

Can tropical macrophytes establish in the Laurentian Great Lakes?

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Abstract Tropical macrophytes sold in the live garden trade are perceived as unlikely to invade temperate regions owing to climate mismatches. Here we study two tropical macrophytes (*Pistia stratiotes* and *Eichhornia crassipes*) not previously considered an invasion risk but which were recently discovered in the Great Lakes, and determine mechanisms that may be responsible for their continued presence including human introduction, reproduction through viable seeds and tolerance of winter conditions. Surveys conducted in 2011 and 2012 revealed recurrent presence of one or both species at some sites. Macrophytes in in situ enclosures failed to survive winter conditions, with plant health declining progressively prior to mortality. Water hyacinth seeds were field-collected, identified using Sanger sequencing, and germinated at 28°C with or without scarification. Germination was highest for scarified versus non-scarified seeds. Human introduction was observed at two sites, one involving both species, the other only

water hyacinth. These species likely persist through a combination of annual reintroduction (both species) and possibly by production of viable seed (water hyacinth). Macrophytes, particularly water hyacinth, that were not previously viewed as a threat to the Great Lakes owing to environmental incompatibility may need to be reassessed.

Keywords Invasive species · Non-indigenous species · Water hyacinth · Water lettuce

Introduction

Climate change is generally perceived as a principal factor affecting ecological communities and the species in them (Hellmann et al., 2008; Thomas, 2010; Burrows et al., 2014). In the northern hemisphere, temperate lakes freeze later in autumn and thaw earlier in spring than they did historically, corresponding with warmer air temperatures (Magnuson et al., 2000). Some native species respond to climate change by shifting their range (e.g. Chen et al., 2011). For example, the northern range boundary of warm- and coolwater sportfishes has shifted northwards 12.9–17.5 km decade⁻¹ coincident with lake warming (Alofs et al., 2014).

Climate change may interact with other ecosystem stressors, including spread of non-native species (Fleming & Dribble, 2015; Havel et al., 2015). Hellmann et al. (2008) identified consequences of

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climate change with respect to non-native species, including changes in transportation and introduction methods, establishment of new invaders, and distributions of current invaders. Available evidence supports these predictions. For example, Thomas (2010) found that 84% of all alien invasive species, i.e. non-native species capable of prolific population spread, tested in the study had expanded their range towards the poles as temperatures increased. Distributional changes may occur either directly or indirectly in response to climate change. Non-native species may benefit directly from increasing carbon dioxide levels or temperature, and indirectly from food web changes (Sorte et al., 2013). Some species adapt readily to changing climate, others less so (Chen et al., 2011; Lawler et al., 2013).

Although many species are introduced to new environments, only a fraction will establish a self-sustaining population. Successful invasion requires that a non-native species interface with a transport pathway and survive and reproduce once introduced to the novel environment (see Blackburn et al., 2011). Pauchard & Alaback (2004) found that the presence of road corridors, along with lower elevations and higher land use promoted alien invasive plant species richness. Alofs & Jackson (2015) observed that abiotic factors, notably growing degree days and lake surface area, were the best predictors of regional spread of three predatory fish species, although biological factors including historical presence of other predatory fishes affected lake-to-lake variation. Aquatic plant distributions are likewise affected by myriad factors including light levels, CO₂ concentrations, substrate type and temperature, among other factors (e.g. Lacoul & Freedman, 2006; Eller et al., 2015).

While contaminated ballast water has been the predominant pathway for introduction of non-native species to the Laurentian Great Lakes during the latter half of the 20th century, no new introductions have been attributed to it since 2006 (Bailey et al., 2011). Other pathways that were once considered relatively minor, including the live garden, bait fish and aquarium trades, may be expected to rise in relative importance (e.g. Drake and Mandrak, 2014). Many non-native species are sold in the live garden and aquarium trades in southern Ontario and in Quebec adjacent to the Great Lakes—Saint Lawrence River (Rixon et al., 2005; Cohen et al., 2007). For example, there are at least 42 stores that sell a total of 60

