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Temperature and herbicide impacts on germination of water chestnut seeds

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ABSTRACT

Water chestnut (*Trapa natans* L.) is a nonnative, annual invasive species that has infested fresh water bodies in the eastern United States (US). Management techniques intended to control or eradicate water chestnut include mechanical or physical removal and herbicide treatments. Information is limited regarding the impact of lake drawdowns on the viability of water chestnut seeds as a complement to other control measures. Experiments were conducted to determine the influence of freezing and simulated drawdowns on the viability of fruit from water chestnut plants growing in herbicide-treated and non-treated areas. In a laboratory experiment, water chestnut fruit frozen in water for 35 d did not germinate. In 2 yr of mesocosm studies, simulated drawdowns did not have a significant influence on water chestnut seed germination, whereas fruit collected from areas treated with herbicide had a significantly different germination rate than those collected from untreated areas. In these studies, the mass of fruit collected from plants in areas treated with herbicide(s) (2,4-D, and/or glyphosate) was significantly less than fruit collected from plants growing in untreated areas. These data suggest that herbicide applications may have a greater or more consistent impact on water chestnut seed viability than lake drawdowns.

Key words: aquatic species, invasive species, seed bank, *Trapa natans*.

INTRODUCTION

Water chestnut (*Trapa natans* L.) is an annual, invasive species that originated in Asia (O'Neill 2006) and forms a floating rosette composed of leaves with serrated edges. The fruit of water chestnut is a nut that has four sharp horns. The seed requires a cold stratification period before germination can occur (Kurihara and Kusima 1991, Cozza et al. 1994), and can exhibit delayed dormancy, thus forming a seed bank in the sediment lasting approximately 5 yr or longer (Kunii 1988, Kishbaugh 2009).

Water chestnut has been observed in areas of the eastern United States (US) including Lake George and Adirondack Lakes, NY (Madsen 1994), Albany County, NY (Methé et al. 1993), the Chesapeake Bay watershed including the Bird and Sassafras Rivers (Naylor 2003), the Hudson River Estuary, NY (Nieder et al. 2009), Lake Champlain, VT and NY since the 1940s (O'Neill 2006, Marsden and Hauser 2009), and in Massachusetts from 1879 to present (Sorrie 2005) Despite this range, water chestnut has only recently become an invasive species in the state of New Jersey. The earliest documented occurrence of water chestnut in New Jersey was a few isolated stands in Lake Musconetcong identified in 2008 (Shannon 2008). These stands expanded to over 30% coverage of the 133-ha lake by the next year.

Several methods have previously been used to manage water chestnut. Chemical management includes the use of the herbicides 2,4-D (Hummel and Kiviat 2004, Kisbaugh 2009) and triclopyr (Hummel and Kiviat 2004, Poovey and Getsinger 2007). Mechanical harvesting is often a more effective approach for controlling water chestnut than with other invasive species, because water chestnut does not reproduce by fragmentation (Galanti and Esposito 1996, Kisbaugh 2009). Weed harvesting, with proper timing, can also reduce the deposition of new seeds to the seed bank of water chestnut (Methé et al. 1993). Nevertheless, weed harvesting may be costly, time consuming, and difficult to conduct in the shallow areas where water chestnut is frequently found. Hand pulling (Kisbaugh 2009) and stem cutting while leaving the rosette in place (Methé et al. 1993) have been shown to be more cost-effective methods for controlling water chestnut than mechanical harvesting, and also significantly reduce seed production. A well-coordinated hand-pulling effort removed 18,000 to 27,000 kg (wet weight) of water chestnut from a New Jersey water body in 1 d (Rector and Nitzsche 2013). A multifaceted program is often warranted to control water chestnut populations sufficiently.

One management measure that is currently not available for use on water chestnut is a biological control agent. Biological control with the leaf beetle (*Galerucella nymphaeae* L.) was investigated but did not substantially impact water chestnut plants (Ding and Blossey 2005). Studies in China documented that *Galerucella birmanica* Jacoby significantly reduced water chestnut leaf canopy; this beetle, however, is not host specific (Ding et al. 2006, Ding et al. 2007, Kisbaugh 2009) and could be problematic in lakes containing beneficial native or endangered plant species.

