

Overwinter Habitat and the Relationship of Overwinter to In-lake Densities of the Milfoil Weevil, *Euhrychiopsis lecontei*, a Eurasian Watermilfoil Biological Control Agent

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ABSTRACT

The native weevil *Euhrychiopsis lecontei* has been associated with declines of Eurasian watermilfoil (*Myriophyllum spicatum*). The weevil spends all summer on submersed plants, producing 3 to 6 generations. In September to November adult weevils move to shore where they overwinter in leaf litter at drier sites near the shoreline. Mean November shoreline densities from 1992-1998 at Lake Auburn (mean = 43 N/m²) and Smith's Bay of Lake Minnetonka, (mean = 125 N/m²) have ranged from zero to over 200 N/m². Overwinter mortality is not severe (survival was typically >60%). Adults collected from terrestrial habitat have developed flight muscles and limited flight has been observed in the spring but submersed adults in summer do not have developed flight muscles. Adults return to the water in spring and females begin to develop and lay eggs after the water temperature reaches 10-15C. Spring (May-June) and Fall (September) in-lake densities in these two lakes have ranged from zero to 40 N/m² and Lake Auburn typically had higher in-lake weevil densities (mean of 15 N/m² compared to 4 N/m² at Smith's Bay). There was no relationship between in-lake and shoreline densities at Lake Auburn, but Smith's Bay spring in-lake densities were correlated with spring shoreline densities. In-lake densities were not correlated between the two lakes but shoreline densities were correlated over time, suggesting that regional climatic factors may influence shoreline densities. Weevils disappeared from Lake Auburn in-lake samples in July 1998; no weevils were found there in shoreline or in-lake samples in 1999. In-lake factors such as fish predation may be more limiting than overwinter conditions.

Key words: biological control, *Myriophyllum spicatum*, conservation, shoreline.

INTRODUCTION

The native milfoil weevil, *Euhrychiopsis lecontei* (Dietz), feeds and develops on Eurasian watermilfoil (*Myriophyllum*

spicatum L.), a nuisance aquatic plant that has infested lakes across North America (Smith and Barko 1990). The weevil is endemic to North America and has been associated with Eurasian watermilfoil declines in a number of states and provinces (Creed and Sheldon 1995, Sheldon 1997, Creed 1998, Lillie 2000, Newman and Biesboer 2000); it is being investigated as a biological control agent for Eurasian watermilfoil. In controlled conditions the weevil has been shown to reduce milfoil buoyancy and cause the plant to sink from the water column (Creed et al. 1992), reduce biomass accumulation and plant height (Creed and Sheldon 1995, Sheldon and Creed 1995), and reduce root and shoot biomass and carbohydrate stores (Newman et al. 1996); reduction in carbohydrate stores due to stem mining by larvae may reduce the plant's ability to overwinter and regrow the next spring (Creed and Sheldon 1995).

Although the milfoil weevil has shown good suppression of Eurasian watermilfoil in controlled conditions and has been associated with numerous Eurasian watermilfoil declines across North America, the weevil has not consistently developed adequate populations to control the plant at many locations (e.g., Jester et al. 1997, Newman et al. 1998). We have noted that the milfoil weevil has failed to develop adequate populations (0.5-1.5 per milfoil stem) to effect control in many Minnesota lakes and that factors that limit populations of the milfoil weevil need to be identified and ameliorated to provide predictable control (Newman et al. 1998, Newman and Biesboer 2000).

Previous publications have described the general life history of the milfoil weevil and its development during the summer submersed phase, however, little information on its overwintering ecology has been published. The adult weevil spends the winter along the shore in leaf litter; in spring adult weevils enter the lake and after a period of feeding develop gametes and begin to reproduce (Mazzei et al. 1999). Females lay single eggs on watermilfoil meristems at an average rate of 1.9 eggs per day (Sheldon and O'Bryan 1996). Eggs hatch in several days, and larvae mine down the stem and the weevil pupates lower on the stem, ≥ 0.5 m below the meristem (Sheldon and O'Bryan 1996). Larvae mine about 15 cm of stem to complete development, which is dependent on temperature (Mazzei et al. 1999); about 310 degree days above 10C are required to complete development from egg to adult (21 d at 25C). At typical Minnesota summer temperatures a maximum of 4-6 generations can be completed each

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summer. In the fall, adult weevils migrate to shore where they overwinter in the leaf litter.

