BIOLOGICAL CONTROL OF
EURASIAN WATERMILFOIL (*Myriophyllum spicatum*) USING THE
NATIVE MILFOIL WEEVIL (*Euhrychiopsis lecontei*)

by

Amy L. Thorstenson

A Thesis

Submitted in partial fulfillment of the requirements of the degree

MASTER OF SCIENCE

IN

NATURAL RESOURCES

College of Natural Resources

UNIVERSITY OF WISCONSIN

Stevens Point, Wisconsin

May 2011

APPROVED BY THE GRADUATE COMMITTEE OF:

Dr. Ronald L. Crunkilton, Committee Chairman
Professor of Fisheries and Water Resources
College of Natural Resources

Dr. Michael A. Bozek
Professor of Fisheries and
Wisconsin Cooperative Fishery Research Unit Leader
College of Natural Resources

Dr. Robert W. Freckmann
Professor Emeritus of Biology
College of Letters and Science

Patrick Sorge
Program and Planning Analyst
Wisconsin Department of Natural Resource

OVERVIEW

The native milfoil weevil, *Euhrychiopsis lecontei* (Dietz), shows potential to be effective biological control of the nuisance aquatic macrophyte Eurasian watermilfoil, *Myriophyllum spicatum* L., in controlled conditions, but field application has shown mixed results. This graduate project was Phase 1 of an anticipated, larger, long-term study to better understand how to use this biological control agent. Two sub-projects are described in Chapter I and Chapter II, with supplementary data presented in Appendices 1 and 2, respectively. Baseline data collected for Phases 2 and 3 of the long-term study are presented in Appendix 3.

Chapter I defines habitat requirements for overwintering success, using multivariate (discriminate analysis) and univariate statistical methods to identify habitat variables that best define weevil hibernation habitat at three lakes in Portage County, Wisconsin: Thomas Lake, a glacial seepage lake; Springville Pond, an impoundment of the Little Plover River; and McDill Pond, an impoundment of the Plover River. Weevil presence and weevil quantity were evaluated relative to numerous habitat variables. Depth of duff material was positively correlated with weevil quantity on Springville Pond, but was inconclusive with multivariate statistics. Percent cover of leaves was positively correlated with weevil quantity on Thomas Lake, but was inconclusive with multivariate statistics. On all three lakes, weevils were never found at sites with zero cm of duff, such as bare sand or mowed, raked lawns. Although not entirely conclusive, the results suggest that management activities that remove duff material from the shoreline, such as mowing and raking, may be disadvantatgeous to weevils. On Thomas Lake and Springville Pond,

distance from water was negatively correlated with weevil quantity. Weevils were most common at 2 – 6 m from shore, but located as far as 8.3 m from shore. Discriminant analysis on Thomas Lake also identified height above water as a significant variable with positive correlation with weevil presence, suggesting that weevils occur more often at higher (and thereby drier) sites. The combined results suggest that higher sites nearer to shore, possibly with more duff material, correlate positively with weevil presence.

Chapter II develops a method for rearing large numbers of weevils to make biological control a practicle option for lake groups. The best chamber type for outdoor weevil rearing was 370-L ‘Freeland poly-tuf’ stock tanks. A 9.6 fold average return rate was produced from four stock tanks stocked initially with 0.19 weevils/L and two Eurasian watermilfoil stems per weevil. Weevils were fed an additional 2.28 milfoil stems per weevil initially introduced at 21 days, and 2.35 milfoil stems per weevil initially introduced at 42 days. An average of 672 weevils per tank was produced.

ACKNOWLEDGEMENTS

Support for planning and guidance came from graduate committee members: Dr. Ron Crunkilton(chair), College of Natural Resources, University of Wisconsin-Stevens Point; Dr. Michael Bozek, Wisconsin Cooperative Fishery Reasearch Unit, University of Wisconsin-Stevens Point; Dr. Robert Freckmann, Professor Emeritus, University of Wisconsin-Stevens Point; and Patrick Sorge, Wisconsin Department of Natural Resources. Funding support came from the Wisconsin Department of Natural Resources Aquatic Invasive Species Grant Program and University of Wisconsin-Stevens Point College of Natural Resources. Additional technical support came from Nancy Turyk, Center for Watershed Science and Education, University of Wisconsin-Stevens Point. Major field assistance came from Charles Boettcher, James Brodzeller, John Mumm, and Adam Skadsen. Major laboratory assistance came from Nathan Thomas and Benjamin Balika. Additional field and laboratory assistance came from the students of the Schmeeckle Reserve and the University of Wisconsin-Stevens Point Water and Environmental Analyasis Lab.

This project would also not have been possible without support from family and friends, but most of all from my husband, Dan, and our faithful dogs, Kessler, Cooper, and Mija. Their enduring patience, understanding, and companionship were vital and deeply appreciated.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

CHAPTER II

LIST OF FIGURES

LIST OF APPENDICES

Appendix 1: CHAPTER I, Supplementary Tables and Figures

Appendix 2: CHAPTER II, Supplementary Tables and Figures

Appendix 3: In-Lake Surveys: Lake Joanis, Thomas Lake, Lake Emily

I. INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a non-native aquatic plant from Eurasia that aggressively invades littoral zones of lakes. Introduced to the United States in the 1940's (Couch and Nelson 1986), it is now found in 45 states and four Canadian provinces (USDA, NRCS 2010). By the end of 2010, 539 waterbodies in Wisconsin had confirmed occurrences of Eurasian watermilfoil (WDNR 2011). The cumulative effect of Eurasian watermilfoil impacts lake ecology, decreases recreational, sporting and aesthetic values of the waterbodies, and decreased property values (Newroth 1985). The magnitude of the problem is so large that several million dollars are spent annually on Eurasian watermilfoil control in the northern tier states (Mullin et al. 2000).

Historically, control options for Eurasian watermilfoil have relied heavily on mechanical harvesting or chemical treatments, which do not provide a long term solution since they require repeated application (Crowell et al. 1994, Getsinger et al. 1997, Parsons et al. 2001). Concerns regarding the potential hazards posed by putting toxic herbicidal chemicals into our public waterways have been expressed by resource managers since Eurasian watermilfoil first emerged as a problem (Blakey 1966). Research examining the effects of herbicides and insecticides at low, residual levels that now commonly contaminate aquatic communities has been limited. Recently, Relyea (2009) found that even residual levels of some pesticides (diazinon, endosulfan) resulted in 24-84% mortality in leopard frogs (*Rana pipiens*), and that mixtures of chemical residuals may be much more toxic (99% mortality in leopard frogs) than the individual chemicals. Moreover, additional concerns regarding chemical use have arisen due to the recent development of flouridone resistance in several biotypes of hydrilla, spurring renewed interest in alternatives to chemical controls (Michel et al. 2004, Netherland et al. 2005).

Declines in Eurasian watermilfoil have been associated with several herbivorous insects: a naturalized moth, *Acentria ephemerella* (Denis & Schiffermüller), a native midge, *Cricotopus myriophylli* (Olivier), and the native milfoil weevil, *Euhrychiopsis lecontei* (Dietz) (Painter and McCabe 1988, Kangasniemi et al. 1993, Julien and Griffiths 1999). Primary focus for biological control has been on the latter (Sheldon and Creed 1995, Newman et al. 1996, Buckingham 1998, Newman 2004, Newman et al. 2006). Research suggests this milfoil weevil has potential to biologically control Eurasian watermilfoil, but more study on factors limiting populations adequate for control is necessary (Creed and Sheldon 1995, Sheldon and Creed 1995, Creed 2000, Jester et al. 2000, Madsen et al. 2000, Newman 2004, Cuda et al. 2008, Reeves et al. 2008).

Biology of *Myriophyllum spicatum*

Eurasian watermilfoil has spread to waterbodies across the U.S. by boaters, recreationalists, and various aquatic industries. Once introduced, Eurasian watermilfoil spreads rapidly via fragmentation (Nichols 1975).

This submersed aquatic plant goes through two flowering periods each summer, after which it fragments into pieces. Subsequently, each fragment may sprout roots and can remain afloat and stay viable for several weeks until it drifts to a suitable site, where it can take root and become another plant (Kimbel 1982, Rawson 1985). As a perennial plant, the lower portions of the stems may remain green during the winter (Reed 1977, Kimbel 1982), allowing the plant to start growing and become well established by April, much sooner than native aquatic plants (Aiken et al. 1979). Then, it grows rapidly, reaching the water surface and then spreading into a dense, tangled canopy, shading out other aquatic plants (Aiken et al. 1979).

The dense canopy of Eurasian watermilfoil alters the physiological and chemical characteristics of littoral zones. It increases dissolved oxygen, carbon dioxide, and pH fluctuations, inhibits water circulation, and promotes localized temperature stratification (Carpenter and Lodge 1986, Engel 1994). Eurasian watermilfoil aggressively out-competes the native aquatic plants, which rapidly decreases the diversity of the lake's plant community (Aiken et al. 1979), which in turn can alter fish communities (Crowder and Cooper 1982, Savino and Stein 1982, Diehl 1988, Dionne and Folt 1991). The tangled canopy at the water surface can become dense enough to hamper recreational activities, clog water intake pipes, and create a stagnant breeding ground for mosquitoes ((Aiken et al.1979, Bates et al. 1985, Newroth 1985).

Biology of *Euhrychiopsis lecontei*

The aquatic milfoil weevil, *Euhrychiopsis lecontei*, is native to North America, is broadly distributed across Wisconsin (Jester et al. 2000), and its lifecycle holds the key to its potential as a biological control for Eurasian watermilfoil. The adult weevil spends the winter hibernating on-shore at the soil-leaf litter interface (Newman et al. 2001). After ice-out, adults move out to milfoil beds to feed on apical stems and begin to lay eggs once water temperature reaches 15°C (May-June) (Newman et al. 2001). In spring, adult flight muscles are well-developed, and they have been documented to fly in spring (back to the milfoil beds), but in summer, flight muscles are atrophied while energy is re-allocated to reproduction (Newman et al. 2001). Females on average lay two to four eggs per day, and may lay multiple eggs on one meristem (Sheldon and O'Bryan 1996a, Sheldon and Jones 2001). Larvae eat the meristem then bore into the stem to feed, mature and pupate (Newman et al. 1996). They spend little time outside of the stem until they are adults. At typical summer lake temperatures of 25°C, the full life cycle can be completed within 21 days, and 3-5 generations may be produced per summer (Mazzei et al. 1999).

Biological Control

There has been a renewed interest in biological control for aquatic plants, due the recent discovery of several biotypes of hydrilla (*Hydrilla verticillata*) becoming resistant to the herbicide flouridone (Cuda et al.

2008). Many nuisance aquatic species are introduced species that arrived in the U.S. without the natural enemies that kept them in control in their native environments (Buckingham 1998). “Classical” biological control seeks to identify and import insects from the plant’s native home to control it where it has been introduced (Cuda et al. 2008, Newman 2004). Once a candidate agent is identified and tested for host-specificity, it may be imported and held in quarantine while assessed for its risk to non-target native species prior to release (Sheldon and Creed 1995).

Alligatorweed (*Alternanthera philoxeroides*) and water hyacinth (*Eichhornia crassipes*) are examples of invasive aquatic plants where classical biological control has been successful (Buckingham 1998). These species are emergent and floating-leaf aquatic plants. However, biological control of submersed aquatic plants has proven to be more difficult because herbivores of submersed aquatic plants must adapt to detecting water-soluble, host-plant cues, rather than volatile cues (Newman 2004). This is one of many challenges presented in an aqueous environment that explains the relatively low number of submersed aquatic specialist herbivores (Newman 2004).

Buckingham (1998) surveyed Eurasian watermilfoil’s native range for classical biological control candidates and identified one that was already native to the U.S. (*Crictopus myriophylli* Oliver), and therefore not necessitating importation. Other candidates identified were not species-specific feeders. Because imported insects may pose an unanticipated risk to native *Myriophyllum* species, and because of current interest from the research community an insect native to the U.S., *Euhrychiopsis lecontei*, Buckingham (1998) recommended against the importation of any non-native species.

E. lecontei's potential as a biological control agent

Native insects are preferred for use in biological control of invasive species due to the reduced risk of impacts to native, non-target plants, especially agricultural crops. Studies on several native or naturalized insects for controlling Eurasian watermilfoil have determined them to be poor candidates for use in biological control because they were: 1) too general in their feeding preferences [e.g. the moth *Acentria ephemerella* (Dennis and Schiffermuller; = *A. nivea* Olivier; = *Acentropus niveus* Olivier); Batra 1977, Buckingham and Ross 1981], 2) incapable of providing control (e.g. *Phytobius leucogaster* (= *Litodactylus leucogaster* Marsham); Buckingham et al. 1981], or 3) too difficult to rear to the high population densities needed (e.g. the milfoil midge *Cricotopus myriophylli* Olivier; Kangasniemi et al. 1993). In contrast, evaluations of studies on *E. lecontei* (hereafter referred to as the milfoil weevil) have found it to be suitable on all three aspects (Sheldon and Creed 1995, Newman 2004).

The milfoil weevil has demonstrated a preference for Eurasian watermilfoil, even when native milfoil species are present, and is not known to cause damage to other aquatic macrophytes (Solarz and

Newman 2001). One reason may be that Eurasian watermilfoil may lack the specific plant defenses that native milfoils posses from coevolving with milfoil weevils, which would give the weevil an advantage against its exotic host (Newman 2004). Adults initially visually target plants with the correct host-plant shape (Reeves et al. 2009), and then respond to the chemical attractants (glycerol and uracil) that are produced at higher concentrations by Eurasian watermilfoil than native milfoil species (Marko et al. 2005).

Control of Eurasian watermilfoil by milfoil weevils is achieved by larval stem-mining, which causes loss of buoyancy, nutrient depletion, and secondary infections. Stem-mining damages the vascular tissue (Newman et al. 1996) and releases cellular gases, which reduces stem buoyancy and causes the plant to sink below the water surface (Creed et al. 1992). This reduces the dense, tangled canopy at the water surface that causes most ecological and public recreation impacts (Sheldon and Creed 1995). Larval stem-mining also reduces the transfer of nutrients and carbohydrates from leaves to stems to roots (Newman et al.1996). Larvae also create openings for secondary infections by pathogens and deposit frass in the stem, which may promote those infections (Creed 2000). For instance, Shearer (2009) found that the endophytic fungus *Mycoleptodiscus terrestris* was only detrimental to Eurasian watermilfoil when the plant was stressed, and suggested that milfoils weevil may be useful in stressing the plant.

Studies have shown *E. lecontei* performs better on Eurasian watermilfoil than on native *Myriophyllum* species. Females will lay over four times as many eggs on Eurasian watermilfoil as on *M. sibiricum* (Sheldon and Creed 1995). Juveniles exhibit faster developmental rates (1-3 d), higher survival rates, and adults emerge from pupal chambers having higher mass than those reared on *M. sibiricum* (Newman et al. 1997, Solarz and Newman 2001). The nutritional quality of Eurasian watermilfoil versus native milfoils may play a significant role in this difference, but this conclusion lacks adequate study (Newman 2004).

Milfoil weevil-related declines

High-density beds of Eurasian watermilfoil in some lakes have exhibited periods of rapid decline in association with the milfoil weevil (Creed and Sheldon 1995, Sheldon 1997, Creed 1998, Lillie 2000), including ten lakes in Vermont (Madsen et al. 2000). Due to a lack of pre-decline data, however, the reasons for these seemingly natural population collapses have generally not been well documented. One of the few well-documented studies of a natural decline of Eurasian watermilfoil reported a reduction from 123 g dry matter/m^2 to 23, 5, 44 and 12, g dm/m^2 in subsequent samples over a 3-year period in a 12 ha man-made lake in Minnesota (Newman and Biesboer 2000). In this system, densities of the weevil were the highest yet reported for Minnesota lakes at 103 weevils per m^2 (1.6 to 2.0 weevils per milfoil stem) at start of the study, when milfoil density was greatest (Newman and Biesboer 2000).

Stocking milfoil weevils in controlled laboratory and field enclosures have shown the herbivore is capable of controlling Eurasian watermilfoil (Creed and Sheldon 1995, Sheldon and Creed 1995, Newman et al. 1996). However, open field trials have shown mixed results. In one supplemental stocking study in Wisconsin, Jester et al. (2000) associated significant within-season declines in Eurasian watermilfoil study plots with weevil densities, ranging from 0.5 to 3 per stem, in six out of 12 treatment lakes. These were open-plot stocking trials and Jester et al. (2000) theorized that one possible reason the other six plots did not reach control-level populations was that the weevils that were stocked may have emigrated from the study plots.

Supplemental stocking has been attempted at numerous lakes throughout the U.S., however, these results have also been mixed (Sheldon 1997, Madsen et al. 2000, Reeves et al. 2008). Estimates of densities required to affect control varies, ranging from as little as 0.25 per stem to $\geq$1.0 per stem, and whether population densities reach levels capable of whole lake control appears to vary from lake to lake (Newman 2004). Currently, there is no prescription for predicting whether the milfoil weevil will be able to reach control levels in a lake, or how long it will take to reach control levels. There is a need for long-term studies of weevil stocking programs.

Lake characteristics affecting weevil success

Suitable overwintering habitat may be critical to supporting sustainable milfoil weevil populations in the long-term. Weevil densities correlate positively with the percentage of natural shoreline adjacent to milfoil beds (Jester 2000). Newman et al. (2001) found that terrestrial overwintering weevil populations were most commonly found from two to six meters from the lake shorelines, and were significantly higher at sites with soil moisture less than 15%, indicating that weevils prefer dry sites close to shore. This suggests that weevils may be less successful on lakes surrounded by low, wet riparian habitats. More studies are needed to better understand overwintering habitat requirements to maximize the chance of weevil success (Jester et al. 2000, Madsen et al. 2000, Newman 2004).

Weevil success may also be limited by predation. Adult weevils are more vulnerable to predation than the larval and pupal life stages that are concealed inside the milfoil stems, and the longevity of the egg-laying adult females are critical to population growth (Ward 2002). Modelling suggests that increasing an adult female's lifespan from five to 10 days can result in an 8-fold increase in end-of-summer populations densities (Ward 2002). Studies on predation by vertebrates have found that while yellow perch (*Perca flavescens*) do not appear to feed on weevils (Creed 2000), sunfish and bluegills (*Lepomis sp.*) do and could limit milfoil weevils from reaching densities capable of suppressing Eurasian watermilfoil

(Ward and Newman 2006). Sunfish catch rates greater than 25-30 sunfish per 24 hr trapnet, as in the case of stunted populations, may result in relatively low weevil densities (<0.1/stem) (Ward and Newman 2006). Because dense Eurasian watermilfoil beds may produce stunted sunfish populations, this may perpetuate the Eurasian watermilfoil problem by increasing predation on milfoil weevils (Engel 1995). To break this cycle, more study on the potential to better understand and ameliorate sunfish predation pressure on weevils is needed.

Predation on weevils by invertebrates is less well studied. Ward and Newman (2006) cite two studies that suggest milfoil weevils do not appear to be vulnerable to most invertebrate predators. In contrast, Tamayo (2003) found a negative correlation between weevils and Hirudinoidea and Hydrachnida densities, suggesting a need for more study on invertebrate predators.

General lake characteristics appear not to influence the distribution of presence and abundance of weevils. When measured at the whole-lake level, temperature, dissolved oxygen, pH, water clarity, nitrogen, chlorophyll a, alkalinity, and conductivity showed no correlation with milfoil weevil densities (Jester et al. 2000). However, because Eurasian watermilfoil is known to alter in-bed pH, dissolved oxygen, carbon dioxide and temperature circulation (Engel 1994), bed-level parameters may prove to be a factor in weevil densities. The size and depth of a milfoil bed are positively correlated with milfoil weevil densities (Jester et al.

2000). Sediment nutrients and plant nutrients may also play a factor in the success of weevils to impact plant vigor; sediment nutrients may allow milfoil to outgrow the impacts of weevil feeding damage, and plant nutrients may affect weevil development, but these relationships are not well understood at this time (Creed 2000). Jester et al. (2000), Reeves et al. (2008), and Creed (2000) all call for further studies on bed-level conditions that may affect milfoil weevil populations.

Integrated use

A relatively new area of exploration in biological control is integrated use, or the coordinated use of multiple control methods. For example, researchers in water hyacinth control have found success by combining one biological control agent with a second agent, or with limited herbicide applications (Van 1988, Haag and Habeck 1991). Combining milfoil weevils with a second biological control agent may also hold promise. Shearer (2009) found that the endophytic fungus *Mycoleptodiscus terrestris* was only detrimental to Eurasian watermilfoil when the plant was stressed, and suggested that milfoil weevils may be useful in creating that stress. Looking back at the mixed results with stocking weevils (Sheldon 1997, Madsen et al. 2000, Reeves et al, 2008), perhaps one of the differences between success or failure was dependent on the presence of a second, unknown agent. This question seems worthy of further study.

Experimentation with carefully coordinated integrations, using targeted applications of mechanical controls, may also hold promise (Newman and Inglis 2009, Sheldon and O'Bryan 1996b). For instance, broad-scale applications of mechanical controls appear incompatible with weevils, since weevils lay their eggs on the tops of milfoil plants and mechanical harvesting, by design, removes the tops of plants. However, mechanical harvesting of less than 15% of the milfoil beds may avoid the detrimental impacts to weevil populations and allow the strategic use of both control methods together (Newman and Inglis 2009).

Although broad-scale use of chemical herbicides is incompatible with milfoil weevils because it removes the food base weevils need to survive, targeted use of the two control methods in separate areas of the same lake may be hold potential. In an unpublished report on Bass Lake in St. Croix County, WI, Jester (2000) found that weevil densities in untreated beds adjacent to chemically treated beds were slightly higher than that of control beds that were far from the treatment areas (0.800 weevils per stem versus 0.617 weevils per stem, respectively). This difference was not significant, but may have been attributable to adult weevils emigrating from treated beds into adjacent beds, or may have been due to the fact that treated beds were usually closer to shore where weevils tend to concentrate (Jester 2000). Additional studies are needed to understand the potential of integrating chemical and biological controls.

Summary

E. lecontei is a native, host-specific herbivore, with a foraging preference for Eurasian watermilfoil. It has been shown to cause feeding damage capable of impacting Eurasian watermilfoil, and produces 3-5 generations per summer which may allow it to achieve population densities sufficient for control. This organism has been linked to numerous natural declines, but has resulted in mixed success in stocking experiments.

There is currently limited guidance pertaining to what features make a lake infested with Eurasian watermilfoil a good candidate for biological control. A number of factors that may impact weevil populations are poorly understood, including overwintering habitat quality, predation, adult longevity, bed-level water quality, sediment nutrients, plant nutrients, and the presence of other biological control agents. Understanding these relationships better will help lake managers predict whether weevils can be successful at a given lake, whether critical habitat needs can be improved to optimize the potential for success, or whether other control methods would be a better choice.

This project was Phase I of a multi-year study seeking to identify key habitat characteristics for supporting milfoil weevils, and to begin to develop management guidance that may increase natural populations of milfoil weevils and presumably their success in controlling Eurasian watermilfoil. Phase I examined overwintering habitat requirements and mass rearing techniques. Subsequent phases in the study will examine information gaps in other factors believed to be critical to weevil success. Suggested areas for study include: 1) defining more precisely what weevil densites are required for control, 2) what water depth becomes limiting for weevils, 3) bed-level conditions that might be limiting to weevils, 4) the presence of a second biological agent, such as *Mycoleptodiscus terrestris*, as an effective integrated control, 5) how to ameliorate predation pressure where sunfish trapnet rates exceed 25-30 sunfish per 24 hr trapnet, and 6) the relatioinship between sediment nutrients, plant nutrients, and the ability of weevils to control milfoil.

II. LITERATURE CITED

Aiken, S.G., P.R. Newroth, and I. Wile. 1979. The biology of Canadian weeds. 34. *Myriophyllum spicatum* L. Can. J. of Plant Sci. 59:201-215.

Bates, A.L., E.R. Burns, and D.H.Webb . 1985. Eurasian watermilfoil (*Myriophyllum spicatum*) in Tennessee Valley: an update on biology and control. pp 104-115 *in* L.W. J. Anderson editor. Proceedings of the First International Symposium on the watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc., Washington D.C.

Batra, S.W.T. 1977. Bionomics of the aquatic moth *Acentropus niveus* a potential biological control agent for Eurasian water-milfoil and hydrilla. J. New York Entomological Society 85:143-152.

Blakey, H.L. 1966. Outlook for weed control activities. J. Aquat. Plant Manage. 5:18-20.

Buckingham, G.R. 1998. Surveys for insects that feed on Eurasian watermilfoil, *Myriophyllum spicatum*, and hydrilla, *Hydrilla verticillata*, in the People's Republic of China, Japan, and Korea. U.S. Army Corps of Engineers Waterways Experiment Station, Technical Report A-98-5, Vicksburg, MS.

Buckingham, G.R., and B.M. Ross. 1981. Notes on the biology and host specificity of Acentria nivea = Acentropus niveus. J. Aquat. Plant Manage. 19:32-36.

Buckingham, G.R., C.A. Bennett, and B.M. Ross. 1981. Investigation of two insect species for control of Eurasian watermilfoil. Technical Report A-81-

4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, USA.

Carpenter, S.R. and D.M. Lodge. 1986. Effects of submersed macrophytes on ecosystem processes. Aquat. Bot. 26:341-370.

Couch, R. and E. Nelson. 1986. *Myriophyllum spicatum* in North America. Proc. 1st International Symposium on watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc. p.8-18.

Creed, R.P., Jr. 2000. The weevil-watermilfoil interaction and different spatial scales: what we know and what we need to know. J. Aquat. Plant Manage. 38:78-81.

Creed, R.P., Jr. 1998. A biographical perspective on watermilfoil declines: additional evidence for the role of herbivorous weevils in promoting declines? J. Aquat. Plant Manage. 36:16-22.

Creed, R.P., Jr. and S.P. Sheldon. 1995. Weevils and watermilfoil: did a North American herbivore cause the decline of an exotic plant? Ecol. Applic. 5:1113-1121.

Creed, R.P., Jr., S.P. Sheldon, and D.M. Cheek. 1992. The effect of herbivore feeding on the buoyancy of Eurasian watermilfoil. J. Aquat. Plant Manage. 30:75-76.

Crowder, L.B. and W.E. Cooper. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecol. 63:1802-1813.

Crowell, W., N. Troelstrup Jr., L. Queen, and J. Perry. 1994. Effects of harvesting on plant communities dominated by Eurasian watermilfoil in Lake Minnetonka, MN. J. Aquat. Plant Manage. 32:56-60.

Cuda, J.P, R. Charudattan, M.J. Grodoqitz, R. M. Newman, J.F. Shearer, M.L. Tamayo, and B. Villegas. 2008. Recent advances in biological control of submersed aquatic weeds. J. of Aquat. Plant Manage. 46:15-32.

Diehl, S. 1988. Foraging efficiency of three freshwater fish: effects of structural complexity and light. Oikos 53:207-214.

Dionne, M. and C.L. Folt. 1991. An experimental analysis of macrophyte growth forms as fish foraging habitat. Can. J. of Fisheries and Aquat. Sci. 48:123-131.

Engel, S. 1995. Eurasian watermilfoil as a fishery management tool. Fisheries, 20:20–25.

Engel, S. 1994. Fish in troubled water: Eurasian watermilfoil as a threat to Wisconsin sport fish. Wisconsin Department of Natural Resources Fish Research Highlights, January 1994.

Getsinger, K.D., E.G. Turner, J.D. Madsen, and M.D. Netherland. 1997. Restoring native vegetation in a Eurasian watermilfoil-dominated plant community using the herbicide triclopyr. Regulat. Rivers Res. Manage. 14(4):357-375.

Haag, K.H. and D.H. Habeck. 1991. Enhanced biological control of waterhyacinth following limited herbicide application. J. of Aquat. Plant Manage. 29:24-28.

Jester, L.L., 2000. Bass Lake milfoil weevil survey. Jester Consulting, Inc. 11 pp.

Jester, L.L., M.A. Bozek, D.R. Helsel, and S.P. Sheldon. 2000. *Euhrychiopsis lecontei* distribution, abundance and experimental augmentation for Eurasian watermilfoil control in Wisconsin lakes. J. of Aquat. Plant Manage. 38:88-97.

Julien, M.H. and M.W. Griffiths. 1999. Biological control of weeds: A world catalogue of agents and their target weeds. 4th Ed. CAB International, Wallingford, UK.

Kangasniemi, B., H. Speier, and P. Newroth. 1993. Review of Eurasian watermilfoil biocontrol by the milfoil midge. In: Proceedings, 27th Annual Meeting of the Aquatic Plant Control Research Program. Misc. Paper A-93-2, Waterways Experiment Station, Vicksburg, MS, pp. 17-22.

Kimbel, J.C. 1982. Factors influencing potential intralake colonization by *Myriophyllum spicatum* L. Aquat. Bot. 14:295-307.

Lillie, R.A. 2000. Temporal and spatial changes in milfoil distribution and biomass associated with weevils in Fish Lake, WI. J. Aquat. Plant Manage. 38:98-104.

Madsen, J.D., H.A. Crosson, K.S. Hamel, M.A. Hilovsky, and C.H. Welling. 2000. Panel discussion - Management of Eurasian watermilfoil in the United States using native insects: State regulatory and management issues. J. Aquat. Plant Manage. 38:121-124.

Marko, D.M., R.M. Newman, and F. Gleason. 2005. Chemically mediated host-plant selection. J. of Chemic. Ecol. 31:2857-2876.

Mazzei, K.C., R.M. Newman, A. Loos, and D.W. Ragsdale. 1999. Developmental rates of the native milfoil weevil, *Euhrychiopsis lecontei*, and damage to Eurasian watermilfoil at constant temperatures. Biol. Control 16:139-143.

Michel , A., R.S. Arias, B.E. Scheffler, S.O. Duke, M.D. Netherland, and F.E. Danyan. 2004. Somatic mutation-mediated evolution of herbicide resistece in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). Mol. Ecol. 13:3229-3237.

Mullin, B. H., L. W. J. Anderson, J. M. DiTomaso, R. E. Eplee, and K. D. Getsinger. 2000. Invasive plant species. Council for Agricultural Science and Technology (CAST), Issue Paper No. 13. 18 pp.

Netherland, M.D., M.V. Hoyer, M.S. Allen, and D. Canfield. 2005. A summary of future management recommendations from the hydrilla summit in Florida. Aquatics 27:4-9.

Newman, R.M. 2004. Biological control of Eurasian watermilfoil by aquatic insects: basic insights from an applied problem. Archiv fur Hydrobiologie 159 (2):145-184.

Newman, R.M. and D.D. Biesboer. 2000. A decline of Eurasian watermilfoil associated the milfoil weevil *Euhrychiopsis lecontei.* J. of Aquat. Plant Manage. 38:105-111.

Newman, R.M., D.W. Ragsdale, A. Milles, and C. Oien. 2001. Overwinter habitat and the relationship of overwinter to in-lake densities of the milfoil weevil *Euhrychiopsis lecontei*, Eurasian watermilfoil biological control agent. J. of Aquat. Plant Manage. 39:63-67.

Newman, R.M., E.M. Gross, W. Wimmer, and P. Sprick. 2006. Life history and developmental performance of the Eurasian milfoil weevil, *Eubrychius velutus* (Coleoptera: Curculionidae). The Coleopterists Bulletin 60(2):170-176.

Newman, R.M., K.L. Holmberg, D.D. Biesboer, and B.G. Penner. 1996. Effects of the potential biological control agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. Aquat. Bot. 53:131-150.

Newman, R.M., M.E. Borman, and S.W. Castro. 1997. Developmental performance of the weevil *Euhrychiopsis lecontei* on native and exotic watermilfoil host-plants. J. of the North Amer. Benthological Soc. 16:627-634.

Newman, R.M. and W.G. Inglis. 2009. Distribution and abundance of the milfoil weevil, *Euhrychiopsis lecontei*, in Lake Minnetonka and relation to milfoil harvesting. J. of Aquat. Plant Manage. 47:21-25.

Newroth, P.R. 1985. A review of Eurasian watermilfoil impacts and management in British Columbia. Pages 139-153 *in* L. W. J. Anderson, editor, Proceedings of the First International Symposium on the watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc., Washington D. C.

Nichols, S.A. 1975. Identification and management of Eurasian watermilfoil in Wisconsin. Wisconsin Academy of Sci., Arts and Letters 63: 116-128.

Painter, D.S. & McCabe, K.J. 1988. Investigation into the disappearance of Eurasian watermilfoil from the Kawartha Lakes. J. of Aquat. Plant Manage. 26:3-12.

Parsons, J.K., K.S. Hamel, J.D. Madsen, and K.D. Getsinger. 2001. The use of 2,4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J. of Aquat. Plant Manage. 39:117-125.

Rawson, R.M. 1985. History of the spread of Eurasian watermilfoil through the Okanogan and Columbia River systems (1978-1984). Pages 35-38 *in* L. W. J. Anderson, editor. Proceedings of the First International Symposium on the watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc., Washington D.C.

Reed, C.F. 1977. History and distribution of Eurasian watermilfoil in United States and Canada. Phytologia 36:417-436.

Reeves, J.I., P.D. Lorch, and M.W. Kershner. 2009. Vision is important for plant location by the phytophagous aquatic specialist *Euhrychiopsis lecontei* Dietz (Coleoptera). J. of Insect Behavior 22:54-64.

Reeves, J.I., P.D. Lorch, M.W. Kershner, and M.A. Hilovsky. 2008. Biological control of Eurasian watermilfoil by *Euhrychiopsis lecontei*: Assessing efficacy and timing of sampling. J. of Aquat. Plant Manage. 46:144-149.

Relyea, R.A. 2009. A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. Oecologia 159:363-376.

Savino, J.F. and R.A. Stein. 1982. Predator-prey interaction between largemouth bass and bluegills as influenced by simulated, submersed vegetation. Transactions of the Amer. Fisheries Soc. 111:255-266.

Shearer, J.F. 2009. *Mycoleptodiscus terrestris*: An endophyte turned latent pathogen of Eurasian watermilfoil. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-11. Vicksburg, MS: U.S. Army Engineer Research and Development Center. <http://el.erdc.usace.army.mil/aqua/aqua.html>.

Sheldon, S.P. 1997. Investigations on the potential use of an aquatic weevil to control Eurasian watermilfoil. Lake Reserv. Manage. 13:79-88.

Sheldon, S.P. and K.N. Jones. 2001. Restricted gene flow according to host plant in an herbivore feeding on native and exotic watermilfoils. Internat. J. of Plant Sci. 162:793-799.

Sheldon, S.P. and L.M. O'Bryan. 1996a. Life history of the weevil *Euhrychiopsis lecontei,* a potential biological control agent of Eurasian watermilfoil. Entomological News 107:16-22.

Sheldon, S.P. and L.M. O'Bryan. 1996b. Effects of harvesting Eurasian watermilfoil on the aquatic weevil *Euhrychiopsis lecontei*. J. Aquat. Plant Manage. 34:76-77.

Sheldon, S.P. and R.P. Creed. 1995. Use of a native insect as a biological control for an introduced weed. Ecol. Applic. 5(4):1122-1132.

Solarz, S.L. and R.M. Newman. 2001. Variation in hostplant preferences and performance by the milfoil weevil, *Euhrychiopsis lecontei* Dietz, exposed to native and exotic watermilfoils. Oecologia 126:66-75.

Tamayo, M.L. 2003. Developmental performance, abundance and habitat of the milfoil weevil, *Euhrychiopsis lecontei*, in Washington State. Dissertation. University of Washington, Seattle, WA.

USDA, NRCS. 2010. The PLANTS Database (http://plants.usda.gov, 30 December 2010). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Van, T.K. 1988. Integrated control of waterhyacinth with *Neochetina* and paclobutrazol. J. of Aquat. Plant Manage. 26: 59-61.

Ward, D.M. 2002. The impact of fish predation on Eurasian watermilfoil herbivores and indirect effects on the macrophyte community. – MS Thesis, University of Minnesota, St. Paul, MN.

Ward, D.M. and R.M. Newman. 2006. Fish predation on Eurasian watermilfoil (*Myriophyllum spicatum*) herbivores and indirect effects on macrophytes. Can. J. of Fisheries and Aquat. Sci. 63:1049-1057.

[WDNR] Wisconsin Department of Natural Resources. 2011. Eurasian water-milfoil in Wisconsin. Web site: http://new.dnr.wi.gov/Default.aspx?Page=12c074b6-b556-492f-8195-575c13870e2e.

