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Cold Tolerance of the Axillary Turions of Two Biotypes of Hydrilla and Northern Watermilfoil

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ABSTRACT

A two-step assay was developed to test the cold tolerance ranges of axillary turions of dioecious and monoecious *Hydrilla* (*Hydrilla verticillata* [L.F] Royle) and *Myriophyllum sibiricum* (Kamarov). In phase one, cold treatments of 0, 28, 63, and 105 d duration were used to test overwintering effects on mortality rates. Phase two consisted of a test of survivorship over 10 weeks in treatments simulating early growing season. These early growing season treatments included rapid and slow temperature increases and long and short daylengths. Survivorship, shoot dry weight, branch number, and root development were measured to determine responses to different temperature increases and daylengths. As overwintering period (phase I) increased from 0 to 105 d, mortality increased from 8% to 98% for dioecious *Hydrilla* and 0% to 48% for monoecious *Hydrilla. M. sibiricum* had low percent mortality across all overwintering treatments (0 to 105 d) and no deaths at 0 and 105 d. Survivorship decreased during the early growing season (phase II) for both *Hydrilla* biotypes and was affected by overwintering period. No dioecious *Hydrilla* turions survived the early growing season after overwintering periods of 63 and 105 d. Monoecious *Hydrilla* turions survival ranged from 67% (63 d) to 42% (105 d). *M. sibiricum* had variable rates of survival in the early growing season after all overwintering treatments, ranging from a minimum of 8% at 0, 28, and 105 d, to a maximum of 67% at 63 d. Shoot dry weight and branching increased with more rapid changes in early growing season temperatures. Using a two-step assay to determine cold tolerance suggests that monoecious *Hydrilla* will likely overwinter in northern latitudes more successfully than dioecious *Hydrilla.*

Key words: cold stress, invasive potential, invasive species, submersed aquatic vegetation, vegetative propagules.

INTRODUCTION

Given the impacts of invasive aquatic plants in the United States, the ability to predict their potential ranges is critical. Invasive aquatic plants negatively impact habitats, interfere with water uses, and displace native plants, costing the United States over $110 million per year (Pimental et al. 2000). Determination of potential geographic distribution is important for developing strategies to minimize spread, such as policies to prevent entry into a region and resources for scouting outbreaks. Computer models such as CLIMEX, developed by CSIRO Entomology in 1985, are most commonly used to predict spread. CLIMEX estimates a species' distribution potential with climatological data from a plant's current range and published reports of individual species' responses to temperature and moisture parameters (Maywald and Sutherst 1985). Values for these model parameters rely on sound empirical estimates of factors that likely limit plant distribution, such as photoperiod requirements or temperature tolerances.

The diversity and abundance of invasive aquatic plants found in the southern U.S. with the potential for northward expansion is of particular concern. For instance, CLIMEX modeling for Minnesota showed that six invasive plant species found in the southern U.S. have a high probability of success in Minnesota: *Cabomba caroliniana* Gray, *Hydrilla verticillata* (L.F.) Royle (monoecious), *Hydrocharis morsus-ranae* L., *Myriophyllum heterophyllum* Michx., *Nymphoides peltata* (Gmel.) Kuntze, and *Trapa natans* L. While much research has been conducted on these species, exact cold tolerance ranges of the plants and therefore the likelihood of northern expansion is uncertain. Predicting potential range expansions of aquatic plants is complicated due to anomalies seen in present distribution, taxon variability, and production of specialized overwintering propagules. Actively extending its range, *Hydrilla* possesses all of these complicating traits (Cook and Luond 1982). According to Balciunas and Chen (1993), low temperature will likely be a primary factor restricting *Hydrilla* distribution and geographical range.

The ability of a plant to survive extended time in cold water, short daylengths, and cold temperatures in an overwintering state and as newly emerging regrowth will likely determine the northern limits of its range if other conditions are appropriate for growth. In the U.S., *Hydrilla* is found as far north as Connecticut, Massachusetts, Washington (Jacano 2002), Maine (Maine Department of Environmental Protection 2002), and Indiana (USGS 2007). Recently, *Hydrilla* was found in an artificial pond in Wisconsin (http://www.datcp.state.wi.us/press_release/result.jsp?prid=2014). Given that *Hydrilla* can survive to 58°N latitude in the Eastern Hemisphere, it can be expected to survive and reproduce in northerly climates in the Western Hemisphere.

Forty-seven biotypes of *Hydrilla* are known globally. Highly polymorphic in response to environmental conditions, each biotype likely differs in overwintering ability. Two biotypes occur in the U.S., one monoecious and the other dioecious. Dioecious biotypes were first reported in 1960 in Florida but have since been found throughout the eastern seaboard as far north as Connecticut (Steward et al. 1984, Les et al. 1997) and across the southern U.S. to California. Monoecious strains are be-

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lieved to have arrived more recently, with initial reports from 1982 in Washington, D.C. Monoecious populations have spread throughout the southeastern U.S. (north to Delaware), and have also been found in California, Washington (McFarland et al. 1998), and Indiana. Factors causing the differences in geographic spread between these two biotypes are unknown, but potentially could include dispersal abilities, environmental limitations, or post-introduction adaptive evolution.

Geographic spread in *Hydrilla* is likely facilitated by the production of vegetative propagules, especially axillary turions (Spencer et al. 1987). Turions, specialized structures that form in the axils of leaves or branches, at the end of branches, or at the terminal nodes of positive geotropic stems, are also important for surviving winter, drawdown, chemical treatment, and other times of stress (Sculthorpe 1967). Turions readily form on floating plant fragments, so vegetative propagation can occur from even small amounts of plant material (Langeland and Sutton 1980). Fragments may be transported on watercraft and trailers, which are likely an important vector for this species as with many aquatic plants. Turions may then form on the floating fragments and grow or overwinter to establish a new population.

More accurate cold tolerance data is needed to determine the risk of *Hydrilla* expansion into northern climates in North America. To determine the survivorship over time of *Hydrilla* axillary turions in winter temperatures, a two-step assay was developed that included an overwintering treatment and early growing season treatments. A taxon native to northern North America, *Myriophyllum sibiricum*, was also tested as a benchmark for assay results. Our objective was first to determine whether the assay could detect differences in turion survival to cold temperature treatment between *Hydrilla* and *Myriophyllum* consistent with their current distributions. Our second objective was to determine whether duration of the overwinter period, and daylength or rate of increase in water temperature during the early growing season are important for determining the likelihood of northern range expansion of axillary turion survival in monoecious and dioecious *Hydrilla*.