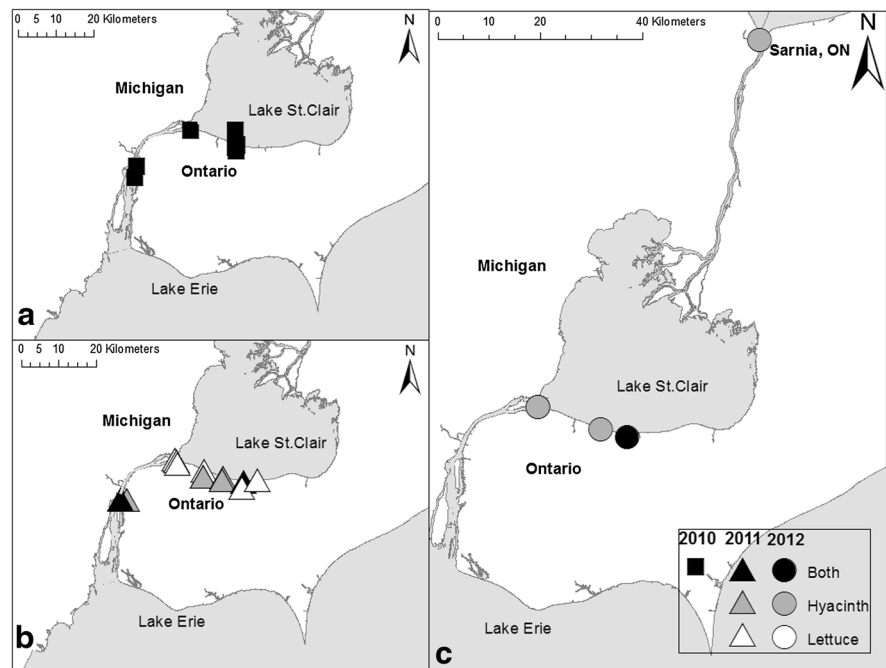
macrophyte species and 308 freshwater animal species in southern Ontario adjacent to Lake St. Clair to the north and Lake Erie to the south (Rixon et al., 2005). In southern Ontario for instance, the Brazilian macrophytes amazon sword (*Echinodorus amazonicus* Rataj) and anacharis (*Egeria densa* (Planch.)) are sold locally but considered invasive elsewhere (e.g. Lehtonen, 2009). Europe is also heavily infested with alien macrophytes, with 96 species recorded across the continent (Hussner, 2012).

Some imported macrophyte species are known to cause harm domestically or globally. For example, water soldier (*Stratiotes aloides* L.) is legally sold in Ontario even though an invasive population occurs in the Trent Severn waterway in the central part of the province and despite intensive eradication efforts to eliminate it (Gagnon, 2011).

Two non-native macrophytes—water lettuce *Pistia stratiotes* L. and water hyacinth *Eichhornia crassipes* (Mart.) Solms—were recently discovered in tributaries flowing into Lake St. Clair (Adebayo et al., 2011; Fig. 1). Both species are invasive in tropical and sub-tropical countries worldwide (e.g. Zhang et al., 2010; Thomaz et al., 2015; Brundu, 2015). These species may impede recreational and commercial boat traffic by forming large single- or mixed-species mats that can span an entire lake and strongly alter chemical conditions and biological communities in invaded lakes (Patel, 2012; Brundu, 2015). Both species are native to South America and were not previously perceived as a threat to Canadian aquatic ecosystems (Rixon et al., 2005) owing to their intolerance of cold temperature (Ramey, 2001). However, these species were sold in 11 and 12%, respectively, of pet stores surveyed in Ontario (Funnell et al., 2009), raising the prospect that some purchased individuals are eventually discarded into regional waterways.

