SEED ECOLOGY OF EURASIAN WATERMILFOIL (*MYRIOPHYLLUM SPICATUM* L.)

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PREFACE

Studies on the seed ecology of Eurasian Watermilfoil during the summer of 1987 were preliminary in nature. Initial results, however, show seed ecology to be an important avenue of research given the increased importance of Eurasian Watermilfoil in Lake George. Partial support for this project was provided by the United Parcel Service to the Rensselaer Fresh Water Institute. The author would like to thank David Smith for collecting samples from Cossayuna Lake, and Lawrence Eichler and Reginald Soracco for their comments on the draft of this report.

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CHAPTER 1

Formation and Germination of Seeds from Lake George and Cossayuna Lake

INTRODUCTION

In all research efforts to date, investigators have indicated that seeds of Myriophyllum species were not important to their propagation (Aiken et al., 1979; Grace and Wetzel, 1978; Haag, 1983). However, numerous seeds are produced by Myriophyllum spicatum; and a significant portion have germinated in laboratory studies (Coble and Vance, 1987; Patten, 1955). The potential importance of propagation by seeds lies in the establishment of a genetically diverse population, with greater adaptiveness to variable environmental conditions. Recent investigators have not attempted a broader examination of the seed ecology of M. spicatum, nor compared seed set and germination between populations.

Our study examined M. spicatum populations in oligotrophic Lake George and eutrophic Cossayuna Lake in order to compare the seed ecology of an older, more established community in a nutrient-enriched environment to that of a younger population in a relatively nutrient-poor environment.

STUDY SITES

Lake George is a large (114 km^2, 58 m maximum depth), oligotrophic lake on the southeastern edge of the Adirondack Mountains of New York State (Figure 1). The M. spicatum

population in Lake George is fairly young, with significant numbers of M. spicatum first observed in 1985 (Rensselaer Fresh Water Institute, 1986). Specimens for this study were collected from Shadow Bay, a small sheltered bay with a dense bed of M. spicatum in 1 to 3 m water depth. Flowering was scattered in this bay, but occurred over a two-month time span (Rensselaer Fresh Water Institute et al., 1988).

Cossayuna Lake, a small (2.7 km^2), shallow (7.6 m maximum depth) eutrophic lake in New York State, is located southeast of Lake George (Figure 1.1). The Cossayuna Lake M. spicatum population is older and more homogeneous than the Lake George population, dating from about 1976 (David Smith, pers. comm.). Attempts to manage nuisance growths of M. spicatum in this lake have included both harvesting and herbicides (e.g., 2,4-D). Plants used in the work reported here were sampled at the southern end of the lake near the outlet, in 1 m of depth. This area is unaffected by control measures (D. Smith, pers. comm.).

METHODS

Flowering spikes were collected and returned to the laboratory where plant stems were grown in aquaria under controlled light and temperature regimes similar to natural conditions. Flowering spikes were kept above the water surface, and maintained until seed set and maturity were achieved. Flowers were treated in this manner to insure that all seeds produced by the flower stalks would not be lost. At this time, flower spikes were counted for female flowers, fruit maturity, and seed set. A subsample of seeds from each population were dried at 50 ^{o}C, and weighed in groups of 5.

Seed germination was tested using 100 seeds from each population. Seeds were surface sterilized for 30 minutes in 5.25% sodium hypochlorite, and rinsed with sterile

distilled water. Twenty seeds were placed into each of five 250 ml flasks with 200 ml of sterile Lake George water. Filtered air was bubbled into the flasks to prevent stagnation. Flasks were placed under 14 h light : 10 h dark conditions, with a light intensity of 400 uE m^{-2} s^{-1}. Temperature was maintained at 25 ^{o}C in the water bath using a constant temperature heating circulator. The number of germinating seeds in each flask was determined weekly for a period of one month.

Statistical tests were applied following Snedecor and Cochran (1980). The nonparametric rank sum test was used for numerical data in preference to the parametric t-test in two-sample statistical tests, because of the small sample size (e.g., seed dry weight), or sample distributions deviated significantly from a normal distribution. Chi-square tests were used to statistically compare percent germination data.

