4-27-2015

Water Chestnut: Field Observations, Competition, and Seed Germination and Viability in Lake Ontario Coastal Wetlands

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by

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A thesis presented to the Faculty of the Department of Environmental Science and Biology of the State University of New York College at Brockport in partial fulfillment of the requirements for the degree of Master of Science

April 27, 2015

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Abstract

Water chestnut (*Trapa natans* L.) has recently invaded an increasing number of sites in New York State, particularly Lake Ontario coastal wetlands. It can severely inhibit ecosystem functioning and can be costly to control. To understand this exotic invasive plant more thoroughly, field observations and experiments were performed. The field observations were made in Lake Ontario coastal wetlands during the 2014 growing season. Percent coverage, time of flowering, time of seed production, and co-occurring species were noted. A competition experiment was performed using water chestnut and white water lily (*Nymphaea odorata* Aiton). They were planted together and in monocultures of differing densities. A greenhouse germination experiment in aquaria was conducted on water chestnut seeds using light and temperature as treatments, and seed-viability was examined to assess development stage and cold-stratification requirements.

Water lily was the better competitor of the two, but water chestnut had very high germination success. Water chestnut germination does not seem to be inhibited by temperature or by exposure to shade. The seeds do, however, need to be mature and cold-stratified (subjected to a period of cold temperatures for dormancy) to germinate. Water chestnut's tolerance to temperature, shade, and water depth has serious implications for Great Lakes wetlands if not controlled. There are a few control methods that could prove to be useful, but more research is needed before they are used in field settings. Early detection and manually pulling small patches of plants is a viable option at present.

Introduction

Background

With increasing globalization and climate change, invasive organisms have had compounding effects on ecosystem health and functioning. Since the 1800s, over 180 exotic species have become established throughout the Great Lakes (Pagnucco *et al*. 2015). Close to half of these exotics have been plants (Mills *et al.* 1993). Exotic plants can often become dominant and form monocultures, which alter habitat structure, reduce biodiversity, and affect nutrient cycling and food webs. Wetlands in particular, as landscape sinks, seem especially vulnerable to invasion (Zedler and Kercher 2004).

Invasive plants, specifically water chestnut (*Trapa natans* L.), can severely inhibit ecosystem functioning and can be costly to control. Water chestnut was introduced to North America in Massachusetts from Eurasia as an ornamental before 1859. It establishes thick, floating leaf beds that compete with, displace, and reduce native vegetation, thereby lowering biodiversity (Methe *et al.* 1993, Strayer *et al.* 2003). The dense beds that water chestnut creates shade and crowd out native vegetation. The reduction of sunlight available to submersed aquatic vegetation affects the survival of previously established vegetation and decreases the amount of oxygen released into the water column via photosynthesis (Caraco and Cole 2002). Water chestnut crowds out useful food sources for wildlife, which results in reduced food quality and availability (Methe *et al.* 1993, Marsden and Hauser 2009). The potential change of the habitat structure formed by submersed vegetation and reduction in dissolved oxygen affect the densities and communities of aquatic macroinvertebrates. The resulting invertebrate community and water conditions affect the diversity and abundances of fish that can spawn and feed in the vegetation (Caraco and Cole 2002, Strayer *et al.* 2003). These habitat changes and adverse environmental effects can be compounded by runoff from surrounding urban and agricultural areas, which results in increased nitrogen levels. Net production of water chestnut increases with increased nitrogen levels, which improves their growth and reproduction (Tsuchiya and Iwakuma 1993).

Water chestnut can cause economic problems in addition to ecological problems. In Lake Champlain, control measures between 1982 and 2004 involved thousands of volunteer hours and more than $5.8 million in state and federal funds (Marsden and Hauser 2009). In the Potomac River, it required upwards of half of a million dollars over the course of nearly a decade to obtain some measure of control (Martin 1955). Mechanical control has been practiced since the 1960s in Sodus Bay of Lake Ontario and is still currently in use (Mills *et al.* 1993).

Records of spread of water chestnut to the Great Lakes are anecdotal, but water chestnut likely escaped from ponds via animal dispersal, was released from aquaria, or was intentionally planted (Mills *et al.* 1993, Marsden and Hauser 2009). Water chestnut has spread to several coastal wetlands around Lake Ontario (personal observation). Because of its potential to disrupt ecosystem functioning and the cost of control, it is important to determine how water chestnut will interact and compete with other species so that managers have a better idea of how to control it and protect Lake Ontario wetlands.

To gain a better understanding of the ecology of water chestnut, I will discuss certain factors that are of interest regarding its increasing range. First, I present a site inventory of where water chestnut has been found in wetlands that are hydrologically connected to Lake Ontario. I will also note personal observations made during the 2014 growing season. Second, I will present results of a competition experiment between water chestnut and a native aquatic plant, *Nymphaea odorata* Aiton, that was performed to observe how water chestnut competes and offer predictions of its impact on the current plant communities in Lake Ontario coastal wetlands. Third, I will present results of germination experiments that were performed to answer specific questions regarding factors that may affect the success of water chestnut germination.

Study Organisms

In aquatic environments, competition is expected to occur between species with similar growth forms because they occupy the same niche (Gopal and Goel 1993). In Lake Ontario coastal wetlands, water chestnut grows alongside the native white water lily (*N. odorata*), and the two species compete for two-dimensional space at the water surface. Both water chestnut and white water lily have long, flexible stems and floating leaves, which form thick, weedy beds. Both plants also prefer water depths of about two meters (Sinden-Hempstead and Killingbeck 1996, Hummel and Kiviat 2004).

White water lilies are perennials with orbicular floating leaves. The leaves are usually a little over 20 cm in diameter (Conrad 1905). Water lilies can be heterophyllous; in addition to floating leaves, some leaves are held slightly above the water surface, which are called aerial leaves. This leaf type seems to occur well into the growing season when there is crowding on the water surface (Villani and Etnier 2008). The leaves are supported by long, flexible stems that grow from a horizontal rhizome. Solitary, white to pink flowers also float and can be up to about 15 cm in diameter. Small tubers may also germinate off the rhizome. Large, smooth, dark brown seeds may germinate immediately after being produced or lie dormant until the following spring (Conrad 1905). The number of seeds produced depends on the size of the plant and nutrient availability.

Water chestnut, *Trapa natans*, is an annual, aquatic, floating-leaved plant. Leaves grow to about 5 cm wide and float due to spongy petioles. Rosettes of leaves, which can grow up to 30 cm in diameter, terminate longs stems (Hummel and Kiviat 2004). Individuals can grow three primary stems and a fourth if one is broken off. Each stem can produce more rosettes vegetatively (Groth *et al.* 1996). The stems are elongate and flexible, and they support additional rosettes and plume-like structures thought to be photosynthetic, adventitious roots. Each plant is anchored by lower roots and the pointed seed case from which it grew, which has four sharp spines with recurved barbs (Hummel and Kiviat 2004). The plant can continue growing if separated from the anchor roots, which causes complications when water chestnut is controlled by machine. Although seed production may be reduced if the rosette is cut, seeds can still be produced until the plant senesces (Methe *et al.* 1993). The flower is single with four white petals and yellow stamens, and it grows from the floating leaves. A one-seeded fruit forms underwater; one rosette can produce 10-15 fruits (Hummel and Kiviat 2004).

Field Study Site

Braddock Bay Fish and Wildlife Management Area, located in Monroe County, NY, is a shallow, marsh-bay complex along the Lake Ontario shoreline (Figure 1). Much of the site is characterized by cattail marsh and open water. Focus was placed on the cove inland from the eastern sand spit, which is located approximately 43^{o}18' N and 77^{o}42' W. Many water lilies and water chestnut were observed in this area previously.

Methods

Site Inventory

Data from studies conducted across the Lake Ontario basin from 2011 through 2015 as part of the U.S. Environmental Protection Agency/Great Lakes Restoration Initiative-funded Coastal Wetland Monitoring project, in which I participated, provided a foundation for an inventory of Lake Ontario wetlands where water chestnut has been observed. Additional data were obtained from state and non-governmental organization (e.g., The Nature Conservancy, iMapInvasives) files on invasive species.