Adebayo et al. (2011) identified these two macrophyte species at four locations on the southern margin of Lake St. Clair in 2010. The macrophytes also were observed the following year in the same region, thus raising the possibility that they may be established. Water lettuce and water hyacinth reproduce mainly asexually, although sexual reproduction may occur infrequently in the latter species in temperate areas (Barrett, 1980). Seed production has not yet been explored as a potential cause for the species' apparent persistence in Lake St. Clair. In addition, the overall distribution of these species in the Great Lakes region

Fig. 1 Locations of water hyacinth and water lettuce populations in **a** 2010, **b** 2011 and **c** 2012. Data for 2010 from Adebayo et al. (2011)



remains unknown. Here we test three non-exclusive hypotheses that may account for the occurrence of these species in consecutive years the Great Lakes: (i) introduced macrophytes survive winter conditions; (ii) introduced macrophytes do not survive winter but produce viable seeds that germinate in subsequent years; or (iii) macrophytes do not survive winter or reproduce but are repeatedly introduced to the same areas by the public.

Methods

Species surveys

We surveyed for water lettuce and water hyacinth by driving a research vessel at low speed within 500 m of the Canadian shoreline of Lake St. Clair (42.304 N, 82.677 W) and Lake Erie (41.843 N, 81.153 W), plus most of Lake Ontario, east to Kingston, Ontario (43.520 N, 76.514 W), and southern Lake Huron as far north as Goderich, Ontario (43.286 N, 81.870 W) in summer 2012. All connecting rivers, tributaries, inlets and streams that were accessible by boat were traversed a minimum of 2 km upstream, or as far as possible in shallow waterways. Field surveys were conducted in 2011 and 2012 encompassing a much

larger area of southern Ontario than studied by Adebayo et al. (2011) in 2010. We identified macrophytes using key features of the species, including colour, the presence on the water surface and, in some cases for water hyacinth, the presence of light purple flowers. Water quality data were collected using a calibrated YSI 85 meter; measurements were made adjacent to the macrophytes and approximately 15–20 cm below the water surface. Water temperature ($^{\circ}\text{C}$), oxygen (% and mg/l), conductivity ($\mu\text{S cm}^{-1}$) and salinity (ppt) were recorded, along with air temperature ($^{\circ}\text{C}$), date, time, and GPS co-ordinates of the site. We also collected sediment from beneath the macrophytes in search of viable plant seeds using a 10-l bucket to skim surficial sediments on the river bed then sealing the container prior to transport.

Winter survival experiments

We tested the hypothesis that plants appear in subsequent years via overwinter survival. Water hyacinth and water lettuce were collected for survival experiments from large populations located in the Puce and Belle rivers in Ontario (42.296 N, 82.782 W; 42.290 N, 82.714 W, respectively). Macrophyte overwinter survival was assessed in enclosures deployed

between October and March at four sites near Lake St. Clair and the Detroit River in both 2011–2012 and 2012–2013. These sites were located in nearshore waters of the Puce River (42.303 N, 82.778 W) and Belle River (42.297 N, 82.710 W), a lagoon off the Detroit River (42.217 N, 83.103 W; 2011 only) and in a pond near Harrow, Ontario (42.103 N, 82.929 W). We selected these locations as they were either known to have had one or both species previously, or, in the case of the pond, because there was no risk of escape. Two enclosures were deployed at each nearshore location, and eight macrophytes (four of each species) were added in autumn. We built enclosures by four 240-cm-long metal *t*-bars with pre-drilled holes, which were inserted a minimum of 60 cm into the nearshore sediment, forming a rectangle of $\sim 90 \times 120$ cm. We lined the bottom and ~ 15 cm of adjacent vertical surfaces of each enclosure with landscape fabric, to prevent the escape of any debris, and fastened the fabric to the bars with zip ties. Plastic fencing with a mesh width of ~ 1 cm² was wrapped around the outside of the bars and secured with zip ties to both the fabric lining and the metal bars. One bar was marked for identification using coloured electrical tape before the enclosure was wrapped with florescent orange snow fence for better visibility.

Plant condition was assessed using a Condition Index developed for this study (see Appendix—Supplementary Material). This index was based on morphological appearance, including colour and rigidity of the leaves and stems, and whether wilting tissues were present. Plant scores ranged from 0 (dead) to 5 (healthy plants). Healthy plants had vibrant green leaves and stems and no sign of wilting or discolouration; plants with a score of 4 were green with rigid leaves but with some chlorosis present; plants with a rating of 3 had rigid leaves and extensive chlorosis but no dark pigmentation; plants with a score of 2 had extensive chlorosis with dark and wilting leaves, while those with a score of 1 were alive but exhibited extreme wilting and very discoloured, brown leaves. Plants with a score of 0 were dead and disintegrated (see Appendix—Supplementary Material). Macrophytes were transported in covered plastic buckets to a laboratory where they were weighed (g), measured (cm) and condition scored prior to being tagged and released into enclosures. Only healthy or very healthy plants were used for overwinter experiments.