The purpose of this paper is to describe the overwinter habitat, life history and population dynamics of the milfoil weevil; to assess the importance of pathogens and parasites to populations of the milfoil weevil; and to relate in-lake density to overwinter shoreline densities of the milfoil weevil, in part to determine if overwinter survival or in-lake population dynamics are more important to the development of weevil densities adequate for reliable control.

METHODS

Spring and fall shoreline soil and litter (overwinter) samples were collected at Smith's Bay of Lake Minnetonka (Hennepin Co.; T117N; R23W; S10,11) and Lake Auburn (Carver Co.; T116N; R24W; S10) from Fall 1993 to Spring 1999. Shoreline soil samples consisted of the top 2.5 to 7.6 cm of soil and leaf litter in a 0.2 m² area. Samples were returned to the laboratory, dried if needed, sifted through a series of gradually decreasing sieves and examined for the presence of adult weevils. In 1994-1996, samples were collected every 2 to 4 weeks from August or September to November and from mid-March to mid-May to determine when the milfoil weevil moved to and from the shoreline overwinter habitat. In subsequent years, samples were collected in March or April and November. In Fall 1993 and 1994, a series of samples was collected every 2 m up to 20 m from the water's edge to determine how far from the water's edge weevils overwintered. A subsample of each of these samples was weighed, dried at 50C to constant mass, and reweighed to determine soil moisture content. In November 1994, 35 samples were taken approximately every 50 m along the entire perimeter of Lake Auburn where known overwintering habitat existed to determine distribution around the lake. Litter sampling was used to estimate weevil population density, timing when insects move into the terrestrial habitat, and when they emerge in the spring to recolonize the aquatic habitat. In 1993 to 1995 samples of overwintering and in-lake weevils were dissected and examined for parasitoids, fat body development, reproductive organ development, presence of sperm and development of flight muscles.

We also attempted to determine how weevils disperse in fall or spring. Based on dissection and observation of collected adults, weevils are capable of flight in the fall and spring. These adults have fully developed flight muscles and have taken flight in the laboratory. In contrast weevils collected from milfoil in mid-summer have much reduced flight muscles and usually cannot be induced to fly, so that mid-summer dispersal appears not to occur. We monitored movement of weevils via pitfall traps (buried 500 ml jar with funnel at soil surface and dichlorovos impregnated plastic to dispatch occupants), and clear "window-pane" traps (plexiglass coated with tanglefoot; 1.2 m by 0.6 m) along the shore of both lakes in Spring 1994-1995. In Spring 1996 we placed three modified Japanese beetle (yellow) traps along the shore (approximately 50 m apart) with a corresponding trap in the water (approximately 10 m out from the shoreline trap) at Lake Auburn and at Smith's Bay. Traps were checked on a weekly basis.

During several years, adult weevils collected from submerged plants or from overwintering (terrestrial) habitats

were held in an incubator or dissected to determine if the adults were parasitized or infected with microsporidians or other pathogens.

In-lake weevil densities were determined from quantitative samples of plant biomass each May or June and September in Lake Auburn and Smith's Bay. Sampling stations were positioned along five transects, 30 m apart, running from shore to near the edge of the plant bed. At Lake Auburn, 6 stations were located every 10 m on each transect from 5 m from shore (0.5 m depth) to 55 m from shore (2.5 m depth) for a total of 30 samples per date. At Smith's Bay, five sampling stations were established along each transect at varying distances apart, starting at 100 m from shore (1 m depth) and extending to 805 m from shore (4 m depth) for a total of 25 samples per date. All plants within a 0.1 m² quadrat were clipped at the sediment interface by SCUBA divers, and placed into a sealable plastic bag underwater. Samples were returned to the laboratory where invertebrates were rinsed off the plants into a 500 µm mesh sieve. External and endophytic invertebrates were removed from each plant (with the aid of a 2× magnifying lens), and plant species were identified and weighed. Milfoil weevil larvae, pupae and adults were enumerated.

RESULTS AND DISCUSSION

Our in-lake and overwinter sampling confirms that adult milfoil weevils leave the water in mid-September to early November and spend the winter in the soil-leaf-litter interface. In spring, adults emerge from the soil and return to the water between ice out and mid-May.

Pitfall traps, although not an efficient method of measuring weevil activity (only 5% of traps contained weevils), showed peak spring emergence at Lake Auburn in 1994 was in the interval of 3 to 10 May; no weevils were collected from the shore after 24 May. Weevils were first collected in pitfall traps on 24 March, before the ice was off the lake and weevils have been collected from Eurasian watermilfoil in the water shortly after ice-out. When temperatures increase in the spring weevils become active and there appears to be an extended period of time when they move from shore into the water.