METHODS

Experimental Approach

A two-step assay was developed to evaluate the effects of cold water temperatures on axillary turions of monoecious and dioecious *Hydrilla verticillata* and *Myriophyllum sibiricum*. *M. sibiricum* has a circumboreal distribution (Ceska and Ceska 1986) and also produces turions as an overwintering strategy (Aiken and Walz 1979). Turions of *M. sibiricum* are important in the reproduction strategy of this species (Weber 1972) and have been shown to survive temperatures below 5 C for more than 105 d in a Minnesota lake (Aiken and Walz 1979). A winter vernalization period of *M. sibiricum* turions is needed for normal plant development (Aiken and Walz 1979); the plant does not grow successfully south of the mean January isotherm of 0 C, suggesting the requirement of a winter cold treatment (Aiken 1981). Germination of *M. sibiricum* turions occurs under the ice and may be triggered by daylength (Weber 1972; Aiken and Walz 1979).

The two steps of the assay correspond to two phases of successful perennation by turions, dormancy during cold temperatures (phase I) and re-emergence with lake warming (phase II). Plants must survive cold water temperatures under ice (typically around 4 C) for extended periods of time and also survive slow temperature increases in the spring. During this early growing season, turions must germinate and have enough stored resources to survive until water temperatures are optimal for growth. Once growth begins, shortened photoperiods in northern climates may be a disadvantage because overall photosynthesis may be decreased. The first step of the assay estimates percent mortality of turions held at 4 C for 0, 28, 63, and 105 d. The second step determines turion survivorship and growth as affected by early growing season daylength and temperature increase.

Turions were subjected to four overwintering treatments approximating conditions found from Minnesota (45°N) southward to its present U.S. range in Florida (25°N): 0, 28, 63, and 105 d. A water temperature of 4 C was used for each of these four treatments to simulate ice-covered lakes typically found in northern climates. Water temperatures may be colder where no ice coverage occurs due to wind action, springs, aeration, or other causes, or warmer depending on human actions, or other factors. At the end of the overwinter treatment (phase I), survivorship was determined for half of these turions, and the other half were further evaluated to assess the effects of early growing season conditions (phase II). This evaluation consisted of a 10-week spring period that simulated early growth conditions typically experienced in natural waterbodies at 0 to 2 m in depth. Factors in the early growing season were rate of temperature increase (fast, slow) and length of photoperiod (long, 14 h; short, 10 h).

Plant Sources and Turion Propagation

M. sibiricum turions, monoecious *Hydrilla* turions, and dioecious *Hydrilla* plants were collected in native environments and transferred to a greenhouse at the University of Minnesota, St. Paul (45°N, 93°W). Dioecious *Hydrilla* plants were shipped from Lewisville, Texas (33°N, 96°W) and arrived on 24 October 2000. Monoecious *Hydrilla* subterranean turions arrived from cultures at North Carolina State University, Raleigh, NC (35°N, 78°W) on 24 October 2000. Dioecious *Hydrilla* was cultured in a 379-L tank in a 50:50 mixture of sterile sand and loam in the greenhouse. The plants underwent a photoperiod of 16-h d and an air temperature of 25 C during the culturing period. Tanks were illuminated by natural sunlight and 400 watt high-pressure sodium lamps, approximately 600 µmol m^{-2} s^{-1}. The monoecious *Hydrilla* turions were placed in water at 25 C in the greenhouse. When the turions failed to grow, they were put through a cold treatment (6 C) of approximately six weeks to attempt to break dormancy. After the cold treatment, the monoecious turions were returned to the greenhouse at 25 C and new growth emerged within two weeks. Germination of *Hydrilla* turions has been found to be between 14 and 35 C, optimally at 25 C (Haller et al. 1976). After new growth reached 10 cm in length, the turions were planted in a 379-L tank in a 50:50 mixture of sterile sand and loam and were allowed to grow to tank capacity. Tanks were cleaned three times/week to remove decaying material and algae; dechlorinated water was added to maintain tank levels. Supplemental nutrients were not provided because plants grew vigorously, and we did not want to trigger algal blooms.

To produce axillary turions, photoperiod cloth was used to create 8-h d for tanks containing monoecious and dioecious *Hydrilla* (Thullen 1990). Shoots of plants were also broken periodically, producing floating fragments to stimulate turion formation (Haller et al. 1976, Thullen 1990).

M. sibiricum turions were collected from Snail Lake, Ramsey County, Minnesota (45°N, 93°W) on 31 October and 6 November 2001. Collections were also taken from Christmas Lake, Hennepin County, Minnesota (44°N, 93°W) on 1 November 2001. The turions were placed in a 284-L tank in the greenhouse at the University of Minnesota, St. Paul, in water maintained at 10 C under a 50% shade cloth to suppress growth and maintain turions.

Phase 1: Overwintering Period

The three taxa of turions underwent four time intervals at 4 C to mimic overwintering periods at different latitudes. One hundred turions were randomly collected from greenhouse cultures of monoecious *Hydrilla*, dioecious *Hydrilla*, and *M. sibiricum* for overwintering treatments of 105 d, acclimatized, and placed at 4C. One hundred turions of each taxon were randomly collected 42 d after the first collection, acclimatized, and placed at 4 C for a 63-d treatment group. The 28-d treatment group was randomly collected from greenhouse cultures 35 d later, acclimatized, and placed at 4 C for 28 d. The 0-d treatment group was randomly collected 28 d later. The greenhouse plant cultures of the *Hydrilla* biotypes produced new turions throughout the 105-d time period so each overwinter period had turions of similar age. The *M. sibiricum* turions were all collected at the same time and therefore were progressively older as they were tested.