Reducing lake water levels to expose the shoreline sediment (i.e., partial lake drawdown) is a management technique that has shown a varied response across several different species of macrophytes (Cooke 1980, Madsen 2000, Phillips et al. 2000, Holdren et al. 2001, New York State

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Department of Environmental Conservation [NYS DEC] 2005). In previous studies, lake drawdowns controlled non-seed-bearing aquatic species, but seed bearers were not affected (Holdren et al. 2001, New Hampshire Department of Environmental Services [NH DES] 2010). The duration of drawdowns and freezing temperatures are crucial factors in determining the success or failure of a drawdown program (Goldsby et al. 1978, NH DES 2010). Minimal information is available in the literature on the impact of drawdowns on water chestnut seed germination.

Lake Musconetcong is a shallow (mean depth 1.1 m, max depth 3.05 m) state-owned lake located in northwestern New Jersey (Princeton Hydro 2011). Several plant species considered to be rare in New Jersey can be found in Lake Musconetcong, including the endangered species, Robbins' pondweed (*Potamogeton robbinsii* Oakes).There are also several aquatic weeds that have been problematic in Lake Musconetcong for decades, including curly leaf pondweed (*Potamogeton crispus* L.), algae (*Oedogonium*, *Bulbochaete*, *Spondylosium*, and *Desmidium* spp.) (Princeton Hydro, pers. comm.), coontail *(Ceratophyllum demersum* L.), and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Princeton Hydro 2006, Shannon 2008). The aquatic weed problem may in part be due to the shallowness of the lake and also a result of excess phosphorus concentrations. The lake has a total maximum daily load for phosphorus, and the majority of the phosphorus load is generated from the outlet of Lake Hopatcong (67%) and stormwater runoff (24%), with 4% of the annual load from internal cycling of the sediments (Princeton Hydro 2006).

The Lake Musconetcong Regional Planning Board (LMRPB) is a nonprofit board created to oversee the environmental health of Lake Musconetcong, including a focus on aquatic invasive species management. The LMRPB has been addressing the issue of water chestnut with a multifaceted attack that includes mechanical harvesting, chemical herbicide treatment, and hand pulling. At the request of the LMRPB, studies were conducted to determine the influence of freezing and simulated drawdowns on the viability of seed germination from the fruit of water chestnut collected from herbicide-treated and untreated areas. These studies were designed to provide information regarding the potential of a partial drawdown as an additional management measure, along with information on impacts of herbicide on seed production.

MATERIALS AND METHODS

Seed source

Lake Musconetcong provides suitable habitat for growth of water chestnut via relatively thick (0.6 m), unconsolidated sediments overlaying 1.3 m of peat (Princeton Hydro 2011). The pH ranges from 7.4 to 8.9 (Princeton Hydro 2011), and nutrient concentrations are high (New Jersey Department of Environmental Protection [NJDEP] 2003, Princeton Hydro 2006, Princeton Hydro 2011). Fruit were collected from Lake Musconetcong in 2010, 2011, and 2012.

Study design

Experiments were conducted over 3 yr (2010, 2011, and 2012) to investigate the germination rate of seeds from water chestnut fruit. The experiment in 2010 tested the effect of 35 d of freezing temperatures versus refrigerated controls. In 2011, a 2 by 2 factorial experiment tested the effect of a simulated lake drawdown environment versus refrigerated controls, with seed source as a second factor, comparing seeds from either herbicide-treated or untreated areas of the lake. In 2012, a 2 by 2 factorial experiment in a mesocosm was conducted with similarities to the 2011 experiment. The differences included: the fruit were from areas of the lake treated once with herbicide (1×) or with multiple herbicide treatments (M×); the substrate in the mesocosm was sediment from the lake bottom; and samples were placed at three depths in the mesocosm.

Water chestnut freeze trial 2010

A completely randomized laboratory experiment was designed to determine the impact of freezing water chestnut fruit on seed germination.

Two hundred three water chestnut fruit were collected from the bottom sediment[1] of Lake Musconetcong with a grab sample on 28 October 2010. Multiple samples were taken from areas of the lake known to have extensive growth of water chestnut. On 1 November 2010, fruit were rinsed, placed in plastic containers (13 cm by 13 cm), immersed in tap water, and stratified in the dark at 4 C. After 107 d the fruit were washed in tap water. One hundred water chestnut fruit were submerged, loosely packed in two sealed plastic containers (13 cm by 13 cm) filled with 700 ml of tap water and 0.5 ml NaClO, and placed in a freezer for 35 d. The other 103 water chestnut fruit were divided into two replications (49 fruit and 54 fruit) and submerged in two sealed plastic containers (13 cm by 13 cm) filled with 700 ml of tap water and 0.5 NaClO and placed in a refrigerator at 4 C. On 23 March 2011, all fruit were washed, placed in four separate growing containers (plastic, 94 cm by 48 cm by 18 cm internal dimensions) with tap water as growing medium in a greenhouse at 18 to 29 C under photosynthetically active radiation (PAR) approximately 25 to 35 mol m^{-2} d^{-1} (Torres and Lopez no date). Germination rate was measured on 28 April 2011.

