



Transoceanic ships as vectors for nonindigenous freshwater bryozoans

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ABSTRACT

Aim The transport of organisms in ships' ballast tanks is a dominant vector for aquatic invasions worldwide. Until recently, efforts to manage this vector have overlooked the potential transport of invertebrate resting stages in the residual waters and sediments within emptied ballast tanks, i.e. NOBOB ('No Ballast On Board') tanks. The resting stages (statoblasts) of freshwater bryozoans are often buoyant and locally abundant and thus can be taken up easily during ballasting operations. They are also resistant to extreme environmental conditions and can generate new colonies after being dormant for decades; as such, they would likely remain viable propagules after lengthy transport in ship ballast tanks. This study quantified the occurrence of freshwater bryozoan statoblasts in ballast tank sediments of transoceanic ships.

Location North American Great Lakes.

Methods We quantified the frequency of occurrence, abundance and diversity of bryozoans (as statoblasts) in residual sediment samples taken from 51 NOBOB tanks of 33 transoceanic ships visiting the Great Lakes from 2000 to 2002.

Results Our study identified 11 species, comprising nearly 12% of the total number of freshwater bryozoans known worldwide. These include two exotic species unrecorded in the Great Lakes (*Fredericella sultana* and *Lophopus crystallinus*), an exotic species already established in the region (*Lophopodella carteri*) and three cosmopolitan species (*Plumatella casmiana*, *P. fungosa* and *P. repens*). Our estimates suggest that a ship with NOBOB tanks may carry up to 10⁶ statoblasts.

Main conclusions The discovery of species unrecorded in the Great Lakes and the potentially large numbers of statoblasts being transported in ship ballast tanks indicate a significant risk of new species introductions. Furthermore, the presence of cosmopolitan species and an exotic species already established in the Great Lakes suggests the strong possibility of cryptic invasions via the introduction of exotic genotypes.

Keywords

Bryozoa, Great Lakes, invasive species, propagule pressure, ship ballast tanks, statoblasts.

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INTRODUCTION

Transoceanic shipping is the dominant vector for exotic species introductions in the North American Great Lakes. Over 60% of the invaders discovered since the opening of the St. Lawrence Seaway in 1959 are attributable to ballast water release (Ricciardi, 2006). Ocean-going vessels have been

required since 1993 to exchange their filled ballast tanks at sea to reduce the abundance and viability of freshwater organisms discharged into the Great Lakes, but ships with un-pumpable residual water in their ballast tanks (reported as 'No Ballast On Board' or NOBOB tanks) were not subject to the same regulation until 2006 in Canada and 2008 in the USA (Government of Canada 2006; Ricciardi & MacIsaac, 2008).

Interestingly, exotic species discovered in the Great Lakes since 1993 have primarily been euryhaline invertebrates, many of which possess resting stages (Ricciardi, 2006). Such species can potentially survive ballast water exchange and NOBOB tank conditions because they are tolerant to salinity fluctuations (Reid & Orlova, 2002; Ellis & MacIsaac, 2009), or their resting stages are resistant to extreme environmental conditions (Grigorovich *et al.*, 2003; Bailey *et al.*, 2005a,b).