We visited each enclosure weekly and assessed the condition (colour and structure) and survival of individual macrophytes, and water quality. Each plant was also measured weekly for number of leaves, root growth (e.g. the presence of white, fresh roots) and the presence of flowers, while fresh weight and length from leaf tip to root tip were measured biweekly. Photographs were taken weekly of each plant to visually document changes in condition over the study interval. We collected sediment from three of the four sites at the end of winter (2011–2012) to check for the presence of seeds that might have been produced by the plants. Sediment (250–500 ml) collected during distribution surveys and winter survival experiments was wet sieved using stacked US Standard Test sieves (2, 1 and 0.5 mm), on a CSC Scientific Sieve Shaker (No. 18480, on setting 0). Sediment from the 1 mm and 0.5 mm sieves was weighed, washed into separate containers, labelled and placed in a refrigerator (~ 3 – 4°C) until processed for seeds. Seeds were recovered from sediment fractions using a dissecting microscope (7–8 \times magnification). Recovered seeds were placed in covered petri dishes (100 mm dia. \times 15 mm) with tap water and stored out of direct sunlight until they could be identified. For quality control, random samples of sediment were rechecked for the presence of any overlooked seeds.

Plant germination experiments

We tested the hypothesis that plants recur in consecutive years via the production of viable seeds. In 2012, we found a local population of water hyacinth during the species survey and monitored it weekly for growth and reproduction. Seed pods were collected from this population and dissected, and mature seeds (i.e. brown in colour) were extracted and either dried or kept moist. These seeds were then either lightly scarified (using 100 and 120 grit sand paper) or not, as previous research indicated that the procedure decreases average time to germination (e.g. Brochet et al., 2010). Four germination treatments were established based on dry/moist seeds and whether seeds had been scarified or not. Moist seeds were kept in tap water and refrigerated at 3– 4°C to ensure they never dried. Three experimental seeds were then placed into standard 100-mm-diameter petri dishes with 15 ml of 0.05 mg l⁻¹ of NaH₂PO₄ growth medium to provide necessary nutrients. In total, we ran ten replicate petri

dishes per treatment. Petri plates were randomly placed into a light and temperature controlled environmental chamber with a cycle of 13 h of light, at 28°C, followed by 11 h of dark at 10°C. The 28°C represented the approximate summer maximum temperature in very shallow waters where macrophytes were found. Light level was reduced immediately prior to (07:00–07:30) and following (20:00–20:30) the 13-h high light period to simulate dawn and dusk. We checked for signs of germination every 07:00 and 19:00 h. 5 ml of solution was added to each plate every 5 days until the completion of the experiment (21 days) to offset evaporation losses, which occurred even though petri plates were covered. Petri plates were photographed during every visit while assessing macrophyte seed germination. We only used seeds collected directly from a large population to ensure experiments were conducted on water hyacinth alone.

Following the germination study, we photographed and labelled each seed prior to molecular characterization of germinated and sequenced plants. Our molecular methods confirmed water hyacinth had indeed produced seeds, which we had collected from sediment and seed pods. DNA was extracted from seeds from the germination experiments, 2011 survival experiment, and from the species surveys using the PowerPlant[®] Pro DNA Isolation Kit from MO BIO Laboratories Inc. The extract was then PCR-amplified using methods of Adebayo et al. (2011). The chloroplast fragment 5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) was used for molecular identification. The primer pair *rbcL*-1F (ATGTCACCA-CAAACAGAAAC) and *rbcL*-724R (CATGTACCTGCAGTAGC) was used to amplify *rbcL* genes. PCRs were performed in 25 µl reaction volume containing ~50 ng of extracted DNA, 0.5 U of Taq polymerase, 1× PCR buffer, 2 mM of Mg²⁺, 0.2 µM of dNTPs and 0.4 µM of each primer (Adebayo et al., 2011). PCR amplification was conducted with an initial denaturing step at 95°C for 5 min, followed by 35 amplification cycles: 95°C for 30 s, 50°C for 30 s, 72°C for 60 s, and a final elongation step at 72°C for 5 min (Adebayo et al., 2011). Samples were then cleaned using the UltraClean[®] PCR clean-up kit from MO BIO Laboratories Inc. and sent to Genome Quebec at McGill University in Montreal, Quebec for Sanger sequencing. Sequences were checked for quality using BioEdit software and those deemed to be of good quality were run through BLAST to determine

