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Euhrychiopsis lecontei Distribution, Abundance, and Experimental Augmentations for Eurasian Watermilfoil Control in Wisconsin Lakes

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ABSTRACT

The specialist aquatic herbivore *Euhrychiopsis lecontei* (Dietz) is currently being researched as a potential biological control agent for Eurasian watermilfoil (*Myriophyllum spicatum* L.). Our research in Wisconsin focused on 1) determining milfoil weevil distribution across lakes, 2) assessing limnological characteristics associated with their abundance, and 3) evaluating milfoil weevil augmentation as a practical management tool for controlling Eurasian watermilfoil. The geographic distribution of the milfoil weevil is widespread with 49 new records of the weevil among Wisconsin lakes containing Eurasian watermilfoil. Among 31 of the Wisconsin lakes that contained the milfoil weevil, their abundance varied from non-detectable to 2.5 weevils per stem of Eurasian watermilfoil. No whole-lake characteristics and only some milfoil bed characteristics such as the percentage of natural shoreline, the depth and distance of the Eurasian watermilfoil bed from shore, the number of apical tips and the percentage of broken apical tips per stem of Eurasian watermilfoil, were significantly correlated with milfoil weevil abundance. Twelve Wisconsin lakes augmented with one of three different treatment levels of weevils (1, 2 or 4 weevils per Eurasian watermilfoil stem) showed some significant damage to the Eurasian watermilfoil in small study plots at the end of the first treatment season. Additional sampling to assess long-term effects of this augmentation is ongoing.

Key words: Myriophyllum spicatum, milfoil weevil, weevil augmentation, biological control.

INTRODUCTION

The exotic aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum* L.) invaded Wisconsin lakes in the 1960s, and continued to spread across the state (Bode et al. 1993). Most Eurasian watermilfoil infestations spread quickly within a lake and often reach nuisance levels; inhibiting recreation, displacing native plant communities, and altering fish and invertebrate communities. Although mechanical harvesting and chemical herbicides are often used as control methods for Eurasian watermilfoil, they provide only short-term relief (Aiken et al. 1979, Smith and Barko 1990) and have potentially negative effects on non-target plants and animals (Engel 1990a,b).

Natural declines of Eurasian watermilfoil in many lakes around the United States have been concurrent with the presence and increased abundance of the aquatic milfoil weevil (*Euhrychiopsis lecontei* Dietz, Creed and Sheldon 1995, Kirschner 1995, Lillie and Helsel 1997, Creed 1998). The milfoil weevil is native to North America and is known to exist in lakes across the northern tier of the United States and southern Canada (Newman and Maher 1995, Sheldon and O'Bryan 1996, Creed 1998), although little is known about its specific distribution. Recent research has demonstrated that the milfoil weevil significantly reduces biomass and density of Eurasian watermilfoil in numerous field and lab experiments (Creed and Sheldon 1993, 1995, Creed et al. 1992, Sheldon and Creed 1995, Newman et al. 1996).

Previous studies have detailed the life history of the milfoil weevil (see Creed et al. 1992, Creed and Sheldon 1993, Solarz and Newman 1996, Newman et al. 1996, 1997, Sheldon

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and O'Bryan 1996) and its effects on Eurasian watermilfoil. The milfoil weevil overwinters in leaf litter along the shoreline as adults and returns to the milfoil bed in the spring to lay eggs in the apical meristems of the milfoil plants. The destruction of the milfoil is caused by the larvae which consume the tissue of the apical tip and burrow into the stem just below the tip. Later larval instars continue burrowing through the stem, consuming vascular tissue and eventually pupating within the stem. The hollow stem loses the ability to transport nutrients and carbohydrates and loses buoyancy in the water column. Research also shows that native aquatic plants are rarely used by the milfoil weevil for rearing and feeding and thus the weevil does not impact their growth (Sheldon and Creed 1995, Solarz and Newman 1996).

Additional research on milfoil weevil distribution, life history requirements and potential use as a control agent is warranted. The objectives of this study were to better document the geographic distribution of the milfoil weevil in the Wisconsin, assess limnological and geographical characteristics associated with its abundance, and evaluate the effectiveness of augmenting milfoil weevil populations as a practical management tool for Eurasian watermilfoil control. In this paper we report new records of the milfoil weevil in Wisconsin, report milfoil weevil abundance and associated variables from a subset of lakes, and report preliminary results on the effectiveness of augmenting milfoil weevil populations as a management tool.

METHODS

Geographic Distribution

The geographic distribution of the milfoil weevil in Wisconsin was studied by assessing the presence of the weevil using two methods in 50 Wisconsin lakes containing Eurasian watermilfoil during the summers of 1996 and 1997. First, we surveyed 44 lakes to assess presence of the milfoil weevil by snorkeling or by boat in surfacing beds and visually searched for milfoil weevil eggs, larvae, pupae or adults. During these surveys, diagnostic evidence of milfoil weevil herbivory aided in the search for actual specimens by helping to identify areas to search more intensively. In each lake, a maximum of four man-hours was spent intensively searching for the milfoil weevil to identify their presence. Second, 6 additional lakes were surveyed during macrophyte sampling used to assess abundance of the milfoil weevil (described later). Adult milfoil weevil specimens were preserved and maintained as voucher specimens for each lake except Little Falls Lake, Mason Lake, and Parker Lake where adult specimens were not collected; only larvae from these lakes were collected and kept as voucher specimens. All specimens are housed in the Museum of Natural History at the University of Wisconsin—Stevens Point.

Milfoil Weevil Abundance

The abundance of the milfoil weevil was evaluated in 31 Wisconsin lakes once between mid-July and mid-August 1996 or 1997. In each lake, four Eurasian watermilfoil beds that had not been harvested or treated with herbicides were selected for sampling. The distance between the four macrophyte beds was maximized to provide an estimate of weevil abundance for the entire lake. A total of 120 apical stems of Eurasian watermilfoil was collected from each lake (4 beds × 3 transects/bed × 5 sampling points/transect × 2 stems/sampling point). Within each Eurasian watermilfoil bed, three equidistant transects (relative to the macrophyte bed width) were sampled. Transects were perpendicular to shore. If Eurasian watermilfoil was distributed throughout the entire lake, then transects ran from the center of the lake to shore. Along each transect, two stems from five equidistant points in the Eurasian watermilfoil bed were sampled. At each sampling point, the top 50 cm of the first two stems of Eurasian watermilfoil touching the snorkeler's hand beneath the surface were collected. Because only the top 50 cm of stem was collected, these are referred to as apical stems herein. These apical stems often included several lateral branches. To minimize seasonal influences on abundance, lakes in southern Wisconsin were sampled first followed by central Wisconsin lakes and finally northern Wisconsin lakes.