III. CHAPTER I

Overwintering habitat requirements of the milfoil weevil, *Euhrychiopsis lecontei*, in Portage County, WI

ABSTRACT

The native milfoil weevil, *Euhrychiopsis lecontei* (Dietz), shows potential to be effective biological control of the nusciance aquatic macrophyte Eurasian watermilfoil, *Myriophyllum spicatum* L.. To better define shoreline habitat requirements for overwintering success, univariate and multivariate (discriminate analysis) statistical methods were used to identify the habitat variables that best define weevil hibernation habitat at three lakes in Portage County, Wisconsin: Thomas Lake, a glacial seepage lake; Springville Pond, an impoundment of the Little Plover River; and McDill Pond, an impoundment of the Plover River. Weevil presence and abundance were evaluated in relation to the presence of milfoil fragments along shore, distance from water, height above water, habitat type, soil texture, soil and duff moisture, soil and duff organic matter, duff depth, and duff composition (percent cover woody debris, deciduous leaves, conifer needles, grass, forbs, rocks, and bare soil). Depth of duff material on shore was found to be positively correlated with weevil quantity on Springville Pond. Percent cover of leaves was positively correlated with weevil quantity on Thomas Lake, but was inconclusive with multivariate statistics. On all three lakes, weevils were never found at sites without duff, such as sites with bare sand or mowed,

raked lawns. The results suggest that management activities that remove duff material from the shoreline, such as mowing and raking, may be disadvantatgeous to weevil populations. On Thomas Lake and Springville Pond, distance from water was also negatively correlated with weevil weevil quantity. Weevils were most common at 2 – 6 m from shore, but were also located as far as 8.3 m from shore. On Thomas Lake, height above water was positively correlated with weevil presence, and on McDill Pond, eleven sites were sampled at less than 50 cm above water and all were negative for weevils, suggesting that weevils occur more often at higher (and thereby drier) sites. The combined results suggest that higher elevation sites nearer to shore, with more duff material, correlate positively with weevil presence.

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 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).

Shoreline 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). 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 affect 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 (1998) found milfoil weevil population density correlated positively with natural shoreline vegetation, and negatively with bare, sand shorelines (Jester 1998). Apparently they also can be successful on natural grass riparian areas (i.e., prairie sites) (Newman et al. 2001). More whole-lake studies are needed to better understand overwintering habitat requirements (Jester et al. 2000, Newman 2001, Newman 2004).

The objectve of this study was to evaluate shoreline habitat characteristics that help discriminate shoreline characteristics where weevils overwinter versus site characteristics where they are absent.

MATERIALS AND METHODS

Study Area - Our study area included three lakes in Portage County, Wisconsin: 1) Thomas Lake (November 2nd - November 7th, 2009) 2) the eastern third of Springville Pond (November 13th - November 21st, 2009) 3) an isolated bay on the eastern side of McDill Pond (April 22nd - May 8th, 2009). All study sites consisted primarily of natural (undisturbed) shoreline.

Thomas Lake is a 13-hectare hard-water seepage lake, with a maximum depth of 9 meters. Frequency of occurrence of *M. spicatum* in vegetated sites (n=51) was 53% in 2008, and naturally-occuring weevil density has ranged from 0.03 – 0.34 weevils per stem from 2004 to 2008.

Springville Pond is a 7-hectare hard-water impoundment of the Little Plover River, with a maximum depth of 4 meters. Frequency of occurrence of *M.*

spicatum within vegetated sites (n=55) was 85% in 2008, and naturally-occuring weevil density has ranged from 0.06 – 4.43 weevils per stem from 2004 to 2008.

McDill Pond is a 106-hectare impoundment of the Plover River, with a maximum depth of 4 meters in the selected study bay. Quantitative survey data for *M. spicatum* and *E. lecontei* in 2008 were unavailable, but spatial distribution of *M. spicatum* beds in the study bay was estimated to be approximately 15% of total area, and weevils were observed to be naturally abundant.

Study Design - Weevil quantity and abundance were measured, as well as shoreline condition, at sample points 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. Terrestrially, two, 1 m^2 circular sample plots were sampled per transect. Because weevils are most commonly found on shore at 2-6 meters from the water, sample plots were centered at 2 and 6 meters from the water, although distance from water varied at some transects due to obstructions or other site-specific features. At each lake, three of the transects (randomly chosen) were also sampled at approximately 10 meters from the water.

Habitat variables were measured at each sample point, including distance from water, height above water, the presence of milfoil fragments at the shoreline, duff layer depth, and duff layer composition. To determine the center of each sample plot, distance from water was measured with incline of the slope. At each sample point, shoreline habitat within the 1 m^2 sample 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 sample plot was considered when characterizing habitat.

To further describe the habitat at each sample point, the duff material was measured and characterized in situ. The depth of the duff layer measured using a meter stick, and composition of the duff materials were characterized by percent cover of various types of material (Table 2). 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 point, soil and duff samples were extracted from each sample plot. Each circular sample was 0.05 m^2, collected to a depth of approximately 5 cm into the soil. Soil and duff material were retained together as composite samples in a re-sealable plastic bag, and kept covered or shaded to keep the samples cool during transport back to the lab. Four, replicate samples were extracted per sample plot, for a total sample area of 0.2 m^2. Data from the four replicate samples were averaged prior to statistical analysis.

Table 1. Qualitative description of each habitat type used to classify habitats during shoreline 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

Table 2. Qualitative descriptions of duff layer composition, recorded as percent cover. (Total Coverage = 100%)

TYPE OF MATERIAL
Woody material (sticks, logs, pinecones)
Deciduous leaf litter
Coniferous leaf litter
Grasses
Forbs
Bare soil (absence of duff)
Rock

To determine the number of weevils per sample circle, 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 3X 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. No 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 and organic matter.

Pearson correlations, non-parametric t-tests, logistic regression, multiple logistic regression, and discriminant analysis were used to differentiate between sites where milfoil weevils were found (weevil sites) and sites they were not found (non-weevil sites), based on quantitative habitat variables. Parametric t-tests were not used, because attempts to normalize the data through arcsin transformations were unsuccessful.

Logistic regression was run on weevil presence/absence relative to each of the site characteristics. Multiple logistic regression was run on weevil presence/absence relative to site characteristis that were significant in univariate analyses. 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 is 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 are close to zero play 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 per site ranged from 0 to 3, with an average of 0.2 N/0.2 m^2. Sites were located at 3 to 20 meters from the waterline, and from 47 to 196 cm above the waterline vertically. Shoreline habitat types included sites having no disturbance to high disturbance, and were most commonly characterized as upland herbacious dominated by either grasses or forbs. Soil texture was rather uniform, with sand present at 49 sample sites, and sandy loam only appearing at four sample sites. Duff depth ranged from 0 to 8 cm of duff material, and composition of duff material 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 2%.

Significant correlations ($P < 0.05$) were found between the quantity of milfoil weevils and two variables: distance from water was negatively correlated with weevil quantity ($R = -0.335$), indicating weevil occurrence decreased as distance from water increased. Percent cover of leaves was positively correlated with milfoil weevil quantity ($R = 0.282$), suggesting that weevil quantity increases as percent cover of leaves increases. Distance from water and percent cover of leaves were negatively colinear ($R = -0.370$, p-value = 0.006), indicating that these two independent variables were correlated, with percent cover of leaves decreasing as distance from water increased.

Non-parametric t-tests showed significant differences between characteristics of weevil sites versus non-weevil sites for percent cover of leaves ($p=0.007$) and distance from water ($p=0.018$). Mean percent cover of leaves at weevil sites was 36%, versus a mean percent cover of 20% at non-weevil sites (a difference of 16%). Mean distance from water at weevil sites was 5.3 m, versus

Table 3. Significant non-parametric t-test results for Thomas Lake. Values represent the mean ±1 standard error with 95% confidence interval in parenthases. Alpha was set at $P \leq 0.05$.

Variable	**Weevils Present (n=13)** Mean ±SE	**Weevils Absent (n=40)** Mean ±SE	**p**
Percent cover of leaves	36 ±5.8 (23-48)	20 ±3.5 (13-27)	0.007
Distance from water (m)	5.3 ±0.4 (4.4-6.2)	9.0 ±0.8 (7.4-10.5)	0.018

a mean distance of 9.0 m at non-weevil sites (a difference of 3.7 m). Most weevils occurred between 4.4 m and 6.2 m from the water (Table 3).

Logistic regression found distance from water and percent cover of leaves were significantly related to the occurrence of weevils (p=0.029 and p=0.037, respectively). However, during 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 included: distance from water (p=0.017) and height above water (p=0.022) (Table 4).

The best discriminant model developed (p=0.011) included only two site location variables: distance from water and height above water (Table 5). The model correctly discriminated between weevil sites and non-weevil sites 75% of the time, and no weevil sites were misclassified as non-weevil sites (Table 6). However, some non-weevil sites were misclassified as weevil sites. The model may be idenitifying suitable habitat that was unoccupied, which would be expected with a low sample size (13 weevil sites vs 40 non-weevil sites).

Springville Pond - Milfoil weevils were found at 17 sites of the 45 sites sampled. The number of weevils found per site ranged from 0 to 5, with an average of 0.6 N/0.2 m^2. Sites were located at 1 to 10 meters from the waterline, and from 37 to 227 cm above the waterline vertically. Shoreline 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 decidous and herbacious vegetation. Soil type was sand on all sites

Table 4. Site characteristics in the final multiple logistic regression analysis ($p<0.001$) for Thomas Lake. Alpha was set at $P \leq 0.05$.

Dependent variable	Independent variables	Coefficients	Wald P
Weevil presence	Distance from water	0.97012	0.0170
	Height above water	-0.06508	0.0216
	Intercept	0.23025	0.8217

Variables included in initial model run:

soil texture

soil/duff moisture

soil/duff organic matter

distance from water

height above water

duff depth

percent cover wood

percent cover deciduous leaves

percent cover grass

percent cover forbs

percent cover bare soil

Table 5. The “best” canonical discriminant function developed for Thomas Lake included the following variables: Distance from water and height above water. Alpha was set at $P \leq 0.05$.

Variable	Structure Coefficient
Distance from water	0.85572
Height above water	-0.09243
Wilke’s Lambda Probability	0.0111

Table 6. Prediction of weevil sites vs. non-weevil sites the “best” canonical discriminant function at Thomas Lake. This function correctly classified all weevil sites, but misclassified some non-weevil sites. The function was likely identifying suitable habitat that was unoccupied, which could be expected with a low population density.

Group	No. of sites	Predicted group membership		Sites correctly predicted
		Weevils	No weevils	(%)
Weevils	13	13	0	100
No weevils	40	20	20	50.0
	Overall percentage of sites correctly classified:			75.0

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 0.34% to 56%, with a mean of 12%.

Significant correlations ($P < 0.05$) were found between milfoil weevils and two variables. Distance from water was negatively correlated with weevil quantity ($R = -0.303$), indicating weevil occurrence decreased as distance from water increased. Duff depth was positively correlated with milfoil weevil quantity ($R = 0.422$), indicating that weevil occurrence increased as duff depth increased. Distance from water and duff depth were negatively colinear ($R = -0.349$, p-value = 0.019); duff depth decreased as distance from water increased.

Logistic regressions also found distance from water and duff depth to be significant variables (p=0.048 and p=0.018, respectively). Non-parametric t-tests found significant differences in duff depth (p=0.015) and distance from water (p=0.031) between weevil sites and non-weevil sites. Mean duff depth at weevil sites was 3.5 cm, versus a mean depth of 2.6 cm at non-weevil sites (a difference of 0.9 cm). Mean distance from water at weevil sites was 3.1 m, versus a mean distance of 4.5 m at non-weevil sites (a difference of 1.4 m). Most weevils occurred between 2.4 m and 3.8 m (Table 7).

The best discriminant model developed for Springville Pond included distance from water and duff depth (Table 8). The model correctly discriminated

Table 7. Significant non-parametric t-test results for Springville Pond. Values represent the mean ±1 standard error with 95% confidence interval in parentheses. Alpha was set at $P \leq 0.05$.

Variable	**Weevils Present (n=17)** Mean ±SE	**Weevils Absent (n=28)** Mean ±SE	**p**
Distance from water (m)	3.1 ±0.3 (2.4-3.8)	4.5 ±0.5 (3.6-5.5)	0.031
Duff depth (cm)	3.5 ±0.3 (3.0-4.1)	2.6 ±0.2 (2.1-3.0)	0.015

Table 8. The “best” canonical discriminant function developed for Springville Pond included the following variables: Distance from water and duff depth. Alpha was set at P Value ≤ 0.05.

Variable	Structure Coefficient
Distance from water	0.74673
Duff depth	-0.88411
Wilke’s Lambda Probability	0.0153

Table 9. Prediction of weevil sites vs. non-weevil sites the “best” canonical discriminant function at Springville Pond. This function misclassified six weevil sites as non-weevil sites.

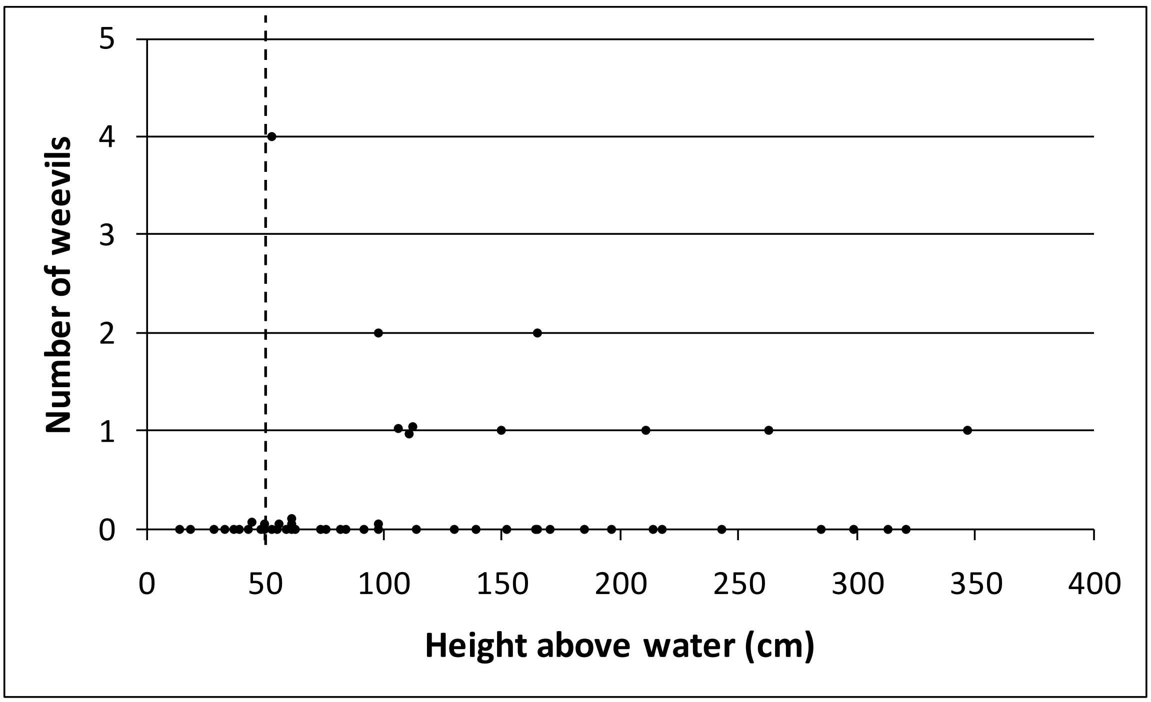
Group	No. of sites	Predicted group membership		Sites correctly predicted
		Weevils	No weevils	(%)
Weevils	17	11	6	64.7
No weevils	28	9	19	67.9
Overall percentage of sites correctly classified:				66.3

between weevil sites and non-weevil sites just 66% of the time, and misclassified six weevil sites as non-weevil sites (Table 9). Although the model was significant ($p=0.015$), it did not do as well at discriminating between sites as the Thomas Lake function did.

McDill Pond - Milfoil weevils were found at 10 sites of the 52 sites sampled. The number of weevils found per site ranged from 0 to 4, with an average of 0.3 $N/0.2\ m^2$. Sites were located at 1 to 10 meters from the waterline, and from 14 to 347 cm above the waterline vertically. Shoreline habitat types included sites having no disturbance to low disturbance, and were most commonly characterized as upland forest dominated by either deciduous or coniferous trees. Soil types ranged from sand to sandy loam. Duff depth ranged from 0 to 10 cm of duff material, and composition of duff material was most commonly dominated by leaves and/or grasses. Percent moisture in soil/duff composite samples ranged widely from 18 to 210%, with a mean of 123%. Percent organic matter also ranged widely, from four percent to 90%, with a mean of 21%.

Of eleven sample sites having heights less than 50 cm above water level, all were negative for milfoil weevils (Figure 1). Also worth noting, fifteen weevils were found in duff depths ranging from 1 cm - 10 cm, but no weevils were ever found at sites where there was no measurable duff. Mean duff depth for weevil sites was 4.0 cm, while non-weevil sites averaged 2.9 cm, yet t-tests did not find this difference to be significant ($p=0.32$), possibly due in part to low sample size.

Figure 1. Relationship between the number of weevils found at a survey point and the height of the survey point above water at McDill Pond. The minimum height above water where weevils were found was 53 cm. Eleven survey points at heights less than 50 cm above water (represented by the dashed line) were sampled and were all negative for milfoil weevils.



DISCUSSION

In this study, four variables were found to be significantly related with weevil presence or abundance: height above water, distance from water, percent cover of leaves, and duff depth. Weevils were not significantly related to many of the other riparian habitat characteristics, 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, and bare soil.

Both logitic regression and discrimnant analysis were able to discrminate 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 greater than 15% soil moisture, suggesting weevils prefer dry sites. The McDill Pond data suggest an apparent minimum height threshold around 50 cm above water, but although height above water was not found to be statistically significant on McDill Pond. Low sample size (n=10 for weevil sites, n=42 for non-weevil sites) may account for the difficulty in discriminating weevil versus non-weevil site characteristics on McDill Pond. Sample timing may also have been a confounding factor: weevils return to the lake between ice-out and mid-May (Newman et al. 2001) and their spring migration may have been underway. In contrast, Springville Pond and Thomas Lake were both sampled in November, after fall migration was likely complete.

Therefore, sample timing was not a confounding factor for the data collected from these two lakes.

Distance from shore was consistently significant and negatively correlated to weevils in both the Thomas Lake and Springville Pond data, suggesting weevils occur more often at sites closer to shore. Weevils most commonly occurred within a few meters from shore, 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 shore as 8.3 m. Newman et al. (2001) found weevils as far as 20 m from shore. This suggests that while weevils most prefer habitat close to the water (and their source of food), 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 is dominated by low, wet areas. If the near shore areas are low, wet habitats, then those areas would not qualify as "good" weevil habitat, due to the aforementioned correlation to soil moisture (Newman et al. 2001).

While sample size and abundance were low in the study, on Thomas Lake, weevil quantity was negatively correlated with distance from water (weevils decreased with distance) and positively correlated with percent cover of leaves (weevils increased with leaves). However, percent cover of leaves and distance from water were negatively colinear (leaves decreased with distance), suggesting the possibility of coincidental correlation to weevils only because leaves and weevils both decreased with distance. Percent cover of leaves was also found to be a significant variable in logistic regression, but was eliminated from both the multiple logistic regression and the discriminant analyses. Because of that colinearity and elimination from multivariate statistics, 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 shorelines 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. While duff depth was significantly correlated with weevils in univariate analysis on Springville Pond, the variable was also negatively colinear with distance from water, leaving the possibility of coincidental correlation to weevils only because duff depth and weevils both decreased with distance. However, duff depth remained a contributing variable in the discriminant function developed (correlation coefficient = -0.88), suggesting that duff depth may truly be a driver in weevil presence and absence and not merely coincidental. It would require additional research to conclusively define the relationship between weevils and duff depth, however, it seems an important variable for lake managers to consider further. Past research (Jester et al. 2000) had found a positive correlation between weevils and “natural” sites, suggesting that “natural” sites offer something that “disturbed” sites do not. While 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 (cover type 10 or 11), with beach, lawn, or landscaping. Only one of the nine sites (or 11%) contained weevils, which happened to be an unraked lawn with some leaf litter. In contrast, 16 (or 44%) of the natural to low disturbance sites 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 possible that higher duff depth is one of the advantages that natural shorelines provide.

While 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 all three lakes, weevils were never found at sites with zero cm of 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 personal contribution in this regard. Raking and mowing of shorelines removes duff, while natural, unraked and unmowed shorelines provide duff material for overwintering.

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 occurences 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 shoreline, nor vice versa. It appears that in-lake weevil occurrence is not a predictor of where they will occur on-shore, therefore shoreline management activities should have a broad focus.

Jester et al. (2000) documented a positive correlation between in-lake weevil densities and “natural” shorelines. The results provided here go further in describing weevil overwintering habitat as sites closer to shore, higher above water, with more duff. While it remains unclear whether it would be important to plant specific types of vegetation (herbacious versus non-herbacious), it is unequivical that minimizing disturbance of vegetation is essential, especially in high, dry, near-shore zones.

Weevils still hold the potential to provide biological control for Eurasian watermilfoil. Further studies should look at: 1) defining more precisely what weevil densites are required for control, 2) what water depth becomes limiting for weevils, 3) bed-level conditions that might be limiting to weevils, 4) the presence of a second biological agent, such as *Mycoleptodiscus terrestris*, as an effective integrated control, 5) how to ameliorate predation pressure where sunfish trapnet rates exceed 25-30 sunfish per 24 hour trapnet, and 6) the relatioinship between sediment nutrients, plant nutrients, and the ability of weevils to control milfoil.

ACKNOWLEDGEMENTS

This project would not have been possible without the cooperation of the landowners on each of the study lakes. Research funding was provided by a grant from the Wisconsin Department of Natural Resources Aquatic Invasive Species Grant Program. Field and laboratory assistants included: Charles Boettcher, James Brodzeller, Benjamin Balika, and Nathan Thomas. Assistance with soils analysis came from Jess Sherman, University of Wisconsin-Stevens Point.

LITERATURE CITED

Blakey, H.L. 1966. Outlook for weed control activities. J. Aquat. Plant Manage. 5:18-20.

Buckingham, G.R. 1998. Surveys for insects that feed on Eurasian watermilfoil, *Myriophyllum spicatum*, and hydrilla, *Hydrilla verticillata*, in the People's Republic of China, Japan, and Korea. U.S. Army Corps of Engineers Waterways Experiment Station, Technical Report A-98-5, Vicksburg, MS. 36 pp.

Creed, R.P., Jr. and S.P. Sheldon. 1995. Weevils and watermilfoil: did a North American herbivore cause the decline of an exotic plant? Ecol. Applic. 5:1113-1121.

Crowell, W., N. Troelstrup, Jr., L. Queen, and J. Perry. 1994. Effects of harvesting on plant communities dominated by Eurasian watermilfoil in Lake Minnetonka, MN. J. Aquat. Plant Manage. 32:56-60.

Cuda, J.P, R. Charudattan, M.J. Grodoqitz, R. M. Newman, J.F. Shearer, M.L. Tamayo, and B. Villegas. 2008. Recent advances in biological control of submersed aquatic weeds. J. Aquat. Plant Manage. 46:15-32.

Getsinger, K.D., E.G. Turner, J.D. Madsen, and M.D. Netherland. 1997. Restoring native vegetation in a Eurasian watermilfoil-dominated plant community using the herbicide triclopyr. Regulat. Rivers Res. Manage. 14(4):357-375.

Jester, L.l., M.A. Bozek, D.R. Helsel, and S.P. Sheldon. 2000. *Euhrychiopsis lecontei* distribution, abundance and experimental augmentation for

Eurasian watermilfoil control in Wisconsin lakes. J. Aquat. Plant Manage. 38: 88-97.

Madsen, J.D., H.A. Crossen, K.S. Hamel, M.A Hilovsky, and C.H. Welling. 2000. Panel discussion: Management of Eurasian watermilfoil in the United States using native insects: State regulatory and management issues. J. Aquat. Plant Manage. 38:121-124.

Michel , A., R.S. Arias, B.E. Scheffler, S.O. Duke, M.D. Netherland, and F.E. Danyan. 2004. Somatic mutation-mediated evolution of herbicide resistece in the nonindigenous invasive plant hydrilla (Hydrilla verticillata). Mol. Ecol. 13:3229-3237.

Mullin, B. H., L. W. J. Anderson, J. M. DiTomaso, R. E. Eplee, and K. D. Getsinger. 2000. Invasive plant species. Council for Agricultural Science and Technology (CAST), Issue Paper No. 13. 18 pp.

Netherland, M.D., M.V. Hoyer, M.S. Allen, and D. Canfield.2005. A summary of future management recommendations from the hydrilla summit in Florida. Aquatics 27:4-9.

Newman, R.M., E.M. Gross, W. Wimmer, and P. Sprick. 2006. Life history and developmental performance of the Eurasian milfoil weevil, *Eubrychius velutus* (Coleoptera: Curculionidae). The Coleopterists Bulletin 60(2):170-176.

Newman, R.M. 2004. Biological control of Eurasian watermilfoil by aquatic insects: basic insights from an applied problem. Archiv fur Hydrobiologie 159(2):145-184.

Newman, R.M., D.W. Ragsdale, A. Milles, and C. Oien. 2001. Overwinter habitat and the relationship of overwinter to in-lake densities of the milfoil weevil *Euhrychiopsis lecontei*, Eurasian watermilfoil biological control agent. J. Aquat. Plant Manage. 39:63-67.

Newman, R.M., K.L. Holmberg, D.D. Biesboer, and B.G. Penner. 1996. Effects of the potential biological control agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. Aquat. Bot. 53:131-150.

Pande, Y.D. and P. Berthet. 1973. Comparison of the Tullgren funnel and soil section methods for surveying Oribatid populations. Oikos 24:273-277.

Parsons, J.K., K.S. Hamel, J.D. Madsen, and K.D. Getsinger; 2001. The use of 2,4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J. Aquat. Plant Manage. 39:117-125.

Relyea, R.A. 2008. A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. Oecologia 159:363-376.

Sheldon, S.P. and R.P. Creed. 1995. Use of a native insect as a biological control for an introduced weed. Ecol. Applic. 5(4):1122-1132.

Woodford, J.E. and M.W. Meyer. 2002. Impact of lakeshore development on green frog abundance. Biol. Conserv. 110:277-284.

IV. CHAPTER II

Mass rearing methods for the milfoil weevil, *Euhrychiopsis lecontei*

ABSTRACT

Research suggests the milfoil weevil, *Euhrychiopsis lecontei*, has the potential to biologically control Eurasian watermilfoil (*Myriophyllum spicatum*). However, the expense of purchasing sufficient quantities of weevils may be cost prohibitive to many lake groups. This project sought to examine how to make biological control a practical option for lake groups by developing a method for rearing large numbers of weevils. The 370-L 'Freeland poly-tuf' stock tank was determined to be the best chamber style for outdoor weevil rearing. An average return rate of 9.6 weevils produced per weevil stocked was produced from four stock tanks stocked initially with 0.19 weevils/L and 2.1 milfoil stems per weevil. Weevils were fed again at 21 days and 42 days, for a total feeding ratio of 6.6 milfoil stems per weevil initially stocked. An average of 672 weevils per tank was produced in 55 days.

INTRODUCTION

Eurasian watermilfoil (EWM), *Myriophyllum spicatum*, is a non-native, aggressively invasive, aquatic plant from the Eurasian continent. Historically, control options have relied primarily on chemical treatments or mechanical harvesting, but this has not provided a long-term solution since they require repeated application (Crowell et al. 1994, Getsinger et al. 1997, Parsons et al.

2001). Declines in Eurasian watermilfoil, an invasive, non-native aquatic macrophyte, have been associated with several herbivorous invertebrates, but primarily the native milfoil weevil, *Euhrychiopsis lecontei* (Dietz), that feeds exclusively on milfoil species (Newman 2004). Evaluation of studies on several other native or naturalized insects have determined them to be either too generalist in their feeding, incapable of providing control, or too difficult to rear to the high population densities needed for control (Sheldon and Creed 1995, Newman 2004). In contrast, evaluation of studies on *E. lecontei* have found it to be suitable on all three aspects (Sheldon and Creed 1995, Newman 2004).

In laboratory cultures, females on average laid 1.9 eggs per day, and may lay multiple eggs on one meristem (Sheldon and O'Bryan 1996, Sheldon and Jones 2001). Larvae eat the meristem, then bore into the stem to feed, mature and pupate (Newman et al. 1996). Larvae mine (i.e. eat) an average total of 15 cm of stem tissue (Mazzei et al. 1999). This mining releases cellular gases and reduces stem buoyancy, causing the plant to sink below the water surface, to the detriment of the plant's health (Creed et al. 1992). The boring and mining also creates openings for secondary infections by pathogens and deposit frass in the stem, which may promote those infections (Creed 2000). Weevils normally pupate within the stem approximately 50 to 75 cm from the meristem, later emerging as adults (Mazzei et al. 1999). At the optimal developmental temperature of 29°C, the full life cycle, egg to adult, takes 17 days (Mazzei et al. 1999). At typical summer lake temperatures of 25°C, the full life cycle can be completed within 21 days (Mazzei et al. 1999). Theoretically, the cycle can be shortened in an artificial rearing situation where temperatures are maintained closer to optimum.

Mass rearing of weevils in predator-free enclosures may result in higher end-of-season populations than naturally-occurring populations or traditional, early-season stocking methods. Whether natural population densities reach levels capable of whole-lake control appears to vary from lake to lake, and estimates of densities required to affect control varies, from as little as 0.25 per stem to $\geq$1.0 per stem (Newman 2004). To increase densities to levels that may control Eurasian watermilfoil, whole-lake supplemental stocking has been attempted in numerous lakes throughout the U.S., with mixed results (Reeves et al. 2008). Present use of milfoil weevils to control Eurasian watermilfoil involves supplemental stocking of large numbers of weevils reared off-site to feed on and subsequently kill Eurasian watermilfoil. The process can be expensive, requiring the purchase of thousands, or tens of thousands [3000 per acre (Madsen et al. 2000)], of weevils at a cost exceeding one dollar per weevil. These stocked weevils are normally introduced to the lake early in the season, leaving the weevils to feed and multiply under natural lake conditions, including predation pressure. Weevil population models have suggested that end of season populations are most critically linked to the survival of adult stage weevils; increasing a female adult's lifespan from five to 10 days can result in an 8-fold increase in end-of-summer population densities (Ward 2002, Newman et al. 2002). Therefore, mass rearing in predator-free enclosures, with reared weevils released to the lake later in the season, could maximize the number of weevils produced by the season.

The objective of this study was to determine the practicality of mass rearing of weevils by lake groups. Mass rearing in artificial chambers is a way to ameliorate two factors that limit weevil reproduction rates: temperature and predators. Mass rearing studies were conducted in 2008 and then repeated in 2009 with revised methodology, based on 2008 results. Several factors were evaluated, including different chamber types, milfoil (food) stem lengths, weevil and milfoil starting densities, and placement of chambers on land versus suspended within a lake.

MATERIALS AND METHODS

Milfoil weevil rearing methods were modeled after Hanson, et al. (1995), with modifications to that methodology based on preliminary testing. Hanson, et al. (1995) reported that an outdoor stock tank performed just as well as their indoor, controlled 20-gal aquariums, with less management time invested. Our preliminary studies found similar results, and investigated a simplified method for outdoor, mass rearing. This study expands on those methods (Table 1). Three outdoor rearing methods were tested:

1) 553 L 'GenFoam' round wading pools (152 cm diam x 38 cm H)
2) 370 L 'Freeland poly-tuf' stock tanks (79cm W x 132cm L x 63cm H)
3) Floating in-lake chambers (the same 370 L stock tanks, suspended in-lake).

Table 1. Study design and feeding schedule for improved rearing of *E. lecontei.* An increased feeding schedule was added in 2009, in stock tank and wading pool rearing chambers. Only *M. spicatum* stems were used as food stems. Stock tanks were divided into three groups by their initial weevil stocking density: 0.09 weevils/L (referred to below as "1X"), 0.19 weevils/L (referred to below as "2X"), and 0.26 weevils/L (referred to below as "3X"). All wading pools were stocked at the same initial weevil stocking rate (0.09 weevils/L). Total hold time for all trials was 55 days.

		2008		2009	
		Milfoil stems fed	Feeding ratio (Total stems per weevil initially stocked)	Milfoil stems fed 2009	Feeding ratio (Total stems per weevil initially stocked)
1X Stock tanks (on land)	Day 0	75		75	
	Day 21	70		120	
	Day 42	70	6.7	150	11.4
2X Stock tanks	Day 0	75		150	
	Day 21	135		150	
	Day 42	135	5.4	165	6.6
3X Stock tanks	Day 0	75		225	
	Day 21	210		225	
	Day 42	210	5.2	255	7.4

Table 1. Continued

		2008		2009	
		Milfoil stems fed	Feeding ratio (Total stems per weevil initially stocked)	Milfoil stems fed 2009	Feeding ratio (Total stems per weevil initially stocked)
1X Stock tanks (in-lake)	Day 0	75		75	
	Day 21	70		120	
	Day 42	70	6.7	150	11.4
Wading pools	Day 0	120		120	
	Day 21	90		180	
	Day 42	90	8.0	240	12.2

Tanks and pools placed on-land were stationed in a fenced, outdoor secured area in Schmeekle Reserve, in Stevens Point, WI, where full sun and access to municipal water supply was available. A 25 cm x 125 cm (Culligan) carbon filter was used to decholorinate the public water supply. Full sun was also important to keep the milfoil stems (food stems) healthy, but water temperatures were checked regularly with simple aquarium thermometers to ensure chambers were not approaching lethal temperatures [34° C (Sheldon 1997)]. Water temperatures were also recorded hourly using continuous recording thermometers to later compare temperature mean and variability of the various chamber types. Fresh water was added as needed to monitor water levels or cool the tanks if temperatures exceeded 29°C. Fiberglass screen (0.033 cm mesh) was used to cover the tanks and pools. While the primary use of the screening was to exclude predator/competitor insects and birds, it also functioned as light shade to reduce peak temperatures in the tanks during sunlight hours.

Floating in-lake chambers were anchored in Joanis Lake (Portage County, WI), which was also the lake planned to be the recipient of the weevils reared by the project. Four 370-L poly stock tanks, the same models as used in the on-land experiments, were used as in-lake chambers in 2009. The drain plugs were removed and covered with Nitex mesh (500 micron) to allow tank water to circulate with the lake water, without allowing predatory insects and small fish to enter the tank. The top of the tank was covered with fiberglass screen (0.033 cm mesh) and secured with bungee cords to exclude predator and competitor insects and birds. The fiberglass screen and Nitex mesh also ensured weevils remained within the rearing tanks. Cylindrical foam tubes (147 cm x 7 cm) were attached to float the tanks at a level that would keep them full of water, but not submerged. Stocking within an exclosure minimized the effects of avian and invertebrate predation, thereby providing a higher rate of weevil reproduction than stocking without an exclosure. Floating the chambers in the lake took advantage of a convenient and steady water supply. These in-lake chambers were stocked with weevils and milfoil at the same rate as the 1X stock tanks on land.

Eurasian watermilfoil stems to be used for food by weevils were collected from Joanis Lake, the same lake that would be the recipient of the weevils reared. To confirm we were using *Myriophyllum spicatum*, and not a hybrid, stem samples were verified by DNA analysis by Ryan Thum, Annis Water Resources Institute, Grand Valley State University. Stems were collected from the deepest milfoil beds, where naturally occurring weevils were not likely to be present, in order to avoid the inadvertent introduction of unaccounted for weevils.

To minimize the introduction of predator or competitor insects, the collected milfoil food stems were laid in a single layer over mesh screen and sprayed with a hose and nozzle at a pressure sufficient to clean the milfoil but not damage it. To ensure that food stems were free of unaccounted weevils or predator/competitor insects, stems were randomly inspected under magnification. Cleaned stems were then floated in a wading pool of clean water, sorted and untangled. Because weevils lay their eggs on apical meristems, only stems with apical meristems were retained for use; stems that had gone to flower, were the wrong species, or had broken tips were discarded. Stems were trimmed to a length sufficient to reach from the base of the rearing chamber to the surface of the chamber's water: 62 cm for stock tanks; 30 cm for wading pools. (In 2009 we tested milfoil stems of both the 62 cm and the 30 cm lengths in the wading pools.) Stems were then bundled together in groups of ten or fifteen stems, and attached at the base to a rock with a rubber-band to weight the stems down and achieve vertical orientation in the rearing chamber. All chambers received an initial stocking of milfoil food bundles, and the stocking was repeated every 21 days to keep the weevils supplied with actively growing milfoil. In 2009, the feedings at Day 21 and Day 42 were increased in an attempt to increase production over that of 2008 (Table 1).

The starter stock of weevils was purchased from EnviroScience, Inc., 3781 Darrow Road, Stow, Ohio, who cultured their weevil stock from Wisconsin-grown weevils. Our preliminary indoor studies conducted with 38 L glass aquariums found that approximately 0.19 weevils per liter of water produced optimal return rates over a 21 day hold time (approximately one generation). Our project would span 55 days (over two generations). Therefore, we tested three stocking rates to bracket this predicted optimum: 0.09 weevils/L, 0.19 weevils/L, and 0.26 weevils/L (hereafter referred to as "1X", "2X", and "3X" stocking rates, respectively).

