Biol Invasions (2013) 15:509–519
DOI 10.1007/s10530-012-0303-0

ORIGINAL PAPER

Recreational boats as a vector of secondary spread for aquatic invasive species and native crustacean zooplankton

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Received: 8 March 2012 / Accepted: 29 July 2012 / Published online: 10 August 2012

Abstract Recreational boats in tow between lakes are a known vector of the spread of aquatic invading species (AIS), but we have no test of the hypothesis that recreational boats are also a vector of secondary spread of AIS among freshwater ecosystems via in-water transport i.e., while boating between interconnected waterways. In this study, we surveyed recreational boaters travelling into Lake Simcoe (44°25′N, 79°20′W), Ontario, Canada, on their recreational activities, boat maintenance, and travel destinations, measured the degree of vessel fouling, and sampled all standing water and attached macrophytes associated with their vessels. A total of 321 zooplankton individuals comprising 15 different species were collected from the standing water in vessels, including veligers of the invasive zebra mussel *Dreissena*. The volume of water collected within the vessels significantly increased the number of zooplankton transported. Zooplankton species from pelagic habitats or with planktonic life stages were collected more frequently than species that occupy littoral or benthic habitats, likely reflecting the recreational activities of boaters. Patterns of boater activities, movements and hygiene habits, suggest recreational boating in the Lake Simcoe region is contributing to the spread of native and invasive species into nearby waterways. Our study validates the widespread assumption that recreational boats are an important in-water vector for the secondary spread of both native and invasive zooplankton species. Future management strategies to reduce the spread of AIS should be aimed at increasing awareness of boater hygiene practices, particularly the frequent draining of standing water.

Keywords Invasive species · Dispersal vector · Secondary spread · Recreational boating · Lake Simcoe · Zooplankton

Introduction

The development of suitable management strategies to protect freshwater lakes from aquatic invasive species (AIS) requires a thorough understanding of the vectors which may facilitate their spread. The discharge of ballast water from transoceanic vessels has been identified as one of the primary human-mediated

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vectors responsible for the release of AIS into freshwater ecosystems (Carlton and Geller 1993; Holeck et al. 2004). For example, the Great Lakes region of North America is considered to be one of the most heavily invaded ecosystems in the world (Ricciardi 2006), and since 1959, the exchange of ballast water in the Great Lakes has been responsible for approximately 67 % of the non-indigenous species introduced (Grigorovich et al. 2003). Other pathways of primary introduction include hull fouling, aquaculture activities, and/or accidental or intentional release of fishes, invertebrates, or macrophytes associated with the aquarium trade (Lodge et al. 1998; Minchin and Gollasch 2002). Once established, an invasive species can secondarily spread from its first location of establishment via a wider range of vectors acting separately or in concert (Minchin and Gollasch 2002; Vander Zanden and Olden 2008).

Recreational boating and its associated activities are thought to be one of the human-mediated vectors to play a large role in the secondary spread of both invasive plants and animals worldwide (reviewed in Minchin et al. 2006). The overland transport of these taxa by trailered boat traffic occurs mainly through the entanglement of fragments or individuals in fishing gear and anchor chains, attachment to boat hulls or trailers, and/or through the transport of standing water (Johnstone et al. 1985; Havel and Stelzleni-Schwent 2000; Johnson et al. 2001; Minchin et al. 2003; Rothlisberger et al. 2010; Stasko et al. 2012). For example, adult zebra mussels (*Dreissena polymorpha*) have been observed directly attached to boat hulls (Minchin et al. 2003) or attached to macrophytes snagged on boat trailers (Johnson et al. 2001), whereas the veligers have been reported in the standing water on trailered boats, such as the bilge, live wells, anchor and bait buckets, and in the engine cooling system (Johnson et al. 2001). However, little attention has been paid to recreational boats that remain in the water, and potentially disperse AIS as they move between interconnected waterways, such as lakes linked by rivers, canals, and locks ("in-water" transport).

