survey data). Naturallyoccurring weevil density has ranged from 0.03 to 0.34 weevils per stem from 2004 to 2009 (Thorstenson 2011).

Springville Pond $(44^{\circ}28'21.21''N \ 89^{\circ}32'37.94''W)$ is a 7-hectare hard-water impoundment of the Little Plover River, with a maximum depth of 4 meters. In 2008, frequency of occurrence within vegetated sites (n = 55) was 85% for *M. spicatum*, and *M. sibiricum* was not detected (unpublished data). The historical data shows the naturally-occurring weevil density has ranged from 0.06 to 4.43 weevils per stem from 2004 to 2006 (Thorstenson 2011).

Study design

Weevil presence and abundance were measured, as well as shoreland condition, at sample sites along transects. Transects were distributed equidistant around each study lake and extended onto shore perpendicular to the shoreline. A minimum of fifteen transects, and as many as 29 transects, were sampled per lake. Two, 1-m² circular sample plots were sampled per transect. Because Newman et al. (2001) found that weevils are most commonly found on shore at 2 to 6 m from the shoreline (where the water met the shore), 1 m² circular plots were centered at 2 and 6 m from the shoreline, although distance from shoreline varied at some transects due to obstructions, saturated soils, or other site-specific features. At each lake, three of the transects (randomly chosen) were also sampled at approximately 10 m from the shoreline (e.g., at 2, 6, and 10 m from shoreline).

Distance from the shoreline to the center of each 1 m^2 circular plot was measured. Habitat variables were measured at each 1 m^2 circular plot, including distance from shoreline, height above water, the presence of milfoil fragments at the shoreline, duff layer depth, and duff layer composition. At each 1 m^2 circular plot, shoreland habitat within the plot was categorized into one of 11 cover types, based on a modified version of the qualitative cover type categories identified in Woodford and Meyer (2002) (Table 1). Only vegetation within, and directly above, the perimeter of the plot was considered when characterizing habitat.

To further describe the habitat at each 1 m^2 circular plot, the duff material was measured and characterized in situ. The depth of the duff layer was measured using a meter stick, and composition of the duff materials was characterized by percent cover of various types of material (woody material, deciduous leaf material, coniferous leaf material,

TABLE 1. QUALITATIVE DESCRIPTION OF EACH HABITAT TYPE (MODIFIED FROM WOODFORD AND MEYER 2002) USED TO CLASSIFY HABITATS DURING SHORELAND HABITAT SURVEYS.

Cover Type	Characteristics
1	Wetlands, dominated by tamarack/black spruce
2	Wetlands, dominated by alder species
3	Wetlands, dominated by herbaceous vegetation
4	Upland forest dominated by $(> 60\%)$ deciduous woody vegetation
5	Upland forest dominated by $(> 60\%)$ coniferous woody vegetation
6	Upland mixed woody and herbaceous
7	Upland herbaceous, dominated by $(> 60\%)$ grasses
8	Upland herbaceous, dominated by $(> 60\%)$ forbs
9	Uplands with no alteration, except for pier access (e.g., foot path)
10	Uplands with moderate housing density, vegetation structure altered significantly, overstory remaining intact
11	Uplands with high house density, vegetation structure removed (e.g., beach, rip rap, seas wall, lawn) to water edge

forbs, grass, rocks, and bare soil). The duff layer was defined as organic materials accumulated on the ground, such as dead grasses, twigs, pinecones, pine needles, and fallen leaves. Erect vegetation, such as standing grasses and goldenrods (*Solidago* spp.), was not included. Occasionally, live vegetation was encountered that sprawled laterally covering the ground surface, such as the basal leaves of hawkweed (*Hieracium* spp.), and was included as duff material.

To determine the presence and abundance of weevils, as well as soil characteristics of the sample plot, we extracted soil and duff samples from each quadrant of the plot. Circular rings 0.05 m² were randomly positioned within each quadrant, and all soil and duff within the ring was collected to a depth of approximately 5 cm into the soil. Thus, four replicate sub-samples of this type were extracted per 1 m² circular plot, and later combined, for a total sample area of 0.2 m². This provided a representative sample of the plot without having to extract the entire 1 m². All weevil numbers are converted to N/m² for statistical analysis.