In order to test for delayed dormancy, on 29 April 2011, nongerminated water chestnut fruit from both the freezing treatment and refrigeration treatments were submerged in two sealed plastic containers filled with 700 ml tap water and 0.5 ml NaClO for a second cold stratification at 4 C. On 2 June 2011, the water chestnut fruit were submerged in tap water in plastic containers exposed to outdoor sunlight and temperatures (June monthly average 22 C and July monthly 26 C). Germination rate was measured on 1 August 2011.

Water chestnut drawdown mesocosm and freeze trials 2011

A 2 by 2 factorial experiment was conducted to assess the effect of simulated lake drawdown conditions (mesocosm)

Table 1. The experimental design for the treatment, number of water chestnut fruit, and replications in the 2011 experiment.

Herbicide treatment	Drawdown treatment	Replication	No. fruit per bag
Untreated	Refrigerator	1	11
Untreated	Refrigerator	2	11
Untreated	Refrigerator	3	11
Untreated	Refrigerator	4	11
Untreated	Mesocosm	1	11
Untreated	Mesocosm	2	11
Untreated	Mesocosm	3	11
Untreated	Mesocosm	4	11
Treated	Refrigerator	1	11
Treated	Refrigerator	2	11
Treated	Refrigerator	3	11
Treated	Refrigerator	4	11
Treated	Mesocosm	1	11
Treated	Mesocosm	2	11
Treated	Mesocosm	3	11
Treated	Mesocosm	4	11
Untreated	24-h freeze	1	11
Untreated	24-h freeze	2	11

versus refrigerated control (as conducted in 2010), and herbicide application at the seed source as the second factor, with seeds from lake areas that were either treated with herbicide or untreated. The response variable was the proportion of seeds that germinated.

On 5 December 2011, fruit were collected from the bottom sediment of Lake Musconetcong, utilizing a modified clam rake, from areas that 1) were mechanically harvested but not treated with herbicide applications or 2) areas of the lake that had been treated with herbicide. Herbicide treatments had been Sculpin-G (2,4-D, ai = 20%) applied at 170 to 227 kg ha^{-1} by broadcast spreader in June and July 2011 and Aquaneat (glyphosate, ai = 53.8%) applied topically at 7 L ha^{-1} by hand spray in August 2011 (Allied Biological 2013). In the treated area the first 13 rake pulls generated zero fruit, but collection continued throughout the area until sufficient fruit were harvested to conduct the experiment. In contrast, for the untreated area each rake pull generated 8 to 10 fruit $pull^{-1}$. Rake pulls were continued until approximately 100 fruit were collected.

On 19 December 2011 the fruit from the treated area (n = 89) and the untreated area (n = 113) were divided into 18 subsamples (Table 1). Several unviable floating fruit were removed and discarded. All subsamples were weighed. Two subsamples of fruit from the untreated area were submerged in two sealed plastic containers filled with 700 ml tap water and 0.5 ml NaClO and placed in a freezer for 24 h before returning to refrigeration for a 24-h freeze experiment. Four control subsamples (two treated and two untreated) were submerged in sealed plastic containers filled with 700 ml of tap water and 0.5 ml NaClO and placed in a refrigerator at 4 C. A 300-gal Rubbermaid structural foam stock tank (1.8 m by 1.6 m by 0.6 m) was utilized for the mesocosm study and filled with 22 cm of a clay substrate (calcium bentonite). The eight subsamples were placed in nylon stockings and buried in the substrate at a depth of 15 cm, with hay bales placed around the stock tank. To mimic lake drawdown conditions, the tank was filled with water and then immediately allowed to drain. On 14 March 2012, all samples (control, freeze, and mesocosm) were submerged in tap water in individual 3-gal buckets in the greenhouse. Germination rate was measured on 19 April 2012. Nongerminated, nonfloating water chestnut fruit were submerged in two sealed plastic containers filled with 700 ml tap water and 0.5 ml NaClO and placed in a refrigerator at 4 C for a second cold stratification. On 5 July 2012 all nongerminated fruit (treated area and untreated) were submerged in tap water in two 3-gal buckets (drawdown and control) in the greenhouse. Germination rate was measured on 7 August 2012.

Temperature was recorded in the mesocosm every 2 h for the duration of the mesocosm study with three temperature sensors[2] and placed in the clay substrate at the same depth as the fruit (15 cm).