seeds' identity. Sequences were also aligned using Codon Code Aligner 2.0 and compared to those reported for other local macrophytes (Adebayo et al., 2011) to determine the number of different haplotypes present in the local populations.

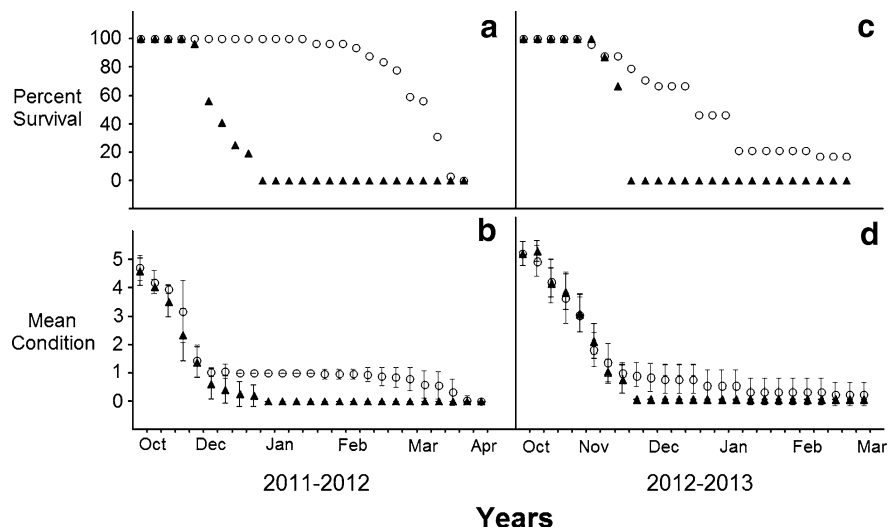
Statistical analyses were performed using IBM SPSS Statistics 20 software. A mixed-model repeated-measures ANCOVA was conducted to identify factors affecting percent survival of macrophytes. Factors tested include species (discrete variable), year (discrete variable), week (repeated measures) and air temperature (continuous variable). The same approach was also performed on data from germination trials, with scarification and drying as discrete variables and time as a covariate.

Results

In 2010, only the southern margin of Lake St. Clair shoreline was surveyed, and both water hyacinth and water lettuce were observed (Fig. 1a). During 2010, the species were found coexisting in three main locations: near a local marina and Turkey Creek in LaSalle, Little River in Windsor, and in Puce River in Lakeshore. We observed one or both species at six locations during our 2011 survey (Fig. 1b). Three of these previously identified sites were identical to those with positive identifications the previous year: Turkey Creek, Little River and Puce River, while new sightings were made at Pike Creek in Tecumseh, as well as Duck Creek and Belle River in Lakeshore. The number of occurrences decreased to three in 2012 (Fig. 1c), all previously identified colonized sites (i.e. Little River, Puce River and Belle River). We also detected a small population of water hyacinth located in the Saint Clair River, near Sarnia, Ontario at the outflow of Lake Huron. This population occurred within a floating boom, suggesting human culturing (see Appendix—Supplementary Material).

