Project LPL-771-01

Effects of Sudden Exposure to Elevated pH on Milfoil Weevil Euhrychiopsis lecontei in Forest Lake
Fond du Lac County, Wisconsin

prepared for:
Wisconsin Department of Natural Resources

by:
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Forest Lake
Improvement Association, Inc

ACKNOWLEDGEMENTS

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Appreciation is extended to EnviroScience, Inc. for their continued efforts to better understand how Forest Lake's water impacts the biological control process and search for solutions; particularly, Heather Zakrajsek and Kerry Kline, the ES biologists, for providing repeated valued consultations, analysis and analytical services, making this research a feasible worthwhile effort.

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INTRODUCTION

Forest Lake is located Fond du Lac County, Wisconsin. Forest Lake is a mesotrophic seepage lake with no inlets or outlets. The lake has a surface area of 50 acres, a water volume of 552 acre feet, a mean depth of 11 feet, a maximum depth of 32 feet. and a watershed area of 0.25 square miles.

According to the Water Quality Index (Lillie and Mason 1983), the surface total phosphorous concentrations indicate a "good" water quality, and Seechi depths and chlorophyll a concentrations indicate a "very good" water quality (Source: 1994-1995-1996 USGS Water Quality Monitoring).

Twenty-five (25) percent of the littoral zone of Forest Lake is heavily infested with Eurasian Watermilfoil (EWM). Studies have shown that biological control of EWM, using the milfoil weevil, Euhrychiopsis lecontei, has been successfully accomplished in selected lakes in Wisconsin, Minnesota, Michigan, and Vermont.

During the summers of 1999 and 2000, a total of 28,000 weevils, E. lecontei, were stocked by EnviroScience, Inc., Ohio, into Forest Lake in the life form of eggs and larvae. The eggs and larvae were contained within healthy disease-free milfoil cuttings, which were attached by wire twists to the tips of Forest Lake EWM plants. Introductions were made into four (4) milfoil areas, and an additional site was set aside as a control area.

Follow-up surveys conducted during the late summers of 1999 and 2000 failed to produce evidence of any life stages of weevils or any damage to milfoil plants. Since weevil disappearance and lack of milfoil damage occurred before the overwintering cycle, inadequate winter survival is not considered a reason for the poor performance.

A three-year Water Quality Monitoring Survey was conducted by the U.S. Geological Survey (USGS) from 1994 to 1996. The USGS survey indicated that the **pH readings taken in the center of the lake were exceptionally high (9.3)**. The high pH readings were considered worthy of investigation as a possible cause of the failure of the milfoil weevils to become established in Forest Lake. The research project to study the effects of Forest Lake's high pH water on the milfoil weevil and on the biological control process was initiated June, 2001.

PROJECT GOALS

Investigate whether ***sudden*** exposure of weevil eggs and larvae to elevated pH is the limiting factor preventing successful biological control of Eurasian watermilfoil.

Goals included:

- Weekly measurement of the pH and temperature in Forest Lake within a transected heavily infested milfoil bed and the lake's center throughout the growing season.
- Weekly lab analysis of weevils in all life forms after their introduction into Forest Lake and lab analysis of watermilfoil damage by the introduced weevils.
- Effect of Forest Lake water and milfoil on weevil reproduction (egg laying).

METHODS AND PROCEDURES

LAKE pH AND TEMPERATURE MEASUREMENTS

Measurements were made in the field to prevent chemical changes during transport for lab testing. Readings were made with a battery powered Oakton pH, conductivity, temperature meter, calibrated weekly with 7.01 and 10.01 pH buffer solutions. To facilitate the penetration into the dense milfoil beds, the meter probe was mounted within a perforated, graduated 7 ft. long, 1 ¼" diameter rigid PVC tube, which slides snuggly through a 12"x12"x2" polyethylene foam block to facilitate depth settings (see photos on pages 4 and 5).

Measurements were taken along three transects, identified by buoys, in heavily infested areas and in the center of the lake. Then pH and temperature readings were taken weekly at 1 ½ ft., 3 ft., 5 ft. and 7 ft. depth increments from May 18, 2001 to September 29, 2001.

WEEVIL INTRODUCTION AND REMOVAL FOR LAB ANALYSIS (Figure 1)

Two thousand (2000) weevils (egg and larvae form) were introduced on June 2, 2001 into the transected 5000 m^2 milfoil area. Introductions were made in the form of milfoil bundles consisting of 10 to 12 stems, each stem with 5 to 7 weevil eggs on their meristems. Bundles were held together with twist ties, which were also used to attach each bundle to existing milfoil plants in Forest Lake. Each week, six (6) bundles and the milfoil they are attached to were cut and removed from the lake, and shipped to EnviroScience Lab in Ohio for laboratory analysis. Bright red ribbons were attached to each bundle to aid in locating them. The Forest Lake plants were cut to a length of > 1.0 meters to ensure capturing any larvae and pupae life forms. Overnight shipments to the Ohio Lab were made on June 11, 18, 25, July 2 and August 18, 2001, in insulated cartons containing ice packs.

Lab analysis by EnviroScience biologists included presence and condition of all weevil life forms, and the condition of the milfoil stems from EnviroScience and milfoil stems from Forest Lake.

EFFECT OF FOREST LAKE WATER ON WEEVIL REPRODUCTION

At the time of weevil introduction on June 2, 2001, EnviroScience biologists gathered forty (40) gallons of Forest Lake water and milfoil cuttings for transport to the EnviroScience Lab to determine the effect of Forest Lake water on weevil reproduction (egg laying).