RESULTS AND DISCUSSION

The Cossayuna Lake M. spicatum population had significantly more female flowers per stalk than did the Lake George population (rank sum $p = 0.028$; Figure 1.2). The median number of female flowers per stalk was 32 for Cossayuna Lake whereas the median for Lake George was 28. The greater number of female flowers could be due to the trophic status of Cossayuna Lake. The more productive environment may allow for greater allocation of resources to flower production. More female flowers per stalk allows greater potential seed production.

Percent seed set in specimens from Cossayuna Lake was significantly higher than in those from Lake George (rank sum $p < 0.0001$; Figure 1.3). The percent seed set observed for the M. spicatum population in Lake George (median 0.0%, mean 1.6%) indicated few seeds were produced in situ,

whereas the observed percent seed set for the Cossayuna Lake population (median 18%, mean 24%) indicated that a large number of seeds were produced *in situ*. The difference in percent of seed set may have resulted from the relative difference in the density of flower stalks in the two populations, but no data has yet been collected on flower stalk density in either lake. *Myriophyllum spicatum*, being wind pollinated and having flower spikes close to the water's surface, should be very sensitive to the density of and average distance between flowers.

No statistically significant difference was found (rank sum $p = 0.86$) between Cossayuna Lake *M. spicatum* seed dry weight (mean = 0.60 mg, n = 50) and Shadow Bay, Lake George *M. spicatum* seed dry weight (mean = 0.62 mg, n = 45). However, a larger sample size for each population might allow a more rigorous statistical comparison. The small size of the seeds creates a technical problem in weighing dried seeds. If the difference in the productivities of the two sites could affect allocation to flower production, it might also be expected to affect the size of seeds produced from these two sites.

Seed germination rates also indicated greater potential success of the Cossayuna Lake seeds (Table 1.1). Cossayuna Lake seeds had a significantly greater rate of germination under light conditions than Lake George seeds (chi-square $p = 0.0004$). The germination rate of Cossayuna Lake seeds incubated in the light (mean = 69%) is comparable to other published reports (77.8% to 97.2%, Coble and Vance, 1987; 85%, Patten, 1955), while Lake George seed germination rates were significantly lower (mean = 41%). Cossayuna Lake seeds incubated in the dark had a germination rate (44%) comparable to Lake George seeds incubated in the light. Both of these rates were significantly less than Cossayuna Lake seeds grown under light conditions (chi-square $p = 0.002$). Although *M. spicatum* seeds do not require light to germinate, light appears to stimulate the germination of some seeds.

Seed production has an as yet undetermined importance to the propagation of M. spicatum under natural conditions. In some populations, such as Lake George, seed production does not appear sufficient to continue or expand the population. In others, such as Cossayuna Lake, there is potential for seeds to contribute to annual propagation of the population, as well as adding to genetic diversity. Although studies to date suggest a low rate of seed germination in situ, laboratory studies show that M. spicatum seeds are quite capable of germinating. Further studies of seed germination are needed to demonstrate the actual ability of seeds to germinate in situ. In general, seeds are not considered to be important to annual propagation of aquatic macrophytes such as M. spicatum, but may provide both genetic diversity and a long-term reservoir for the species in a given location.

Table 1.1. Germination of Myriophyllum spicatum seeds from Cossayuna Lake (light and dark treatments) and Lake George (light treatment only) after one month of incubation at 25 °C. Values in parentheses are percentages of totals. Yate's corrected chi-square p-value for two-by-two comparisons are given below.

Population tested	Number Germinated	Number Ungerminated	Total
1. Lake George	41 (41)	59 (59)	100
2. Cossayuna Lake (Light Treatment)	55 (69)	25 (31)	80*
3. Cossayuna Lake (Dark Treatment)	44 (44)	56 (56)	100

Yate's corrected chi-square p-values for two-by-two comparisons:

	Population		
Population	1	2	3
1. Lake George	---	0.0004	0.77
2. Cossayuna Lake (Light Treatment)	---	---	0.002
3. Cossayuna Lake (Dark Treatment)	---	---	---

*One flask of the Cossayuna Lake light treatment had unusual amounts of fungal growth, resulting in an apparent reduction of seed germination. This flask was deleted from the analysis.

Figure 1.1. Map showing the location of Cossayuna Lake (1) and Lake George (2) in New York State; the sampling location in Cossayuna Lake; and the sampling site on Lake George.