The purchased weevils arrived as eggs and early instar larvae attached to inoculated milfoil stems in sealed plastic bags. The estimated total number of weevils (n = 2505) was provided by EnviroScience, Inc. and verified upon receipt by laboratory enumeration examinations, as follows: A random sampling of 86 inoculated stems were examined under magnification to count the number of eggs and larvae on each stem, generating an estimated average of 4.56 weevils/stem; with a total shipment of 506 stems, our estimated total number of weevils received was 2306 (95% confidence interval, 1972 – 2640), or within 8% of the EnviroScience estimate of 2505 weevils. Inoculated stems were then selected randomly to accumulate the number of weevils needed to stock each rearing chamber. (Thus, the number of weevils initially stocked to each rearing chamber is an estimate, based on the estimated average number of weevils per stem.) Next, inoculated stems were affixed with twist-ties to the bundled food stems awaiting them in the rearing chambers, allowing the tiny larvae to easily crawl onto new, healthy milfoil stems.

Chambers were maintained for approximately 55 days, theoretically allowing enough time for the production of two generations at ambient temperature. Prior to releasing the weevils to their recipient lake, subsamples were extracted to estimate total production. A 10% subsample of the weevil-containing food stems were randomly selected, preserved in 80% isopropyl alcohol, and refrigerated for later laboratory enumeration.

The preserved subsample stems were floated in a glass pan and examined over a light table with 3x OptiVisor® glass binocular magnifiers. Each stem was carefully examined for weevil larvae, adults, and eggs and the total number of weevils recorded. The assistance of a 30x Carson Magniscope ™

was used for identification of specimens when needed. Specimen vouchers were preserved in sample vials in 80% isopropyl alcohol.

RETURN RATE AND TOTAL PRODUCTION CALCULATIONS

The output (total number of weevils produced) of each rearing chamber was extrapolated from the number of weevils counted in the 10% subsamples and the total number of stems in each tank. To calculate the return rate for each chamber, the total output was divided by input (weevil produced/weevil stocked). To estimate the average return rate of each chamber type, we summed the output of each chamber (of that type) and divided by the number of chambers (of that type).

RESULTS

Productivity was measured in two ways: output (total weevils produced per tank), and return rate (total output divided by total input). Stock tank output was highest (780 weevils) from the 3X stock tanks in 2009, where the feeding ratio was 7.4 stems per weevil initially stocked (Table 2). At that feeding ratio, mean weevil output in the 2X stock tanks increased by 20% from 550 to 658 weevils, and mean weevil output in the 3X stock tanks increased by 42% from 548 to 780 weevils. In contrast, when the feeding ratio was increased in the 1X stock tanks from 6.7 to 11.4 stems per weevil initially stocked, mean weevil output decreased by 33%, from 368 weevils to 247 weevils.

Table 2. Weevil production results for stock tanks, 2008 and 2009. Input is the initial number of weevils stocked. Feeding ratio is the total number of milfoil stems fed per weevil initially stocked. Output is the total number of weevils produced. Return rate is the ratio of output:input. Estimated output and return rates based on examination of 10% subsamples extracted. Feeding rates (of milfoil stems) were increased in 2009 to increase output. In-lake tanks (1X) were also used to evaluate using lake water as an alternative water supply. All stock tanks were 370 L.

	INPUT		OUTPUT 2008	RETURN RATE 2008			OUTPUT 2009	RETURN RATE 2009	
	Weevils	Feeding ratio[1] (2008)	Weevils per tank ($\bar{x}$)	Return rate per tank ($\bar{x}$)	Return rate per tank (Range)	Feeding ratio[1] (2009)	Weevils per tank ($\bar{x}$)	Return rate per tank ($\bar{x}$)	Return rate per tank (Range)
1x stock tanks	33								
2008 (5 tanks)	(0.09/L)	6.7	367	11.3	5.4 – 15.6	--	--	--	--
2009 (6 tanks)		--	--	--	--	11.4	247	8.2	0.0 – 12.5
2X stock tanks (4 tanks)	70 (0.19/L)	5.4	550	8.6	5.2 – 12.7	6.6	658	9.6	2.7 – 18.3
3X stock tanks (4 tanks)	95 (0.26/L)	5.2	548	5.7	3.1 – 8.0	7.4	780	8.2	4.2 – 15.1
1X in-lake stock tanks (4 tanks)	34 (0.09/L)	6.7	--	--	--	11.4	303	8.9	6.2 – 12.4

[1] Total milfoil stems per weevil initially stocked.

The highest weevil return rates for all stock tanks (1X, 2X, and 3X) were seen at a feeding ratio approximately seven milfoil stems per weevil initially stocked. At this optimal feeding rate, the weevil return rate from the 1X and 2X tanks was 8.2 and 9.6, respectively; 3X tanks increased from 5.7 to 8.2 (Fig. 1). Increasing the feeding ratio in the 1X stock tanks from approximately seven stems per weevil initially stocked to over 11 stems resulted in a decreased weevil return rate, dropping from 11.4 to 8.2.

In-lake tanks (stocked at the 1X rate) performed similarly to the 1X tanks on land. Examining the temperature data from the continuous-reading thermometer deployed in an in-lake chamber found that the chambers exhibited a more stable temperature regime, and stayed closer to optimal temperatures (84F) for weevil reproduction, than the other chambers. This suggests that in-lake chambers may have a more favorable temperature regime than chambers on land, which could result in increased production, although this was not seen in this study.

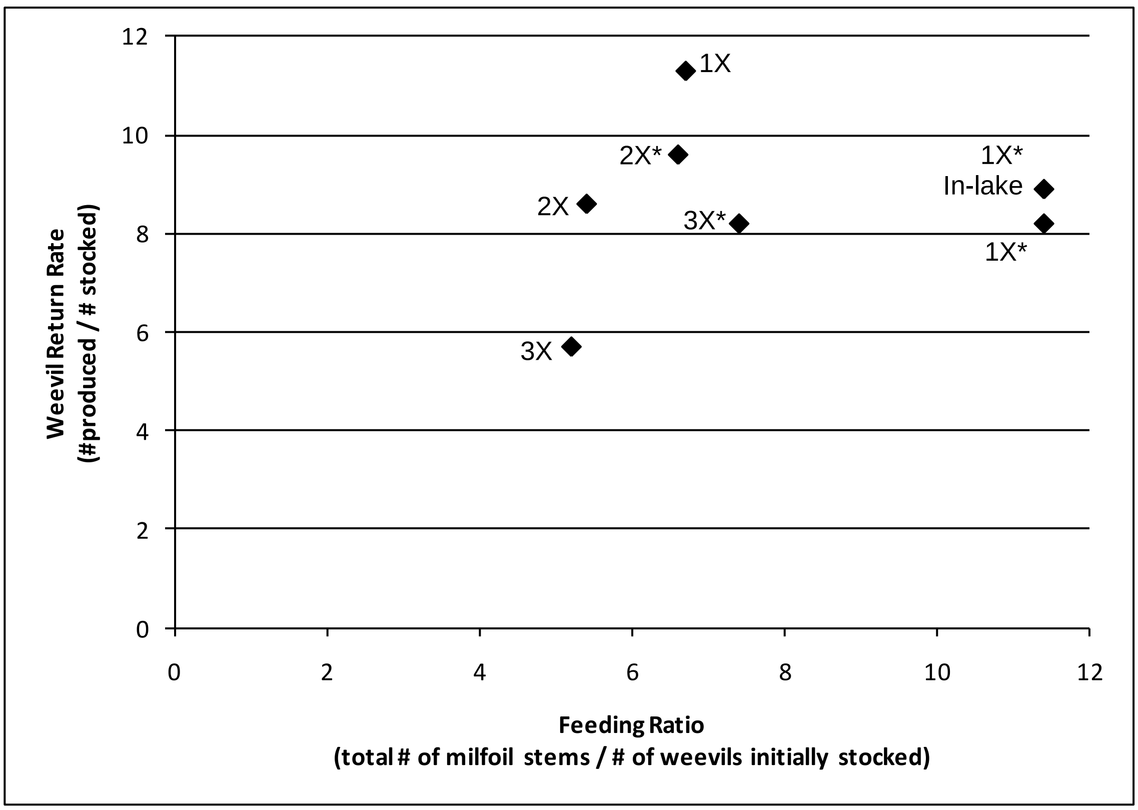
Total output in wading pools ranged from 18 to 126 weevils at the end of each trial. Wading pool return rate with short milfoil stem lengths (30 cm) ranged from 0.0 to 5.6 in 2008, and averaged half that of the stock tanks (Table 3). There was no improvement in 2009 at increased feeding rates. Wading pools with long stem lengths (62 cm) and increased feeding rates in 2009 also had low return rates (0.8 average).

Table 3. Weevil production results for wading pools, 2008 and 2009. Input is the number of weevils initially stocked. Feeding ratio is the total number of milfoil stems fed per weevil initially stocked. Output is the total number of weevils produced per wading pool. Return rate is the ratio of output:input. Estimated output and return rates based on examination of 10% subsamples extracted from a subset of tanks. All pools were 553 L. In 2009, feeding rates were increased over 2008, and milfoil stem lengths varied in an attempt to increase production.

		INPUT		OUTPUT	RETURN RATE	
	Milfoil stem length	Weevils	Feeding ratio[1]	Weevils (Ave)	Return rate (Ave)	Return rate (Range)
Wading pools w/ short stems 2008 (4 pools)	30 cm	41 (0.07/L)	7.3	126	3.1	0 – 5.6
Wading pools w/ short stems 2009 (4 pools)	30 cm	44 (0.08/L)	12.3	18	0.4	0 – 1.4
Wading pools w/ long stems 2009 (4 pools)	62 cm	44 (0.08/L)	12.3	35	0.8	0 – 3.0

[1] Total milfoil stems per weevil initially stocked.

Figure 1. Comparison of mean weevil return rates vs. feeding ratio (total number of milfoil stems per number of weevils initially stocked) in stock tanks, for 2008 and 2009. Feeding rates were increased in 2009 (*) to increase production, resulting in a higher feeding ratio (total number of milfoil stems per weevil initially stocked). (Note: The in-lake stock tank is included, since it is the same chamber type. The 1X, 2X, and 3X tanks were all on land.)



COST/BENEFIT ANALYSIS

There are benefits and drawbacks to each rearing chamber approach (Table 4). Wading pools were the most inexpensive chamber style ($10 ea), but they performed the poorest. The stock tanks, which consistently produced well, had high initial costs ($75 ea), but were durable and could be reused for many seasons. Cost estimates are based on 2009 production numbers, including the cost of new equipment and all paid labor (Table 5). Because of the poor performance of some of the experimental chamber types, our total return rate for the project (averaged across all chamber styles) was approximately 6.4 weevils produced per weevil stocked. In spite of that, our cost per weevil was $0.81, which is below the purchase cost $1.30/weevil of our original 1,215 weevils. If these cost estimates are re-figured for a hypothetical lake association scenario using only the optimal chamber types and stocking rates and where all equipment is purchased new and labor is volunteer-based, the cost per weevil drops to an estimated $0.31/weevil (Table 6). In subsequent years when equipment is reused and labor is volunteer-based, the cost drops /o $0.14/weevil.

DISCUSSION

The best weevil return rate for all stock tanks (1X, 2X, and 3X) occurred at a feeding ratio of approximately 7 total milfoil stems per weevil initially stocked. Increasing the number of total food stems beyond this level did not increase weevil return rates, but rather decreased it. It is possible that the extra stems may have been of no benefit to the weevils and were perhaps increasing the

Table 4. Comparison of rearing chamber types.

Chamber type	Cost	Predicted return rate[1]	Predicted output[2]	Pupation Success	Temperature fluctuation	Other notes
Stock tanks (1X stocking rate)	Expensive ($75)	11.3	395	High	Moderate (did not reach fatal[3])	
Stock tanks (2X stocking rate)	Expensive ($75)	9.6	672	High	Moderate (did not reach fatal[3])	Requires twice as many food stems as 1X stocking rate.
Stock tanks (3X stocking rate)	Expensive ($75)	8.2	861	High	Moderate (did not reach fatal[3])	Requires three times as many food stems as 1X stocking rate.
Stock tanks (In-lake)	Expensive ($75)	(same as on land)	(same as on land)	High	Lowest (mean closest to optimum[4])	May save cost/labor of external water supply.
Wading Pool	Inexpensive ($10)	1.4	75	Very low	High (did not reach fatal[3])	Short or long food stems both performed poorly. Harder to secure to keep out predators.

[1] Number weevils produced per weevil stocked
[2] Total number of weevils per chamber
[3] Temperatures fatal to weevils = 34°C
[4] Temperatures optimal to weevils = 27°C

Table 5. Estimates of weevil rearing costs. (Project actuals)

Cost estimate for our project		
Total labor	358 hrs	$2610
Purchased starter weevil stock	1215 weevils	$1590*
Equipment	Tubs/pools/ screen/boards	$2073
	Our total cost	$6273
	Weevils produced	7739†
	Our cost per weevil	**$0.81***

* Includes the costs of shipping

† Includes low-output chambers, for an average return rate of 6.4 weevils produced per weevil stocked

Table 6. Projected weevil rearing costs for lake groups.

Cost estimate for lake group scenarios		
Paid labor, minimum wage	358 hrs	$2596
Purchased starter weevil stock	1215 weevils	$1590*
Equipment	Tubs/pools/ screen/boards	$2073
	Total cost	$6259
	Weevils produced	11664†
	Cost per weevil, year 1	**$0.54***
	Cost per weevil, year 2 (re-used equipment)	**$0.36***
	Cost per weevil, year 1 (volunteer labor)	**$0.31***
	Cost per weevil, year 2 (volunteer labor, re-used equipment)	**$0.14***

* Includes the costs of shipping

† Includes only 2X stock tanks, with projected return rate of 9.6 weevils produced per weevil stocked.

amount of decomposing organic material that might degrade water quality in the tank. Additional milfoil stems did not translate into higher return rate, therefore, it was not worth the added labor involved with cleaning and bundling more food stems.

The 2X stocking density (0.19 weevils/L) produced twice as many weevils as the 1X stocking density (0.08 weevils/L), with half the equipment as would be needed to produce the same number of weevils from standard tanks. In contrast, the 3X tanks failed to produce three times as many weevils as the 1X tanks, even at the optimal feeding ratio (approximately 7 stems per weevil intitially stocked), suggesting a point of diminishing returns. Perhaps at the 3X stocking density the chamber becomes overcrowded with weevils, or the number of food stems required to sustain the weevils begins to impact the water quality. In any event, the 3X weevil stocking density did not produce weevils at the rate expected.

In-lake chambers (1X stocking rate) perfomed similarly to the 1X stock tanks on land, with an average return rate of 8.9 weevils produced per weevil initially stocked. (Note that the in-lake stock tanks were also fed at the same high ratio as the 1X stock tanks on land.) No significant problems with predator or competitor organisms were observed using this method. However, the sturdy design of the chambers made them suitable loafing platforms for ducks. The droppings deposited by the ducks resulted in higher amounts of filamentous algae than was found in the other chambers.

Wading pools did not improve in production in 2009 with the increased feeding ratio, with either long stems (average return rate of 0.8) or short stems (average return rate of 0.4), but in fact showed worse production than in 2008. Mean weevil production decreased by 80% from 126 weevils in 2008 to 26 weevils in 2009. Stem inspections found few signs of larval or pupation damage to stems, suggesting poor success at pupation or reproduction. Hundreds non-weevil insects were also found in the chambers, which may have resulted in competition or predation.

Ensuring that weevil rearing chambers are predator-free may be critical to maximizing weevil production. Newman et al. (2002) modelled the effect of predation on weevil populations, finding that extending a female weevil's longevity by five days can lead to an eight-fold increase in the end-of-season population. Although this study was not designed to examine the impacts of invertebrate competition or predation on weevil production, some invertebrate data was collected to investigate potential problems with low-performing chambers. In 2009, an estimated 770 non-weevil insects were found in a single wading pool, while only 130 non-weevil insects were found in an on-land stock tank and 10 non-weevil insects were found in an in-lake stock tank. Since all chambers were stocked with milfoil from the same source and cleaned with the same methods, it seems that some feature about the wading pools allows for greater chance of colonization by aquatic insects. Because of their flexible construction, the pools are simply harder to seal, or the greater surface area may allow for greater potential for insect immigation whenever pools are temporarily left uncovered. Mayfly larvae (*Caenis sp.*), which are known to be scrapers of periphyton, were found in great numbers in the wading pool sample, but it is unknown how they may have impacted weevil reproduction (i.e. whether they prey on or compete with weevils). Some predatory nymphs, such as those in the famiily Odonata and Trichoptera, were also found in the wading pool sample, which could have been feeding on small, early instar weevils.

Milfoil stem length and positioning appears to be an important factor in weevil production. Stock tanks used milfoil stems 62 cm long, that reached from the bottom of the chamber to the water surface, and stem inspections found heavy damage from larval and pupation, indicating good reproduction and pupation success. In contrast, the short stems (30 cm) used in the low-profile wading pools had low weevil production, probably because weevils tend to pupate at around 50 cm from the stem tip. Increasing the stem length to 62 cm did not improve weevil production. Although the 62-cm stems were long enough to allow pupation, in such a low profile chamber (as the wading pools were) the stems were long enough to “trail” along the water surface, which may negatively impact weevil success (Mazzei et al. 1999). Trailing stems on the water surface accumulate heat on sunny days, causing surface water temperatures to exceed those conducive to weevil reproduction and development [>31°C (Sheldon 1997, Mazzei et al. 1999)]. Temperatures in the shaded water 50 cm below the surface mat remain within ranges optimal for weevils [27°C (Sheldon 1997, Mazzei et al. 1999)]. Weevils in natural lake settings will tend to migrate to progressively deeper (and therefore cooler) water as milfoil reaches the surface throughout the summer (Pers. observations, Lillie 2000). This migration is likely a survival strategy to avoid unfavorable thermal conditions in surface mats. Because chambers on land have relatively small water volumes compared to lake volumes, water in chambers may be susceptible to overheating, especially near the surface. Shallow chambers, such as wading pools, appear to be especially problematic in this regard.

Based on cost estimates, utilizing biological control of Eurasian watermilfoil may be much more affordable for lake associations in the future. Mass rearing in outdoor chambers holds the potential for turning 1,000 brood stock weevils into 8,000 – 10,000 stockable weevils, at one-tenth to one-half the cost of purchased stock. However, there are some drawbacks to consider: 1) Rearing weevils is labor intensive (approximately 34 weevils produced for every hour of labor); 2) dedicated volunteer labor is needed to keep costs down; 3) careful, methodical, and consistent technique is required to produce high weevil numbers; 4) mass rearing results in a two-month delay in release date compared to standard weevil stocking, which in turn leads to a delay in milfoil control. This nearly ten-fold greater number of weevil released may mean a faster increase in the weevil population density, but the results are delayed until the following season. Some lake associations may find this delay unacceptable, but other more patient lake associations may not.

An additional consideration before using this mass rearing method, is the long-term success of the weevil population. Regardless of the method used to rear and stock the weevils, they still need natural shorelines that are high and dry, with accumulated duff material, to successfully overwinter (Newman et al. 2001, Thorstenson 2011, Jester et al. 2000). Lake associations that invest the money and labor into this mass rearing method should be fully committed to providing the overwintering habitat needed for the weevils to come back in strong numbers each spring.

CONCLUSION

The best rearing method developed used the 370 L ‘Freeland poly-tuf’ stock tanks, with stems long enough to reach from the bottom of the chamber to the surface of the water (about 62 cm). Tanks may be stationed on land or floated in a lake. The most efficient initial stocking densities were 0.19 weevils/L (70 weevils per stock tank) and approximately 7 milfoil stems total per weevil initially stocked (465 stems total). At an estimated 9.6-fold return rate, this should produce approximately 672 weevils per stock tank. An example calculation:

4 tanks (2X stocking rate) + 280 weevils in + 1,860 EWM stems in ➔ 2,688 weevils out

While the cost savings of mass rearing may make biological control more attainable to more lake associations, the drawbacks should be carefully considered. The mass rearing method would be best suited to a lake association with patient members and that has more volunteers than money.

ACKNOWLEGEMENTS

Funding support comes from the Wisconsin Department of Natural Resources Aquatic Invasive Species Grant Program and the University of Wisconsin-Stevens Point College of Natural Resources. Field assistance came from Charles Boettcher, James Brodzeller, John Mumm, Adam Skadsen, and other students with the UW-Stevens Point Center for Watershed Science and Education and Schmeekle Reserve, Stevens Point, WI. Additional field assistance came from volunteers with Woodlands Church, Plover, WI, and Golden Sands Resource Conservation & Development Council, Inc., Stevens Point, WI. Assistance with logistical coordination came from Schmeekle Reserve and City of Stevens Point.

LITERATURE CITED

Creed, R.P., Jr. 2000. The weevil-watermilfoil interaction and different spatial scales: what we know and what we need to know. J. Aquat. Plant Manage. 38, 78-81.

Creed, R.P., Jr., S.P. Sheldon, and D.M. Cheek. 1992. The effect of herbivore feeding on the buoyancy of Eurasian watermilfoil. J. Aquat. Plant Manage. 30, 75-76.

Crowell, W., N. Troelstrup Jr., L. Queen, and J. Perry. 1994. Effects of harvesting on plant communities dominated by Eurasian watermilfoil in Lake Minnetonka, MN. J. Aquat. Plant Manage. 32, 56-60.

Getsinger, K.D., E.G. Turner, J.D. Madsen, and M.D. Netherland. 1997. Restoring native vegetation in a Eurasian watermilfoil-dominated plant community using the herbicide triclopyr. Regulat. Rivers Res. Manage. 14(4), 357-375.

Hanson, T., C. Eliopoulos, and A. Walker. 1995. Field Collection, Laboratory Rearing and In-lake Introductions of the Herbivorous Aquatic Weevil, Euhrychiopsis lecontei, in Vermont. Vermont Department of Environmental Conservation, Waterbury, VT. 41 pp.

Jester, L.l., M.A. Bozek, D.R. Helsel, and S.P. Sheldon. 2000. *Euhrychiopsis lecontei* distribution, abundance and experimental augmentation for Eurasian watermilfoil control in Wisconsin lakes. J. Aquat. Plant Manage. 38, 88-97.

Lillie, R.A. 2000. Temporal and spatial changes in milfoil distribution and biomass associated with weevils in Fish Lake, WI. J. Aquat. Plant Manage. 38, 98-104.

Madsen, J.D., H.A. Crossen, K.S. Hamel, M.A Hilovsky, and C.H. Welling. 2000. Panel discussion: Management of Eurasian watermilfoil in the United States using native insects: State regulatory and management issues. J. Aquat. Plant Manage. 38, 121-124.

Mazzei, K.C., Newman, R.M., Loos, A., Ragsdale D.W. 1999. Developmental rates of the native milfoil weevil, *Euhrychiopsis lecontei*, and damage to Eurasian watermilfoil at constant temperatures. Biological Control 16, 139-143.

Newman, R.M. 2004. Biological control of Eurasian watermilfoil by aquatic insects: basic insights from an applied problem. Archiv fur Hydrobiologie 159(2), 145-184.

Newman, R.M., D.W. Ragsdale, A. Milles, and C. Oien. 2001. Overwinter habitat and the relationship of overwinter to in-lake densities of the milfoil weevil *Euhrychiopsis lecontei*, Eurasian watermilfoil biological control agent. J. Aquat. Plant Manage. 39, 63-67.

Newman, R.M., D.W. Ragsdale, and D.D. Biesboer, 2002. Factors influencing the control of Eurasian watermilfoil with native or naturalized insects: final report for 1999-2001 to the Minnesota Department of Natural Resources, Ecological Services, St. Paul, MN, 71 pp.

Newman, R.M., K.L. Holmberg, D.D. Biesboer, and B.G. Penner. 1996. Effects of the potential biological control agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. Aquat. Bot. 53, 131-150.

Parsons, J.K., K.S. Hamel, J.D. Madsen, and K.D. Getsinger; 2001. The use of 2,4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J. Aquat. Plant Manage. 39, 117-125.

Reeves, Sheldon, S.P. and K.N. Jones. 2001. Restricted gene flow according to host plant in an herbivore feeding on native and exotic watermilfoils. Internat. J. of Plant Sci. 162, 793-799.

Reeves, J.I., P.D. Lorch, M.W. Kershner, and M.A. Hilovsky. 2008. Biological control of Eurasian watermilfoil by *Euhrychiopsis lecontei*: Assessing efficacy and timing of sampling. J. of Aquat. Plant Manage. 46, 144-149.

Sheldon, S.P. 1997. Investigations on the potential use of an aquatic weevil to control Eurasian watermilfoil. Lake reserve. Manage. 13, 79-88.

Sheldon, S.P. and L.M. O'Bryan. 1996. Life history of the weevil *Euhrychiopsis lecontei,* a potential biological control agent of Eurasian watermilfoil. Entomological News 107, 16-22.

Sheldon, S.P. and R.P. Creed. 1995. Use of a native insect as a biological control for an introduced weed. Ecol. Applic. 5(4), 1122-1132.

Thorstenson, A.L. 2011. Biological control of eurasian watermilfoil (*Myriophyllum spicatum*) using the native milfoil weevil (*Euhrychiopsis lecontei*). M.S. Thesis. University of Wisconsin-Stevens Point, Stevens Point, WI.

Ward, D.M., 2002. The impact of fish predation on Eurasian watermilfoil herbivores and indirect effects on the macrophyte community. – MS Thesis, University of Minnesota, St. Paul, MN.

APPENDIX 1

CHAPTER I

Supplementary Tables and Figures

Appendix 1.1 Thomas Lake: Shoreline habitat data at 53 sample points.

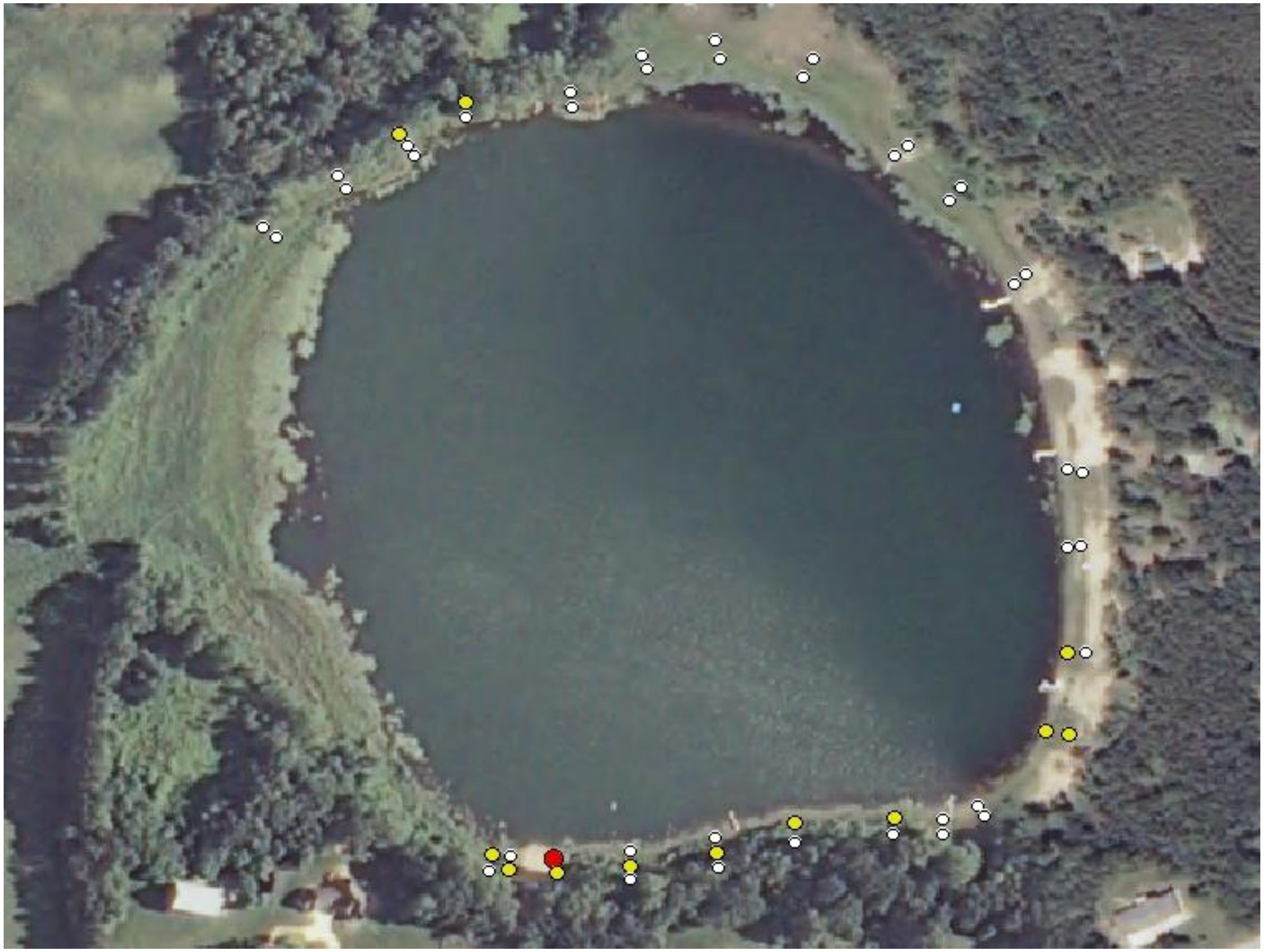
SiteID	Weevil Qty	Weevil Presence Yes = 1 No = 0	Ordinat'n (°)	EWM Rank	EWM Frags	Soil Texture	% Soil Moisture	% Soil Organic Matter	Dist from Water (m)	Ht Above Water (cm)	Hab Code	Duff Depth (cm)	% wood	% leaves	% grass	% forbs	% bare
L1	3	1	196	0	1	S	21.41	0.88	3.4	64	8	4	0	40	60	0	20
L2	1	1	196	0	1	SL	26.49	4.28	5.9	108	7	7	10	30	60	0	5
L3	0	0	196	0	1	S	23.03	1.63	4.2	54	8	4	0	5	95	0	0
L4	0	0	196	0	1	S	22.42	1.62	6.2	70	8	2	0	10	80	5	5
R2	0	0	301	2	1	SL	21.55	1.06	5.9	94	8	4	0	30	65	0	0
R3	1	1	301	2	1	SL	17.91	3.87	8.3	115	7	8	0	40	50	10	0
TSHT10A	1	1	127	0	1	S	39.54	3.30	4.4	58	8	2	0	60	40	0	0
TSHT10B	1	1	127	0	1	S	23.74	4.13	6.4	72	8	1	0	5	80	0	0
TSHT11A	0	0	137	2	1	S	28.81	1.76	5.2	55	8	2	0	50	20	0	0
TSHT11B	0	0	137	2	1	S	20.72	1.76	7.2	73	8	1	0	10	30	0	10
TSHT11LA	0	0	137	0	1	S	19.27	1.01	5.4	53	11	0.5	0	5	10	2	10
TSHT11LB	0	0	137	0	1	S	14.04	1.98	8.4	72	11	1	0	15	25	3	0
TSHT12A	1	1	149	2	0	S	29.69	2.25	3.9	51	7	3	0	60	30	10	0
TSHT12B	0	0	149	2	0	S	27.74	2.73	5.9	67	8	3	0	20	70	0	0
TSHT13A	1	1	161	0	1	S	13.50	1.10	3.9	62	8	1	0	15	15	5	0
TSHT13B	0	0	161	0	1	S	13.20	2.01	5.9	88	8	2	0	72	15	10	10
TSHT14A	0	0	174	2	1	S	12.76	1.42	4.0	53	8	1	5	30	50	0	0
TSHT14B	1	1	174	2	1	S	10.36	1.01	6.0	83	8	4	5	20	60	0	0
TSHT14C	0	0	174	2	1	S	17.84	2.55	13.0	196	11	2	0	10	85	0	0
TSHT15A	0	0	187	0	1	S	17.32	1.10	3.6	53	8	2	0	30	40	10	0
TSHT15B	1	1	187	0	1	S	11.67	1.13	5.6	93	8	1	10	20	75	0	0
TSHT15C	0	0	187	0	1	S	24.16	2.98	10.0	135	5	5	0	95	5	0	0
TSHT16A	1	1	202	2	1	S	32.80	1.69	5.9	69	8	1	0	25	65	5	5
TSHT16B	0	0	202	2	1	S	30.62	3.83	7.9	94	7	3	5	70	25	5	5
TSHT1A	0	0	11	0	1	S	26.51	1.98	14.0	73	8	3	0	5	95	0	0
TSHT1B	0	0	11	0	1	S	34.55	4.07	16.0	85	8	3	0	5	95	0	5
TSHT25A	0	0	299	1	1	SL	45.10	4.02	17.0	67	8	4	0	0	100	0	45
TSHT25B	0	0	299	1	1	S	25.67	4.32	19.0	99	8	5	0	20	80	0	0
TSHT26A	0	0	309	1	0	S	12.79	1.61	4.4	57	8	2	0	30	60	0	55

Appendix 1.1 Continued

SiteID	Weevil Quantity	Weevil Presence Yes = 1 No = 0	Ordination (°)	EWM Rank	EWM Frags	Soil Texture	% Soil Moisture	% Soil Organic Matter	Dist from Water (m)	Ht Above Water (cm)	Habitat Code	Duff Depth (cm)	% wood	% leaves	% grass	% forbs	% bare
TSHT26B	0	0	309	1	0	S	14.58	1.45	6.4	75	8	2	0	20	60	10	0
TSHT27A	0	0	320	1	1	S	18.22	1.03	2.8	55	8	7	5	30	70	0	40
TSHT27B	0	0	320	1	1	S	29.03	2.87	4.8	72	8	5	0	20	70	10	0
TSHT27C	1	1	320	1	1	S	11.48	2.24	6.8	112	8	5	0	60	30	0	10
TSHT28A	0	0	329	1	1	S	19.45	1.27	2.9	48	7	6	0	60	35	5	55
TSHT28B	1	1	329	1	1	S	12.44	2.55	4.9	83	7	2	5	70	10	5	0
TSHT29A	0	0	345	1	1	S	18.47	1.80	3.0	49	7	3	0	5	85	10	83
TSHT29B	0	0	345	1	1	S	16.91	1.52	5.0	73	8	4	0	5	75.2	20	13
TSHT2A	0	0	24	0.5	0	S	42.33	3.24	18.0	79	8	4	0	0	90	10	20
TSHT2B	0	0	24	0.5	0	S	31.46	7.79	20.0	104	8	3	0	5	95	0	5
TSHT30A	0	0	358	2	1	S	28.78	1.43	9.0	59	8	4	0	0	100	0	0
TSHT30B	0	0	358	2	1	S	19.74	4.18	11.0	73	8	2	0	5	95	0	5
TSHT3A	0	0	37	2	0	S	26.31	3.52	10.8	64	8	3	5	5	75	10	0
TSHT3B	0	0	37	2	0	S	20.47	2.96	12.8	78	8	4	0	5	5	85	0
TSHT4A	0	0	49	3	0	S	25.90	9.99	14.0	68	7	3	15	10	75	0	0
TSHT4B	0	0	49	3	0	S	8.83	1.03	16.0	91	8	4	5	20	75	10	0
TSHT5A	0	0	64	1	1	S	48.09	6.27	7.5	47	8	0	5	5	40	10	0
TSHT5B	0	0	64	1	1	S	12.00	1.17	12.5	77	8	4	0	10	90	0	10
TSHT7A	0	0	91	1	0	S	12.78	0.84	6.2	60	8	2	5	20	10	10	10
TSHT7B	0	0	91	1	0	S	9.59	1.47	11.2	110	8	6	0	5	95	0	0
TSHT8A	0	0	104	2	1	S	11.00	1.19	5.2	59	8	1	5	40	15	0	0
TSHT8B	0	0	104	2	1	S	5.77	1.31	10.2	105	8	3	0	5	95	0	0
TSHT9A	1	1	118	0.5	1	S	24.28	0.98	3.5	50	8	3	0	20	40	30	0
TSHT9B	0	0	118	0.5	1	S	12.15	0.84	5.5	64	8	2	0	5	30	10	10
Min	0	0	11	0	0		5.77	0.84	2.8	47	5	0	0	0	5	0	0
Max	3	1	358	3	1		48.09	9.99	20.0	196	11	8	15	95	100	85	0
Mean	0.2	0.2	179	1.1	0.8		21.52	2.45	8.2	76	8	3	1	23	57	6	0

S = Sand
LS = Loamy Sand

Appendix 1.2 Thomas Lake: On-shore distribution of weevils, Nov 2-7, 2009.   A total of 15 weevils were collected from 13 of the 53 survey sites.



Appendix 1.3 Thomas Lake: Significant Pearson correlations between **weevil quantity** and site characteristics. Alpha was set at $P \leq 0.05$. ($^{\alpha}$ns denotes a non-significant correlation.)

VARIABLE	NUMBER OF OBSERVATIONS	CORRELATION COEFFICIENT	P^{α}
Direction	53	-	ns
Milfoil abundance	53	-	ns
Soil texture	53	-	ns
% Soil moisture	53	-	ns
% Soil organic matter	53	-	ns
Distance from water	53	- 0.3349	0.0142
Height above water	53	-	ns
Duff depth	53	-	ns
% Wood	53	-	ns
% Leaves	53	0.2822	0.0407
% Grass	53	-	ns
% Forbs	53	-	ns
% Bare	53	-	ns

Appendix 1.4 Thomas Lake: T-test results for sites where weevils were present vs. absent. Bold variables are statistically significant variables. Alpha was set at P ≤ 0.05.