On June 8, 2001, three (3) 20-gallon tanks were set up at the lab facility. Tank A used EnviroScience water with Forest Lake milfoil. Tank B used Forest Lake water and Forest Lake milfoil. Tank C used Forest Lake water with EnviroScience milfoil. Each tank contained 160 milfoil plants and 100 adult weevils of unknown sex. Tank temperatures were 24.2°C +/- 2°.

EnviroScience water source was 7 pH Akron city water aerated for a minimum of 10 hours before use. The effect of Forest Lake water on weevil reproduction was made by counting the number of layed eggs on the 3rd, 4th and 5th day after tank set-up and adult weevil introduction.

EURASIAN WATER MILFOIL AREAS

Source: DNR Aerial Photos

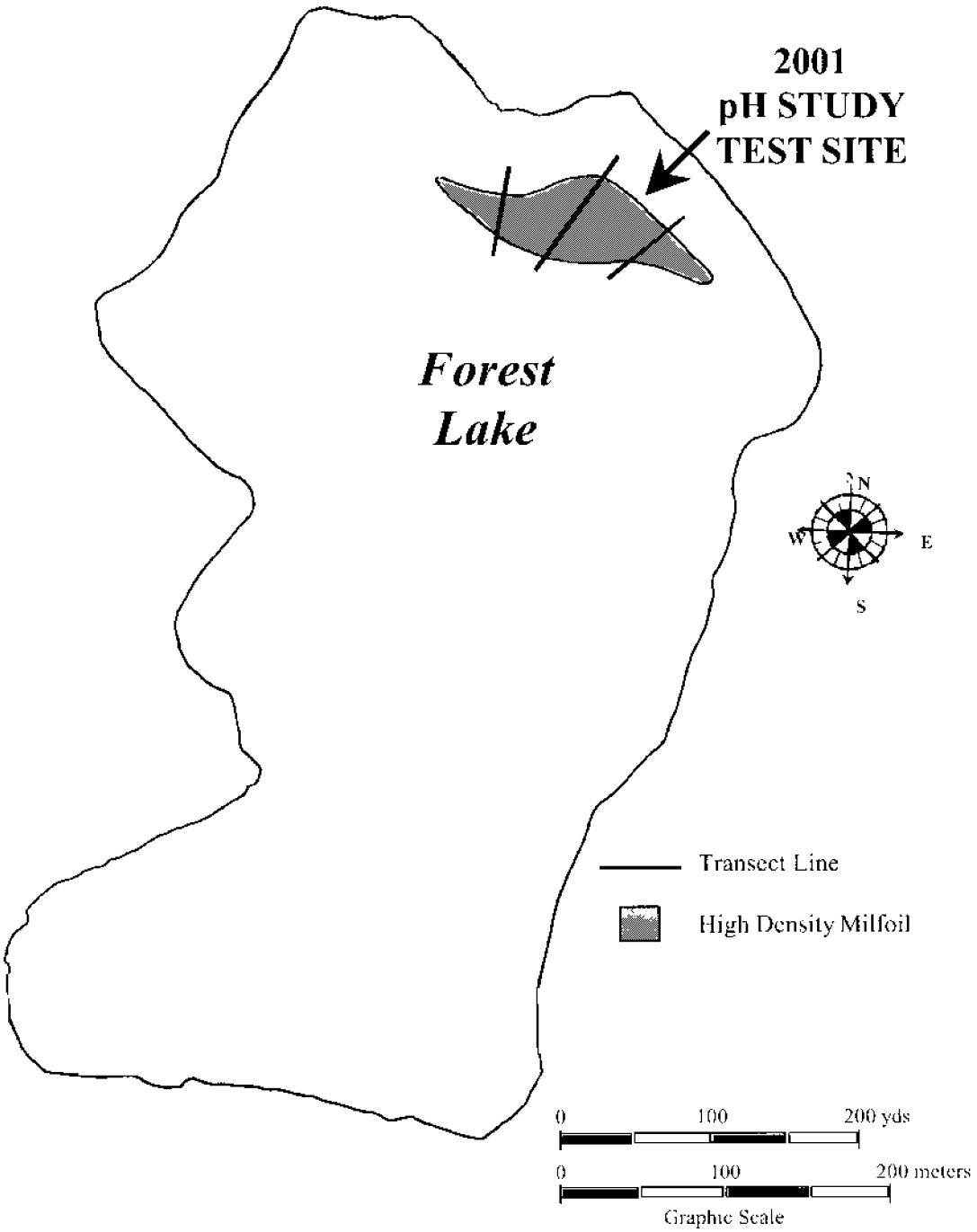
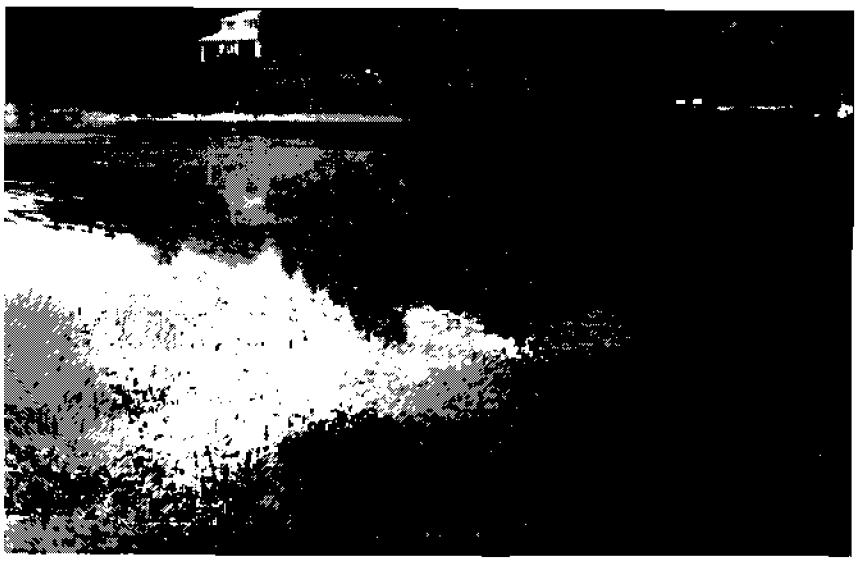
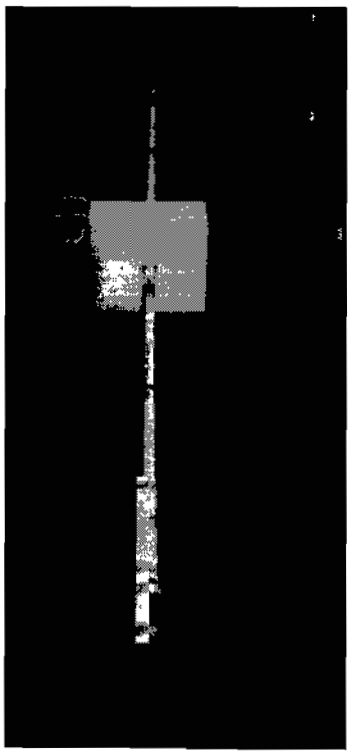


