Euhrychiopsis leconti and *Myriophylum spicatum*

Establishing a balance In Candlewood Lake: Response to

Stocking Weevils in Eurasian watermilfoil weed beds.

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Abstract

The relationship between North American weevils, *Euhrychiopsis lecontei*, and Eruasian watermilfoil, *Myriophylum spicatum,* in Candlewood Lake was investigated. Approximately nine thousand *E. lecontei eggs and larvae* were stocked in three sites along the northern part of Lake Candlewood. Plant samples were taken throughout the summer of 2008 to investigate the presence of weevils and to record any damage. Analysis of the sites showed that there significantly more weevils and more damage done to milfoil in the stocked sites encouraging further studies.

Introduction

Euraisan watermilfoil, *Myriophyllum spicatum* (Haloragaceae), here after referred to as milfoil, was introduced into the United States sometime during the 1940s. Details of how the plant was introduced; including, the exact timing and origin of introduction remain in dispute (Smith and Barko 1990). Regardless of this disagreement in origin milfoil has spread throughout North America. Milfoil has been found in 44 states and 3 Canadian provinces (Creed; Non indigenous Aquatic Species Database 1997).

Milfoil is a completely aquatic rooted perennial, found in waters from 1 to 10 meters deep (Smith and Barko 1990). When the water temperature reaches about 15C° milfoil grows rapidly (Adams et al. 1974; Smith and Barko). In lakes with greater clarity milfoil is able to grow in deeper water. Regardless of depth, the plants form a canopy which in turn reduces the penetration of sun light. This reduced light possibly reduces the chance of survival for other aquatic plants competing in the same area. Once the milfoil reaches the water's surface, it flowers (Patten 1956; Smith and Barko 1990).

Eurasian Watermilfoil found its way into Candlewood lake during the 1970s (Tarsi 2007). During the past two decades this invasive aquatic plant has been a nuisance. Milfoil has negatively impacted the ecological environment. Milfoil beds have a direct correlation with decreased water quality (CAES and CLA 2005); decrease in water quality has been recorded in other lakes(Grace and Wetzel 1978; Confrancesco 2004). Recreational use and property values around Candlewood Lake are also affected by the beds. Milfoil is able to alter the local aquatic conditions, by increasing the water temperature at the surface, as observed by both sample collectors, and by shading deeper water and restricting water movement due to the dense tangled mats the vine like stems form. Milfoil also reproduces quickly and easily resulting in an increase in established bed sizes and the creation of new beds. One method of reproduction is fragmentation. Many people using the lake can unintentionally disrupt the plant; fragments broken from the original plant then establish themselves as viable new plants, expanding their area.

Studies in other lakes have shown that *Euhrychiopsis lecontei*, (<u>Curculionidae</u>) an aquatic weevil, is able to reduce the biomass of Eurasian watermilfoil. Weevils were introduced into Candlewood Lake to study their effectiveness as a biological control based on the finding of earlier studies completed in aquarium, pool and field experiments (Creed et al., 1992; Creed and Sheldon, 1993, 1994b, 1995; Sheldon and Creed, 1995; Newman et al., 1996; Creed and Sheldon 2003). The life cycle of the aquatic weevil is dependent on water temperature, but generally the development times averaged 4 days for the egg stage, 13 days for larval stage, and 10 to 13 day for the pupae stage in water 21.5 to 24.0° C(Sheldon and O'Bryan 1996). The studies also suggested that the weevils prefer the invasive Eurasian watermilfoil to native species suggesting minimal damage to other aquatic plants (Solarz and Newman 1996; Newman, Borman and Castro 1997; Creed and Sheldon 2003). This background encouraged further study to investigate the effectiveness of weevils as a biological control in Candlewood Lake.

The purpose of this experiment was to determine whether sites stocked with weevils had more damage to milfoil than the sites without the stocked weevils. The results indicate that stocking the sites resulted in more damage to the milfoil.

Materials and Methods

The areas selected for further investigation were determined by plant surveys conducted between 2005 and 2006 by The Connecticut Agriculture Experiment Station (Greg Bugbee, Roslyn Selsky, Robert Capers and Michelle Marco 2006). Suitable sites, bordering Sherman and New Fairfield, were located on Candlewood Lake as determined by the size and shape of the milfoil bed, water traffic, and the shore line environment. The beds needed to be large enough for two transects, perpendicular to the shore, and long enough to allow five sample positions. The beds chosen were located in coves and had little to no boat traffic, and the shore line environment was similar. The six sites had shore lines that appeared natural. The immediate shore line consisted of areas containing underbrush, rocks, and shrubs of various species. This buffer zone was approximately a meter wide, beyond which was manicured lawn.

Each site used in the study had two transects. Three of the six sites chosen were natural control sites; the only weevils found in theses sites were the naturally occurring population. The other three sites had one transect stocked with weevils, while the other transect was the natural population.