We observed a sharp decrease in percent survival of water lettuce at the beginning of the study (Fig. 2a). Water hyacinth experienced significantly higher survival ($P < 0.0001$) throughout most of the study period, although it, too, died by the end of March 2012. Decline in condition index preceded death of the plants. Both species experienced a sharp decrease in mean condition at the beginning of the study, with water lettuce reaching a score of zero in January 2012,

Fig. 2 Performance of water lettuce and water hyacinth in enclosure experiments: **a** percent survival in 2011–2012; **b** mean (\pm standard deviation) condition of macrophytes in 2011–2012; **c** percent survival in 2012–2013 and **d** mean condition in 2012–2013. *White circles* represent means for water hyacinth specimens, *while dark triangles* represent means for water lettuce



while water hyacinth performed slightly better, reaching zero at the end of March (Fig. 2b). Winter 2012–2013 was harsher than the preceding one (Table 1). Yet, survival and condition index results were largely similar to those of the previous winter. Survival of water lettuce declined rapidly at the beginning of the study period and reached 0% by late November 2013 (Fig. 2c). As in 2011–2012, water hyacinth experienced higher survival throughout most of the study but eventually reached complete mortality by mid-February 2013. Condition of both species declined earlier than in 2011–2012: water lettuce and water hyacinth reached a condition score of zero by December and March, respectively (Fig. 2d). Results of a mixed-model repeated-measures ANCOVA

indicated all factors (species, year, week and air temperature) had significant effects on survival of macrophytes ($P < 0.0001$; $F = 285.5, 61.5, 738.4, 159.5$, respectively) (Table 2).

Germination was high particularly for scarified treatments, where 67 and 53%, respectively, of moist and dry seeds germinated (Fig. 3). Treatments involving non-scarified seeds had no germination. Greater than 99% of all seeds that germinated did so within the first seven days of the experiment. Water hyacinth seed germination success was significantly affected by scarification and by time (ANCOVA, $p < 0.01$; $F = 17.7, F = 45.7$, respectively; Table 2). The effect of seed drying was not significant ($P = 0.306$; $F = 1.3$) (Table 2).

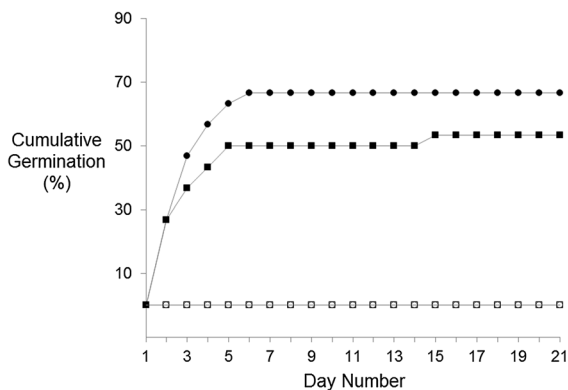
Table 1 Mean seasonal water quality measures in enclosures during the 2011–2012 and 2012–2013 winter exposure experiments

Year	Date	Air temp. (°C)	Water temperature (°C)	Oxygen (%)	Conductivity ($\mu\text{S cm}^{-1}$)	NaCl (ppt)	Depth (cm)
2011–2012	10/28/11	9.3	9.7	60	367	0.24	32
	11/03/11–12/16/11	5.8	6.5	55	306	0.23	38
	12/22/11–01/25/12	1.8	2.1	64	301	0.26	54
	02/02/12–03/09/12	3.1	3.5	64	436	0.37	35
	03/14/12–04/11/12	11.0	11.7	73	486	0.32	36
2012–2013	10/11/12	12.3	NA	NA	NA	NA	0
	10/18/12–12/07/12	9.2	9.5	54	912	0.66	9
	12/13/12–01/24/13	−1.0	2.8	54	606	0.49	11
	02/01/13–03/13/13	−2.3	4.2	85	398	0.32	16
	03/20/13–03/27/13	2.3	4.6	47	559	0.48	20

Table 2 Effects on survival and germination by factors including species (water hyacinth or water lettuce), year (2011–2012, 2012–2013), duration of exposure (1–25 weeks),

temperature, Time (1–21 days), Drying (moist or dry) and scarification (scarified or non-scarified)