In-water transport of AIS through recreational boating may be a significant vector for their secondary spread. While the dispersal of AIS can occur naturally through interconnected waterways, in-water transport may dramatically increase the rate and spatial scale of secondary spread (e.g. Boltovskoy et al. 2006). Compared to other vectors, in-water transport may also promote longer species survival during transport and thus increase the likelihood for dispersal, and ultimately successful establishment, through vast networks of locks, streams, and canals. For example, the survival of AIS transported long-distances overland via trailered boats may be limited to a few days or less during the heat of summer months, due to direct exposure to warm air or the highly variable conditions (e.g. high temperatures, low dissolved oxygen) found in dwindling volumes of water in live wells, bilges, or anchor buckets (Havel and Stelzleni-Schwent 2000; Johnson et al. 2001). In contrast, since boats are more likely to travel over smaller spatial scales through in-water routes, the timescale of AIS exposure to onboard conditions is shorter, increasing the probability of being released alive into a new waterway. For boats travelling greater distances (e.g., >100 km) through interconnected waterways, continuous operation of the vessel may provide a greater opportunity to accumulate water and organisms, resulting in more natural conditions in the standing water onboard, and potentially a greater number of propagules delivered to non-invaded sites. In addition, the construction and engineering of navigable waterways (e.g. canals and locks) generally homogenizes their water quality (Galil and Minchin 2006), and thus transport through such dispersal corridors may increase the success of AIS establishment, since the environmental conditions in a new site are more likely to be similar to those in the location of origin.

The assumption that recreational boating contributes to the secondary spread of AIS via in-water transport is frequently used in studies modelling the spread of invaders through freshwater ecosystems (e.g. Bossenbroek et al. 2001; Havel et al. 2002; Kraft et al. 2002; Leung et al. 2006), yet few studies have been conducted to gather empirical evidence to support this hypothesis. In this study, we conducted a field test to validate whether recreational boats are a vector of secondary spread for AIS via in-water transport. To accomplish this goal, we received the individual permission of surveyed boaters to gather information on their recreational activities, boat maintenance, and travel destinations. We measured the degree of vessel fouling, and sampled all standing water and attached macrophytes contained within or on the vessels. We hypothesized that more species would be transported within recreational boats if a greater volume of standing water accumulated within the boat, if the boats travelled longer distances, and/or if boats exhibited a greater degree of fouling. In addition, we hypothesized that boaters' recreational activities would determine the types of zooplankton taxa (e.g. pelagic, littoral, benthic) transported. To our knowledge, this is the first observational study to provide empirical evidence for the in-water transport of AIS via recreational boating, a useful and necessary exercise to guide the development of future management strategies for the prevention of AIS dispersal among freshwater ecosystems.

Materials and methods

Study site

The Trent-Severn Waterway may be an important pathway for the dispersal of AIS. A lock and canal system in central Ontario, it spans 386 km and connects Lake Ontario to Georgian Bay and Lake Huron through a series of 4 rivers and 17 lakes (Helleiner 1981). This waterway is divided into 7 distinct regions based on the amount and kinds of recreational activity, including boater traffic (Helleiner 1981). Lake Simcoe (44°25′N, 79°20′W) is the largest inland lake in southern Ontario, exclusive of the Laurentian Great Lakes, and is one of the 7 regions of the Trent-Severn Waterway. The Lake Simcoe Region spans the Talbot River in the Kawarthas of central Ontario through to Big Chute and the Marine Railway located just east of Georgian Bay (Helleiner 1981). Lake Simcoe supports diverse tourism and recreational activities and a year-round sport fish industry, which collectively generates hundreds of millions of dollars in annual revenue for local and provincial economies (LSEMS 2008). Since 1984, the Lake Simcoe basin has been invaded by nine non-indigenous species; of particular concern are the Eurasian watermilfoil (*Myriophyllum spicatum*) (LSEMS 2008), zebra (*Dreissena polymorpha*) (Evans et al. 2011) and quagga (*D. bugensis*) mussels (Ozersky et al. 2011), and the spiny water flea (*Bythotrephes longimanus*) (NEK, unpubl. data), due to their effects on water quality and overall ecosystem health. Estimates suggest there are approximately 1.2 million recreational boaters in Ontario (GLC 2000), and ~120,000 vessels travel through the locks into and out of Lake Simcoe each year (Parks Canada 2012). Given its size, number of invasive species, popularity, and proximity to the Great Lakes, Lake Simcoe has the potential to become an invasion hub (Muirhead and MacIsaac 2005), facilitating a "stepping stone" like spread of species (Floerl and Inglis 2005; Floerl et al. 2009), and put adjacent non-invaded waterways at risk. Thus, Lake Simcoe is an ideal model system to study the impact of recreational boater traffic on facilitating the secondary spread of AIS.

