

PHYSIOLOGICAL ECOLOGY OF SEED
GERMINATION IN MYRIOPHYLLUM SPICATUM L.

Undergraduate Thesis to:
Biology Department at Rensselaer

prepared by

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FWI Report #90-15
May 1990

PHYSIOLOGICAL ECOLOGY OF SEED GERMINATION IN
MYRIOPHYLLUM SPICATUM L.

by

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A Thesis Submitted to the Undergraduate
Faculty of Rensselaer Polytechnic Institute
in Partial Fulfillment of the
Requirements for the Degree of
Bachelors of Science

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Troy, New York

May 1990

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ACKNOWLEDGEMENT

The author wishes to express many thanks to Dr. John Madsen, for without his help and guidance this project would have never been and I would still be a helpless undergraduate. I would also like to thank Dr. Charles Boylen for giving me a chance and a place to get my "feet wet", in the research experience. Many thanks goes to the staff of the Fresh Water Institute for their help with the field work and in understanding what it all means.

I am especially grateful to my parents who helped me to get here in the first place and I would like to give a special thanks to Denise Hyde who taught me, "you have to go after the things in life you really want - and yet, you don't always get what you want."

ABSTRACT

Myriophyllum spicatum L. (Eurasian watermilfoil) is an aquatic nuisance plant that recently invaded Lake George, NY, and is now found at over 40 sites in the lake. Very little is known about the sexual reproductive capabilities of this species and *in situ* germination of its seeds has never been observed.

Laboratory studies were conducted to determine the time needed to sterilize the seeds and the light and temperature requirements necessary for maximum seed germination. The effects of sedimentation on seed germination was also examined due to poor seed survival in zones of high deposition. *In situ* studies included the germination of seeds at depths of 1 m, 3 m, and 5 m and the survival of fragments at the same depths. Both methods of plant spread were examined in two different bays within the lake over a one month growing period.

Significant germination of seeds was observed in the northern bay, but not in the southern, probably due to sedimentation. Significant survival of fragments was observed in both bays, with a much higher survival rate than seed germination. Warmer temperatures were

found to be necessary for successful seed germination in laboratory studies, but light was determined not to be a limiting factor on its own. Seeds buried under more than two centimeters of sediment were shown to experience significant decreases in germination.

Sexual reproduction by seeds may represent a long term survival adaptation for this species. It could provide genetic diversity within the plant's own community and may represent a means of reestablishment to a disturbed area where a large seed bank is present from a previously established Eurasian watermilfoil community.

PART 1

INTRODUCTION & HISTORICAL REVIEW

Myriophyllum spicatum L. (Eurasian watermilfoil), a member of the family Haloragaceae, is an herbaceous, aquatic, submersed macrophyte with long flexuous stems and thin dissected leaves. It is a perennial, overwintering under the ice cover either as a root crown, or as a group of bright green stems and in the spring, these stems elongate, and by early July have reached the water's surface. The floral spike is produced at the apical end of the stem, with staminate flowers above the pistillate flowers. Flowering occurs once, or twice annually, usually in mid to late June and in August (Kimbel, 1982). Flowers are four-merous, with the fruits splitting into nutlets containing only one seed. A Lake George, NY milfoil population produced a median of 7 whorls of female flowers, or 28 total flowers, for a total possible seed set of 112 seeds per stalk (Madsen & Boylen, 1988). The percentage of seed set may vary tremendously both between lake sites and between years. Despite the high seed production, no seedlings have been observed in nature, nor has any seed germination been measured *in situ*.

Eurasian watermilfoil (abbreviated herein to milfoil) is considered an aquatic "weed" because it forms dense bed communities which can reach sizes that may be acres in area, while displacing the native vegetation (Madsen *et al.*, 1989). These dense stands curtail recreational activities by creating habitats favorable for the production of blood-sucking insects and substantially degrade beach quality by the decaying of washed up vegetation. They can clog industrial and potable water supply intakes and alter temperature profiles in the shallow water of a lake by as much as 10 degrees Celsius per meter (Aiken *et al.*, 1979). However, milfoil can also be beneficial to an aquatic ecosystem. It may harbor invertebrates which are valuable food sources for fish and waterfowl. Its dense growth can provide cover for fish, and recycle nutrients from the sediment into the water column. Unfortunately, this last quality may also increase productivity of a lake so much, that the lake suffers and degrades in its trophic state (Nichols & Shaw, 1986).

Milfoil was first introduced in North America in the 1880's in the Chesapeake Bay, probably carried from its native habitat in the ballast of ships. Dispersal throughout the

United States (Fig. 1.1) has been related to the aquarium and aquatic nursery trade. Shorter distances of dispersal, such as that throughout New York State (Fig. 1.2), has been ascribed to transport of plant fragments on boats or boat trailers moving from lake to lake, and by moving water (Nichols & Shaw, 1986).

Milfoil naturally spreads by three basic mechanisms: 1) above-sediment runners and below - sediment rhizomes, causing the expansion of a given bed outward, resulting in a widening periphery; 2) fragments of shoot material, both naturally and artificially produced, colonizing new areas within a lake, or carried to other lakes; 3) seeds, carried by a variety of means, colonizing new sites or creating denser beds.

Vegetative propagation is prevalent in milfoil and is highly visible, due to the intense capacity of this plant to spread by vegetative means, and because of this, sexual reproduction has been considered of limited importance. As a result, little is known about the sexual reproduction of this species and even less is known of *in situ* seed germination of this plant. For many aquatic plants seeds are

Fig. 1.1 Map showing the location of *Myriophyllum spicatum* L. in the United States and the years in which it was first sighted in each state.