Dependent variable	Independent variable	Degrees of freedom	F	P<
Survival	Species	56.750	285.5	0.0001
	Year	2698.7	61.40	0.0001
	Week	2703.1	738.4	0.0001
	Temperature	2703.1	159.9	0.0001
Germination	Time	1.0000	45.69	0.0001
	Drying	1.0000	1.301	0.3060
	Scarification	1.0000	17.69	0.0080

**Fig. 3** Germination results for water hyacinth seeds in four treatment groups, scarified moist (*shaded circle*), scarified dry (*shaded square*), non-scarified moist (*open circle*) and non-scarified dry (*open square*) at 28°C

Over 250 seeds were collected and assayed using Sanger sequencing for species identification; of these, only 13 (5%) were confirmed as water hyacinth after BLASTN query against GenBank. No water lettuce seeds were found. Most seeds provided poor-quality sequences and were not identified (56%), while another 7% were not matched at all. Many sequences belonged to other non-native species, including Eurasian flowering rush (*Butomus umbellatus* L., 20%), European stinging nettle (*Urtica dioica* L., 2%), Mediterranean rose loosestrife (*Lythrum junceum* Banks & Sol., <1%) and Korean pentactina (*Pentactina rupicola* Nakai, <1%). Approximately 9% of sequences were either of unknown origin or were native species. Water hyacinth seeds assayed originated from three locations: the pond used in the 2011 survival experiment (0.4%); Pike Creek from the 2011 survey (0.4%) and a population in Belle River from the 2012 species survey (4.3%). All water hyacinth

sequences obtained from this study belonged to a single haplotype.

Discussion

Our study suggests that water lettuce and possibly water hyacinth are dependent on reintroduction for their continued persistence in the Great Lakes. Rixon et al. (2005) predicted that neither species would be a threat to the Great Lakes based on their environmental requirements. Despite this, both species have recurred in three consecutive years in the same coastal waters of Lake St. Clair. The most plausible explanation for this pattern for water lettuce is annual reintroduction by homeowners living on connected waterways, as we obtained no evidence of seed production or survival in either mild or severe winters with experimentally exposed macrophytes (Fig. 2). The situation is more complex for water hyacinth, as we observed production of seeds in nature that experiments revealed were capable of germination if scarified and incubated at relatively high temperature. However, like water lettuce, no water hyacinth survived winter exposure. We also observed water lettuce and water hyacinth in coastal waters around Lake St. Clair that homeowners had admitted to introducing.

Global distributions for permanent water lettuce and water hyacinth populations appear to be limited to locations south of 60° latitude in the northern hemisphere (Téllez et al., 2008). The most northern populations have been observed in Stockholm, Sweden and in Montreal, Canada (Verlaque, 2013). Both of these locations had live individuals, though the populations were not recorded as established. Most countries—including many throughout Africa, the

USA and Australia—that support introduced populations of one or both species experience tropical or subtropical temperatures (e.g. Pérez et al., 2011).

Even if water hyacinth persistence in the Great Lakes is not dependent on seed production, these seeds could nevertheless provide an essential supplement to plants reintroduced annually and thereby contribute to persistence of the species. Some populations in southern Ontario were observed during July, when water temperature had not yet reached the minimum required for germination (20°C; Ramey, 2001), suggesting that the plants were introduced. However, populations observed in September could be a combination of these original plants augmented by seeds that germinated when water temperature increased sufficiently (Bolsenga & Herdendorf, 1993). With up to 67% of seeds we collected seemingly viable (Fig. 3), it is possible that water hyacinth can build a seed bank in areas where it occurs. Water temperature reached 19.3°C in autumn 2012–2013 at the site of enclosure experiments, which is very close to the minimum germination temperature of both species studied.

While water hyacinth produces seed banks in other invaded countries (Pérez et al., 2011), seed production appears to be extremely limited in temperate areas (Barrett, 1980). Water hyacinth seeds can remain dormant and viable for up to 20 years (Ramey, 2001), rendering eradication difficult once seed production has begun. Since our study demonstrated that the species is capable of producing viable seeds, it is theoretically possible—if unlikely—that these populations could behave like annual terrestrial plants and die off each winter, only to recover the following year from a seed bank. However, if water hyacinth is dependent on annual introduction, the problem persists only as long as human-mediated introduction occurs, and even then only for warmer months of the year. Interestingly, all of the water hyacinth examined genetically from the Great Lakes belong to the same clone reported by Adebayo et al. (2011), suggesting that there was either only one introduction of the plant or that environmental conditions select for a single clone. Zhang et al. (2010) also found very low genetic diversity in populations of water hyacinth introduced to Asia, Africa, Europe, North America, and Central America and the Caribbean.