Recreational boater survey

Recreational boaters in the Trent-Severn Waterway passing through Couchiching ($n = 54$) and Gamebridge ($n = 9$) locks towards Lake Simcoe, were surveyed over the course of 10 days during August and September 2010. Lock locations were chosen as they are the last obstacles boaters must cross in order to enter Lake Simcoe by water. For the purposes of this study, Lake Couchiching was considered part of Lake Simcoe as there is no barrier of flow between them. Of the 65 boaters who were approached only 2 refused to partake in the survey, resulting in a total of 63 boaters surveyed. Boaters were queried anonymously about their recreational activities (fishing and hunting, water sports, sightseeing/cruising, and/or camping), boat maintenance (visual inspections, draining, drying, and cleaning) and travel details (how frequently the boat was used, number of different lakes or waterways generally visited, length of time boat is in the water, current and total number of lock passages entered, length of time in Lake Simcoe) (after Qureshi and Bailey 2010). Boaters were also queried as to the starting location of their trip in order to discern the geographic origin of each boat, despite boaters being surveyed when passing through the locks in only one direction (into Lake Simcoe). A few days into the sampling, an additional question regarding the longest trip boaters made during the season was added to the list and was used in all subsequent surveys, resulting in 37 boater responses to this additional question.

To determine the general level of "cleanliness" of boats and the potential for AIS to be transported via direct attachment to boats, qualitative observations were made regarding the overall fouling on both the exterior and interior of each vessel. A fouling score (none = 0, low = 1, moderate = 2, and high = 3) was assessed for the hull, floorboards, anchor and anchor line of each boat (after Ashton et al. 2006). The resulting scores from each boat area were then combined to give an overall fouling score for the boat (possible range: 0–12). A macrophyte fouling score (none = 0, few = 1, many = 2, clumps = 3) was also assessed for each of the same boat areas (after Johnson et al. 2001), and combined for an overall macrophyte score for the boat.

Zooplankton collection

To examine whether AIS might be carried in the standing water on boats, any location on the vessel which contained standing water was sampled, including the bilge, live well, engine cooling water, bait buckets, ballast, or anchor box. If the bilge had an automatic bilge pump, the pump was activated and water was collected in a graduated bucket as it exited the side of the vessel (after Darbyson et al. 2009). Otherwise, bilge water was manually extracted using either a bilge pump or turkey baster (Havel and Stelzleni-Schwent 2000; Darbyson et al. 2009). The turkey baster and bilge pump were also used to collect all other samples of water. The total volume of water collected was recorded and then condensed for preservation in a 500 mL PET jar containing 70 % ethanol. Samples were condensed through a 63 µm mesh filter (after Johnson et al. 2001), using lake water that had also been filtered with 63 µm mesh. The sample itself did not exceed 30 % of the final volume of the solution (Black and Dodson 2003). All sampling equipment was rinsed with filtered lake water between collections to reduce the chance of contamination. Where possible, any visible macrophytes were also collected from the vessel and stored in 70 % ethanol for later examination of attached animals including adult zebra mussels.

Water samples were initially scanned for the presence of zooplankton under a dissecting microscope. Samples containing zooplankton were sorted and all individuals then enumerated and identified to species, or to life stage for immature copepods, following the classification of Witty (2004).

Data analysis

We examined three factors potentially influencing the abundance of zooplankton collected in the standing water found on recreational boats: the volume of water transported by the boat, the geographic origin of the boat on its current trip, and the daytime habitat of the zooplankton collected (we were unable to statistically compare zooplankton abundance and vessel or macrophyte fouling due to low sample sizes). Linear regression analysis was conducted to test whether an increase in the amount of water increased the number of zooplankton collected in the boat. Numbers of zooplankton were log(x + 1)-transformed, and the volume of water log-transformed, prior to analysis to meet assumptions of normality and homogeneity of variances. We examined whether the starting location of the boat influenced the abundance of zooplankton (no. L^{-1}) collected. We used a two-sample *t* test for equal variances to test the difference in mean zooplankton abundance between boats originating within versus outside the Lake Simcoe region. Homogeneity of variances was indicated by a Bartlett's test prior to analysis. We used non-parametric Kruskal–Wallis rank sum tests to examine whether the daytime habitat occupied by zooplankton species influenced the mean abundance of zooplankton (no. L^{-1}) collected. Zooplankton habitat categories included pelagic (offshore, in the epi-, meta-, and/or hypolimnion), littoral (nearshore, often associated with macrophytes), and benthic (near or offshore, associated with the sediment). A non-parametric test was used due to the presence of heterogeneous variances, as identified by a Bartlett's test. Post hoc testing was conducted using pair-wise Wilcox rank sum tests for each comparison. Differences were considered significant at $\alpha = 0.05$. All statistical analyses were conducted using the software package R (v. 2.14.1, R Development Core Team 2011).