A portable chiller maintained a 379-L tank at 4 C in the greenhouse for the overwintering period of the experiment. Dechlorinated water was added to the tank three times/week. A 50% shade cloth covered the tank throughout the experiment. After collection from greenhouse cultures, each taxa of turions and each length of overwintering treatment was placed in a separate mesh bag. With the exception of the 0-d cold treatment, bags were placed in a tank at 12 C, maintained by a Blue M constant-flow portable cooling unit, for a 24-h acclimation. Bags (excluding the 0-d treatment) were then moved to a 4 C tank for the overwintering treatment.

On 1 March 2002, 105 d after initiation of the overwintering treatment, all bags of turions were removed from the 4 C tank simultaneously, and the 0-d treatment turions of each taxa were collected from the culture tanks. Each bag of 100 turions, 12 bags total (three taxon by 4 overwintering periods), was randomized into two equal groups. One group was evaluated for survivorship without further treatment and the other underwent early growing season treatments.

Survivorship of turions after overwintering was determined by their ability to sprout when grown for 10 weeks in 25 C water. Turions of each taxon and overwintering period were placed in separate 600-ml flasks on a greenhouse bench under 50% shade cloth to prevent overheating (12 flasks total). Dechlorinated water was added to the flasks at least three times/week. Emergence was recorded when the tip of the turion began to elongate as new green growth appeared. Turions that showed no elongation or were decomposing after 70 d were counted as dead.

Turion mortality for the three taxon (*Hydrilla* biotypes and *M. sibiricum*) and four overwintering periods (105, 63, 28, and 0 d; n = 624) were compared using Chi-square contingency tests (SAS 2001). For turions that did not undergo a cold treatment (0 d), dormancy may not have been broken, thereby preventing emergence. For these turions, percent mortality was adjusted to the percent mortality from phase II. So, if 0-d turions had higher mortality in phase I than phase II, then phase II mortality percentage was used for the calculation. Three chi-square tests were conducted: (1) overall mortality at all four overwintering treatments for all three taxon; (2) mortality of all three taxon at specific overwintering treatments; and (3) mortality of each taxon across all four overwintering treatments.

Phase II: Early Growing Season Emergence under Rapid and Slow Temperature Increases, Long and Short Daylength

To determine how emergence of turions is affected by different conditions during the early growing season corresponding to the latitudinal gradient, phase II turions (n = 576), were randomized into four groups to be placed in four different tanks (Coleman Star milk coolers). Equal numbers of turions per taxa and overwintering period were placed in each tank. Frames were placed inside each tank to hold 144 cone containers in a 12-cone by 12-cone grid (51 cm by 51 cm). Each container was 3.5 cm in diameter and 17 cm long and held one turion. Containers were filled with a 50:50 mix of sand and loam soil and surrounded by window screen to 20 cm above top of container to ensure separation between individuals. The containers were randomly assigned to locations in each tank. Tank conditions were: (1) rapid temperature increase, 14-h photoperiod; (2) slow temperature increase, 14-h photoperiod; (3) slow temperature increase, 10-h photoperiod; and (4) rapid temperature increase, 10-h photoperiod. The slow temperature increase mimicked Minnesota spring conditions and gradually raised the temperature from 4 C to 15 C over 42 d and to 25 C at 70 d (http://waterontheweb.org/). The rapid temperature increase raised the temperature from 4 C to 15 C in 14 d and to 25 C at 42 d; it remained at 25 C until the end of the experiment.

After 70 d in the early growing season, containers were removed and all plant material was collected. Each turion was recorded as live if new growth was present. These plants represent overall survivorship because they did not die throughout overwintering and early growing season treatments. For each live turion, shoot number, shoot length, root number, root length, and turion length were measured. Shoots were cut and separated from turions and roots, collected, bagged separately, and dried in a forced-air drying oven at 80 C for at least 48 h. Individual shoot, root, and turion dry mass were weighed for each plant. If no growth occurred or all growth was dead, a turion was counted as dead. Turion length was measured for each dead turion. Percent mortality during the early growing season (i.e., additional mortality) was determined by subtracting the % mortality observed in Phase 1 of the assay from % mortality observed in Phase II.

Binomial logistic regressions were used to analyze mortality during the early growing season and overall survivorship among monoecious *Hydrilla*, dioecious *Hydrilla*, and *M. sibiricum.* Factors were species, length of overwintering treatment (from Phase I), daylength, and rate of temperature increase. All logistic regressions were run in SAS version 8.2 (SAS 2001). Likelihood ratio chi-square, which measures the deviance in fitting each term last in the model, was used to determine the probability that the effect of each factor level was equal.

T-tests were done to examine differences in turion size between the monoecious and dioecious Hydrilla biotypes and alive and dead turions within each biotype using SAS version 8.2 (SAS 2001). Analysis of variance (ANOVA) was used to determine the effects that length of overwintering treatment, species, daylength, and temperature increase had on the average number of branches per plant and the average shoot biomass per plant. ANOVA procedures were performed in SAS version 8.2 (SAS 2001). The model followed a split-plot design with daylength and temperature increase as whole plot treatments and overwintering treatment and species as split plot treatments. All factors were considered fixed. A binomial logistic regression was used to determine differences in live turion presence or absence of roots, as described in the previous paragraph. Results for all tests were considered significant if $p < 0.05$.

RESULTS

Factors Affecting Turion Mortality

Overall, overwintering (phase I) mortality rates differed among the three taxa and across the four overwintering treatments (df = 5, $\chi^2 = 236.26$, $p < 0.001$). For each taxon, turion mortality differed among overwintering treatments. *M. sibiricum* had low percent mortality across all overwintering treatments (0 to 105 d) and no deaths at 0 and 105 d (Figure 1; df = 3, $\chi^2 = 8.81$, p = 0.0319). Monoecious *Hydrilla* percent mortality increased with overwintering treatment duration, from 8% at 0 d to 48% at 105 d (df = 3, $\chi^2 = 41.17$, $p < 0.001$). Dioecious *Hydrilla* showed the greatest increase in percent mortality with increasing overwintering treatment from 0% at 0 d to 98% at 105 d (df = 3, $\chi^2 = 179.26$, $p < 0.001$).