We found no evidence that either species can survive winter conditions in southern Ontario, even

though weather conditions were very mild during the first winter of our in situ survival experiments (Fig. 2a, b). This result is consistent with known environmental requirements for growth and reproduction of both water lettuce and water hyacinth (Ramey, 2001). This may change in the future if global climate change renders the region more suitable for both species (You et al., 2013). Water hyacinth is the most likely to survive winters in this area in the future, as it has a lower minimum temperature relative to water lettuce, 12 versus 15°C, respectively (Ramey, 2001; Bradley et al., 2010). Winter temperature has already risen in Toronto, Ontario from an average daily low air temperature of −7°C in February 1990 to −5.5°C in February 2013 (Environment Canada, 2013). It is important to note that we remain far from a suitable temperature required for survival of either species, though evolution of some degree of cold tolerance should not be discounted (Chown et al., 2015). However, the absence of genetic variability in surveyed individuals indicates that evolution may require introduction of different genotypes from source populations.

Many municipalities in the USA south of the Great Lakes are already affected by infestations of water lettuce and/or water hyacinth in local waterways (Ramey, 2001). Water hyacinth, in particular, is also an invasion threat in Europe, especially Spain and Portugal (Téllez et al., 2008). Both species are globally invasive and can pose a threat to sub-tropical and possibly some temperate locations. Countries in Africa that have not been invaded are more likely to be limited by propagule supply than by thermal constraints. Southeast Asia is also vulnerable to invasion by both species (Zhang et al., 2010), as environmental conditions there are very similar to those in the species' native range.

In this study, we determined the likelihood that water hyacinth and water lettuce can establish in the Great Lakes by assessing whether the species can reproduce and survive winter conditions. Our field survival experiments included both mild and very severe conditions, and thus likely encapsulate the range of conditions the species are likely to encounter. The experiments were also conducted with both species present together in all enclosures. This makes it statistically impossible to determine if the species could survive individually, as we commonly observed them coexisting, making this set-up representative of

situations common in nature (Barrett, 1989; Adebayo et al., 2011).

Although the Great Lakes region has experienced increasingly warmer winters over the past 15 decades (Magnuson et al., 2000), conditions are not suitable for winter survival of either species. In addition, we found no viable water lettuce seeds, although they are produced by water hyacinth in the Great Lakes. Additional study is required to demonstrate whether extant seed banks are responsible for subsequent appearance of water hyacinth in the region. Personal communication with local residents suggests that live individuals of both species are periodically introduced to connecting waterways within the Great Lakes. Residents with backyard ponds may find that prolific clonal growth by these species quickly overwhelms their water gardens, and some may then make the unwise decision to release macrophytes into surrounding tributaries or directly into the lakes. Similar problems have been recorded for bait fishes that were no longer wanted by their owners. For example, the round goby (*Neogobius melanostomus*) (Pallas) may have been intentionally released into Lake Huron tributaries by anglers (Dillon & Stepien, 2001). Education and perhaps regulation may reduce the occurrence of such incidents.

In conclusion, the main reason for the reoccurrence of water lettuce in consecutive years on Lake St. Clair appears to be annual release by local residents. Water hyacinth was also introduced by residents, though the possibility exists that these individuals may be supplemented by germination of seeds produced via sexual reproduction. Neither species is capable of surviving winters in the Lake St. Clair region at present. Confirmation that human introduction is involved in persistence of these species in the region will require surveys of individuals to determine the fate of plants purchased for ornamental ponds, and demonstration that both species lack seed banks in lake or river sediments. We believe it prudent for environmental managers to assess the complement of macrophyte species sold in their jurisdictions, and conduct risk assessments to determine whether they pose a current or future invasion risk if released into the wild.

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