Only two weevils were collected in the window pane traps (total both lakes, spring and fall two years); so this therefore does not appear to be an effective way to collect flying weevils. The Japanese beetle traps were not much more effective; five weevils (total) were collected in these traps in the spring. One weevil, however, was collected at Smith's Bay in the trap placed in the water 10 m out from shore, indicating that at least some weevils fly over the water to disperse or locate milfoil beds.

In the fall, weevils were found in pitfall traps and shoreline samples as early as mid-September. Peak shoreline densities were usually reached by mid-November and appeared to be near locations where mats of milfoil had drifted to shore. Intensive collections at Lake Auburn in Fall 1993 and 1994 indicated the highest densities of overwintering weevils were within 1 m of the water's edge although in 1993 weevils were common up to 6 m from shore and some weevils were found up to 20 m from the shoreline. In Fall 1994, when shoreline weevil densities were much lower (Figure 1), weevils were found only in the first two meters from the shoreline. Weevils appear to like drier sites; densities in soil with >15% moisture

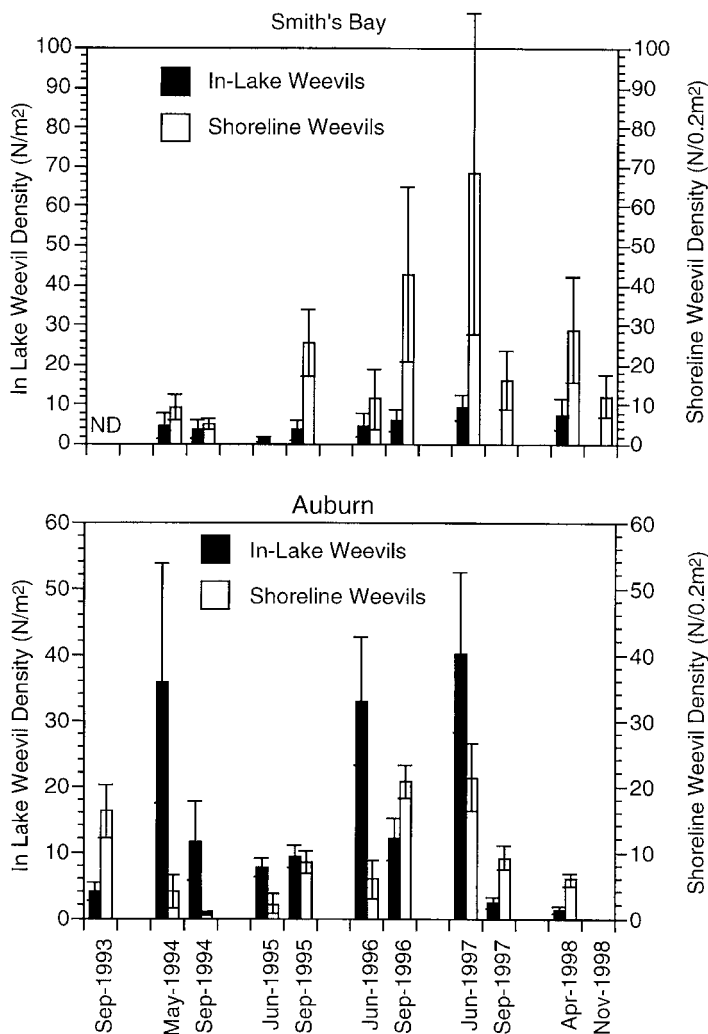


Figure 1. Mean number of *E. lecontei* adults per 0.2 m² shoreline litter samples at the peak for each sampling period and the corresponding in-lake density (N/m²; larvae, pupae and adults) in spring and fall samples at Lake Auburn and Smith's Bay. Vertical bars are 1 SE. Shoreline densities in April 1999 were 0 N/0.2 m² in Lake Auburn and 10 ± 3.6 N/0.2 m² at Smith's Bay.

(16.2 ± 1.7 N/m²) were significantly lower than in sites with <15% moisture (110.7 ± 6.7 N/m²). Weevils were not found at soil depths >5 cm, indicating they overwinter in the soil-leaf-litter interface rather than burrow into the soil.