After unloading cargo, foreign ships with NOBOB tanks may take on ballast water prior to visiting another port within the Great Lakes basin (Colautti *et al.*, 2003). The resulting mixture of fresh ballast water with residual water and tank sediments containing organisms and resting stages may subsequently be released at another port upon arrival, and thus poses a previously unrecognized risk of invasion (Colautti *et al.*, 2003; Duggan *et al.*, 2005; Ricciardi & MacIsaac, 2008). Each year between 1994 and 2000, about half of the transoceanic ships with NOBOB tanks entering the Great Lakes engaged in the uptake and discharge of ballast water at multiple ports within the basin (Colautti *et al.*, 2003). Recent studies have quantified the density and viability of invertebrate resting stages found in the residual sediments of NOBOB tanks, enumerating ubiquitous groups such as copepods, rotifers, cladocerans, dinoflagellates, diatoms, euglenoid flagellates and ciliates to the lowest taxon possible (e.g. Hallegraeff & Bolch, 1991; Kelly, 1993; Bailey *et al.*, 2003, 2005a). Although resting stages of invertebrate taxa are more abundant in residual sediments than active invertebrates are in residual water, the latter group of organisms may experience greater opportunities for discharge than taxa buried in sediments (MacIsaac *et al.*, 2002; Duggan *et al.*, 2005). Furthermore, burial in sediments decreases the hatching success of resting stages, and only a portion of those resting stages delivered are viable (Bailey *et al.*, 2005a). Nevertheless, in a recent study, more than 10% of all active and dormant propagules found in NOBOB ballast sediments comprised taxa that have already invaded the Great Lakes (Duggan *et al.*, 2005). Moreover, resting stages of other common taxonomic groups occur frequently in these tanks, but their species composition has been largely ignored in most studies. For example, Bailey *et al.* (2005a) noted the presence of resting stages of freshwater bryozoans in over 60% of ships sampled in their study, although they did not attempt to identify them. Therefore, we expect that transoceanic ships with NOBOB tanks carry a much greater abundance and diversity of freshwater invertebrate taxa in tank sediments than heretofore recognized, including those entering the Great Lakes before current ballast water regulations. This expectation prompted us to determine the frequency, abundance and diversity of freshwater bryozoans occurring as resting stages in NOBOB tank sediment samples taken from transoceanic ships entering the Great Lakes prior to 2006.

Freshwater bryozoans are common colonial invertebrates that live attached to submerged substrates in inland waters worldwide (Wood, 2001a; Massard & Geimer, 2008a,b). Several species are major fouling pests of aquaculture facilities

and water supply systems (Aprosi, 1988; Dubost *et al.*, 1996; Wood & Marsh, 1999; Wood, 2005a). Their global diversity has been recently estimated at 94 species comprising 24 genera and 10 families, most of which are in the class Phylactolaemata (Massard & Geimer, 2008a,b). Phylactolaemate bryozoans produce asexual resting stages (statoblasts), which are dormant buds comprised of sclerotized valves encapsulating nutrient-rich yolk and germinal tissue. There are two general types: floatoblasts, which are typically buoyant; and sessoblasts, which are fixed to substrates. The floatoblasts of some species are actually not buoyant (e.g. those produced by *Fredericella* spp.), and in other species (e.g. *Lophopodella carteri*), the floatoblasts achieve buoyancy only after a period of desiccation; but in most species (e.g. most *Plumatella* spp.), they are buoyant immediately upon release from the parent colony (Bushnell, 1966; Wood & Marsh, 1996; Wood, 2001a).

Statoblasts of phylactolaemate bryozoans exhibit both facultative dormancy (quiescence) and obligate dormancy (diapause). Dormancy and germination may be induced by changes in ambient light, temperature or water chemistry. Statoblasts of various species are also resistant to extreme temperatures, periods of drought, and exposure to organic pollution, heavy metals, toxins, pesticides, herbicides, and even weak hydrochloric acid. Freezing and desiccation may be tolerated by some species for months or even years (Bushnell & Rao, 1974). Statoblasts generally remain viable after being stored for up to 2 years in 1–2% saline solution and will germinate quickly upon return to fresh water (Wood, 2005b). Some freshwater species (e.g. *Plumatella repens*, *P. fungosa*, *Fredericella sultana* and *Paludicella articulata*) thrive in slightly brackish water (Everitt, 1975; Massard & Geimer, 2008b). Given their properties and hardiness, statoblasts appear to be prime candidates for being taken up and transported as viable propagules in the ballast tanks of transoceanic ships.

METHODS

We analysed samples from 51 NOBOB tanks in 33 transoceanic ships arriving in the Great Lakes from December 2000 to December 2002, a period prior to the revised ballast regulations that require saltwater flushing of residual ballast in NOBOB tanks. Each ship was boarded to collect residual sediments from NOBOB tanks. Using sterile spatulas, 4 kg of residual sediments were collected from at least five areas of each tank where drainage was minimal (Bailey *et al.*, 2003). From each 4 kg sample, we took four 40 g subsamples (wet weight) and preserved them in 95% ethanol, as per Bailey *et al.* (2005a). We analysed a total of 177 replicate (40 g) samples of tank sediments, a subset of those taken by Bailey *et al.* (2005a), comprising an average of 3.5 replicates per tank. Bryozoan statoblasts were separated from sediment via a Ludox[®] HS 40 protocol in the laboratory (Burgess, 2001). Identifications and counts of statoblasts were primarily made under a dissecting microscope (45×) and subsequently under a compound microscope (400×), when higher magnification was necessary.