Water chestnut drawdown mesocosm trial 2012

A 2 by 2 factorial experiment was conducted following the procedure developed in 2011 with three exceptions. In the 2012 experiment fruit were taken from areas of the lake that had been either treated once with herbicide or treated multiple times with herbicide. No fruit came from untreated lake areas. Additionally, fruit were placed at three depths in the mesocosm, and the substrate was bottom sediment from Lake Musconetcong.

On 28 August 2012 water chestnut fruit were removed from plants collected by a mechanical harvester in Lake Musconetcong. Eighty-nine fruit were collected from plants in areas of the lake that were treated once with herbicide (1×) on 19 June 2012, and 104 fruit were collected from areas that had been retreated after the initial June treatment on 23 July 2012 and also potentially on 7 August 2012. All fruit collected from the retreated area is considered as multiple treated (M×), as it could not be determined whether an individual fruit was collected from an area that received the third treatment on 7 August 2012. Applications during the summer of 2012 were with Sculpin G (2,4-D, ai = 20%) applied at 170 to 227 kg ha^{-1} by broadcast spreader in June Platoon (2,4-D, ai = 46.8%) applied at 9.5 to 14 L ha^{-1} by broadcast spray in July, and Aquaneat (glyphosate, ai = 53.8%) applied topically at 7 L ha^{-1} by hand sprayer in August (Allied Biological 2013).

The mesocosm described previously in the 2011 trial was utilized and filled to a depth of 34 cm with bottom sediment from Lake Musconetcong. On 13 December 2012 fruit were rinsed with tap water and randomly separated to four treatments: refrigerated control (R), surface (S), 7-cm depth and 15-cm depth. Each treatment contained three replications (Table 2). Control (R) fruit was refrigerated following methods developed in 2010 experiment. Fruit for the drawdown experiment were buried in the mesocosm substrate at the specified depths. On 3 April 2013 water chestnut fruit were removed from the mesocosm and refrigerator and placed in tap water in individual 11.4-L plastic buckets in a greenhouse. Germination was measured on 3 June 2013.

Temperature was recorded during the experiment with three temperature sensors.[2] One sensor was placed at the surface, one at a depth of 7 cm, and one at 15 cm.

Table 2. The experimental design for the treatment, number of water chestnut fruit, and replications in the 2012 experiment.

Herbicide treatment	Depth in mesocosm (cm)	Replication	No. fruit per bag
1×	Refrigerator	1	7
1×	Refrigerator	2	7
1×	Refrigerator	3	8
1×	Surface	1	7
1×	Surface	2	7
1×	Surface	3	8
1×	7	1	7
1×	7	2	7
1×	7	3	8
1×	15	1	7
1×	15	2	8
1×	15	3	8
M×	Refrigerator	1	9
M×	Refrigerator	2	9
M×	Refrigerator	3	8
M×	Surface	1	9
M×	Surface	2	9
M×	Surface	3	8
M×	7	1	9
M×	7	2	9
M×	7	3	8
M×	15	1	9
M×	15	2	9
M×	15	3	8

Statistical analyses

Germination data for 2010 were analyzed by contingency table with Fisher's exact test to determine if there was an association between germination counts and temperature treatment (35-d freeze or refrigerated control) (PROC FREQ, SAS/STAT, SAS Institute, Cary, NC).

Germination data for 2011 were analyzed with logistic regression where the dependent variable was the proportion of seeds germinated, and independent variables were the source of the seeds, drawdown treatment, and the interaction of these factors. The source of the seeds variable indicated if seeds came from treated or untreated areas of the lake; the drawdown treatment variable indicated if seeds were subjected to refrigerated control or drawdown mesocosm treatment. A generalized linear model was employed with a logit link function and a binomial distribution (Johnston no date, SAS Institute 2013). Differences in treatment means were determined by pairwise comparisons ($\alpha = 0.05$) (PROC GENMOD, SAS/STAT, SAS Institute, Cary, NC). The 1-d freeze experiment data were analyzed by contingency table with Fisher's exact test to determine if there was an association between germination counts and temperature treatment (1-d freeze or refrigerated control).

Germination data for 2012 were analyzed similarly to 2011 data by logistic regression; however, because there were zero counts of germinated seeds for most treatments, treatment effects could not be reliably determined with this procedure. A contingency table with Fisher's exact test was then used to determine if there was an association between germination counts and the combinations of seed source (areas of the lake once treated with herbicide or areas treated twice or more) and drawdown treatment (refrigerated control or drawdown mesocosm).