Results

Recreational boater survey

While all boaters surveyed reported using their vessels for cruising and sightseeing, few reported using their vessels for fishing and hunting (Table 1). Most boaters used their boats frequently, kept their vessels in the water at all times, and conducted their activities within Lake Simcoe (Table 1). When not in use, most boaters stored their boats in the water (82.5 %, $n = 52$) rather than on land (17.5 %, $n = 11$). While most boaters

Table 1 Summary of responses (percent out of 63 total responses) from recreational boaters surveyed in August and September 2010, entering Lake Simcoe, Ontario, Canada

Survey question	Response category			
Boating activities	Fishing/hunting	Water sports	Cruising/sightseeing	Camping
	25.4	30.2	100.0	84.1
Length of time boat remains in the water	Always	3 weeks	≤1 week	Unsure
	82.5	1.6	14.3	1.6
Length of time boat remains in Lake Simcoe	Always	Indefinitely	1–2 weeks	≤1 week
	22.6	53.2	4.8	19.4
How often boat is used	Very often	Often	Infrequently	Occasionally
	55.6	22.2	3.2	19.0
Boat maintenance	Always	Sometimes	Never	Not applicable
Visual inspection	57.1	14.3	28.6	0
Drain bilge	44.4	25.4	25.4	4.8
Drain live well	1.6	0	0	98.4
Drain engine cooling water	4.8	3.2	52.4	39.7
Flush motor intake with hot water	0	1.6	93.7	4.8
Dry 5 days	12.7	3.2	0	84.1
High pressure rinse	14.3	19.0	66.7	0
Hot water rinse	3.2	1.6	95.2	0
Clean anchor/anchor line	68.3	7.9	17.5	6.3
Clean downrigger cables/fishing line	12.7	0	7.9	79.4
Number of different lakes or waterways generally visited	>6	4–6	2–3	1
	41.3	14.2	41.3	3.2

The response categories associated with the ‘boating activities’ survey question are non-exclusive and thus do not sum to 100 %

performed basic tasks to maintain the cleanliness of their boats (i.e. visual inspections, cleaning anchors, anchor lines, and fishing equipment), many did not conduct more thorough cleaning tasks (i.e. high pressure wash, hot water rinse, draining any and/or all standing water) (Table 1).

The majority of recreational boaters surveyed began their trip within the Lake Simcoe region (66.2 %, $n = 41$) and travelled out to other waterways. Of the 33.8 % ($n = 21$) of boats originating outside the Lake Simcoe region, the majority of these originated from areas far from the lake (38.1 % travelled <30 km; 61.9 % travelled ≥30 km). The furthest distance boats would travel throughout the course of the season ranged from 0–719 km, with a mean distance of 152.7 ± 29.8 (SE) km. At the time of the survey, boaters had passed an average of 8.1 ± 2.2 (SE) locks; the total number of locks to be passed averaged 10.5 ± 2.2 (SE), indicating most boaters were on their way back into Lake Simcoe at the end of their trip. Throughout the season, most boaters would travel either 2–3 or more than 6 different waterways; few boaters travelled only one waterway or between 4–6 different waterways (Table 1).

Most recreational boats displayed some level of fouling: 47.6 % of boats had an overall fouling score of 1 or 2, while 34.9 % of boats had an overall fouling score between 3 and 6. The remaining 17.5 % of boats displayed no degree of fouling with an overall score of zero. Few boats were found with macrophytes fouling their vessels; only 7 boats had a macrophyte fouling score of 1, and only 1 boat had a score of 2. However, only four samples of macrophytes were able to be collected from the vessels (most macrophytes observed were in poor condition), and were usually attached to the anchor line. No adult zebra mussels or other zooplankton species were found attached to the macrophytes.