Three of the ships were sampled multiple times over the 2-year period. We treated these samples as independent data points, because Bailey *et al.* (2005a) found that the similarity between such samples was as low as that of samples taken from 1000 randomly paired ships, according to comparisons made using Sorensen's coefficient of similarity (Krebs, 1999). Similarly, Bailey *et al.* (2005a) also compared the similarity between tanks within a ship arriving at a particular time to that of 1000 randomly paired tanks from different ships; in this case, tanks within a ship were more similar than tanks between ships, and therefore tanks within ships were not treated as independent.

A simple linear regression was performed in R (version 2.6.2) to analyse the relationship between statoblast abundance (\log_{10} -transformed) and statoblast diversity per sampled ship. The regression analysis used pooled data from all 40 g replicates from each tank and all tanks from each ship. Replicate and tank numbers varied across ships; hence, sampling effort varied for different data points.

Hatching experiments were not systematically carried out, but some statoblasts were hatched during the course of experiments examining the viability of resting stages of common taxa collected from tank sediments (see Bailey *et al.*, 2005a for methodology).

RESULTS AND DISCUSSION

Propagule abundance and diversity

We retrieved an average of 2.3 ± 0.4 (95% C.I.) statoblasts per 40 g sample, i.e. a mean density of 0.06 ± 0.01 statoblasts g^{-1} . Given that a ship with NOBOB tanks carried 15 (range = 0.1–

65.0) t of residual sediment at the time of our study (Duggan *et al.*, 2005), it could have transported as many as 10^6 statoblasts. Ships with NOBOB tanks account for the vast majority of vessels visiting the Great Lakes. On average, 484 ± 75 (SD) ships with NOBOB tanks entered the system each year between 1994 and 2000, of which about half [249 ± 69 (SD)] took on Great Lakes water and subsequently discharged it at another port within the same basin (Colautti *et al.*, 2003). Collectively, these ships carried 10^9 g of tank sediments and thus had the opportunity to discharge up to 10^8 statoblasts into the Great Lakes annually. This finding adds substantially to previous estimates of 10^9 active propagules and 10^8 dormant propagules for all invertebrate groups in tank sediments of ships entering the Great Lakes annually (Bailey *et al.*, 2005a; Duggan *et al.*, 2005).

In total, we collected 409 statoblasts belonging to 11 species (Table 1), which comprise nearly 12% of the world's freshwater bryozoan diversity (Massard & Geimer, 2008b). Three families were present, but one (Plumatellidae) accounted for 7 of the 11 identified species. We identified an average of 3.0 ± 0.4 (95% CI) species per tank for which we had four 40 g replicates ($n = 38$). For ships ($n = 5$) with fully replicated samples from two tanks, mean bryozoan species richness for pooled tanks was 4.6 ± 1.3 (95% CI) species.

Species richness increased with statoblast abundance per sampled ship (Fig. 1). Because our sampling effort was not uniform across ships, Fig. 1 is of course confounded by this variable; however, its implication is noteworthy. Our maximum sampling effort for a given ship was a 480 g sample. Given an average of 10^7 g of residual sediment per ship (Duggan *et al.*, 2005), and a relationship between diversity and abundance that is not asymptotic over the range sampled

Table 1 Per cent occurrence and abundance by taxon of 409 bryozoan statoblasts collected from 33 ships visiting the Great Lakes from December 2000 to December 2002. Native species are those that have an evolutionary history in the Great Lakes basin and have been present there since before European colonization, while exotic ones have no such history and have not been present on such a time scale (Ricciardi, 2006). Cryptogenic species are those that are not demonstrably native or introduced, and thus their origins are highly uncertain (Carlton, 1996).