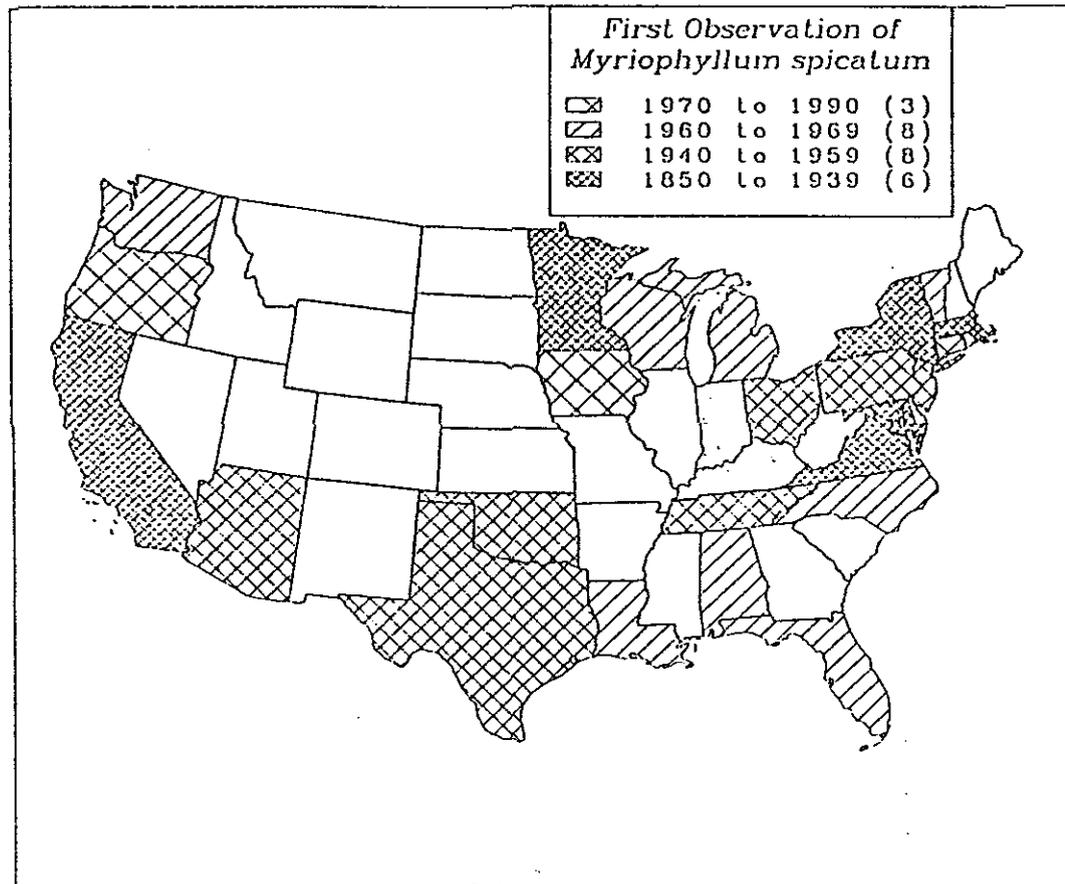
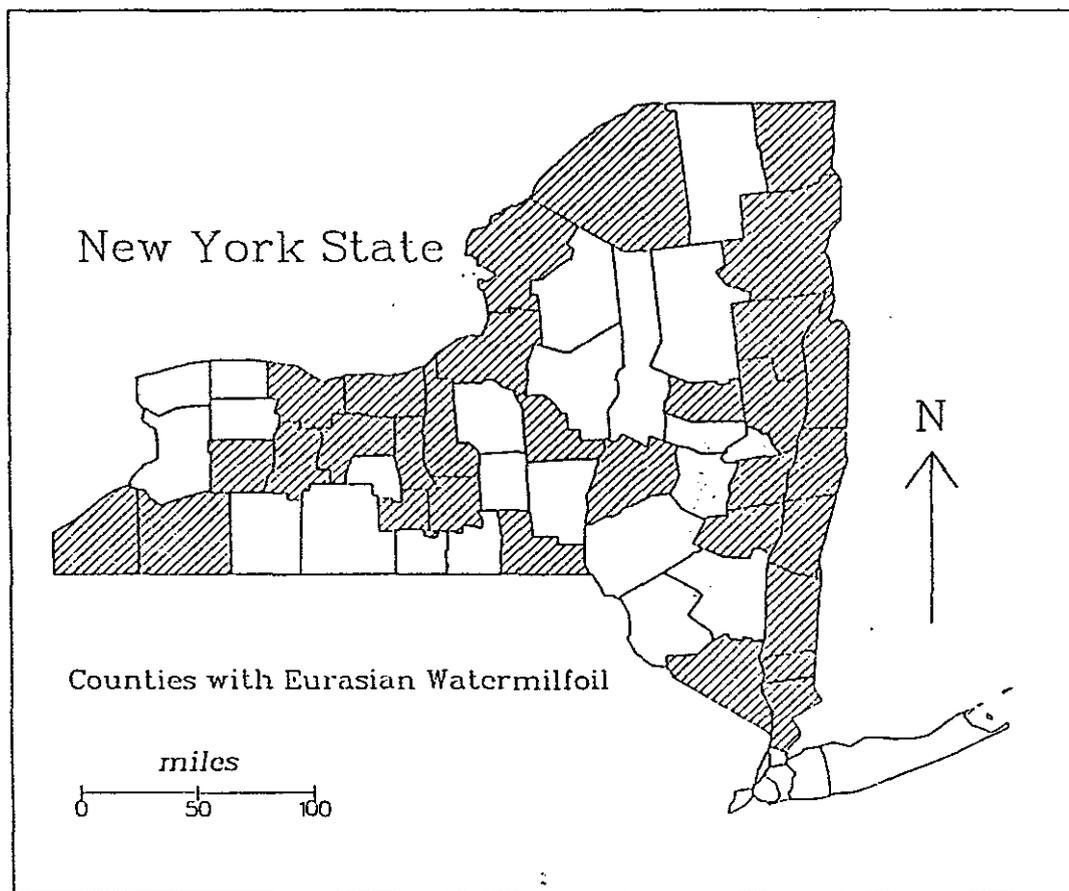


Fig. 1.2 Map showing the counties in New York State which have Eurasian watermilfoil.



used for long-term dormancy and dispersal over larger distances, and may lie dormant for many years. The importance of sexually produced seeds lies in furnishing genetic diversity and recombination within this species (Madsen & Adams, 1988). If milfoil is able to create a large seed bank and survive long periods of dormancy and disturbance, then viable germinating seeds would tend to decrease the effectiveness of weed management activities such as herbicide treatments, lake drawdown and dredging (McDougall, 1983).

Local variation in the density of ecologically active seeds can be related to many environmental gradients such as depth, sediment texture, irradiance, temperature, and disturbance. It has been shown that a maximum density of milfoil coincides with the distribution of fine organic ooze and diminishes to nearly total absence on sand and rocky surfaces (Patten, 1956). The effects of depth and sediment texture on the species composition may be as important as their effect on the ecophysiology of the species in determining the size and composition of the seed bank at a particular site (Haag, 1983).

This study examined the environmental

gradients that affect the reproductive mechanisms, particularly seed germination, by which milfoil colonizes new areas within a lake, or other lakes. Seeds were collected from dense beds of milfoil and transferred to controlled study areas *in situ*, at depths of 1, 3 and 5 meters and observed for a one month period. At the end of that month the seeds were collected and the percent germination of seeds was determined. Seeds collected from the dense milfoil beds were also transferred to the laboratory and were germinated under controlled temperature and photoperiods. The effect of sedimentation on germination was also observed, as well as survival of vegetative fragments over a one month growth period.