Zooplankton transport

Of the 63 boaters surveyed, 21 boats contained standing water that was able to be sampled; these areas included the bilge ($n = 19$), an anchor bucket ($n = 1$), or a small area just behind the engine ($n = 1$). A total of 321 zooplankton comprising at least 15 different species were collected, which included 9 veligers of the invasive mussel, *Dreissena* sp. (Table 2). The invasive spiny water flea, *Bythotrephes longimanus*, was not found. The majority of boats (15 out of 21) originated within the Lake Simcoe region and contained the majority of zooplankton individuals collected (284 out of 321). On average, 14.9 ± 5.6 (SE) crustacean zooplankton and 0.4 ± 0.3 (SE) *Dreissena* veligers were collected per boat. Accounting for the volume of standing water collected in boats, the mean abundance (no. L^{-1}) of crustacean zooplankton was 13.0 ± 2.8 (SE), and the mean abundance of *Dreissena* veligers was 0.2 ± 0.04 (SE). The resulting probability of finding a *Dreissena* veliger in any given boat was 4.8 %, whereas the probability of finding any individual zooplankton was 25.4 % (inclusive of *Dreissena* sp.).

The volume of standing water in a boat had a positive effect on the number of zooplankton (Fig. 1). Log-transformed numbers of zooplankton was significantly, although weakly, related to the log-transformed volume of water collected from recreational boats ($Y = 0.453 \times X + 0.673$, $F_{(1,19)} = 7.170$, $p = 0.015$, $r^2 = 0.27$). There was no significant difference in mean zooplankton abundance (Student's $t = 0.735$, $df = 19$, $p = 0.471$) between boats originating within [mean±SE (n): 6.1 ± 2.8 (15)] versus outside [mean±SE (n): 10.8 ± 7.5 (6)] the Lake Simcoe region. The daytime habitat occupied by zooplankton had a significant effect on mean zooplankton abundance ($\chi^2 = 27.3, df = 2, p < 0.0001$) (Fig. 2). Mean zooplankton abundance collected in recreational boats was significantly higher for species that occupied a pelagic habitat than for either littoral or benthic habitats ($p < 0.0001$ for both comparisons), indicating pelagic zooplankton species are transported in recreational boats more frequently than species from any other habitat. There was no significant difference in mean zooplankton abundance between species from littoral or benthic habitats ($p = 0.7094$).

Discussion

In the Trent-Severn Waterway, in Ontario, Canada, recreational boats are indeed transporting live zooplankton

Table 2 Classification, daytime habitat, and number of all zooplankton individuals found in water samples ($n = 21$) collected from recreational boats travelling into Lake Simcoe, Ontario, Canada

Group name	Species name	Daytime habitat	Number collected in all water samples
Cladocera	*Bosmina freyi*	Pelagic[a,b]	7
	Ceriodaphnia lacustris	Pelagic[a]	1
	Chydorus sphaericus	Littoral[a]	1
	Daphnia mendotae	Pelagic[a,c]	3
	Diaphanosoma birgei	Pelagic[c]	2
	Eubosmina longispina	Pelagic[d]	1
	Polyphemus pediculus	Littoral[a]	1
Cyclopoida	Nauplii		96
	Copepodites		70
	Diacyclops bicuspidatus thomasi	Pelagic[a]	15
	Macrocyclops albidus	Littoral[c]	16
	Mesocyclops edax	Pelagic[a]	4
	Paracyclops poppei	Littoral[c]	22
	Tropocyclops extensus	Pelagic[e]	24
Calanoida	Nauplii		15
	Copepodites		10
	Leptodiaptomus minutus	Pelagic[c]	17
	Skistodiaptomus oregonensis	Pelagic[a]	2
Harpacticoida		Benthic[c]	5
Bivalvia	*Dreissena* sp.[f]	Pelagic[g]	9

Pelagic habitat definition includes species found in all three water layers (hypo-, meta- and/or epilimnion)

[a] Barnett et al. (2007)

[b] Nicholls and Tudorancea (2001)

[c] Balcer et al. (1984)

[d] Keller and Conlon (1994)

[e] Hudson and Lesko (2003)

[f] Invasive species

[g] Evans et al. (2011)

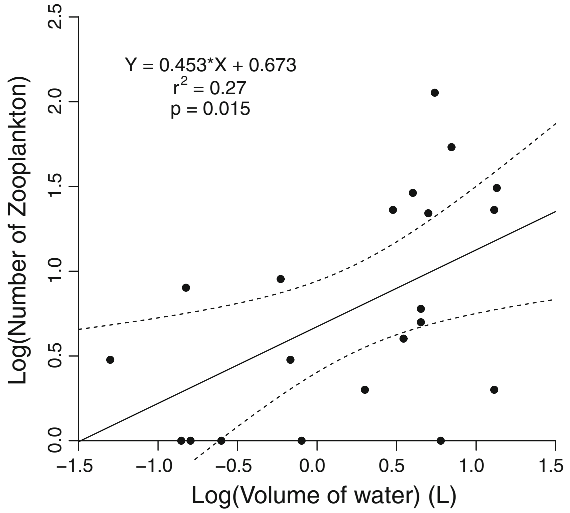

Fig. 1 Linear regression (±95 % CI) of log(x + 1)-transformed zooplankton (numbers of individuals) versus the log-transformed volume of water (L) collected from recreational boats in Lake Simcoe, Ontario