	Per cent occurrence	Per cent abundance	Recorded in the Great Lakes?	Status of species in the Great Lakes
Plumatellidae	100	96.8		
<i>Hyalinella punctata</i> *	36.4	6.1	Yes	Native
<i>Plumatella casmiana</i>	21.2	1.7	Yes	Cryptogenic
<i>Plumatella cf. fungosa</i>	75.8	35.2	Yes	Cryptogenic
<i>Plumatella cf. repens</i>	15.2	1.7	Yes	Cryptogenic
<i>Plumatella</i> sp. (<i>fungosa-repens</i> group)	63.6	14.4		
<i>Plumatella cf. nitens</i>	45.5	7.1	Yes	Native
<i>Plumatella emarginata</i>	51.5	11.7	Yes	Native
<i>Plumatella reticulata</i>	39.4	9.0	Yes	Native
unidentified plumatellids	57.6	9.8		
Lophopodidae	15.1	2.2		
<i>Lophopodella carteri</i> *	9.1	0.7	Yes	Exotic
<i>Lophopus crystallinus</i> *	6.1	1.5	No	Exotic
Fredericellidae	12.1	1.0		
<i>Fredericella indica</i> *	3.0	0.2	Yes	Native
<i>Fredericella sultana</i> *	9.1	0.7	No	Exotic

Values in boldface type are total values for each family.

*Species with non-buoyant statoblasts.

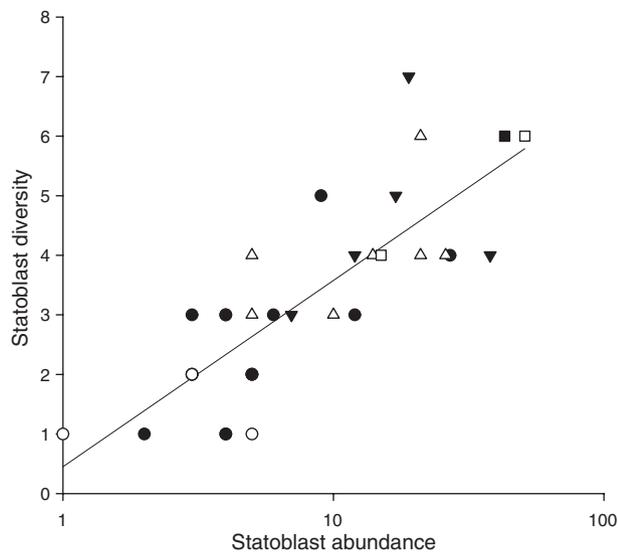


Figure 1 Statoblast diversity as a function of statoblast abundance per ship. Circles are pooled replicates from one tank; triangles are pooled replicates from two tanks; squares are pooled replicates from three tanks. Closed symbols are ships for which all tanks had four 40 g replicates. Open symbols are ships for which at least one tank had fewer than four 40 g replicates. Line fit by least-squares regression: $y = 0.45 + 1.36 \log x$ ($R_{adj}^2 = 0.64$, $P < 0.0001$, $n = 33$).

(Fig. 1), these ships likely transported a much greater number of species than we discovered in our surveys. This is astonishing, as our relatively small sampling effort has revealed a substantial fraction of global freshwater bryozoan diversity being transported into the Great Lakes basin.

Our samples may have contained an even greater diversity than we recognized, because we were not able to identify all statoblasts to species with a high level of confidence. Although we are quite confident in the presence of the species that we identified, it is possible that a few statoblasts identified as a particular species could be another, more obscure species. Within the past several years, new plumatellid species have been described that closely resemble *P. repens* and *P. fungosa* but cannot be identified easily without scanning electron microscopy, which was not used in our study. Some of our statoblasts had features typical of *P. repens* and *P. fungosa*, but others could also belong to *P. rugosa*, *P. similirepens*, *P. bushnell*, *P. nodulosa* or *P. vaihiriae* (Wood *et al.*, 1998; Wood & Marsh, 1999; Wood, 2001b). Statoblast identification can be difficult without access to a range of specimens from one colony and when the morphology of the colony and (when relevant) the sessoblasts (a type of statoblast fixed to the substrate) cannot be assessed simultaneously.

Statoblast viability

Because freshwater bryozoans are sexually active for only a single brief period during the year and larvae usually settle within an hour (Wood, 2001a), statoblasts are far more

plausible propagules for transport in ship ballast tanks. Their general hardiness to all manner of extreme environmental conditions (Bushnell & Rao, 1974; Wood, 2005b) should enhance the likelihood of propagules remaining viable and being spread in transoceanic ships. Although no comprehensive experiments were performed on the germination of statoblasts recovered from our samples, a few trials were attempted. Two statoblasts of one species (*Plumatella casmi-ana*) and a third statoblast of an unknown *Plumatella* sp., each from different ships, were successfully hatched in the laboratory (S.A. Bailey, unpublished data).