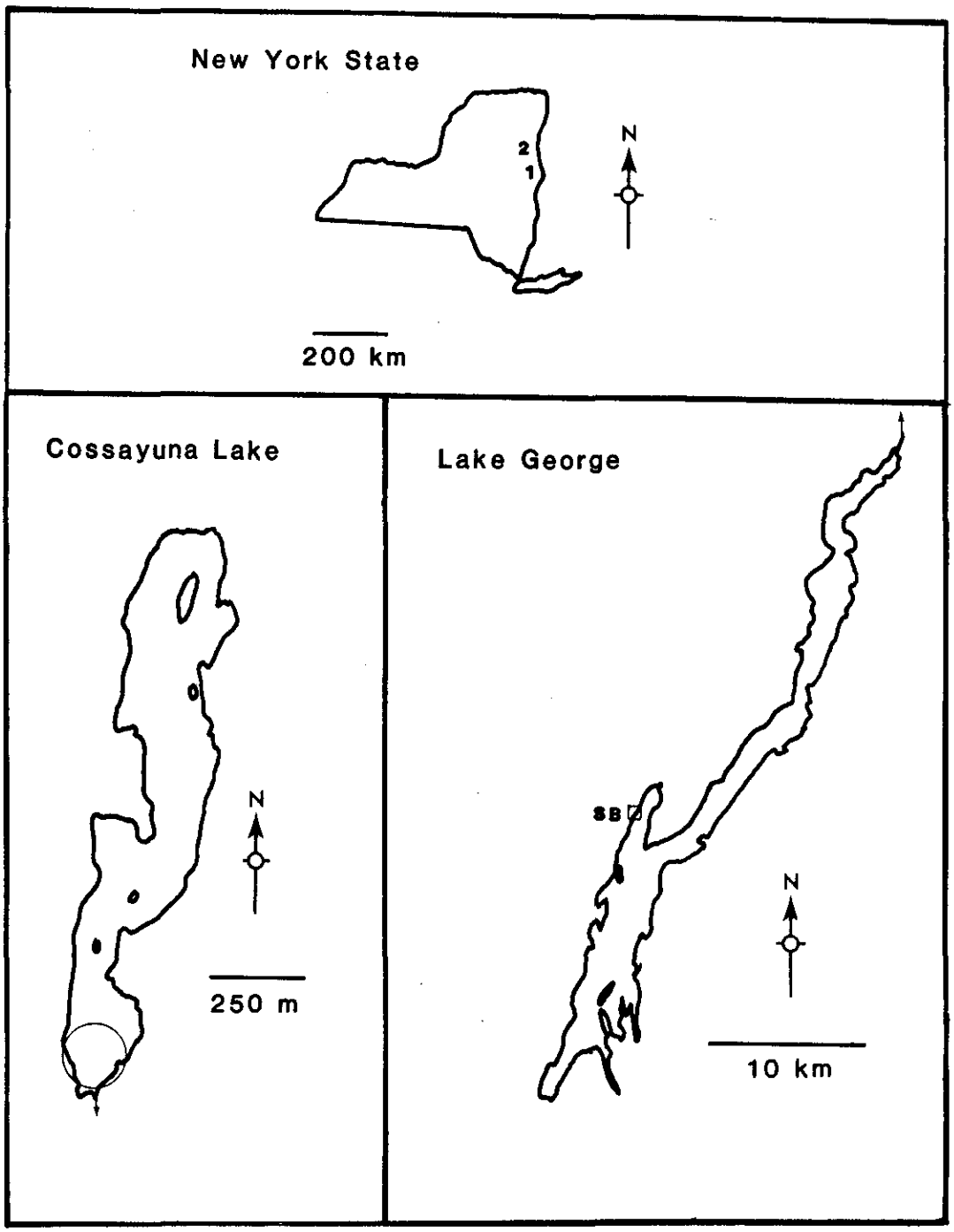


Figure 1.2. Histogram of the number of female flowers per stalk for the populations at Lake George and Cossayuna Lake. "M" = median for population.