Differences in turion percent mortality were observed among taxa for each overwintering treatment except 28 d. The 0-d overwintering treatment resulted in no mortality for *M. sibiricum* and dioecious *Hydrilla*, but 8% for monoecious *Hydrilla* (df = 2, $\chi^2 = 8.21$, p = 0.0165). After the 63-d overwintering treatment, 8% of *M. sibiricum* died, while 20% of monoecious *Hydrilla* and 92% of dioecious *Hydrilla* died (df = 2, $\chi^2 = 87.33$, $p < 0.001$). None of the *M. sibiricum* were dead after a 105-d overwintering treatment, compared to 48% of monoecious *Hydrilla* and 98% of dioecious *Hydrilla* turions (df = 2, $\chi^2 = 100.12$, $p < 0.001$).

Additional mortality occurring during the early growing season (i.e., after the overwintering period) varied among species (df = 3, $\chi^2 = 16.727$, p = 0.0008) and by duration of overwintering they had experienced (df = 2, $\chi^2 = 64.081$, $p < 0.001$; Figure 2). Additional early growing season mortality increased with length of overwintering treatment for dioecious and monoecious *Hydrilla* but not for *M. sibiricum.* Dioecious *Hydrilla* had relatively low additional mortality, averaging 2 to 25%, in conditions where turions remained after the overwintering treatment. Additional early growing season mortality increased on average with overwintering treatment for monoecious *Hydrilla*, ranging from an additional 6.5% at 0 d to 37.25% at the 63-d overwintering treatment. *M. sibiricum* average additional mortality rates were generally higher (42 to 75%) than for *Hydrilla* and were unrelated to the four overwintering treatments.

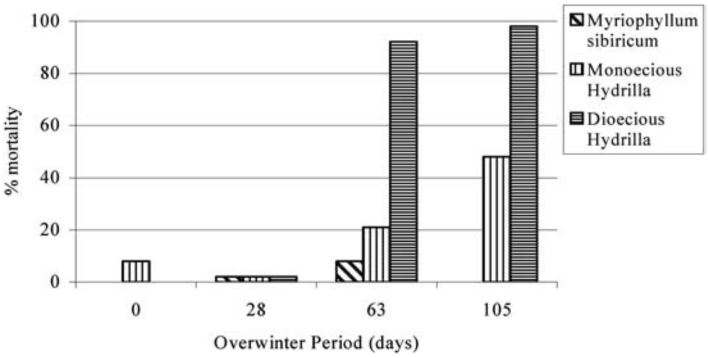

Figure 1. Mortality rates for *Myriophyllum sibiricum*, monoecious *Hydrilla*, and dioecious *Hydrilla* after overwinter periods of 0, 28, 63, and 105 d at 4 C.

Factors Affecting Overall Survivorship

Overall rates of survival after both the overwintering treatment and the early growing season varied with species (df = 2, $\chi^2 = 32.63$, $p < 0.001$) and length of time in overwintering treatment (df = 3, $\chi^2 = 101.38$, $p < 0.001$; Figure 3). Monoecious *Hydrilla* turions, mean = 9.608 mm (SE = ±0.2), were larger in size than dioecious *Hydrilla*, mean = 8.962 mm (SE = ±0.18; df = 317, t-value = 2.47, p = 0.014). Live monoecious *Hydrilla* turions, mean = 9.930 mm (SE = ±0.26), were larger than dead monoecious turions, mean = 9.035 mm (SE = ±0.25; df = 157, t-value = 2.23, p = 0.0269). The same pattern was observed in dioecious *Hydrilla* turions: alive mean = 9.6375 mm (SE = ±0.26); dead mean = 8.2692 mm (SE = ±0.21; df = 156, t-value = 4.08, $p < 0.0001$). Dioecious *Hydrilla* had high survival rates after both phases for 0- and 28-d overwintering treatments, 80 to 100% and 58 to 100%, respectively, and no survival at 63- and 105-d treatments. Monoecious *Hydrilla* had declining survival with length of time in overwintering treatment but remained at higher levels than dioecious *Hydrilla.* Maximum survivability for monoecious *Hydrilla* was 92% at 0- and 28-d overwintering treatments, 67% at 63 d, and 42% at 105 d. *M. sibiricum* had variable rates of survival in all overwintering treatments, ranging from a minimum of 8% at 0, 28, and 105 d, to a maximum of 67% at 63 d. Daylength and temperature increase did not have an effect on survival rates for any species (df = 1, $\chi^2 = 0.3321$, p = 0.5644). Greater shoot dry weight and branching was seen under rapid early growing season temperature increases compared to slow increases (Figures 4 and 5).