In the laboratory, the stems were inspected for the presence of milfoil weevil eggs, larvae, pupae, and adults and for damage by the weevils. All apical tips (i.e., apical meristems) and portions of stems with damage were inspected with a dissecting microscope between 10× and 20× power. Stems damaged by herbivory were sliced open length-wise with a razor blade and larvae and pupae were extracted (Creed and Sheldon 1995). The number of weevils in each life stage (egg, larva, pupa, adult) on each stem was recorded and all were preserved. Abundance was recorded as the number of milfoil weevils (all lifestages combined) per apical stem of Eurasian watermilfoil and confidence intervals were calculated. Abundance was also recorded as the number of milfoil weevils (all lifestages combined) per apical tip of Eurasian watermilfoil by dividing the number of milfoil weevils collected by the number of tips (both damaged and undamaged) collected.

Independent variables that might explain variation in weevil abundance were collected and tested for correlations with weevil abundance. Some variables were whole-lake characteristics (lake-level variables) and other variables were measured in, or adjacent to the bed of Eurasian watermilfoil being sampled (bed-level variables). Lake-level variables collected during weevil abundance sampling included water temperature, dissolved oxygen and Secchi depth each taken at the lake's deepest point. Additional lake-level variables were acquired from the Wisconsin Department of Natural Resources and the Environmental Protection Agency STORET database. These variables included lake location, maximum and mean depth, area, and type (seepage, drainage or spring-fed), time of the Eurasian watermilfoil invasion, and water chemistry variables collected within a few years of weevil abundance sampling such as total phosphorus, total alkalinity, total Kjeldahl nitrogen, nitrate plus nitrite, chlorophyll a, pH, and conductivity.

Bed-level variables collected during weevil abundance sampling included the distance to shore from the deep and shallow edges of the bed and the depth of each transect sampling point. Riparian conditions were visually estimated using percentages of each shoreline type present at the land-water interface (natural shore, mowed lawn to water's edge, sand, or seawall/rip-rap) parallel to the sampled bed or at the end of a transect if the entire littoral zone was sampled. Specific Eurasian watermilfoil characteristics such as biomass, density, stem length and number of apical tips were also measured in a subset of lakes as part of the final study objective (see below).

Independent variables were not normally distributed and thus were analyzed for significant associations with milfoil weevil abundance using Spearman rank correlation analysis ($p \leq 0.05$). Associations with lake-level variables were tested using the mean number of milfoil weevils per apical stem for the entire lake (i.e., across all four Eurasian watermilfoil beds) while bed-level variables were tested using the mean milfoil weevil per apical stem for that bed.

Milfoil Weevil Population Augmentation

In 12 Wisconsin lakes, existing milfoil weevil populations were augmented with additional weevils in designated plots to determine the effectiveness of stocking weevils to control Eurasian watermilfoil. In each lake, four plots, 2 m × 6 m, were established and in July 1997 small bundles of Eurasian watermilfoil containing estimated numbers of milfoil weevil eggs and larvae were tied onto existing Eurasian watermilfoil plants in three of the four plots. The fourth plot was a reference and was not augmented. In each lake existing weevil abundance was augmented to obtain a final treatment level of 1, 2 or 4 weevils per stem of milfoil. Past research suggested that approximately two weevils per stem resulted in significant Eurasian watermilfoil declines (Newman et al. 1996). The actual number of weevils released in each plot ranged from 115 to almost 10,000. Treatment levels were randomly assigned to the lakes unless the augmentation sites were found to have greater than the randomly assigned augmentation level. In these cases, higher augmentation levels were randomly assigned when possible. Plots within a lake were replicates and plots among lakes were treatments. Milfoil weevil eggs and larvae were cultured at Middlebury College in Vermont from adults collected in Wisconsin. Adults were shipped to Vermont in coolers, on ice via overnight express; eggs and larvae were returned in the same manner. The rectangular plots were set parallel to shore with approximately 9 m between the plots. While we tried to keep depth constant among lakes, depth of the plots ranged from 1.0 m to 3.0 m depending on the Eurasian watermilfoil depth in each lake, but plot depth within each lake remained constant. Although boat traffic was encouraged to stay away from the plot areas, neither enclosures nor exclosures were established.

Prior to augmentation, milfoil weevil abundance was estimated in June 1997 to determine the number of weevils needed to bring populations up to the desired treatment level. Approximately five weeks post-augmentation (i.e., late August 1997), milfoil weevil abundance was measured again among the plots to assess population levels. Milfoil weevil abundance was measured among the plots again in June and August 1998.

Macrophytes were sampled in the plot areas using a 0.15 m^2 quadrat sampler and scuba. In 1996, the year before augmentation, 16 samples were collected along the augmentation plot contour. In 1997, approximately five weeks post-augmentation, three randomly selected samples were collected from each of the four plots for a total of 12 samples. Macrophytes were collected during peak plant biomass in August of both years and measured for biomass, stem density, stem length, number of apical tips and the percentage of broken tips as well as the biomass of native macrophytes.

In the lab, samples were processed by "floating" plants lengthwise in an aquaculture raceway. The number of plants in each sample was counted to determine plant density and converted to number of plants per square meter. A sub-sample of 30 plants per sample was used to get average plant length (cm), number of tips per plant, and the number of broken tips per plant. Plants covered with calcium carbonate deposits were soaked in a 5% hydrochloric acid bath and rinsed with tap water to remove the deposits. Macrophytes other than Eurasian watermilfoil were separated and identified to species. All plants of each species were then air dried for 30 minutes and measured for wet weight. To determine dry weight biomass, plants from each species and each sampling site were placed in a pre-dried, tared paper bag, dried at 106C to a constant weight (approximately 56 hours), and weighed.

Wilcoxon Signed-Rank Tests were used to determine significant differences in Eurasian watermilfoil variables between pre-augmentation (1996) to post-augmentation (1997) and between reference and treatment plots. Data for only one variable, the percentage of broken tips, was normally distributed, thus a parametric t-test was performed. Alpha was set at $p \leq 0.05$ for all tests. Additional research will determine changes in Eurasian watermilfoil within the augmentation plots in future years.

RESULTS

Milfoil Weevil Distribution

The milfoil weevil was widely distributed across Wisconsin in lakes containing Eurasian watermilfoil (Figure 1, Table 1). The milfoil weevil was found in 49 lakes where it was not previously recorded and was found in nearly every lake sampled except Silver Lake in Waupaca County. After searching for four man-hours in Silver Lake, no milfoil weevils at any life-stage were found and there was no evidence of milfoil weevil herbivory. In most other lakes, a milfoil weevil adult was collected within ten minutes of searching. In three lakes where adults were not found, Mason and Parker Lakes in Adams County and Little Falls Lake in St. Croix County, only larval weevils were found during weevil abundance sampling.