PART 2

STUDY SITE

2.1 Site Description

Lake George is located in northeastern New York State, on the southeastern edge of the Adirondack Park. It is a large (114 km²), deep (58 m z_{max}), and relatively clear (Secchi disk depth 9.2 m) oligotrophic lake, with a circumneutral pH (7.58) and low alkalinity (15-20 mg CaCO₃ l⁻¹) (Madsen *et al.*, 1988). The milfoil population in Lake George is fairly young, with significant populations first observed in 1985 (Madsen & Boylen, 1989). Huddle Bay, a large, shallow embayment in the south basin of the lake, and Northwest Bay, a large, intermediate depth embayment in the north basin were used as *in situ* study sites in this experiment. Flowering stalks collected for laboratory seed experiments were collected from milfoil plants in Shadow Bay (a small bay in the northwest section of Lake George), and returned to the laboratory until the seeds reached maturity, upon which they were harvested.

METHODS

2.2 Seed collection

Flowering stalks were collected from Northwest Bay along with approximately 20 cm of vegetative stem material. They were returned to the laboratory and incubated in aquaria with Lake George water under controlled light (400 $\mu\text{E m}^{-2} \text{ s}^{-1}$, 14 h light : 10 h dark) and temperature (20 \pm 2 C). Flowering stalks were maintained until seed set and maturity were achieved, a period of approximately four weeks. Pollination was assumed to have occurred prior to collection. Seeds were collected from the emergent fruiting spikes of the milfoil plants; were quantified and were allowed to overwinter at 4 C for four months.

2.3 Sterilization time

Optimum sterilization time, for laboratory germination experiments, was determined using 500 mature seeds. Five groups of 100 seeds, representing sterilization times of 0, 5, 15, 25, and 35 minutes, were surface sterilized in 5.25% sodium hypochlorite, rinsed with sterilized distilled water, and were placed into sterile Petri dishes. The dishes were placed under 14 h light : 10 h dark daylength conditions, with a light intensity of 200 $\mu\text{E m}^{-2}$

s⁻¹. Temperature was maintained at 20 C in a temperature controlled growth room. The number of germinating seeds in each dish was counted daily for a period of two weeks. The optimum sterilization time determined was to be used in laboratory seed germination experiments.

The presence/absence of algal, fungi, and bacterial contamination during sterilization was also determined, using 50 seeds. Five groups of 10 seeds, representing the sterilization times of 0, 5, 15, 25, and 35 minutes, were surface sterilized in 5.25% sodium hypochlorite, rinsed with sterilized distilled water and were placed into Petri dishes containing nutrient agar. The dishes were placed into an incubator, at 35 C, for a period of three days. The dishes were then examined for the presence of any bacteria, algae, and fungus growth, under a Bausch & Lomb dissecting microscope. The absence of contaminant growth was to help establish the optimum sterilization time for the laboratory seed germination experiments.

2.4 Laboratory seed germination

A total of 1,500 seeds were surface sterilized for 25 minutes in 5.25% sodium hypochlorite as established in the sterilization

procedure. Temperature and daylength treatments were performed on groups of 500 seeds, which were germinated in Petri dishes containing sterilized distilled water. Three daylength periods were examined (10 h light : 14 h dark; 14 h light : 10 h dark; 0 h light : 24 h dark) in combination with five temperature regimes (5, 10, 15, 20, and 25 C). Five plates containing 20 seeds each were placed at each temperature treatment and five groups of five plates were placed at each daylength, for a total of 500 seeds at each daylength treatment. The dishes were placed in growth chambers to maintain constant temperatures and the light intensity was set at $200 \text{ uE m}^{-2} \text{ s}^{-1}$. At the end of the experimental period (established at one week during sterilization experiments), the seeds were removed and analyzed for germination, which was indicated by the emergence of a root tip from a cracking seed coat.

2.5 *In situ* seed germination

In situ germination containers were prepared using plastic cups, which were filled with fine sandy sediment; weighted trays (to keep the cups on the lake bottom), which were covered with fine mesh screening to contain the diminutive seeds. A marker buoy was attached to each tray in order to locate and recover it.

Ten seeds were placed into each cup, (10 cups per tray, 2 trays per depth) covered with screening and placed at depths of 1, 3 and 5 meters outside of a milfoil bed in both Huddle and Northwest Bays. After a one month germination period, the trays were collected and the number of germinated seeds was counted. Percent germination was calculated for seeds from each depth and from each embayment, to determine effects of various conditions on seed germination.

2.6 Effect of burial on germination

The effect of burial by sedimentation on the germination of seeds was examined. Containers, 15 cm high, were filled with sandy sediment and were placed in aquaria in the laboratory. A 17 cm long strip of dialysis tubing was divided into one centimeter sections and into each section 5 milfoil seeds were placed. A total of six strips were prepared and placed into the containers, with 13 cm of the dialysis tubing being covered with sediment and 3 cm being above the surface, and one section at the sediment - water interface. Temperature was maintained at 20 C and light intensity at $400 \text{ uE m}^{-2} \text{ s}^{-1}$, with daylength at 14 h light : 10 h dark. After a one week germination period the tubing was removed and the number of germinated seeds in each section was determined.

2.7 *In situ* fragment survival

In situ fragment survival experiments used two different types of vegetative propagules: autofragments, abscising shoot fragments that develop roots at the nodes before separating from the parent plant; and allofragments, plant fragments with meristematic tissue but lacking preformed roots, released by artificial abscission. Containment bags were made from fine mesh screening and were weighted and marked, so they would stay on the lake bottom, but could be found one month later. Fragments, 10 cm long, were collected from milfoil plants and were placed into the containment bags. A subset of 20 fragments were collected, dried at 70 C to constant weight, and weighed for an average at the initial point in time. Ten fragments were placed into each bag, and 8 bags were placed at each depth (1, 3 and 5 meters). Four of these bags contained autofragments and four contained allofragments. After a one month growth period the bags were collected and the number of surviving fragments, as well as the weight and length of each fragment, was determined. Percent survival was calculated from the number of fragments recovered and the weight gained by each fragment was determined.

Data was used to compare survival of fragments
from each depth and embayment.

PART 3

RESULTS

3.1 Sterilization time

Maximum seed germination was observed after seven days for all treatments in 5.25% sodium hypochlorite (Fig. 3.1). Germination was highest for seeds treated for 25 minutes with sodium hypochlorite, with 63 out of 100 seeds germinating. A decreasing order in the number of germinating seeds then followed for the remaining treatments of 15, 35, 0, and 5 minutes in sodium hypochlorite, respectively.