Field Observations

Field observations were conducted in Braddock Bay, Monroe County, NY during the 2014 growing season on six dates: 30 May, 7 June, 14 June, 19 June, 3 July, and 25 July. Water chestnut control in the form of hand pulling occurred in August, preventing further observation. A patch of mixed water chestnut and water lily, roughly 1,200m^2 in size, was identified and observed throughout the summer to compare phenology of the two species and to make sure development in the competition experiment was similar to a natural setting. Between five and ten 1m^2 quadrats were randomly placed within this patch. Approximate percent coverage, number of leaves and rosettes, presence of flowering, water temperature, water depth, and co-occurring species were determined and noted.

Data Analyses

The percent coverage of water lily and water chestnut, number of water lily leaves, number of water chestnut rosettes, and water depth were averaged for the site for each date of observation. The percent coverages of both species and numbers of leaves and rosettes were compared over time in a scatter plot. The average water depth and water temperature for each date were also plotted to show that they remained relatively steady. They were tested for normality and analyzed with a *t*-test.

Competition Experiment

A competition experiment was conducted outdoors near The College at Brockport State University of New York aquaculture ponds during the 2014 growing season. The following factorial design was used to grow water chestnut seeds and white water lily rhizomes, respectively, in the following ratios: 0:2, 0:5, 2:0, 2:2, 2:5, 5:0, 5:2, 5:5. There were three replicates of these eight treatments.

The plants were grown in 265-liter, round, sturdy, rubber stock tubs that contained about 37 liters of organic soil, which was collected from the edge of a marsh near Braddock Bay on 10 May 2014, and filled with pond water from the aquaculture ponds on the same day. Lily rhizomes were obtained from Southern Tier Consulting, Inc. on 2 May 2014 and stored in a cool, dark room (about 13^{o}C) until being planted. Water chestnut seeds were collected manually from Braddock Bay, NY in on 14 April 2014 and stored in a refrigerator at 5^{o}C until being planted.

The experiment began 20 May 2014. Each seed and rhizome was weighed to the nearest 0.01 gram and then placed in the stock tubs. Fifteen extra rhizomes and sixteen extra seeds were placed in a drying oven, dried at 15.5^{o}C for at least 48 hours, and weighed again to verify whether the wet weight was a good variable for measuring growth. Various measurements were taken throughout the growing season: 27 May, 30 May, 3 June, 5 June, 7 June, 10 June, 12 June, 17 June, 19 June, 21 June, 24 June, 27 June, 3 July, 11 July, 19 July, 25 July, 5 August, 15 August, 19 August, and 28 August. Plant height was recorded until the water surface was reached. Percent coverage was recorded for each species. Occurrence of flowering was also noted. The experiment was terminated 1 September 2014.

Water lily leaves and water chestnut rosettes were considered comparable units. Leaf and rosette diameters were measured, and the plants were then placed in plastic zip bags and stored in a refrigerator until the wet weight could be measured. After the wet weights were measured, two leaves were taken from each plant and scanned to determine the area. The leaves and the remainder of the plants were placed in a drying oven at 15.5^{o}C for at least 48 hours, and the dry weights were then measured. Specific leaf area was calculated. Final observations and counts of water lily seeds could not be made because of observer error.

Data Analyses

The final, dried biomass was used in all data analyses. The final, dried biomass of both species, percent coverage of both species, number of water chestnut rosettes, number of water lily leaves, specific leaf area, and number of water chestnut seeds produced were first analyzed for normality using the Shapiro-Wilkes test and frequency histograms. They were then analyzed using Spitters' reciprocal-yield model (1983). This involved multiple linear regressions in the following forms for each of the variables:

$$1/X_t = a_{t0} + a_{tt}d_t + a_{tn}d_n$$

$$1/X_n = a_{n0} + a_{nn}d_n + a_{nt}d_t$$

In these equations, X_t and X_n may represent the following variables: final dried biomass, percent coverage, number of rosettes or leaves, specific leaf area (SLA), and number of seeds produced. The subscript t refers to *Trapa natans* and the subscript n refers to *Nymphaea odorata*. The respective planting densities for *Trapa natans* and *Nymphaea odorata* are represented by d_t and d_n. The intercepts of the equations, a_{t0} and a_{n0}, represent the inverse of the maximum value of each variable of an isolated plant. The coefficients a_{tt} and a_{nn} represent intraspecific competition, a_{tn} represents interspecific competition as *Nymphaea odorata* affects *Trapa natans*, and a_{nt} represents interspecific competition as *Trapa natans* affects *Nymphaea odorata*. A ratio was determined using these coefficients to show which form of competition was greater by dividing the interspecific competition coefficient by the intraspecific competition coefficient. A resulting number greater than one would indicate greater importance of interspecific competition. A resulting number less than one would indicate greater importance of intraspecific competition.

The relative growth rate (RGR) of both species was calculated using the formula:

$$RGR = \frac{(\ln X_2 - \ln X_1)}{(t_2 - t_1)}$$

where X_2 and X_1 are the percent coverages at t_2 and t_1 in days. Percent coverages were used instead of dried weight due to the number of plants available. A univariate analysis of variance (ANOVA) in a General Linear Model was performed to determine whether there was a significant difference in the relative growth rate of each species between different treatments.

Water Chestnut Germination Experiment

A germination experiment was conducted from December 2013 to March 2014 in the greenhouse using light and temperature as treatments. Twelve rectangular, 75.7-liter aquaria were divided into lit and shaded halves using layers of landscaping fabric. They were subjected to one of three temperature ranges: 10-14^{o}C, 17-19^{o}C, and 21-25^{o}C, resulting in four replicates of each temperature range. The aquaria in the coldest range were left at room temperature (set at 10^{o}C), while the warmer temperature ranges were reached and maintained using submersed 100-watt and 200-watt fish-tank heaters. The water depths were maintained by manually supplying tap water about every two days. Seeds were collected from Braddock Bay in October 2013, rinsed with tap water and distilled water, placed in a container filled with distilled water, and stored in a refrigerator at 5^{o}C for eight weeks. Eight seeds were placed in each half of a tank. HOBO® Temperature/Light Pendant® data loggers, which were weighted and placed in the middle of each tank half, recorded light intensity and temperature (to ± 0.53°C) every four hours. The number of seeds germinated was recorded for all treatments. At the end of the experiment, where possible, the number of branches, the number of rosettes, seed case size, stem length, total leaf length, inflated petiole length, and petiole widths were recorded. The measured leaves were scanned and the images analyzed to determine surface leaf area. The leaves were then dried for at least 48 hours in a drying oven at 15.5^{o}C and massed. Due to decomposition, these measurements were not possible across all treatments, specifically the warmest range of temperatures and two replicates of the middle range. Some replicates of the lowest temperature range did not have mature plants to measure.

Data Analyses

The averaged time (in days) that it took for seeds to germinate, number of seeds (as a percentage) that did germinate, and growth rates for each replicate were analyzed for normality using IBM SPSS Software. The variables Days to Germination and Growth Rate were log-transformed and Number Germinated was arcsine-transformed. Transformation did not improve the normality tests, so analyses were performed on the original data. Because there were missing data in some of the warmer-ranged replicates, only time until germination and percent of seeds germinated were analyzed. A dissimilarity matrix was made using the Bray-Curtis statistic. A cluster analysis and non-metric multidimensional ordination were performed on the Bray-Curtis matrix. A two-factor analysis of similarity (ANOSIM) was performed on the untransformed data as the non-parametric alternative to the multivariate analysis of variance (MANOVA) test.

Water Chestnut Seed-Viability Experiment

A water chestnut germination experiment was started on 29 September 2014 to test germination success while varying two factors: different stages of seed development and cold-stratification of seeds. Water chestnut seeds were picked from mature plants in Braddock Bay on 25 July 2014. They were separated into four size classes based on the height of the nutlet to represent four stages in development. A fifth stage of development, fully mature seeds, was represented by seeds produced in the greenhouse from 13 water chestnut plants that were collected from Braddock Bay on 25 August 2014 and seeds collected on 14 April 2014. Half of each of the first four size groups was cold-stratified for nine weeks. The remaining seeds were stored in a cool, dark room at 13^{o}C. Previously cold-stratified seeds, which were collected 14 April 2014, were used as the cold-stratified, fully mature seed group.