Weevil densities in soil samples ranged from 0 N/m² (Spring 1999) to 107 ± 26 N/m² (Spring 1997) at Lake Auburn and from 25 ± 7 N/m² (Fall 1994) to 341 ± 202 N/m² (Spring 1997) at Smith's Bay. Shoreline densities at Smith's Bay were generally higher than those at Lake Auburn (mean of 125 N/m² and 43 N/m² respectively); densities at both lakes were highest in the Fall of 1996 and Spring of 1997 (Figure 1). Weevils disappeared from bi-weekly in-lake samples at Lake Auburn in July 1998 (and all of 1999, pers. observ.) and were rare in Fall 1998 soil samples and absent in Spring 1999 soil samples. Adult weevils were found in the lake in Spring 2000 (personal observation), indicating recolonization from elsewhere, perhaps the other side of the lake or another nearby lake.

Comparison of fall to spring shoreline densities provides a rough estimate of overwinter mortality. Although there was considerable variability among these survival estimates (survival ranged from 0 to 300% and averaged 100%), it is clear that overwinter mortality is not extreme. With the exception of 1998-1999, when the low density of weevils at Lake Auburn in the fall disappeared in the spring, the other survival estimates were >24% and most were >60%. Survival approaching 50% is comparable to other Coleoptera whose overwintering success has been studied (Grafius and Collins 1986, Charlet 1989, 1991) and thus winter survival, at least generally at our sites, does not appear to be a limiting factor.

No parasitoids were found to emerge from overwintered adults and we found that fall shore-collected weevils from the soil could be kept overwinter in soil in a refrigerator at 4C (80% survival); weevils collected from the water in the fall did not overwinter well in the refrigerator (10% survival) and were attacked by fungi (*Beauveria bassiana* (Balsamo) Vuillemin). Adults collected from the water may not be physiologically acclimated to survive extended cold or may require additional conditioning to induce diapause.

Dissections of weevils collected from submersed plants in 1999 indicated that few individuals were infected with microsporidia (0 of 104 dissected at Smith's Bay; 2 of 95 at Ceniako Lake). Other protozoans (gregarines) were found (12 of 104 at Smith's Bay; 1 of 95 at Ceniako Lake), but these are considered commensal and the low number found in individual weevils suggests they are not pathogenic (Tanada and Kaya 1993). Female weevils collected in fall or spring from the soil do not have well-developed ovaries but most (>75%) were mated (Figure 2). Fat reserves are depleted by spring but increase over the summer (first in males and later toward fall in females). Females can begin egg laying after a week or two in 15C or warmer water (Mazzei et al. 1999) and females throughout the summer had well developed ovaries. Flight muscles appear atrophied in summer, but most overwintering weevils have developed flight muscles and can fly. Our observations suggest that in summer weevils put energy into reproduction. The last summer generation probably does not attempt to reproduce (but many females have mated), instead directing energy into fat-body reserves and flight muscle de-

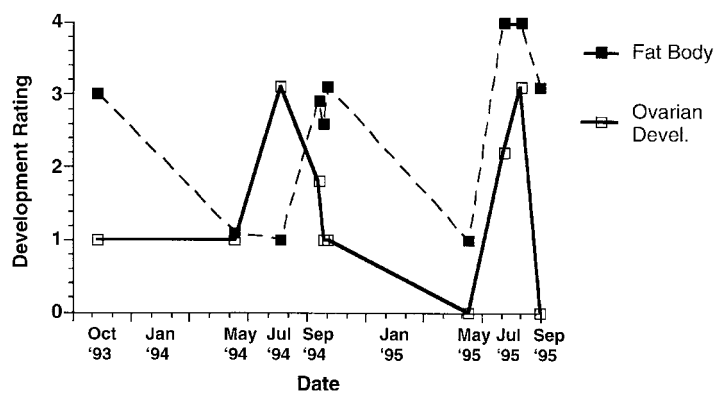


Figure 2. Fat body and ovarian developmental status of *E. lecontei* adults. Developmental ratings are on a scale of 0 (no fat body or eggs and ovarian development) to 4 (dense fat bodies or well developed ovaries with mature oocytes in each ovariole).

velopment. This allocation prepares the weevil to overwinter and to disperse to other sites either in spring or in fall. Buckingham and Passoa (1985) also found that waterhyacinth weevils (*Neochetina eichhorniae* Warner and *N. bruchi* (Hustache)) either developed flight muscles or eggs, but rarely both. Muda et al. (1981) reported a similar phenomenon for rice water weevils; overwintering weevils had degenerated flight muscles which regenerated in spring but were quickly lost when the weevils returned to rice fields and began to oviposit. Because the milfoil weevil produces several generations per summer but spends a long winter as an adult in the soil it appears that the trade-off between reproduction and dispersal or overwinter preparation is more distinct than for other weevils.