Lakes with new records of the milfoil weevil were variable in type, size and location (Table 1). Both seepage and drainage lakes were found to harbor weevils, lake size ranged from 1.2 ha (Lower Kelly Lake) to almost 3,000 ha (Big Green Lake) and maximum lake depth ranged from 0.6 m (Goose Pond) to 71.9 m (Big Green Lake). The lakes were also widely distributed geographically around the state (Figure 1). General characteristics of Eurasian watermilfoil beds in lakes with the milfoil weevil also varied. Many lakes had very dense and broad beds of milfoil while others had narrow bands of Eurasian watermilfoil in the littoral zone, or sporadic distributions. Mean depth of Eurasian watermilfoil beds varied as well as the height of the plants in the water column.

Milfoil Weevil Abundance and Correlated Variables

Milfoil weevil abundance across 31 lakes varied from 0.0 (non-detectable) to 2.5 weevils per apical stem with a mean

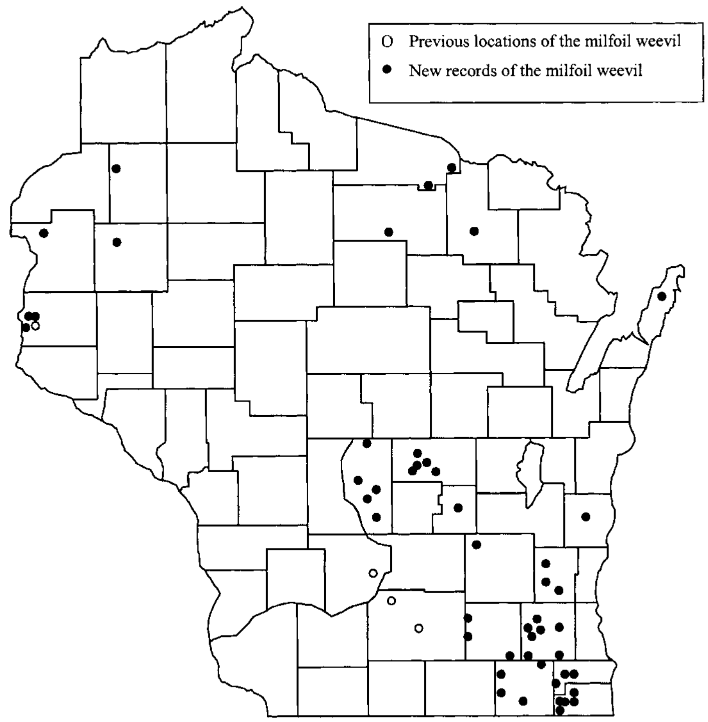

Figure 1. Known distribution of the milfoil weevil in Wisconsin. Previous locations referenced in Lillie (1991), Newman and Maher (1995), and Lillie and Helsel (1997).

of 0.65 weevils per apical stem (Figure 2, Table 2). Milfoil weevil larvae and eggs were found to be more abundant than adults or pupae in 30 of the 31 lakes. While adults never made up more than 36.4% and pupae never made up more than 25% of weevil lifestages collected in any one lake, larval abundance reached as high as 100% and eggs as high as 84.6% of weevils collected in some lakes.

In 19 of 31 lakes, all life stages of the milfoil weevil were collected during abundance sampling. Within lakes, milfoil weevil abundance also varied among Eurasian watermilfoil beds with a mean difference of 0.81 weevils per apical stem between the highest and lowest abundance found among beds within one lake (Table 3). The greatest difference between beds in one lake was 2.3 weevils per apical stem in Lake Wingra.

Milfoil weevil abundance was not significantly correlated with any lake-level variables such as latitude of the lake, time since the invasion of Eurasian watermilfoil, lake depth (maximum or mean), lake type, or lake size. Milfoil weevil abundance also did not significantly correlate with water quality variables such as summer water temperature, dissolved oxygen, Secchi disc depth, total phosphorus, NO_2 + NO_3 nitrogen, total Kjeldahl nitrogen, chlorophyll a, alkalinity, pH, nor conductivity.

Milfoil weevil abundance was significantly correlated with some bed-level variables (Table 4). Abundance was positively correlated with the percentage of broken apical tips ($r = 0.54$), distance from shore to the middle ($r = 0.25$) and deep ($r = 0.28$) edge of the Eurasian watermilfoil bed, percentage of natural shoreline ($r = 0.21$), and number of apical tips per Eurasian watermilfoil plant ($r = 0.60$). Weevil abundance was also negatively correlated with depth of the Eurasian watermilfoil bed ($r = -0.30$) and the percentage of sandy shoreline ($r = -0.29$).

The percentage of broken apical tips of Eurasian watermilfoil was also correlated with some lake-level and bed-level variables including lake surface area ($r = 0.49$), maximum depth ($r = 0.50$), distance from shore to the middle of the Eurasian watermilfoil bed (0.27), and depth of the bed ($r = 0.81$).

First-year Results of Milfoil Weevil Augmentation

Although augmentation was designed to bring weevil abundance up to the desired treatment level in the plots, the actual observed weevil abundance five weeks post-augmentation was well below treatment levels in most lakes (Table 5). Survival of weevils after shipping was high (approximately 75%), and would not account for declines. Analysis of first year post-augmentation data indicates that many milfoil variables collected in plots decreased significantly from pre-augmentation. Moreover, significant declines were more common in lakes that received the highest treatment level (4 weevils per stem) (Table 5). For instance, 60% of lakes given the lowest treatment level (1 weevil per stem) experienced significant declines in Eurasian watermilfoil biomass while 67% of lakes given the medium treatment level (2 weevils per stem) and 100% of lakes given the highest treatment level experienced significant declines in Eurasian watermilfoil biomass. Almost the same was true for Eurasian watermilfoil stem density: 40% of lakes given the lowest treatment level, 67% of lakes given the medium treatment level and 75% of lakes given the highest treatment level showed significant declines in stem density.

When comparing post-augmentation Eurasian watermilfoil measurements between augmented plots and reference plots, far fewer significant differences were detected (Table 6). Only one lake showed a significant decrease in Eurasian watermilfoil biomass as compared to the reference plot and no lakes showed a significant difference in Eurasian watermilfoil stem density between reference plots and treatment plots.

While significant declines were apparent from the data in both treatment and reference plots, these observations were not always apparent in the field. Although some lakes had extensive larval damage in and around the plots several weeks after augmentation, there did not appear to be a visually noticeable decline of Eurasian watermilfoil in most plot areas. Three lakes did experience substantial declines in Eurasian watermilfoil that were visually apparent (Mukwonago, Kusel, and Lower Spring). However, all three lakes were in the midst of a natural Eurasian watermilfoil decline at the onset of weevil augmentations.