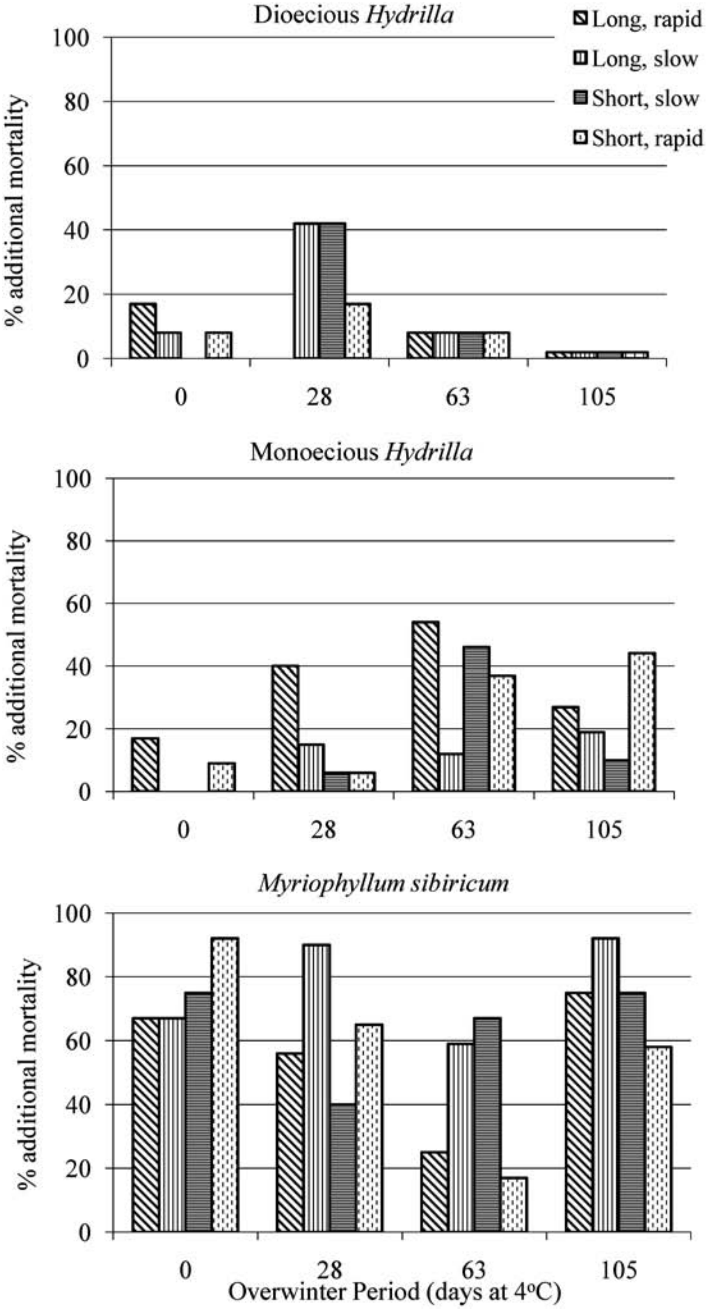

Figure 2. Additional mortality after the early growing season and overwinter period for dioecious *Hydrilla*, monoecious *Hydrilla*, and *Myriophyllum sibiricum*. The four tank conditions are: long daylength, rapid temperature increase; long daylength, slow temperature increase; short daylength, slow temperature increase; short daylength, rapid temperature increase.

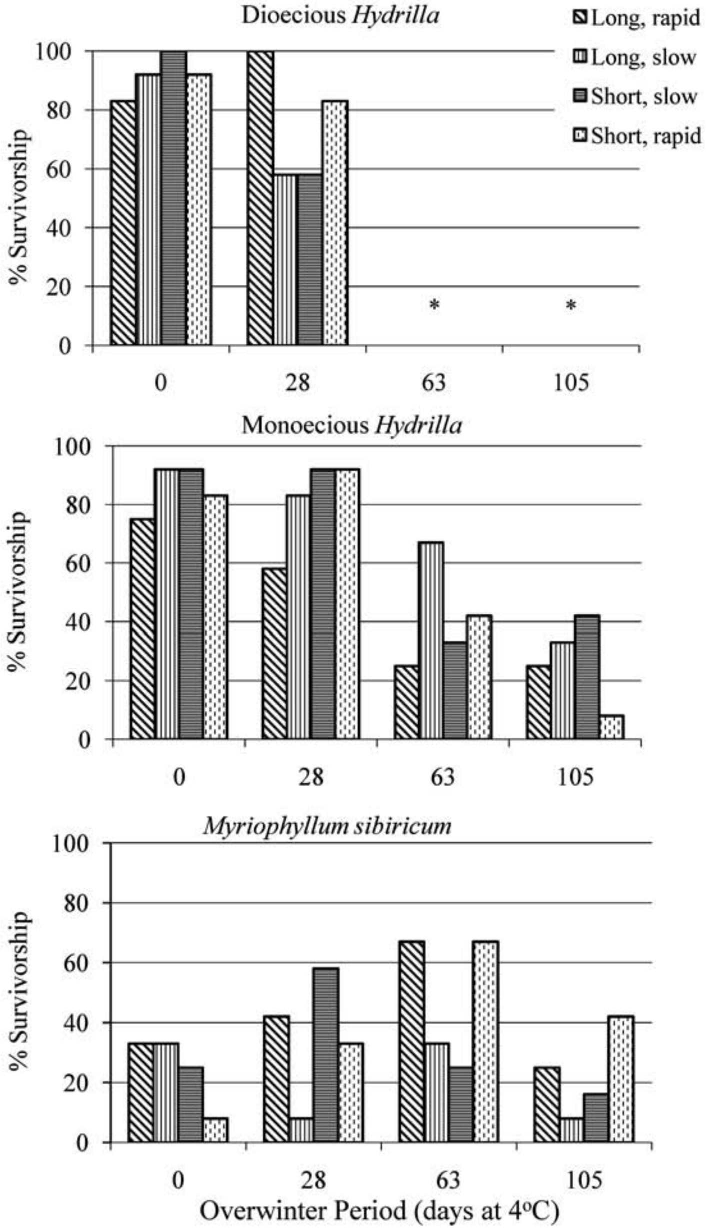

Figure 3. Survivorship after the early growing season for dioecious *Hydrilla*, monoecious *Hydrilla*, and *Myriophyllum sibiricum* after overwinter periods of 0, 28, 63, or 105 d at 4 C. An * indicates no live turions. The four tank conditions are: long daylength, rapid temperature increase; long daylength, slow temperature increase; short daylength, slow temperature increase; short daylength, rapid temperature increase.

Shoot and Root Production in Early Growing Season (Phase II)

Average aboveground biomass per turion in the early growing season differed with length of overwintering treatment (F = 5.75, p = 0.0044). An interaction between species and length of overwintering treatment affecting average aboveground biomass was also observed (F = 3.00, p = 0.0258). Biomass production was similar for dioecious and monoecious *Hydrilla* at 0 d (14.1 mg/turion and 12.6 mg/turion, respectively) and 28-d overwintering treatment (9.4 mg/turion and 11.1 mg/turion, respectively; Figure 4). Monoecious *Hydrilla* average shoot biomass production continued to decrease between 28- and 63-d overwintering treatment groups, from 7.4 mg/turion at 63 d to 5.0 mg/turion at 105 d. No live turions remained for dioecious *Hydrilla* beyond 28 d. Shoot biomass production for *M. sibiricum* was lower than either *Hydrilla* biotype in all four overwintering treatment groups.