To determine the number of weevils per sample, weevils were extracted from the soil/duff samples with the use of Tullgren funnels (Pande and Berthet 1973). The soil/duff composite samples were kept at 4 C until processing. The Tullgren funnel used an overhead 25 watt incandescent light bulb to gradually dry the sample and force the organisms to emigrate from the duff material onto a screen retaining the sample, then drop down through the screen into a funnel leading into a collection jar filled with 80% isopropyl alcohol. Collection jars were then inspected using light tables and $3 \times$ magnification to identify and count the milfoil weevils present. To evaluate the efficacy of the Tullgren funnel extraction method, three percent of the processed samples (samples that had already been in the Tullgren funnels) were examined manually over light tables to search for any weevils remaining in the samples. Of the three percent examined, 22% of those were known to have produced weevils in the Tullgren funnels. No additional weevils were found during manual examinations.

The soil/duff composite samples were characterized relative to percent moisture, texture, and the amount of dry organic matter (%). Before the samples were dried in the Tullgren funnels, wet weights were measured. Samples remained in Tullgren funnels until dry to the touch (24 to 96 hrs). Dry weights were then taken to be used in the calculation of percent moisture. The dry samples were later characterized for texture by hydrometer method (Dane and Topp 2002), and for organic matter by loss on ignition (LOI) method (Schulte and Hopkins 1996).

Non-parametric t tests (Kruskal-Wallis), logistic regression, multiple logistic regression, and discriminant analysis were used to differentiate between sites where milfoil weevils were found and sites they were not found, based on quantitative habitat variables. Multiple logistic regression tests were run using Number Cruncher Statistical Systems (NCSS) (Hintz 2004). All other tests were run using SAS software, version 9.2 (SAS Institute, Inc. 2008).

Non-parametric t tests tests for a statistically significant difference between sites where weevils are present versus absent, based on available measurements, where the measurements may not be normally distributed. Logistic regression was run to measure the relationship between weevil presence/absence relative to each of the continuous independent variables. Multiple logistic regression was run on weevil presence/absence relative to site characteristics that were significant in univariate analyses (logistic regression of a single variable). Variables that were not significant in multiple logistic regression were systematically eliminated before re-running the regression to develop a significant logistic equation.

Discriminant analysis was used to discriminate between sites where weevils were present versus absent based on habitat measurements. All continuous site variables were used in the initial analysis. Resultant structure coefficients that were close to zero played an insignificant role in the predictive model, and were, therefore, dropped from subsequent analyses to reduce colinearity. Structure coefficients closer to |1.0| were used in subsequent analyses in various combinations to develop a significant model that best predicted where weevils were present vs. where weevils were absent, with the highest correct classification results.

RESULTS

Thomas Lake

Milfoil weevils were found at 13 of the 53 sites sampled. The number of weevils found ranged from 0 to 15 per m², with a mean of 1.4 weevils per m². Sites were located at 3 tp 20 m from the shoreline ($\bar{x} = 8.2$ m), and 47 to 196 cm above the water vertically ($\bar{x} = 76$ cm). Weevils were found at 3 to 8 m from the shoreline ($\bar{x} = 5.3$ m), and from 50 to 115 cm above water ($\bar{x} = 78$ cm). Average weevil density was 1.6 per m² at the sites closest (2 m) to the shoreline. Shoreland habitat types included sites having no disturbance to high disturbance, and were most commonly characterized as upland herbaceous dominated by either grasses or forbs. Soil texture was rather uniform, with sand present at 49

	Weevils Present	Weevils Absent	
Variable	x	x	Р
Thomas Lake	n = 13	n = 40	
Percent cover of leaves	36 (23-48)	20 (13-27)	0.007
Distance from shoreline (m)	5.3(4.4-6.2)	9.0 (7.4-10.5)	0.018
Springville Pond	n = 17	n = 28	
Duff depth (cm)	3.5(3.0-4.1)	2.6(2.1-3.0)	0.015
Distance from shoreline (m)	3.1 (2.4-3.8)	4.5 (3.6-5.5)	0.031

sample sites, and sandy loam only appearing at four sample sites. Duff depth ranged from 0 to 8 cm, and composition of duff was most commonly dominated by grasses and/or deciduous tree leaves. Percent moisture in soil/duff composite samples ranged widely from 6% to 48%, with a mean of 22%. Percent organic matter ranged from less than 1% to 10%, with a mean of 22%.

