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Short Communication

Water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) in the Great Lakes: playing with fire?

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Abstract

The Laurentian Great Lakes have been successfully invaded by at least 182 nonindigenous species. Here we report on two new species, water hyacinth *Eichhornia crassipes* and water lettuce *Pistia stratiotes*, that were found at a number of locations in Lake St. Clair and Detroit River during autumn 2010. Both species are commonly sold in the water garden and aquarium trade in southern Ontario and elsewhere. While it is not clear whether these species are established or can establish in the Great Lakes, the historic assumption that neither of these subtropical to tropical plants pose an invasion risk must be questioned in the light of changing environmental conditions associated with climate warming that may render Great Lakes' habitats more suitable for these species and increase the likelihood of their successful establishment.

Key words: nonindigenous, alien, macrophyte, *Eichhornia crassipes*, *Pistia stratiotes*

Introduction

The Laurentian Great Lakes have a long legacy of species introductions. The Great Lakes Aquatic Nonindigenous Species Information System currently lists 182 nonindigenous species (NIS) as established in the Great Lakes (NOAA 2010). Many of the more problematic NIS in the system are invertebrates or fishes, although 55 introduced, wetland or aquatic plant species are currently established in the basin (NOAA 2010). The predominant vector of introduction of NIS to the Great Lakes over the past 60 years has been the discharge of contaminated ballast water, which accounts for at least 55% of established NIS, most of which have been introduced from European sources (e.g. Kelly et al. 2009). Historically, a number of other possible vectors, including connecting channels and the aquarium, human food, and live garden trades, appeared much less important. However, connecting channels have attracted significant attention recently, as bighead *Hypophthalmichthys nobilis* (Richardson, 1845) and silver carp *H. molitrix* (Valenciennes, 1844) are poised to enter the Great Lakes *via* Chicago-area canals that link the Mississippi River and Lake Michigan (see Cooke and Hill 2010). In addition, a variety of fishes and molluscs are sold commercially in the aquarium and water garden trades that potentially could survive if released into the Great Lakes (Rixon et al. 2005; Gertzen et al. 2008).

Aquarium and water garden (i.e. pond) shops in the lower Great Lakes region also sell at least 19 species of macrophytes, including a number of species considered to be highly problematic in some areas where they have been introduced (Rixon et al. 2005). These taxa include *Ceratophyllum demersum* L. 1753, *Egeria densa* Planch. 1857, *Myriophyllum aquaticum* (Vell.) Verdc., *Cabomba caroliniana* Gray 1837, *Pistia stratiotes* L. 1753 and *Eichhornia crassipes* (Mart.) Solms 1883. Rixon et al. (2005) indicated that the former four plants could overwinter in the Great Lakes, and, indeed, *C. demersum* is native to the system. The same study suggested that water hyacinth and water lettuce could not survive Great Lakes' winters.

Commercial sale of invasive NIS may portend subsequent release and establishment in the wild, as Duggan et al. (2006) observed a positive correlation between popularity (i.e. frequency) of fishes sold in aquarium stores and their establishment in the wild. Only one of the aforementioned plants, water lettuce, has been reported in the Great Lakes proper, at a single location in Metzer Marsh in western Lake Erie in 2000, although the species did not persist at that site (USGS 2010; D. Wilcox, pers. comm.). Water lettuce is possibly a South American native (Cordo et al. 1981; USGS 2010) that occurs on all continents except Antarctica (Holm et al. 1977; Dray and Center 2002). The species has been recorded in waterways adjacent to the Laurentian Great Lakes including Bull Creek, adjacent to the Erie Canal, New York, and in the Rideau River, Ontario.

Water hyacinth is a South American native that has attained a very broad global distribution in tropical and semi-tropical countries. Its established distribution in North America is limited mainly to the southeastern United States and California, although non-permanent populations occur farther north in Illinois, Wisconsin, New York and Pennsylvania (USGS 2010).