VARIABLE	WEEVIL PRESENCE	N	95% LCL	MEAN	95% UCL	STD DEV	STD ERR	NON-PARAMETRIC T-TEST CHI SQUARE	NON-PARAMETRIC T-TEST P
Milfoil abundance	No	40	0.88	1.162	1.44	0.873	0.138	1.6418	0.2001
	Yes	13	0.26	0.808	1.35	0.902	0.250		
Direction	No	40	137	174	210	114	17.954	1.0705	0.3008
	Yes	13	155	199	243	72.8	20.194		
Soil/duff % organic matter	No	40	1.90	2.515	3.13	1.934	0.306	0.0346	0.8524
	Yes	13	1.49	2.263	3.03	1.272	0.353		
Soil/duff % moisture	No	40	18.65	21.749	24.85	9.700	1.534	0.0427	0.8362
	Yes	13	15.56	21.178	26.79	9.294	2.578		
Distance from water (m)	No	40	7.39	8.950	10.51	4.876	0.771	5.5601	**0.0184**
	Yes	13	4.42	5.300	6.18	1.458	0.404		
Ht above water (cm)	No	40	67.42	76.200	84.98	27.467	4.343	0.2464	0.6196
	Yes	13	64.75	78.461	92.17	22.692	6.294		
Duff layer depth(cm)	No	40	2.53	3.038	3.54	1.571	0.248	0.0217	0.8830
	Yes	13	1.83	3.231	4.63	2.315	0.642		
Woody debris % cover	No	40	0.00	0.014	0.02	0.030	0.005	0.5735	0.4489
	Yes	13	-0.00	0.023	0.05	0.039	0.011		
Deciduous leaf % cover	No	40	0.13	0.198	0.27	0.221	0.035	7.1091	**0.0077**
	Yes	13	0.23	0.358	0.48	0.209	0.058		
Grass % cover	No	40	0.50	0.606	0.71	0.318	0.050	2.5802	0.1082
	Yes	13	0.34	0.473	0.61	0.219	0.061		
Forbs % cover	No	40	0.01	0.059	0.10	0.138	0.022	0.0033	0.9545
	Yes	13	0.00	0.050	0.10	0.084	0.023		
Bare soil % cover	No	40	0.06	0.132	0.20	0.218	0.035	0.2430	0.6220
	Yes	13	0.00	0.104	0.21	0.174	0.048		

Appendix 1.5 Springville Pond: Shoreline habitat data at 45 sample points.

Site_ID	Weevil Qty	Weevil Presence Yes = 1 No = 2	Location North = 1 South = 2	EWM Rank	EWM Frags	Soil Texture	% Soil Moist	% Soil Organ Matter	Dist from Water (m)	Ht above Water (cm)	Hab Code	Duff Depth (cm)	% wood	% leaves	% grass	% forbs	% needles	% bare
SSHT10A	1	1	2	1	1	S	8.35	1.32	2.0	72	6	4	5	75	20	0	0	0
SSHT10B	0	0	2	1	1	S	12.91	3.08	4.0	117	9	4	10	80	10	0	10	0
SSHT11A	2	1	2	1	0	S	31.02	7.63	2.0	141	6	4	5	80	15	0	0	0
SSHT11B	1	1	2	1	0	S	24.46	6.76	4.0	195	6	4	0	20	80	0	0	0
SSHT12A	0	0	2	1	1	S	28.35	7.84	2.0	91	6	3	5	80	15	0	0	0
SSHT12B	0	0	2	1	1	S	66.12	17.92	4.0	147	6	3	0	95	3	0	2	0
SSHT12C	0	0	2	1	1	S	6.12	0.34	2.0	64	6	2	10	90	0	0	0	0
SSHT13A	0	0	2	2	0	S	30.97	6.58	2.5	72	6	2	5	85	5	0	0	5
SSHT13B	0	0	2	1	0	S	26.73	7.95	4.5	109	6	4	10	70	20	0	0	0
SSHT13C	0	0	2	1	0	S	28.21	7.24	10.0	161	6	4	10	60	30	0	0	0
SSHT14A	5	1	2	2	1	S	72.01	8.71	2.0	43	6	4	0	85	10	5	0	0
SSHT14B	0	0	2	2	1	S	52.30	9.89	4.0	49	6	5	2	98	0	0	0	0
SSHT14LA	0	0	2	2	1	S	40.00	3.10	2.0	48	11	3	0	90	5	5	0	0
SSHT14LB	1	1	2	2	1	S	35.17	5.47	4.0	58	11	2	0	75	25	0	0	0
SSHT16A	2	1	2	1	0	S	41.16	12.26	2.5	148	6	3	5	90	0	0	5	0
SSHT16B	1	1	2	1	0	S	37.77	19.81	5.0	189	5	2	5	75	0	0	20	0
SSHT17A	0	0	2	1	1	S	83.79	55.77	2.4	121	11	2	80	20	0	0	0	0
SSHT17B	0	0	2	1	1	S	68.77	41.63	5.4	206	11	2	90	10	0	0	0	0
SSHT18A	0	0	2	0	1	S	55.75	8.49	2.0	104	8	4	5	85	10	0	0	0
SSHT18B	0	0	2	0	1	S	21.86	6.79	4.0	158	8	2	0	60	20	5	0	15
SSHT19A	0	0	2	0	0	S	34.00	5.19	4.4	98	6	1	0	80	0	0	20	0
SSHT19B	0	0	2	0	0	S	34.66	18.40	6.4	227	5	3	5	55	0	5	35	0
SSHT1A	3	1	2	1	1	S	29.93	9.23	3.0	50	5	6	20	65	15	0	0	0
SSHT1B	0	0	2	1	1	S	12.44	1.33	4.5	152	7	2	0	55	45	0	0	0
SSHT20A	0	0	2	0	0	S	63.94	40.94	5.9	101	5	2	0	98	0	0	2	0
SSHT20B	0	0	2	0	0	S	55.64	19.48	7.9	164	5	1	2	18	0	0	80	0
SSHT23A	0	0	1	0	0	S	19.83	8.07	2.0	130	6	4	2	16	80	0	2	0
SSHT23B	1	1	1	0	0	S	29.63	4.04	4.0	172	5	2	0	80	18	0	2	0
SSHT23C	0	0	1	0	0	S	25.00	8.36	10.0	205	6	2	10	70	0	0	20	0
SSHT24A	0	0	1	0	0	S	42.49	19.48	2.0	61	5	4	5	75	20	0	0	0
SSHT24B	1	1	1	0	0	LS	43.80	16.18	4.0	97	5	3	5	65	30	0	0	0
SSHT25A	1	1	1	0	0	S	11.51	2.60	2.0	64	5	2	5	75	20	0	0	0

Appendix 1.5 Continued

Site_ID	Weevil Qty	Weevil Presence Yes = 1 No = 2	Location North = 1 South = 2	EWM Rank	EWM Frags	Soil Texture	% Soil Moisture	% Soil Organic Matter	Dist from Water (m)	Ht above Water (cm)	Habitat Code	Duff Depth (cm)	% wood	% leaves	% grass	% forbs	% needles	% bare
SSHT25B	0	0	1	0	0	S	17.12	4.82	4.0	92	5	4	5	80	15	0	0	0
SSHT26A	1	1	1	0	0	S	58.86	33.11	2.0	51	5	5	0	30	70	0	0	0
SSHT26B	0	0	1	0	0	S	27.70	15.64	4.0	75	5	3	5	60	30	5	0	0
SSHT27A	0	0	1	1	0	S	54.07	8.17	2.0	37	11	2	5	80	15	0	0	0
SSHT27B	0	0	1	1	0	S	51.02	9.71	4.0	50	11	1	0	20	80	0	0	0
SSHT2A	0	0	2	1	1	S	25.89	6.85	5.9	54	11	1	0	12	88	0	0	0
SSHT2B	0	0	2	1	1	S	17.87	3.69	10.4	132	11	1	0	20	80	0	0	0
SSHT32A	2	1	1	1	1	S	39.02	15.83	1.5	62	5	3	5	91	5	0	0	2
SSHT32B	0	0	1	1	1	S	21.44	5.58	5.2	138	11	1	1	74	21	0	1	3
SSHT33A	1	1	1	1	1	S	83.26	20.28	2.0	49	5	4	7	92	1	0	0	0
SSHT33B	1	1	1	1	1	S	34.68	13.18	4.0	68	6	4	5	89	5	0	1	0
SSHT3A	2	1	2	1	1	S	19.83	4.07	2.0	60	5	4	5	52	30	18	0	0
SSHT3B	2	1	2	1	1	S	21.08	5.45	6.5	112	6	4	0	75	25	0	0	0
Min	0	0	1	0	0		6.12	0.34	1.5	37	5	1	0	10	0	0	0	0
Max	5	1	2	2	1.		83.79	55.77	10.4	227	11	6	90	98	88	18	80	15
Mean	0.6	0.4	1.7	0.8	0.5		36.60	12.00	4.0	107	6.8	2.9	8	66	21	1	4	1

S = Sand
LS = Loamy Sand

Appendix 1.6 Springville Pond: On-shore distribution of weevils, November, 2009. A total of 28 weevils were collected from 17 of the 45 survey sites.

Appendix 1.7 Springville Pond: Significant Pearson correlations between **weevil quantity** and site characteristics. Alpha was set at $P \leq 0.05$. ($^{\alpha}$ns denotes a non-significant correlation.)

VARIABLE	NUMBER OF OBSERVATIONS	CORRELATION COEFFICIENT	P^{α}
Milfoil abundance	45	-	ns
Soil texture	45	-	ns
% Soil moisture	45	-	ns
% Soil organic matter	45	-	ns
Distance from water	45	- 0.3027	0.0433
Height above water	45	-	ns
Duff depth	45	0.4216	0.0039
% Wood	45	-	ns
% Leaves	45	-	ns
% Grass	45	-	ns
% Forbs	45	-	ns
% Needles	45	-	ns
% Bare	45	-	ns

Appendix 1.8 Springville Pond: T-test results for sites where weevils were present vs. absent. Bold variables are statistically significant variables. Alpha was set at $P \leq 0.05$.

								NON-PARAMETRIC T-TEST	
VARIABLE	WEEVIL PRESENCE	N	95% LCL	MEAN	95% UCL	STD DEV	STD ERR	CHI SQUARE	P
Milfoil abundance	No	28	0.46	0.714	0.97	0.659	0.124	0.8493	0.3568
	Yes	17	0.57	0.882	1.19	0.600	0.146		
Soil/duff % organic matter	No	28	7.50	12.583	17.67	13.12	2.48	0.0049	0.9440
	Yes	17	6.73	10.937	15.14	8.17	1.98		
Soil/duff % moisture	No	28	28.94	36.607	44.27	19.772	3.736	0.0548	0.8149
	Yes	17	26.41	36.561	46.71	19.738	4.787		
Distance from water (m)	No	28	3.57	4.544	5.52	2.505	0.473	4.6673	**0.0307**
	Yes	17	2.38	3.090	3.80	1.381	0.335		
Ht above water (cm)	No	28	92.91	113.000	133.00	51.725	9.775	1.2630	0.2611
	Yes	17	68.78	95.941	123.10	52.827	12.812		
Duff layer depth(cm)	No	28	2.11	2.571	3.04	1.200	0.227	5.8852	**0.0153**
	Yes	17	2.95	3.529	4.11	1.125	0.273		
Woody debris % cover	No	28	0.01	0.095	0.18	0.217	0.041	0.0731	0.7869
	Yes	17	0.02	0.042	0.07	0.048	0.012		
Deciduous leaf % cover	No	28	0.51	0.620	0.73	0.292	0.055	0.8401	0.3594
	Yes	17	0.61	0.714	0.82	0.204	0.049		
Grass % cover	No	28	0.10	0.211	0.32	0.278	0.053	0.7044	0.4013
	Yes	17	0.10	0.217	0.33	0.225	0.055		
Forbs % cover	No	28	0.00	0.007	0.01	0.018	0.003	0.0252	0.8738
	Yes	17	-0.01	0.014	0.04	0.045	0.011		
Needles % cover	No	28	0.00	0.061	0.13	0.166	0.032	0.0557	0.4554
	Yes	17	-0.01	0.017	0.04	0.049	0.012		
Bare soil % cover	No	28	0.00	0.008	0.02	0.030	0.006	0.3800	0.5376
	Yes	17	0.00	0.001	0.003	0.005	0.001		

Appendix 1.9 McDill Pond: Shoreline habitat data at 52 sample points.

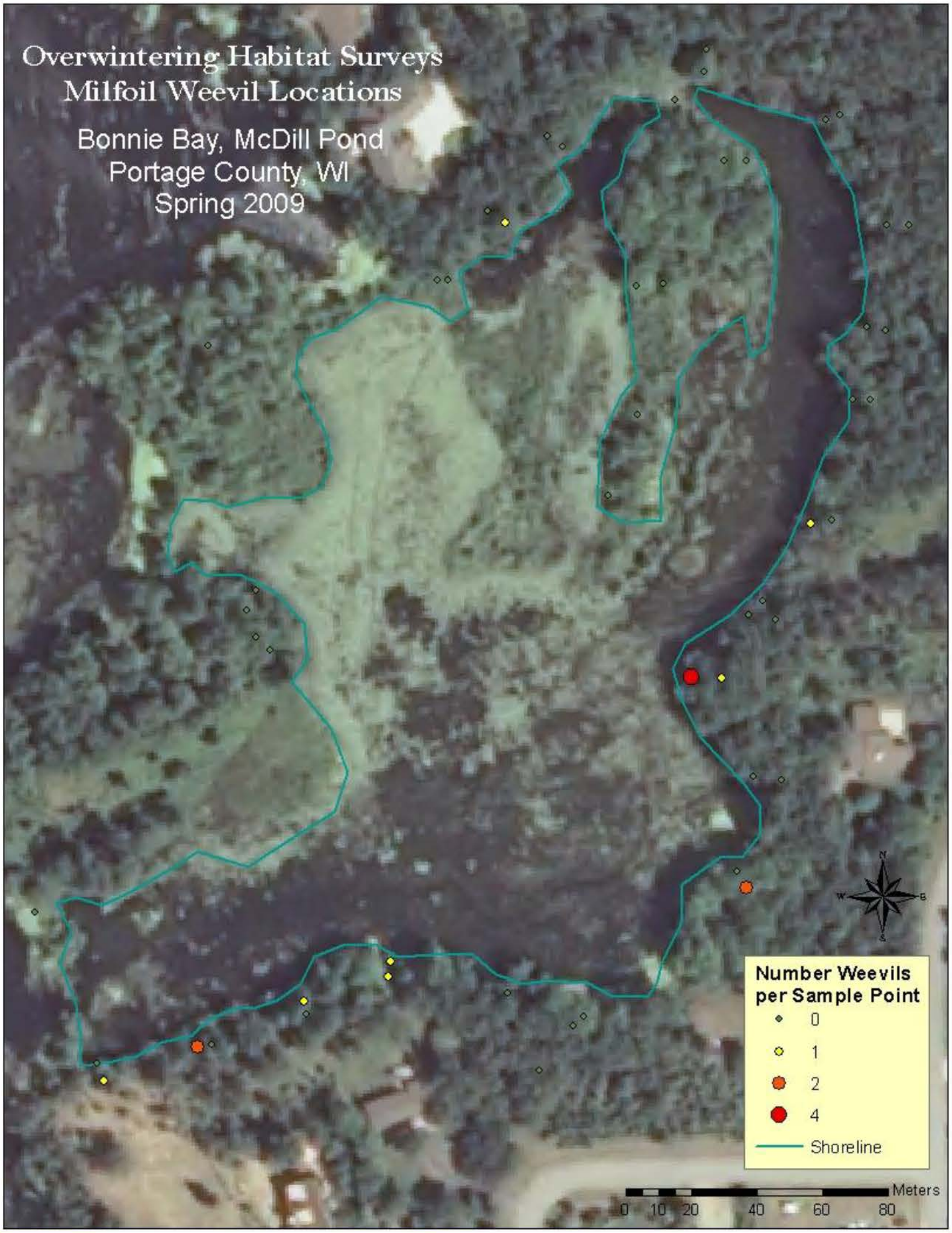
Site ID	Weevil Qty	Weevil Presence 1=Yes 0=No	Soil Texture	% Soil Organic Matter	% Soil Moisture	Dist From Water (m)	Ht Above Water (cm)	Habitat Code	Duff Depth (cm)	% wood	% leaves	% grass	% forbs	% needles	% bare soil
msht10a	0	0	LS	19.35	89.44	2	18	5	4	10	40	40	0	10	0
msht10b	0	0	LS	10.98	31.33	6	59	5	3	0	90	5	0	5	0
msht11a	0	0	LS	10.40	49.40	4	48	5	3	0	60	40	0	0	0
msht11b	0	0	LS	8.90	30.90	10	76	5	3	0	60	40	0	0	0
msht15a	0	0	LS	8.08	79.30	2	55	3	2	5	10	85	0	0	0
msht17a	0	0	S	5.67	28.71	2	139	5	4	5	60	20	10	2	3
msht17b	1	1	LS	8.86	44.42	6	347	6	5	30	45	10	0	5	10
msht18b	0	0	S	3.75	19.70	4	165	9	0	5	50	40	0	0	50
msht18r	2	1	S	10.59	41.42	4	165	5	7	10	80	10	0	0	0
msht19a	1	1	S	9.73	33.20	2	106	4	2	5	15	10	0	65	5
msht19b	0	0	S	7.52	33.14	6	285	4	2	5	60	30	0	5	0
msht1a	0	0	S	11.49	92.39	2	37	3	7	0	10	90	0	0	0
msht20a	1	1	LS	15.92	43.11	2	112	7	4	5	75	20	0	5	0
msht20b	1	1	LS	13.69	35.66	6	263	7	4	0	80	10	5	5	0
msht21a	0	0	S	25.21	64.50	2	98	5	3	0	90	0	0	5	5
msht21b	0	0	S	7.41	20.67	6	299	5	3	5	55	40	0	0	0
msht22a	0	0	LS	10.64	51.24	2	114	5	2	5	65	30	0	0	0
msht22b	0	0	S	6.31	28.48	6	313	5	2	0	80	10	10	0	0
msht24a	0	0	LS	40.18	112.66	2	61	6	2	5	20	0	5	70	0
msht24b	2	1	LS	8.13	46.66	6	98	5	1	15	55	0	0	30	0
msht25a	0	0	SL	52.56	126.17	2	44	4	4	0	60	40	0	0	0
msht25b	0	0	LS	11.50	39.12	6	164	4	4	10	70	10	0	10	0
msht26a	4	1	LS	16.27	122.93	2	53	4	4	0	10	90	0	0	0
msht26b	1	1	S	11.84	38.22	6	150	4	1	10	5	5	0	80	0
msht27a	0	0	S	7.73	36.25	2	92	4	2	10	40	20	0	20	10
msht27b	0	0	LS	18.43	66.65	6	321	4	1	15	50	0	20	40	20
msht27r	0	0	LS	29.67	89.40	1	50	4	2	10	40	20	0	20	10
msht28a	0	0	LS	17.94	69.71	2	61	5	2	15	65	5	10	5	0
msht28b	1	1	LS	16.13	33.26	6	211	5	2	15	25	50	10	0	0
msht29a	0	0	S	17.80	113.61	2	98	4	1	5	40	5	0	40	10
msht29b	0	0	LS	62.18	97.80	6	243	4	2	15	45	0	0	40	0

Appendix 1.9 Continued

Site ID	Weevil Quantity	Weevil Presence 1=Yes 0=No	Soil Texture	% Soil Organic Matter	% Soil Moisture	Dist From Water (m)	Ht Above Water (cm)	Habitat Code	Duff Depth (cm)	% wood	% leaves	% grass	% forbs	% needles	% bare soil
msht2a	0	0	S	4.71	26.95	2	73	4	4	0	30	30	0	20	20
msht2b	0	0	LS	32.97	52.15	6	214	4	3	5	10	5	0	80	0
msht30a	0	0	S	7.97	20.60	2	50	9	0	5	10	0	25	60	0
msht30b	0	0	LS	15.49	42.07	6	170	9	2	5	45	0	0	50	0
msht31a	0	0	LS	44.52	90.39	2	82	5	2	15	35	10	0	40	0
msht31b	0	0	LS	28.16	79.14	6	218	5	2	20	55	20	0	50	0
msht32a	0	0	LS	59.64	118.45	2	56	5	8	5	40	36	15	50	0
msht32b	0	0	LS	24.52	74.83	6	185	4	5	30	40	10	0	15	0
msht33a	0	0	S	8.50	23.59	2	84	3	4	0	30	70	0	0	0
msht33b	0	0	S	3.88	8.19	6	196	8	2	5	65	30	0	0	0
msht34a	0	0	S	5.04	73.99	2	53	5	6	0	20	80	0	0	0
msht34b	0	0	S	4.47	57.35	6	63	5	4	0	40	60	0	0	0
msht39a	0	0		79.00	210.27	2	14	1	2	10	20	70	0	0	0
msht3a	1	1	LS	22.17	90.66	2	111	4	10	10	60	0	0	30	0
msht3b	0	0	LS	22.07	66.42	6	130	4	5	5	80	0	0	15	0
msht40a	0	0	LS	90.07	172.23	4	33	1	1	5	90	5	0	0	0
msht41a	0	0	S	20.94	49.76	2	39	3	4	40	30	30	0	0	0
msht41b	0	0	S	8.81	22.17	6	61	5	3	5	60	35	0	0	0
msht4a	0	0	LS	38.80	72.20	2	43	3	3	0	10	90	0	0	0
msht4b	0	0	S	6.86	66.82	6	152	4	3	0	30	60	0	10	0
msht6a	0	0	SL	63.16	137.35	9	28	4	2	10	10	5	0	75	0
Min	0	0		3.75	8.19	1	14	1	0	0	5	0	0	0	0
Max	4	1		90.07	210.27	10	347	9	10	40	90	90	25	80	50
Mean	0.3	0.2		21.09	64.71	4	123	4.7	3	8	45	27	2	18	3

S = Sand
LS = Loamy Sand

Appendix 1.10 McDill Pond: On-shore distribution of weevils on Spring Slough, April 22 – May8, 2009. A total of 15 weevils were collected from 10 of the 52 survey sites.



Appendix 1.11 McDill Pond: Significant Pearson correlations between milfoil weevil quantity and site characteristics. Alpha was set at $P \leq 0.05$.

VARIABLE	NUMBER OF OBSERVATIONS	CORRELATION COEFFICIENT	P^{α}
Soil texture	51	-	ns
% Soil moisture	52	-	ns
% Soil organic matter	52	-	ns
Distance from water	52	-	ns
Height above water	52	-	ns
Duff depth	52	-	ns
% Wood	52	-	ns
% Leaves	52	-	ns
% Grass	52	-	ns
% Forbs	52	-	ns
% Needles	52	-	ns
% Bare	52	-	ns

$^{\alpha}$ns denotes a non-significant correlation

Appendix 1.12 McDill Pond: T-test results for sites where weevils were present vs. absent. Alpha was set at $P \leq 0.05$.

VARIABLE	WEEVIL PRESENCE	N	95% LCL	MEAN	95% UCL	STD DEV	STD ERR	NON-PARAMETRIC T-TEST CHI SQUARE	P
Soil texture rank	No	41	1.42	1.610	1.79	0.586	0.092	0.3401	0.5598
	Yes	10	1.35	1.700	2.05	0.483	0.153		
Soil/duff % organic matter	No	42	16.16	22.935	29.71	21.755	3.356	0.0911	0.7628
	Yes	10	10.21	13.332	16.45	4.362	1.379		
Soil/duff % moisture	No	42	54.28	67.324	80.36	41.843	6.457	0.5871	0.4436
	Yes	10	34.03	51.782	69.54	24.820	7.849		
Distance from water (m)	No	42	3.29	4.009	4.73	2.306	0.356	0.1756	0.6752
	Yes	10	2.78	4.200	5.62	1.989	0.629		
Ht above water (cm)	No	42	87.09	113.900	140.72	86.049	13.278	3.4518	0.0632
	Yes	10	97.98	161.600	225.22	88.940	28.125		
Duff layer depth(cm)	No	42	2.42	2.929	3.44	1.644	0.254	0.9778	0.3227
	Yes	10	1.98	4.000	6.02	2.828	0.894		
Woody debris % cover	No	42	0.04	0.069	0.09	0.083	0.013	1.7892	0.1810
	Yes	10	0.04	0.100	0.16	0.088	0.028		
Deciduous leaf % cover	No	42	0.38	0.455	0.53	0.233	0.036	0.0005	0.9814
	Yes	10	0.24	0.450	0.66	0.294	0.093		
Grass % cover	No	42	0.20	0.289	0.37	0.272	0.042	0.8321	0.3617
	Yes	10	0.00	0.205	0.41	0.283	0.090		
Forbs % cover	No	42	0.00	0.023	0.04	0.058	0.009	0.0112	0.9158
	Yes	10	-0.01	0.015	0.04	0.034	0.011		
Pine needle % cover	No	42	0.10	0.175	0.25	0.240	0.037	0.2932	0.5882
	Yes	10	0.01	0.220	0.43	0.292	0.092		
Bare soil % cover	No	42	0.00	0.031	0.06	0.089	0.014	0.0011	0.9731
	Yes	10	-0.01	0.015	0.04	0.03	0.011		

Appendix 1.13 The best canonical discriminant function developed for McDill Pond included the following variables: Soil Texture, % Organic Matter, Height Above Water, Duff Depth, and % Woody Material. Alpha was set at $P \leq 0.05$.

Variable	Structure Coefficient
Soil Texture	0.1587
% Organic Matter	-0.4446
Ht Above Water	0.5146
Duff Depth	0.5330
Wilke’s Lambda Probability	0.1416

.

Appendix 1.14 Prediction of weevil sites vs. non-weevil sites using the best canonical function for McDill Pond. The function was not highly significant and misclassified four weevil sites as non-weevil sites, but this may be due in some part to small sample size (Weevil sites = 10, Non-weevil sites = 42).

Group	No. of sites	Predicted group membership		Sites correctly predicted
		Weevil	No weevils	(%)
Weevils	10	6	4	60.0
No weevils	42	14	27	65.8
Overall percentage of sites correctly classified:				62.9

APPENDIX 2

CHAPTER II

Supplementary Tables and Figures

Appendix 2.1 Results of rearing 2008.

Chamber type	Chamb #	Date IN	Date OUT	# Days in Tank	# milfoil stems INPUT				# weevils INPUT	10% subsample	Weevils on inspected stems (Subsample inspections)				# weevils OUTPUT					Return Rate
					Day 0	Day 21	Day 42	Total	(eggs & larvae)	(# stems Inspected)	Adults	Pupae	Larvae	Eggs	Adults	Pupae	Larvae	Eggs	Total	
1X Stock	T2	6/12	8/7	56	75	70	70	215	32											
1X Stock	T3	6/12	8/7	56	75	70	70	215	32											
1X Stock	T5	6/12	8/7	56	75	70	70	215	32	21.5	0	10	20	1	0	100	200	10	310	9.7
1X Stock	T8	6/12	8/7	56	75	70	70	215	32	21.5	0	2	34	4	0	20	340	40	400	12.5
1X Stock	T9	6/12	8/7	56	75	70	70	215	32											
1X Stock	T10	6/12	8/7	56	75	70	70	215	32											
1X Stock	T11	6/12	8/7	56	75	70	70	215	32											
1X Stock	T12	6/12	8/7	56	75	70	70	215	32											
1X Stock	T17	6/12	8/7	56	75	100	70	245	31											
1X Stock	T18	6/12	8/7	56	75	100	70	245	32	30	1		17	3	8	0	139	25	172	5.4
1X Stock	T19	6/12	8/6	55	75	100	70	245	34	30	4		56	5	33	0	457	41	531	15.6
1X Stock	T20	6/12	8/6	55	75	100	70	245	32	30	3		41	8	25	0	335	65	425	13.3
1X Stock	T21	6/12	8/7	56	75	100	70	245	32											
1X Stock	T22	6/12	8/7	56	75	100	70	245	32											
1X Stock	T23	6/12	8/7	56	75	100	70	245	32											
1X Stock	T24	6/12	8/7	56	75	100	70	245	35											
1X Stock	T25	6/12	8/7	56	75	70	70	215	32											
1X Stock	T26	6/12	8/7	56	75	70	70	215	32											
1X Stock	T27	6/12	8/7	56	75	70	70	215	32											
2X Stock	T6	6/12	8/8	57	75	135	135	345	64	34.5	0	2	22	9	0	20	220	90	330	5.2
2X Stock	T13	6/12	8/8	57	75	165	135	375	64	37.5	1	16	40	3	10	160	400	30	600	9.4
2X Stock	T15	6/12	8/8	57	75	165	135	375	64	37.5	0	30	15	1	0	300	150	10	460	7.2
2X Stock	T16	6/12	8/8	57	75	165	135	375	64	37.5	5	18	51	7	50	180	510	70	810	12.7
3X Stock	T1	6/12			75	210	210	495	96	49.5	0	12	15	3	0	120	150	30	300	3.1
3X Stock	T4	6/12	8/8	57	75	210	210	495	96	49.5	0	20	43	14	0	200	430	140	770	8.0
3X Stock	T7	6/12	8/8	57	75	210	210	495	96	49.5	1	17	43	8	10	170	430	80	690	7.2
3X Stock	T14	6/12			75	240	210	525	96	52.5	2	8	25	8	20	80	250	80	430	4.5

Appendix 2.1 Continued. Results of rearing 2008.

Chamber type	Chamb #	Date IN	Date OUT	# Days in Tank	# milfoil stems INPUT				# weevils INPUT	10% subsample	Weevils on inspected stems (Subsample inspections)				# weevils OUTPUT					Return Rate
					Day 0	Day 21	Day 42	Total	(eggs & larvae)	(# stems Inspected)	Adults	Pupae	Larvae	Eggs	Adults	Pupae	Larvae	Eggs	Total	
Pools	P1	6/12	8/7	56	120	120	90	330	41											
Pools	P2	6/12	8/7	56	120	120	90	330	41	33	0		7	0	0	0	70	0	70	1.7
Pools	P3	6/12	8/7	56	120	120	90	330	41	33	1		19	3	10	0	190	30	230	5.6
Pools	P4	6/12	8/7	56	120	120	90	330	41											
Pools	P5	6/12	8/7	56	120	120	90	330	41	33	0	3	15	5	0	30	150	50	230	5.6
Pools	P6	6/12	8/7	56	120	120	90	330	41											
Pools	P7	6/12	8/7	56	120	120	90	330	41	33	0	3	6	1	0	30	60	10	100	2.4
Pools	P8	6/12	8/7	56	120	120	90	330	41											
Pools	P9	6/12	8/8	57	120	120	90	330	68											
Pools	P10	6/12	8/7	56	120	120	90	330	41											
Pools	P11	6/12	8/7	56	120	75	90	285	46											
Pools	P12	6/12	8/7	56	120	120	90	330	41											
Pools	P13	6/12	8/7	56	120	75	90	285	46											
Pools	P14	6/12	8/7	56	120	75	90	285	41											
Pools	P15	6/12	8/7	56	120	75	90	285	41											
Pools	P16	6/12	8/7	56	120	75	90	285	41											
Pools	P17	6/12	8/7	56	120	75	90	285	41											
Pools	P18	6/12	8/7	56	120	75	90	285	41											
Pools	P19	6/12	8/7	56	120	75	90	285	41											
Pools	P21	6/12	8/7	56	120	75	90	285	41											
Pools	P22	6/12	8/7	56	120	75	90	285	41											
Pools	P23	6/12	8/7	56	120	75	90	285	41											
Pools	P24	6/12	8/7	56	120	75	90	285	41	29	0	0	0	0	0	0	0	0	0	0.0

Appendix 2.2 Results of rearing 2009.

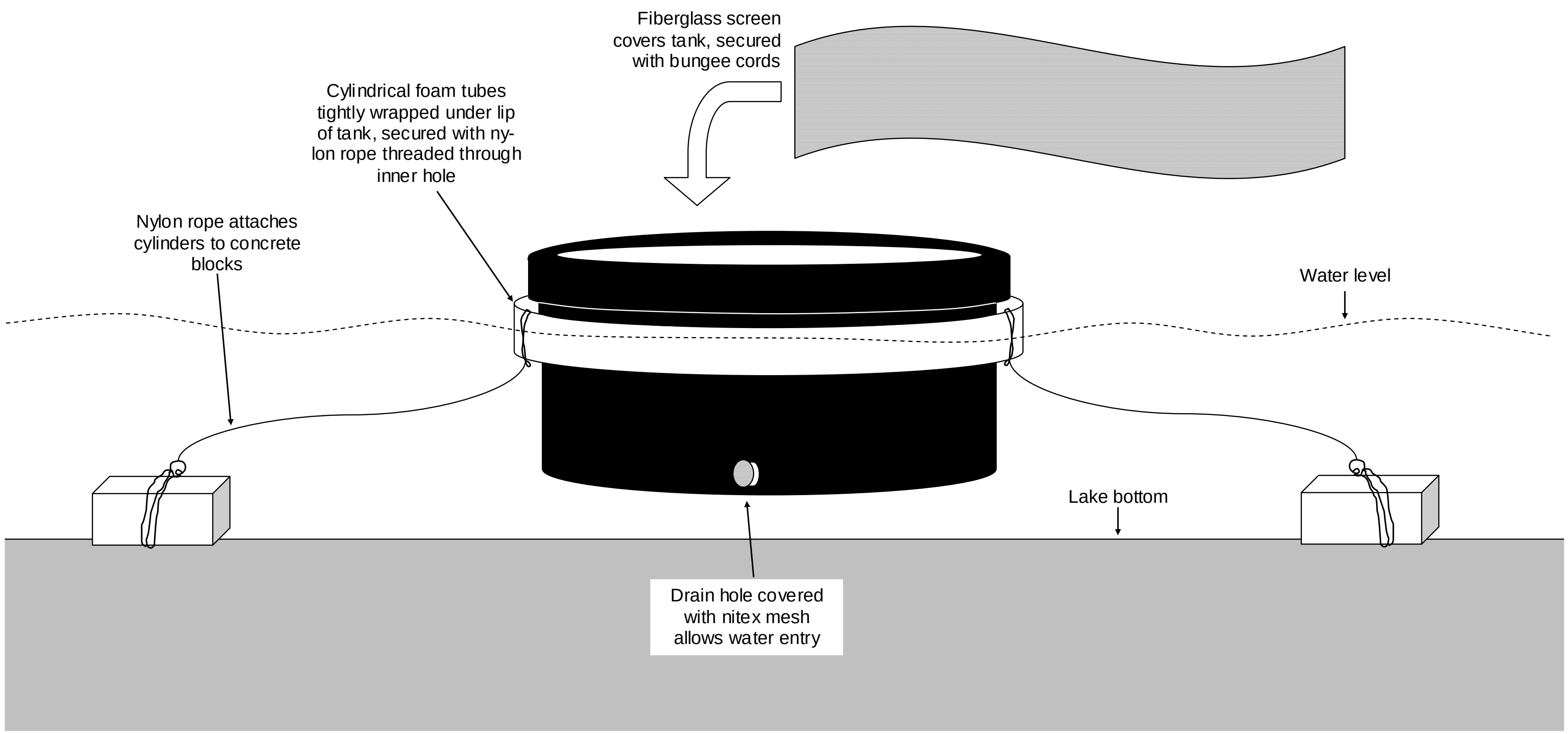
Chamber Type	Chamb #	Date IN	Date OUT	# Days in tank	# milfoil stems INPUT Day 0	Day 21	Day 42	Total	# weevils INPUT (eggs & larvae)	10% subsample (# stems inspected)	Weevils on inspected stems (Subsample inspections) Adults	Pupae	Larvae	Eggs	# weevils OUTPUT Adults	Pupae	Larvae	Eggs	Total	Return Rate
1X Stock	T1	6/10	8/4	55	75	120	150	345	28	34.5	1	7	14	13	10	70	140	130	350	12.5
1X Stock	T2	6/10	8/4	55	75	120	150	345	28											
1X Stock	T3	6/10	8/4	55	75	120	150	345	28											
1X Stock	T4	6/10	8/4	55	75	120	150	345	24											
1X Stock	T5	6/10	8/4	55	75	120	150	345	24											
1X Stock	T6	6/10	8/4	55	75	120	150	345	24	34.5	0	6	12	1	0	60	120	10	190	7.9
1X Stock	T7	6/10	8/4	55	75	120	150	345	28											
1X Stock	T8	6/10	8/4	55	75	120	150	345	28											
1X Stock	T9	6/10	8/4	55	75	120	150	345	28	34.5	1	7	13	7	10	70	130	70	280	10.0
1X Stock	T10	6/10	8/4	55	75	120	150	345	39											
1X Stock	T11	6/10	8/4	55	75	120	150	345	35	34.5	0	0	0	0	0	0	0	0	0	0.0
1X Stock	T12	6/10	8/4	55	75	120	150	345	35											
1X Stock	T13	6/10	8/4	55	75	120	150	345	35	34.5	2	8	16	10	20	80	160	100	360	10.3
1X Stock	T14	6/10	8/4	55	75	120	150	345	35											
1X Stock	T15	6/10	8/4	55	75	120	150	345	35	34.5	1	11	12	6	10	110	120	60	300	8.6
2X Stock	T16	6/10	8/4	55	150	150	165	465	63	46.5	1	23	30	2	10	230	300	20	560	8.9
2X Stock	T17	6/10	8/4	55	150	150	165	465	70	46.5	0	0	18	1	0	0	180	10	190	2.7
2X Stock	T18	6/10	8/4	55	150	150	165	465	70	46.5	7	12	80	29	70	120	800	290	1280	18.3
2X Stock	T19	6/10	8/4	55	150	150	165	465	70	46.5	0	7	51	2	0	70	510	20	600	8.6
3X Stock	T20	6/10	8/4	55	225	225	255	705	95	70.5	1	20	113	9	10	200	1130	90	1430	15.1
3X Stock	T21	6/10	8/4	55	225	225	255	705	95	70.5	1	1	37	1	10	10	370	10	400	4.2
3X Stock	T22	6/10	8/4	55	225	225	255	705	95	70.5	1	20	50	3	10	200	500	30	740	7.8
3X Stock	T23	6/10	8/4	55	225	225	255	705	95	70.5	0	14	41	0	0	140	410	0	550	5.8

Appendix 2.2 Continued. Results of rearing 2009.