Non-parametric t tests showed significant differences between characteristics of sites with weevils versus sites without for percent cover of leaves (P = 0.007) and distance from shoreline (P = 0.018). Mean percent cover of leaves at sites with weevils was 36%, versus a mean percent cover of 20% at sites without (a difference of 16%). Mean distance from shoreline at sites with weevils was 5.3 m, versus a mean distance of 9.0 m at sites without (a difference of 3.7 m). Most weevils occurred between 4.4 m and 6.2 m from the shoreline (Table 2).

Logistic regression found distance from shoreline and percent cover of leaves were significantly correlated with the occurrence of weevils (P = 0.029 and P = 0.037, respectively); probability of weevil presence decreased as distance increased, and probability of weevil presence increased as percent cover of leaves increased. However, for multiple logistic regression, percent cover of leaves was not significant (P = 0.160) and was eliminated from that model. The variables remaining in the final multiple logistic equation were distance from shoreline (P = 0.017) and height above water (P = 0.022) (Table 3).

The best discriminant model developed (P = 0.011) included only two site location variables: distance from shoreline and height above water (Table 4). The model correctly discriminated between sites with weevils and sites without 75% of the time, and no sites with weevils were misclassified as sites without (Table 5). However, some sites without weevils were misclassified as sites with weevils. The model may be identifying suitable habitat that was

Table 3. Site characteristics in the final multiple logistic regression analysis (P <0.001) for Thomas Lake. Alpha was set at P $\leq0.05.$

Dependent Variable	Independent Variables	Coefficients	Р
Weevil presence	Distance from shoreline	0.97012	0.017
-	Height above water	-0.06508	0.022
	Intercept	0.23025	0.822

Variables included in initial model run: soil texture, soil/duff moisture, soil/duff organic matter, distance from shoreline, height above water, duff depth, percent cover wood, percent cover, deciduous leaves, percent cover grass, percent cover forbs, percent cover bare soil.

Table 4. The "best" canonical discriminant function developed for Thomas Lake used distance from shoreline and height above water. Alpha was set at P $\,<\,0.05.$

Variable	Structure Coefficient
Distance from shoreline	0.85572
Height above water	-0.09243
Wilke's lambda probability	0.011

unoccupied, which would be expected with a low sample size (13 sites with weevils vs. 40 sites without).

Springville Pond

Milfoil weevils were found at 17 sites of the 45 sites sampled. The number of weevils found ranged from 0 to 25 per m², with an average of 3.1 weevils per m². Sites were located at 1 to 10 m from the shoreline ($\bar{x} = 4$ m), and 37 to 227 cm above the water vertically ($\bar{x} = 107$). We evils were found at 2 to 6 m from shoreline ($\bar{x} = 3$ m), and from 43 to 195 cm above the water ($\bar{x} = 96$ cm). Average weevil density was 4.8 per m^2 at the sites closest (2 m) to the shoreline, and 1.7 per m^2 at the sites farther (6 m) from the shoreline. Shoreland habitat types included sites having no disturbance to high disturbance, and were most commonly characterized as upland forest dominated by either coniferous trees or mixed deciduous and herbaceous vegetation. Soil type was sand on all sites except one, where loamy sand occurred. Duff depth ranged from 1 to 6 cm, and composition of duff material was most commonly dominated by leaves and/or grasses. Percent moisture in soil/duff composite samples ranged widely from 6 to 84%, with a mean of 37%. Percent organic matter also ranged widely, from < 1 to 56%, with a mean of 12%.