Seeds were placed in the aquaria, which had been previously filled with tap water, at 0900 on 29 September 2014 and monitored for one month. Each treatment was placed in its own aquarium. The quantity of seeds collected at each development stage determined the size of each treatment. The first size class, measuring less than one centimeter in height, included 21 cold-stratified seeds and 21 non-stratified seeds placed in respective aquaria. The second size class, measuring between 1.0 and 1.4 cm in height, included 30 cold-stratified seeds and 30 non-stratified seeds placed in respective aquaria. The third size class, measuring between 1.5 and 1.9 cm in height, included 26 cold-stratified seeds and 25 non-stratified seeds in respective aquariums. The fourth size class, measuring at least 2.0 centimeters in height, included 20 cold-stratified seeds and 21 non-stratified seeds placed in respective aquaria. The fifth group, mature seeds that had already fallen from the plants, included 30 cold-stratified and 30 non-stratified seeds in respective aquaria.

Throughout the month, occurrence of germination was noted. Statistical tests were not performed on the data due to the outcome of the experiment. After a month of monitoring, the percent of seeds that successfully germinated was calculated for each size class and stratification treatment.

Results

Site Inventory

Water chestnut has been observed in waterways near the Hudson Valley and Long Island since at least the mid-1900s (iMapInvasives 2015). It has been actively controlled in Sodus Bay since the 1970s (Mills *et al.* 1993). Oswego seems to be the next area to have been infested; observations were made around 2000. Water chestnut was then observed to the west on Tonawanda Creek and further east and north in Lake Ontario wetlands near Pulaski between 2008 and 2010. In 2011, observations of water chestnut were made in wetlands located north of Rochester and on the coast of Jefferson County west of Watertown (iMapInvasives 2015) (Figure 2). Water chestnut has also been observed across the Canadian border on Wolf Island (personal communication with Justin White, Ducks Unlimited Canada).

When the GLRI coastal wetland monitoring began, water chestnut was only observed at sites near Sodus Bay. One site had water chestnut in 2011, two more in 2012, seven more in 2013, and four more in 2014 (Table 1). Water chestnut was not found in Floodwood Pond, located on the eastern shore, when it was sampled in 2011, but the site was infested in 2013. Braddock Bay, in Monroe County, did not have water chestnut in 2012 but the site was infested in 2013. Third Creek, near Sodus Bay, did not have water chestnut when it was sampled in 2011 but did in 2014. Water chestnut arrived on Wolfe Island between 2009 and 2011 (personal communication with Justin White, Ducks Unlimited Canada) (Figure 3).

Field Observations

On the first day of observation, 30 May 2014, only water lily leaves were at the water surface. These were relatively small, about six centimeters in diameter, and purple-green in color. Some water chestnut rosettes were at the water surface by the next day of observation, 7 June, although they consisted of only three or four leaves. Some of the water lily leaves had begun to turn green and photosynthesize. Flowering began about mid-June. The water lilies bloomed about two weeks earlier (14 June) than the water chestnuts (3 July). After the initial growing period, the water lily coverage and number of leaves remained relatively steady while the percent coverage of water chestnut and number of rosettes increased throughout the season (Figure 4, Figure 5). The number of water chestnut rosettes decreased on the last observation date. The water temperatures and depths varied slightly throughout the season but remained steady (Figure 6, Figure 7). Co-occurring species (in no particular order) included *Myriophyllum spicatum*, *Potamogeton crispus*, *Ceratophyllum demersum*, *Spirodella polyrrhiza*, *Lemna minor*, and *Stuckenia pectinata*.

Competition Experiment

Water lilies reached the water surface soon after planting at the end of May. Water chestnut reached the water surface in various tubs around 10 June. Toward the end of June, it became very difficult to distinguish between the individual water chestnut plants. Lily blooms were first observed on 24 June. Water chestnut blooms were first observed on 3 July. Some lily leaves began to grow in an aerial form at the beginning of July. Some water chestnut rosettes also grew in an aerial form in mid-July. Seed development of both species was observed in mid-July as well. Water chestnut rosettes became fragile toward the end of August, which aided in the decision to end the experiment.

Regressions of *Trapa natans* percent coverage, *Nymphaea odorata* percent coverage, number of rosettes, and number of leaves had relatively good fits (r^2=0.715, 0.508, 0.628, 0.801, respectively) and were statistically significant (p=0.000, 0.005, 0.001, 0.000, respectively). Interspecific competition from water lily was 2.27 times more effective than intraspecific competition on water chestnut percent coverage (Table 2). There was little effect from water chestnut on water lily percent coverage or number of leaves. The regressions modeling the biomass of *Trapa natans*, the biomass of *Nymphaea odorata*, and seed production of *Trapa natans*, while statistically significant or approaching significance (p=0.041, 0.061, and 0.032, respectively), did not show strong relations (r^2=0.346, 0.311, and 0.369, respectively), although they may still be biologically significant. In the regression equation of the water chestnut biomass, the ratio of the interspecific coefficient to the intraspecific coefficient was 2.5, meaning that one water lily had the effect of 2.5 water chestnut plants on water chestnut biomass. The ratio for water lily biomass was 0:0, which means that water lilies were not exerting a competitive effect against other water lilies at those planting densities. The ratio for water chestnut seed production was -135, meaning that the competitive effect of one lily on water chestnut seed production was equivalent to 135 water chestnut plants. The negative ratio indicates a facilitative, rather than competitive, effect. Analysis of the multiple regressions showed poor fit to the model for the specific leaf area (SLA) of either species (Table 2).

There was no significant difference among any of the calculated relative growth rates. It seems that the RGR was higher when there were greater plant densities. In mixtures, the RGR increased when the second species' density was two. The RGR then decreased when the second species' density was five (Figure 8, Figure 9).

Water Chestnut Germination Experiment

Averages of the water temperatures and relative light intensities were all significantly different from each other, so each combination could be considered a separate treatment. A total of 92 seeds germinated out of the 192 seeds placed in the tanks, which is 48% successful germination. About 49% of the seeds placed in the lit treatments germinated and 47% of the seeds placed in the shaded treatments germinated. About 34% of the seeds in the cold range, 60% in the middle range, and 50% in the hot range germinated (Table 3). It took five days for the first seeds to germinate, which occurred in the warmest temperature range. After eight days, the first seeds in the middle range had germinated. After 15 days, seeds in the coldest range began to germinate. The middle range yielded the greatest number of germinated seeds, followed by the hot range and then the cold range (Figure 10).

The percent of seeds that germinated in the cold treatment was determined to be statistically lower than the percent of seeds that germinated in the warmer two treatments. The cluster analysis resulted in two main clusters at a similarity of 75%

(Figure 11). The nMDS ordination showed similar groupings (Figure 12). The groupings observed in the cluster analysis and nMDS ordination appear to be a result of the temperature treatment—the middle and hot temperature ranges group together, and the cold temperature range forms the other cluster. The 2D stress level of 0.01 indicates excellent representation of the data by the ordination grouping. The cluster and nMDS analyses show consistency between both representations.

The ANOSIM analysis tested for differences between the three temperature ranges and between the two light groups. The middle and hot temperature ranges had similar results, while the cold temperature range had different results. The global R statistic for the temperature ranges was 0.536 with a 0.1% significance level. For the cold and middle and cold and hot ranges, the R statistic was 0.865. The R statistic was only 0.089 for the middle and hot ranges. The lit and shaded treatments did not have significantly different results. The global R statistic for the light groups was 0.071 with a 23.3% significance level (Table 3).