Table 3. Water chestnut seed germination rates from the frozen treatments and refrigerated (control) treatments in the 2010 experiment. Fisher exact test confirmed the association between treatment and germination ($P < 0.0001$).

Treatment	Number germinated	Number not germinated	Total
Frozen	0	100	100
Refrigerated	54	49	103
Total	54	149	203

Fruit mass data were pooled across 2011 and 2012, and an analysis of variance was conducted to determine differences in fruit mass for herbicide treatments (untreated, treated, once treated, or twice treated). Analysis was conducted with a general linear model (PROC GLM, SAS/STAT, SAS Institute, Cary, NC), and residuals were checked for normality and equal variance among treatments. Differences in means for treatments were determined with Tukey-adjusted pairwise comparisons ($\alpha = 0.05$), and linear contrasts were used to compare means of fruit mass between treatments within years. The effect of years on fruit mass could not be determined statistically, because herbicide treatments varied across the years.

RESULTS AND DISCUSSION

Water chestnut freeze trial 2010

In 2010, 52% of the refrigerated control seeds germinated within 5 wk, whereas none of the seeds subjected to a 35-d freeze germinated (Table 3). Fisher's exact test confirmed the association of treatment and germination counts ($P < 0.0001$). The fruit returned to refrigeration after freezing to test for potential delayed dormancy showed no additional germination.

Germination rates for the refrigerated controls were similar to Menegus et al. (1992), who reported a 50% germination rate of water chestnut seeds after a period of cold storage in water. Cozza et al. (1994) observed a 68% germination rate for cold storage seeds.

Water chestnut drawdown mesocosm trial 2011

For the logistic model predicting the proportion of seeds germinating, seed source (treated or untreated areas of the lake) was significant ($P < 0.0001$), whereas drawdown treatment (refrigerated control or drawdown mesocosm) and the interaction factor were not ($P \geq 0.7$) (Fig. 1). Mean values for the proportion of seed germinating were significantly greater for untreated areas of the lake than for herbicide-treated areas, with mean germination rates of 75 and 77% for seeds from untreated areas, and mean germination rates of 7 and 5% for seeds from treated areas. The reduction in germination of fruit from treated areas suggests that herbicide application may provide a greater influence on the successive year's crop of water chestnut than would a lake drawdown.