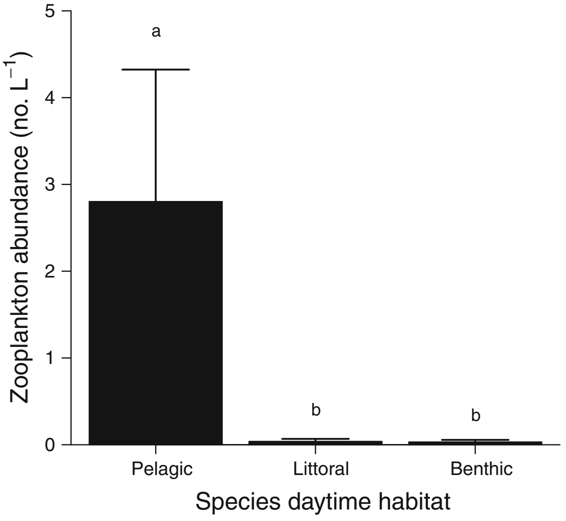

Fig. 2 Comparison of the mean (±SE) abundance (no L^{-1}) of zooplankton collected from recreational boats among three different daytime habitats (pelagic, littoral, benthic) occupied by zooplankton (as listed in Table 2). Different letters over bars indicate significant differences in daytime habitat occupied by zooplankton ($p < 0.05$)

species among waterways, facilitating the dispersal of both native and invasive zooplankton species. A total of 321 individuals comprising at least 15 different species were primarily transported via entrapment in bilge water, with a greater amount of standing water resulting in a greater quantity of zooplankton being transported. The majority of recreational boaters surveyed kept their vessel in the water for the entire season, originating within but travelling outwards from the Lake Simcoe region through multiple different waterways throughout the season. Regardless of their origin, recreational boats transported similar zooplankton abundances throughout the Trent-Severn Waterway. A considerable proportion of boaters were found to seldom or never conduct effective boat hygiene practices known to prevent the spread of AIS (e.g. Rothlisberger et al. 2010), and had moderate to heavily fouled vessels at the time of the survey. Lake Simcoe has a high ratio of inbound to outbound boater traffic (1:53, Muirhead and MacIsaac 2005), and together with the results of our analyses and surveys, suggests recreational boating in the Lake Simcoe region is an important vector of secondary spread for native and invasive zooplankton.

Of the four previous studies examining the transfer of species through freshwater ecosystems by recreational boats conducted in North America (Havel and Stelzleni-Schwent 2000; Johnson et al. 2001; Rothlisberger et al. 2010; Stasko et al. 2012), our findings are most comparable to those of Havel and Stelzleni-Schwent (2000), who found *Daphnia lumholtzi* in live wells, and Johnson et al. (2001), who found *Dreissena* sp. veligers in the standing water, on recreational boats. The mean abundance of veligers we collected in boats from Lake Simcoe (0.2 ± 0.04 veligers L^{-1}) was much less than that reported for boats on Lake St. Clair in Michigan, USA (5.9 ± 13 veligers L^{-1}) (Johnson et al. 2001). Differences in veliger abundance between Lakes Simcoe (mean±SE: 0.4 ± 0.2 veligers L^{-1} in 2007; NEK, unpubl. data) and St. Clair (mean±SD: 25 ± 22 veligers L^{-1}; Johnson et al. 2001), would influence the probability of a veliger entering, and thus being transported, in the standing water of a recreational boat. However, similar to the results of Havel and Stelzleni-Schwent (2000) and Rothlisberger et al. (2010), the majority of species we collected from recreational boats were native species. The most frequently found native zooplankton species in the standing water on recreational boats generally reflected their rank abundance as reported for Lake Simcoe from 1986–1992; for example, of the ten most common species or life stages found in our study (excluding *Dreissena* sp. veligers), seven were also among the ten most common found by Nicholls and Tudorancea (2001). Slight differences in results could be due to the use of different sampling locations within the lake between their study and ours, or changes in zooplankton community composition in Lake Simcoe over time. Overall, the presence of many native zooplankton species within recreational boats suggests that human-mediated transport is an effective vector of dispersal for native species as well as AIS, and is contributing to the homogenization of fauna among freshwater ecosystems in southern Ontario, Canada.