FIGURE 1

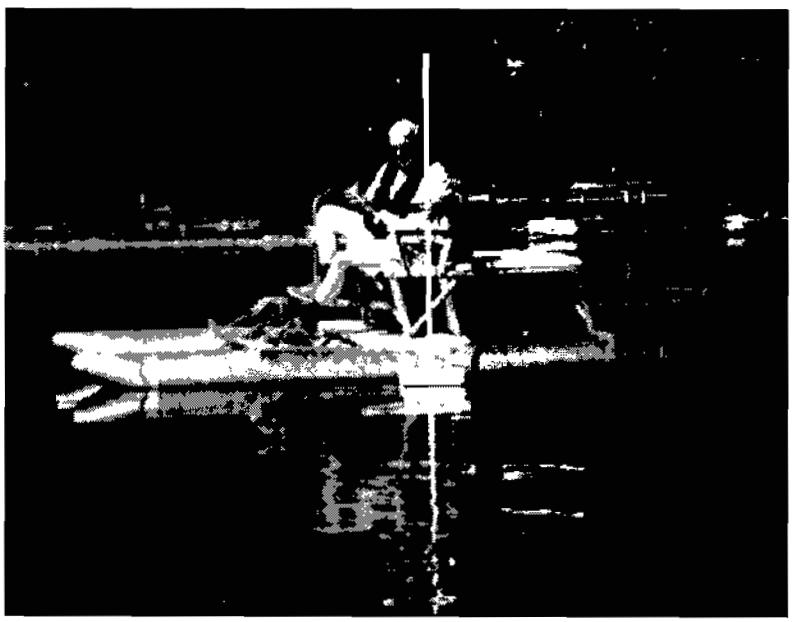


Test site of milfoil infestations.

Ribbon earmarked milfoil stems containing weevil eggs and larvae, prior to lake introduction.



Assembly of pH meter probe within graduated and perforated rigid PVC tube.



Taking pH and temperature readings within milfoil infestations

RESULTS & DISCUSSIONS

pH OF FOREST LAKE (Figure 2)

The pH readings taken weekly from May 18, 2001 to September 29, 2001 showed that the pH on May 18 was 8.59 (+/- .05, n=9) and reached a maximum of 9.67 (+/- .11) on July 14. Whenever the pH probe penetrated the sediment in the heavy milfoil areas at the 7 ft. depth, a pH of 6 to 7 was recorded.

Prior to their introduction into Forest Lake on June 2, the EnviroScience water used to transport the cultured milfoil, weevil eggs, and larvae was tested and indicated a pH of 7. On that date, Forest Lake's pH was 8.59 and, as a result, the weevil eggs and larvae were suddenly subjected to an environment 59 times more alkaline than their culture medium when they were placed in the lake. Since the pH scale is logarithmic, there is a ten-fold difference in hydrogen ion concentration for each pH unit, e.g., pH 8 is 10 times more alkaline than pH 7, pH 9 is 100 times more alkaline than pH 7. Previous weevil introductions were made on July 2, 1999 and June 29, 2000, at which time the lake measured a pH of 8.92. This high pH may have attributed to the weevils not surviving at that time also.

Although other invertebrates are subjected to the same pH regime, they thrive and survive, because over time they have adjusted and adapted and are not subjected to rapid changes in pH.

Most pH readings (81%) were taken between 12:00 noon and 6:00 pm. Diurnal changes in pH, which are largely the result of the photosynthesis and respiratory processes, were minimal. Time of day reading variations were as follows:

June 10, 2001	12:00 noon - pH 9.08	10:00 pm - pH 8.97	
June 17, 2001	6:00 am - pH 9.34	1:00 pm - pH 9.38	6:00 pm - pH 9.38

It was expected that the pH levels within the milfoil beds would decline with increasing water depths (due to reduced photosynthesis at higher depths). However, the anticipated variations of the pH due to increased depth in the heavy milfoil beds did not occur. This may indicate that although the milfoil infestations are very dense, there is sufficient circulation and water movement to dilute and distribute the products of photosynthesis and respiration. Also surprising were the results showing that the pH readings in the lake center were basically identical to those in the milfoil beds (+/- .05, n=16).