The ANOSIM analysis results further supported groupings illustrated by the cluster and nMDS analyses, suggesting importance of the temperature treatments and not the light treatment. While the global R statistic for the temperature ranges was not particularly high, it was significant (p=0.001). Within the temperature treatment, there were significant differences between the cold range and warmer two ranges but not between the middle and hot range. This result matched the earlier results. The global R statistic for the light groups was low and not significant (p=0.233), indicating no difference between the lit and shaded treatments.

Water Chestnut Seed Viability Experiment

Only the mature, cold-stratified seeds germinated. In this group, 90% (27) of the seeds germinated and 87% (26) germinated during the first three days of the experiment. The remainder of the seeds in this group became moldy. In all other groups, the seeds became soft, rotted, and did not germinate.

Discussion

Competition

Water chestnut has shown a recent rapid range expansion, appearing in coastal wetlands along much of Lake Ontario. Although it has been known in Sodus Bay and Oswego since at least 2000, as well as other inland sites, occurrences near Rochester and the coastline of Jefferson County are relatively new. About 47% of the 483 observations provided by iMapInvasives occurred within the past five years, since 2010 (iMapInvasives 2015). Given this recent, rapid expansion, competition with a morphologically similar plant that occupies the same space in the water column is expected (Gopal and Goel 1993). Water chestnut and white water lily both compete for two-dimensional space at the water surface rather than three-dimensional space in the water column. Therefore, competitive effects were more easily observed in each species' percent coverage and number of leaves/rosettes, as seen by the significance of those particular multiple regressions. The lily, as a *k*-selected species, was the better competitor. However, water chestnut is an efficient invader because it quickly forms a weedy bed and has very high germination success.

Temperature and Light Effects on Water Chestnut Germination

The results of the germination experiment are somewhat contrary to growth requirements reported in literature. Temperature did not significantly affect the percentage of seeds that germinated, only how long it took for them to germinate. This result is similar to that found by Kurihara and Ikusima (1991), who reported that culture temperature did not affect the germination rate of the closely related *Trapa bispinosa*. However, Hummel and Kiviat (2004) reported that *T. natans* requires full sun. The shade treatment used in this germination experiment did not affect water chestnut germination, although continuously dark conditions did support a slower germination rate in *Trapa bispinosa* (Kurihara and Ikusima 1991). *T. natans* may thus be shade-tolerant to an extent and may be able to survive in a wider range of conditions than previously thought.

Such tolerance may also have an implication on future competition between water chestnut and native aquatic vegetation. If water chestnut can indeed survive a wider range of light conditions, it follows that it can establish in more places within a wetland system and form thick, weedy beds that have close to 100% cover, which adversely impacts submersed vegetation and associated wildlife, across a wider range (Methe *et al.* 1993, Strayer *et al.* 2003, Hummel and Kiviat 2004). Although germination was not affected by the shade treatment, additional traits should be examined, including flowering and seed production. The light treatments used in this experiment may not have been extreme enough (i.e. shaded darkly enough) to show any effect on germination success or rate.

Water Chestnut Seed-Viability

It seems that water chestnut seeds will not germinate unless they have been cold-stratified and are mature enough to fall from the plant. Germination of mature seeds should not occur the same season in which they were produced. If the plant were to extend its range south, beyond a cold winter, it should not survive.

Water chestnut is reported to be native to tropical as well as temperate regions (Muenscher 1944). Although results from the aforementioned seed viability experiment support the constraint of water chestnut to waters that experience a period of cold temperature, other genetic strains of this species may not experience such restrictions. The spread of such a strain would be disastrous to waterways and water bodies currently outside of water chestnut's range, as well as the Great Lakes basin. Predicted global temperature increases of 2.3 to 4.5^{o}C by 2100 could impede thermal barriers in the Great Lakes that would keep a tropical strain out (Pagnucco *et al.* 2015). Further studies could be conducted on water chestnut growth and cold-stratification requirements in its native range (i.e., where temperatures would be sufficiently cold for stratification to occur). Water depth as a result of lake-level regulation could also affect the growth and range of water chestnut and should be investigated.

Control

Even without the threat of a tropical strain, water chestnut's success could have serious implications for Lake Ontario coastal wetlands and, eventually, the entire Great Lakes basin if the species should so spread. A total of 879 distinct wetlands, totaling 25,847 hectares, would be put at risk in Lake Ontario and the

Upper St. Lawrence River alone (Wilcox *et al.* 2005). Once established, water chestnut would form weedy beds of rosettes at the water surface, allowing only shade-tolerant submersed plants, like *Myriophyllum spicatum*, *Elodea canadensis*, and *Ceratophyllum demersum*, to inhabit the water column. The reduction of photosynthesis in the water column would lower the amount of dissolved oxygen available to aquatic invertebrates, changing the diversity and species that live in the vegetation (Methe *et al.* 1993, Caraco and Cole 2002, Strayer *et al.* 2003). The lowered dissolved oxygen and altered invertebrate community would also negatively impact the fish that feed and spawn in the wetlands, and different and fewer fish would upset recreational fishermen. The change in plant community would reduce food sources for water birds and would discourage birds and birders. Weedy beds at the water surface would inhibit any boating, paddling, swimming, and wading activities that would normally take place. Not only would the wetland ecosystem be threatened, but societal recreation would as well.

Although costly in the short-term, the smaller populations of water chestnut in Lake Ontario coastal wetlands should be controlled, thereby greatly reducing source populations. Chemical control could have unintentional negative effects on non-target species and water quality (Methe *et al*. 1993, Vander Zanden 2010). However, other means of control have potential. Cutting the stems of rosettes 10 cm below the water surface disrupts the rosettes' normal growth and reduced plant vigor and seed production (Methe *et al.* 1993). In an experimental study, various frequencies and amplitudes of ultrasound applied directly to water chestnuts resulted in mortality rates of 100%, although the study was preliminary in nature and further research is needed

(Wu and Wu 2005). Biological control is also a potential control method. *Galerucella birmanica*, an Asian leaf beetle, feeds on water chestnut and could develop a sustaining population on an infestation (Ding *et al*. 2006, Ding *et al*. 2007). However, biological control requires a vast amount of testing in quarantined lab settings and confidence in the organisms before any sort of field test can take place, which makes commencement of this potential control method lengthy and time-consuming. The water lily beetle, *G. nymphaeae*, is a native beetle that also feeds on water chestnut and is morphologically similar to *G. birmanica*. Although it would exert less pressure on water chestnut than *G. birmanica*, it may inhibit water chestnut populations. Natural predators could interfere with both species (Ding and Blossey 2005). Given the results of the my seed viability experiment, hand pulling the plants prior to the seeds maturing is also a viable option for small, accessible populations. This practice would have to be repeated every year, however, since the seeds can remain viable in the seedbank for up to 12 years (Hummel and Kiviat 2004). Planting white water lily, or a similar species, may also help control water chestnut populations when utilized in conjunction with hand pulling because it is a good competitor. However, it can become weedy; more research would have to be conducted.

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Table 1. The years that coastal wetland sites where water chestnut has been observed were sampled. Observations in Buck Pond and Salmon River were made by employees of The Nature Conservancy. Observations on Wolfe Island were communicated via personal communication with an employee of Ducks Unlimited Canada.

Site	First Sampled	Water Chestnut Observed
Braddock Bay	2012	2013
Buck Pond	2013	2013
Catfish Creek	2013	2013
East Sodus Bay	2012	2012
Floodwood Pond	2011	2013
Little Sandy Creek	2013	2013
Maxwell Creek	2011	2011
North Colwell Pond	2014	2014
South Sodus Bay	2012	2012
Red Creek	2011	2014
Salmon River	2014	2014
Sherwin Bay Marsh	2013	2013
South Colwell Pond	2012	2013
Sterling Creek	2014	2014
Third Creek	2011	2014
Wolfe Island	2009	2011

Table 2. Multiple regression analysis of competition between *Trapa natans* and *Nymphaea odorata*.