Chamber Type	Chamb #	Date IN	Date OUT	# Days in tank	# milfoil stems INPUT				# weevils INPUT	10% subsample	Weevils on inspected stems (Subsample inspections)				# weevils OUTPUT					Return Rate
					Day 0	Day 21	Day 42	Total	(eggs & larvae)	(# stems inspected)	Adults	Pupae	Larvae	Eggs	Adults	Pupae	Larvae	Eggs	Total	
In-lake Stock	T24	6/11	8/4	54	75	120	150	345	34	34.5	1	1	19	0	10	10	190	0	210	6.2
In-lake Stock	T25	6/11	8/4	54	75	120	150	345	34	34.5	0	4	20	6	0	40	200	60	300	8.8
In-lake Stock	T26	6/11	8/4	54	75	120	150	345	34	34.5	1	5	18	18	10	50	180	180	420	12.4
In-lake Stock	T27	6/11	8/4	54	75	120	150	345	34	34.5	0	1	25	2	0	10	250	20	280	8.2
Pools	P1S	6/10	8/4	55	120	180	240	540	44	54	0	0	0	0	0	0	0	0	0	0.0
Pools	P2S	6/10	8/4	55	120	180	240	540	44	54	0	0	0	1	0	0	0	10	10	0.2
Pools	P3S	6/10	8/4	55	120	180	240	540	44	54	0	0	0	0	0	0	0	0	0	0.0
Pools	P4S	6/10	8/4	55	120	180	240	540	44	54	0	0	5	1	0	0	50	10	60	1.4
Pools	P5L	6/10	8/4	55	120	180	240	540	44	54	0	0	0	0	0	0	0	0	0	0.0
Pools	P6L	6/10	8/4	55	120	180	240	540	44	54	0	0	0	1	0	0	0	10	10	0.2
Pools **C**	P7L	6/10	8/4	55	120	180	240	540	44	54	0	0	4	9	0	0	40	90	130	3.0
Pools	P8L	6/10	8/4	55	120	180	240	540	44	54	0	0	0	0	0	0	0	0	0	0.0

S = Short bundles (surfacing stems)
L = Long bundles (stems just as long as Tub stems, trailing on surface)
C = Compromised screen integrity

Appendix 2.3 Diagram of floating in-lake stock tanks.



Appendix 2.4 Predator and competitor insects recorded in weevil rearing chambers, 2009.

Weevil Rearing Chamber Evaluation
Miscellaneous Insects Recorded

Order	Suborder	Family	Genus	Food Habits	Risk Level*	# Specimens Recorded**	Estimated Total Production	**Weevil Production**
Tub 9: Stock Tank, Schmeekle Reserve Study Plot								
Diptera		Chironomidae	(larvae, pupae)	species dependent (herb/detr/omni/carn)	low (carn species may pose risk)	11	110	
Diptera		Culicidae	(larvae, pupae)	microorganisms, algae, detritus	none	1	10	
Trichoptera		(various)		family dependent	unknown	1	10	
					Total Miscellaneous Insects:	**13**	**130**	280
Tub 24: Stock Tank, Floated in Lake Joanis								
Miscellaneous Insects Recorded:								
Ephemeroptera		Caenidae	Caenis	collecting, gathering, scraping algae, periphyton, detritous	unknown	1	10	
					Total Miscellaneous Insects:	**1**	**10**	210
Pool 9: Wading Pool, Schmeekle Reserve Study Plot								
Miscellaneous Insects Recorded:								
Hemiptera		Pleidae	Neoplea			1	10	
Hemiptera		Corixidae		detr	none	2	20	
Diptera		Chironomidae	(larvae, pupae)	species dependent (herb/detr/omni/carn)	low	15	150	
Diptera		Culicidae	(larvae, pupae)	microorganisms, algae, detritus	none	7	70	

Appendix 2.4 Continued. Predator and competitor insects recorded in weevil rearing chambers.

Order	Suborder	Family	Genus	Food Habits	Risk Level*	# Specimens Recorded**	Estimated Total Production	**Weevil Production**
Pool 9 (continued)								
Diptera		Chaoboridae	Chaoborus	mosquito larvae	none	1	10	
Ephemeroptera		Caenidae	Caenis	collecting, gathering, scraping algae, periphyton, detritous	unknown	2	20	
Odonata	Zygoptera	Coenagrionidae	Engallagma	pred	high	1	10	
Odonata	Anisoptera	Libellulidae	Sympetrum	pred	high	2	20	
Odonata	Anisoptera	Libellulidae	Erythemis	pred	high	1	10	
Coleoptera		Dytiscidae	Ilybius	pred	high	6	60	
Coleoptera		Dytiscidae	Laccophilus (larv)	pred	high	1	10	
Coleoptera		Dytiscidae	Hydroporus oblitus	pred	high	3	30	
Trichoptera		(various)		family dependent	unknown	30	300	
Trichoptera		Leptoceridae	Ceraclea	omni, non-portable case	low	2	20	
Trichoptera		Polycentropodidae	Cernotina	carn, non-portable case	low	1	10	
Trichoptera		Rhyacophilidae	Rhyacophila	carn - free-living	high	1	10	
Lepidoptera				herb, competitive	high	1	10	
					Total Miscellaneous Insects:	**77**	**770**	130

* = Relative risk to rearing weevils

** = Specimens extracted from a 10% subsample.

N/A = Not applicable. Euhrychiopsis is the subject organism

carn = carnivore

detr = detritivore

herb = herbivore

omni = omnivore

APPENDIX 3

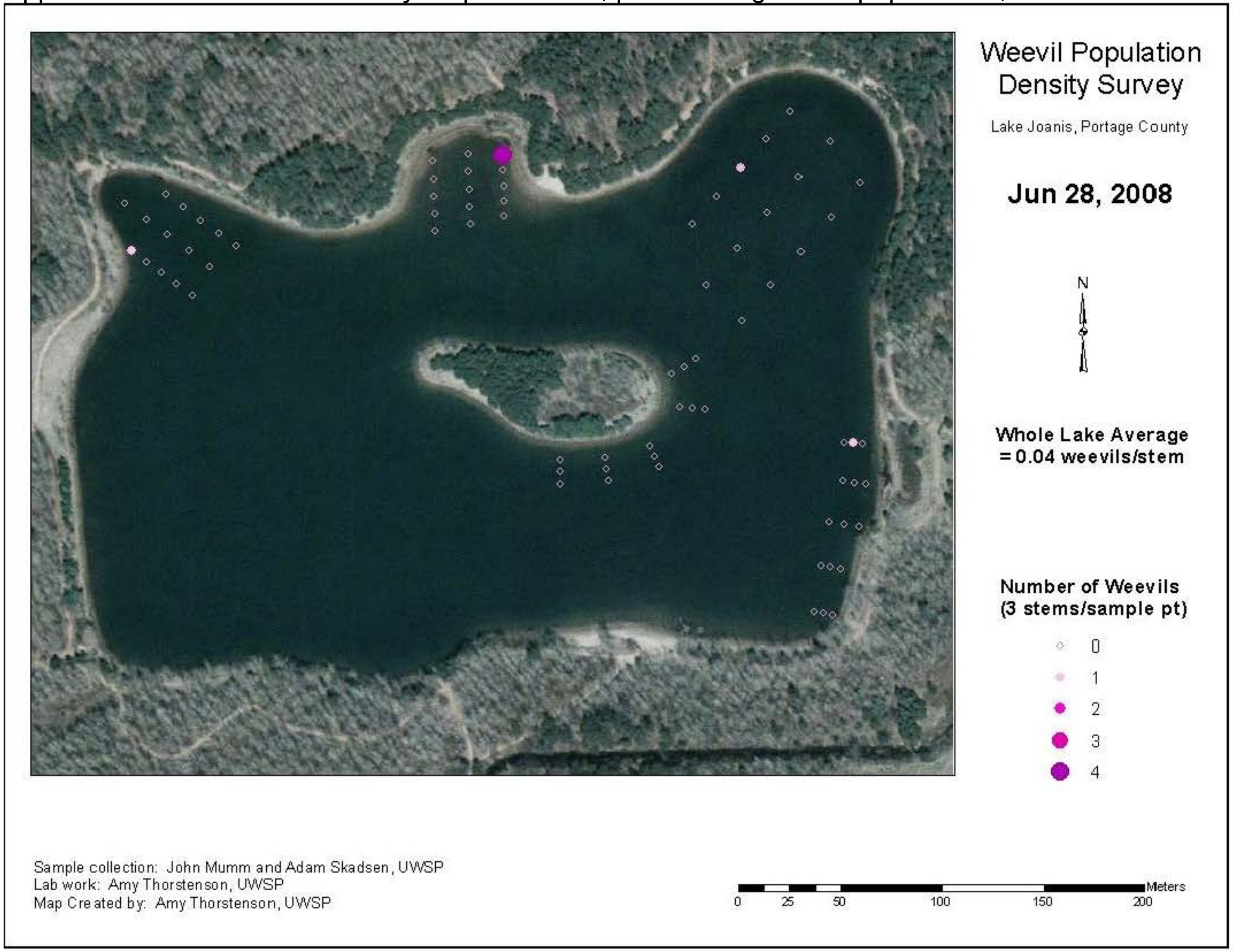
IN-LAKE SURVEYS

Lake Joanis

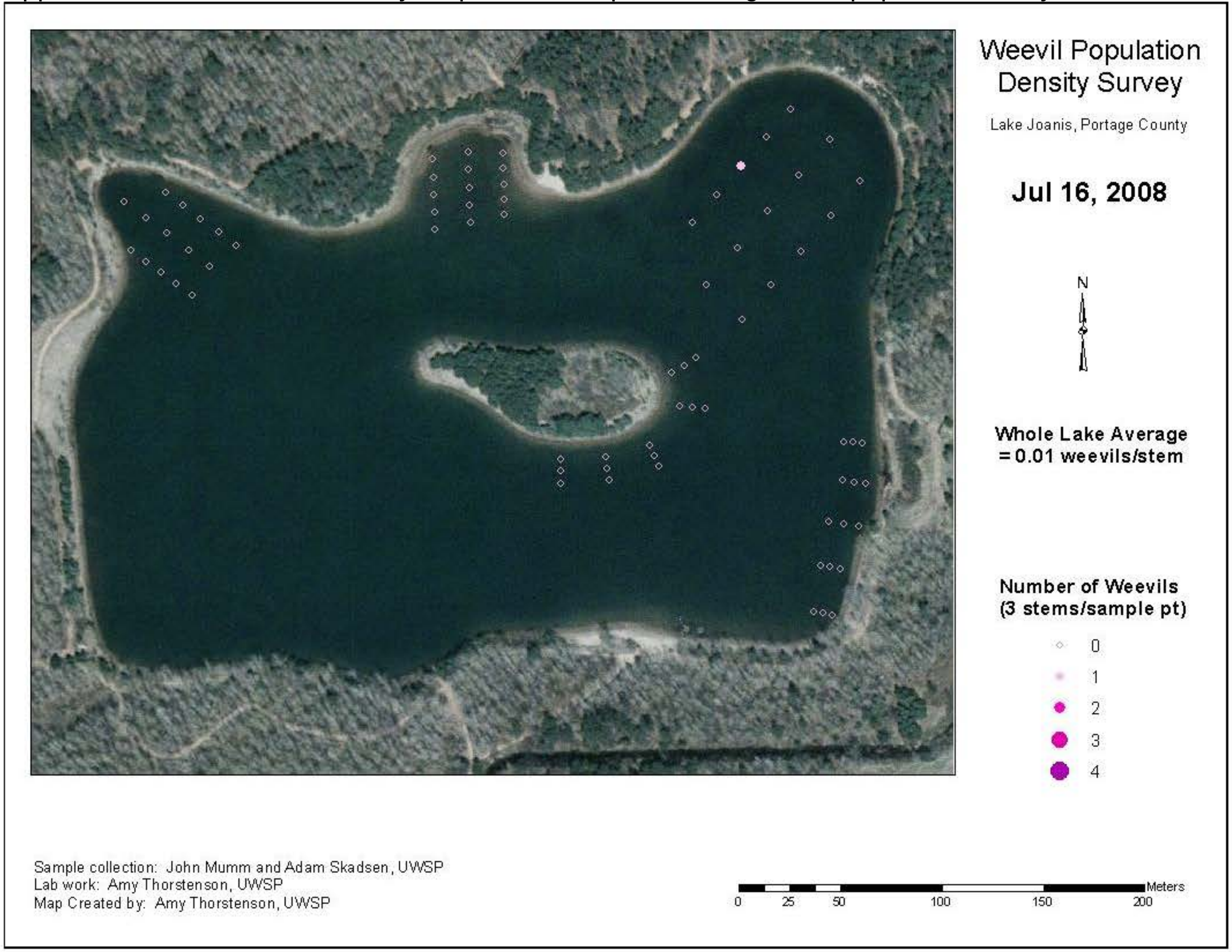
Thomas Lake

Lake Emily

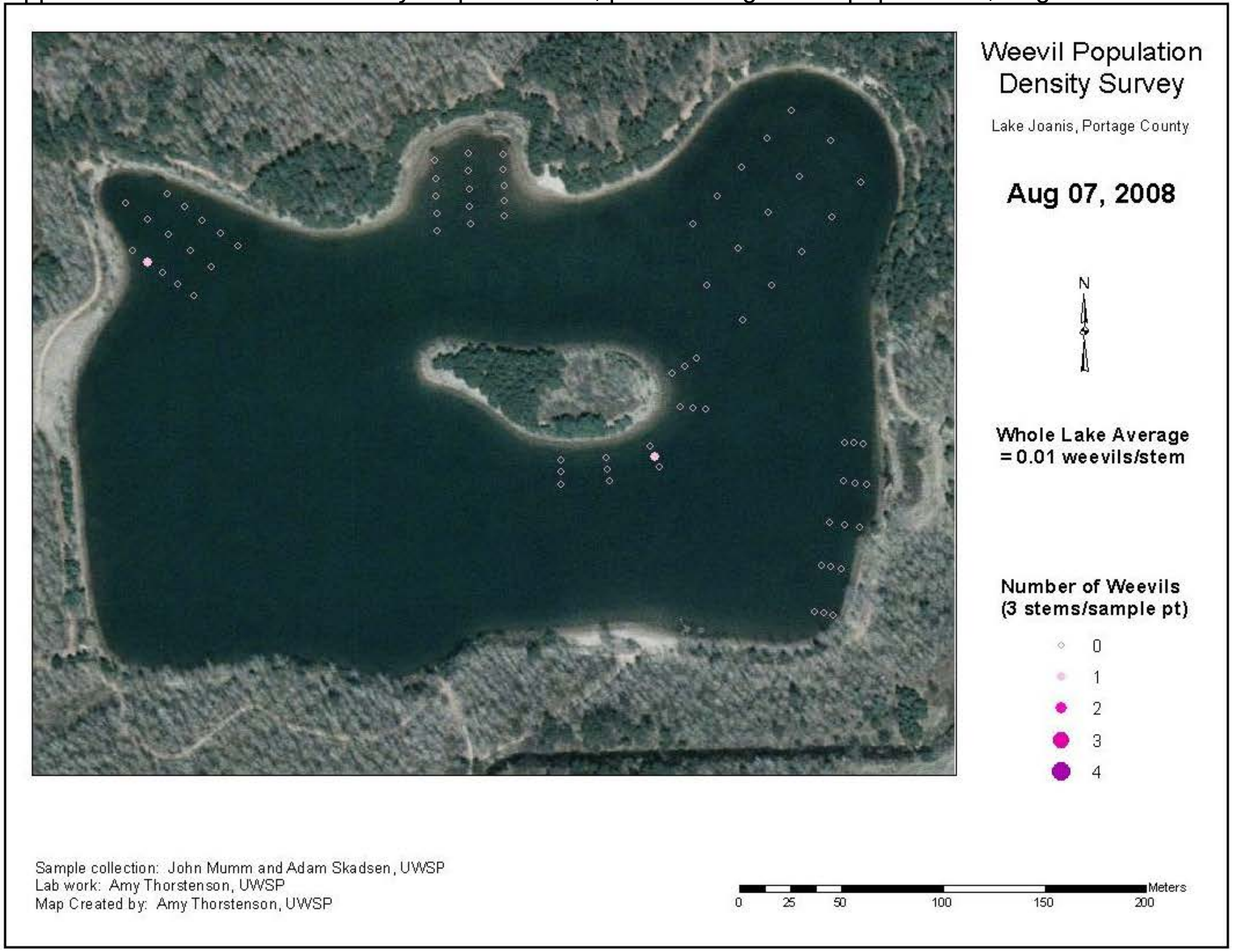
Appendix 3.1 Lake Joanis survey map of natural, pre-stocking weevil populations, June 2008.



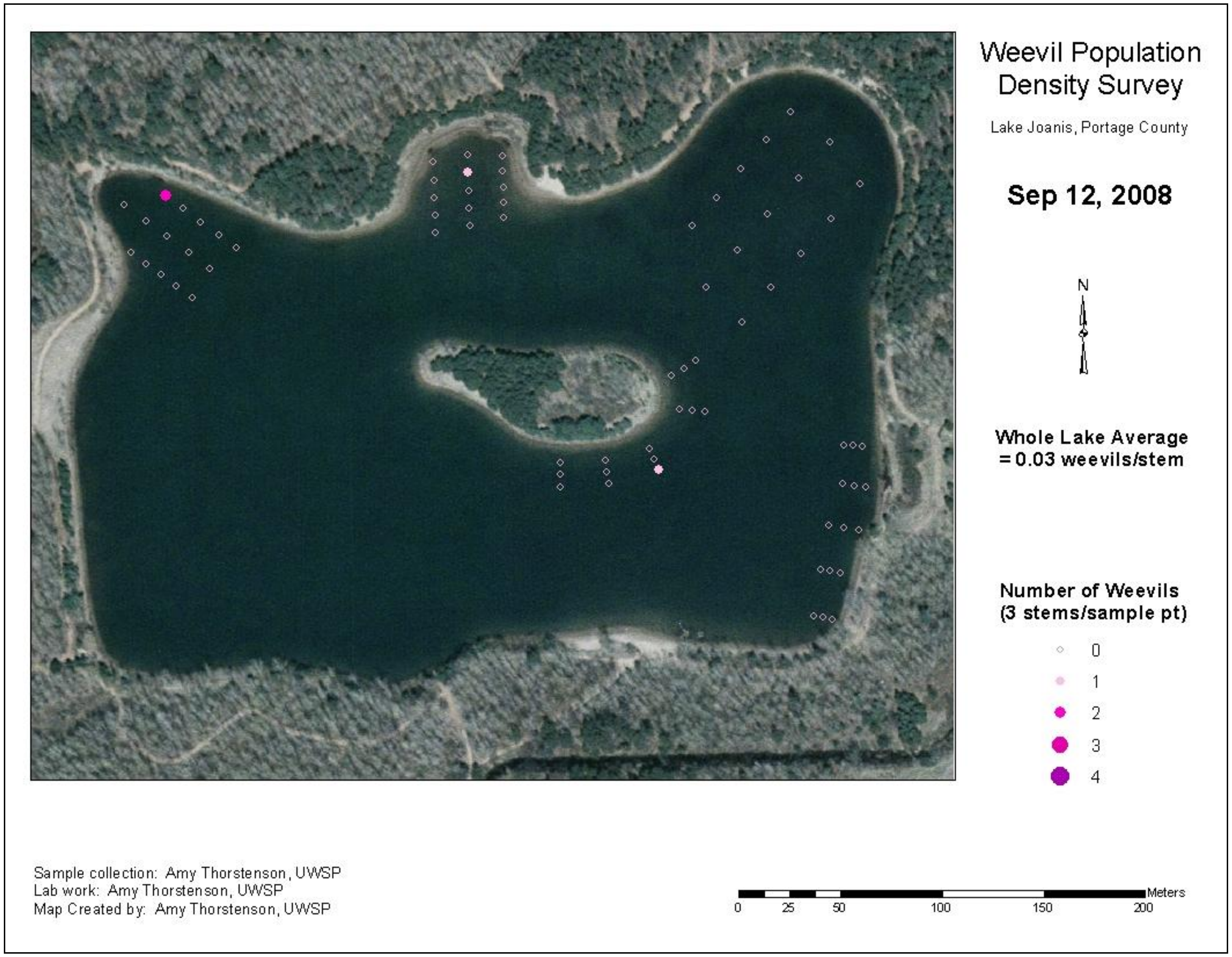
Appendix 3.2 Lake Joanis survey map of natural, pre-stocking weevil populations, July 2008.



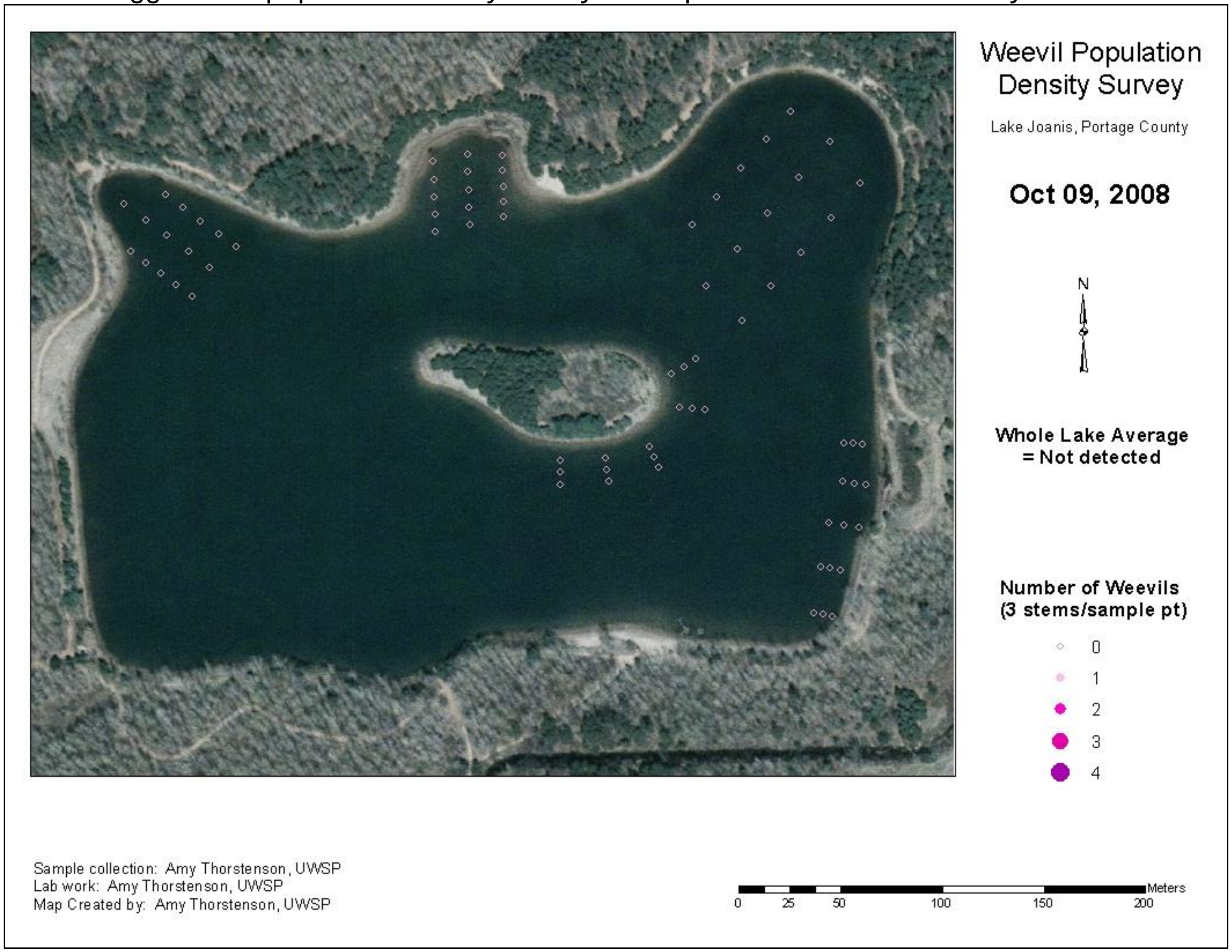
Appendix 3.3 Lake Joanis survey map of natural, pre-stocking weevil populations, Aug 2008.



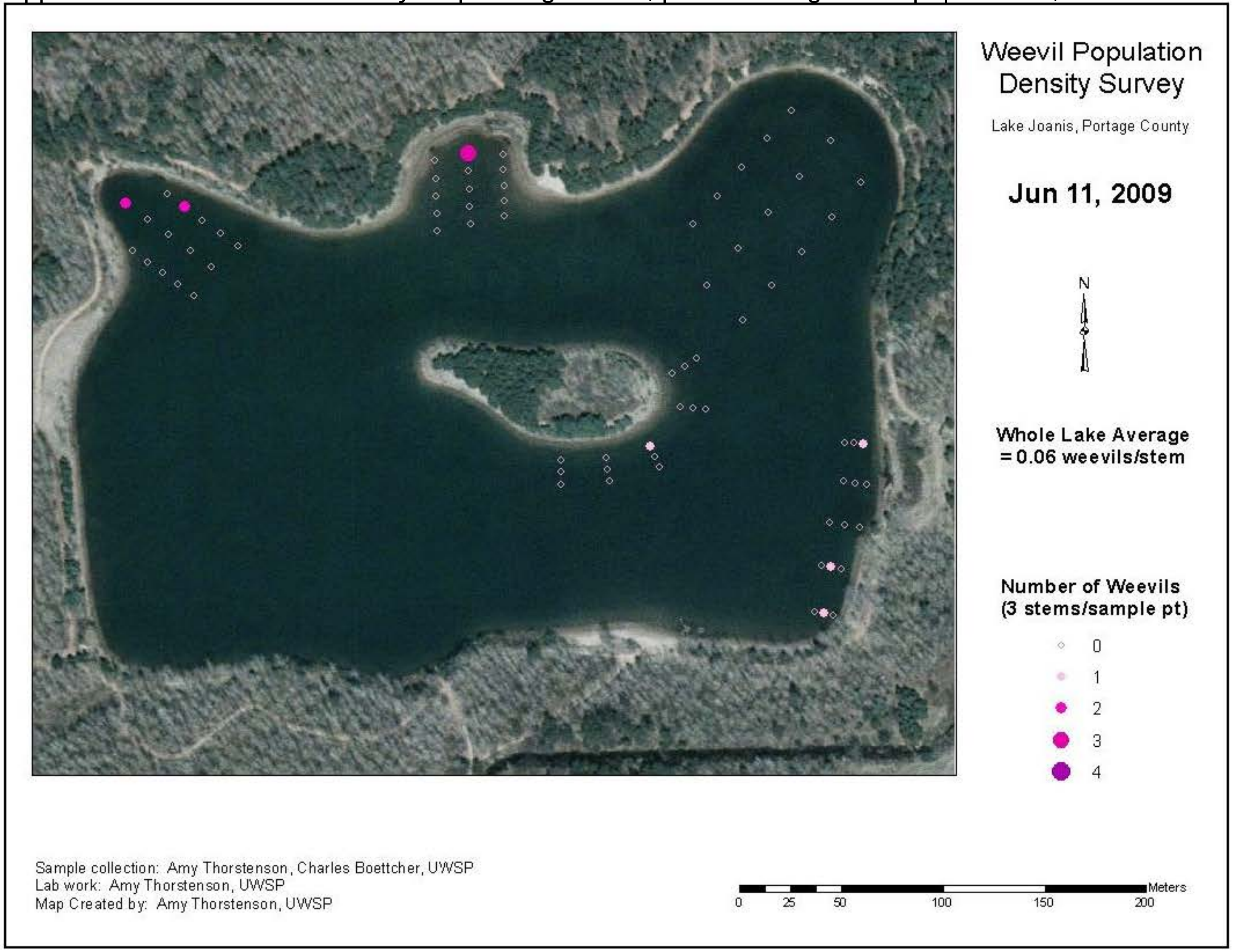
Appendix 3.4 Lake Joanis survey map of augmented, post-stocking weevil populations, September 2008. Approximately 13,041 weevils were stocked to the NE bed and Island bed on August 4-8, 2008. Weevils fly to shore in September – October to hibernate for the winter, which may be one reason no significant increase in weevil population density was detected.



Appendix 3.5 Lake Joanis survey map of augmented, post-stocking weevil populations, October 2008. Weevils fly to shore in September – October to hibernate for the winter, which may be a confounding variable in this survey. These results suggest that population density surveys in September and October may be of little value.



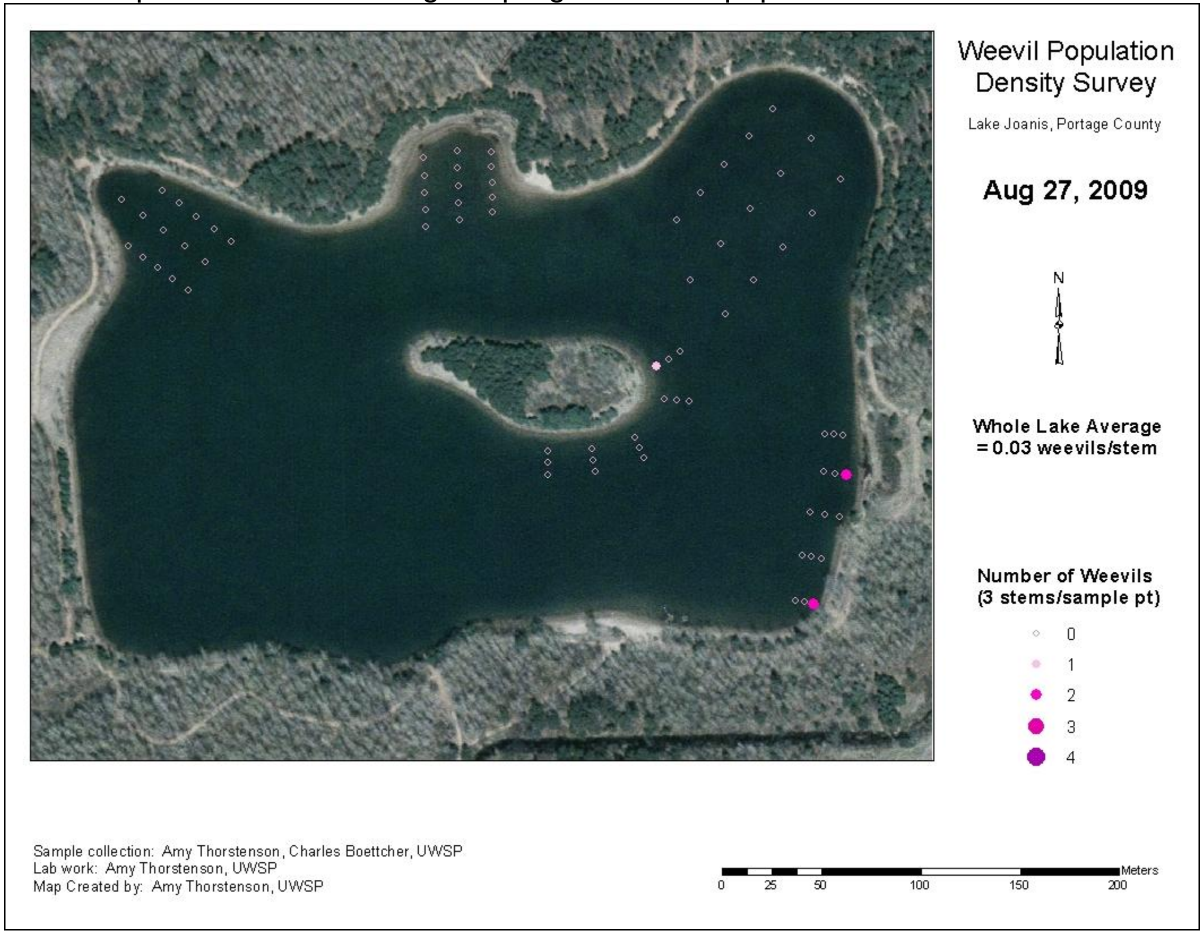
Appendix 3.6 Lake Joanis survey map of augmented, post-stocking weevil populations, June 2009.



Appendix 3.7 Lake Joanis survey map of augmented, post-stocking weevil populations, July 9, 2009.

Appendix 3.8 Lake Joanis survey map of augmented, post-stocking weevil populations, July 28, 2009.

Appendix 3.9 Lake Joanis survey map of augmented, post-stocking weevil populations, August 27, 2009. A second stocking of approximately 9,994 weevils was released to the NW bed, August 4, 2009. Weevils fly to shore in September – October to hibernate for the winter, but prepare for this migration ahead of time by ceasing to spend energy on reproduction and storing up fat reserves. Without the juvenile stage weevils represented in the population, a survey as late as August 27th may be too late in the year for a representative survey of weevil densities. Surveys in 2010 and 2011 will be important for monitoring the progress of the population.



Appendix 3.10 Lake Joanis survey of natural, pre-stocking weevil populations, June 2008. Samples collected by Adam Skadsen and John Mumm, UWSP. Samples examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Lake Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 6/27/2008

Whole Lake Average: 0.04

Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	% Stems w/ Weevil Damage: larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	Average Per Stem: # Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
10/08 - 06/09	NW	0.5 - 14.8	natural	29	17.59	0%	0.24	2.69	3%	3%	10%	3%	10%	0.00	0.03	0.00	0.00	0.03
09/08 - 06/09	N	2.0 - 6.6	natural	17	15.00	0%	0.29	3.00	24%	6%	29%	6%	29%	0.12	0.06	0.06	0.00	0.24
09/08 - 06/09	NE	0.5 - 15.0	natural	26	16.83	0%	0.26	2.09	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
10/08 - 05/09	Island	0.5 - 13.1	natural	32	20.00	0%	0.38	2.69	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
10/08 - 05/09	SE	0.5 - 14.8	natural	36	19.50	0%	0.14	3.00	6%	3%	3%	0%	6%	0.03	0.00	0.00	0.00	0.03

Survey Notes: All samples preserved.

Appendix 3.11 Lake Joanis survey of natural, pre-stocking weevil populations, July 2008. Samples collected by Adam Skadsen and John Mumm, UWSP. Samples examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Lake Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 7/16-18/2008

Whole Lake Average: 0.01

									%Stems w/ Weevil Damage					Average Per Stem				Ave
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	%Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	Weevils Per Stem (All Life Stages)
10/08 - 04/09	NW	0.5 - 14.8	natural	30	17.19	0%	0.13	2.61	3%	3%	3%	3%	0%	0.00	0.00	0.00	0.00	0.00
10/08 - 04/09	N	2.0 - 6.6	natural	3	11.33	0%	0.00	1.67	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
10/08 - 04/09	NE	0.5 - 15.0	natural	33	13.60	12%	0.23	1.87	6%	3%	6%	6%	9%	0.00	0.00	0.03	0.00	0.03
10/08 - 04/09	Island	0.5 - 13.1	natural	42	16.85	0%	0.10	2.69	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
10/08 - 04/09	SE	0.5 - 14.8	natural	23	16.65	0%	0.04	2.74	0%	0%	0%	4%	4%	0.00	0.00	0.00	0.00	0.00

Survey Notes: All samples preserved.