An examination of contamination by bacteria, algae, and fungus showed no contamination by bacteria at the 15 and 35 minute treatments, with minimal contamination at the 25 minute period (Table 1). Complete contamination by bacteria occurred at the 0 minute treatment, with partial contamination at the 5 minute treatment. Fungus contamination was least at the 35 minute treatment, followed by the 25 minute treatment. Fungus was then present at the remaining treatments in all Petri dishes, as well as some other contaminant (?) that could not be identified.

No contamination by algae was discovered in

Fig. 3.1 Number of seeds germinated versus days needed to germinate for sterilization time treatments.

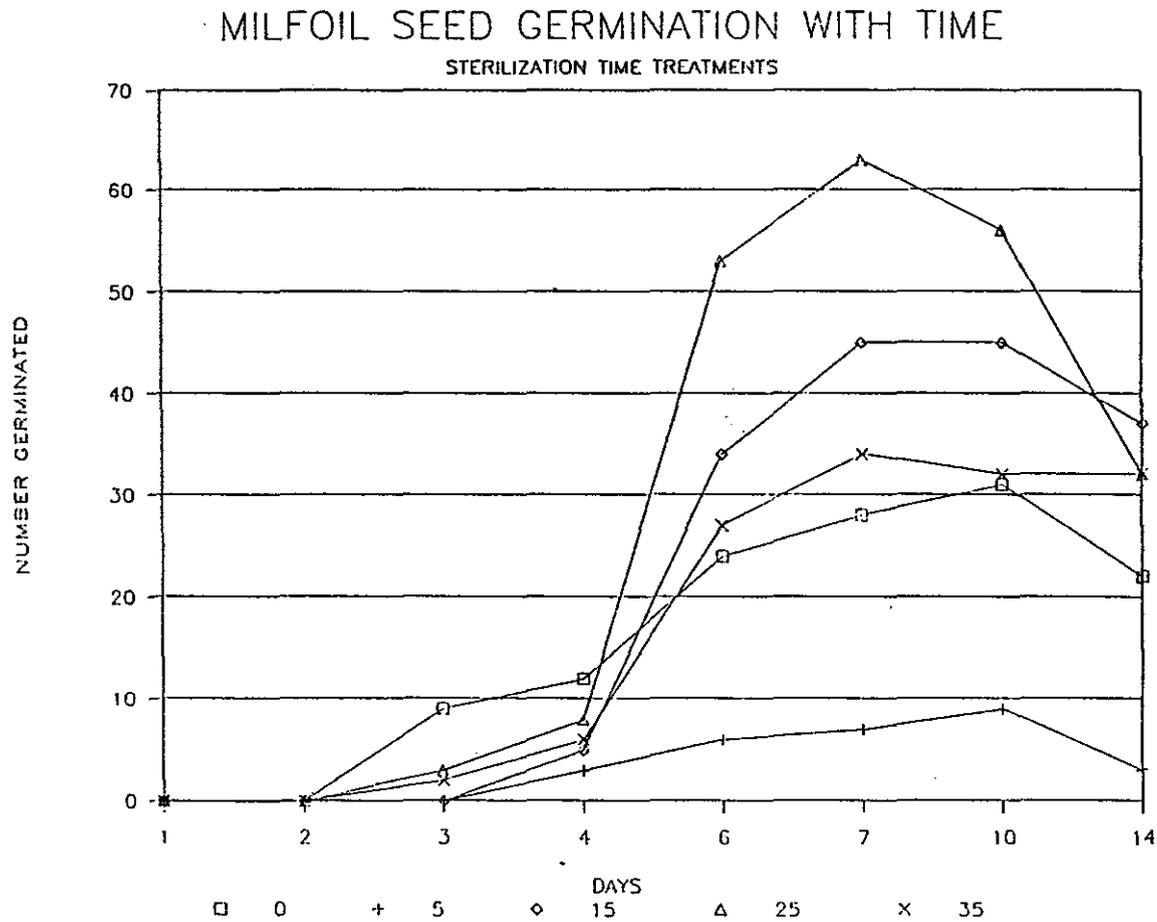


Table 1. Determination of Bacterial, Algal, and Fungi Contamination of Milfoil Seeds.

Minutes in 5.25% Sodium Hypochlorite:
0, 5, 15, 25, 35

Bacteria: (+ Present : - Absent)

		Treatment 1				
		0	5	15	25	35
Dish						
	1	+	-	-	-	-
	2	+	-	-	-	-

		Treatment 2				
		0	5	15	25	35
Dish						
	1	+	+	-	-	-
	2	+	+	-	+	-

Algae / Fungi
F = Fungi ? = Something 0 = Nothing

		Treatment				
		0	5	15	25	35
Dish						
	1	?	?	F	F	F
	2	F	F	F	?	0
	3	?	F	F	0	F
	4	F	F	F	F	0
	5	F	F	F	F	?

any of the dishes containing germinating seeds.

3.2 Laboratory seed germination

Maximum seed germination was observed at a temperature of 20 C and daylength of 14 h light : 10 h dark (Fig. 3.2). Almost no germination was observed for all daylengths at the temperature regimes of 5 and 10 C. Chi-square tests for seed germination indicated that there was a highly significant difference in germination for all temperatures exposed to the daylength of 14 h light : 10 h dark ($p < 0.0001$). The effect of daylength at 10 h light : 14 h dark was also highly significant across all temperatures based on χ^2 tests ($p < 0.0001$). The effect of absence of light (dark treatment) on seed germination across all temperature regimes was highly significant based on χ^2 tests ($p < 0.0001$). Seed germination in the presence of light was greater for the three higher temperatures (15, 20, and 25 C), than germination in the dark treatment within each temperature regime. The highest percent germination was observed in the 20 C temperature treatment for all daylength periods.