On August 25, 2001, Forest Lake experienced a heavy 4" rainfall. On the following day, the lake was found to have a substantial increase to pH 10.09. This appears to indicate that the watershed has a relatively alkaline bedrock and carbon rich soil, which may more than counterbalance acids from the atmosphere. This phenomenon will be further investigated. During 1994 through 1996 USGS water quality monitoring studies reported in late July and August the following pH readings: 1994 - pH 8.6, 1995 - pH 8.9, and 1996 - pH 9.3, as compared to 2001 – pH 9.78, which appears to indicate a growth in pH over the years.

FOREST LAKE MEAN pH
IN MILFOIL BEDS
n=9

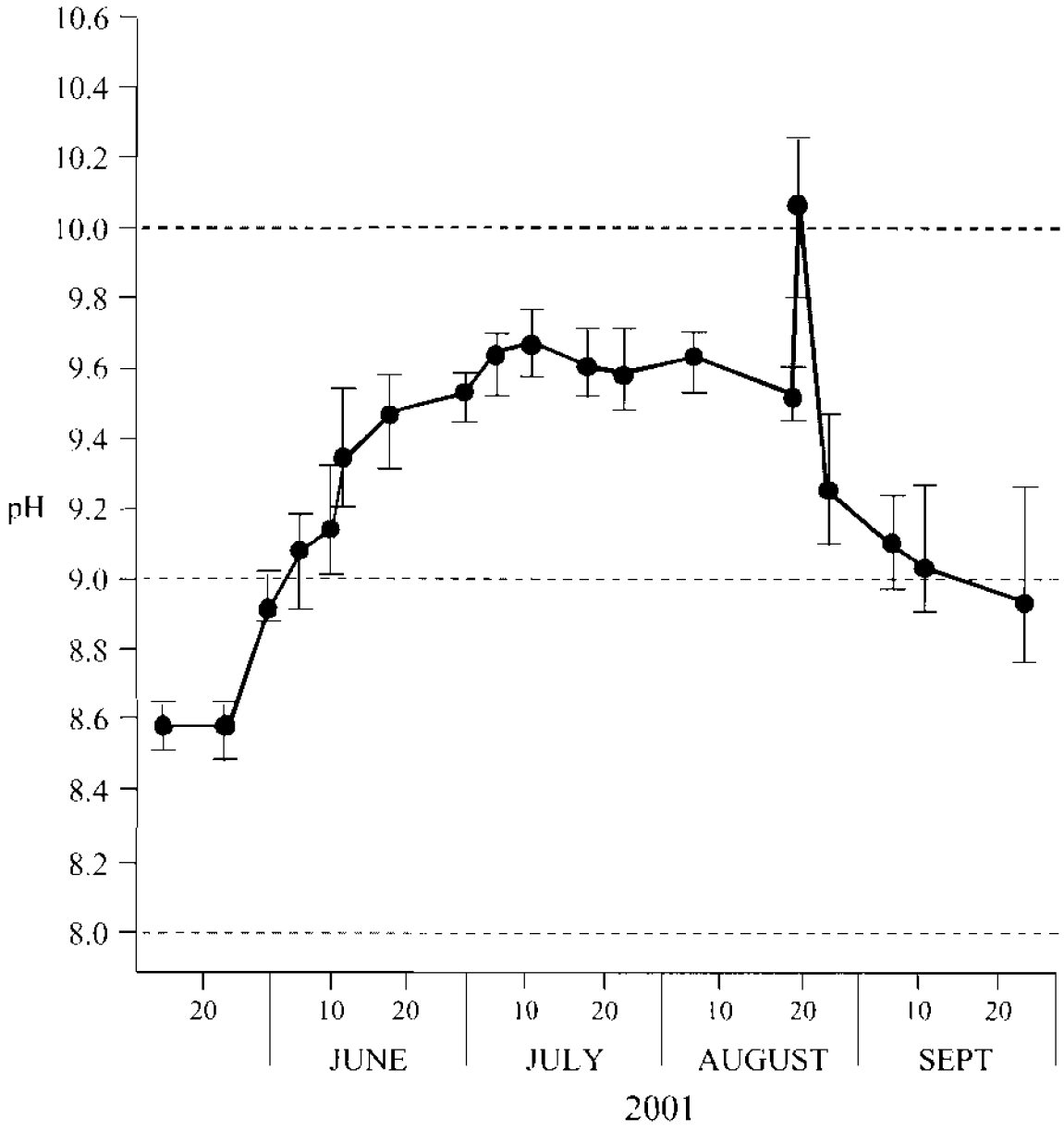


FIGURE 2

FOREST LAKE WATER TEMPERATURE (Figure 3)

At the time of weevil introduction on June 2, 2001, the lake's water temperature was 15.1°C. It then increased to a maximum of 29.1°C on July 24, and then decreased to 16.5°C by September 29. In the milfoil areas, the surface temperature measured at the 1.5 ft. depth was an average .7°C (n=62) higher than the temperature at the 5 ft. depth. Median temperature within the milfoil beds was .2°C (0-.8, n=19) higher than the median temperature at the lake's center.

MERISTEM DAMAGE (Figure 4)

Meristem damage, which is indicative of adults feeding and the feeding of first instar larvae after hatching, was noted on both the introduced milfoil stems and the Forest Lake stems they were attached to. The introduced stems showed significant meristem damage: 64% damage the very first week, 82% by week 2, 98% by week 3, 69% by week 4, and 100% by week 5.

The adjacent Forest Lake stems showed minor meristem damage: 4% on week 1, 3% on week 2, 1% on week 3, 12% on week 4, and 10% on week 5. This indicates that a small number of the first instar larvae had transferred to the Forest Lake stems.