Non-parametric *t* tests found significant differences in duff depth (P = 0.015) and distance from shoreline (P = 0.031) between sites with weevils and sites without. Mean duff depth at sites with weevils was 3.5 cm, versus a mean depth of 2.6 cm at sites without (a difference of 0.9 cm). Mean distance from shoreline at sites with weevils was 3.1 m, versus a mean distance of 4.5 m at sites without (a difference of 1.4 m). Most weevils occurred between 2.4 m and 3.8 m from the shoreline (Table 2).

Logistic regressions found distance from shoreline and duff depth to be significantly correlated variables (P = 0.048 and P = 0.018, respectively); probability of weevil presence decreased as distance from shoreline increased, and probability of weevil presence increased as duff depth increased.

TABLE 5. PREDICTION OF SITES WITH WEEVILS VS. SITES WITHOUT THE "BEST" CANONICAL DISCRIMINANT FUNCTION AT THOMAS LAKE. THIS FUNCTION CORRECTLY CLASSIFIED ALL SITES WITH WEEVILS, BUT MISCLASSIFIED SOME SITES WITHOUT WEEVILS.

		Predicted Group Membership		
Group	No. of Sites	Weevils	No Weevils	Sites Correctly Predicted (%)
Weevils	13	13	0	100
No weevils	40	20	20	50.0
Overall per	75.0			

Table 6. The "best" canonical discriminant function developed for Springville Pond was composed of distance from shoreline and duff depth. Alpha was set at P < 0.05.

Variable	Structure Coefficient
Distance from shoreline	0.74673
Duff depth	-0.88411
Wilke's lambda probability	0.0153

The best discriminant model developed for Springville Pond included distance from shoreline and duff depth (Table 6). The model correctly discriminated between sites with weevils and sites without just 66% of the time, and misclassified six sites with weevils as sites without weevils (Table 7). Although the model was significant (P = 0.015), it did not do as well at discriminating between sites as the Thomas Lake function did.

DISCUSSION

Four variables were found to be significantly related with weevil presence or abundance: height above water, distance from shoreline, percent cover of leaves, and duff depth. Weevils were not significantly related to many of the other riparian habitat characteristics measured, including: the presence of milfoil fragments along shore; habitat type; soil texture; soil and duff moisture; soil and duff organic matter; and percent cover of woody debris, conifer needles, grass, forbs, rocks, or bare soil.

Both logistic regression and discriminant analysis were able to discriminate characteristics between sites having weevils versus those that did not. Height above water positively discriminated between sites with weevils and those without on Thomas Lake, and this may relate to the moisture threshold reported by Newman et al. (2001). Newman et al. (2001) found weevil densities were significantly lower at sites with > 15% soil moisture, suggesting weevils prefer dry sites. Data from McDill Pond, an impoundment in Portage County, WI, with naturally high weevil populations, suggest an apparent minimum height threshold around 50 cm; no weevils were found at sites where height above water was < 50 cm (n = 11) (Thorstenson 2011). Although height above water was not found to be statistically significant on McDill Pond due to low sample size (n = 10 for sites with weevils, n = 42 for sites without), the cumulative evidence suggests that low, wet sites are not ideal weevil habitat.

Distance from shoreline was consistently significant and negatively correlated to weevils in both the Thomas Lake and Springville Pond data, suggesting weevils occur more

TABLE 7. THE "BEST" CANONICAL DISCRIMINANT FUNCTION AT SPRINGVILLE POND TO DISTINGUISH SITES WITH AND WITHOUT WEEVILS. THIS MODEL MISCLASSIFIED SIX SITES WITH WEEVILS AS SITES WITHOUT WEEVILS.

		Predicted Group Membership		
Group	No. of Sites	Weevils	No Weevils	Sites Correctly Predicted (%)
Weevils	17	11	6	64.7
No weevils	28	9	19	67.9
Overall per	66.3			

often at sites closer to the shoreline. Weevils most commonly occurred within a few meters from the shoreline, as was the case for Newman et al. (2001). Mean distances at Thomas Lake and Springville Pond were 5.3 m and 3.1 m, respectively, however, we also found weevils as far from the shoreline as 8.3 m. Newman et al. (2001) found weevils as far as 20 m from the shoreline. This suggests that while most weevils prefer overwinter habitat close to the water (and their summer habitat), habitat protection must extend beyond just a few meters. Wisconsin law requires shoreland buffers of 10.6 m. This may be adequate to protect most weevil habitat, but not all, and certainly not where the near shore zone includes low, wet areas unsuitable for weevils. In such cases, the shoreland buffer must be extended beyond the required 10.6 m to include high and dry habitat for weevils.