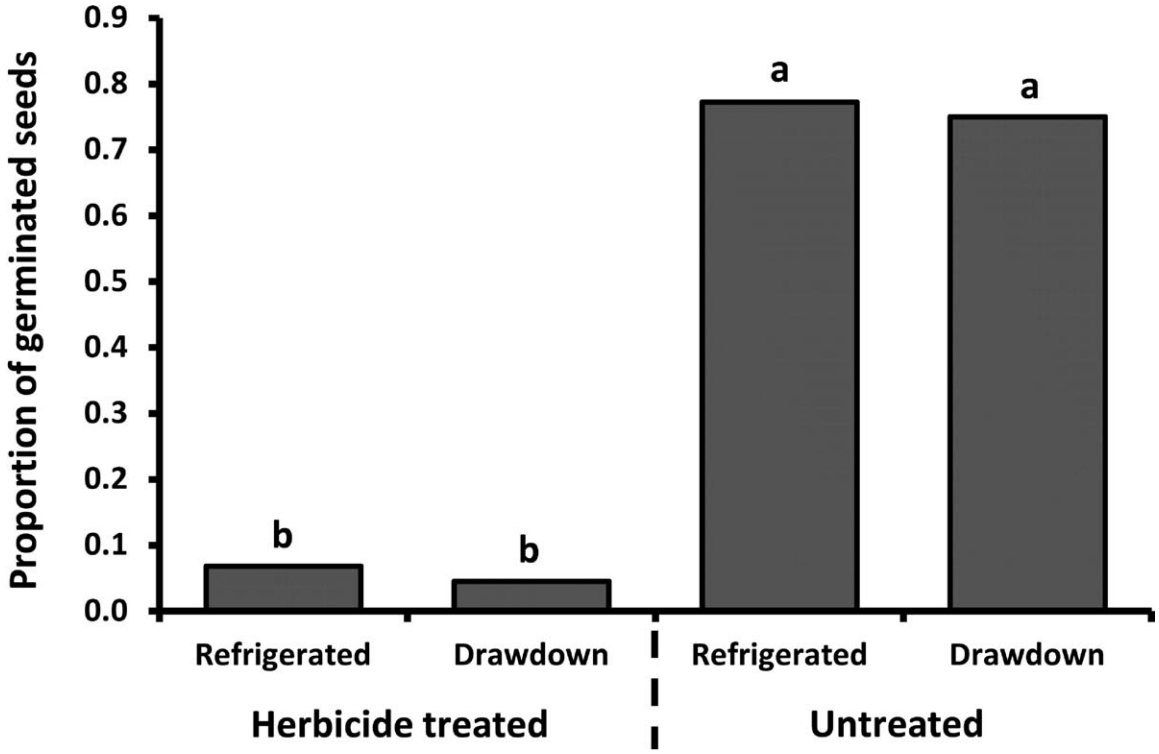

Figure 1. Proportion of *Trapa natans* seeds that germinated in relation to seed source (herbicide-treated areas of the lake or untreated areas) and drawdown treatment (refrigerated control or drawdown mesocosm). Means with the same letter are not statistically different ($\alpha = 0.05$).

A 1-d freeze did not have a significant effect on water chestnut seed germination (73% germination, $n = 22$) compared to the refrigerated control (77% germination, $n = 44$) ($P = 0.8$).

Water chestnut drawdown mesocosm trials 2012

Of 89 seeds collected from once-treated areas in 2012, only 3 germinated (3.4%), and all these were in the mesocosm drawdown treatment (Table 4). None of the 104 seeds collected from multiple-treated lake areas germinated. Fisher's exact test showed no significant association between germination and the combination of seed source (once-treated or multiple-treated areas of the lake) and drawdown treatment (refrigerated control or drawdown mesocosm) ($P = 0.19$). These results suggest that the herbicide treatments applied to the areas in the source lake impacted seed viability; however, it is difficult to measure the magnitude of this impact due to a lack of seeds from plants in the untreated areas.

Table 4. Water chestnut seed germination rates from lake areas treated with herbicide once (once-treated) or two or more times (multiple-treated), and for controls (refrigerator) or seeds subjected to drawdown mesocosms (mesocosms) in 2012.

Herbicide treatment[1]	Drawdown treatment	Germination rate[2] (%)
Once treated[3]	Refrigerator	0
Once treated	Mesocosm	4.5
Multiple treated[4]	Refrigerator	0
Multiple treated	Mesocosm	0

[1]Association of treatment-drawdown combinations and germination counts not significant by Fisher's exact test $P = 0.19$.
[2]$n = 22$ for once-treated refrigerated; 67 for once-treated mesocosm; 26 for twice-treated refrigerated; 78 for twice-treated mesocosm.
[3]Once-treated application was Sculpin G (2,4-D, ai = 20%) applied at 170 to 226.8 kg ha^{-1} by broadcast spreader in June.
[4]Multiple-treated applications were treated with the above herbicide and with Platoon (2,4-D, ai = 46.8%) applied at 9.5 to 4.3 L ha^{-1} by broadcast spray (July) and potentially Aquaneat (glyphosate, ai = 53.8%) applied topically at 7 L ha^{-1} by hand spray (August). Treatment data provided by Allied Biological (2013).

Temperature

The average temperature varied during the 2 yr of the mesocosm trial (Table 5). In 2011, there were 12 consecutive d below freezing at a substrate depth of 15 cm and 44 d in 2012. There were interannual variations between 2011 and 2012, such as cooler mean January (2011) and warmer March

Table 5. Summary of temperature data for burial depth of data loggers in sediment in mesocosms for 2011 and 2012 studies. Temperature was recorded every 2 h from 27 December 2011 to 14 March 2012 during the 2011 study. For the 2012 study temperature was recorded every 2 h from 12 December 2012 to 31 March 2013. The temperature loggers were buried at the same depths as the water chestnut samples.

	2011	2012		
	Depth (in cm)	Depth (in cm)		
	15	Surface	7	15
Longest stretch of consecutive days at or below 0 C	12	8	27	45
Total number of days at or below 0 C	20	57	58	45