MILFOIL STEM DAMAGE BY LARVAE (Figure 5)

Damage within the milfoil stems is caused by the later instar larvae entering the stem and consuming the entire cortex of the stem, until it progresses to begin its pupae stage. The mining of the cortex causes the milfoil to lose its ability to translocate carbohydrates and nutrients and lose its buoyancy.

The introduced stems showed immediate damage by the larvae: 80% on week 1, 100% on week 2, 99% on week 3, and 100% on week 4.

Forest Lake stems showed very little larvae damage, until the 4th and 5th week: 4% on week 1, 11% on week 2, 5% on week 3, 34% on week 4, and 35% on week 5.

Pupae were not found on either the Forest Lake stems or introduced stems, which is an indication that the larvae are not surviving through their instar stages to reach their pupae life form and consequently into adults.

FOREST LAKE MEAN-TEMP °C
(avg. 1 ½, 3, 5 ft. Depths) IN MILFOIL AREAS
n=9

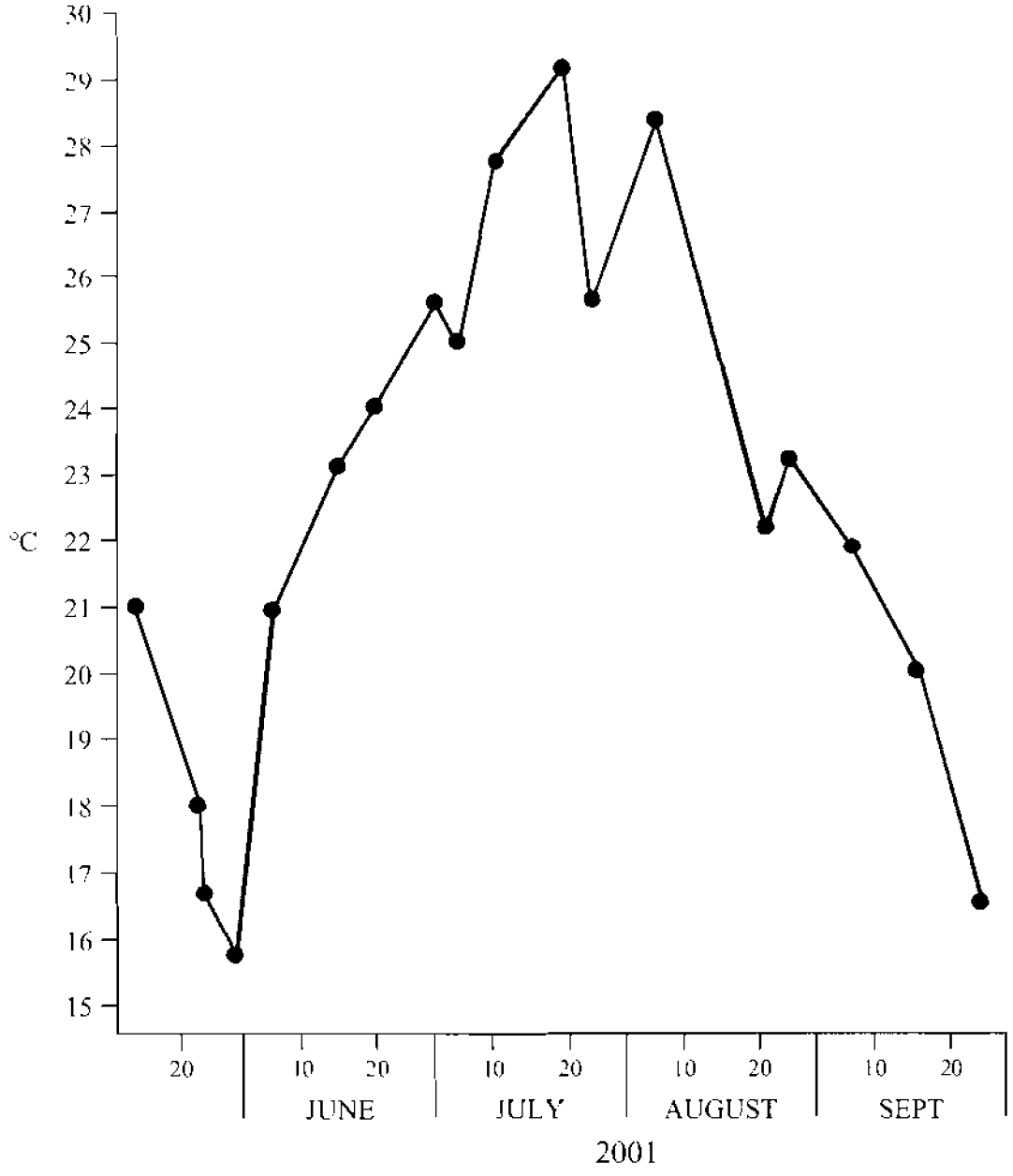
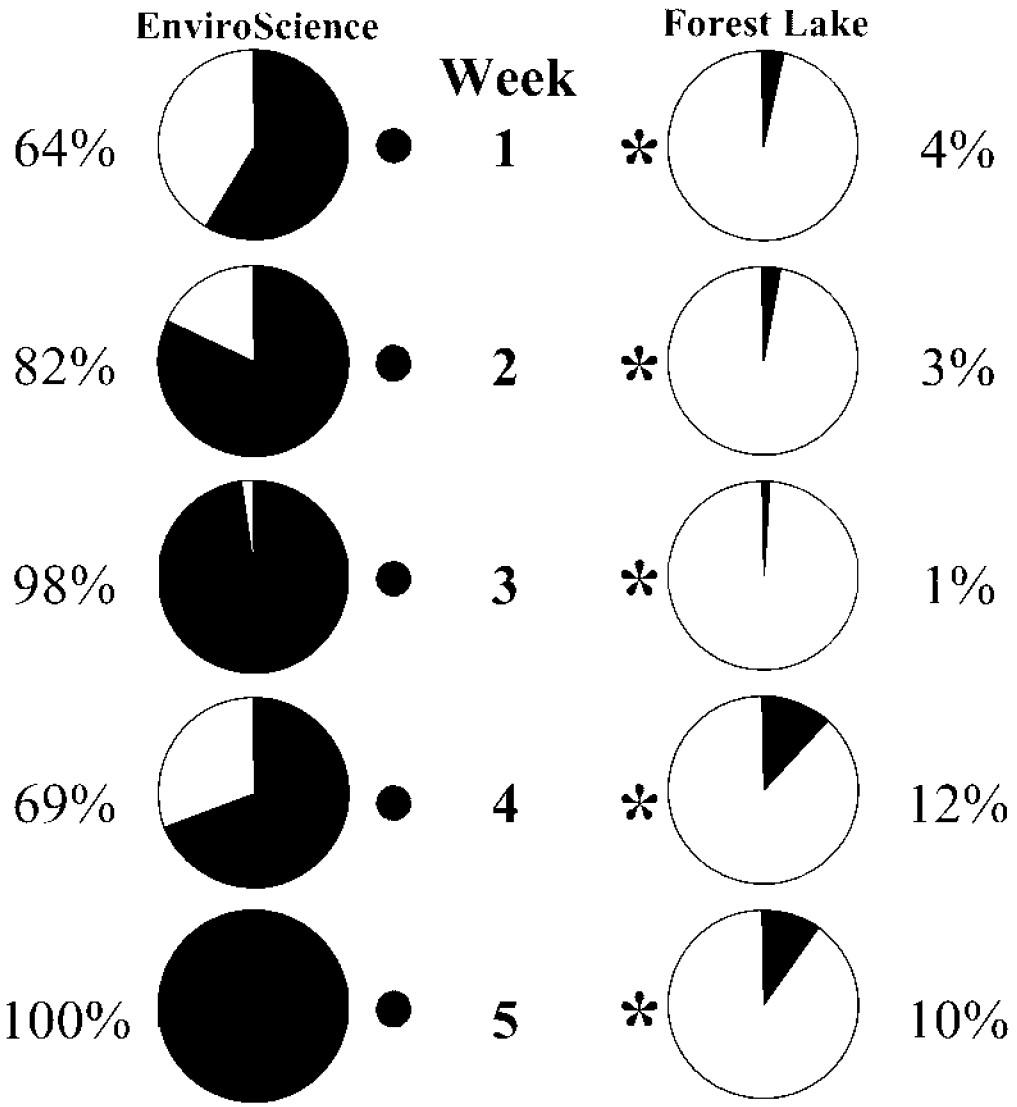