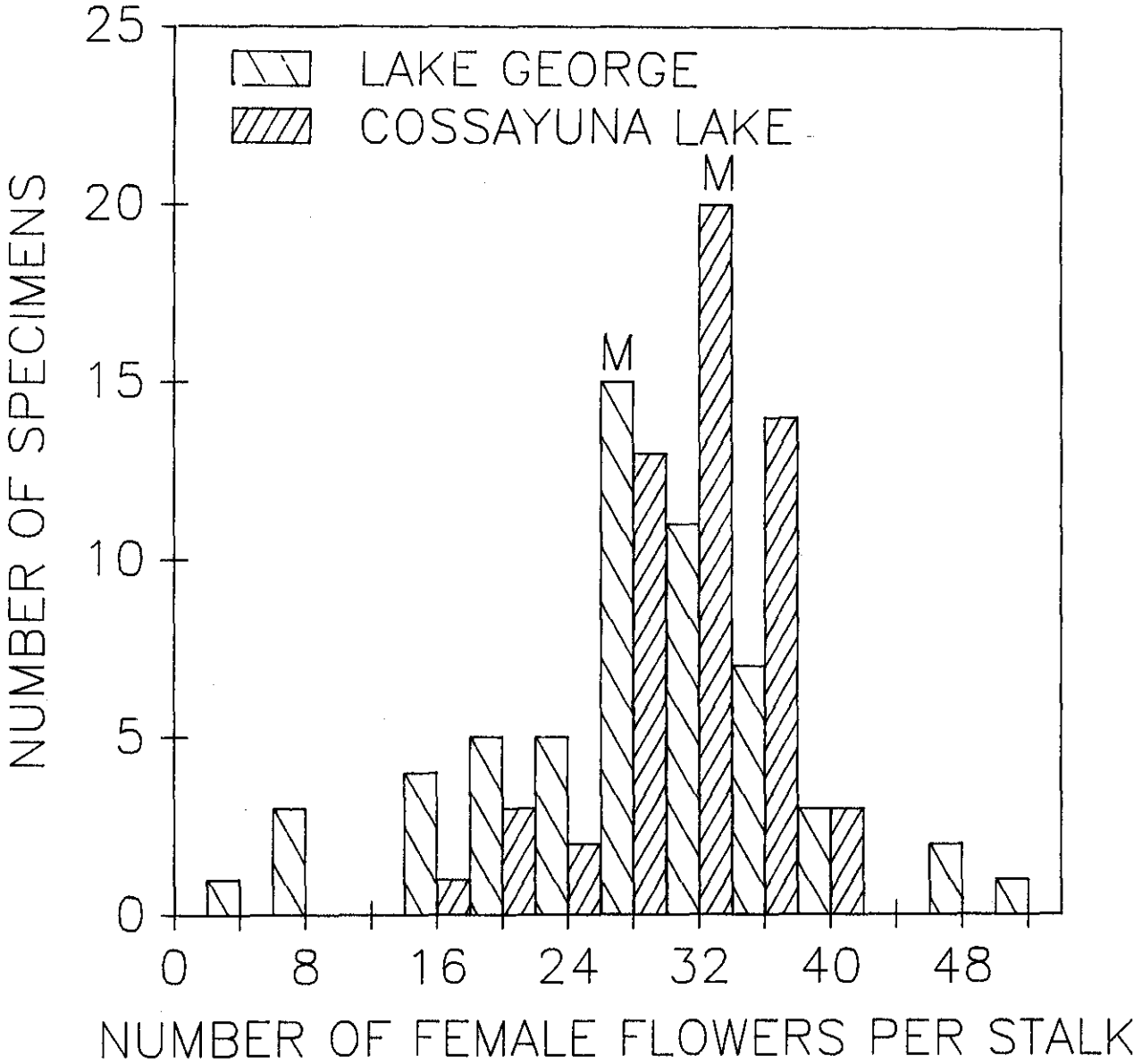
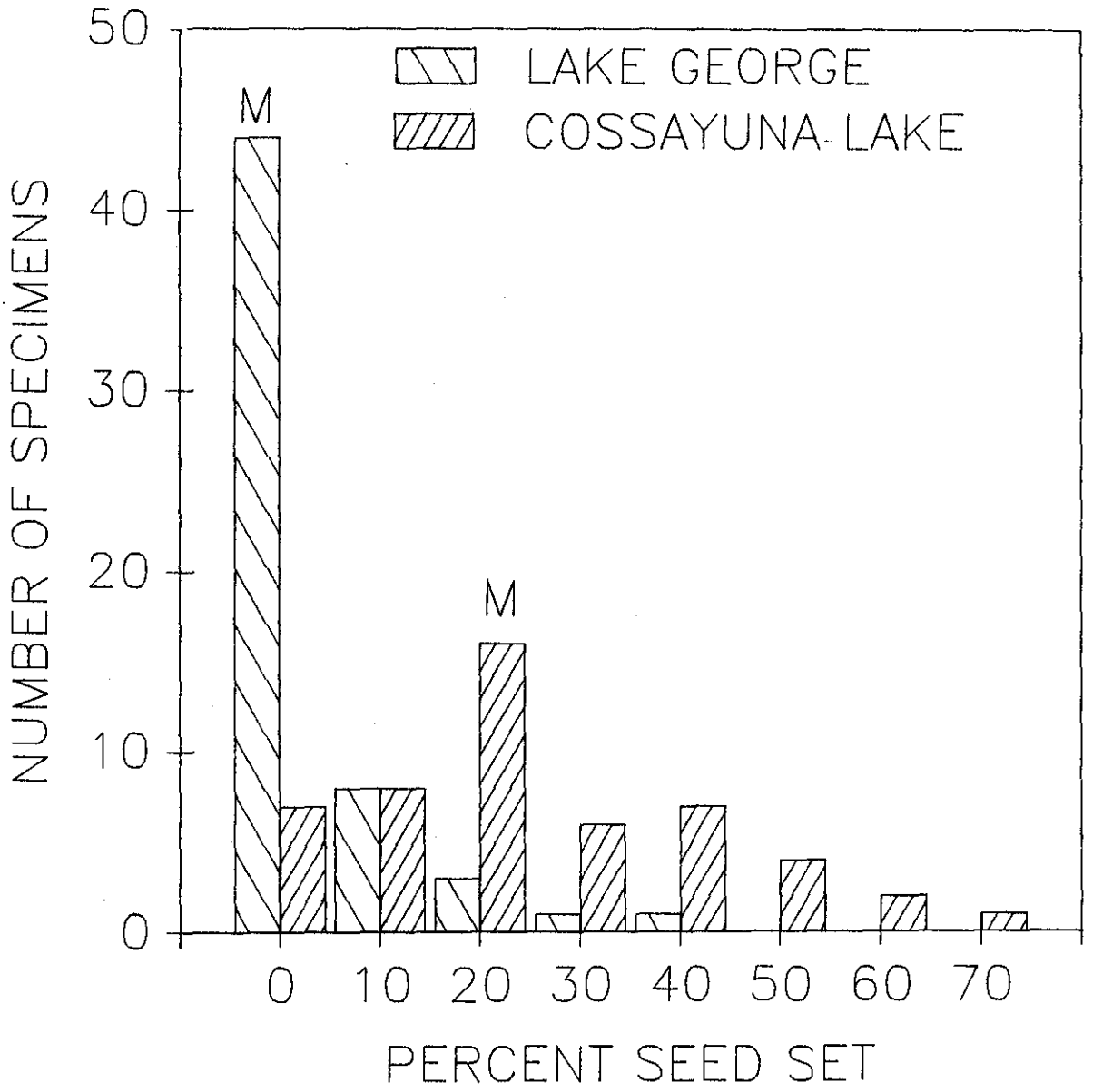