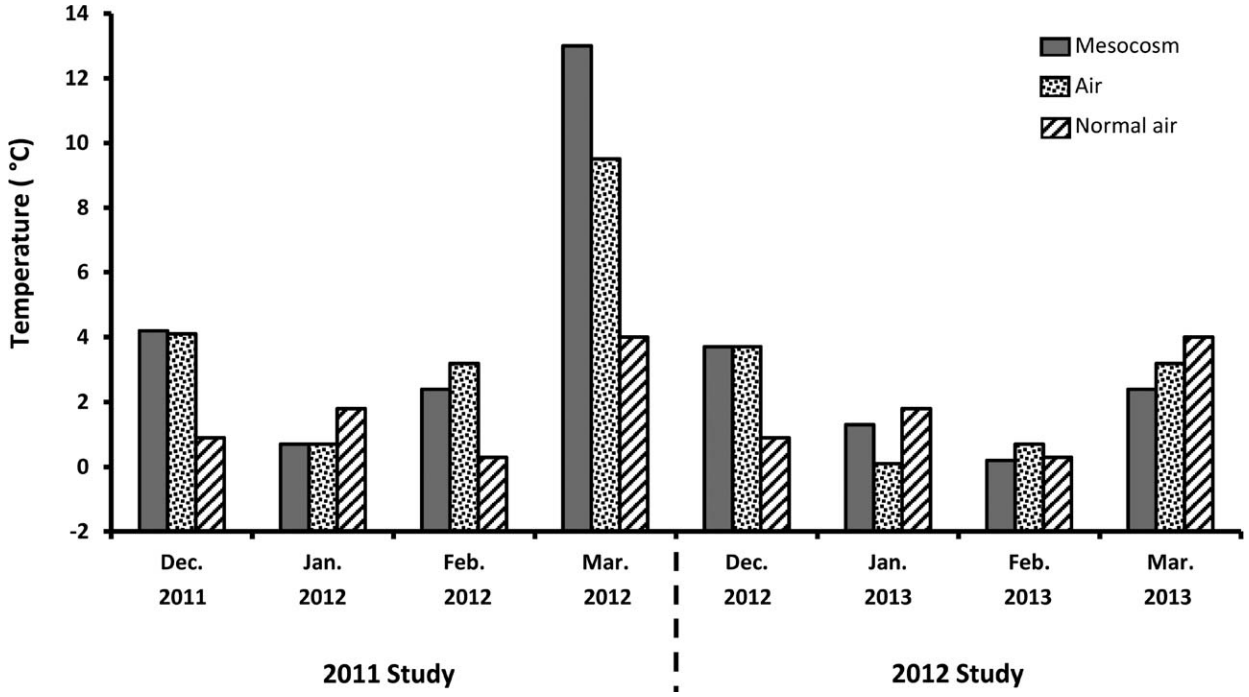

Figure 2. Comparison among monthly average temperatures in mesocosms, monthly average air temperature for northern New Jersey in 2011 and 2012, and 30-yr normal air temperature averages for northern New Jersey. Air temperature averages for 2011 and 2012 were downloaded from the Office of the State Climatologist at Rutgers University. Thirty-year normal air temperatures are based on values from 1981 to 2010 and are preliminary.

(2012) temperatures (Office of the State Climatologist at Rutgers University 2014) (Fig. 2).

Temperature has considerable effect on water chestnut fruit, by instituting and breaking dormancy (Kurihara and Kusima 1991, Menegus et al. 1992, Cozza et al. 1994) as well as altering germination rates (Kurihara and Kusima 1991, Cozza et al. 1994). During partial drawdowns newly dewatered shorelines may expose plants, fruit, or seeds to freezing.

Winter temperature variability is one reason the impact of drawdowns as an aquatic weed management measure can be variable, as typically a sustained period of freezing is required to provide the necessary environment to achieve control (Bellaud 2009). Furthermore, temperature differences were observed at different depths in the mesocosm substrate in 2012, suggesting that water chestnut fruit may be exposed to varying temperatures depending on burial depth. Water chestnut fruit will often be at, or near, the surface, where they may be exposed to broader temperature fluctuations than at lower sediment depths where temperatures may be more consistent for longer periods of time (Table 5).

Mass of fruits

Mean mass of water chestnut fruits varied significantly by seed source ($P < 0.0001$) (Fig. 3). In 2011, mean fruit mass were 3.16 g fruit^{-1} for treated areas and 3.88 g fruit^{-1} for untreated areas. In 2012, mean fruit mass were 5.69 g fruit^{-1} for once-treated areas and 3.62 g fruit^{-1} for multiple-treated areas. Within years, linear contrasts indicated significant differences in means of fruit mass between herbicide treatments (herbicide treated and untreated areas for 2011, and once-treated and multiple-treated areas for 2012 ($P = 0.0047$, $P < 0.0001$, respectively). It was not possible to compare mass of the fruits between years statistically, because treatments varied across years. Observation of the fruit from the multiple-treated source area showed numerous fruit that were twisted, deformed, or missing horns, deviating from the signature water chestnut fruit morphology and potentially related to the lower mass.

