The Effects of Desiccation and Freezing on the Growth and Survival of Eurasian Milfoil

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Note: The results presented in this report are preliminary in nature and still under investigation. No part of this report should be distributed without permission of the author.

Introduction

Draw downs of Candlewood Lake have been the main method used to control the growth of the invasive aquatic species Eurasian milfoil (*Myriophyllum spicatum*). The yearly outcomes of the draw downs have been inconsistent, suggesting that multiple variables, both biological and environmental or climatological are interacting. While it is not possible to predict future climatological events, such as the duration or timing of winter freezes, it is possible to determine how sensitive milfoil is to desiccation and freezing.

Few plant species can survive if they dry and lose fifty percent of their tissue water to the air. A few marine algae, particularly red algae, are genetically programmed to survive dry air when exposed to low tides. Milfoil, an aquatic species that did not evolve to cope with daily tidal changes would not be expected to be desiccation tolerant.

Freezing of most animal and plant cells causes large ice crystals to form that damage the membranes associated with cells. Damage to the cell membranes can lead to cell death. Some terrestrial plants are genetically programmed to survive freezing by making specialized proteins, generically called antifreeze proteins, or make complex carbohydrates (sugar compounds) that protect the cells. The binding of additional water resulting from the presence of these molecules can slow down the rate of tissue freezing resulting in less cell damage. These proteins and carbohydrates act much like the way salt on roadways decreases the freezing point. While freeze tolerance has been documented in some terrestrial plants, it has never been reported for aquatic species. It is therefore unlikely that milfoil is freeze tolerant.

To kill milfoil by desiccation or freezing, the root system must be substantially damaged. The entire stem system can die but will regenerate if the roots survive. Therefore, the effectiveness of draw downs needs to be evaluated on root survival. Milfoil roots are fibrous, do not penetrate the soil deeply, and spread out (Figure 1).

Figure 1: Left: A milfoil plant consisting of multiple stems, each approximately eight feet long.
Right: Root mass of the plant shown in the left picture.

In this preliminary study, two main questions were examined:

1. Can it be verified that either desiccation or freezing to -5^{o}C (23^{o} F) actually damages milfoil root cells, how fast does the damage occur and how severe is the damage?
2. If milfoil roots are air dried or frozen for various periods of time, will the root systems survive and regenerate stems once the treatment is reversed?

The results of this study collectively indicate that Eurasian milfoil collected in the spring is very susceptible to desiccation and/or freezing, and does not recover and re-grow once damaged.

Materials and Methods:

Collection. One hundred mature M*yriophyllum spicatum* were harvested by divers with intact root systems from the shoreline of Candlewood Point of Candlewood Lake (given lat/long) on June 6, 2009. Plants and Candlewood lake water were transported back to the WCSU greenhouse where the stems were trimmed from the 8-10 foot long plants with scissors. Plants were allowed to recover for three weeks in a 100 gallon tank containing Candlewood lake water. Only specimens with visible stem regrowth were used.

Water conditions. Temperature was held at 23 ^{o}C with a light intensity at the surface of the water (12pm noon reading) of of 0.3 µEinsteins of photosynthetically active irradiation/ meter ^{2} / sec
Treatment. Plants were removed from the tank and placed on sheets of aluminum foil and placed in a refrigerator (2 ^{o}C) to air dry to equilibrium or in a freezer at -5^{o}C for 24 hr to three weeks in duration.

Relative Electrolyte Release (REL). Assessment of root cell damage to the desiccation or freezing treatments was examined by the magnitude of electrolyte release. After the treatment period, roots were placed in vials containing MilliQ water and agitated by rocking for 18-24 hr. Damaged root cells would be expected to release their electrolytes into the bathing solution. Conductivity of the bathing solution, measured in µSeimens, was measured for each sample. Samples were then autoclaved which will lyse all root cells releasing all electrolytes. The conductivity after the treatment / conductivity after autoclaving is termed Relative Electrolyte Release and is an indication of root cell damage.