Evidence of benthic disturbance during ballasting

We found dozens of non-buoyant and sessile resting stages in our samples. Five of our 11 species (*F. sultana*, *F. indica*, *H. punctata*, *L. carteri* and *L. crystallinus*) have statoblasts that are not buoyant upon release from the parent colony, although statoblasts of *L. carteri* and *L. crystallinus* can become buoyant after desiccation (Wood & Marsh, 1996; Wood & Okamura, 2005). In one sample, we identified an intact *Plumatella* sp. sessoblast. Furthermore, we found the resting stages (gemmules) of a freshwater sponge, *Eunapius fragilis*, a cosmopolitan species that cements its gemmules in a pavement layer on submerged solid surfaces. We found fragments of gemmule pavements from multiple samples in a single ship. Collectively, the presence of non-buoyant statoblasts, a sessoblast, and gemmule pavements strongly suggest sediment disturbance during ballast uptake in shallow water ports, an activity that could facilitate a broader diversity of benthic species to be taken up by ships.

Exotic and cryptogenic species transported to the Great Lakes

Most of the identified bryozoans have broad global distributions (Table 2), with some notable exceptions. *Plumatella nitens* is known to occur only within a narrow north-central range in North America that encompasses the Great Lakes (Wood, 1996). *Plumatella fungosa* is common in Europe (Wood & Okamura, 2005), where it is a notorious fouling pest (e.g. Pourcher & d'Hondt, 1987; Aprosi, 1988; Thome *et al.*, 1989). It is generally uncommon in North America (Wood, 2001a), although it is locally abundant in the Great Lakes basin (Ricciardi & Reising, 1994). *Lophopodella carteri* is primarily a subtropical species native to southeast Asia but is now found on all continents except South America and Antarctica (Massard & Geimer, 2008b) and is already established in the Great Lakes (Ricciardi & Reising, 1994).

We identified two species, *Fredericella sultana* and *Lophopus crystallinus*, that have not been recorded in the Great Lakes. Historically, specimens of *Fredericella* from North American inland waters were considered to be *Fredericella sultana* but are in fact *F. indica* Annandale; the former is common in Europe (Wood & Okamura, 2005) but is known only from one location in western North America, whereas *F. indica* is

Table 2 Known distributions of bryozoan species in this study, including exotic and native ranges. Distributional information from Massard & Geimer (2008a,b), Wood & Okamura (2005), and Wood (2002).

Species	Current distribution (exotic and native ranges)
<i>Fredericella indica</i>	Nearctic (common); Palaearctic; Oriental
<i>Fredericella sultana</i>	Nearctic (western N. America, rare); Palaearctic; Oriental; Australasia
<i>Hyalinella punctata</i>	Nearctic; Neotropical; Palaearctic
<i>Lophopodella carteri</i>	All regions except Neotropical and Antarctica
<i>Lophopus crystallinus</i>	Palaearctic
<i>Plumatella casmiana</i>	All regions except Australia and Antarctica
<i>Plumatella emarginata</i>	All regions except Antarctica
<i>Plumatella fungosa</i>	Palaearctic; Nearctic (eastern N. America); Australia
<i>Plumatella nitens</i>	Nearctic (confined to northern US and southern Canada)
<i>Plumatella repens</i>	All regions except Pacific islands and Antarctica
<i>Plumatella reticulata</i>	Nearctic; Palaearctic (Europe, uncommon); Afrotropical (Israel)

common throughout North America and Europe (Wood, 2001a, 2002). The presence of *L. crystallinus* is noteworthy because it is considered native to Europe and has not been found in the Great Lakes (Wood, 2001a). It has long been thought to be rare, but recent evidence from Europe suggests otherwise (Hill *et al.*, 2007). Given that colonization could result from a single statoblast, the presence of these exotic species adds to the evidence that the discharge of untreated NOBOB tanks posed a broader invasion risk to the Great Lakes than previously believed (Bailey *et al.*, 2003, 2005a,b; Duggan *et al.*, 2005).

Apart from the discovery of these non-indigenous species, two additional findings point to increased invasion risk. First, the presence of *P. nitens*, a species native to the Great Lakes and apparently restricted to the American midwest (Wood, 1996, 2001a; Massard & Geimer, 2008b), suggests inter-port dispersal of statoblasts by ship traffic within the Great Lakes basin. Such dispersal could contribute to the invasion success of exotic species in general by increasing the likelihood that such species are introduced to sites having favourable environmental conditions.