Figure 1.3. Histogram of percent seed set for populations from Lake George and Cossayuna Lake. "M" = median for population.



CHAPTER 2

Germination Experiments: Daylength and the Light Requirement

INTRODUCTION

The propagation of most submersed aquatic plants is generally considered to be dominated by vegetative processes (Sculthorpe, 1967). In the case of Myriophyllum sibiricum (ex. M. exalbescens), no seedlings have been found in two lakes for which it is reported as a dominant species (Weber, 1972; Haag, 1983).

Myriophyllum spicatum propagates vigorously by vegetative means, both through fragments and stolons or root crowns (Madsen et al., 1988; Nichols and Shaw, 1986). The plant may produce numerous seeds in nature, but to date no indication of successful natural seed germination or seedling survival has been noted (Patten, 1956; Aiken et al., 1979). Despite the absence of observed seed germination in nature, the seeds germinate well in the laboratory under a variety of conditions (Coble and Vance, 1987; Godmaire and Nalewajko, 1986).

Although M. spicatum is an economically significant plant, having a major impact as a nuisance aquatic plant, there is little information on the physiological response of M. spicatum seeds to environmental parameters. The purpose of the two experiments reported here was to examine light requirements for germination of M. spicatum seeds, both in terms of photoperiod and the absolute requirement for light versus incubation in the absence of light.