The frequency and number of species transported will influence the risk of invasion (Johnson and Padilla, 1996). In our study, the probability of finding a *Dreissena* veliger in the bilge water was fairly low (<5 %), but was similar to that calculated for the overland transport of a zebra mussel by an individual boater at Ensign boat launch, Michigan, USA (Johnson et al. 2001). While appearing as an uncommon occurrence, this seemingly small probability becomes more important when combined with the amount of boater activity occurring over the course of a season. For example, combining the probability of collecting a *Dreissena* sp. veliger in the bilge water (4.8 %) with the frequency of boat passages though both the Couchiching and Gamebridge locks [9457 passes for 2010; (Parks Canada 2012)], suggests that approximately 450 boats will transport at least one veliger in a single season. Boater traffic in these two locks represents only a fraction of the total number of boating activity on Lake Simcoe each season, suggesting that potentially thousands of dispersal events occur within the region every year.

The method of transport within recreational boats likely has an impact on the survival of zooplankton and thus the probability of their spread. Stasko et al. (2012) found that zooplankton transported via attachment to canoe hulls were able to establish viable populations after exposure to portage conditions of up to 30 min. Ricciardi et al. (1995) estimated that adult mussels could to survive ~3–5 days under temperate summer conditions when transported overland on small trailered boats. Havel and Stelzleni-Schwent (2000) found *Daphnia* sp. could survive for at least 3 days in small volumes of water (≤100 mL) in the covered live wells of recreational boats, and that variations in food levels had little effect on *Daphnia* survivorship over this period. However, zooplankton likely cannot live for long periods of time in unprotected live wells during the peak of summer, when water temperature may exceed their upper thermal limits (Havel and Stelzleni-Schwent 2000). In contrast, the primary method of zooplankton transport in our study was via bilge water. Transport via bilge water, which is enclosed and located beneath the deck of the vessel, could provide a more suitable environment for zooplankton to travel longer distances, as it would protect any zooplankton from UV radiation and likely maintain the bilge water at a cooler temperature than an exposed live well. Many zooplankton, including *Daphnia* sp., are susceptible to UV radiation and cannot survive intense exposure (Heubner et al. 2006). Similarly, zebra mussel veligers are more susceptible to UV radiation than adults due to the transparency of the shell (Chalker-Scott et al. 1994; Seaver et al. 2009) and may benefit from the cover of the bilge. However, while zooplankton may be protected from some environmental conditions, they would still be exposed to the contaminants which commonly drain into the bilge, such as oil, fuels and metals (Penny and Suominen-Yeh 2006). Compared to the other sub-vectors we identified in our study (anchor bucket, area behind the engine), the bilge was the mostly likely area from which zooplankton would be released into a new waterway, as many boats had a bilge pump that would expel water automatically when it reached a certain volume threshold, draining as frequently as it was filled, or when the boat was stored. Given that the frequency of boat use in Lake Simcoe was high (i.e., 77.8 % of respondents used their boats >10 times per year), any zooplankton entrained in bilge water would likely be released within a few days, increasing the likelihood of their dispersal into a new waterway. On the other hand, in late summer or early autumn, many zooplankton species form resting eggs that can tolerate desiccation and/or other harsh conditions likely encountered in bilge water (Dodson et al. 2010). Thus even boats which are used infrequently, or travel longer distances, could still facilitate AIS spread via dispersal of resting eggs.

Recreational boaters in the Lake Simcoe region are transporting species that occupy the pelagic zone of lakes or possess a planktonic life stage more frequently than from any other area in the lake. This pattern is not surprising given that most of the water in the bilge originates from surface splashing and small cracks in the hull (KW, personal observation). The collection of *Dreissena* sp. veligers on recreational vessels was expected, since the larval stage of development is the most common stage for these mussels to be transported and they can accumulate in many different locations which collect standing water on a boat (Johnson et al. 2001). Overall, the presence of any given zooplankton species collected on vessels likely depends on the recreational activities of the individual boaters. For example, at the time of year during which our survey was conducted, most boats in the Lake Simcoe region were used for sightseeing or cruising through open waters rather than fishing, potentially explaining the higher number of pelagic species entrained within vessels. Infrequent use of fishing equipment (e.g. fishing lines, anchors and anchor lines, minnow seines), particularly in the nearshore areas of Lake Simcoe, may explain the low numbers of littoral and benthic zooplankton species, as well as snagged macrophytes, collected on recreational boats.