On July 2, 2008 Enviroscience Inc, based in Stow, Ohio stocked three transects with approximately 9,000 weevil eggs and larvae; 3,000 per transect. Enviroscience, Inc. has been treating lakes with weevils since 1995 under the trade name Middfoil. The day of stocking was warm and sunny, the water temperature was about 25° C, and there was no wind. The sites were stocked with eggs of weevils known to be native to this area. The method of stocking was to snorkel through the weed bed and tie bundles of 10 to 25 stems of milfoil containing the eggs and larvae to milfoil growing in the lake.

The labeling of each transect followed the pattern, position 1 is closest to the shore and position 5 is farthest from shore. There is a general correlation to water depth and amount of milfoil present, but we did not measure the exact depths at each position. The general pattern was the same at each site. The shallowest positions and the deepest positions contained fewer plants than middle positions. The water depth at position one, for all the transects, was the shallowest, and estimated to be between 1 to 1.5 meter deep. There was an increased depth to the fifth and farthest position from shore, where the average depth was estimated to be between 3 and 6 meters deep.

Samples were taken every two weeks. We snorkeled through the beds and broke the stems and retained the top .5m. Ten random stems were collected from each location and gently placed in plastic freezer bags (Glad Ziplock®) while under water. Each bag had been pre-labeled for the site, transect and position. The samples were drained of excess water and placed on ice until they were analyzed. This technique was repeated at all six collection sites.

The 600 stems were taken back to the lab and analyzed for damage. Each stem was individually examined in a white tray with water. Starting at the meristem and investigating the length of the stem, any sign of egg, larval damage, pupae, or adults was recorded

Eggs, if any, are present at the meristem tips of the underwater stem. The eggs are attached to the plant, but don't appear to cause any harm. The larvae burrow into the plant and destroy the stem tissue about 7 cm from the tip causing the stem to die back, which is indicative of a stem mining beetle. Continuing down the stem the last instar migrates to areas of larger diameter to reenter the plant to finish pupating into an adult. The adults are free swimming, but usually found on the leaves or stems.

After collecting the raw data it had to be analyzed and transformed into meaningful information. Statistical analyses consisted of one- and two-way Analysis of Variance (ANOVA). These tests were conducted using StataSE software, version 9. A confidence level of 95% (i.e. $p \leq 0.05$) was considered significant.

Results

The results are based on examining 3700 stems of milfoil throughout the summer of 2008. There were 2754 stems examined from the control sites and 946 stems from the stocked sites. This large amount of zeros in the data violates the critical assumption of normality required for standard parametric statistics. Tables 1 – 4, display the raw data. The actual means show a very low population and low damage in both the control and stocked sites. These low populations required advanced statistical analysis to clarify if there were indeed any significant differences. The raw data gives a decreasing slope rather than the normal bell shaped curve one would expect to find; therefore, data was rank transformed and ANOVA was done on the ranks (Conover and Iman 1981). This method has the added benefit of allowing the use of 2-way analysis of variance, for which there is no non-parametric analog. Although the statistical analysis was done on ranks, the summary tables 1 through 4 located at the end of the report are based on the unranked data.

Tables 5 and 6 show the p-values for all categories studied. A p-value less than .05 is considered significant. Any p-value less than .05 creates confidence that there is at least 95% probability that there is a significant difference. Hyphens indicate no statistical difference found between the two factors compared. The factor “Position” refers to the same location along a transect as compared to other sites and the same transect location.

Graph 1 shows there was a significant difference between the number of eggs, the number of larvae between the different sites. The error bars indicate one standard error. Significant differences were found comparing the larval damage between the sites and the number of larvae found at different positions along the transect. The only exception was pupae where there p-value indicated that there was no significant difference.

Discussion

The significant differences between the control and stocked sites are encouraging. This correspondence between higher numbers of larval weevils and increased damage is supported by previous studies (Creed et al., 1992; Creed and Sheldon, 1993, 1994b, 1995; Sheldon and Creed, 1995; Newman et al., 1996; Creed and Sheldon 2003; Newman 2004). While this part of the study is encouraging, more research needs to be done to realize the possible effectiveness of the weevil, *Euhrychiopsis leconti,* as a biological control against milfoil, *Myriophylum spicatum*, in Candlewood Lake. While the stocking has increased the number of weevils, the numbers are still too low to be effective against the densely packed areas of milfoil. Studies have shown that the ratio of weevil to milfoil should be one or two weevils per stem to cause significant damage to the milfoil bed (Jester 2000). The numbers in Lake Candlewood are closer to one weevil per hundred stems.

It is too early to know if the stocking has lead to a higher population of weevils after overwintering. Research is needed to monitor the stocked sites to investigate if there maintained higher numbers of weevils. The survival of the adults might be impacted by the deeper than normal draw down scheduled for this year. This study was not designed to measure or record the impact, if any, of the lake draw down.