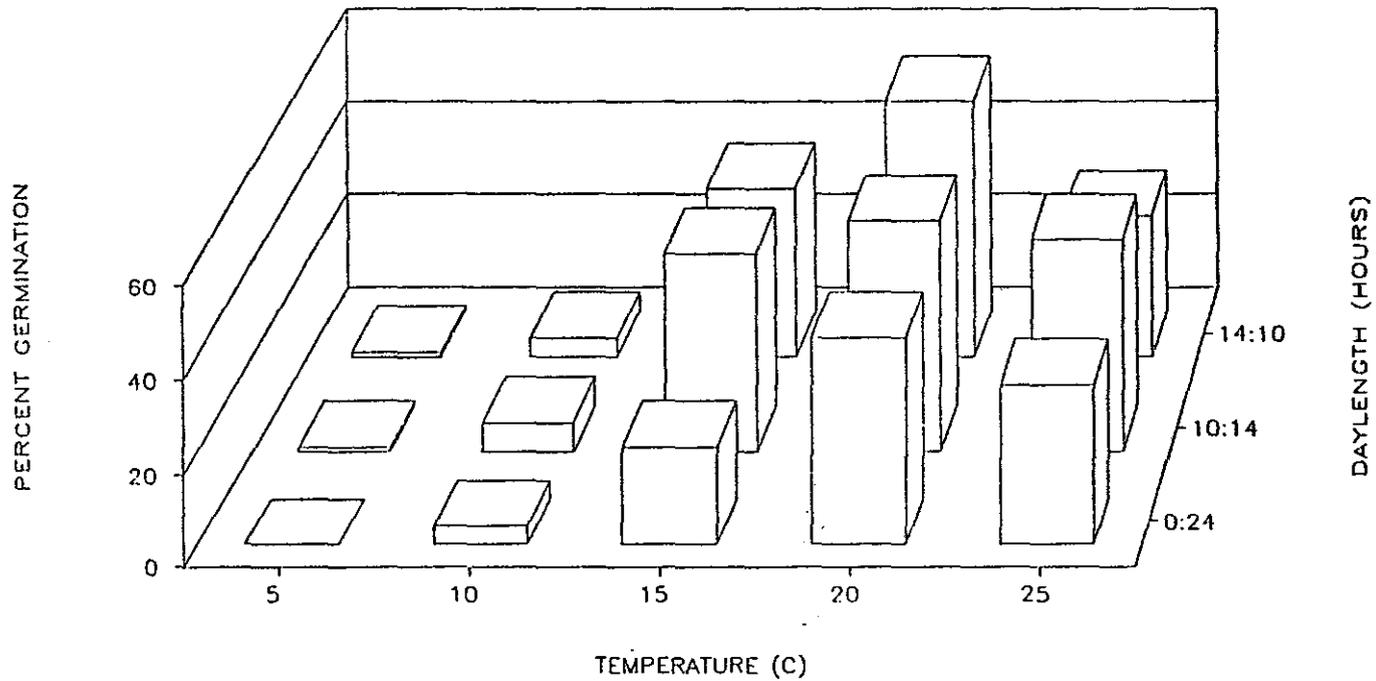


Fig. 3.2 Percent seeds germinated during laboratory experiment determining temperature and daylength requirements.

3.3 *In situ* seed germination

Percent seed germination in Northwest Bay was significantly greater than seed germination in Huddle Bay (χ^2 $p < 0.0001$; Fig. 3.3). Seed germination at all three depths (1, 3, and 5 meters) in Northwest Bay was significantly higher than seed germination in Huddle Bay at all three depths based on χ^2 tests ($p < 0.0001$; Fig. 3.4). In both bays the highest rate of seed germination was observed at the 5 meter depth, with no significant difference in germination between the 1 and 3 meter depths.

3.4 Effect of burial on germination

Percent germination of seeds was significantly greater for seeds buried under less than 2 cm of sediment, or for seeds not buried at all, based on χ^2 tests ($p < 0.001$; Fig. 3.5). Germination was significantly reduced, to less than 15%, once the depth of seed burial exceeded 2 cm. Seeds placed above the sediment surface showed the greatest percent germination, with up to 60% germinating.

Fig. 3.3 Histogram of percent seed germination at the two bays used as study sites.

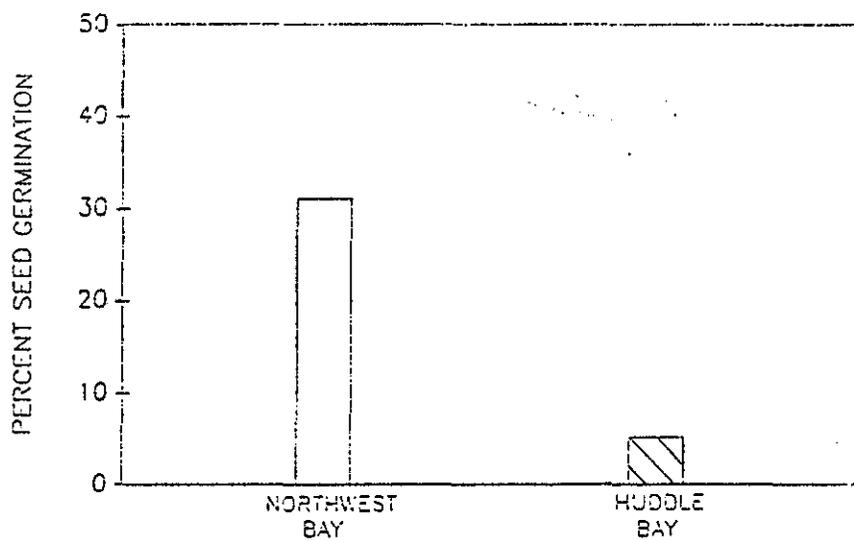


Fig. 3.4 Histogram of percent seed germination for each bay at depths of 1, 3, and 5 meters.