Introduced aquatic plants can cause myriad changes in invaded ecosystems, including reduced water flow and a dramatic increase light attenuation, with consequent effects on primary and secondary production (e.g. Carpenter and Lodge 1986). In addition, mass accumulations of aquatic plants may strongly interfere with recreational and commercial vessel navigation, fisheries, and human health (e.g. Opande et al. 2004; Hershner and Havens 2008; Villamagna and Murphy 2010).

In this report, we describe the presence of water lettuce and water hyacinth in the lower Great Lakes.

Methods

Following a report from a citizen, Tim Duckett, regarding the suspected presence of water lettuce and water hyacinth in a river flowing into Lake St. Clair, we conducted surveys on 28-29 October 2010 to visually determine occurrence of both species at seven sites and 11 locations in total in major rivers and creeks adjacent to Windsor, Ontario (Table 1; Figure 1). We examined waterway margins on foot, while littoral zones were surveyed by a boat capable of manoeuvring in shallow waters. Each location was surveyed for ca. 2 hours, with longitude, latitude and water temperature recorded. Mature plants were collected in plastic containers, while young leaves were preserved in 95% ethanol and subsequently used for barcoding and species identity confirmation. We also collected 5L of water at each site where these macrophytes occurred, and buckets of surface sediment from Puce River and Turkey Creek to screen for seeds. In the laboratory, sediment was passed through a 0.7 mm sieve; matter retained on the sieve was hand-processed for seeds, which were examined under a microscope at 16x magnification. Water hyacinth reproduces largely via clonal growth, though sexual reproduction also may occur – with consequent production of seeds – albeit at reduced frequency in introduced, temperate populations (Barrett 1980; Zhang et al. 2010).

Images of plants were sent to Ted Center, U.S. Department of Agriculture, for confirmation of identification. In addition, three to four individuals of each species from each location of occurrence were utilized for DNA barcoding. However, many reactions failed owing to poor DNA isolation. Water hyacinth was more difficult to process than water lettuce owing to its rigid cell wall structures. Both plant species were barcoded for molecular identification using two chloroplast gene fragments, RNA polymerase C (rpoC1) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) (Newmaster et al. 2006; Kress and Erickson 2007).

Total genomic DNA was extracted from young leaves according to the proteinase K method (Waters et al. 2000). The primer pairs, rpoC1-2F (GGCAAAGAGGGAAGATTTCG) and rpoC1-4R (CCATAAGCATATCTTGAG TTGG) (Sass et al. 2007), and rbcL-1F (ATGTCACCACAAACAGAAAC) and rbcL-724R (CATGTACCTGCAGTAGC) (Asmussen and Chase 2001) were used to amplify rpoC1 and rbcL genes, respectively. PCR amplifications were performed in a 25 µL reaction volume containing ~50 ng of genomic DNA, 0.5 U of *Taq* polymerase, 1 x PCR buffer, 2 mM of Mg^{2+}, 0.2 µM of dNTPs, and 0.4 µM of each primer. PCR 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. All PCR products were verified on 1% agarose gel and subsequently purified using Agencourt® CleanSEQ protocol