Appendix 3.12 Lake Joanis survey of natural, pre-stocking weevil populations, August 2008. Samples collected by Adam Skadsen and John Mumm, UWSP. Samples examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 8/7/2008

Whole Lake Average: 0.01

									% Stems w/ Weevil Damage					Average Per Stem				Ave
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristems present	% Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	Weevils Per Stem (All Life Stages)
6/8-7/22/09	NW	0.5 - 14.8	Natural	40	18.97	0.05	0.00	3.18	0.00	0.00	0.03	0.00	3%	0.00	0.03	0.00	0.00	0.03
6/8-7/22/09	N	2.0 - 6.6	Natural	13	15.75	0.17	0.00	2.58	0.03	0.03	0.03	0.03	8%	0.00	0.00	0.00	0.00	0.00
6/8-7/22/09	NE	0.5 - 15.0	Natural	38	17.82	0.08	0.21	1.79	0.00	0.00	0.00	0.00	0%	0.00	0.00	0.00	0.00	0.00
6/8-7/22/09	Island	0.5 - 13.1	Natural	45	17.80	0.31	0.11	2.22	0.00	0.00	0.00	0.00	0%	0.02	0.00	0.00	0.00	0.02
6/8-7/22/09	SE	0.5 - 14.8	Natural	26	16.81	0.23	0.27	3.12	0.00	0.00	0.00	0.00	0%	0.00	0.00	0.00	0.00	0.00

Survey Notes: All samples preserved.

Appendix 3.13 Lake Joanis survey of augmented, post-stocking weevil populations, September 2008. Sample collection and stem examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected. Approximately 13,041 weevils were stocked to the NE bed and Island bed on August 4-8, 2008. Weevils fly to shore in September – October to hibernate for the winter, which may be one reason no significant increase in weevil population density was detected.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 9/12/2008

Whole Lake Average: 0.03

									% Stems w/ Weevil Damage					Average Per Stem				
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
7/24/09	NW	0.5 - 14.8	natural	31	18.71	0.06	0.06	3.58	0.00	0.03	0.00	0.00	3%	0.00	0.06	0.00	0.00	0.06
7/24/08	N	2.0 - 6.6	natural	16	18.31	0.69	0.31	4.25	0.00	0.06	0.06	0.00	13%	0.00	0.19	0.00	0.00	0.19
1/7-8/7/09	NE	0.5 - 15.0	natural	30	17.60	0.87	0.13	2.17	0.00	0.03	0.00	0.00	3%	0.00	0.00	0.00	0.00	0.00
1/7-8/7/09	Island	0.5 - 13.1	natural	29	19.38	0.28	0.31	3.69	0.00	0.00	0.03	0.07	10%	0.00	0.03	0.00	0.00	0.03
8/7/09	SE	0.5 - 14.8	natural	29	19.03	0.55	0.10	3.06	0.00	0.00	0.03	0.00	3%	0.00	0.00	0.00	0.00	0.00

Survey Notes: All samples preserved.

Appendix 3.14 Lake Joanis survey of augmented, post-stocking weevil populations, October 2008. Sample collection and stem examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected. Weevils fly to shore in September – October to hibernate for the winter, which may be a confounding variable in this survey. These results suggest that population density surveys in September and October may be of little value.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 10/9/2008

Whole Lake Average: 0.00

									% Stems w/ Weevil Damage					Average Per Stem				
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
8/6/09	NW	0.5 - 14.8	natural	29	17.45	0.10	0.14	3.72	0.03	0.00	0.03	0.07	7%	0.00	0.00	0.00	0.00	0.00
8/6/09	N	2.0 - 6.6	natural	10	18.50	0.70	0.10	3.00	0.00	0.00	0.00	0.10	10%	0.00	0.00	0.00	0.00	0.00
8/6 - 8/14/09	NE	0.5 - 15.0	natural	13	18.08	0.69	0.31	3.23	0.00	0.00	0.00	0.00	0%	0.00	0.00	0.00	0.00	0.00
8/6 - 8/14/09	ISLAND	0.5 - 13.1	natural	22	15.55	0.32	0.14	2.23	0.00	0.00	0.00	0.05	5%	0.00	0.00	0.00	0.00	0.00
8/14/09	SE	0.5 - 14.8	natural	21	15.24	0.48	0.52	3.19	0.14	0.05	0.14	0.00	14%	0.00	0.00	0.00	0.00	0.00

Survey Notes: All samples preserved.

Appendix 3.15 Lake Joanis survey of augmented, post-stocking weevil populations, June 2009. Samples collected by Amy Thorstenson and Charles Boettcher, UWSP. Stem examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 6/11/2009 Whole Lake Average: 0.06

Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	% Stems w/ Weevil Damage: larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	Average Per Stem: # Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
6/24-7/1/09	NW	0.5 - 14.8	natural	44	17.95	0.16	0.80	3.36	5%	2%	7%	20%	25%	0.09	0.00	0.00	0.00	0.09
6/24-7/1/09	N	2.0 - 6.6	natural	20	17.35	0.10	0.40	3.45	30%	10%	15%	25%	40%	0.00	0.15	0.00	0.00	0.15
6/24-7/1/09	NE	0.5 - 15.0	natural	33	18.15	0.45	0.21	3.00	0%	6%	3%	0%	9%	0.00	0.00	0.00	0.00	0.00
6/24-7/1/09	Island	0.5 - 13.1	natural	40	19.48	0.25	0.33	3.83	0%	3%	0%	0%	3%	0.00	0.00	0.03	0.00	0.03
6/24-7/1/09	SE	0.5 - 14.8	natural	37	19.16	0.24	0.49	3.57	22%	0%	16%	16%	27%	0.00	0.08	0.00	0.00	0.08

Survey Notes: All samples preserved.

Appendix 3.16 Lake Joanis survey of augmented, post-stocking weevil populations, July 9, 2009. Samples collected by Amy Thorstenson and Charles Boettcher, UWSP. Stem examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 7/9/2009 Whole Lake Average: 0.03

Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	%Stems w/ Weevil Damage: larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	%Stems w/ Damage	Average Per Stem: # Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
9/2/09	NW	0.5 - 14.8	natural	39	19.59	0.00	0.26	2.62	0%	5%	5%	0%	5%	0.00	0.00	0.05	0.00	0.05
9/2/09	N	2.0 - 6.6	natural	28	14.89	0.21	0.07	2.21	0%	4%	4%	0%	4%	0.00	0.00	0.00	0.00	0.00
9/2/09	NE	0.5 - 15.0	natural	42	18.12	0.00	0.10	2.31	2%	0%	2%	0%	0%	0.00	0.00	0.00	0.00	0.00
9/2/09	Island	0.5 - 13.1	natural	45	17.56	0.27	0.13	2.84	0%	0%	0%	7%	2%	0.00	0.00	0.05	0.00	0.05
9/2/09	SE	0.5 - 14.8	natural	40	18.93	0.05	0.15	3.15	18%	10%	15%	15%	25%	0.06	0.11	0.00	0.00	0.17

Survey Notes: All samples preserved.

Appendix 3.17 Lake Joanis survey of augmented, post-stocking weevil populations, July 28, 2009. Samples collected by Amy Thorstenson and Charles Boettcher, UWSP. Stem examination by Amy Thorstenson, UWSP. A second stocking of approximately 9,994 weevils were stocked to the NW bed on August 4, 2009. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 7/28/2009 Whole lake average: 0.02

Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	% Stems w/ Weevil Damage: larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	Average Per Stem: # Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life
9/21-10/12/09	NW	0.5 - 14.8	natural	46	19.91	0.02	0.11	3.87	2%	0%	7%	17%	24%	0.00	0.04	0.00	0.00	0.04
9/21-10/12/09	N	2.0 - 6.6	natural	32	17.63	0.25	0.19	3.06	0%	3%	9%	0%	9%	0.00	0.00	0.00	0.00	0.00
9/21-10/12/09	NE	0.5 - 15.0	natural	45	20.71	0.02	0.11	2.58	2%	0%	0%	0%	2%	0.00	0.00	0.00	0.00	0.00
9/21-10/12/09	Island	0.5 - 13.1	natural	45	20.36	0.31	0.24	3.91	2%	0%	2%	0%	2%	0.00	0.00	0.00	0.00	0.00
9/21-10/12/09	SE	0.5 - 14.8	natural	45	27.91	0.13	0.31	3.44	7%	2%	4%	7%	13%	0.04	0.00	0.00	0.02	0.07

Survey Notes: All samples preserved.

Appendix 3.18 Lake Joanis survey of augmented, post-stocking weevil populations, August 27, 2009. Samples collected by Amy Thorstenson and Charles Boettcher, UWSP. Stem examination by Amy Thorstenson, UWSP. A second stocking of approximately 9,994 weevils were stocked to the NW bed on August 4, 2009. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Joanis** COMPARISONS BETWEEN BEDS

Sample Date: 8/27/2009

Whole lake average: 0.03

									% Stems w/ Weevil Damage					Average Per Stem				
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
10/9/09 - 4/9/10	NW	0.5 - 14.8	natural	38	25.08	0.18	0.24	4.21	0%	0%	3%	0%	3%	0.00	0.00	0.00	0.00	0.00
10/9/09 - 4/9/10	N	2.0 - 6.6	natural	30	18.63	0.47	0.17	4.07	0%	3%	0%	0%	3%	0.00	0.00	0.00	0.00	0.00
10/9/09 - 4/9/10	NE	0.5 - 15.0	natural	43	19.53	0.07	0.07	2.44	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
10/9/09 - 4/9/10	Island	0.5 - 13.1	natural	40	29.33	0.25	0.25	3.55	3%	0%	3%	0%	3%	0.03	0.00	0.00	0.00	0.03
10/9/09 - 4/9/10	SE	0.5 - 14.8	natural	42	18.21	0.12	0.40	4.74	7%	7%	10%	7%	14%	0.07	0.00	0.02	0.00	0.10

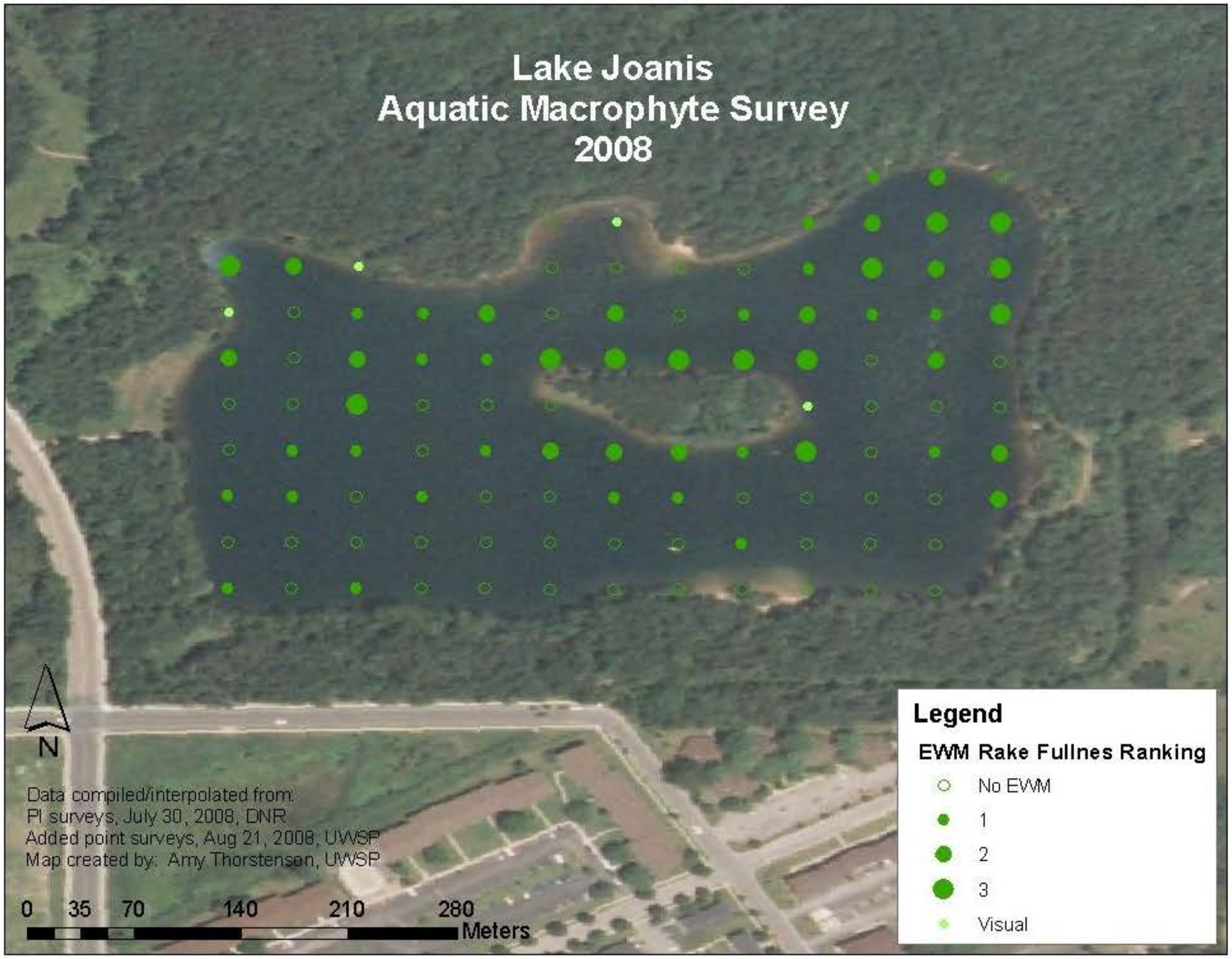
Survey Notes: All samples preserved.

Appendix 3.19 Lake Joanis weevil population monitoring, summary table, whole lake averages (# weevils/stem). Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

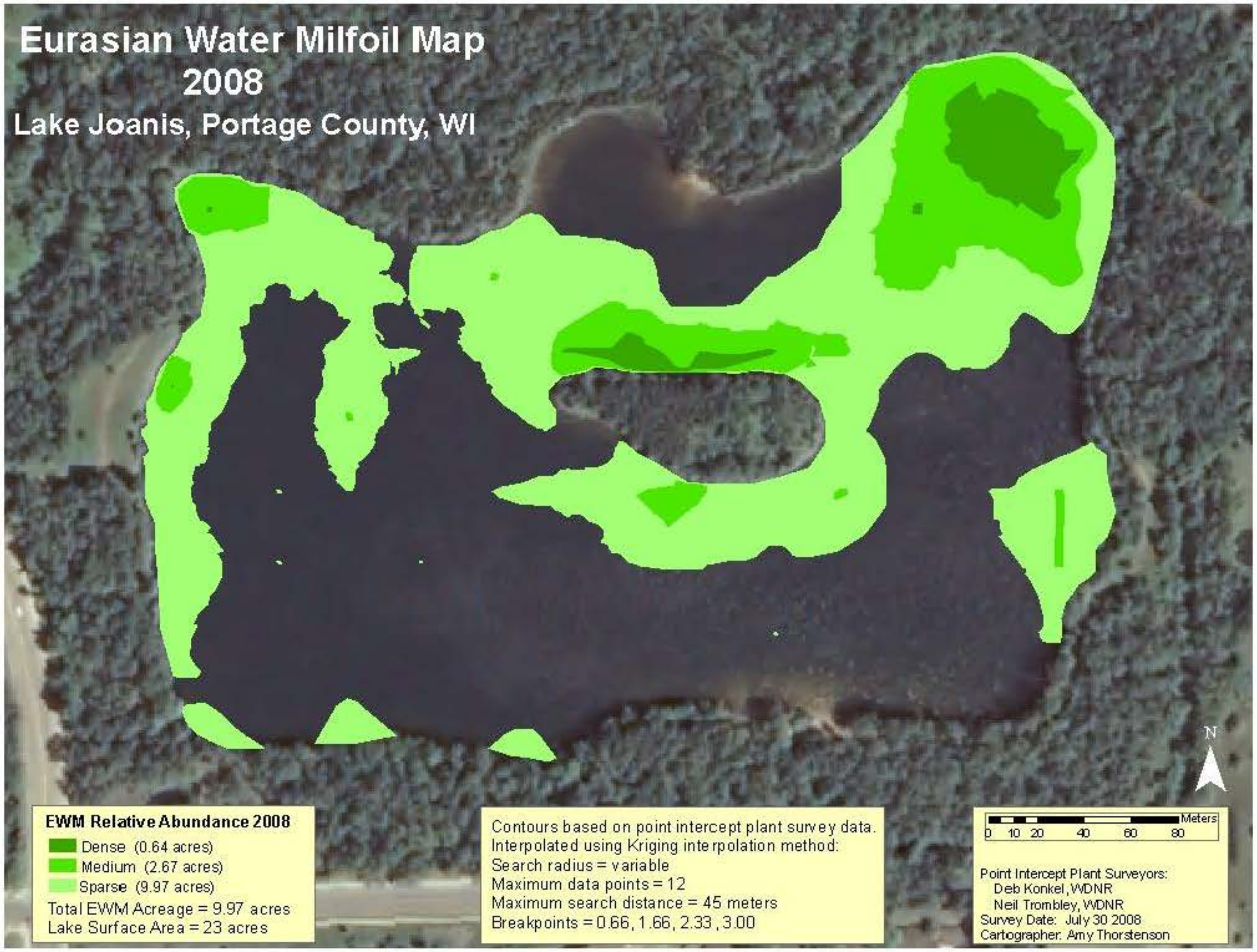
Month	2008	2009
June	0.04	0.06*
July	0.01	0.03*
Aug†	0.01	0.02*

† July 28, 2009 survey used for comparison to Aug 7, 2008 survey.
* Surveys of augmented populations.

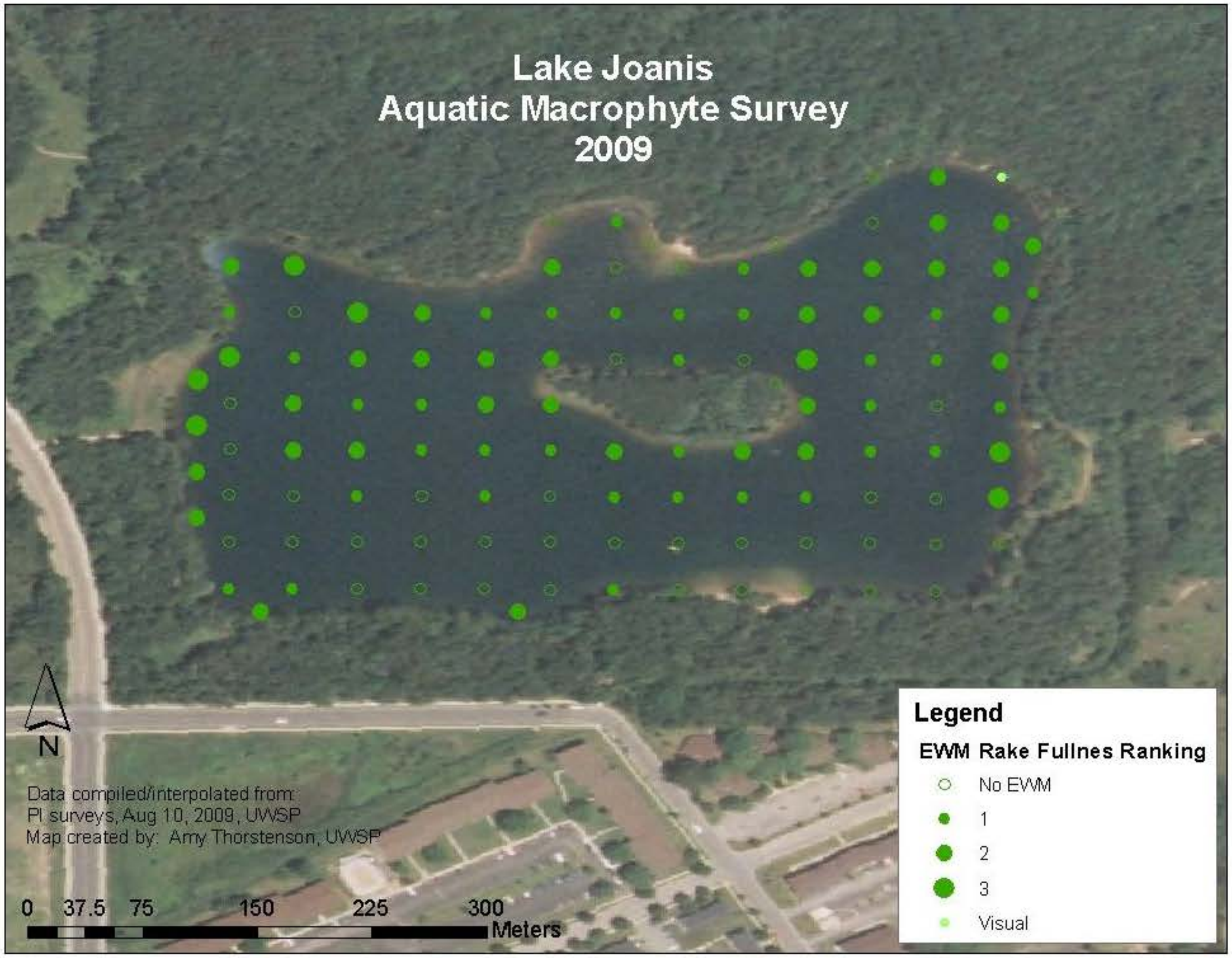
Appendix 3.20 Lake Joanis point intercept survey map for Eurasian watermilfoil, 2008.



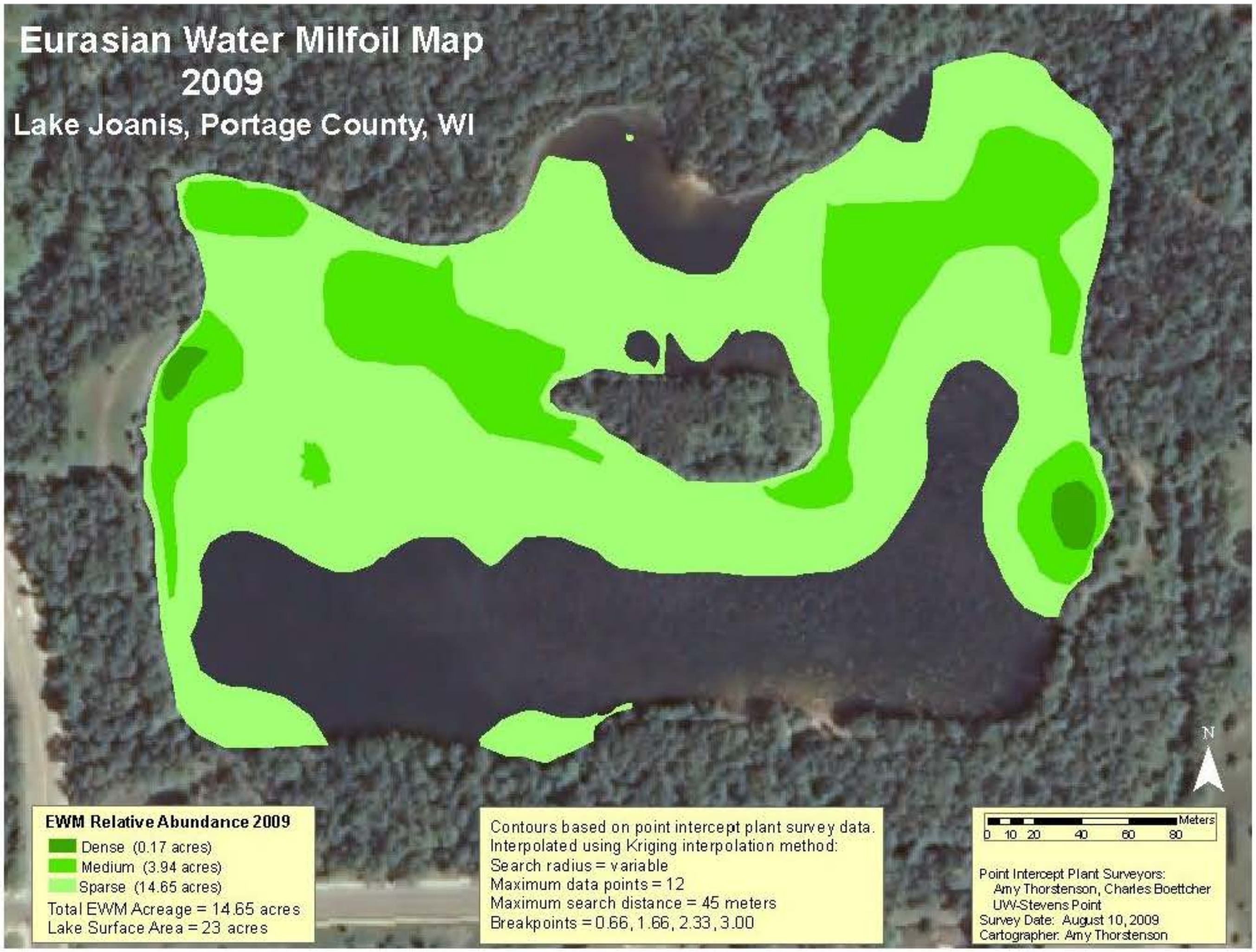
Appendix 3.21 Lake Joanis Eurasian Water Milfoil map based on interpolations of point intercept survey data, 2008.



Appendix 3.22 Lake Joanis point intercept survey map for Eurasian watermilfoil, 2009.



Appendix 3.23 Lake Joanis EWM map based on interpolations of point intercept survey data, 2009.



Appendix 3.24 Lake Joanis point Intercept macrophyte survey results, 2008. Survey conducted by Deborah Konkel and Neil Trombley, WDNR, using standard WDNR point intercept method and survey grid.

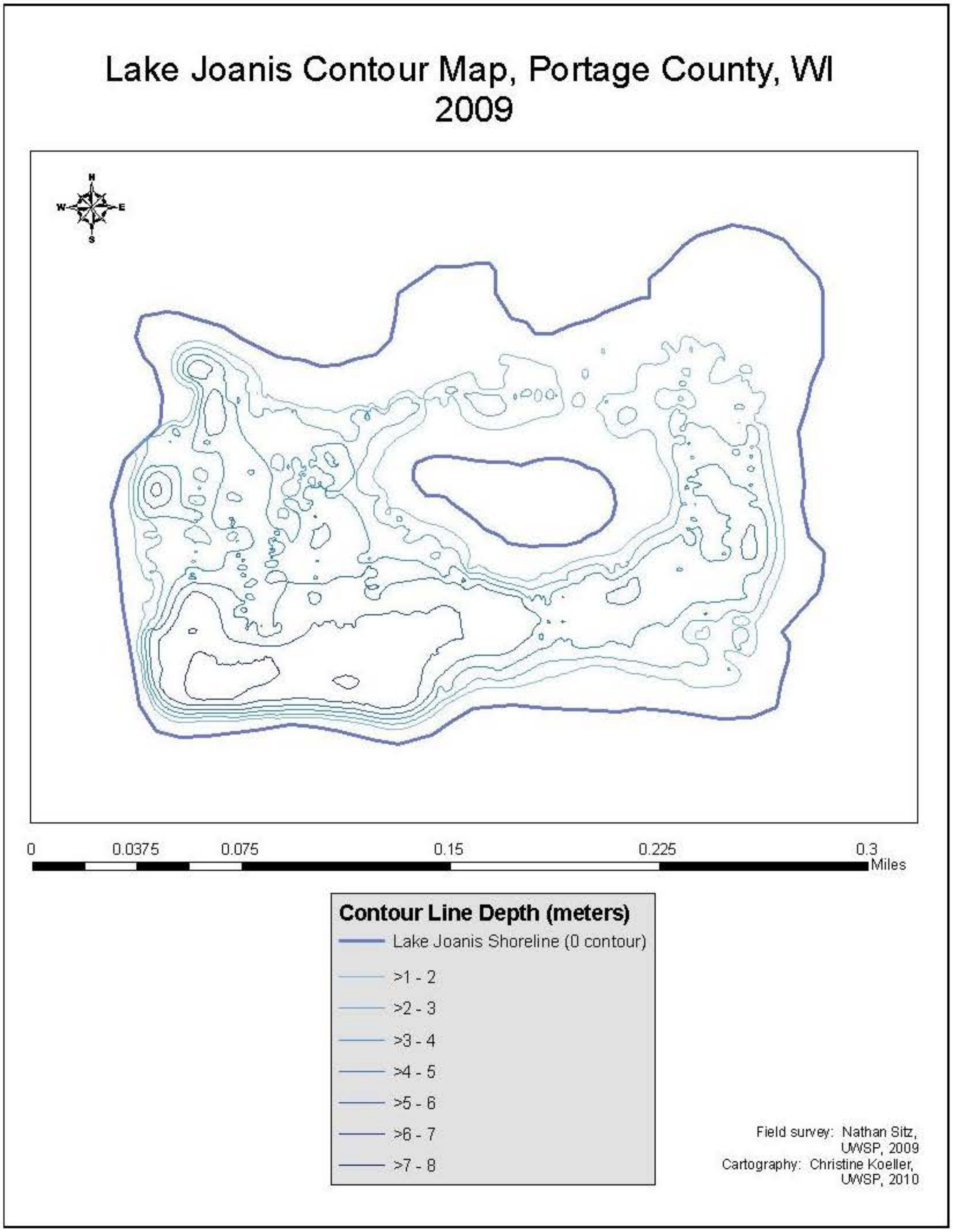
	STATS	Total vegetation	*Myriophyllum spicatum*, Eurasian water milfoil	*Potamogeton crispus*, Curly-leaf pondweed	Chara	Eleocharis acicularis	Elodea canadensis	Juncus canadensis	Lythrum salicaria	Myriophyllum sibiricum	Najas flexilis	Nitella	Phalaris arundinacea	Ponterderia cordata	Potamogeton amplifolius	Potamogeton foliosus	Potamogeton illinoensus	Potamogeton nodosus	Potamogeton pectinatus	Potamogeton pusillus	Potamogeton spirillus	Scirpus validus	Spartina pectinata	Typha angustifolia	Vallisneria americana
Lake Name	Joanis																								
County	Portage																								
WBIC	3000096																								
Survey Date	07/30/08																								
	INDIVIDUAL SPECIES STATS:																								
	Frequency of occurrence within vegetated areas (%)		55.32	2.13	6.38	5.32	2.13	1.06		6.38	18.09	20.21	1.06	1.06	36.17	2.13	6.38		1.06	11.70	2.13		2.13		11.70
	Frequency of occurrence at sites shallower than max depth of plants		49.06	1.89	5.66	4.72	1.89	0.94		5.66	16.04	17.92	0.94	0.94	32.08	1.89	5.66		0.94	10.38	1.89		1.89		10.38
	Relative Frequency (%)		28.7	1.1	3.3	2.8	1.1	0.6		3.3	9.4	10.5	0.6	0.6	18.8	1.1	3.3		0.6	6.1	1.1		1.1		6.1
	Relative Frequency (squared)	0.15	0.08	0.00	0.00	0.00	0.00	0.00		0.00	0.01	0.01	0.00	0.00	0.04	0.00	0.00		0.00	0.00	0.00		0.00		0.00
	Number of sites where species found		52	2	6	5	2	1		6	17	19	1	1	34	2	6		1	11	2		2		11
	Average Rake Fullness		1.85	1.00	1.00	2.00	1.50	2.00		1.00	1.12	1.58	1.00	1.00	1.68	1.00	1.33		1.00	1.18	1.00		1.00		1.00
	#visual sightings		4						3					4	1			1				3		3	1
	present (visual or collected)		present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present	present

SUMMARY STATS:	
Total number of points sampled	106
Total number of sites with vegetation	94
Total number of sites shallower than maximum depth of plants	106
Frequency of occurrence at sites shallower than max depth of plants	88.68
Simpson Diversity Index	0.85
Maximum depth of plants (ft)	23.00
Number of sites sampled using rake on Rope (R)	50
Number of sites sampled using rake on Pole (P)	56
Average number of all species per site (shallower than max depth)	1.71
Average number of all species per site (veg. sites only)	1.93
Average number of native spp per site (shallower than max depth)	1.14
Average number of native spp per site (veg. sites only)	1.55
Species Richness	19
Species Richness (including visuals)	23

Appendix 3.25 Lake Joanis point Intercept macrophyte survey results, 2009. Survey conducted by Amy Thorstenson and Charles Boettcher, UWSP, using standard WDNR point intercept method and sample grid.

	STATS	Total vegetation	*Myriophyllum spicatum*,Eurasian water milfoil	Chara ,Muskgrasses	Eleocharis acicularis,needle spikerush	Elodea canadensis,Common waterweed	Juncus palocarpus f. submersus,Brown-fruited rush	Najas gracillima,Northern naiad	Najas marina,Spiny naiad	Nitella sp.,Nitella	Potamogeton illinoensis,Illinois pondweed	Potamogeton nodosus,Long-leaf pondweed	Potamogeton obtusifolius,Blunt-leaf pondweed	Potamogeton pusillus,Small pondweed	Potamogeton spirillus,Spiral-fruited pondweed	Stuckenia pectinata,Sago pondweed	Vallisneria americana,Wild celerv
Lake Name	Joanis																
County	Portage																
WBIC	3000096																
Survey Date	08/10/09																
	INDIVIDUAL SPECIES STATS:																
	Frequency of occurrence within vegetated areas (%)		77.17	10.87		3.26	2.17	1.09	1.09	21.74	46.74	1.09	2.17	2.17	1.09	1.09	9.78
	Frequency of occurrence at sites shallower than max depth of plants		65.14	9.17		2.75	1.83	0.92	0.92	18.35	39.45	0.92	1.83	1.83	0.92	0.92	8.26
	Relative Frequency (%)		42.5	6.0		1.8	1.2	0.6	0.6	12.0	25.7	0.6	1.2	1.2	0.6	0.6	5.4
	Relative Frequency (squared)	0.27	0.18	0.00		0.00	0.00	0.00	0.00	0.01	0.07	0.00	0.00	0.00	0.00	0.00	0.00
	Number of sites where species found		71	10		3	2	1	1	20	43	1	2	2	1	1	9
	Average Rake Fullness		1.70	1.20		1.00	1.00	1.00	1.00	1.40	1.37	1.00	1.00	1.00	1.00	1.00	1.00
	#visual sightings		1		1						1						1
	present (visual or collected)		present	present	present	present	present	present	present	present	present	present	present	present	present	present	present
	SUMMARY STATS:																
	Total number of points sampled	**109**															
	Total number of sites with vegetation	**92**															
	Total number of sites shallower than maximum depth of plants	**109**															
	Frequency of occurrence at sites shallower than max depth of plants	**84.40**															
	Simpson Diversity Index	**0.73**															
	Maximum depth of plants (ft)	**20.00**															
	Number of sites sampled using rake on Rope (R)	**118**															
	Number of sites sampled using rake on Pole (P)	**0**															
	Average number of all species per site (shallower than max depth)	**1.53**															
	Average number of all species per site (veg. sites only)	**1.82**															
	Average number of native species per site (shallower than max deptl	**0.88**															
	Average number of native species per site (veg. sites only)	**1.41**															
	Species Richness	**14**															
	Species Richness (including visuals)	**15**															

Appendix 3.26 Lake Joanis depth contour mapping.



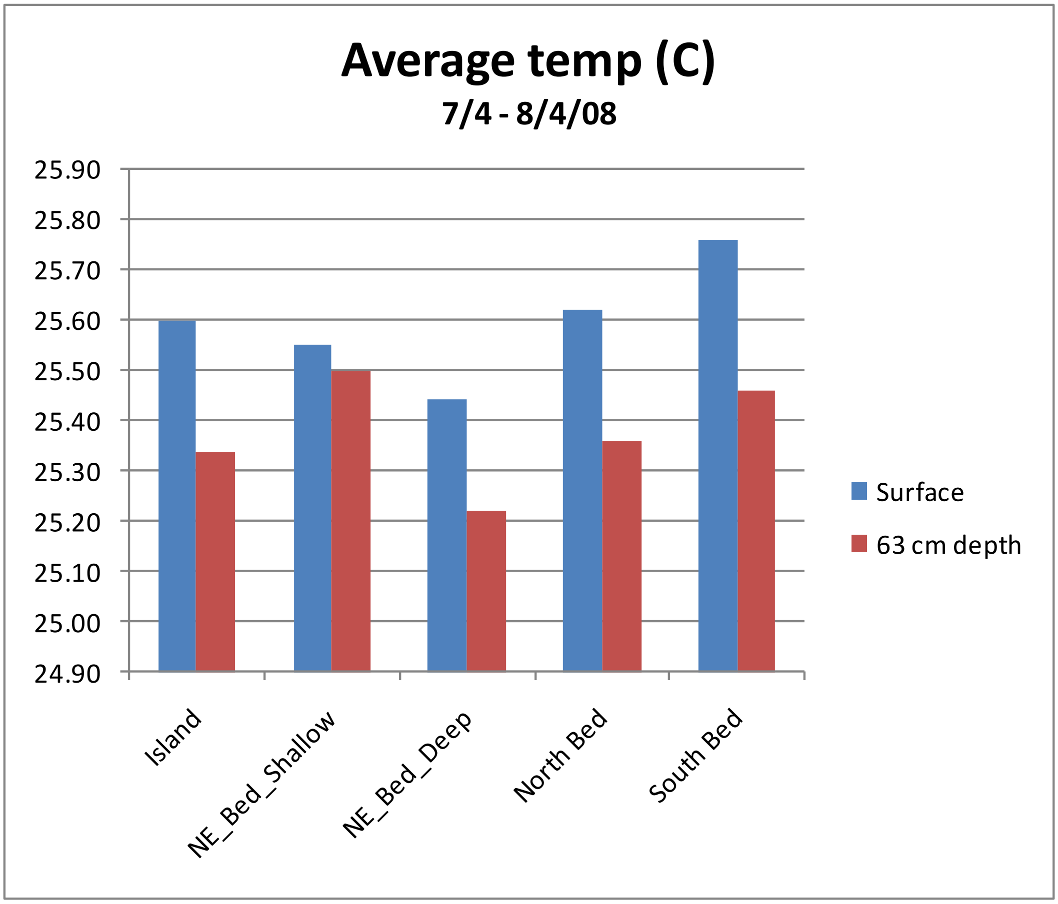
Appendix 3.27 Lake Joanis milfoil bed characterization: sediment and depth data, based on 2008 weevil survey data.