Shoreline and in-lake densities were generally not correlated; a weak correlation of Smith's spring in-lake and shoreline density was noted ($P < 0.1$), but no other correlations were significant. There was also no significant correlation between Auburn and Smith's Bay in-lake densities over time, but shoreline densities were significantly correlated (Bonferroni corrected $P < 0.01$), suggesting some regional climatic effect on shoreline, but not in-lake, densities.

In-lake densities were generally much higher at Lake Auburn than Smith's Bay (Figure 1). It should be noted, however, that the sampling stations at Smith's Bay extend over 800 m from shore (weevils have never been found at these stations but have occasionally been found at the next station, 585 m from shore) whereas the stations at Lake Auburn only extend 55 m from shore. Densities closer to shore at Smith's Bay are more comparable to the Lake Auburn densities. Weevil densities at the farthest and thus deepest stations at Auburn are also typically much lower than stations nearer to shore, suggesting that depth or proximity to the edge of the plant bed may limit weevil populations. Tamayo et al. (2000) found that milfoil beds with weevils were shallower than beds without weevils and milfoil weevil abundance has been negatively associated with depth (Jester et al. 2000, Johnson et al. 2000). This effect is not due to a greater distance from shore preventing weevil access to plants because Jester et al. (2000) also found that weevil abundance was positively correlated with distance from shore to the middle and deep edges of the plant bed, but was not related to distance to the shallow edge of the bed. Thus weevil populations may be higher in large shallow expanses of milfoil rather than steep shorelines with plants below the surface (Jester et al. 2000). Lillie (2000) also found the highest densities of weevils and greatest damage in the shallow and middle portions of beds and much lower densities at the deep edges. Deeper plants may provide less refuge for the weevil than plants that approach the surface, both from access to fish predation and to wave action. Deeper plants may also be less accessible to adults that would need to dive to reach the plants.

The highest in-lake densities (all stages) at Lake Auburn were in the spring samples in 1994, 1996 and 1997 (33-40 N/m²) and fall density was higher than spring density only in 1995. The highest densities at Smith's Bay were in spring 1997 and 1998, however, fall densities were similar to or higher than spring densities in 1994-1996. The much larger source area (bay area with milfoil) of in-lake weevils at Smith's Bay likely explains the higher overwinter densities seen there compared to Lake Auburn despite the lower in-lake densities.

These results suggest that at least at Smith's Bay and Lake Auburn, overwinter conditions are not limiting weevil populations; overwinter mortality is not extreme and parasitoids, pathogens and terrestrial predators appear unimportant. The failure to build population densities adequate to control Eurasian watermilfoil appears more related to in-lake factors. The lakes that have developed high populations with some degree of control have shown increases in in-lake densities over the summer (Newman and Biesboer 2000), whereas lakes with low populations (even lower than Lake Auburn and Smith's Bay) fail to have increasing weevil densities over the summer. Given the possibility of producing 4 or more generations per summer (Mazzei et al. 1999), in-lake densities should increase over the summer unless some factor is limiting their abundance. Predation by sunfish may be limiting weevil populations at some of our sites (Sutter and Newman 1997, Newman et al. 1998, Newman and Biesboer 2000), although we have not investigated the role invertebrate predators may play in adult or larval weevil survival.

This is not to say that overwinter habitat is unimportant. Jester et al. (2000) found in-lake weevil densities in Wisconsin were positively correlated with percent natural shoreline and negatively related to percent sand shore. At a larger scale, availability of overwinter habitat will likely become limiting. Our sampling sites were all along natural shoreline and we have not examined other habitats such as lawns. Cenaiko Lake, where we have documented a Eurasian watermilfoil decline due to weevils, has steep banks covered with prairie (few trees) and maintains high weevil populations (Newman and Biesboer 2000) so it appears that forested areas are not essential but undisturbed grasses may be. It is also possible that rising fall or winter lake levels will flood out overwintering weevils and it is clear that dry sites are required for overwintering.

More research to determine the factors limiting milfoil weevil populations is needed. In-lake factors such as predation (vertebrate and invertebrate) or plant quality deserve further investigation as do broader scale evaluations of overwintering habitat. Identification and amelioration of these limiting factors will be needed to provide predictable control of Eurasian watermilfoil with this native weevil.

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