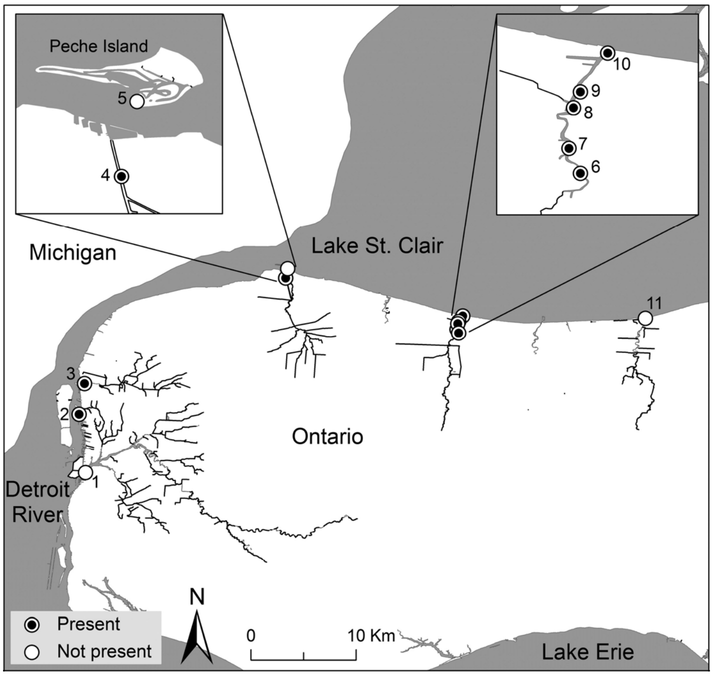

Figure 1. Sampling locations for water hyacinth and water lettuce in Lake Saint Clair and Detroit River in October 2010. Sites where both species were present (filled symbols or absent (open symbols) are indicated.

Table 1. Sampling sites in Ontario where surveys for water hyacinth and water lettuce were conducted on Lake St. Clair and the Detroit River. Site number refers to locations in Figure 1. Both species were either present (P) or absent (A) at each surveyed location. Sites are ordered by presence/absence.

Location	Site	Coordinates	Present
Puce River	6	N 42°18'10" W 82°46'41"	P
	7	N 42°17'53" W 82°46'53"	P
	8	N 42°17'28" W 82°46'58"	P
	9	N 42°17'46" W 82°46'56"	P
	10	N 42°17'17" W 82°46'53"	P
Little River	4	N 42°20'07" W 82°55'46"	P
Turkey Creek	3	N 42°14'41" W 83°06'06"	P
Island View Marina	2	N 42°13'07" W 83°06'23"	P
Peche Island	5	N 42°20'36" W 82°55'40"	A
Ruscom River	11	N 42°18'03" W 82°37'18"	A
Canard River	1	N 42°10'07" W 83°06'03"	A

(Agencourt). Sequencing was performed on purified PCR products using the forward primers for each gene, BigDye Terminator 3.1 sequencing chemistry and an ABI 3130XL automated sequencer. Sequences were annotated using the BLASTN algorithm on NCBI website *http://blast.ncbi.nlm.nih.gov/Blast.cgi*. All sequences were aligned using BioEdit software. Polymorphisms at different locations were assessed using DnaSP software. rbcL and rpoC sequences for both species were deposited in the GenBank barcoding databases (accession numbers HQ702899 - HQ702907 for rbcL gene and HQ702908 - HQ702915 for rpoC1 gene).

Results and discussion

Air and water temperatures on collection dates ranged between 8-10°C and 10-11°C, respectively. Water lettuce (Figure 2A) and water hyacinth (Figure 2B) were found at four of the seven surveyed sites in rivers connected to Lake St. Clair and in the Detroit River, and the species always co-occurred (Table 1; Figure 1). These species have been observed in the Puce River for the last two and three years, respectively (Tim Duckett, pers. comm.). Previous studies also have reported co-occurrence of these taxa (e.g. Agami and Reddy 1990). Species identification was confirmed by Ted Center using photographic images and by molecular analysis. In total, eight water lettuce individuals sampled across all seven locations in four rivers, and one water hyacinth individual collected from Puce River, were successfully sequenced for both markers. The BLASTN searches using the rbcL gene confirmed identities of both species with 100% certainty. We observed only one haplotype for water lettuce across all locations in all rivers, suggesting that they may be derived from a single stock. Low observed genetic diversity is consistent with observations for a number of other introduced, nonindigenous plants (see Zhang et al. 2010).

Recovered plants appeared as moderately to very healthy, with all water hyacinth and most water lettuce exhibiting no signs of dieback (ie. chlorosis or wilt; Figure 2). Plant abundance was lower (<10 plants) at Turkey Creek, Harbour View Marina, and Little River than at other sites where the species were found (Figure 1). Both species were also found stranded on the beach in Lake St. Clair, adjacent to the Puce River, and water lettuce was observed floating into the lake from the river mouth.