FIGURE 3

% MERISTEM DAMAGE

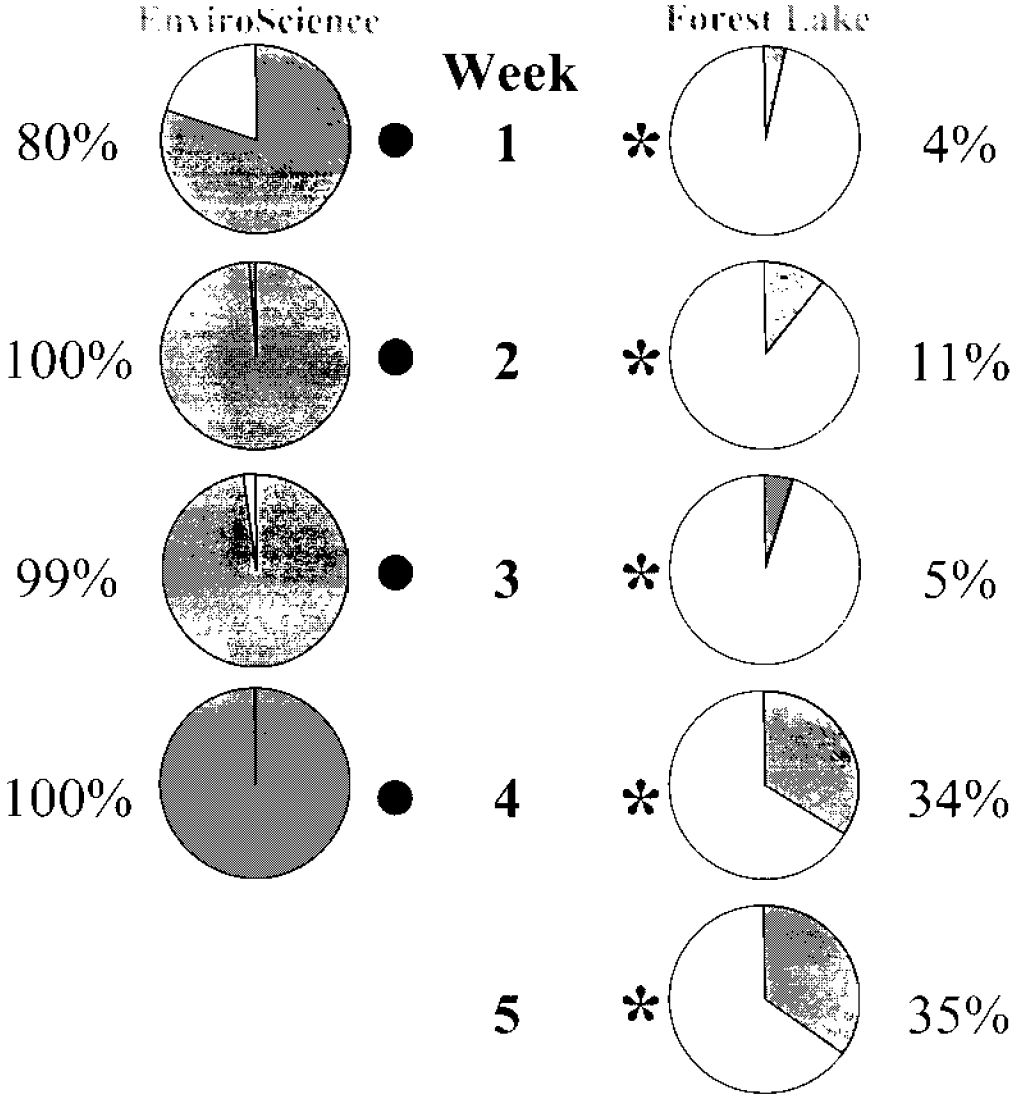


* Existing Forest Lake Milfoil

● Milfoil Stems containing cultured weevils (eggs & larvae) EnviroScience supplied 6 bundles removed per week, containing 7 stems each, from Forest Lake plants and 7 stems from EnviroScience Milfoil

FIGURE 4

% STEM DAMAGE



* Existing Forest Lake Milfoil

● Milfoil Stems containing cultured weevils (eggs & larvae) EnviroScience supplied 6 bundles removed per week, containing 7 stems each, from Forest Lake plants and 7 stems from EnviroScience Milfoil

FIGURE 5

EFFECT OF pH ON WEEVIL REPRODUCTION (Figure 6)

For biological control of milfoil to succeed, the weevils must not only survive, they must also reproduce and proliferate sufficiently to offset the losses from predation, parasites, diseases, overwintering, and the weevil life span.

TANK A

Using the 7 pH water used for culturing weevils, along with 160 Forest Lake milfoil stems and 100 adult weevils of unknown sex identity, Tank A produced 180 eggs by the 3rd day of tank set-up, 468 eggs by the 4th day, and 648 eggs by the 5th day. This was reported to approximate the typical culturing output of 666 eggs by EnviroScience.

TANK B

Utilizing Forest Lake's water of pH 8.59 and 160 Forest Lake milfoil stems and 100 adult weevils previously kept in 7 pH water, Tank B produced 252 eggs by the 3rd day. However, the egg count remained at 252 eggs on the 4th and 5th day. One can surmise that either the weevils did not survive, or their reproductive processes were severely damaged by the Forest Lake water.

TANK C

Using Forest Lake's water, along with 160 milfoil stems from EnviroScience and 100 adult weevils previously kept in pH 7 water, Tank C produced 108 eggs by the 3rd day, 144 eggs by the 4th day, and 288 eggs by the 5th day. The data indicates that the Forest Lake milfoil is not the cause of the poor weevil egg laying reproduction in Forest Lake.