1/Variable	Intercept	Intraspecific Coefficient	Interspecific Coefficient	Ratio	r	r^2	F	p-value
T. natans biomass	0.013	0.002	0.005	2.50	0.588	0.346	3.964	0.041
N. odorata biomass	0.003	0.001	0.000	0.00	0.557	0.311	3.379	0.061
T. natans coverage	0.045	0.011	0.025	2.27	0.845	0.715	18.783	0.000
N. odorata coverage	0.009	0.027	-0.004	-0.15	0.713	0.508	7.757	0.005
T. natans rosettes	0.127	0.030	0.037	1.23	0.793	0.628	12.679	0.001
N. odorata leaves	0.035	0.025	0.003	0.12	0.895	0.801	30.16	0.000
T. natans seeds	0.072	-0.002	0.270	-135	0.607	0.369	4.378	0.032
T. natans SLA	0.009	-0.0000236	-0.0000355	1.50	0.097	0.009	0.072	0.931
N. odorata SLA	0.006	-0.0000960	-0.0000325	0.34	0.316	0.100	0.834	0.454

Table 3. The percent of seeds that germinated in each temperature range and light treatment.

	% germinated		
	lit	shaded	total
Cool Range	31.25	37.5	34.375
Middle Range	59.375	59.375	59.375
Hot Range	56.25	43.75	50

Table 4. ANOSIM test for differences between temperature ranges and light treatments. C=cold (10-14 ^{O}C), M=middle (17-19^{O}C), H=hot (21-24 ^{O}C).

	Temperature Range		Light Treatment
Global R	0.536		0.071
Significance Level (%)	0.1		23.3
	Groups	**R Statistic**	
	C, M	0.865	
	C, H	0.865	
	M, H	0.089	

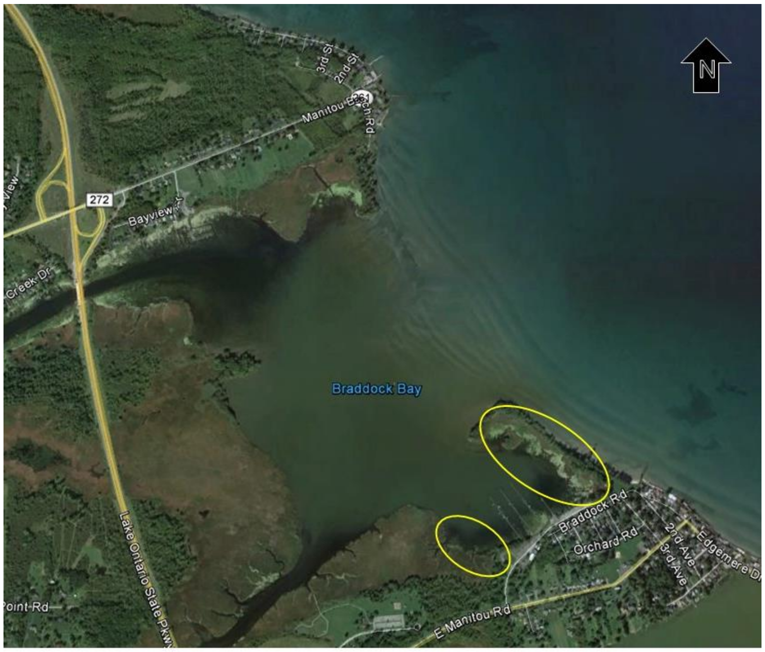

Figure 1. Braddock Bay in Monroe County, New York. The ovals outline where water chestnut has been found. Field observations were made on the inland side of the eastern sand spit, within the larger oval.

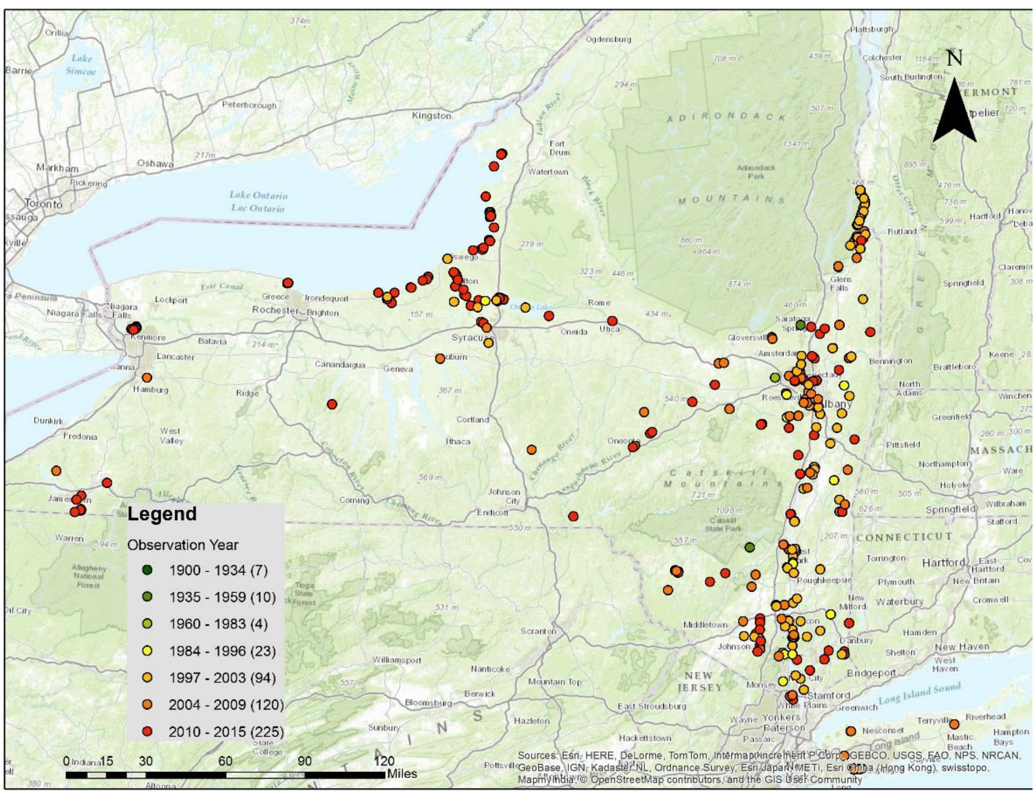

Figure 2. Observations of water chestnut (including approximate locations) from iMapInvasives symbolized by the range of years within which the observation was made. The number of observations made within those ranges are indicated within parentheses.