DISCUSSION

Milfoil Weevil Distribution

This study shows that the milfoil weevil is geographically widespread throughout Wisconsin as 49 new records of the milfoil weevil from southern, central and northern parts of the state were found. More importantly, because nearly every lake surveyed in this study actually contained the weevil, it is likely that the milfoil weevil is widespread across most Wis-

TABLE 1. NEW RECORDS OF *EUHRYCHIOPSIS LECONTEI* IN WISCONSIN.

Lake name	County	Type[a]	Location	Surface area (hectares)	Max. depth (m)	Date of collection
Alpine Lake	Waushara	DG	T19N R11E Sec. 4	22.4	5.5	17 July 1997
Bark Lake	Washington	SE	T9N R19E Sec. 26	24.8	10.4	9 June 1997
Bass Bay	Waukesha	DG	T5N R20E Sec. 15	40.0	7.0	12 July 1997
Bass Lake	St. Croix	SE	T30N R19E Secs. 23,26	166.8	10.7	10 June 1998
Beaver Dam Lk.	Barron	SE	T35N R13W Secs. 5, 6, 7, 8 T35N R14W Sec. 1	444.8	32.3	12 June 1996
Beulah lake	Walworth	DG	T4N R18E Sec. 4	333.6	17.7	21 July 1997
Big Cedar Lake	Washington	SE	T10N R19E Sec. 5; T11N R19E Secs. 20,20,30,31,32	372.8	32.0	25 June 1996
Big Green Lake	Green Lake	DG	T15,16N R12,13E	2938.4	71.9	08 August 1997
Big Sand Lake	Vilas	DG	T41N R12E Secs. 2,3,4,9; T42 R12E Secs. 34,35	563.2	19.8	25 June 1996
Camp Lake	Kenosha	DG	T1N R20E Secs. 20,21,28,29	184.4	5.2	02 August 1996
Crooked Lake	Waukesha	DG	T7N R17E Sec. 23	23.2	4.9	17 Sept. 1996
Crystal Lake	Sheboygan	SE	T16N R21E Secs. 31, 32	60.8	18.6	30 July 1997
Delavan Lake	Walworth	DG	T2N R16E Secs. 21, 22, 27, 28, 32, 33	828.8	17.1	28 July 1997
Eagle Lake	Racine	DG	T3N R20E Secs. 21,22,27,28	208	3.6	06 June 1996
Elizabeth Lake	Kenosha	DG	T1N R19E Secs. 28, 29, 32, 33	255.2	9.7	13 August 1997
Fox Lake	Dodge	DG	T13N R13E	1050.0	5.8	11 August 1997
Friendship Lake	Adams	DG	T17N R6E Sec. 5	46.0	4.9	27 June 1997
George Lake	Kenosha	DG	T1N R21E Secs. 20,29	23.6	4.9	02 August 1996
Gilbert Lake	Waushara	SE	T20N R11E Secs. 10,11,14,15	56.4	19.8	10 June 1996
Goose Pond	St. Croix	SE	T28N R20W Sec. 8	5.6	0.6	10 June 1998
Johns Lake	Waushara	SE	T19N R11E Sec. 32	29.2	12.5	24 June 1998
Jordan Lake	Adams	SE	T15N R7E Sec. 34	85.2	24.1	27 June 1997
Kangaroo Lake	Door	DG	T29N R27E Sec. 1	449.2	3.6	10 July 1997
Kusel Lake	Waushara	SE	T20N R11E Secs. 26,27,34,35	31.6	8.8	10 June 1996
Lac La Belle	Waukesha	DG	T8N R17E	465.6	13.7	29 July 1997
Little Falls Lake	St. Croix	SE	T29N R19W Secs. 4,8,9	68.8	5.5	14 August 1996
Long Trade Lake	Polk	DG	T36N R18W Sec. 49	61.2	4.0	06 August 1996
Lorraine Lake	Walworth	SE	T3N R15E Sec. 29	53.2	2.4	06 June 1996
Lower Kelly Lake	Waukesha	SE	T6N R20E Sec. 36	1.2	11.0	02 June 1997
Lower Spring Lake	Jefferson	DG	T5N R16E Secs. 22,23	41.6	3.3	06 June 1996
Marie Lake	Kenosha	DG	T1N R19E Sec. 21, 28	118.8	10.0	13 August 1997
Manson Lake	Oneida	DG	T36N R7E Secs. 32,33	94.4	16.4	25 June 1996
Mason Lake	Adams	DG	T13N R7E Secs. 25, 26, 35, 36 T13N R8E Secs. 30, 31	34.2	2.7	13 August 1996
Metonga Lake	Forest	DG	T35N R13E Sec. 8	862.8	24.1	14 August 1997
Mukwonago Pond	Waukesha	SP	T5N R18E Sec. 29	6.4	1.5	18 June 1996
Nancy Lake	Washburn	SE	T42N R13W Secs. 27,28,33	308.8	11.9	12 June 1996
North Lake	Waukesha	DG	T8N R18E Secs. 16,17,20,21	174.8	23.8	14 August 1996
Paddock Lake	Kenosha	DG	T1N R20E Sec. 2	44.8	9.7	02 August 1996
Parker Lake	Adams	SE	T15N R7E Sec. 14	23.6	9.1	27 June 1997
Pearl Lake	Waushara	SE	T19N R12E Sec. 30	36.8	15.2	10 June 1996
Pike Lake	Washington	DG	T10N R18E Secs. 22,23,26,27	208.8	13.7	12 August 1996
Pine Lake	Waukesha	SP	T8N R18E Secs. 27, 28, 32	281.2	25.9	02 June 1998
Ripley Lake	Jefferson	SE	T6N R13E Sec. 7,8	167.2	13.4	31 July 1997
Rock Lake	Jefferson	DG	T7N R13E Secs. 2,10,11,14,15	548.4	17.1	13 August 1996
Sherwood Lake	Adams	DG	T20N R6E Secs. 16, 17	98.4	8.2	12 August 1997
Whitewater Lake	Walworth	DG	T3N R15E Sec. 3 T4N R15E Secs. 25, 26, 27, 34, 35	256.0	11.6	06 June 1996
Wind Lake	Racine	DG	T4N R20E Secs. 3,4,8,9,10,16,17	374.4	15.2	29 July 1996
Wolf Lake	Racine		T2N R20E Secs. 15, 22	46.0		08 August 1996
Yellow Birch Lk.	Vilas	DG	T40N R10E Secs. 21,22	80.8	7.0	13 August 1997

[a]DG = Drainage lake, SE = Seepage lake, SP = Spring Lake.

consin lakes containing Eurasian watermilfoil. In the only lake where weevils were not found, Silver Lake, the weevils may exist at a very low abundance, making it difficult to detect in our sampling. The higher numbers of lakes with the milfoil weevil in the southeast and central parts of the state merely reflect the greater distribution of Eurasian watermilfoil in those areas and extra effort by the WDNR in collecting specimens there.