Furthermore, this study did not look at the possible predation on weevils by fish. There are conflicting reports, but the most recent suggests that weevils are preyed upon by littoral fish (Newman 2004) including blue gill and pumpkin seeds.

The dynamics of a living system will make the control of milfoil a challenging task, further studies may indicate that the northern weevil will indeed help to control, but not eliminate, this invasive species. Other research has implied that milfoil is too aggressive to be controlled by one method alone.

This research started in May when Andy Oguma and I went to New Haven to the Connecticut Agricultural Station and Dooly Pond. We went to observe weevils in the tanks, learn how to snorkel through milfoil and collect samples. After gathering our samples we went back to the CAS and looked for eggs.

Then in mid June we accompanied Dr. Marko to survey possible study sites on Candlewood Lake, we collected data for the GPS, milfoil stems and a biomass sample. On July 2nd we assisted with the stocking of the sites. Then we collected Milfoil twice a month until the second week in September for a total of 3700 samples collected and observed.

The fact that every life stage except pupae was more abundant at the stocked sites is curious. There was a possible error in the data analysis. At the beginning of the study I was looking for pupae lower on the stem. Over the summer, during one sample analysis I noticed a bump on a couple of plants near the flower and I discounted it as a seed. During my last collection I noticed the bumps again, but that time I peeled back the bump and realized that the bumps were indeed pupa, higher on the plant than expected. This change in location could possibly be explained because the plants had a large diameter at the flowering tips.

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Table 1. Weevil Density at Control Sites

	Stems examined	Mean (per site)	SD
Eggs	2754	.0044	.0807
Larvae	2754	.0029	.0538
Pupae	2754	.0015	.0466
Adults	2754	.0015	.0381

Table 2. Weevil Density at Stocked Sites

	Stems examined	Mean (per site)	SD
Eggs	945	.0095	.1075
Larvae	946	.0095	.0971
Pupae	946	.0011	.0325
Adults	946	.0063	.0918

Table 3. Weevil Damage Density at Control Sites

	Stems examined	Mean (per site)	SD
Larvae	2753	.0534	.2249
Adults	2754	.0007	.0269

Table 4. Weevil Damage at Stocked Sites

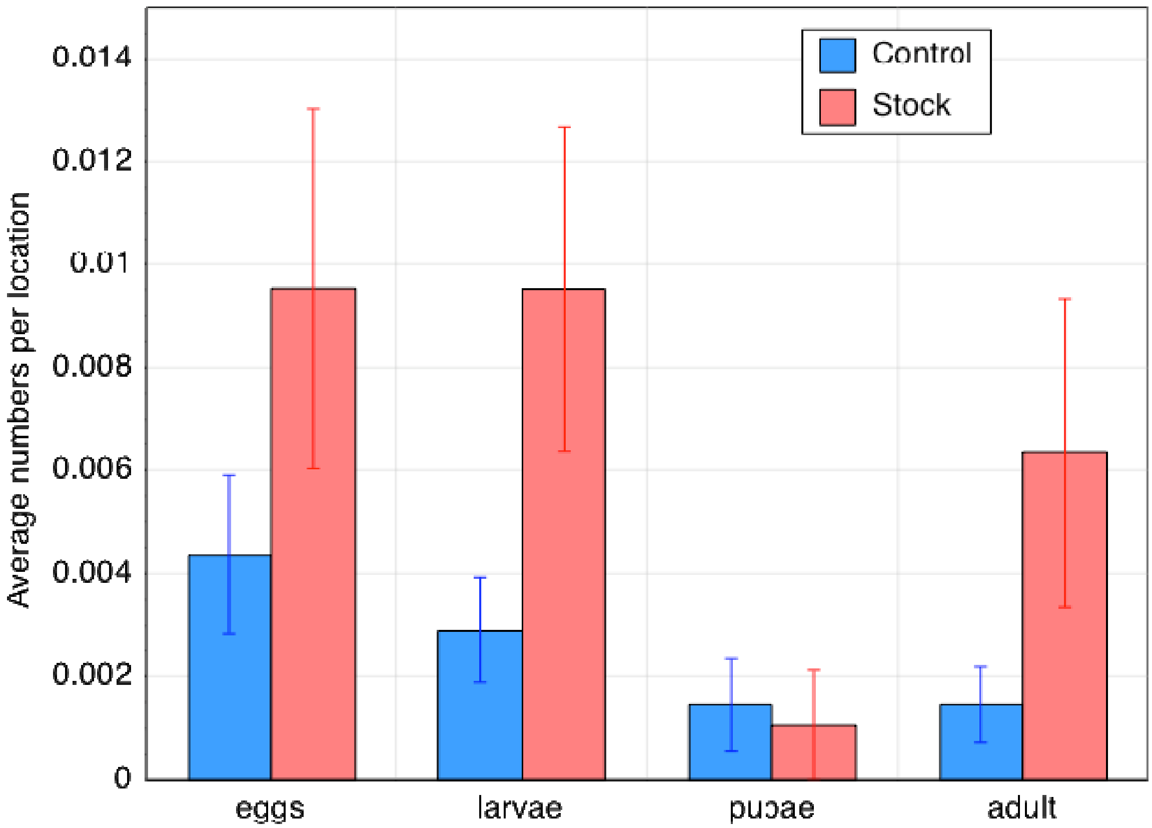
	Stems examined	Mean (per site)	SD
Larvae	946	.0877	.2831
Adults	946	.0063	.0794