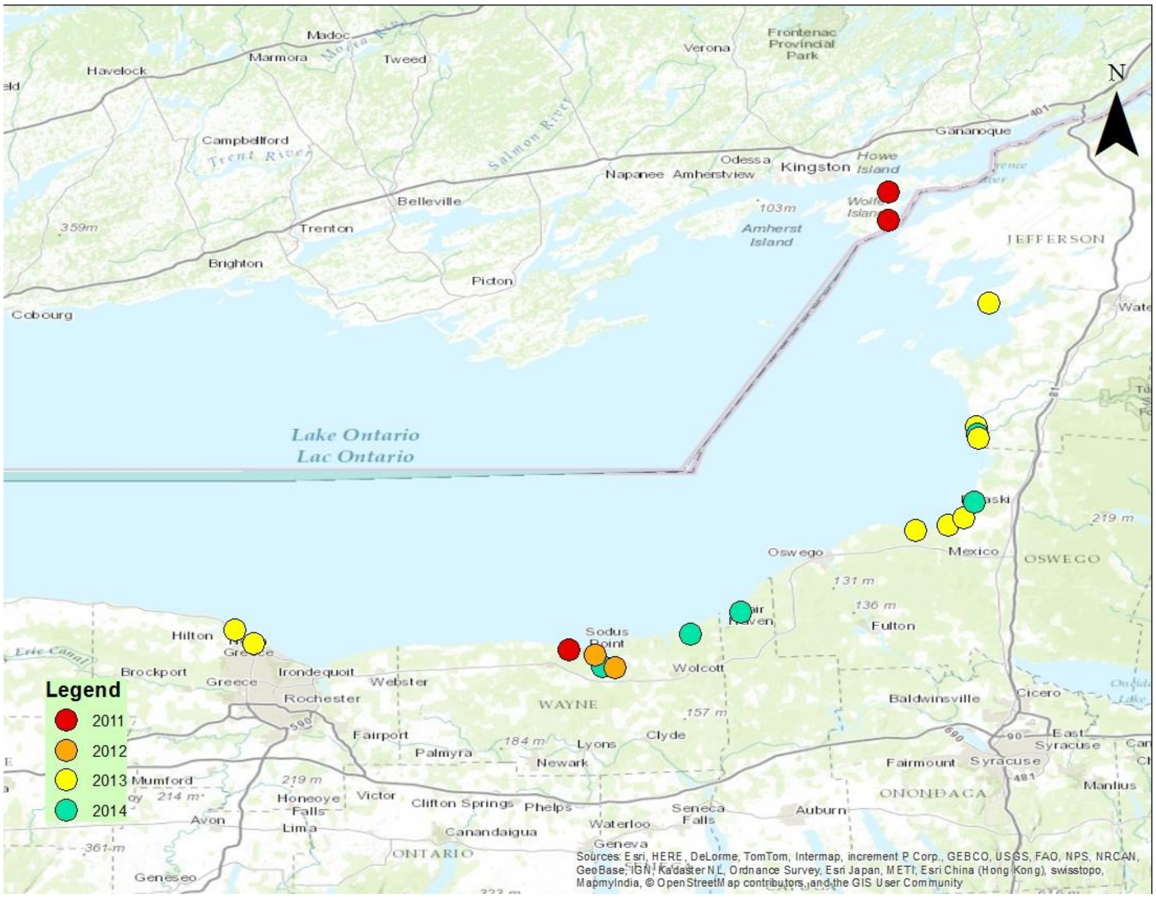

Figure 3. Lake Ontario coastal wetlands infested with water chestnut. The sites are symbolized using the year water chestnut was observed during monitoring projects. However, water chestnut has been observed in Sodus Bay since the 1970's.

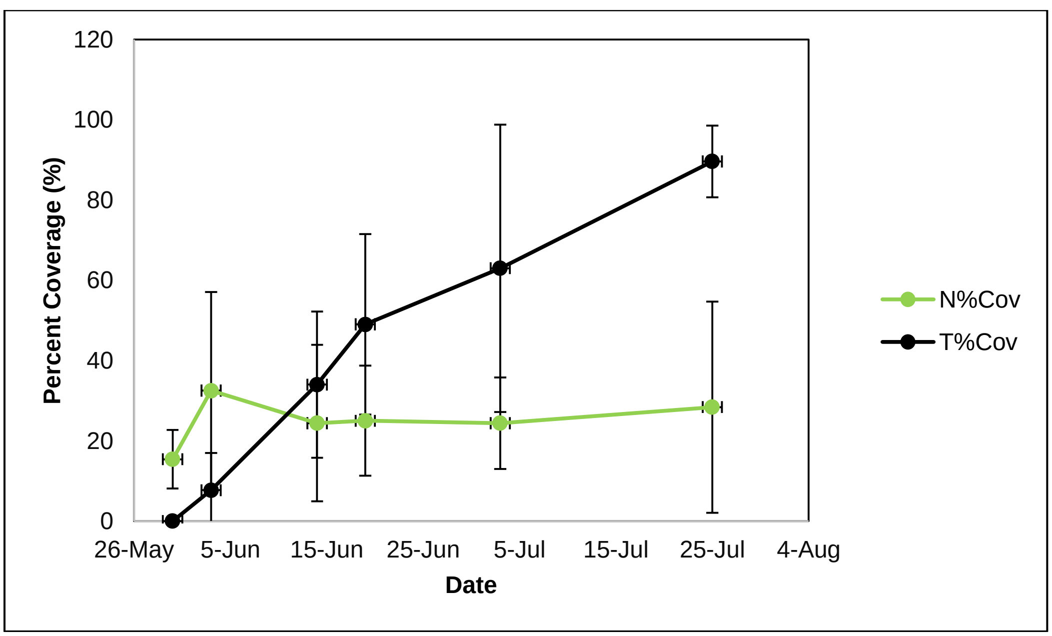

Figure 4. The percent coverage, with error bars equal to the standard deviation, of *Nymphaea odorata* and *Trapa natans* in 1m^2 quadrats during the growing season of 2014 in Braddock Bay, Monroe County, NY.

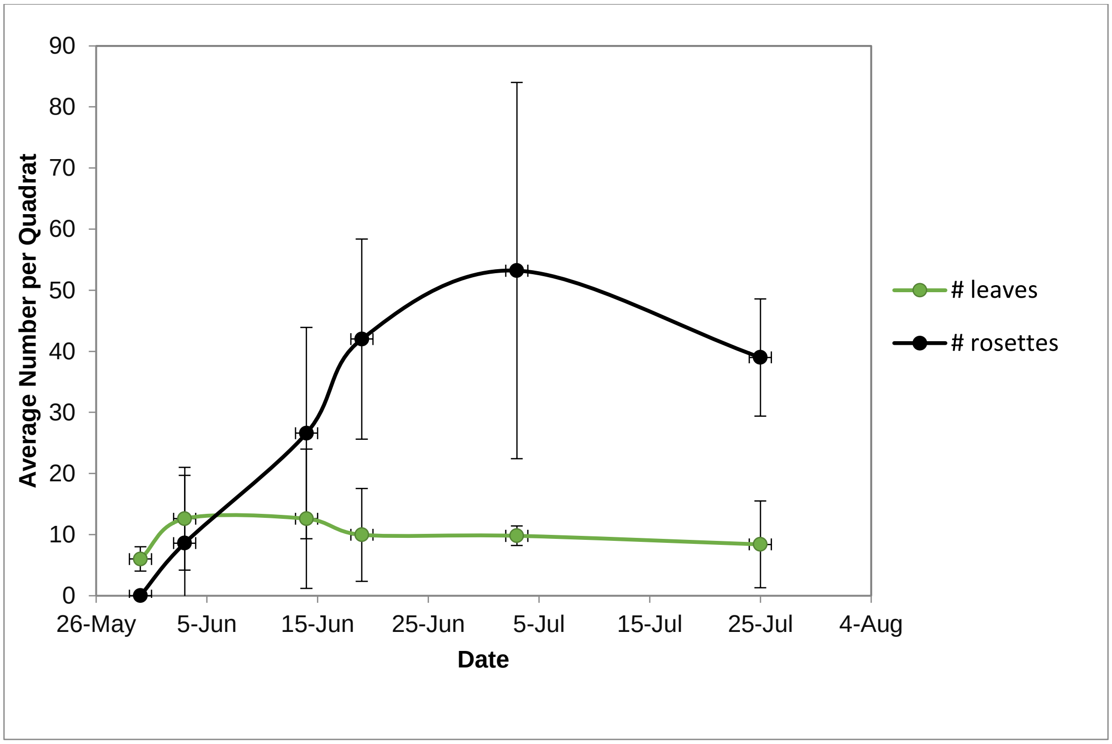

Figure 5. The number of *Nymphaea odorata* leaves or *Trapa natans* rosettes, with error bars equal to the standard deviation, in 1m^2 quadrats during the 2014 growing season in Braddock Bay, Monroe County, NY.

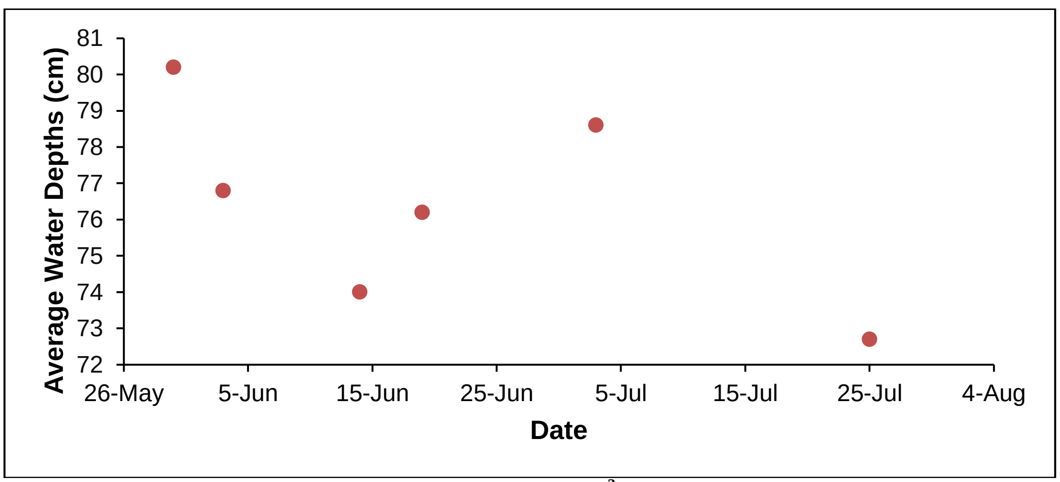

Figure 6. Average water depths (cm) from the center of 1m^2 quadrats during the 2014 growing season in Braddock Bay, Monroe County, NY.