Growth Recovery. Separate groups of treated plants were placed in a 100 gallon recovery tank containing Candlewood lake water. Water temperature was 23 o C, pH averaged 7.8, conductivity indicating general ion content of the water averaged 220 µS, nitrate concentration average at 0.3 mg/L and orthophosphate averaged 0.20 mg/L. Every seven days, plants were removed from the water, blotted dry, weighed and returned to the growth tank. The per cent change in mass was plotted over time.

Results

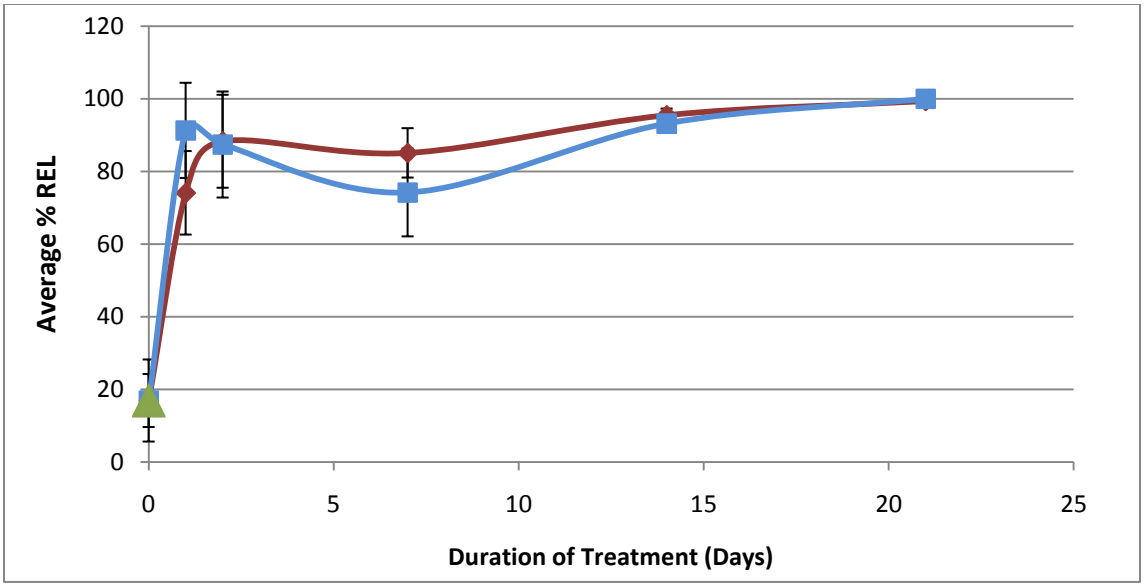
Damage to plant roots, stems or leaves is typically measured by a technique call relative electrolyte release (REL). Plant cells contain a number of various salts or electrolytes. If a cell is damaged, the electrolytes leak out of the plant cells into any fluid that surrounds the tissue. The percentage of total electrolytes inside a cell that leak out after a treatment can easily be measured and is expressed as % REL. The greater the percentage REL, the greater the cellular damage.

Sets of milfoil roots, with the stems cut approximately 6 inches above the root mass, were either desiccated (dried to equilibrium with the air in the refrigerator) at 2^{o}C (36^{o}F) or frozen at -5 ^{o}C (23^{o}F) (Figure 2).

Figure 2. Left: Large root masses, like that shown in Figure 1 were separated into smaller pieces. The stems were cut approximately 9-12 inches above the root mass.
Right: Root masses were placed on sheets of aluminum foil and either placed in a refrigerator at 2^{o}C for 1-21 days to desiccate the root masses, or in a freezer at -5^{o}C.

When roots are dried in air at 2°C or frozen at -5° C, substantial damage is done to the roots within 24 hours (Figure 3). With increasing time, additional damage is done until 100% of the root cells are damaged (three weeks of treatment = 100% REL).

Figure 3: % REL is a measure of how severely root cells have been damaged by a treatment, such as desiccation or freezing. Average % REL in milfoil roots is shown for treatments ranging from 1 to 21 days. The blue line represents freezing at -5°C, the red line drying roots in air at 20°C. Notice that for both treatments, % REL is in the 75-80 % range after just 24 hours, representing a substantial amount of root damage. Each data point represents approximately ten replicate plants. The error bars represent standard deviation about the mean.