Bed Name	Sediment type average	Depth average (m)	Depth std dev (m)
NW	sand	3.6	1.4
N	sand	1.2	0.6
NE	sand	3.5	0.4
Island	sand	2.7	0.9
SE	sand	2.6	1.3

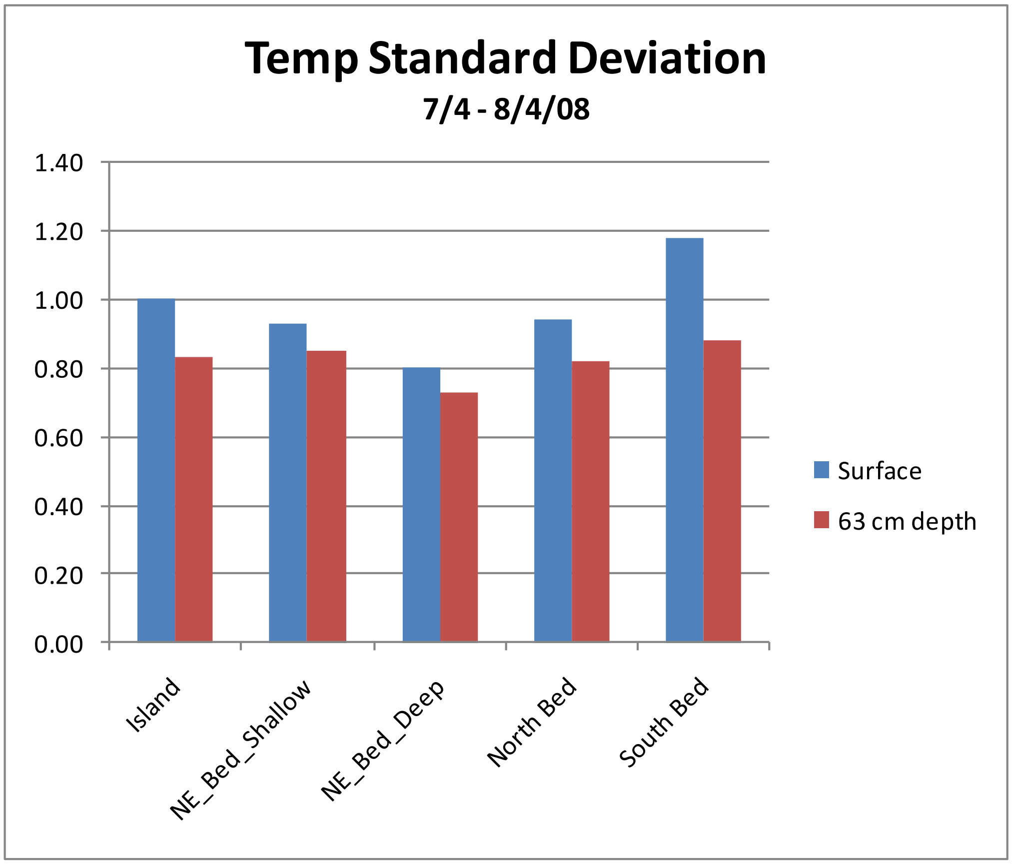
Appendix 3.28 Lake Joanis milfoil bed characterization: water temperature, dissolved oxygen, and secchi depth. All measurements collected during weevil population surveys from center sample point of weevil survey beds.

Sample Date	Bed	Water Temperature (C)	Dissolved Oxygen (mg/L)	Secchi disk depth (m)
6/27/2008	NW	24.8	7.02	6
	N	24.9	9.10	6
	NE	24.9	8.49	5
	Island	23.0	6.89	5
	SE	23.5	7.64	12
7/16-18/2008	NW			
	N			
	NE	25.3		7
	Island	24.9	7.42	7.5
	SE	24.3	8.01	5
8/7/2008	NW	25.7	8.67	18
	N	26.2	7.25	6
	NE	25.3	9.03	4
	Island	25.8	8.73	
	SE			
8/27/2009	NW	14.8	22.20	8.03
	N	4.5	21.80	9.27
	NE	10.0	22.20	7.93
	Island	8.3	22.20	8.13
	SE	9.8	22.20	9.25

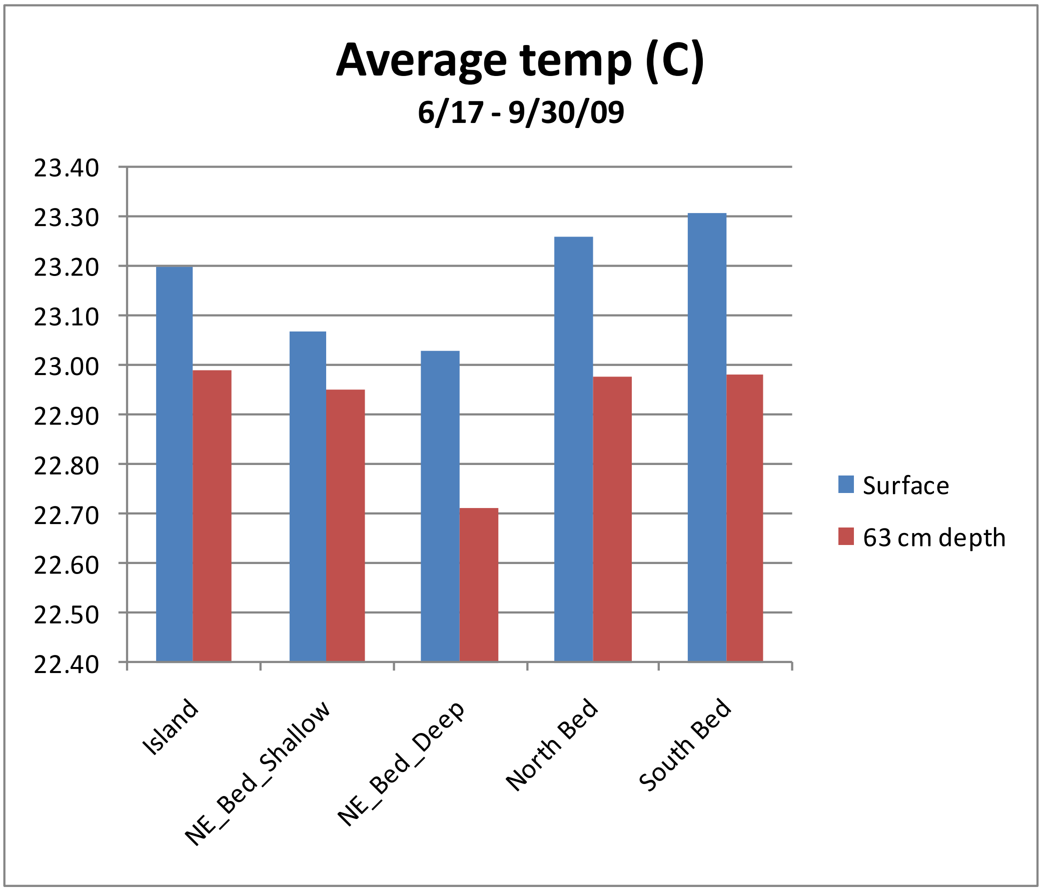
Appendix 3.29 Lake Joanis milfoil bed characterization: seasonal water temperature data, 2008. Data collected from continuously recording thermometers, placed in milfoil beds 1) at milfoil tips, and 2) at 63 cm below milfoil tips.



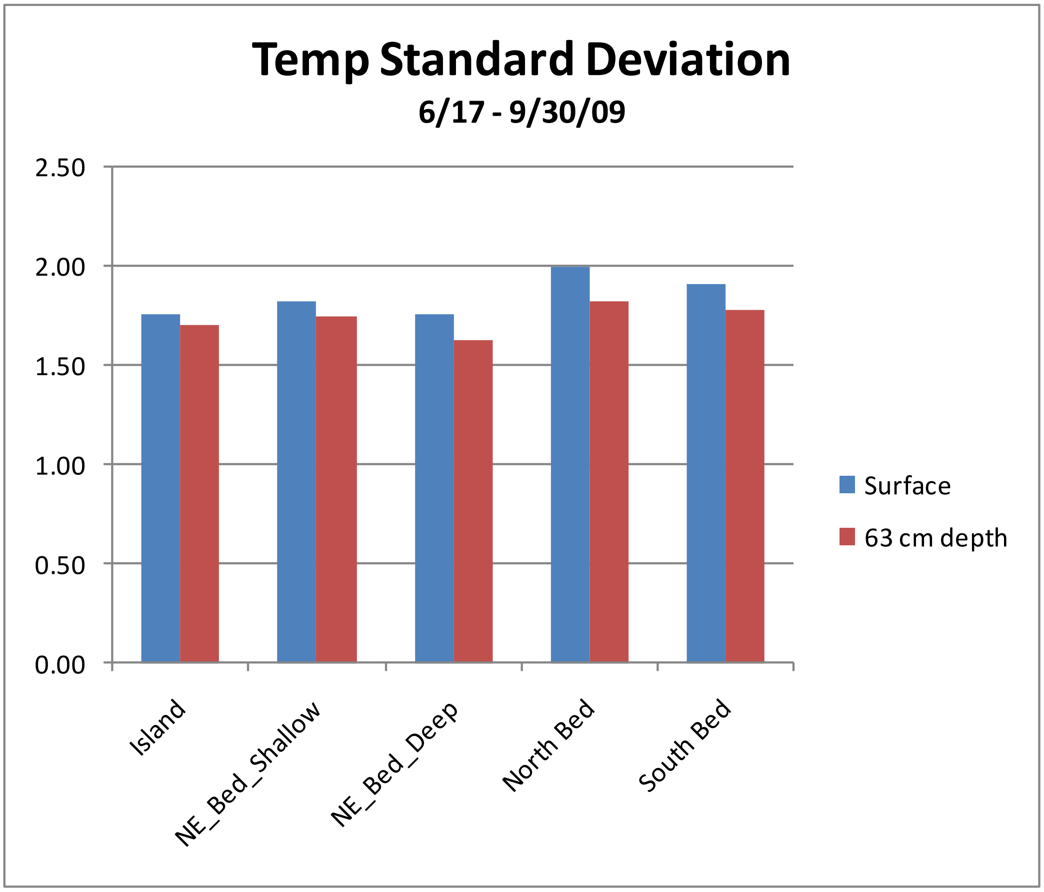
Appendix 3.30 Lake Joanis milfoil bed characterization: seasonal water temperature data, 2008. Data collected from continuously recording thermometers, placed in milfoil beds 1) at milfoil tips, and 2) at 63 cm below milfoil tips.



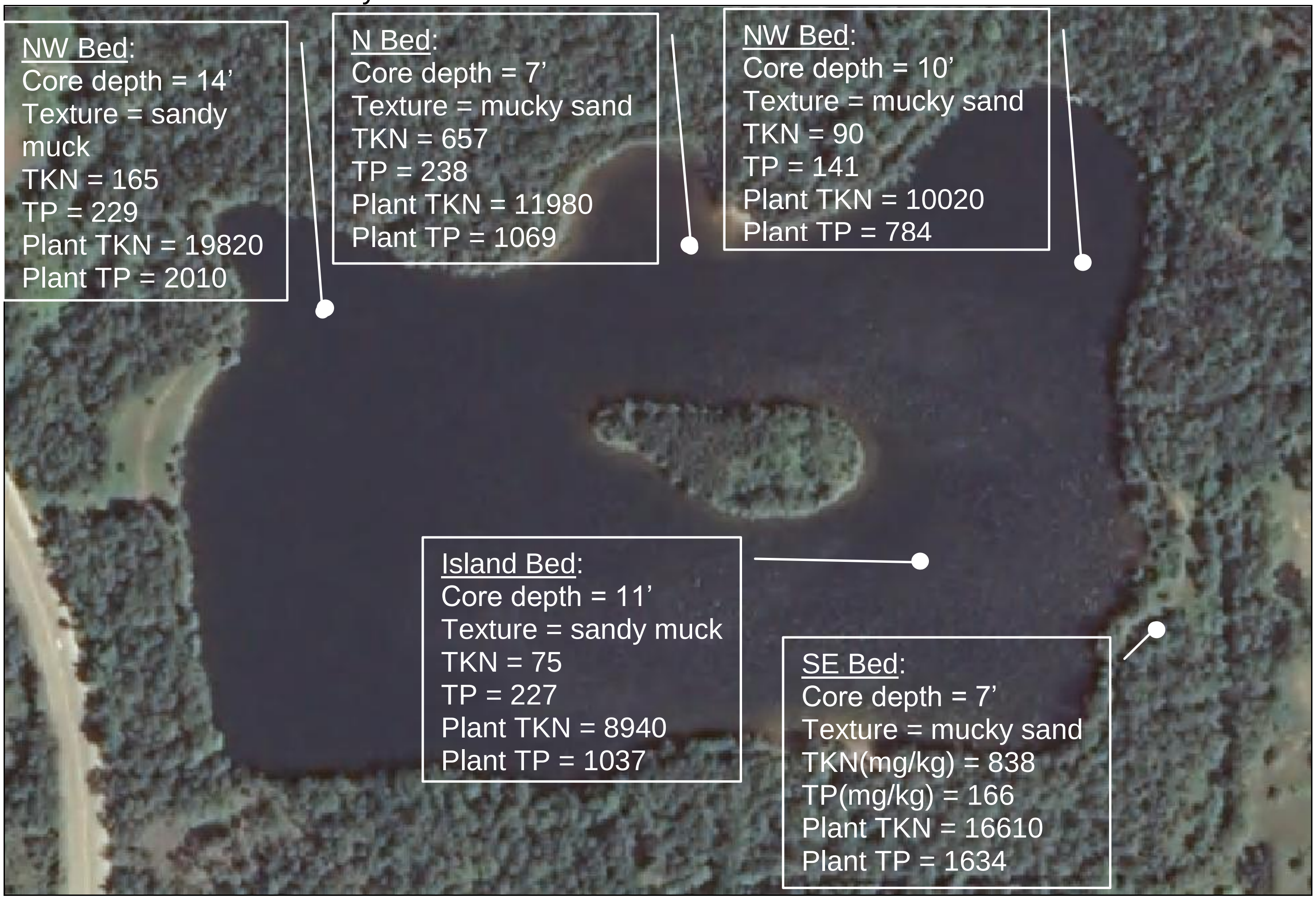
Appendix 3.31 Lake Joanis milfoil bed characterization: seasonal water temperature data, 2009. Data collected from continuously recording thermometers, placed in milfoil beds 1) at milfoil tips, and 2) at 63 cm below milfoil tips. In both 2008 and 2009, the SE Bed appears to exhibit temperatures closest to 29C, the optimal temperature for weevil reproduction. The SE Bed is also the bed where weevil surveys seem to be showing the strongest growth in weevil population densities.



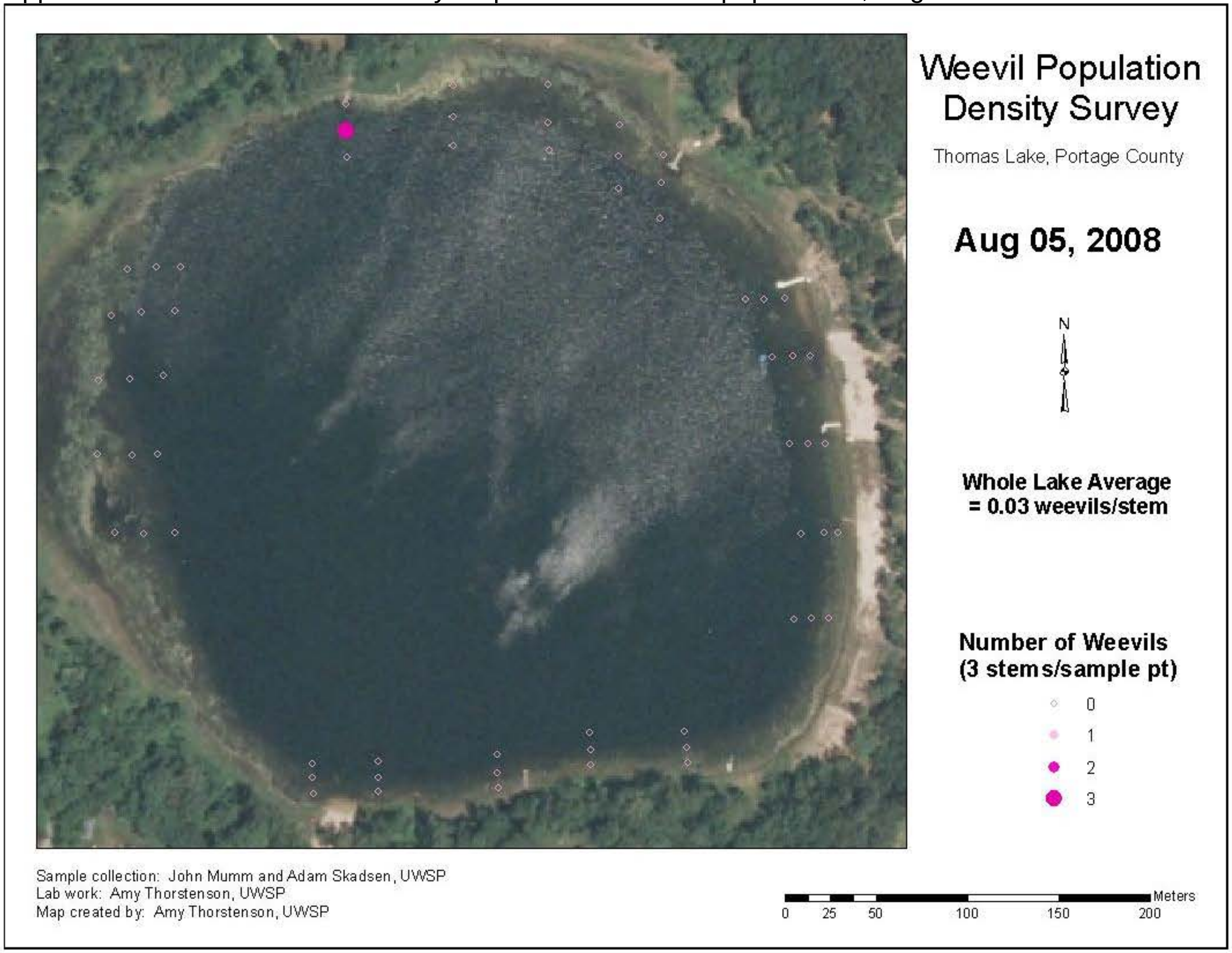
Appendix 3.32 Lake Joanis milfoil bed characterization: seasonal water temperature data, 2009. Data collected from continuously recording thermometers, placed in milfoil beds 1) at milfoil tips, and 2) at 63 cm below milfoil tips.



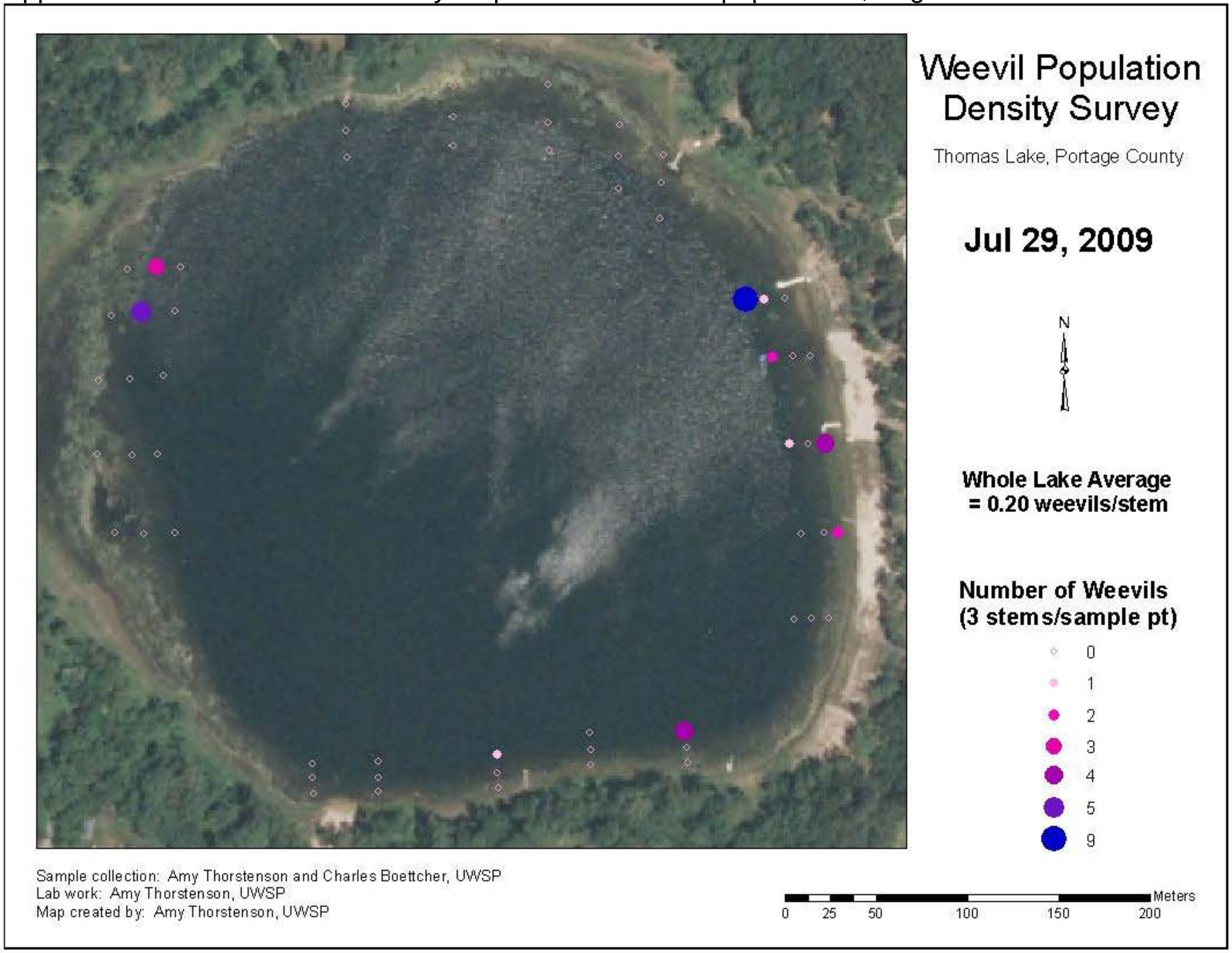
Appendix 3.33 Lake Joanis nutrient analyses results. Sediment sample cores taken 2/25/09 by James Brodzeller and Charles Boettcher, UWSP. Three replicate samples taken per sample point. Reported values are averages (mg/kg). *M. spicatum* plant tissue samples collected 9/5/08 by Amy Thorstenson, UWSP. All sample analyses run by the UWSP Water & Environmental Analysis Lab.



Appendix 3.34 Thomas Lake survey map of natural weevil populations, August 2008.



Appendix 3.35 Thomas Lake survey map of natural weevil populations, August 2009.



Appendix 3.36 Thomas Lake survey of natural weevil populations, August 2008. Samples collected by Adam Skadsen and John Mumm, UWSP. Sample examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected.

Waterbody: **Thomas** COMPARISONS BETWEEN BEDS

Sample Date: 8/5/2008

Whole lake average: 0.03

Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	% Stems w/ Weevil Damage: larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	Average Per Stem: # Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
9/19-9/29	S	0-12	resid/nat	33	17.50	33%	0.00	1.35	0.00	0.03	0.00	0.06	9%	0.00	0.00	0.00	0.00	0.00
9/19-9/29	E	0-12	resid/beach	20	14.80	40%	0.65	1.75	0.00	0.00	0.10	0.05	10%	0.00	0.00	0.00	0.00	0.00
9/19-9/29	N	0-12	resid/nat	30	13.10	63%	0.27	1.80	0.00	0.00	0.00	0.00	0%	0.10	0.00	0.00	0.00	0.10
9/19-9/29	W	0-12	natural	29	16.42	34%	0.19	1.77	0.00	0.00	0.00	0.00	0%	0.00	0.00	0.00	0.00	0.00

Survey Notes: All samples preserved.

Appendix 3.37 Thomas Lake survey of natural weevil populations, August 2009. Samples collected by Amy Thorstenson and Charles Boettcher, UWSP. Stem examination by Amy Thorstenson, UWSP. Three, 63-cm stem samples were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected. The frequency of fused (deformed) milfoil leaflets was also recorded in 2009, to track the spatial distribution of this deformity that may indicate exposure to chemical herbicides.

Waterbody: **Thomas** COMPARISONS BETWEEN BEDS

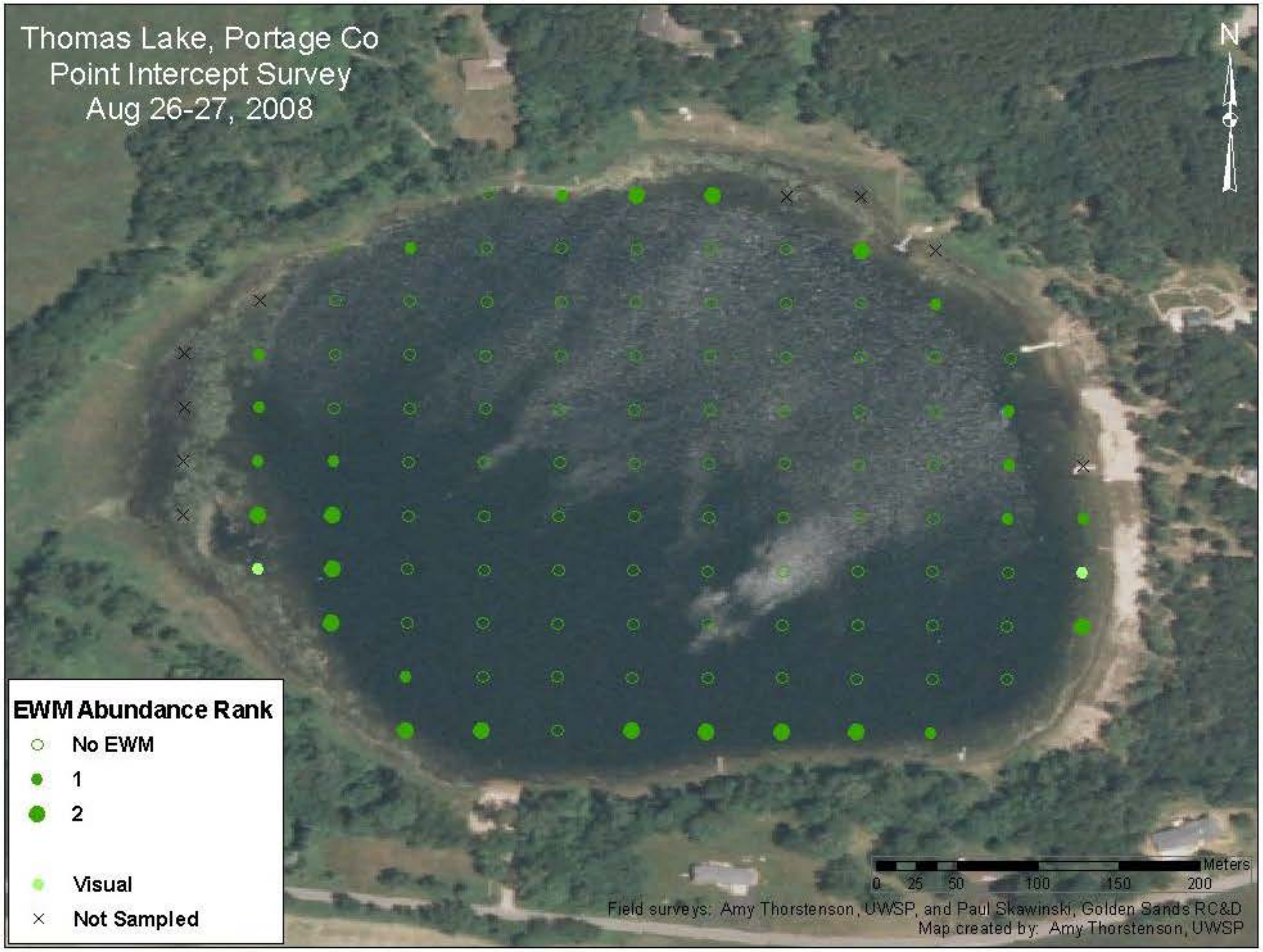
Sample Date: 7/29/2009

Whole lake average: 0.20

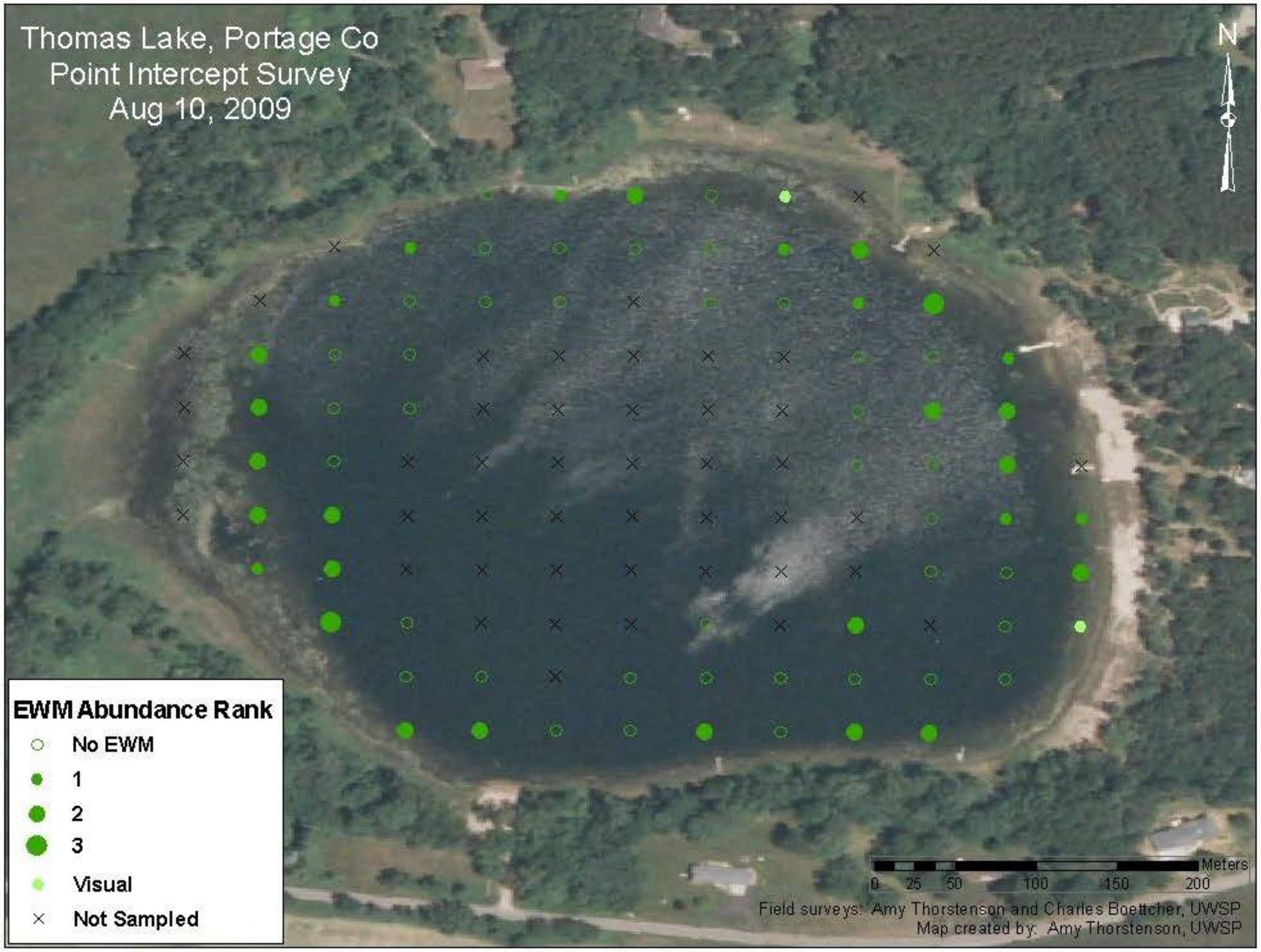
										% Stems w/ Weevil Damage					Average Per Stem				Ave Weevils Per Stem (All Life Stages)
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Fused leaflets	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	
4/20 - 5/10/10	S	0-12	resid/nat	36	21.44	39%	42%	0.19	2.92	0.11	0.00	0.11	0.03	14%	0.06	0.08	0.00	0.00	0.14
10/7/09 - 5/10/10	E	0-12	resid/beach	45	20.11	31%	38%	0.51	3.02	0.36	0.11	0.38	0.16	49%	0.02	0.18	0.18	0.04	0.42
2/18 - 5/7/10	N	0-12	resid/nat	42	19.05	69%	26%	0.00	3.31	0.10	0.02	0.07	0.02	10%	0.00	0.00	0.00	0.00	0.00
2/16 - 5/12/10	W	0-12	natural	39	26.26	59%	56%	0.08	3.67	0.00	0.03	0.05	0.05	5%	0.13	0.00	0.05	0.03	0.21

Survey Notes: All samples preserved.

Appendix 3.38 Thomas Lake point intercept survey map for Eurasian watermilfoil, 2008.



Appendix 3.39 Thomas Lake point intercept survey map for Eurasian watermilfoil, 2009.



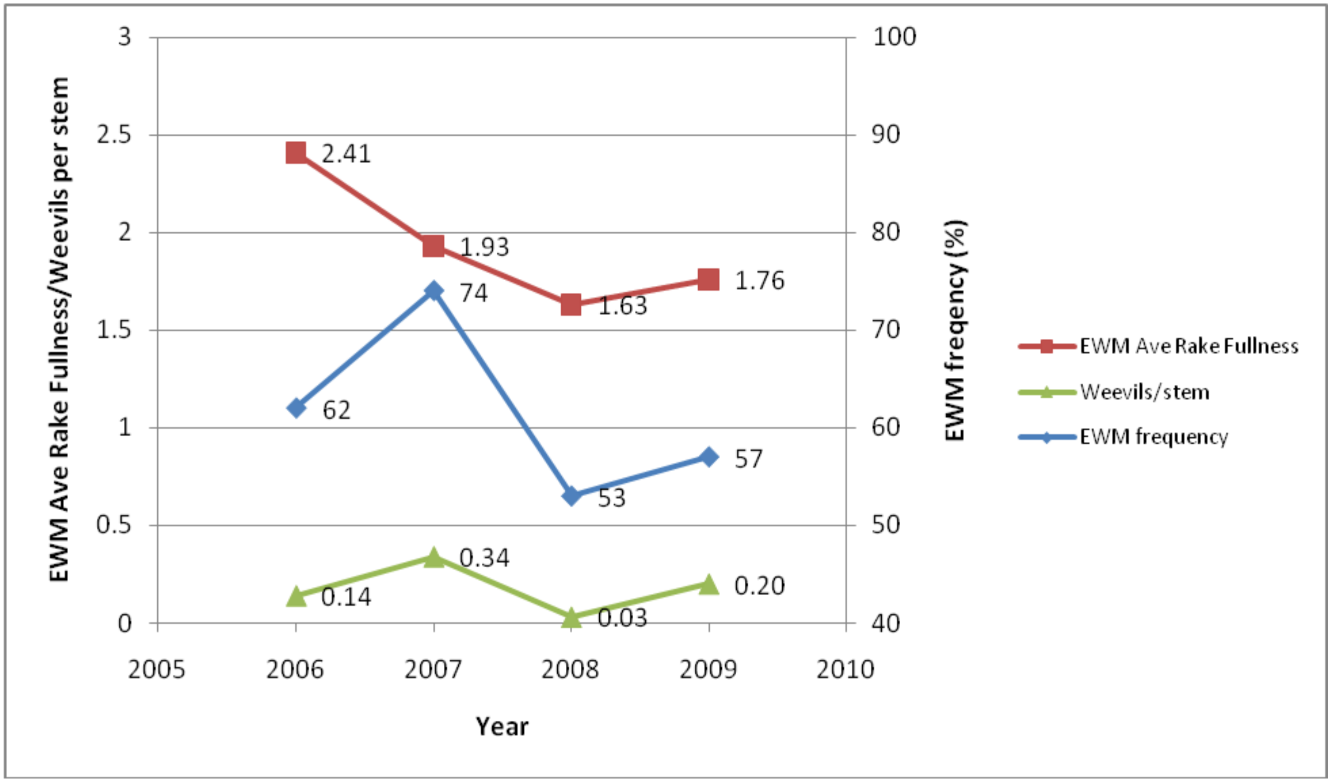
Appendix 3.40 Thomas Lake point Intercept survey result, 2008. Survey conducted by Amy Thorstenson, UWSP, and Paul Skawinski, Golden Sands RC&D Council, Inc., using standard WDNR point intercept method and sample grid.