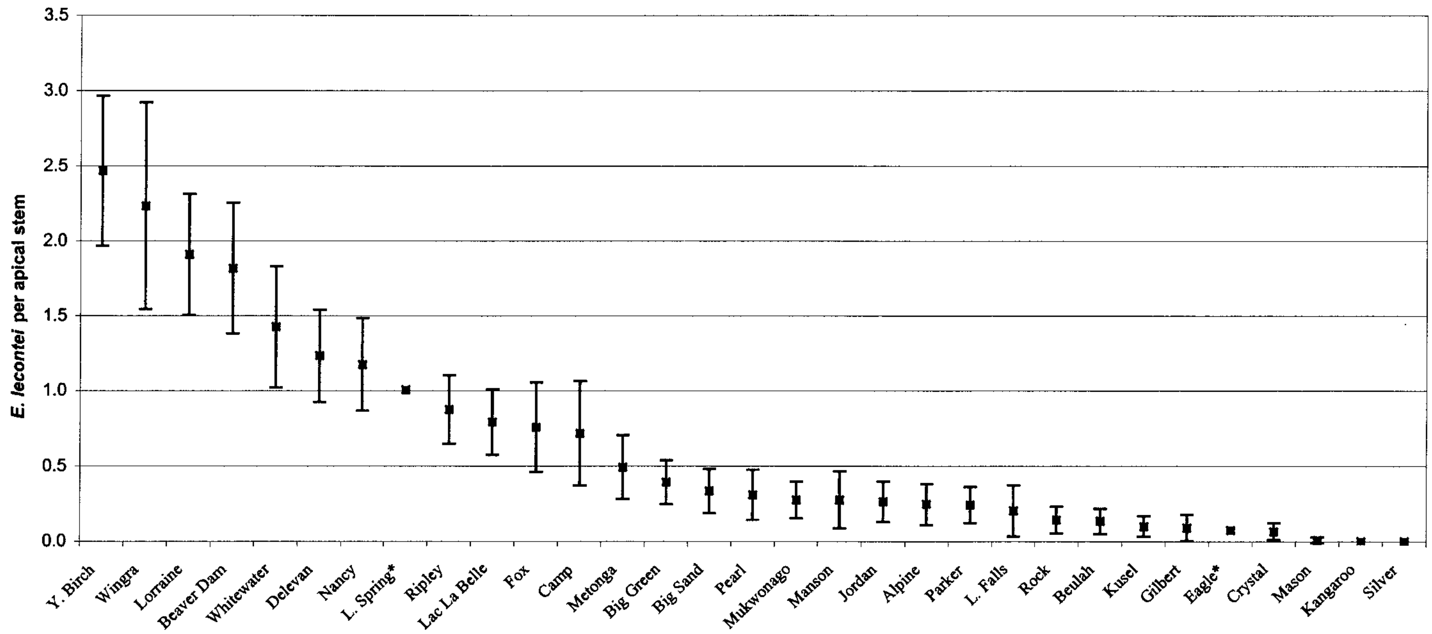

Figure 2. Abundance of the milfoil weevil in Wisconsin lakes prior to augmentation: all weevil lifestages combined. Values indicate the mean abundance of the milfoil weevil per apical stem ±95% confidence intervals. *Confidence intervals were not calculated for Lower Spring and Eagle Lakes due to a different sampling method.

The widespread distribution of the milfoil weevil was not expected. Newman and Maher (1995) found the milfoil weevil in only 10 of 25 lakes sampled in Minnesota and Wisconsin. In Washington, only 21 of 51 lakes surveyed were found to harbor the milfoil weevil (Tamayo et al. 1999). Generally, other states have not searched extensively for the milfoil weevil but, at least 12 Vermont, 7 Massachusetts, 1 New York, 2 Connecticut, 2 Michigan, 3 Ohio, 3 Illinois, and 5 British Columbia lakes are known to harbor the milfoil weevil (Creed 1998, Sallie Sheldon pers. comm.).

Declines of Eurasian watermilfoil in some lakes are being attributed to milfoil weevil populations due to specific evidence of damage to the Eurasian watermilfoil typical of milfoil weevil herbivory, concurrent with relatively high milfoil weevil populations (Lillie and Helsel 1997, Creed and Sheldon 1995, Kirschner 1995). Creed (1998) also showed significantly more declines of Eurasian watermilfoil have occurred within the known geographic range of the milfoil weevil than would be expected by chance (Creed 1998). Moreover, we observed at least ten Wisconsin lakes found to harbor the milfoil weevil in this study or previous surveys (Newman and Maher 1995, Lillie 1991) that have experienced Eurasian watermilfoil declines: Big Green Lake (Green Lake Co.), Devil's Lake (Sauk Co.), Fish Lake and Lake Wingra (Dane Co.), Long-Trade Lake (Polk Co.), Kusel Lake (Waushara Co.), Whitewater Lake (Walworth Co.), Yellow Birch Lake (Vilas Co.), Mukwonago Pond and Wind Lake (Waukesha Co.). Interestingly, Carpenter (1980) reported that the duration of peak abundance of Eurasian watermilfoil in many lakes is approximately 10 years before declines are evident. However, the definitive causes of Eurasian watermilfoil declines have not been established (Lillie and Helsel 1997, Carpenter 1980) but warrants further investigation into predator-prey cycles between Eurasian watermilfoil and the milfoil weevil.

Factors Influencing Milfoil Weevil Abundance

Milfoil weevil abundance varied greatly across the 31 lakes sampled as well as among different Eurasian watermilfoil beds within lakes. Our results indicate that some variables we measured within the Eurasian watermilfoil beds may influence milfoil weevil abundance more than the large-scale geographical and limnological variables. Milfoil weevil abundance was positively correlated with the percentage of broken apical tips. This relationship has biological relevance because herbivory damage to the apical tip and stem just below the tip occurs as larvae burrow into the stem consuming apical and vascular tissues. Fragile tips were often observed in conjunction with past or present larval herbivory and these tips were often broken prior to sampling or during handling. Due to the significant positive correlation between the percentage of damaged tips and the abundance of the milfoil weevil and the biological relevance of the correlation, the amount of milfoil tip damage was clearly an indication of milfoil weevil abundance.