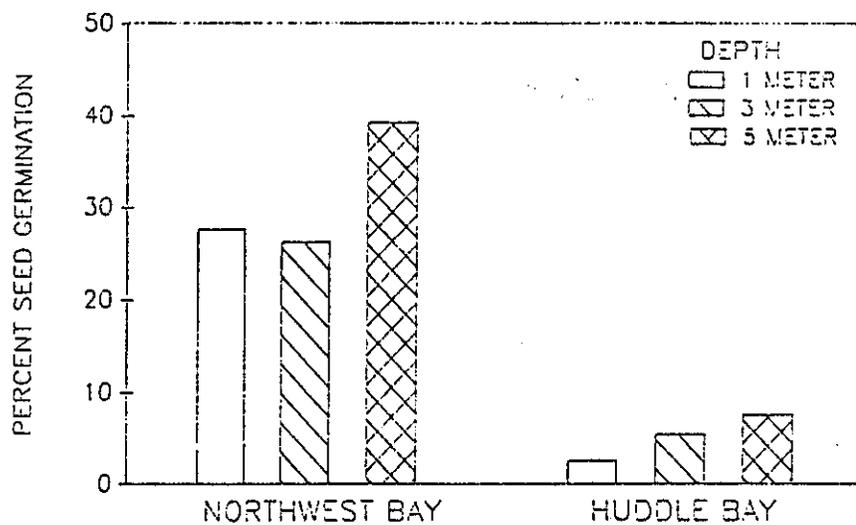
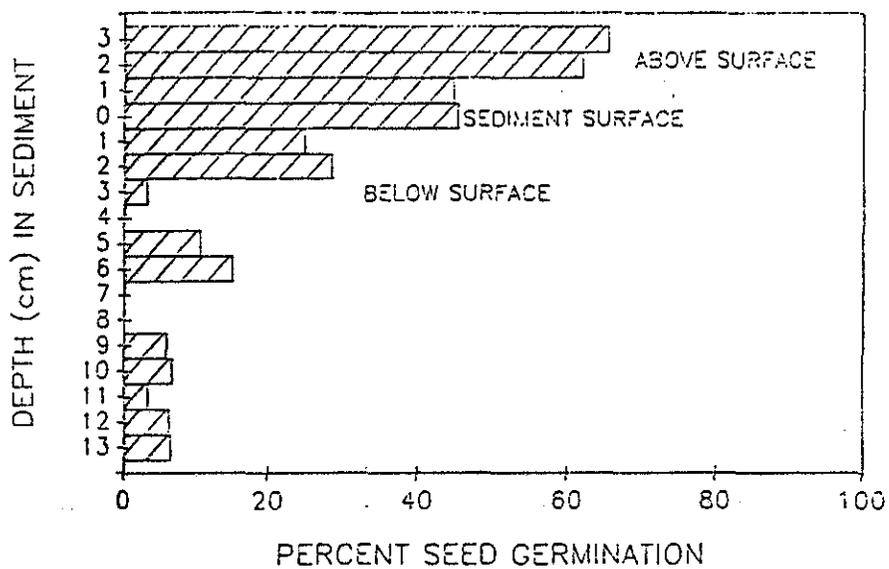


Fig. 3.5 Graph showing the percent seed germination versus the depth the seeds were buried in sediment.



3.5 *In situ* fragment survival

Overall survivorship of fragments in both Northwest and Huddle Bays was highly significant based on χ^2 tests ($p = 0.008$; Fig. 3.6). A comparison of the depths at each bay indicated that higher survivorship was achieved at the shallower depths, with little difference in survival between allo- and autofragments (Fig. 3.7). A noticeable decrease in survival of fragments was observed at the 3 meter site in Huddle Bay, but survivorship still exceeded 60%.

Fig. 3.6 Histogram of percent survivorship for fragments at the two bays used as study sites.

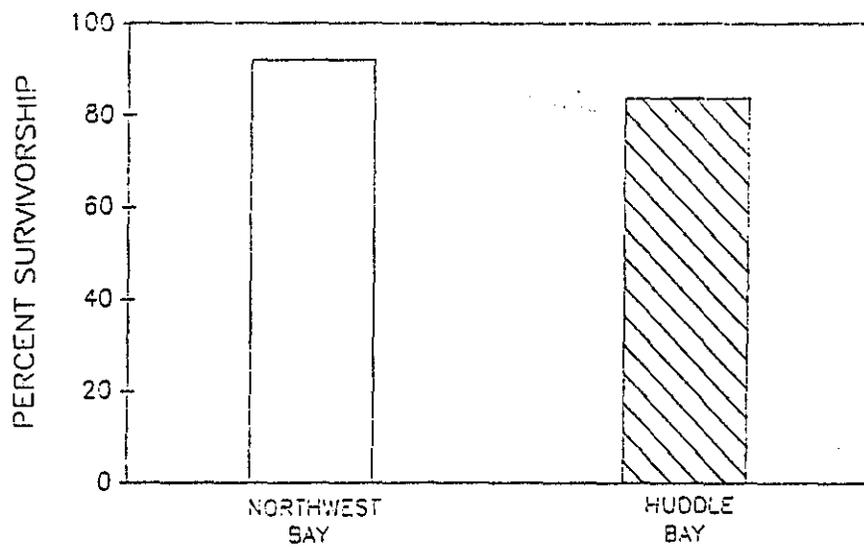
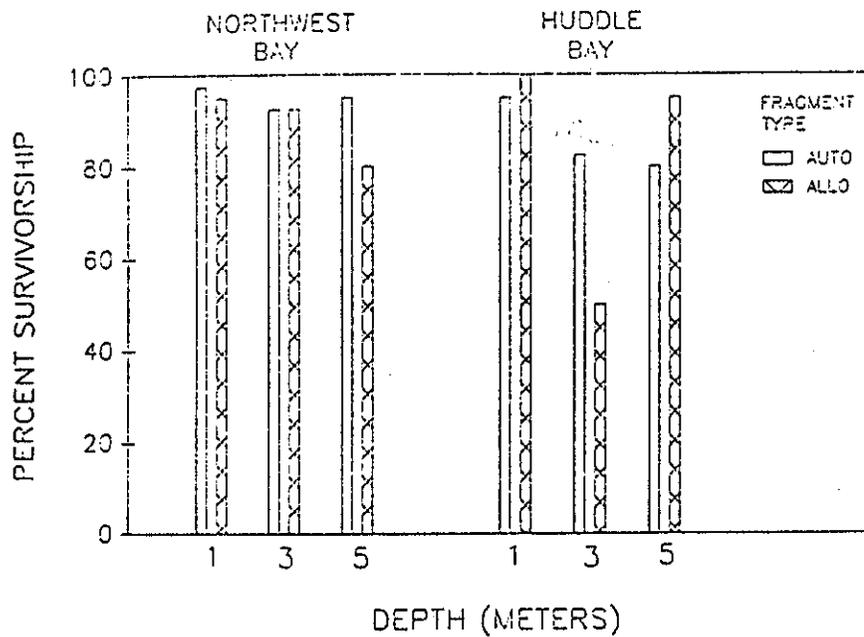


Fig. 3.7 Histogram of percent survivorship of fragments for each bay at depths of 1, 3, and 5 meters.



DISCUSSION

4.1 Sterilization time

Sterilization time was established at 25 minutes in 5.25% sodium hypochlorite by comparing the maximum number of seeds that germinated, with the treatment that showed the least amount of bacterial, algal, and fungi contamination. During sterilization procedures the amount of time needed for the maximum number of seeds to germinate was also determined, that being seven days. Once these two values were determined they were used as the set standard upon which the laboratory germination experiments were carried out.