Verification that the freezing (Figure 4) and desiccation (Figure 5) treatments damage root cells sufficiently to inhibit recovery and subsequent re-growth was obtained when samples treated for 1-3 weeks were placed in a recovery tank of Candlewood Lake water in a greenhouse. The mass of the individual plants in each treatment were measured over a four week period. Control milfoil plants each increased in mass over the time period, but not only did treated milfoil plants fail to grow, they each lost mass over time indicating necrosis or complete death of the root system. Identical results were found for roots treated for one, two or three weeks, indicating that the duration of the treatment is not a major factor in damaging the roots (Figure 4).

Figure 4: Percent change in plant mass over time. Every seven days, the mass of each plant was compared to the week before (% change in mass). While control plants grew over the following four weeks. Plants that remained frozen for 1-3 weeks decreased in mass, indicating progressive necrosis (death of tissue) over the measurement period.

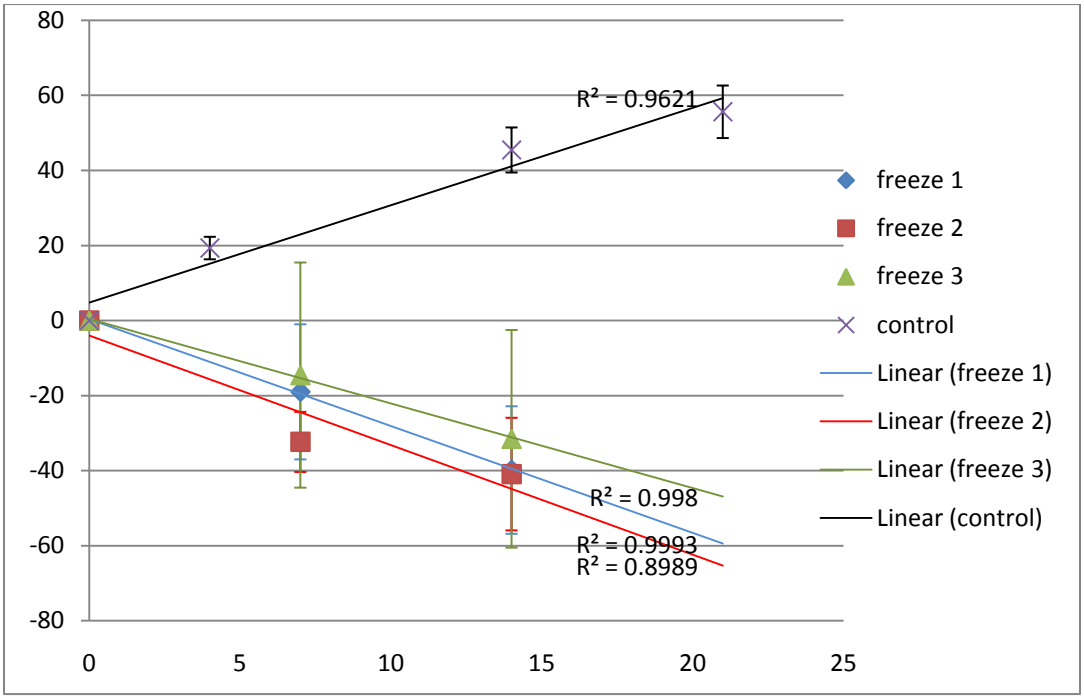
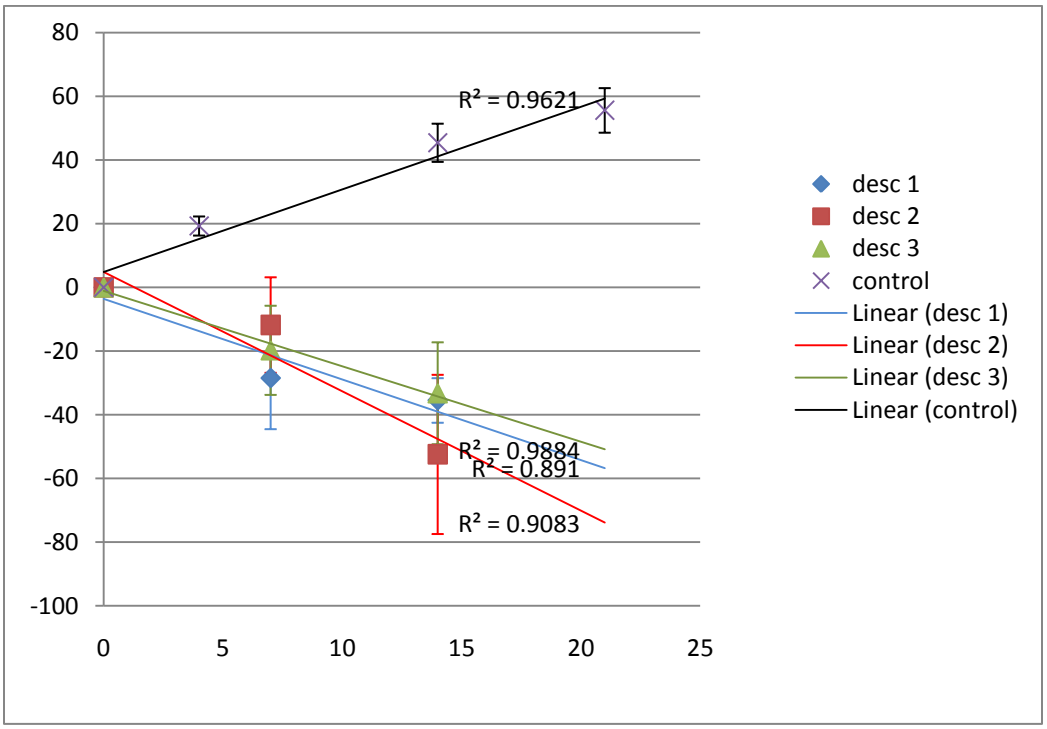