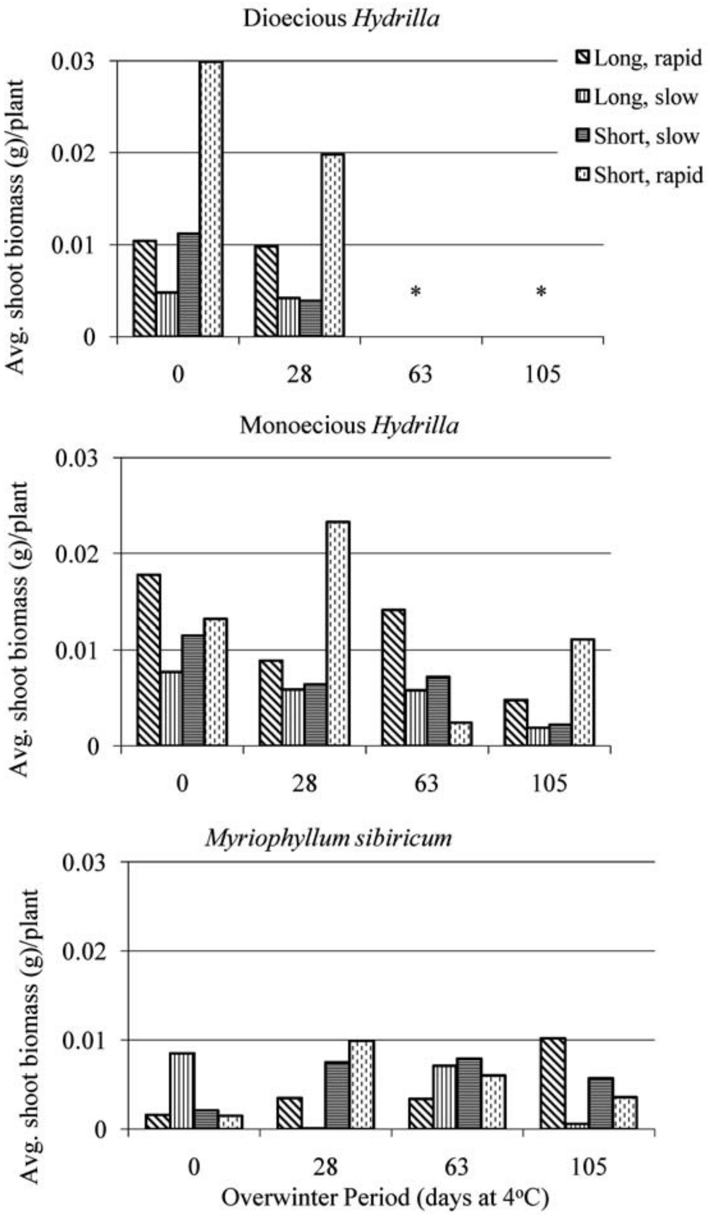

Figure 4. Average shoot biomass (g) per plant for dioecious *Hydrilla*, monoecious *Hydrilla*, and *Myriophyllum sibiricum* after the early growing season. An * indicates no live turions. The four tank conditions are: long daylength, rapid temperature increase; long daylength, slow temperature increase; short daylength, slow temperature increase; short daylength, rapid temperature increase.

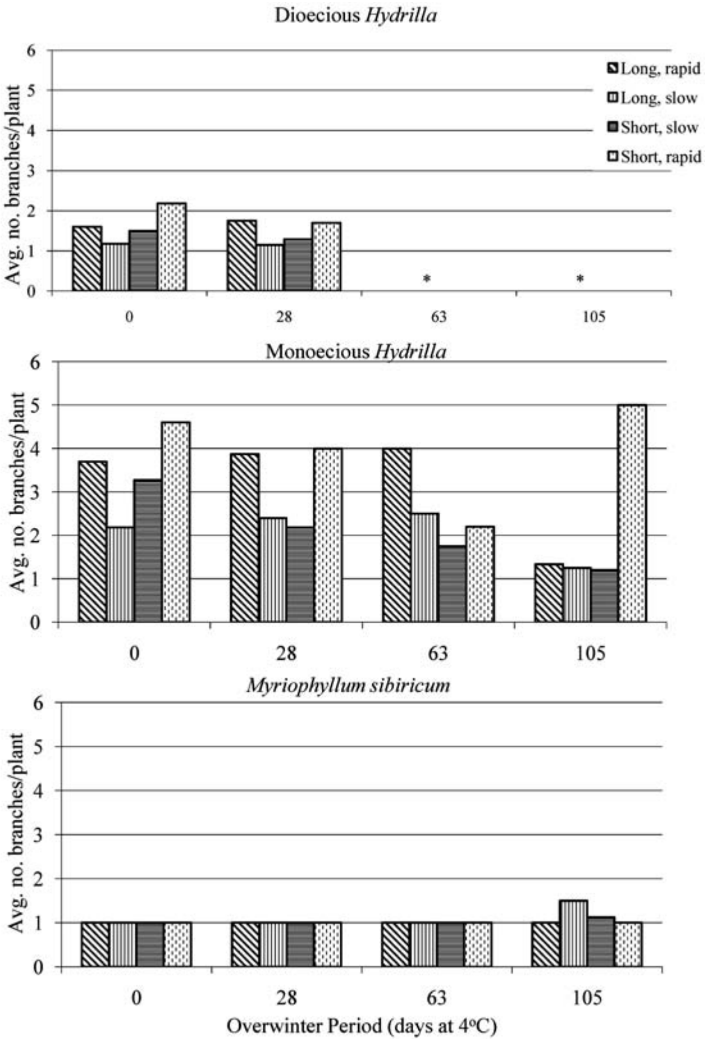

Figure 5. Average number of branches per plant for dioecious *Hydrilla*, monoecious *Hydrilla*, and *Myriophyllum sibiricum* after the early growing season. An * indicates no live turions. The four tank conditions are: long daylength, rapid temperature increase; long daylength, slow temperature increase; short daylength, slow temperature increase; short daylength, rapid temperature increase.

Number of branches a turion produced in the early growing season depended on the temperature increase the species underwent (F = 7.40, p = 0.0033), and the species and length of time in overwintering treatment (F = 2.66, p = 0.0413). Dioecious and monoecious *Hydrilla* had the highest number of average branches per plant under the rapid temperature increase conditions (Figure 5). Rate of temperature increase did not affect *M. sibiricum.* Average number of branches per plant ranged from a maximum of 2 to a minimum of 1 for dioecious *Hydrilla* in the 0- and 28-d overwintering treatment groups, respectively. Monoecious *Hydrilla* produced a maximum average of 5 branches per plant in the 105-d overwintering treatment and a minimum of 1 branch per plant, also in the 105-d overwintering treatment group. Average number of branches per plant was 1 for *M. sibiricum* in 0-, 28-, and 63-d overwintering treatment groups and a maximum of 1.5 in the 105-d overwintering treatment group.