Milfoil weevil abundance was also positively correlated with the distance from shore to the middle and deep edges of the Eurasian watermilfoil bed and negatively correlated with the depth of the Eurasian watermilfoil bed. These relationships indicate that weevils are more abundant in large, shallow expanses of Eurasian watermilfoil rather than deep (and perhaps non-surfacing) Eurasian watermilfoil closer to shore. This relation may characterize the type of Eurasian watermilfoil bed where the milfoil weevil has the best reproductive success. Milfoil weevil abundance was also positively correlated with the number of apical tips per Eurasian watermilfoil plant. However, it is unknown which response is the dependent variable in this relationship. On one hand, weevils may reproduce more effectively in milfoil beds with high-

TABLE 2. MEAN MILFOIL WEEVIL ABUNDANCE ±95% CONFIDENCE LIMITS IN WISCONSIN LAKES. WEEVILS PER APICAL STEM (TOP ~ 50 CM OF STEM) AND WEEVILS PER APICAL TIP (I.E., APICAL MERISTEM) INCLUDE ALL LIFESTAGES COMBINED. THE LAST FOUR COLUMNS REPORT THE PERCENTAGE OF WEEVILS IN EACH OF FOUR LIFESTAGES FOR EACH LAKE. WEEVILS PER APICAL STEM AND TIP REPRESENT THE MEAN OF ALL STEMS COLLECTED PER LAKE.

Lake	County	Weevils per apical stem	Weevils per apical tip	Percent adults	Percent larvae	Percent pupae	Percent eggs
Alpine	Waushara	0.2 ± 0.13	0.1	6.9	34.5	0.0	58.6
Beaver Dam	Barron	1.8 ± 0.44	0.5	6.4	42.7	10.1	40.8
Beulah	Walworth	0.1 ± 0.08	0.1	6.3	75.0	12.5	6.3
Big Green	Green Lake	0.4 ± 0.15	0.1	2.1	34.0	6.4	57.4
Big Sand	Vilas	0.3 ± 0.15	0.1	10.0	40.0	17.5	32.5
Camp	Kenosha	0.7 ± 0.35	0.1	15.1	16.3	1.2	67.4
Crystal	Sheboygan	0.1 ± 0.06	0.0	12.5	50.0	0.0	37.5
Delavan	Walworth	1.2 ± 0.31	0.2	16.9	16.2	11.5	55.4
Eagle	Racine	0.1[a]	0.0	0.0	66.7	0.0	33.3
Fox	Dodge	0.8 ± 0.30	0.1	0.0	56.0	4.4	39.6
Gilbert	Waushara	0.1 ± 0.09	0.0	0.0	81.8	0.0	18.2
Jordan	Adams	0.3 ± 0.13	0.1	6.5	58.1	12.9	22.6
Kangaroo	Door	0.0	0.0	0.0	0.0	0.0	0.0
Kusel	Waushara	0.1 ± 0.07	0.0	33.3	25.0	25.0	16.7
L. Spring	Jefferson	1.0[a]	0.3	7.4	73.6	19.0	0.0
Lac La Belle	Waukesha	0.8 ± 0.22	0.1	2.1	50.5	5.3	42.1
Little Falls	St. Croix	0.2 ± 0.17	0.1	0.0	15.4	0.0	84.6
Lorraine	Walworth	1.9 ± 0.41	0.4	9.2	41.9	21.0	27.9
Manson	Oneida	0.3 ± 0.19	0.1	6.1	57.6	9.1	27.3
Mason	Adams	<0.1 ± 0.02	0.0	0.0	100.0	0.0	0.0
Metonga	Forest	0.5 ± 0.21	0.1	3.4	59.3	3.4	33.9
Mukwonago	Waukesha	0.3 ± 0.12	0.0	36.4	54.5	9.1	0.0
Nancy	Washburn	1.2 ± 0.31	0.4	3.5	52.5	6.4	37.6
Parker	Adams	0.2 ± 0.12	0.0	3.4	48.3	10.3	37.9
Pearl	Waushara	0.3 ± 0.17	0.1	0.0	70.3	2.7	27.0
Ripley	Jefferson	0.9 ± 0.23	0.2	1.9	21.0	8.6	68.6
Rock	Jefferson	0.1 ± 0.09	0.1	11.8	64.7	11.8	11.8
Silver	Waupaca	0.0	0.0	0.0	0.0	0.0	0.0
Whitewater	Walworth	1.4 ± 0.40	0.3	8.8	41.5	7.0	42.7
Wingra	Dane	2.2 ± 0.69	0.5	4.1	18.7	3.4	73.9
Y. Birch	Vilas	2.5 ± 0.50	0.5	3.1	49.1	2.4	45.4
		mean = 0.65	mean = 0.15	mean = 7.01	mean = 45.65	mean = 7.13	mean = 33.77

[a]Lakes were sampled with different sampling method; confidence interval could not be calculated.

er numbers of apical tips because there are more oviposition sites. In contrast, perhaps the Eurasian watermilfoil plants respond to high weevil abundance and greater amounts of tip damage by growing new apical tips.

At the bed-level, milfoil weevil abundance was positively correlated with the percentage of natural shoreline. Milfoil weevils overwinter in the leaf litter and mud along the shore within a few meters of the water (Newman et al. 1997). It is possible that the milfoil weevil is more abundant along natural shorelines because they are more successful at overwintering in these areas. Habitats other than a natural shore, such as a seawall, rock rip-rap, sand, or mown grass, may not offer enough protection or burrowing capabilities for hibernating adult weevils. Unfortunately, more specific characteristics of the natural shorelines were not measured in this study and the majority of beds sampled were adjacent to natural shorelines. More research needs to be conducted regarding the importance of overwintering success in weevil distribution and population dynamics.

Variables measured at the lake-level were not correlated with milfoil weevil abundance. Time since the Eurasian watermilfoil invasion was not a factor in milfoil weevil abundance although we speculated that the longer Eurasian watermilfoil had resided in a lake (i.e., years), the more time the weevil had to increase its populations in a predator response to increased prey. However, factors such as the rate of Eurasian watermilfoil expansion following the initial invasion and the amount of chemical or mechanical control used in the lake may affect the ability of the milfoil weevil to effectively colonize and increase in abundance in even long-residing Eurasian watermilfoil beds. Variables related to water chemistry were not significantly correlated with milfoil weevil abundance. Water temperature and dissolved oxygen may be related to (or limiting) milfoil weevil abundance within the habitat of individual macrophyte beds, however these variables were only collected from the lake as a whole instead at the bed-level.

Herbivory by the milfoil weevil on Eurasian watermilfoil has been shown to significantly reduce its standing biomass (Creed et al. 1992, Creed and Sheldon 1993, 1995, Sheldon and Creed 1995, Newman et al. 1996). However, Eurasian watermilfoil biomass, density and other variables measured in

TABLE 3. MILFOIL WEEVIL ABUNDANCE IN DIFFERENT EURASIAN WATERMILFOIL BEDS IN 31 WISCONSIN LAKES. MEAN VALUES ARE THE MEAN OF THE FOUR BEDS ±95% CONFIDENCE INTERVAL.