Figure5: Percent change in plant mass over time. Every seven days, the mass of each plant was compared to the week before (% change in mass). While control plants grew over the following four weeks. Plants that remained dried for 1-3 weeks decreased in mass, indicating progressive necrosis (death of tissue) over the measurement period.



Conclusions

Some very interesting trends are beginning to emerge concerning the sensitivity of Eurasian milfoil to environmental stress as a means of managing or controlling this invasive species.

If milfoil roots are dried in air or frozen in air to -5°C (23 ° F), for as little as 24-48 hours, substantial damage to the root cells occurs, as measured by percent relative electrolyte leakage (Figure 3). A small increase in root damage is observed by increasing the treatment to a duration of 1,2 or 3 weeks.

Root systems treated for one, two or three weeks did not recover and resume growth over a four week recovery period (Figure 4). In fact, the root masses not only do not recover, they continuously decompose over the four week period of measurement.

These results collectively suggest that spring collected plants are highly sensitive to damage by drying or freezing, and that Eurasian milfoil may not be genetically programmed to survive such treatment.

There are numerous unanswered questions, including:

1. Are plant systems collected in the fall, prior to winter cold treatments similar in response to the spring collected plants? It is possible that milfoil is genetically programmed to survive freezing and or drying, but that this capability is not expressed in plants until the fall. This study will be conducted in Fall 2009.

2. Are plants treated for 24-48 hours able to recover growth once the treatment has been reversed? If plants cannot recover growth after these brief treatments, the possibility of damaging milfoil with minimal durations of adverse environmental conditions may allow recommendations to be made concerning the timing and/or duration of draw downs. This experiment is currently underway.

3. Is the damage to roots the same if roots are frozen in water (wet soil) versus air (dry soil)? Preliminary results suggest a small protection to the roots if frozen in water.

4. Does the rate of freezing and thawing affect the amount of root damage?

5. What is the typical level of water and temperature in the soil during a draw down? This question addresses a major climatological issue as it relates to control of milfoil. It cannot be assumed that if the air temperature is in the freezing range that the soil temperature around the roots is in the freezing range. Soil temperature could be affected by soil water content, duration of the freeze, and snow or ice insulation on top of the soil. This question will be examined in Winter 2009/2010 by monitoring soil conditions during the draw down.