In the early growing season, root development differed by species (df = 2, $\chi^2 = 20.81$, $p < 0.001$) and overwintering treatment (df = 3, $\chi^2 = 40.01$, $p < 0.001$), regardless of early growing season conditions. Dioecious *Hydrilla* had the highest number of turions with roots after 0- and 28-d overwintering treatments in the early growing season, a maximum of 8 turions with roots and 4 turions with roots, respectively, out of groups of 12 (Figure 6). Monoecious *Hydrilla* ranged from a maximum of 5 turions with roots in the 0-d overwintering treatment group and a minimum of 1 turion with roots in the 28- and 105-d treatment groups. Number of turions with

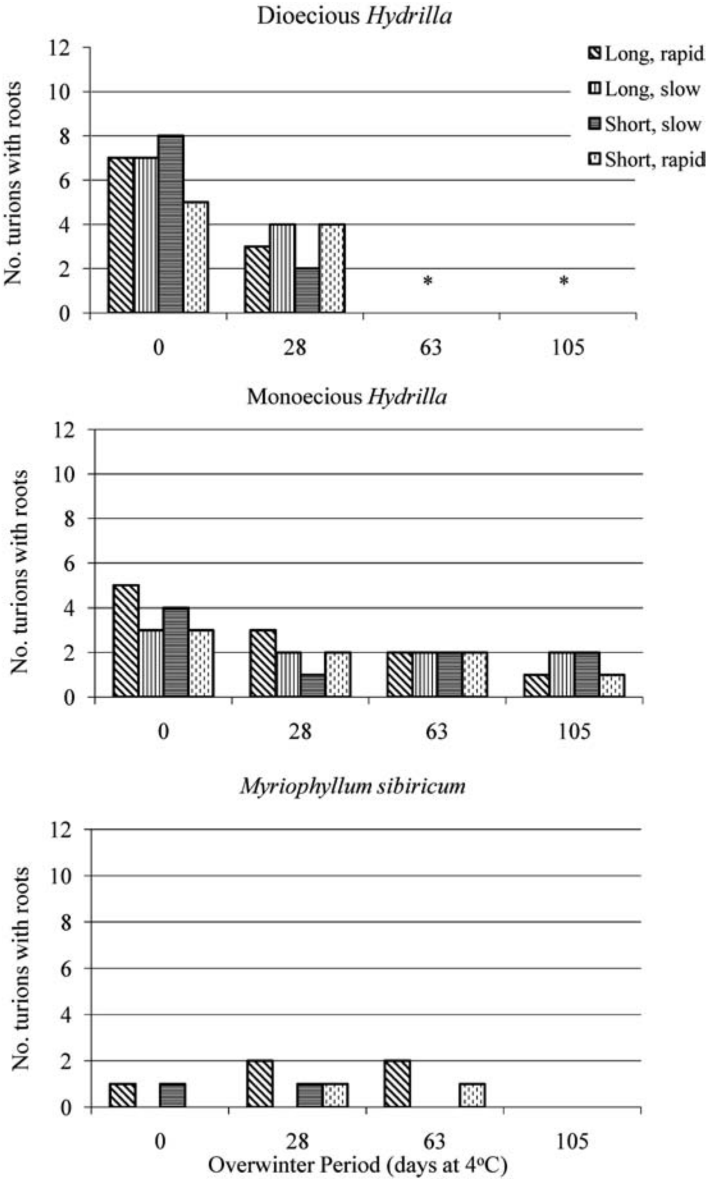

Figure 6. Number of dioecious *Hydrilla*, monoecious *Hydrilla*, and *Myriophyllum sibiricum* with roots after the early growing season. An * indicates no live turions. The four tank conditions are: long daylength, rapid temperature increase; long daylength, slow temperature increase; short daylength, slow temperature increase; short daylength, rapid temperature increase.

roots varied for *M. sibiricum*, from a minimum of 0 turions with roots in all overwintering treatment groups to a maximum of 2 turions in the 28- and 63-d treatment groups.

DISCUSSION

The two-stage cold tolerance assay developed was able to distinguish varying survival abilities between a local, cold tolerant species (*M. sibiricum*) and two biotypes of *Hydrilla*. The ability to determine cold tolerance will lead to estimates on winter survival and range expansion. *M. sibiricum*, a circumboreal species known to require a cold period to break dormancy, showed higher survival ability than monoecious and dioecious *Hydrilla*, whose cold tolerance limits were in question. The two phases in the assay, overwintering and early growing season, made the prediction of survivorship more precise than overwintering alone. Predictions of monoecious *Hydrilla* survival decreased by 37% when both the overwintering and the early growing season were considered versus overwintering alone.

Our two-step assay suggests that if all other growing conditions are suitable, duration of overwintering period is likely the main factor that will determine the northward expansion of monoecious and dioecious *Hydrilla* in North America. For these biotypes, threshold levels for length of overwintering treatment were much lower for dioecious *Hydrilla* than monoecious *Hydrilla*. Survivorship for both biotypes in the early growing season was primarily affected by length of overwintering treatment, not temperature increase or daylength conditions. Nevertheless, rapid temperature increases during the early growing season compared to slow increases promoted more growth in monoecious and dioecious *Hydrilla*.

Monoecious *Hydrilla* has a greater tolerance to long duration overwintering periods than dioecious *Hydrilla*, but both are lower than *M. sibiricum*. The low mortality rates of *M. sibiricum* in all overwintering durations were expected because it is indigenous to cold climates (Ceska and Ceska 1986). Collections of *M. sibiricum* with turions have been made at 68°N suggesting the ability to survive long overwintering periods (Aiken 1981). While *Hydrilla* has been collected at 58°N, the monoecious and dioecious biotypes found in the U.S. are not from these areas (Balciunas and Chen 1993). In our assay, overwintering periods longer than 28 d duration resulted in high rates of mortality for dioecious *Hydrilla*, suggesting turion survival will be restricted to more southerly locations. Sastroutomo (1980) also found no germination of *Hydrilla* turions after 60 d in cold water in Japan (biotype unknown). However, 52% of monoecious *Hydrilla* germinated even after 105 d in the current study, suggesting survival in climates similar to Minnesota. Assays of turion survival could be improved if dormant turions (or tubers) are more effectively distinguished from those that have died (Vermaat and Hootsmans 1994). In the case of *Hydrilla*, we found viability tests that we ran difficult to interpret, although a large number were obviously decomposing after the overwintering period.