The predatory cladoceran *Bythotrephes longimanus* is an invasive zooplankter of current concern in the Lake Simcoe region, as its invasion in North American lakes is typically associated with reductions in zooplankton abundance and diversity (Yan et al. 2001; Barbiero and Tuchman 2004; Strecker et al. 2006), with the potential to impact overall ecosystem functioning (Strecker et al. 2011). Weisz and Yan (2010) reported correlations between the number of people on a lake and the presence of *Bythotrephes*, suggesting that recreational boats may be its primary mechanism of spread. The Lake Simcoe watershed houses roughly 12 000 cottages and is home to over 350 000 residents, with an additional 50 000 cottagers present in summer months (LSEMS 2008), suggesting Lake Simcoe could be an active hub for the spread of *Bythotrephes*. However, contrary to expectation, *Bythotrephes longimanus* was not collected from recreational boats in our study. In Lake Simcoe, *Bythotrephes* generally has two peaks in population abundance, with the first occurring at the end of July and early August and the second occurring during October and November (NEK, unpubl. data). At the end of August and early September, when our study occurred, *Bythotrephes* lake densities are typically $<5.0\times10^{-3}$ (no. L^{-1}) (NEK, unpubl. data), potentially explaining their absence on recreational boats. *Bythotrephes* also foul fishing equipment (Kerfoot et al. 2011); however, we did not sample any, since the majority of boaters surveyed did not fish on their boats at this time of year (KW, personal observation). However, the presence of native pelagic zooplankton species found in recreational boats does suggest that *Bythotrephes* could be transported in this manner when its population abundance is high, particularly in July and August when boater traffic is also high. Since *Bythotrephes* reproduces parthenogenetically, only a single individual entrained within recreational vessels would be theoretically required to establish a new population, potentially facilitating its spread.

Attached macrophytes were observed on eight recreational boats, and were collected from four, although no adult mussels were present on any of the plants. However, the presence of macrophytes attached to vessels suggests that it is possible to transport adult molluscs, which was the primary method of transport observed at boat launches in Michigan, USA (Johnson et al. 2001). Macrophytes were all observed attached to boats with an average overall fouling score of 4.5. This pattern suggests vessel fouling could be an additional factor influencing the spread of AIS through the Lake Simcoe region, with "dirtier" vessels more likely to transport attached macrophytes with associated AIS. In addition to transporting attached AIS, recreational boaters may be facilitating the dispersal of invasive macrophytes such as *Cabomba caroliniana* (Jacobs and MacIsaac 2009) and *Myriophyllum spicatum,* the Eurasian watermilfoil (Crowell et al. 1999 as cited by Kerr et al. 2005). Gravity models have suggested that Lake Simcoe is one of the most at-risk lakes for the invasion of *C. caroliniana*, based on its size and boater activity (Jacobs and MacIsaac 2009).

Despite the presence of existing invading species prevention programs designed to raise awareness of the ecological impacts associated with the spread and establishment of AIS (OFAH 2012), recreational boaters in the Trent-Severn Waterway continue to transport (at least one) AIS and thus pose a significant risk to surrounding waterways. We recommend that future conservation efforts should focus on increasing awareness of effective boater hygiene practices in order to reduce the spread of AIS in the Lake Simcoe region. For example, during our survey, we observed that many boaters were unaware of the water that was present in their bilge, often stating it was empty when upwards of 13 L was then found. Most of the macrophytes collected were snagged on the propeller and/or anchor line. Decreasing the amount of water that boats are carrying in their bilge, draining any standing water prior to entering every new waterway and/or lock chamber, as well as removing all attached macrophytes, would substantially reduce the number of species and individuals being transported among waterways, and are thus behaviours that should be emphasized in all future education programs. Finally, most boater traffic originates within Lake Simcoe and travels outwards to other lakes, making these boats the primary group of concern for increasing the invasion risk to surrounding waterways. Since these boats remain in the water while travelling through the lock system and between various waterways, it may be beneficial for the staff assisting in lock operations to be involved in ensuring that the boaters comply with recommended boat hygiene practices.

Acknowledgments We thank Lynne Witty of Laurentian University for her taxonomical expertise and technical support, and Parks Canada for allowing sampling at the various locks. Funding was provided by the Ontario Ministry of Natural Resources and York University to NDY, and from a Natural Sciences and Engineering Research Council Post-doctoral Fellowship to NK.

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