On Thomas Lake, weevil presence was negatively correlated with distance from shoreline (probability of weevil presence decreased with distance) and positively correlated with percent cover of leaves (probability of weevil presence increased with leaves). However, percent cover of leaves was eliminated from both the multiple logistic regression and the discriminant analyses, making it is difficult to discern whether percent cover of leaves was truly an important driver in weevil presence or absence, or if it is simply a coincidental occurrence in the near shore zone that weevils appear to prefer. Newman and Biesboer (2000) documented a weevil-associated milfoil decline on Cenaiko Lake, MN; a lake with shoreland dominated by prairie, suggesting that trees (and deciduous leaf duff) are not required by weevils (Newman et al. 2001).

Duff depth seemed to be important in explaining weevil distribution. Like percent cover of leaves, duff depth could also be a coincidental correlation to weevils only because duff depth and weevil presence both decreased with distance. However, duff depth remained a contributing variable in the discriminant function developed on Spring-ville Pond (Structure Coefficient = -0.88), suggesting that duff depth may truly be a driver in weevil presence and absence and not merely coincidental. Duff depth seems an important variable to consider further.

For example, Jester et al. (2000) found a positive correlation between weevil abundance and "natural" sites, suggesting that "natural" sites offer something that "disturbed" sites do not. Although our study was not designed to analyze the value of "natural" sites versus "disturbed" sites, the results on Springville Pond were interesting in this regard. Nine of the sites sampled on Springville Pond were characterized as moderately to highly disturbed sites (habitat type 10 or 11), with beach, lawn, or landscaping. Only one of the nine sites (11%) contained weevils, which happened to be an unraked lawn with some leaf litter. In contrast, 16 of the 36 natural to low disturbance sites (44%)contained weevils. Overall, 96% of the weevils were collected from natural to low disturbance sites, where mean duff depth was 3.1 cm, while the moderately to highly disturbed sites averaged only 1.7 cm. It is likely that higher duff depth is one of the advantages that natural shorelands provide for weevils.

Whereas the results of Thomas Lake make it unclear whether duff composition is significantly related to weevil presence, the Springville Pond results seem to indicate that the presence of duff material is likely related, and the more the better. On both lakes, weevils were never found at sites with no duff, such as bare sand or mowed, raked lawns. Depth of duff layer is one variable that can be easily altered through management, and lake residents can make a direct contribution in this regard. Raking and mowing of shorelands removes duff, while natural, unraked, unmowed shorelands provide duff material for weevils to overwinter in.

Managing shorelands for weevil habitat should be approached on a lakewide basis, however. In-lake weevil population data available for Thomas Lake (from another 2009 study) was compared to on-shore weevil occurrences in this study, and no spatial relationship could be discerned. The milfoil bed with the most weevils did not have the most weevils on the adjacent shore, nor vice versa. Newman and Inglis (2009) also did not find a relationship between in-lake weevil density and shoreland habitat within a lake. It appears that in-lake weevil occurrence is not a predictor of where they will occur on-shore, suggesting shoreland management activities should be applied widely.

Jester et al. (2000) documented a positive correlation between in-lake weevil densities and "natural" shorelands across lakes, and Newman et al. (2001) found that weevils correlated positively with sites that are both drier and closer to the shoreline. The results provided here concur with those and go further, suggesting that weevils also prefer sites with deeper duff, but are not significantly related to many of the additional riparian habitat characteristics we measured. This suggests that weevil overwintering habitat requirements may actually be rather broad. While it remains unclear whether it would be important to plant specific types of vegetation (herbaceous versus non-herbaceous), it is unequivical that minimizing disturbance of shoreland vegetation is essential, especially in high, dry, near-shore zones.

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