Monoecious *Hydrilla* produces greater numbers of branches than dioecious *Hydrilla* in the early growing season, but overall shoot biomass is similar in the two biotypes. McFarland and Barko (1987) also found monoecious Hydrilla produced greater densities of shoots than dioecious Hydrilla on different sediment types. Both biotypes produce more branches and shoot biomass under rapid temperature increases than slow, but this effect was masked in the analysis of the three taxa because of *M. sibiricum*'s low turion survival in the early growing season. Optimal temperatures for growth occurred sooner under rapid temperature increases resulting in greater shoot branching and biomass in both *Hydrilla* biotypes. Previous research has found that optimal growth occurs above 15 C for *Hydrilla* biotypes (Steward and Van 1987). In addition, monoecious *Hydrilla* likely produces more branches than dioecious *Hydrilla* because the monoecious biotype can emerge at lower temperatures (Haller et al.

1976, Steward and Van 1987). Steward and Van (1987) found that while growth was initially retarded at low temperatures where emergence occurs, monoecious *Hydrilla* was able to grow rapidly when the temperature increased in the spring. Even though monoecious *Hydrilla* has a head start on dioecious *Hydrilla* in the spring, biomass production is eventually similar for both biotypes at temperatures above 22 C. Likewise, Steward and Van (1987) also found at the end of the early growing season both biotypes produced similar amounts of biomass after short overwintering periods. Biomass production at long overwintering periods is reduced on average by as much as 50% compared to short periods for monoecious *Hydrilla*. Furthermore, slower temperature increases typical of northern U.S. climates further diminish biomass production of monoecious *Hydrilla*, even though these conditions do not cause turion mortality.

Empirical data on overwintering abilities of *Hydrilla* biotypes can assist in refining predictions of potential northward expansion. Although dioecious *Hydrilla* has high turion survivorship at overwintering treatments of 0 and 28 d, it most likely cannot sustain populations from axillary turions in areas where cold water temperatures (4 C) persist for more than 63 d. In contrast, monoecious *Hydrilla* can persist at these low water temperatures for at least 105 d. Larger turion sizes associated with monoecious *Hydrilla* in this study may provide greater amounts of reserves than dioecious *Hydrilla*, enabling it to survive longer overwintering periods. A compilation of published data on ice cover duration for lakes in the northern United States and southern Canada, ranging from 41.76°N to 50.12°N (Table 1; Benson and Magnuson 2000, State of the Canadian Cryosphere 2002) indicates that dioecious *Hydrilla* may survive at latitudes near 45°N during years with minimum ice cover duration. However, stable populations of dioecious *Hydrilla* are more likely south of 41°N, based on average ice duration. Monoecious *Hydrilla* will likely survive the average ice duration of these lakes to at least 45°N and years with minimum duration to at least 46°N. However, much variation is seen in ice cover days at similar latitudes depending on individual lake characteristics and activities. More useful to managers is actual survival after a given number of overwintering days. Survival predictions based on number of days at 4 C can also assist in determination of when control measures should be implemented and to the amount of regrowth expected in the spring given the fall population size.

Future geographic ranges for *Hydrilla* will be difficult to predict given the variability in lake phenology due to size, elevation, human activities, and water flow (Walsh et al. 1998). Marginal numbers of turions produced in warm winters may persist and propagate until conditions in a subsequent year are colder for longer durations. Over time, overwintering propagules besides axillary turions (e.g., subterranean turions) may be produced that may have greater cold tolerance. Marginal populations are especially likely to persist where power plant discharge keeps water temperatures high during the winter or other human activities affect water temperatures. These marginal populations could then provide a new front for additional invasions. In addition to ecosystem variability, genotypic variability will limit certainty of geographic projections. In particular, if *Hydrilla* biotypes vary in photosynthetic capacity, their ability to withstand temperature extremes may likewise vary (Pilon and Santamaria 2001).

Knowledge of tolerances and survival parameters for specific species enable predictions to be based on the plant's response to climate, not on climatic factors alone. Given the predicted change in global climates and differences in individual lake phenology, determination of a plant's success based on empirical factors (e.g., temperature tolerance and photosynthetic capacity) will be useful to managers and regulators. Information gained on growth and competitive ability of plants in the early growing season will allow better prediction of survivability and invasive potential. As new introductions of aquatic plant species continue, new *Hydrilla* biotypes or other species, results from this assay can help focus efforts in minimizing spread. Determining the overwintering duration that a species can survive will demonstrate more clearly whether a species is at risk to expand into colder regions.

Table 1. Ice cover duration for selected lakes in the northern United States and southern Canada. Data are from the Global Lake and River Ice Phenology web site (Benson and Magnuson 2000) and the State of the Canadian Cryosphere web site (2002).

				Ice cover duration (d)			
Lake	State/province	Latitude (°N)	Longitude (°W)	Min	Max	Avg	Years of data
Mohonk	NY	41.76	74.16	71	137	108	55
Mendota	WI	43.00	89.00	21	161	105	145
Monona	WI	43.05	89.37	41	160	105	147
Oneida	NY	43.24	76.14	54	122	99	21
Bay of Quinte	Ontario	44.12	77.53	45	148	1124	46
Mirror	NY	44.29	73.99	110	169	143	89
Placid	NY	44.30	‘73.99	101	164	132	73
Green Bay	MI	45.00	87.64	0	152	97	65
Lake Utopia	New Brunswick	45.10	66.73	68	140	108	34
Shell Lake	WI	45.73	91.90	109	171	143	88
Maple Lake	WI	46.13	89.73	122	174	150	24
Lac Saint-Jean	Quebec	48.60	72.10	135	177	154	23
Pelican lake	Ontario	50.12	91.90	138	189	164	49

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