	STATS	Total vegetation	*Myriophyllum spicatum*, Eurasian water milfoil	*Potamogeton crispus*, Curly-leaf pondweed	filamentous algae	Ceratophyllum demersum, Coontail	Myriophyllum sibiricum, Northern water milfoil	Najas gracillima, Northern naiad	Nymphaea odorata, White water lily	Potamogeton diversifolius, Water-thread pondweed	Potamogeton illinoensis, Illinois pondweed	Potamogeton pusillus, Small pondweed	Potamogeton zosteriformis, Flat-stem pondweed	Stuckenia pectinata, Sago pondweed	Potamogeton robbinsii, Robbins pondweed	Unknown spp. 1
Lake Name	Thomas Lake															
County	Portage															
WBIC	200300															
Survey Date	08/26/08															
	INDIVIDUAL SPECIES STATS:															
	Frequency of occurrence within vegetated areas (%)		52.94	1.96	1.96	86.27	5.88	1.96	17.65	3.92	1.96	1.96	21.57	9.80	21.57	1.96
	Frequency of occurrence at sites shallower than maximum depth of plants		45.00	1.67	1.67	73.33	5.00	1.67	15.00	3.33	1.67	1.67	18.33	8.33	18.33	1.67
	Relative Frequency (%)		22.9	0.8	0.8	37.3	2.5	0.8	7.6	1.7	0.8	0.8	9.3	4.2	9.3	0.8
	Relative Frequency (squared)	0.22	0.05	0.00	0.00	0.14	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00
	Number of sites where species found		27	1	1	44	3	1	9	2	1	1	11	5	11	1
	Average Rake Fullness		1.63	1.00	1.00	1.66	1.00	2.00	1.11	1.50	1.00	2.00	1.55	1.40	1.36	1.00
	#visual sightings															
	present (visual or collected)		present	present	present	present	present	present	present	present	present	present	present	present	present	present
	SUMMARY STATS:															
	Total number of points sampled	**67**														
	Total number of sites with vegetation	**51**														
	Total number of sites shallower than maximum depth of plants	**60**														
	Frequency of occurrence at sites shallower than maximum depth of plants	**85.00**														
	Simpson Diversity Index	**0.78**														
	Maximum depth of plants (ft)	**19.70**														
	Number of sites sampled using rake on Rope (R)	**48**														
	Number of sites sampled using rake on Pole (P)	**14**														
	Average number of all species per site (shallower than max depth)	**1.97**														
	Average number of all species per site (veg. sites only)	**2.31**														
	Average number of native species per site (shallower than max depth)	**1.48**														
	Average number of native species per site (veg. sites only)	**1.88**														
	Species Richness	**14**														
	Species Richness (including visuals)	**14**														

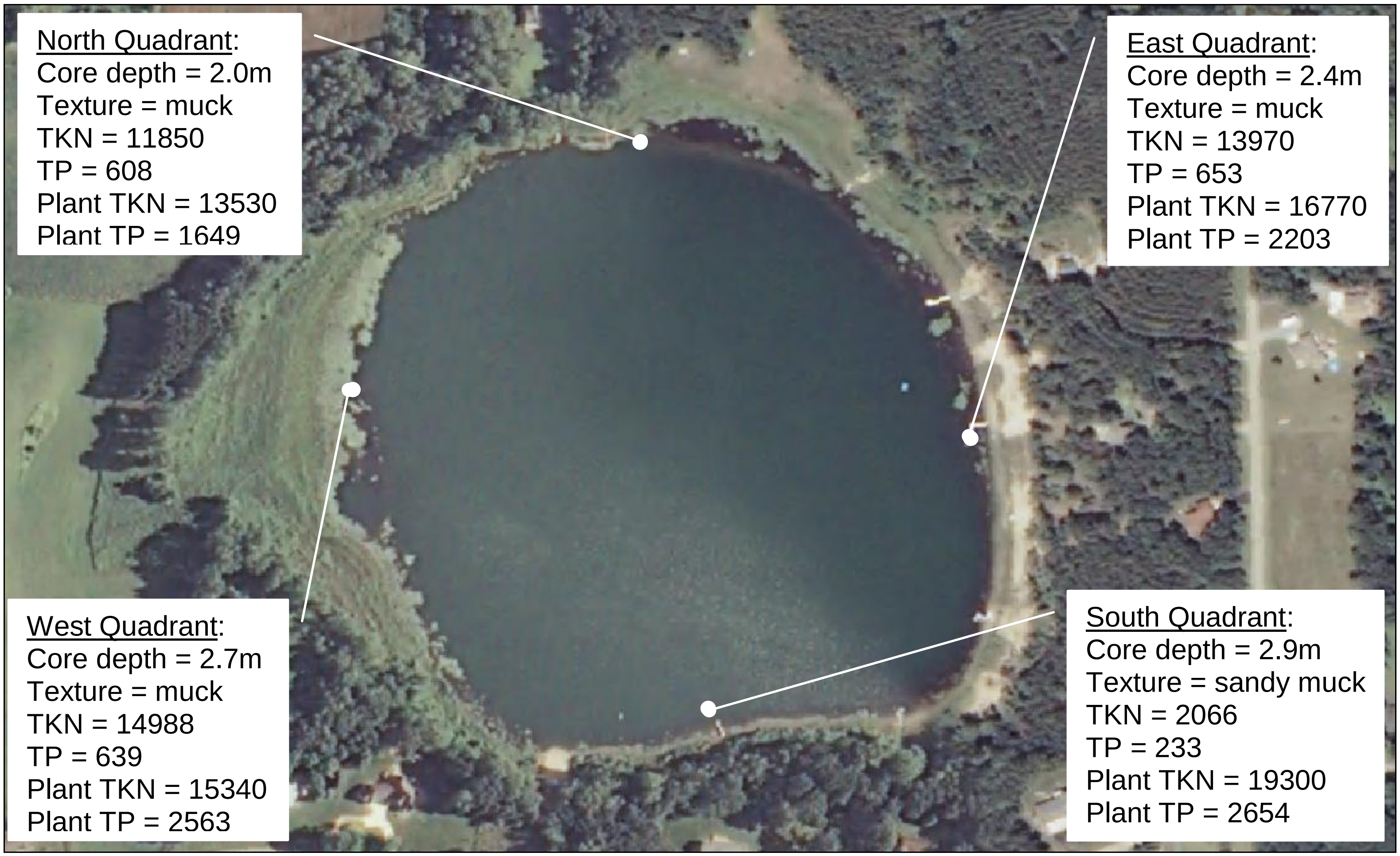
Appendix 3.41 Thomas Lake point Intercept survey results, 2009. Survey conducted by Amy Thorstenson and Charles Boettcher, UWSP, using standard WDNR point intercept method and sample grid.

	STATS	Total vegetation	*Myriophyllum spicatum*, Eurasian water milfoil	*Potamogeton crispus*, Curly-leaf pondweed	filamentous algae	Ceratophyllum demersum,Coontail	Chara ,Muskgrasses	Myriophyllum sibiricum,Northern water milfoil	Najas flexilis,Bushy pondweed	Nymphaea odorata,White water lily	Potamogeton illinoensis,Illinois pondweed	Potamogeton zosteriformis,Flat-stem pondweed	Stuckenia pectinata,Sago pondweed	Typha angustifolia,Narrow-leaved cattail	Potamogeton Pectinatus	Fern Leaf Pondweed
Lake Name	Thomas Lake															
County	Portage															
WBIC	200300															
Survey Date	08/12/09															
	INDIVIDUAL SPECIES STATS:															
	Frequency of occurrence within vegetated areas (%)		56.86	1.96	1.96	68.63	3.92		1.96	9.80	1.96	23.53			3.92	19.61
	Frequency of occurrence at sites shallower than maximum depth of plants		52.73	1.82	1.82	63.64	3.64		1.82	9.09	1.82	21.82			3.64	18.18
	Relative Frequency (%)		29.3	1.0	1.0	35.4	2.0		1.0	5.1	1.0	12.1			2.0	10.1
	Relative Frequency (squared)	0.24	0.09	0.00	0.00	0.12	0.00		0.00	0.00	0.00	0.01			0.00	0.01
	Number of sites where species found		29	1	1	35	2		1	5	1	12			2	10
	Average Rake Fullness		1.76	1.00	1.00	1.43	2.00		1.00	1.00	1.00	1.17			1.00	1.10
	#visual sightings		2			2		2	1	11	2	5	1	3	1	1
	present (visual or collected)		present	present	present	present	present	present	present	present	present	present	present	present	present	present
	SUMMARY STATS:															
	Total number of points sampled	**68**														
	Total number of sites with vegetation	**51**														
	Total number of sites shallower than maximum depth of plants	**55**														
	Frequency of occurrence at sites shallower than maximum depth of plants	**92.73**														
	Simpson Diversity Index	**0.76**														
	Maximum depth of plants (ft)	**17.25**														
	Number of sites sampled using rake on Rope (R)	**70**														
	Number of sites sampled using rake on Pole (P)	**0**														
	Average number of all species per site (shallower than max depth)	**1.80**														
	Average number of all species per site (veg. sites only)	**1.94**														
	Average number of native species per site (shallower than max depth)	**1.24**														
	Average number of native species per site (veg. sites only)	**1.57**														
	Species Richness	**11**														
	Species Richness (including visuals)	**14**														

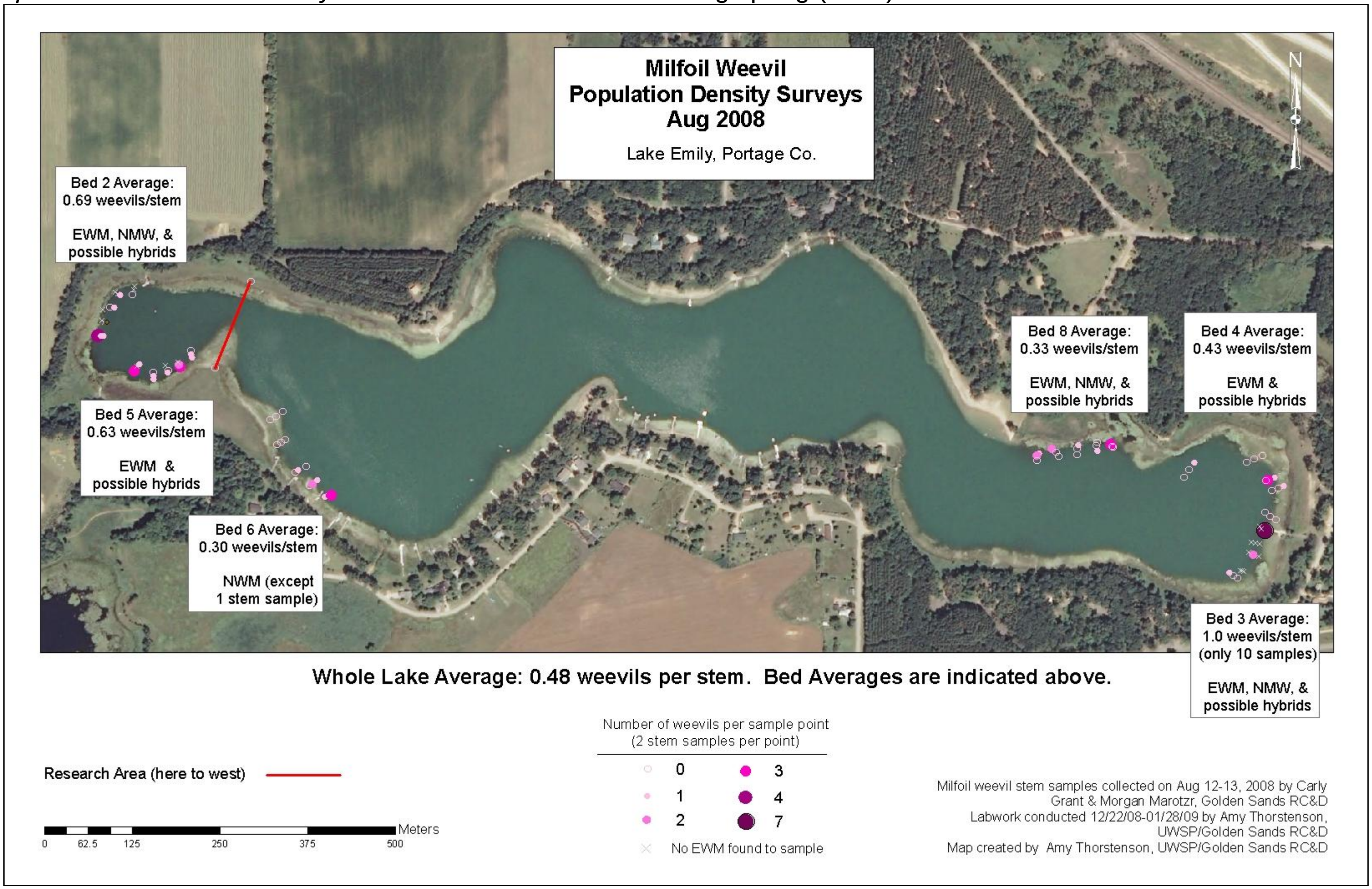
Appendix 3.42 Lake Thomas trends in EWM and milfoil weevil populations over time. Milfoil and weevil trends are graphed here, utilizing historical data as far back as 2006. Note: Weevil surveys in 2006 and 2007 conducted by random stem collection, surveys in 2008 and 2009 conducted by transect method. Weevil populations appeared to have crashed as milfoil crashed. This is how the predator-prey relationship would be expected to respond together (pers. observations, Lillie 2000).



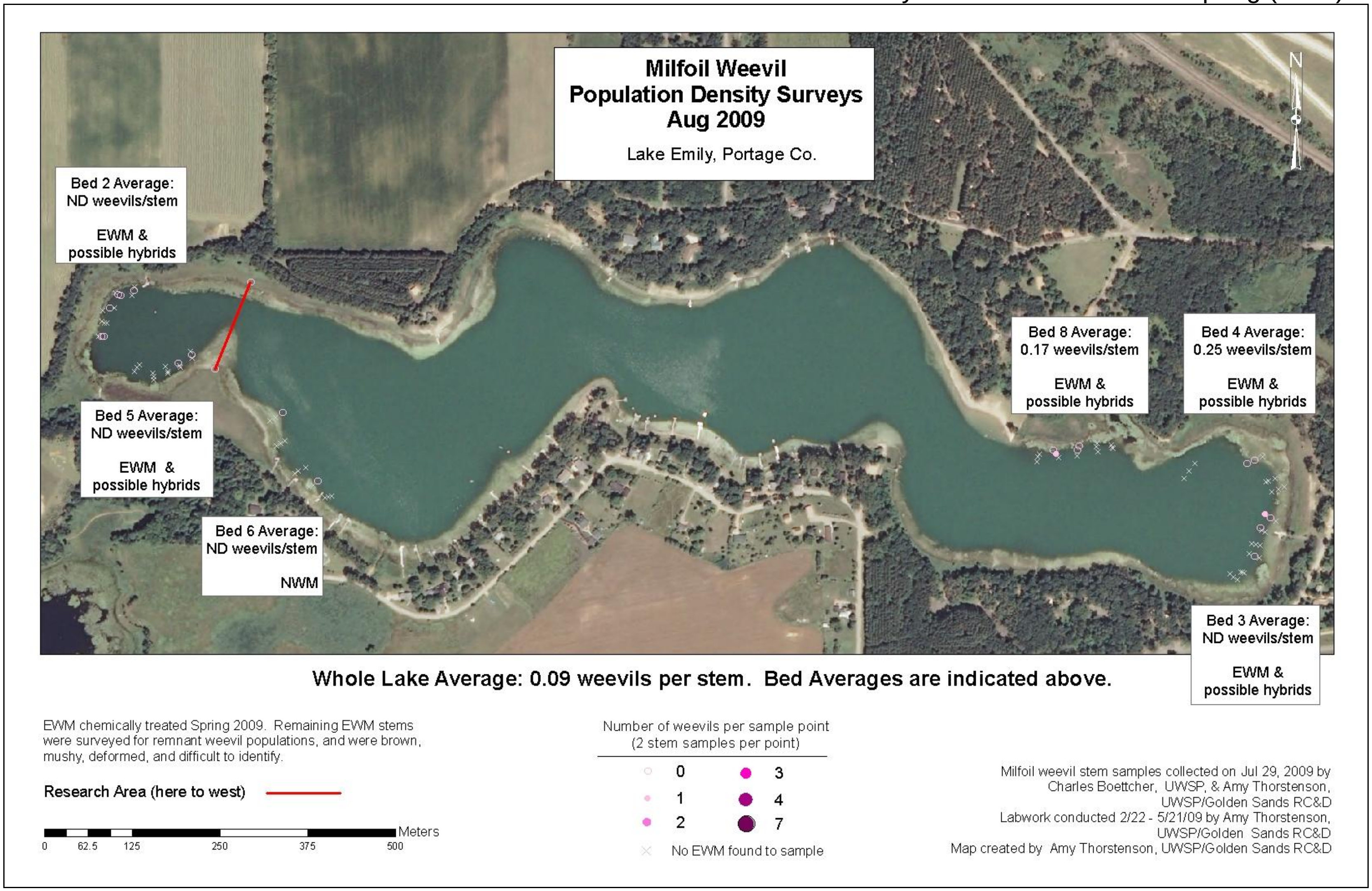
Appendix 3.43 Thomas Lake nutrient survey results. All results reported in mg/kg (ppm). Sediment sample cores taken 2/27/09 by James Brodzeller, Charles Boettcher, and Amy Thorstenson, UWSP. Three replicate samples taken per sample point. Reported values are averages. *M. spicatum* plant tissue samples collected 9/5/08 by Amy Thorstenson, UWSP. All sample analyses run by the UWSP Water & Environmental Analysis Lab.



Appendix 3.44 Lake Emily survey map of natural weevil population densities, August 2008. A chemical treatment of *M. spicatum* was conducted by the lake association the following spring (2009).



Appendix 3.45 Lake Emily survey map of natural weevil population densities, August 2009. These surveys sampled the residual milfoil stems that remained after the chemical treatment conducted by the lake association in spring (2009).



Appendix 3.46 Lake Emily survey of natural weevil population densities, August 2008. Stem samples collected by Carly Grant and Morgan Marotz, UWSP. Three stem samples 63-cm in length were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected. A chemical treatment of *M. spicatum* was conducted by the lake association the following spring (2009).

Waterbody: **Emily** COMPARISONS BETWEEN BEDS

Sample Date: 8/12 - 8/13/2008 Whole lake average: 0.48

									% Stems w/ Weevil Damage					Average Per Stem				
Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	# Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
1/21/09	2	3-11	nat/resid	13	19.69	15%	0.92	3.77	0.62	0.15	0.77	0.00	77%	0.15	0.38	0.08	0.08	0.69
1/21/09	3	4-11	natural	10	17.80	60%	0.30	1.80	0.60	0.20	0.60	0.60	70%	0.40	0.10	0.50	0.00	1.00
12/18/08	4	4-13	nat/park	30	19.87	23%	0.30	2.90	0.23	0.03	0.30	0.13	33%	0.20	0.23	0.00	0.00	0.43
12/18/08	5	3-13	natural	24	19.63	8%	0.88	2.88	0.71	0.21	0.71	0.50	83%	0.17	0.38	0.04	0.04	0.63
12/22/08	6	4-11	resid	30	19.13	17%	0.97	2.67	0.37	0.17	0.47	0.27	53%	0.10	0.13	0.03	0.03	0.30
12/22/08	8	3-14	nat/beach	30	19.70	7%	0.50	3.03	0.53	0.13	0.57	0.13	60%	0.00	0.30	0.00	0.03	0.33

Survey Notes: All samples preserved.

Appendix 3.47 Lake Emily survey of natural weevil population densities, August 2009. Stem samples collected by Amy Thorstenson and Charles Boettcher, UWSP. A maximum of three stem samples 63-cm in length were taken per sample point. Whole Lake Average based on total number of weevils (all life stages) divided by total number of stem samples collected. These surveys sampled residual milfoil stems remaining after the chemical treatment of M. spicatum conducted by the lake association in spring (2009). Residual stems were scarce and in poor condition.

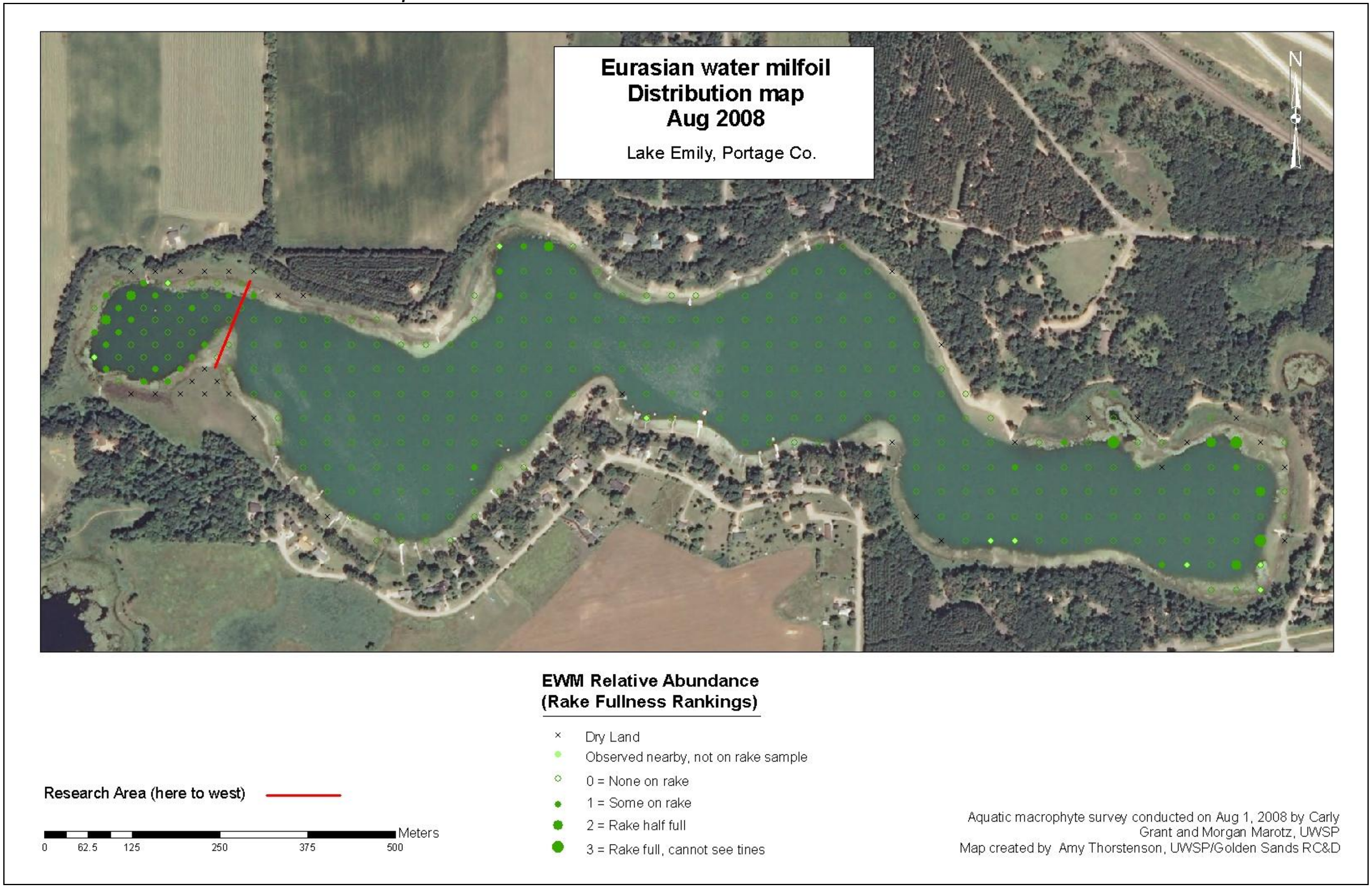
Waterbody: **Emily** COMPARISONS BETWEEN BEDS

Sample Date: 7/29/2009 Whole lake average: 0.09

Lab Date	Bed No.	Depth Range (ft)	Land Cover @ Shore	Total Stems	Ave Length (in)	Ave Algae Covered	Ave # Broken Tips	Ave # Apical Tips	% Stems w/ Weevil Damage: larval pinholes present	pupal holes present	larval tunnels present	damaged meristem present	% Stems w/ Damage	Average Per Stem: # Eggs	# Larvae	# Pupae	# Adults	Ave Weevils Per Stem (All Life Stages)
2/22/10	2	3-11	nat/resid	8	20.00	0%	1.00	3.00	13%	13%	0%	0%	13%	0.00	0.00	0.00	0.00	0.00
5/21/10	3	4-11	natural	1	0.00	0%	0.00	0.00	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
5/21/10	4	4-13	nat/park	4	8.00	0%	1.00	3.00	0%	0%	0%	0%	0%	0.00	0.25	0.00	0.00	0.25
2/22/10	5	3-13	natural	2	12.00	0%	0.00	2.00	0%	50%	0%	0%	50%	0.00	0.00	0.00	0.00	0.00
2/22/10	6	4-11	resid	2	6.00	0%	1.00	1.00	0%	0%	0%	0%	0%	0.00	0.00	0.00	0.00	0.00
3/1/10	8	3-14	nat/beach	6	12.00	0%	0.50	1.50	0%	0%	0%	0%	0%	0.00	0.17	0.00	0.00	0.17

Survey Notes: All samples preserved. Residual EWM stems could not be found at every sample point. Only 1 or 2 stems could be found at some sample points. Stems were darkened, malformed, and generally in poor condition from the chemical treatment in spring.

Appendix 3.48 Lake Emily point intercept survey map, August 2008. Survey grid density was doubled in the Study Bay to better define the extent of the *M. spicatum* bed.



Appendix 3.49 Lake Emily point intercept survey map, August 2009. A chemical treatment of *M. spicatum* was conducted by the lake association spring (2009). No residual *M. spicatum* beds were found anywhere in the lake during August PI surveys, even though the survey grid density was doubled in the Study Bay to improve detection of *M. spicatum*.

Appendix 3.50 Lake Emily point Intercept macrophyte survey results, 2008. Surveys conducted by Carly Grant and Morgan Marotz, UWSP, using standard WDNR point intercept method and sample grid. A chemical treatment of *M. spicatum* was conducted by the lake association the following spring (2009).

	STATS	Total vegetation	Myriophyllum spicatum ,Eurasian water mil	Potamogeton crispus ,Curly-leaf pondweed	filamentous algae	Ceratophyllum demersum,Coontail	Chara ,Muskgrasses	Elodea canadensis,Common waterweed	Myriophyllum sibiricum,Northern water milfoil	Najas flexilis,Bushy pondweed	Nitella sp.,Nitella	Nymphaea odorata,White water lily	Potamogeton gramineus,Variable pondweed	Potamogeton illinoensis,Illinois pondweed	Potamogeton zosteriformis,Flat-stem pondweed	Stuckenia pectinata,Sago pondweed	Vallisneria americana,Wild celery	sp1 - Blue green algae
Lake Name	Lake Emily																	
County	Portage																	
WBIC	189800																	
Survey Date	08/01/08																	
	INDIVIDUAL SPECIES STATS:																	
	Frequency of occurrence within vegetated areas (%)		18.67		1.33	6.00	76.00	1.33	2.67	8.00	4.00	4.00	9.33	2.00	8.67	8.00	32.67	5.33
	Frequency of occurrence at sites shallower than maximum depth of plants		14.58		1.04	4.69	59.38	1.04	2.08	6.25	3.13	3.13	7.29	1.56	6.77	6.25	25.52	4.17
	Relative Frequency (%)		9.93		0.71	3.19	40.43	0.71	1.4	4.3	2.1	2.1	5.0	1.1	4.6	4.3	17.4	2.8
	Relative Frequency (squared)	0.21	0.01		0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
	Number of sites where species found		28		2	9	114	2	4	12	6	6	14	3	13	12	49	8
	Average Rake Fullness		1.43		1.50	1.11	1.39	1.00	1.25	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.10	1.13
	#visual sightings		7						1				3		1	1		
	present (visual or collected)		present		present	present	present	present	present	present	present	present	present	present	present	present	present	present

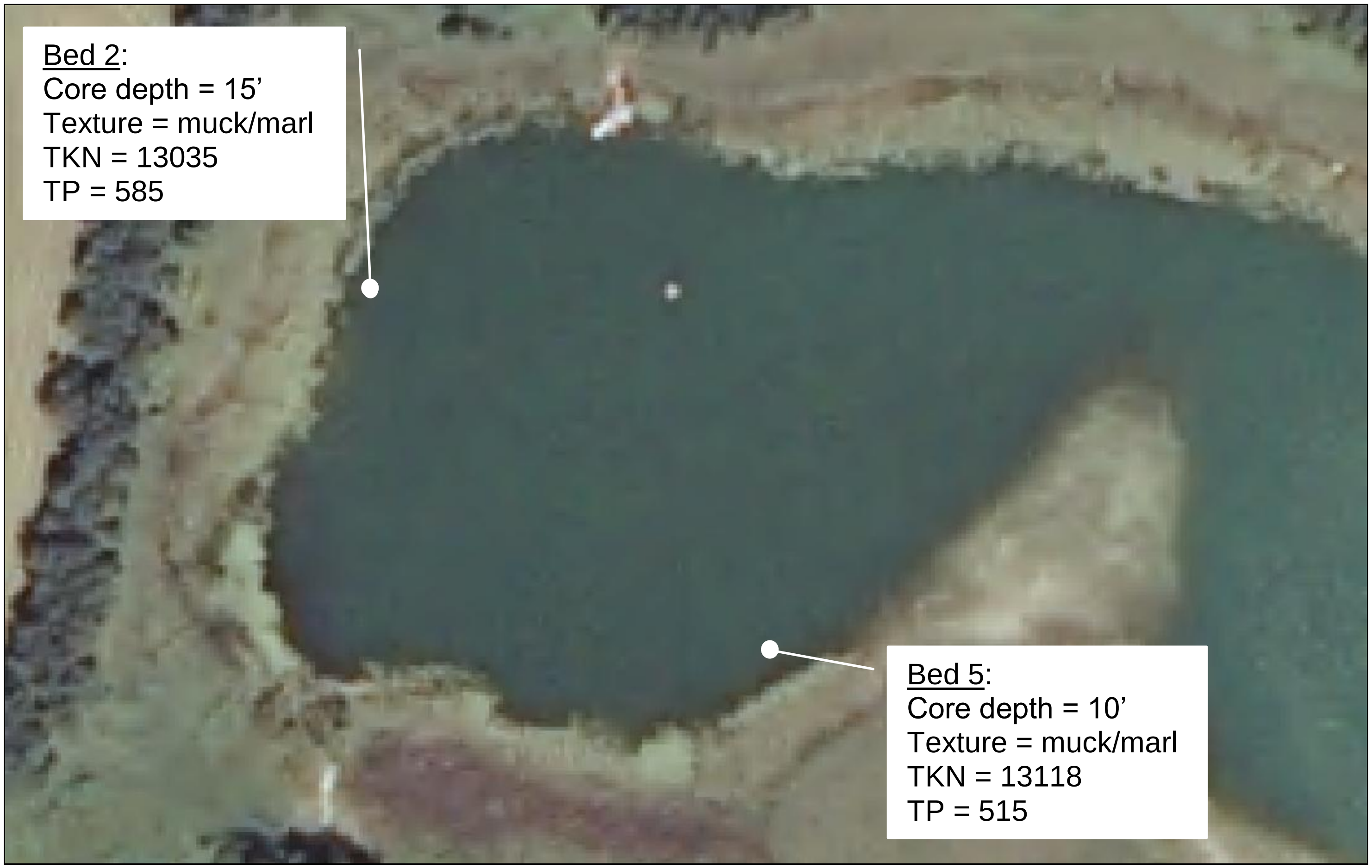
	SUMMARY STATS:	
	Total number of points sampled	**194**
	Total number of sites with vegetation	**150**
	Total number of sites shallower than maximum depth of plants	**192**
	Frequency of occurrence at sites shallower than maximum depth of plants	**78.13**
	Simpson Diversity Index	**0.79**
	Maximum depth of plants (ft)	**9.00**
	Number of sites sampled using rake on Rope (R)	**351**
	Number of sites sampled using rake on Pole (P)	**0**
	Average number of all species per site (shallower than max depth)	**1.42**
	Average number of all species per site (veg. sites only)	**1.78**
	Average number of native species per site (shallower than max depth)	**1.26**
	Average number of native species per site (veg. sites only)	**1.67**
	Species Richness	**15**
	Species Richness (including visuals)	**15**

Appendix 3.51 Lake Emily point Intercept macrophyte survey results, 2009. Surveys conducted by Amy Thorstenson and Charles Boettcher, UWSP, using standard WDNR point intercept method and sample grid. A chemical treatment of *M. spicatum* was conducted by the lake association in spring (2009).

	STATS	Total vegetation	*Myriophyllum spicatum* ,Eurasian water milfo	*Potamogeton crispus* ,Curly-leaf pondweed	Ceratophyllum demersum,Coontail	Chara ,Muskgrasses	Najas flexilis,Bushy pondweed	Nitella sp.,Nitella	Nymphaea odorata,White water lily	Potamogeton friesii,Frie's pondweed	Potamogeton illinoensis,Illinois pondweed	Potamogeton pusillus,Small pondweed	Potamogeton zosteriformis,Flat-stem pondweed	Schoenoplectus acutus,Hardstem bulrush	Schoenoplectus tabernaemontani,Softstem bulrush	Stuckenia pectinata,Sago pondweed	Utricularia vulgaris,Common	Vallisneria americana,Wild celery
Lake Name	Emily																	
County	Portage																	
WBIC	189800																	
Survey Date	08/17/09																	
	INDIVIDUAL SPECIES STATS:																	
	Frequency of occurrence within vegetated areas (%)			0.69	6.21	73.10	20.69	21.38	3.45	0.69	10.34		14.48			0.69	0.69	32.41
	Frequency of occurrence at sites shallower than maximum depth of plants			0.60	5.42	63.86	18.07	18.67	3.01	0.60	9.04		12.65			0.60	0.60	28.31
	Relative Frequency (%)			0.4	3.4	39.6	11.2	11.6	1.9	0.4	5.6		7.8			0.4	0.4	17.5
	Relative Frequency (squared)	0.22		0.00	0.00	0.16	0.01	0.01	0.00	0.00	0.00		0.01			0.00	0.00	0.03
	Number of sites where species found			1	9	106	30	31	5	1	15		21			1	1	47
	Average Rake Fullness			2.00	1.22	1.60	1.10	1.81	1.20	1.00	1.00		1.00			1.00	1.00	1.26
	#visual sightings					5	1		8		3	1	2	1	3			3
	present (visual or collected)			present	present	present	present	present	present	present	present	present	present	present	present	present	present	present

SUMMARY STATS:	
Total number of points sampled	**166**
Total number of sites with vegetation	**145**
Total number of sites shallower than maximum depth of plants	**166**
Frequency of occurrence at sites shallower than maximum depth of plants	**87.35**
Simpson Diversity Index	**0.78**
Maximum depth of plants (ft)	**23.50**
Number of sites sampled using rake on Rope (R)	**166**
Number of sites sampled using rake on Pole (P)	**0**
Average number of all species per site (shallower than max depth)	**1.61**
Average number of all species per site (veg. sites only)	**1.85**
Average number of native species per site (shallower than max depth)	**1.61**
Average number of native species per site (veg. sites only)	**1.84**
Species Richness	**12**
Species Richness (including visuals)	**15**

Appendix 3.52 Lake Emily nutrient analyses results (study Bay only). Sediment sample cores taken 1/12/09 by James Brodzeller, Charles Boettcher, and Amy Thorstenson, UWSP. Three replicate samples taken per sample point. Reported values are averages (mg/kg). Sample analyses run by the UWSP Water & Environmental Analysis Lab.



Appendix 3.53 Results of DNA testing of milfoil beds on Lake Joanis, Thomas Lake, and Lake Emily. Milfoil stem samples collected by Amy Thorstenson, UWSP, during 2009 aquatic macrophyte surveys. DNA analysis by Grand Valley State University, Michigan, MI.

Lake	Species	Sample collection
Lake Joanis	*M. spicatum*	8/10/09
Thomas Lake	*M. spicatum*	8/12/09
Lake Emily	Hybrid	8/17/09

Appendix 3.54 Management decision tree for using milfoil weevils and other methods to control Eurasian watermilfoil.