Table 5. Results of ANOVA of weevil numbers. Numbers presented are p-values. Dash (—) indicates no significance.

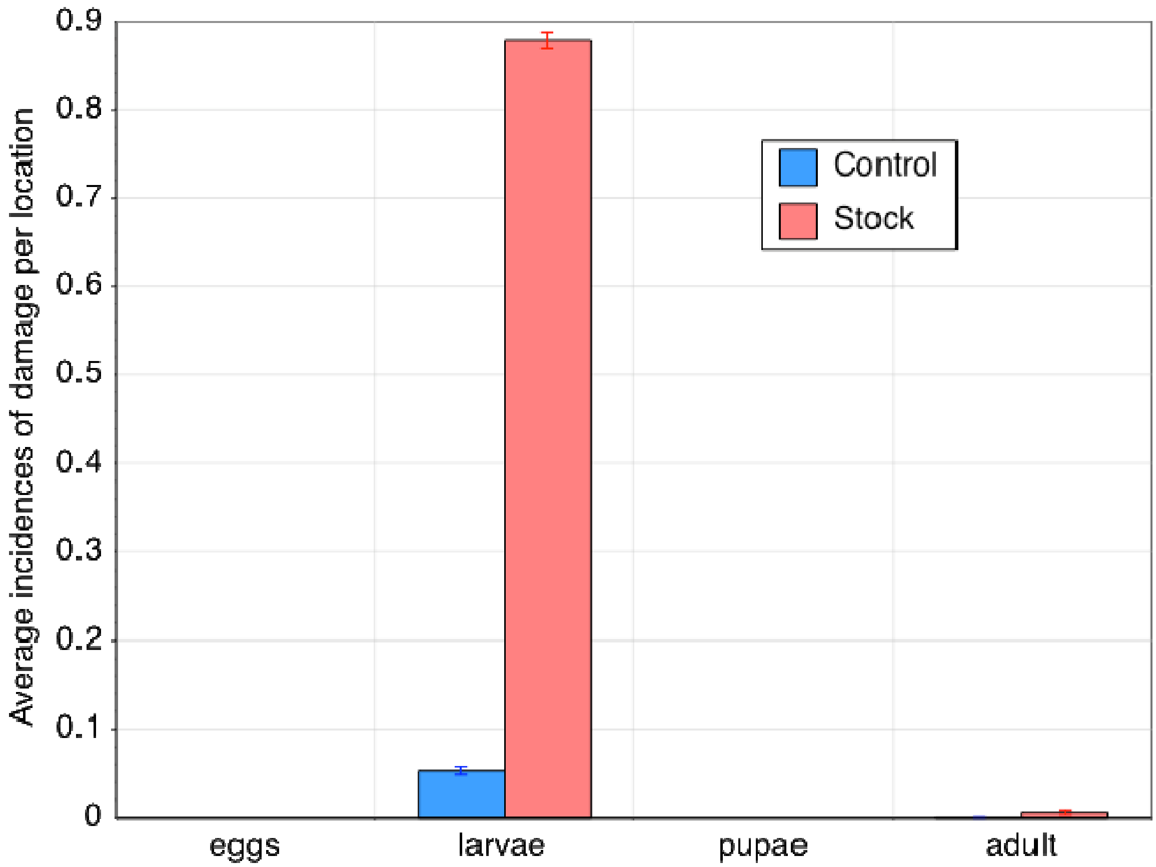
	Site	Position	Control/Stock
Eggs	0.012	—	0.042
Larvae	<0.001	0.006	0.0095
Pupae	—	—	—
Adults	—	—	.0389

Table 6. Analysis of weevil damage

	Site	Position	Control/Stock
Larvae	<0.001	—	0.0002
Adults	<0.001	0.009	0.0013



Graph 1. This graph compares the averages for all life stages of the Weevil. It shows a significant difference in populations for every stage except pupae. The line through the bar graph represents a standard error of one.



Graph 2. The graph compares the incidences of damage of the stocked sites to that of the control. The larvae control site has an incident of damage about .005 and the stocked larvae sites have an incident of damage of about .88.