Second, there is a strong potential for cryptic (genetic) invasions in the Great Lakes from exotic species already recorded as established (*Lophopodella carteri*) and from 'cosmopolitan' species such as *Plumatella casmiana*, *P. fungosa*, *P. repens* and *Fredericella indica*, typically listed as native in the Great Lakes region. These three plumatellid species are best categorized as cryptogenic (i.e. of unknown origin, *sensu* Carlton, 1996). *Plumatella fungosa* is among the most common and widespread bryozoans in Europe but has a scattered distribution in eastern North America. *Plumatella repens* was once considered the most common and widely distributed

phylactolaemate bryozoan in the world, but scanning electron microscopy has revealed surface features on statoblasts that differentiate multiple species including three from North America previously identified as *P. repens* (Wood, 2001b); *P. repens* occurs very infrequently in North America but is common throughout Europe (Wood, 2001b; Wood & Okamura, 2005). As we cannot be fully certain of the identity of statoblasts resembling *P. repens* and *P. fungosa*, we have designated them as "*Plumatella cf. repens*" and "*Plumatella cf. fungosa*". In cases where statoblasts exhibited morphologies that were not easily assigned to either *P. cf. repens* or *P. cf. fungosa*, we identified statoblasts as being within the "*fungosa-repens* group" (Table 1), which could comprise any of the five aforementioned species that resemble *P. fungosa* and *P. repens*.

Ballast water transport as a vector of bryozoan invasions worldwide

Extrapolated beyond the Great Lakes, our results suggest the ship-mediated dispersal of statoblasts from multiple sources to many disparate locations over long distances (i.e. mass dispersal, *sensu* Wilson *et al.*, 2009), a process that can enhance the genetic diversity of introduced populations and thus increase the spread of non-indigenous species (Roman, 2006). Yet, freshwater bryozoans are very rarely implicated in transoceanic invasions (but see Wood & Okamura, 1999). They are absent from most exotic species lists for large freshwater systems; for example, only *Lophopodella carteri* is noted as an invasive bryozoan in the Great Lakes (Ricciardi, 2006), although the three plumatellid species mentioned previously are cryptogenic. We believe this is due largely to (1) the obscurity and unresolved taxonomic problems of many species and (2) the presumption of natural cosmopolitanism of species that are reported from several disjunct locations around the world. Many introductions of such species may have occurred prior to the 20th century, when statoblasts (which can survive prolonged periods of desiccation; Bushnell & Rao, 1974) were likely moved in the soil and cobble that was loaded and discharged as dry ballast by overseas vessels at ports.

While large-scale disjunct distributions of freshwater bryozoans have, by default, been attributed primarily to the flight patterns of waterfowl and to spotty field sampling (Bushnell, 1973; Wood, 2002), our findings reveal the potential importance of transoceanic shipping to their global dispersal. This vector may have already contributed to an incalculable number of cryptic invasions of bryozoans worldwide. However, its role in further introductions to the Great Lakes may have been diminished by recent management changes for vessels with NOBOB tanks (Ricciardi & MacIsaac, 2008). These tanks must now be flushed on the open ocean, prior to entry into the St. Lawrence Seaway. Flushing would serve a number of functions, including the purging of statoblasts at sea, and a possible reduction in viability of retained statoblasts when exposed to open ocean seawater in tanks – although this remains to be tested. We have observed an apparent decline in the quantity of

residual sediment carried in NOBOB tanks since mandatory flushing commenced in 2006 (S. Bailey, pers. obs.), which should lead to fewer statoblasts being carried into the lakes.

ACKNOWLEDGEMENTS

We thank the Shipping Federation of Canada for facilitating this study. We are especially grateful to Tim Wood for helping us to identify bryozoan species and to three anonymous reviewers for their helpful comments. Funding was provided by NSERC Discovery Grants and the Canadian Aquatic Invasive Species Network to HJM and AR.

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BIOSKETCH

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All authors are interested in the ecology of freshwater invasions, with a common research focus on the Great Lakes. S.B. and H.J.M. conceived this study and collected the samples. R.K. and A.R. identified samples, analysed data and led the writing.

Editor: David Richardson