METHODS AND MATERIALS

Seeds were collected from Cossayuna Lake, a small eutrophic lake in Washington County, New York which has had a dense population of M. spicatum since approximately 1976 (Chapter 1). Seeds were stored in lake water at 4°C, without light, for a period of at least six weeks to ensure that they would germinate.

In the first experiment which examined the effect of photoperiod, seed germination was monitored for a period of two weeks under three photoperiod treatments: 10 hours light, 14 hours dark; 12 hours light, 12 hours dark; and 14 hours light, 10 hours dark. These photoperiods represent the range available at the latitude at which the seeds were obtained. Light intensity for this experiment was approximately 400 uE m^{-2} s^{-1}. Seeds were surface sterilized in 5.25% sodium hypochlorite for 30 minutes, and rinsed with two aliquots of sterile distilled water before being placed in culture flasks. Sterilization was done to ensure the proper germination of seeds, without interference from fungi or bacteria. Ten 250 ml flasks with 200 ml of autoclaved distilled water were inoculated with twenty sterile seeds, and placed in a water bath at 25°C. The number of germinated and ungerminated seeds were counted at the end of the second week (after 14 days).

The second 48-day experiment examined the requirement for light in seed germination. Four treatments were used in this experiment: 1) Light available from the first day; 2) seeds initially kept in the dark until day 7, then exposed to light conditions; 3) seeds initially kept in the dark until day 14, then exposed to light conditions; and 4) seeds initially kept in the dark until day 21, and then exposed to light conditions. For each treatment, twenty surface-sterilized seeds were placed in sterile petri dishes with sufficient autoclaved distilled water to fill the dish. Five petri dishes were used for each treatment, for a total of one hundred seeds per treatment. Light

intensity in this experiment was approximately 400 uE m^{-2} s^{-1}, with a photoperiod of 14 h light : 10 h dark. The number of germinated and ungerminated seeds in each petri dish were counted every seven days for those seeds exposed to light conditions. Seeds in the initial dark treatments were not examined until the time for the treatment to be exposed to light conditions.

Statistical analyses included both two-by-two and three-by-two Chi-square analyses, and Fisher's exact test on a two-by-two table, according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Photoperiod

In the experiment examining the effects of photoperiod on seed germination at a single temperature (25°C), the seed germination rates varied from 59% to 67% (Table 2.1). However, there was no significant difference in these germination rates based on the chi-square test of the three-by-two table ($p=0.19$). Chi-square and Fisher's exact tests on the possible two-by-two tables also indicate that the levels were not significantly different. Therefore, daylength or photoperiod effects are not significant at 25°C. However, daylength may play a significant role in germination rates at lower temperatures, as might be found in the early spring.

Initial Dark Period

Results of the effect of exposure to different initial dark periods are shown in Figure 2.1. Seeds exposed immediately to the light (treatment #1) reached maximum germination within 7 days, with 75% of the seeds germinating. The percentage of germinated seeds remained

constant for the following 35 days. For each of the initial dark period treatments (treatments #2 through #4), the initial postdark germination rates were significantly lower than when light was provided from the beginning of the incubation, ranging from 24% to 35% ($p<0.001$). However, the postdark germination rates for the three initial dark treatments were not significantly different from each other (Chi-square $p=0.22$). In each of the initial dark treatments, the germination rate increased to its maximum within seven days of exposure to light and the overall germination rates decreased with increased length of initial dark treatment. Although initial dark treatments of 7 and 14 days (treatments #2 and #3) did not have significantly lower final germination rates than treatment #1 ($p=0.31$), the final germination rate of the longest initial dark period tested (treatment #4, the 21 day dark treatment) was significantly lower than treatment #1 ($p=0.006$ for two-by-two Chi-square table), but not significantly less than the 7 or 14 day initial dark treatments (treatments #2 and #3). The 7 and 14 day dark treatments did not have significantly lower final germination rates than treatment #1.

The initial dark treatment experiment highlights several important aspects of M. spicatum seed physiological ecology, that may aid in interpreting field observations. Some seeds of M. spicatum will germinate in the dark, regardless of light treatment, given the proper temperature and pretreatment conditions. An additional number of seeds are stimulated by the presence of light, but the percentage of seeds stimulated by the presence of light decreases with the time of storage in the dark at warm temperatures. Therefore, burial of seeds in sediments should not prevent germination of seeds from only the absence of light.