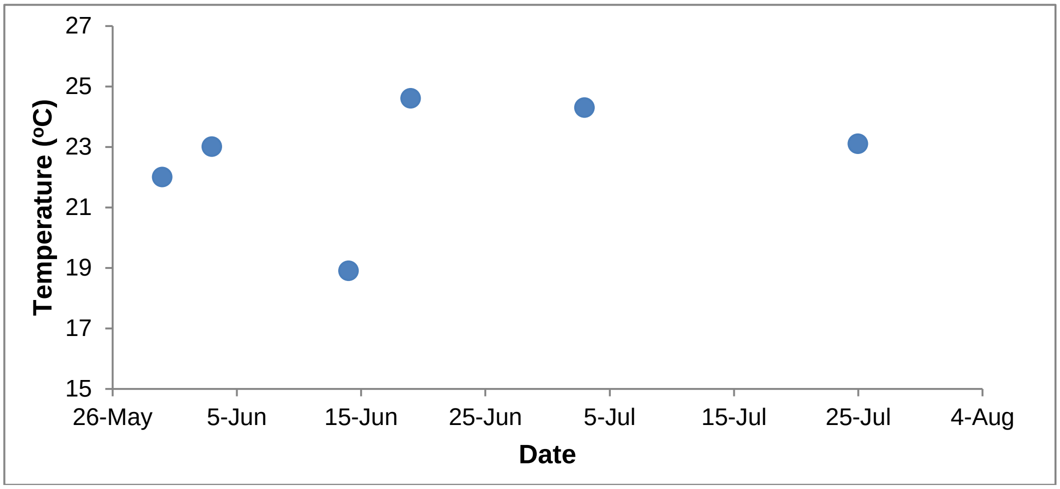

Figure 7. Water temperature (C°) during the 2014 growing season in Braddock Bay, Monroe County, NY.

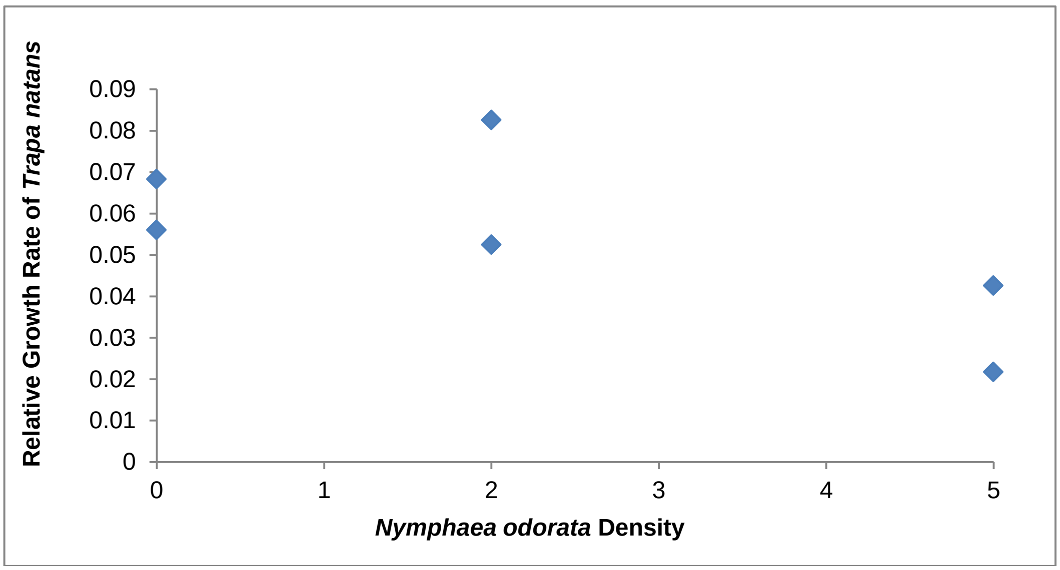

Figure 8. The relative growth rate of 2 or 5 *Trapa natans* plants when planted with 0, 2, or 5 *Nymphaea odorata* plants. The lesser data points are from the planting density of 2 *T. natans* plants. The greater data points are from the planting density of 5 *T. natans* plants.

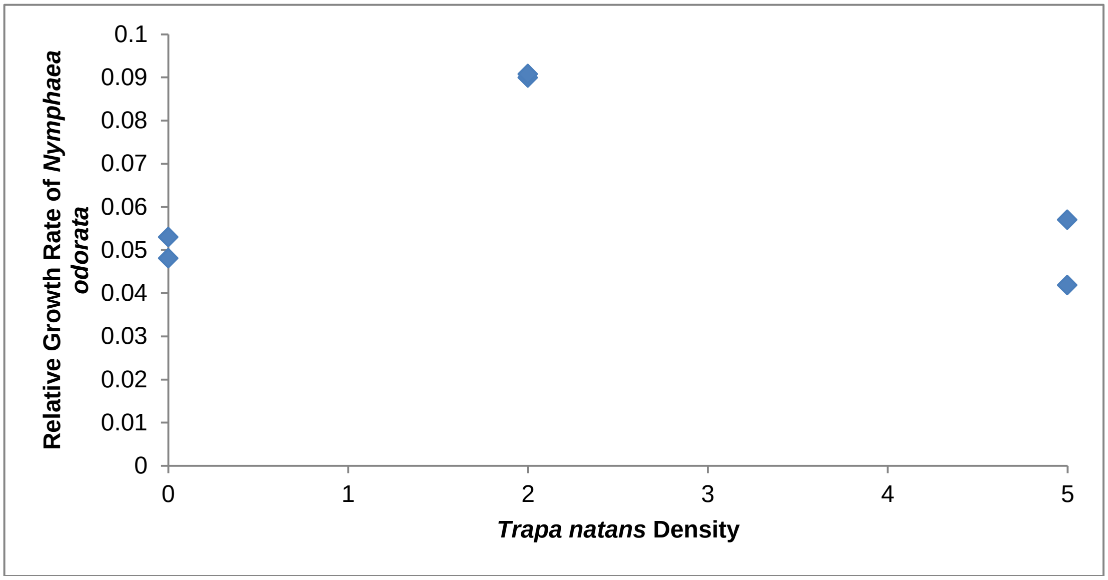

Figure 9. The relative growth rate of 2 or 5 *Nymphaea odorata* plants when planted with 0, 2, or 5 *Trapa natans* plants. The lesser data points are from the planting density of 2 *T. natans* plants. The greater data points are from the planting density of 5 *T. natans* plants.