4.2 Laboratory seed germination

Seeds which were exposed to the colder temperature treatments (5 and 10 C) exhibited the lowest rates of germination across all daylength exposures. This trend would conform to the requirements of most aquatic plant species, since most seeds need to overwinter at colder temperatures, being stimulated to germinate by some factor such as warm temperatures. Milfoil seeds require a temperature greater than 10 C before they begin to show significant germination rates. The

requirement for water temperature greater than 10 C may have adaptive significance. *Potamogeton pectinatus* tubers required temperatures of at least 15 C to germinate (Madsen & Adams, 1988a), which corresponded to the temperature at which it demonstrates positive carbon balance (Madsen & Adams, 1988b). Also, laboratory photosynthesis studies showed that daily carbon balance of *M. spicatum* was lower at 15 C than at 20 C (Madsen & Boylen, 1990).

Seeds exposed to the warmer temperatures of 15, 20, and 25 C and exposed to dark treatments showed an average of 40% germination. It has been shown before that some seeds of milfoil will germinate in the dark, regardless of light treatment, given the proper temperature and pretreatment conditions (Madsen & Boylen, 1988). Therefore, the absence of light alone does not prevent seed germination and a combination of another factor(s) plus light limitation must be responsible for the lack of seed germination in nature.

Both 14 h light : 10 h dark and 10 h light : 14 h dark daylength treatments showed significant seed germination at the warmer temperature regimes of 15, 20, and 25 C, with percent germination around 50%. Maximum germination was achieved at 20 C and 14 h light

: 10 h dark and this corresponds to the temperature and daylength experienced at Lake George during the late spring and early summer months. Therefore, it would be expected that seed germination should have occurred in Lake George, unless other limiting factors were present to prevent germination.

4.3 *In situ* seed germination

A comparison of percent seed germination in Northwest Bay and Huddle Bay illustrates the requirements for proper conditions to be present in order for seeds to germinate. Huddle Bay, which is in the southern portion of the lake, experiences heavy use and recreational activity and is affected by runoff from a nearby brook/marsh. There is a constant churning of the water and sediment particles are in continuous motion during most of the day. During the course of the experiment over 3 cm of sediment was deposited on top of the containers and seeds. Milfoil seeds were shown to exhibit significant germination if covered with 2 cm of sediment or less. It is likely that buried seeds remain ungerminated if buried in sediment. Upon disturbance of the sediment and exposure to lighted conditions, the dormant seed may be

induced to germinate and colonize the disturbed site (McMillan, 1987). Submersed aquatic plants reflect a particularly strong degree of dormancy and may remain this way until proper conditions prevail (Haag, 1983). Seeds in Northwest Bay exhibited far greater germination rates, over 30%, compared to seeds from Huddle Bay, only 5%. Northwest Bay, located in the northern portion of the lake, experiences far less recreational activity and exhibits less sedimentation from streams and marshes, than bays located in the southern part of the lake. Seeds deposited in water not subject to the effects of severe wavewashing and sedimentation, may experience significant germination rates (McDougall, 1983).

The graph comparing the germination rates by depth in Northwest Bay and Huddle Bay indicates that seeds prefer the 5 meter depth for germination in both bays. In Northwest Bay there is a 10% difference in germination rates between the 5 meter site and the 1 meter and 3 meter sites. In Huddle Bay the 5 meter site experienced the greatest germination rate, followed by the 3 meter and then the 1 meter sites. This trend, of deeper sites showing the greatest germination rates, once again reflects the need by the seeds for calm, undisturbed areas to germinate. The 5 meter depth provided

the undisturbed conditions the seeds required to germinate, while the 1 meter and 3 meter sites had too much wave and recreational action for the seeds to experience significant germination rates. However, milfoil seeds germinating at a depth of 5 meters will probably not form dense beds. Natural progression in the colonization of milfoil within a lake dictates that after scattered plants have established themselves new plants will increase the density within the established area. Milfoil has been shown to grow at depths between 1 and 4 meters, with optimal growth at 3 meters (Madsen *et al.*, 1988), so even though maximum germination occurred at the 5 meter depth colonization by seedlings is doubtful.

These results coupled with the laboratory results that showed significant seed germination in dark treatments at warm temperatures, tend to disagree with previous findings which claimed that light qualities and quantities strongly influence the germination rate of milfoil seeds (Coble and Vance, 1987). These results, as well as those by Madsen & Boylen (1988), show that absence of light is not the only factor limiting the germination of seeds. Viable seeds may have multiple chilling requirements, or be buried too deeply, cues such as sediment texture

(nutrient content) or the proper redox potential may be lacking, or some process such as breakdown of fruits by bacterial action or gut passage through birds may need to occur before seeds will germinate (Haag, 1983).

4.4 Effect of burial on germination

The figure depicting seed germination after burial in sediment showed that seeds buried greater than 2 cm below the surface exhibited a significant decrease in germination. Seeds that were placed above the sediment surface showed germination rates greater than 50%, while those seeds located between the sediment surface, but above 2 cm of sediment, showed germination rates greater than 30%. Once the seeds were placed below 2 cm of sediment germination rates dropped below 10%. Therefore, sedimentation is a key factor limiting the germination of milfoil seeds and as stated before, seeds located in Huddle Bay experienced the lowest rates of germination, but the highest rates of sedimentation. Many factors may be limiting the germination of these buried seeds such as the lack of sufficient light, mechanical action, oxygen tension / redox, absence of bacterial action on seeds (due to anaerobic conditions), or the decrease in temperature experienced at deeper depths in sediment. The laboratory experiment was done in

fine, sandy sediment and under controlled temperature conditions, so some of these factors were eliminated, yet significant germination still did not occur below two centimeters of sediment.

4.5 *In situ* fragment survival

Percent survivorship, of both allo- and autofragments, in both bays illustrates the common opinion that plant fragments are generally the most important mechanism for propagation and dispersal of this aquatic "weed" (Madsen & Boylen, 1988; Kimbel, 1982; Nichols & Shaw, 1986). Plant fragments in Northwest Bay exhibited significant survivorship, over 90%, while those in Huddle Bay also displayed significant survivorship, with over 80% surviving the month long experiment. Fragments can survive well on the nutrient content of oligotrophic lake water, but are affected by other factors as well. Factors important to fragment survival include: adequate light intensities in order to maintain a daily positive carbon balance, fragment settling rates, light compensation points, and reserve stored carbon pools (Madsen *et al.*, 1988).

A comparison of fragment survivorship at each depth, in each bay, showed that fragments

preferred shallower waters with higher light levels. Although fragments at all depths showed significant survivorship (most over 80%), fragments at both 1 meter sites had the highest levels of survivorship, followed by the 3 meter sites and finally, the 5 meter sites. These trends tend to follow the requirements established for fragment survival, since the 1 meter sites provided the greatest amount of factors, that were mentioned before. Limited survivorship was observed at the 3 meter site in Huddle Bay, but this was probably due to disturbance caused by extreme recreational activity during the experiment.