LABORATORY TANK ANALYSIS OF WEEVIL REPRODUCTION

WEEVIL EGGS LAID BY 100 ADULT WEEVILS/TANK

Introduced June 8, 2001

Eggs counted on June 11, 12, 13

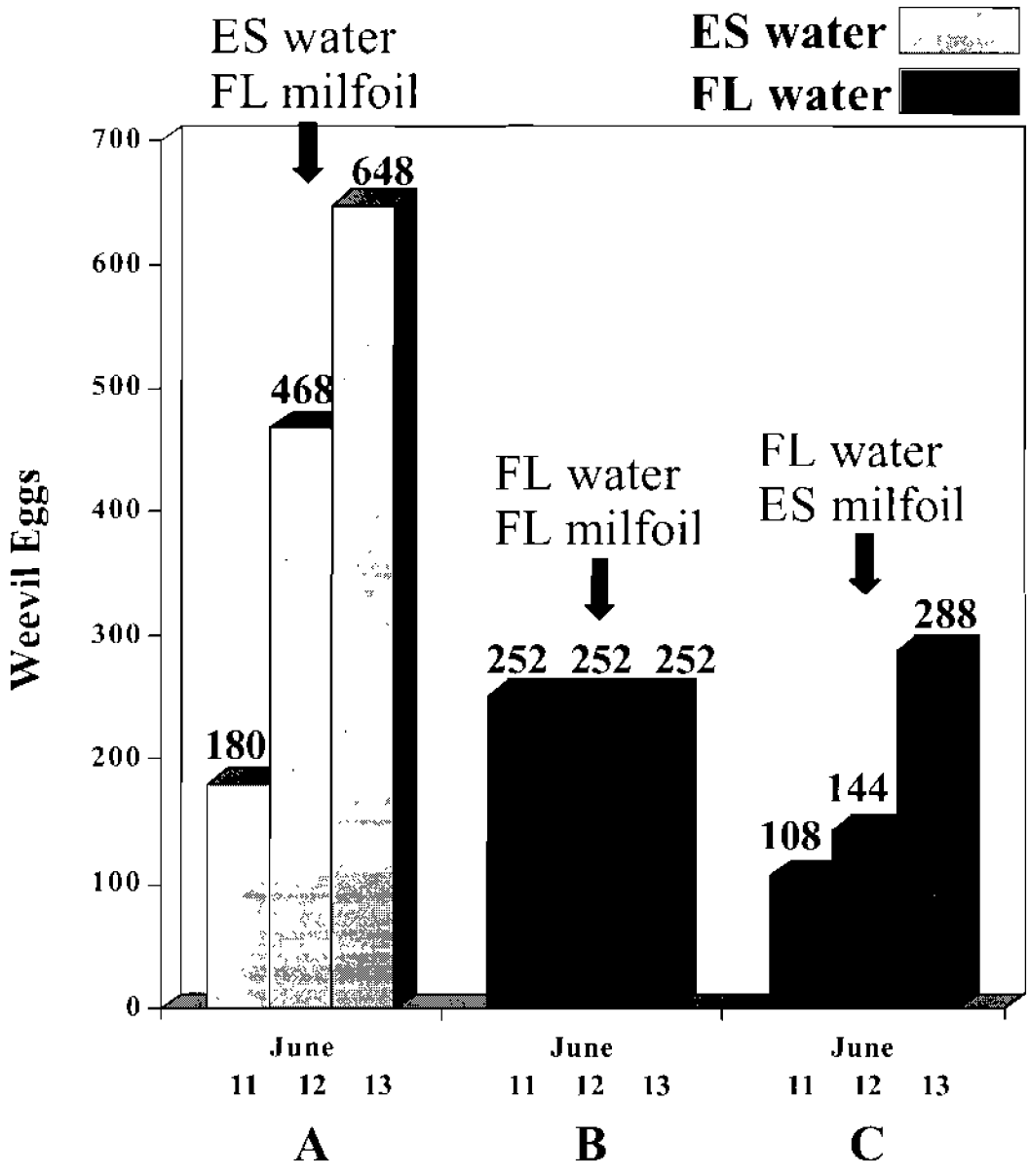


FIGURE 6

FUTURE RESEARCH DIRECTION

As the accompanying data and graphics illustrate, Forest Lake's pH at the time of weevil egg and weevil introduction was 59 times more alkaline than the medium the weevils were cultured in.

It appears that the ***sudden*** exposure of weevils into the significantly higher pH Forest Lake water may be adversely affecting egg laying and hatching and development from late instar larvae into pupae and adult life form; and, causing increased mortality. As a result, the weevils did not survive or proliferate to a sufficient population for successful biological control of the Eurasian watermilfoil in Forest Lake.

To alleviate the deleterious stress caused by the rapid exposure to the Forest Lake environment, the project will be expanded during 2002 to study the effect of ***slowly*** acclimating weevils (egg and larvae) to Forest Lake water prior to lake introduction. Further lab studies will also be conducted to study the effect of slow acclimation on weevil reproduction (egg laying). The acclimation process will be similar to that used successfully by the aquarium industry for acclimating fish and invertebrates to environments with differing pH and temperatures.

AMENDMENT

Lake Management Grant LPL-771-01

Effect of Acclimating Milfoil Weevils to High pH Forest Lake Water Prior to Their Introduction to Forest Lake

Fond du Lac County, Wisconsin

prepared for:
Wisconsin Department of Natural Resources

by:
Casey Kendziorski
Vice President, Forest Lake Improvement Association, Inc.

December 2001

Forest Lake
Improvement Association, Inc.

INTRODUCTION

Findings during the 2001 investigation on the milfoil weevil indicate that Forest Lake's high pH may be detrimental to the survival of the milfoil weevil. This amendment proposes to eliminate the sudden or rapid exposure to the high pH by slowly acclimating the milfoil eggs and larvae to the elevated pH prior to their introduction into Forest Lake.

The amplification of the pH research study may have significant implications towards achieving a successful biological control of Forest Lake's milfoil infestations. It will also make a significant contribution towards a clearer understanding of the factors leading to an increase in successful bio-controls nationwide.

PROJECT GOALS

The project goals will be to study the effect of slow acclimation on the milfoil weevil.

Activities will include:

1. Continuation of weekly pH and temperature measurements in Forest Lake.
2. Weekly lab analysis of milfoil damage and the stocked weevils in all life forms which are subject to acclimation.
3. Weevil reproduction (egg laying) investigation in the EnviroScience Laboratory using adults who have been acclimated to Forest Lake water.

METHODS AND PROCEDURES

WEEVIL INTRODUCTION, ACCLIMATION, AND ANALYSIS

Four thousand (4,000) eggs (egg and larvae form) will be introduced into heavy milfoil infestations. Two thousand (2,000) weevils will be slowly acclimated to Forest Lake high pH water prior to their lake introduction. They will be marked with red ribbons. Another two thousand (2,000) weevils will be introduced without the acclimation process and marked with yellow ribbons.

The milfoil with the cultured eggs and larvae will be applied as bundles, consisting of ten (10) to twelve (12) milfoil stems, each stem with five (5) to seven (7) eggs on their meristems. At the time of introduction, each bundle will be attached to existing milfoil plants in Forest Lake.

Six (6) bundles each of red and yellow ribbon-marked bundles will be removed weekly for four (4) weeks and expeditiously shipped to the EnviroScience Lab for laboratory analysis of milfoil damage and presence of weevil life forms.

WEEVIL REPRODUCTION (EGG LAYING) STUDY

EnviroScience biologists will gather approximately 60 gallons of Forest Lake water and milfoil cuttings and transport same to the EnviroScience Lab in Ohio.

In 20-gallon tanks, the lab will slowly acclimate their culture water (7 pH) and the weevils they contain, to the water collected from Forest Lake. Eggs laid will be counted on the 3rd and 4th days, as well as the day after tank set-up.

SCHEDULE

	by	
pH measurements of Forest Lake – Weekly	Forest Lake	May 15, 2002 to October, 2002
Collection of Forest Lake water and milfoil for egg laying reproduction study	EnviroScience	June 1, 2002
Introduction of acclimated and non-acclimated weevils into Forest Lake	EnviroScience	June 1, 2002
Weekly removal of acclimated and non-acclimated weevils and milfoil and shipment to EnviroScience Lab for analysis	Forest Lake	June 8, 2002 to July 8, 2002
Lab and field report findings	EnviroScience	September 1, 2002
Final report	Forest Lake	December 1, 2002