	Weevils per apical stem					
Lake	Bed 1	Bed 2	Bed 3[a]	Bed 4[a]	Mean	Difference[b]
Alpine	0.0	0.0	0.7	0.3	0.25 ± 0.325	0.7
Beaver Dam	2.6	2.6	1.0	1.0	1.80 ± 0.905	1.6
Beulah	0.0	0.0	0.2	0.3	0.12 ± 0.147	0.3
Big Green	0.0	0.2	0.8	0.6	0.40 ± 0.358	0.8
Big Sand	0.1	0.7	0.0	0.6	0.35 ± 0.344	0.7
Camp	0.7	0.2	0.7	1.2	0.70 ± 0.400	1.0
Crystal	0.2	0.1	0.0	0.0	0.07 ± 0.094	0.2
Delavan	0.2	2.1	1.0	1.6	1.22 ± 0.802	1.9
Eagle	0.2	<0.01	0.0	<0.01	0.05 ± 0.098	0.2
Fox	0.3	1.3	0.1	1.4	0.77 ± 0.657	1.3
Gilbert	<0.01	0.2	0.1	0.0	0.07 ± 0.094	0.2
Jordan	0.3	0.2	0.4	0.2	0.27 ± 0.094	0.2
Kangaroo	0.0	0.0	0.0	0.0	0.00	0.0
Kusel	0.1	0.1	0.0	0.2	0.10 ± 0.080	0.2
Lower Spring	0.2	2.0	0.7	1.1	1.00 ± 0.746	1.8
Lac La Belle	0.5	0.4	1.1	1.2	0.80 ± 0.400	0.8
Little Falls	0.1	0.3	na	na	0.20	0.2
Lorraine	1.2	2.0	1.8	2.6	1.90 ± 0.566	1.4
Manson	0.0	0.5	0.6	<0.01	0.27 ± 0.314	0.6
Mason	<0.01	0.0	0.0	0.0	<0.01	<0.01
Metonga	0.1	0.1	0.2	1.6	0.50 ± 0.720	1.5
Mukwonago	0.3	0.2	<0.01	0.6	0.27 ± 0.245	0.6
Nancy	2.2	0.5	0.9	1.1	1.17 ± 0.713	1.7
Parker	0.1	0.1	0.4	0.4	0.25 ± 0.170	0.3
Pearl	0.4	0.7	0.1	0.1	0.32 ± 0.281	0.6
Ripley	0.7	0.7	1.0	1.1	0.87 ± 0.202	0.4
Rock	0.2	0.3	0.0	0.1	0.15 ± 0.126	0.3
Silver	0.0	0.0	0.0	0.0	0.00	0
Whitewater	1.6	0.8	2.2	1.0	1.40 ± 0.620	1.4
Wingra	3.2	2.2	0.9	2.6	2.22 ± 0.955	2.3
Yellow Birch	1.9	1.6	2.7	3.6	2.45 ± 0.878	2.0
						mean = 0.81

[a]Only two Eurasian watermilfoil beds were sampled on Little Falls Lake.
[b]Difference between beds with the highest and lowest abundance.

12 lakes (and used as pre-augmentation variables) were not related to natural weevil abundance in those lakes. Unfortunately, a trend in Eurasian watermilfoil density and biomass could not be detected as only one year of data were collected, thus the dynamics of a predator-prey cycle between Eurasian watermilfoil and natural milfoil weevil abundance were not measurable in this study. It is possible that lakes with higher milfoil weevil abundance, also had lower Eurasian watermilfoil biomass compared to previous years. In this study, the two lakes with the highest measured milfoil weevil abundance (Lake Wingra and Yellow Birch Lake) have experienced dramatic declines in Eurasian watermilfoil in the past. In fact, Eurasian watermilfoil was very difficult to find in Yellow Birch Lake (there was barely enough to sample) and it is possible that the milfoil weevil is currently controlling the Eurasian watermilfoil in this lake.

Variation in milfoil weevil abundance across lakes could be caused and compounded by many variables not measured in this study. Milfoil weevil populations, like most other organisms, may fluctuate for a variety of reasons. In fact, large fluctuations in populations are not unusual in common species (Pielou 1974). Milfoil weevil populations within a lake could be explained by predator-prey cycles with an increase in Eurasian watermilfoil followed by an increase in the milfoil weevil and then subsequent decrease in Eurasian watermilfoil and decrease in the milfoil weevil. This relationship is even more probable due to the fact that the milfoil weevil is primarily species-specific and thus a decline in Eurasian watermilfoil would most certainly mean a subsequent decline in the milfoil weevil. Lillie, WDNR (pers. comm.) reported large fluctuations in milfoil weevil populations from an ongoing study on Fish Lake in Dane Co., Wisconsin. Occupancy rates of the milfoil weevil in Eurasian watermilfoil stems fluctuated over the years from 18% in 1992, to 3% in 1995, and back to 18% in 1997. Furthermore, Fish Lake Eurasian watermilfoil biomass seems to fluctuate in response to milfoil weevil herbivory. Lillie reported a "lag" between milfoil weevil abundance and Eurasian watermilfoil biomass which suggests the presence of predator-prey-induced oscillations. The data collected in this study does not allow us to decipher

TABLE 4. SPEARMAN RANK CORRELATIONS BETWEEN OBSERVED MILFOIL WEEVIL ABUNDANCE AND CHARACTERISTICS OF MACROPHYTE BEDS IN WHICH THE WEEVILS WERE COLLECTED.

Variable	Correlation coefficient	P[a]
Percent broken tips	0.54002	0.0001
Depth of Eurasian watermilfoil	-0.30400	0.0007
Distance from shallow bed edge to shore	—	ns
Distance from middle of bed to shore	0.24957	0.0092
Distance from deep bed edge to shore	0.28417	0.0029
Percent natural shore	0.21407	0.0334
Percent mowed grass shore	—	ns
Percent sand shore	-0.28563	0.0042
Percent wall or rip-rap shore	—	ns
Biomass of Eurasian watermilfoil	—	ns
Biomass of native macrophytes	—	ns
Stem length of Eurasian watermilfoil	—	ns
Stem density of Eurasian watermilfoil	—	ns
No. of apical tips per Eurasian watermilfoil plant	0.59828	0.0399
No. of apical tips m^{-2}	—	ns

[a]ns denotes a non-significant correlation.

where milfoil weevil abundance is located on the predator-prey curve for a given lake. Their abundance may be on the rise in one lake, at the apex in another and on the decline in yet another.