4.6 Dispersal of species

Although optimum germination of seeds in Lake George was only 40%, this may indicate that seed production and germination might not appear to be sufficient enough to contribute to the spread of the population in this lake. There are lakes that exhibit higher seed set percentages and germination rates (Madsen & Boylen, 1989), and seed germination may contribute to the annual propagation and expansion of the populations in these lakes. Seeds may remain dormant for prolonged periods of time and germinate only when favorable conditions prevail, such as suitable depth,

water temperature, adequate substrate, water quality and protection from excessive wave exposure. Therefore, milfoil seeds may represent a long term survival adaptation for this species.

Survivorship of stem fragments obtained an optimum of 90%, indicating that fragment formation and dispersal is probably the dominant means by which this species invades and colonizes within a lake and other lakes. No significant difference was observed between survivorship of allo- and autofragments. Therefore, if a plant abscises its stem and releases a fragment for colonization, there is a considerable chance that the fragment will disperse and reestablish itself in another area. This also means that human interaction with this plant, by artificially producing fragments, will increase the percent of formation and dispersal of fragments, but is not the sole cause, or major contributor to this event.

PART 5

CONCLUSION

Seed germination of milfoil does indeed occur *in situ*, but is affected by many factors. Laboratory studies showed that temperature and daylength are both factors that affect the percent of seeds that will germinate, but dark conditions alone will not prevent seeds from germinating. Sedimentation causes seeds to show a significant decrease in germination, probably because it induces many other limiting factors upon the successful germination of the seeds, but is an area that needs further study in order to determine its overall impact on the inhibition of germination. The seed of *M. spicatum* is well endowed with remarkable survival adaptations mediated through its germination habit, adaptations which insure that seedlings are produced only at times when favorable conditions for maturation prevail, and also assure that rapid germination will proceed concurrent with the inception of such conditions (Patten, 1956). The seed of milfoil does not tend to germinate soon after its development unless exposed to certain conditioning treatments. Under natural conditions, then, it is likely that low winter temperatures and

occasional freezing subdue germination, at the same time increase after- ripening so that prompt germination is assured with spring warming (Patten, 1956).

The survival of *M. spicatum* seedlings *in situ* is likely restricted by factors such as grazing, competition and shading from other aquatic plants, water depth and exposure to wave action. Under suitable conditions, milfoil seeds have the requirements needed to germinate and grow to mature size (McDougall, 1983). There are several plausible explanations that may account for the low numbers or lack of seedlings found in nature. Many seedlings may have been overlooked since it is difficult to identify them amongst the extensive and intertwined growth of germinating turions, rhizomes and root crowns of other plants as well as within a milfoil bed itself. Also, seedlings may not be capable of competing with plants from turions which germinate and grow rapidly and in profuse numbers early in spring (Scribailo & Posluszny, 1985).

In general, milfoil has a strong potential to develop a large seed bank and seeds could provide genetic diversity and may represent a long term adaptation to survival for this species. Sexual reproduction by seeds has the capabilities to provide a means of

reestablishment following disturbance and may lead to local changes in density during years favorable for seed production and germination.

PART 6

LITERATURE CITED

- Aiken, S.G., P.R. Newroth, and I. Wile. 1979. The biology of Canadian weeds 34. *Myriophyllum spicatum* L. Can. J. Plant Sci. 59:201-215.
- Coble, T.A., and B.D. Vance. 1987. Seed germination in *Myriophyllum spicatum* L. J. Aquat. Plant Manage. 25:8-10.
- Haag, R.W. 1983. Emergence of seedlings of aquatic macrophytes from lake sediments. Can. J. Bot. 61:148-156.
- Kimbel, J.C. 1982. Factors influencing potential intralake colonization by *Myriophyllum spicatum* L. Aquat. Bot. 14:295-307.
- Madsen, J.D., and M.S. Adams. 1988a. The germination of *Potamogeton pectinatus* tubers: environmental control by temperature and light. Can. J. Bot. 66:2523-2526.
- Madsen, J.D., and M.S. Adams. 1988b. The seasonal biomass and productivity of the submerged macrophytes in a polluted Wisconsin stream. Freshwater Bio. 20:41-50.
- Madsen, J.D., and C.W. Boylen. 1988. Seed Ecology of Eurasian watermilfoil (*Myriophyllum spicatum* L.). Rensselaer Fresh Water Institute Report 88-7, Rensselaer Polytechnic Institute, Troy, New York. November 1988. 25 pp.
- Madsen, J.D., and C.W. Boylen. 1990. The physiological ecology of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and native macrophytes in Lake George: depth distribution of biomass and photosynthesis. Rensselaer Fresh Water Institute Report 89-6, Rensselaer Polytechnic Institute, Troy, New York. February 1990. 52 pp.

- Madsen, J.D., L.W. Eichler, and C.W. Boylen. 1988. Vegetative spread of Eurasian watermilfoil in Lake George, New York. J. Aquat. Plant Manage. 26:47-50.
- Madsen, J.D., and C.W. Boylen. 1989. Eurasian watermilfoil seed ecology from an oligotrophic and eutrophic Lake. J. Aquat. Plant Manage. 27:119-121.
- Madsen, J.D., L.W. Eichler, C.W. Boylen, J.W. Sutherland, J.A. Bloomfield, and K.M. Roy. 1989. The Lake George Aquatic Plant Survey Final Report. NYS Dept. of Environ. Conserv., Albany, NY. June, 1989.
- McDougall, I.A. 1983. A study of the germination potential in *Myriophyllum spicatum* L. seeds. Studies on Aquatic Macrophytes Part XXIV, Province of British Columbia Ministry of Environment, Victoria, Canada. Water Management Branch. October, 1983. 31 pp.
- McMillan, C. 1987. Seed germination and seedling morphology of the seagrass, *Halophila engelmannii* (Hydrocharitaceae). Aq. Bot. 28:179-188.
- Nichols, S.A., and B.H. Shaw. 1986. Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Flodea canadensis*. Hydrobio. 131:3-21.
- Patten, B.C., Jr. 1955. Germination of the seed of *Myriophyllum spicatum* L. Bulletin of the Torrey Botanical Club. 82:50-56.
- Patten, B.C., Jr. 1956. Notes on the biology of *Myriophyllum spicatum* L. in a New Jersey lake. Bulletin of the Torrey Botanical Club. 83:5-18.
- Scribailo, R.W., and U. Posluszny. 1985. The reproductive biology of *Hydrocharis morsusraeae*. II. Seed and seedling morphology. Can. J. Bot. 63:492-496.