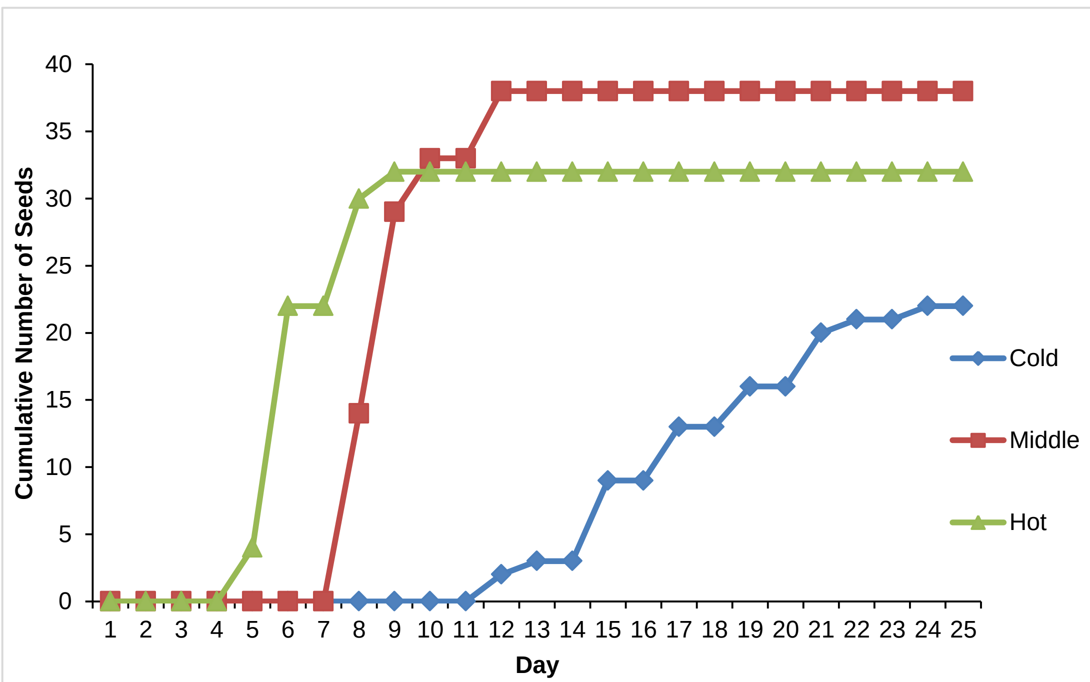

Figure 10. The number of seeds germinated in each temperature range throughout the 25-day germination experiment. Replicates for each temperature treatment were combined.

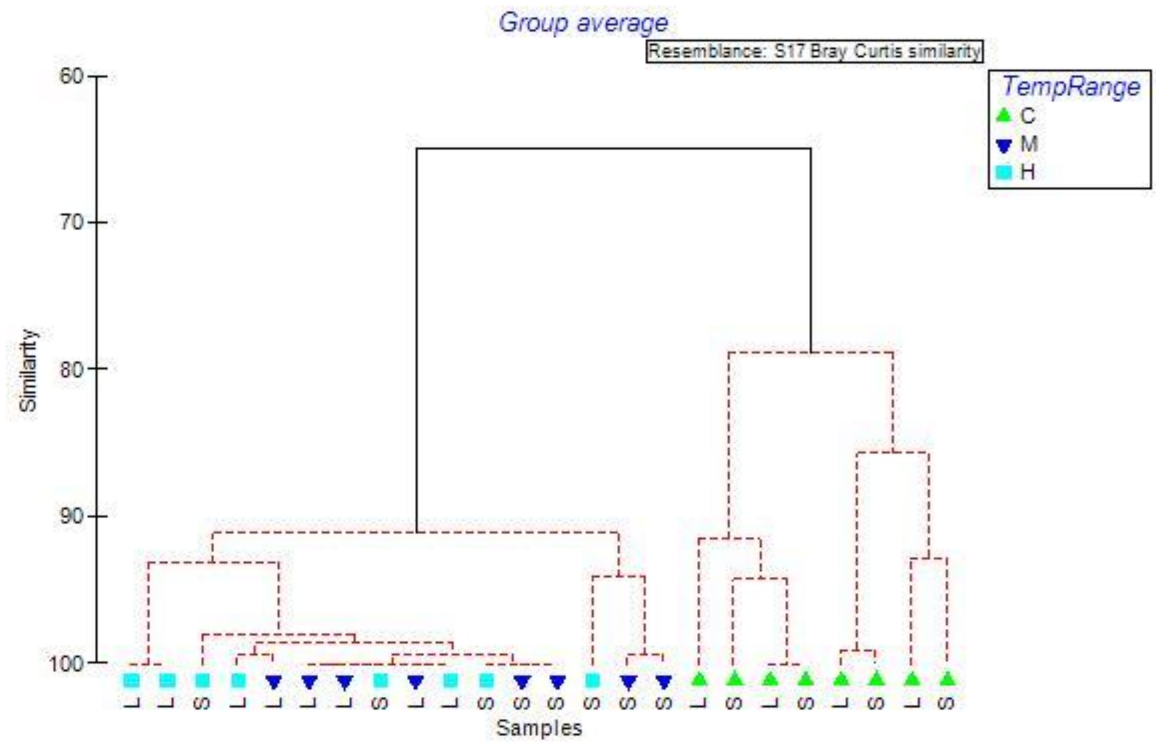

Figure 11. Cluster analysis dendrogram of the germination experiment. Temperature ranges marked by shape and color, L=lit and S=shaded. Two clusters formed around a similarity of 75.

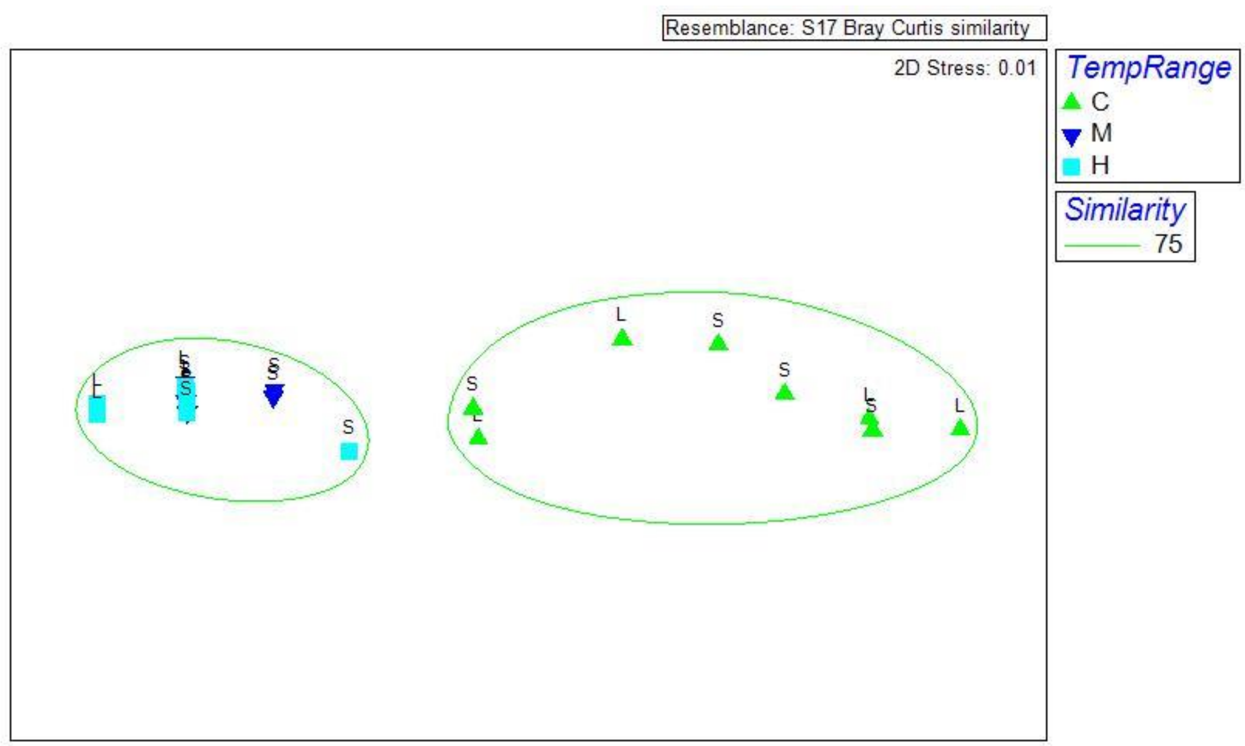

Figure 12. The nMDS ordination of the three temperature ranges (10-14^{O}C, 17-19 ^{O}C, 21-24 ^{O}C), and two light treatments (lit or shaded) of the germination experiment with the distance 75 cluster overlay.