Milfoil Weevil Augmentation

Preliminary (i.e., first year) post-augmentation data shows that five-weeks after weevil augmentation Eurasian watermilfoil biomass, stem density, stem length and tips per stem in the stocked plots of many lakes were significantly lower than pre-augmentation (the year before augmentation) levels. Although weevil abundance did not reach desired treatment levels in most lakes, those lakes having the highest treatment level (4 weevils per stem) showed a greater number of significant declines versus lakes with lower treatment levels. It is possible, given the weevil's life cycle, that the stocked weevils could have had an effect on the Eurasian watermilfoil and moved out of the plot areas before post-augmentation sampling five weeks later. However, despite the fact that there were far fewer significant changes in Eurasian watermilfoil between augmented plots and reference plots, lakes with higher levels of weevils overall showed more declines. It is very important to note that the apparent discrepancy in results can simply be explained by the fact that all plots were open and thus weevils were able to move among the treatment and reference plots. As a result, reference plots in the proximity of treatment plots were an in-effective control. In the initial design of the project, the plots were intended to gauge the rate at which weevils migrated out of treatment plots rather than for comparison to treatment plots. Following study design workshops, participants concluded that no truly effective control plots could be established.

Weevil abundance was lower in reference than in treated plots in only half of the lakes (Table 6). Also, Eurasian watermilfoil declines in lakes that were stocked at lower levels did not exhibit significant declines in the post-augmentation period and thus would unlikely be different from reference plots. For these reasons, pre- and post-augmentation comparisons might offer a better indication of weevil effectiveness.

Research on the effects of the weevil augmentations is preliminary at this point. Data on Eurasian watermilfoil was collected only five weeks after the augmentation and thus it is too early to generalize these results. Data collected over the next two-three years should show how well the weevils overwintered, how many returned to the augmented areas, and the effect of augmentation on Eurasian watermilfoil. Data gathered from this study may help agencies implement Eurasian watermilfoil management plans that integrate the use of biocontrol agents such as the milfoil weevil. Additional research is necessary to determine where and in what kind of lakes and in what types of Eurasian watermilfoil beds the milfoil weevil might establish large, effective populations. Al-

TABLE 5. OBSERVED WEEVIL ABUNDANCE AMONG TREATED PLOTS PRE-AUGMENTATION AND 5 WEEKS POST-AUGMENTATION AND THE PERCENT CHANGE IN EURASIAN WATERMILFOIL MEASUREMENTS AFTER MILFOIL WEEVIL AUGMENTATION. A POSITIVE PERCENTAGE INDICATES A SIGNIFICANT INCREASE IN THAT VARIABLE FROM PRE-AUGMENTATION (1996) TO POST-AUGMENTATION (1997) WHILE A NEGATIVE PERCENTAGE INDICATES A SIGNIFICANT DECLINE. WILCOXON SIGNED-RANK TEST, $P \leq 0.05$. NO SIGNIFICANT DIFFERENCE IS INDICATED WITH "N.S."

Lake	Observed weevils per stem pre-augmentation[a]	Treatment level (milfoil weevils per stem)	Observed weevils per stem post-augmentation[a]	EWM biomass	EWM stem density	EWM stem length	Apical tips per EWM stem	Native macrophyte biomass
Eagle	0.10	1	0.1	-76%	-43%	-21%	-13%	n.s.
Kangaroo	0.0	1	0	-43%	-27%	-30%	n.s.	-100%
Nancy	0.52	1	0.6	n.s.	n.s.	n.s.	+23%	n.s.
Pearl	0.38	1	0.3	n.s.	n.s.	n.s.	n.s.	-73%
Whitewater	0.87	1	1.775	-41%	n.s.	-20%	n.s.	n.s.
Beaver Dam	1.35	2	0.35	n.s.	n.s.	n.s.	n.s.	+1473%
Gilbert	0.37	2	0	-39%	-16%	-37%	-31%	n.s.
Lorraine	1.72	2	1.925	-57%	-43%	n.s.	-52%	n.s.
Big Sand	1.2	4	0.85	-49%	-39%	-33%	-51%	n.s.
Kusel	0.88	4	0.975	- 87%	-49%	-61%	-40%	n.s.
L. Spring	1.43	4	1.775	-73%	-64%	-60%	-57%	+3555%
Mukwonago	3.11	4	0.45	-55%	+71%	-52%	n.s.	n.s.

[a]In treated plots.

Table 6. Observed weevil abundance among treated and reference plots 5 weeks post-augmentation and the percent changes between augmented plots and reference plots approximately 5 weeks after milfoil weevil augmentation. Percentages indicate a significant difference in the augmented plots as compared to the reference plot. Wilcoxon Signed-Rank Test, $p \leq 0.05$ No significant difference is indicated with "n.s."

Lake	Treatment level (milfoil weevils per stem)	Observed weevils per stem in treated and (reference) plots	Eurasian watermilfoil biomass	Eurasian watermilfoil stem density	Eurasian watermilfoil stem length	Apical tips per Eurasian watermilfoil stem	Native macrophyte biomass
Eagle	1	0.1 (0.2)	n.s.	n.s.	n.s.	n.s.	n.s.
Kangaroo	1	0 (0)	n.s.	n.s.	n.s.	n.s.	n.s.
Nancy	1	0.6 (0)	n.s.	n.s.	n.s.	n.s.	n.s.
Pearl	1	0.3 (0.25)	n.s.	n.s.	n.s.	n.s.	n.s.
Whitewater	1	1.775 (3.2)	n.s.	n.s.	n.s.	n.s.	n.s.
Beaver Dam	2	0.35 (0.25)	+236%	n.s.	-26%	n.s.	n.s.
Gilbert	2	0 (0)	n.s.	n.s.	n.s.	n.s.	n.s.
Lorraine	2	1.925 (0.95)	n.s.	n.s.	n.s.	n.s.	n.s.
Big Sand	4	0.85 (0.7)	-46%	n.s.	n.s.	n.s.	+1825%
Kusel	4	0.975 (0.35)	n.s.	n.s.	-9%	n.s.	n.s.
L. Spring	4	1.775 (2.25)	n.s.	n.s.	n.s.	n.s.	-85%
Mukwonago	4	0.45 (0.15)	n.s.	n.s.	n.s.	n.s.	n.s.

though this study suggests that augmenting milfoil weevil populations to 4 weevils per stem of Eurasian watermilfoil may cause a decline in the Eurasian watermilfoil in the immediate area of stocking, how far these effects extend beyond the augmentation plots is unknown. If successful, it would be critical to determine the number of weevils necessary for Eurasian watermilfoil control within a specific size of macrophyte bed so that lake resource managers and property owners can effectively implement this method or use it in conjunction with other control methods.

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