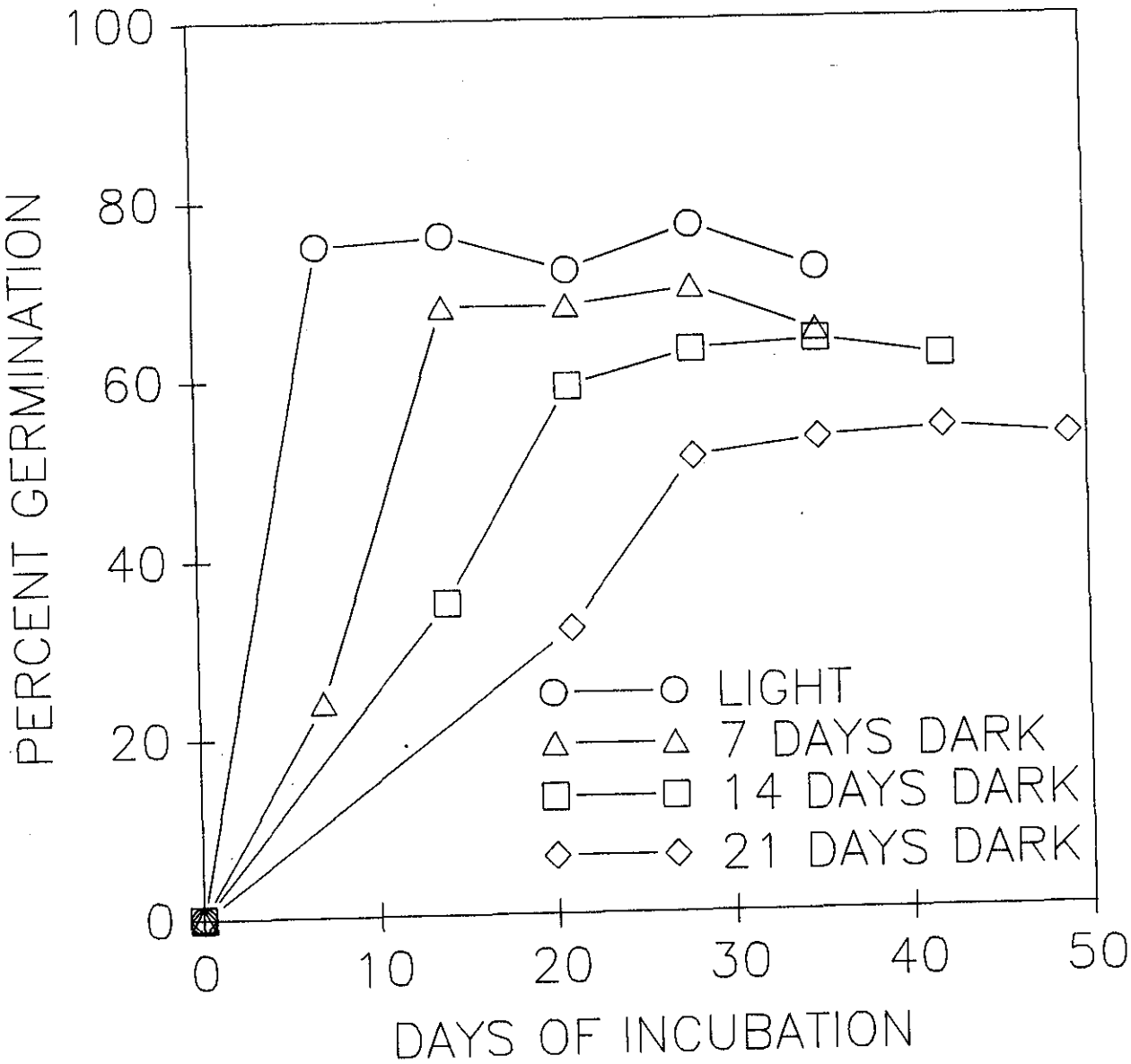
More laboratory and field experiments need to be done to resolve the apparent paradox between laboratory and field observations of M. spicatum seed formation and germination.

Table 2.1. Germination rates for M. spicatum seeds incubated at 25 °C for two weeks under three photoperiod treatments. The numbers presented are actual counts, the numbers in parentheses are percentages. Yate's Chi-square value and p-value are given below.