Groth et al. (1996) observed that the mass of the fruit was correlated with seed size and that seed size may also impact the early stages of development, including the seed's ability to compete during those early stages. Therefore, fruit in this study that were found to be significantly smaller in size may have contained seeds with a lesser competitive ability. Other literature indicates that, at least for some terrestrial species, seed mass and emergence are at least partially regulated by the influence of environmental factors on the parent plant (Stanton 1984).

Decreasing the existing population may be insufficient as a management measure, in light of a seed bank. Water chestnut fruit have been known to form a considerable seed bank (Methé et al 1993) with some reports of seeds remaining viable in the sediments to 10 yr (Kisbaugh 2009). Water chestnut is capable of increasing the number of ramets under low density conditions, thereby quickly achieving higher densities (Kurihara and Kusima 1991, Groth et al. 1996) and greater total fruit production per plant (Groth et al. 1996). A strategy that could provide a reduction of the seed bank, at least along the shoreline, through the use of partial lake drawdown was therefore worthy of investigation. Water chestnut fruit frozen in water

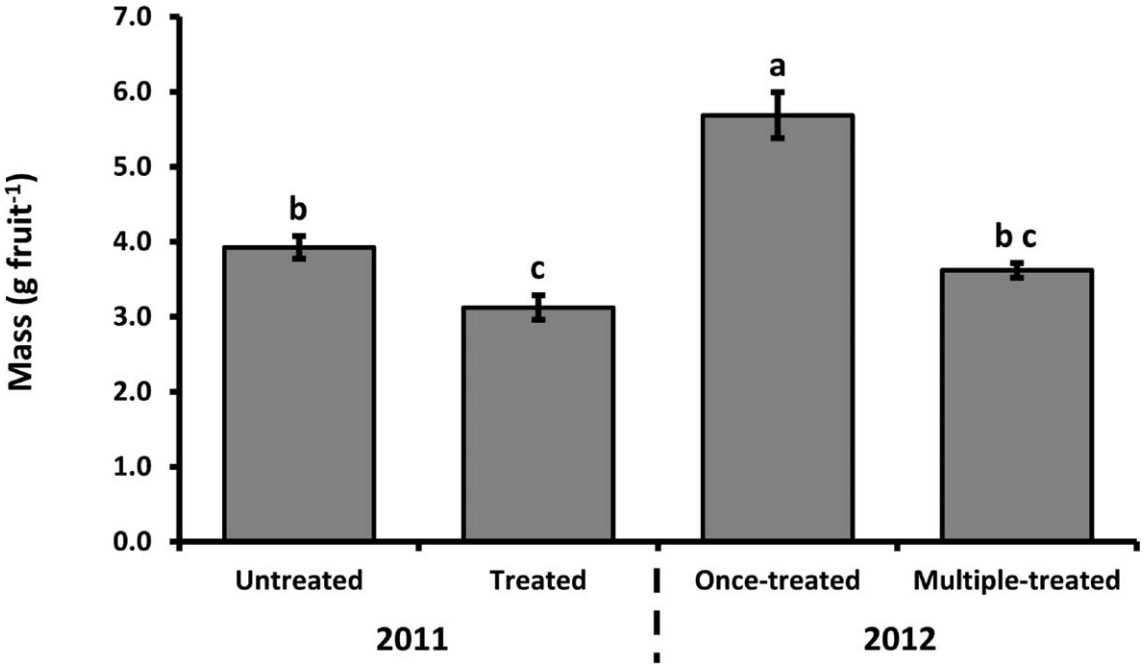

Figure 3. Mass of *Trapa natans* fruit in relation to seed source (untreated areas of the lake or herbicide treated areas in 2011, and areas once treated with herbicide or multiple-treated areas in 2012). Means with the same letter are not statistically different by Tukey-adjusted comparisons ($\alpha = 0.05$). Error bars indicate standard error of the mean.

for 35 d had a zero germination rate, but this may not mimic drawdown conditions. These studies provide data indicating drawdowns may be a viable management method in sufficiently cold conditions.

The results of these studies indicate that herbicide application may have a significant impact on the surviving plants' ability to produce viable fruit. The seeds may either be nonviable or of smaller mass and lack vigor. Although as noted by Poovey and Getsinger (2007), herbicide applications may not provide complete control of water chestnut, this study indicates that herbicide applications may provide additional control in subsequent years through impacts on seed germination.

SOURCES OF MATERIALS

[1]Petite Ponar model SKU 1728-G40, Wildlife Supply, 8645 Gene Lassere Blvd. Yulee, FL. 32097

[2]One Wire Viewer: Maxim Integrated 160 Rio Robles, San Jose 95134

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