A central question with the discovery of these plants in or adjacent to the Great Lakes is whether they can establish, or are established, in the basin. Observations of both species in the Puce River over multiple years would seemingly require production and subsequent germination of viable seeds, survival by some colonies during winter freezing followed by clonal growth, or repeated stocking events in different years, possibly at multiple sites. Recolonization of water hyacinth *via* seeds seems unlikely given the absence of genetic diversity in introduced populations, and low seed production in temperate areas (Barrett 1980; Spencer Barrett, pers. comm.). In addition, we recovered no seeds of either species from sediment collected from areas with extant populations of these species, although lack of comprehensive sampling precludes a conclusion that they are not produced as only ca. 3.8kg of sediment was collected, sieved and examined.

Figure 2. Images of water lettuce (Figure 1A) and water hyacinth (Figure 1B) collected from the Puce River, Ontario adjacent to Lake Saint Clair.

Presence of a refugium from freezing conditions during winter also seems unlikely in the areas surveyed as there are no major thermal effluent inputs in the area where plants were recovered. Water lettuce is highly vulnerable to low temperatures, with populations in south Florida experiencing high winter mortality when temperature approached freezing (Dewald and Lounibos 1990). Populations in a thermally enhanced stream in Slovenia experienced loss of leaves and decline in rosette size during winter, although plants were never exposed to freezing conditions (Šajna et al. 2007). However, the species has been recorded in a number of locations in Europe that experience freezing conditions (see Šajna et al. 2007). Water hyacinth range (Owens and Madsen 1995, and references therein) and growth (Center and Spencer 1981; Rodríguez-Gallego 2004; Wilson et al. 2005) also are influenced by cold temperatures (~5 - 8.1°C). Owens and Madsen (1995) determined that regrowth following exposure to freezing temperatures was typically higher for rooted water hyacinth than for floating plants. Owens and Madsen (1995) speculated that winter mortality can influence the degree of population regrowth and infestation the following year, particularly along northern range boundaries. Thus, it seems improbable these species overwintered as adult plants in the Great Lakes.

It is possible that the plants may be introduced frequently by citizens seeking to dispose of excessive production from their personal water gardens. Water gardens are very popular in southern Ontario, and both species are sold in commercial trade (Rixon et al. 2005). However, if this vector were responsible, the observed distribution would require repetitive introductions at one and possibly multiple sites.

Even if the plants are not presently established in the basin, their repeated occurrence in surveyed waterways may pose localized navigational problems and precipitate ecological changes. It is possible, and perhaps probable, that additional introduced macrophytes may be established in the Great Lakes but hitherto have escaped formal reporting. We expect that under-reporting would be most likely for submerged species (e.g. *Hydrilla verticillata*, *Cabomba caroliniana*, *Egeria densa*), which may not be detected until their population densities impede navigation. *Cabomba* is present in the Great Lakes watershed and is dispersing slowly in Ontario (Jacobs and MacIsaac 2009).

Climate change and nonindigenous species

Species distributions may change in response to variation in key environmental drivers, notably those associated with climate warming (Baskin 1998; Parmesan 2006). The sale of potentially invasive macrophyte plants by the water garden trade in the Great Lakes basin is a risk factor for unanticipated aquatic introductions. Considering that water hyacinth and water lettuce are amongst the most commonly occurring macrophyte species sold in aquarium shops in southern Ontario (in 30% and 20% of stores, respectively), opportunities clearly exist for intentional or accidental release into the wild (Rixon et al. 2005). Lakes in the northern hemisphere have experienced later ice formation and earlier breakup in conjunction with climate warming (Magnuson et al. 2000). Winter temperature is predicted to be up to 4-6°C warmer in southern Ontario over the coming century (Colombo et al. 2007). If these forecasts are accurate, habitats previously unsuitable for completion of species' life cycles may become increasingly suitable, and establishment of some NIS may be anticipated (Hellmann et al. 2007; Dytham 2009). Such a scenario suggests that a review of species offered for sale by live garden and aquarium trades is warranted, perhaps including formal risk assessment under a scenario of warmer water temperature regimes during critical seasons (Champion et al. 2010; Andreu and Vilà 2010).

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