Daylength	Ungerminated	Germinated	Total
10 h light: 14 h dark (Treat. 1)	68 (34)	132 (66)	200 (100)
12 h light: 12 h dark (Treat. 2)	82 (41)	118 (59)	200 (100)
14 h light: 10 h dark (Treat. 3)	66 (33)	134 (67)	200 (100)

Yate's chi-square = 3.30, p-value = 0.192

Figure 2.1. Germination rates of M. spicatum seeds exposed to four initial dark period treatments versus days of incubation. The first percentages for initial dark period treatments were determined immediately after the initial dark treatment ended, prior to any exposure to light.



CHAPTER 3

Notes on Viability Testing with Tetrazolium

INTRODUCTION

As part of the investigation of the seed ecology of different populations of Eurasian Watermilfoil (Myriophyllum spicatum L.), a rapid method for determining seed viability was sought that would be insensitive to the effects of dormancy and other inhibitions to germination. For terrestrial plant seeds, such a viability test involves staining seeds with a tetrazolium dye (Moore, 1962, 1969, 1976). The use of these dyes for seed viability testing is widely recognized for agronomic crops, but is seldom used for wild populations, nor for aquatic plants. Previous studies on wild plants compared both species and populations of emergent marsh vegetation, with some success (Statler and Batson, 1973; Statler, 1973). Therefore, a series of tetrazolium staining techniques were attempted with Eurasian Watermilfoil seeds, using an agronomic crop seed for a standard.

METHODS

Seeds used in these experiments were M. spicatum seeds from Cossayuna Lake (see Chapters 1 and 2) as the study material, and Radish seed (Raphanus sativus var. Crimson Giant) as the control.

Both types of seeds were germinated in petri dishes, with M. spicatum seeds stored in lake water at 4°C for six weeks prior to testing. Seeds were sterilized with 5.25% sodium hypochlorite for 30 minutes, and rinsed with sterile distilled water. Light conditions for germination were

14 h light : 10 h dark at 400 uE m^{-2} s^{-1}, with the ambient air temperature being approximately 18 ^{o}C.

For tetrazolium testing, seeds were imbibed for at least 30 hours. The seeds were then bisected longitudinally, with each half placed in a separate container. The seeds were treated at both 0.1% and 1% concentrations of tetrazolium chloride, with time lengths varying from one to 24 hours. After this incubation, seeds were flushed with distilled water, and stored at 4^{o}C until examined. For each test treatment, 10 seeds were bisected, stained, and each half was examined.

RESULTS AND DISCUSSION

Myriophyllum spicatum seed germination rates ranged from 60% to 75% in the light (Chapter 2), and the germination rates of radish seeds averaged 85%. Theoretically, the number of seeds stained by tetrazolium dye should equal or exceed the number of germinated seeds.

In Table 4.1, the general results of tetrazolium staining on the two seed types is presented. Myriophyllum spicatum seeds did not stain adequately to be counted at any treatment level, whereas radish seeds stained quite well with the 0.1% tetrazolium solution with an incubation time of 4 hours. Also, the test results on a relatively small number of radish seeds correlated well with the germination rates for this type of seeds with approximately 80% of radish seeds testing viable.

In conclusion, M. spicatum is not a strong reactant to the tetrazolium dye, and thus is not a good candidate for tetrazolium test procedures. Also, the extremely small size of the seeds makes bisecting the seeds difficult and tedious, and prevents easy identification of stained parts. Our general recommendation is that seed germination tests are the best method to determine the approximate seed viability of Eurasian Watermilfoil seeds.

Table 4.1. Tetrazolium staining tests on radish and Eurasian Watermilfoil seeds for varying time lengths and tetrazolium concentrations. Color indicates the intensity of staining, the count is the sum of the two halves, with the average percentage given in parentheses.

	Radish		Eurasian Watermilfoil	
Treatment	Color	Count	Color	Count
		0.1% Tetrazolium		
1 hour	faint	14 (70)	none	----
2 hours	faint	13 (65)	none	----
4 hours	good	18 (90)	none	----
12 hours	dark	14 (70)	none	----
		1.0% Tetrazolium		
2 hours	too dark	----	none	----
4 hours	too dark	----	none	----
12 hours	too dark	----	none	----
24 hours	too dark	----	none	----

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