

Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes

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Abstract: Most ships entering the Great Lakes carry cargo and declare “no-ballast-on board” (NOBOB) status. Approximately 250 of these vessels annually load Great Lakes’ ballast water when they offload inbound cargo and then discharge this water (which has now mixed with residual water previously present in the tanks) when they load outbound cargo. This procedure potentially allows nonindigenous species present in ballast residuals to invade the Great Lakes. We collected residual sediment, water, and associated organisms from 38 NOBOB ships entering the Great Lakes. We recorded seven established Great Lakes’ nonindigenous species, including some discovered since ballast water exchange was implemented. Occurrences of species not yet invaded indicate that this vector provides further opportunity for invasion. Collectively, NOBOB vessels appear to constitute a greater risk than ballasted vessels, as they make up a greater proportion of the traffic entering the lakes (~90%), and they do not undergo ballast exchange. Invertebrates in residual water appear to have a greater opportunity for discharge than those in sediments, although most in the water fraction have already invaded this system. Invertebrate numbers in residual freshwater ballast could be dramatically lowered if these vessels flushed with open-ocean water prior to entering the Great Lakes.

Résumé : La plupart des navires qui pénètrent dans les Grands Lacs sont chargés de marchandises et déclarent n’avoir pas d’eau de ballastage à bord (statut NOBOB). Environ 250 de ces navires chaque année se chargent d’eau de ballastage en provenance des Grands Lacs lors du débarquement des marchandises à destination locale et déchargent cette eau (maintenant mêlée à l’eau résiduelle déjà présente dans les ballasts) lors du chargement des marchandises destinées à l’exportation. Cette procédure permet potentiellement aux espèces non indigènes présentes dans les eaux de ballastage résiduelles d’envahir les Grands Lacs. Nous avons prélevé des sédiments et de l’eau résiduels ainsi que les organismes associés dans 38 navires NOBOB arrivant dans les Grands Lacs. Nous y avons retrouvé sept espèces non indigènes déjà établies dans les Grands Lacs, dont certaines découvertes depuis que la politique d’échange des eaux de ballastage est en vigueur. La présence d’espèces qui n’ont pas encore envahi les Grands Lacs indique que ce véhicule de transmission procure des occasions pour des envahissements futurs. Dans leur ensemble, les navires NOBOB semble constituer un risque plus élevé que les navires qui portent de l’eau de ballastage, parce qu’ils représentent un proportion plus importante (~90 %) du trafic maritime qui pénètre dans les Grands Lacs et qu’ils ne font pas d’échange de leurs eaux de ballastage. Les invertébrés dans les eaux résiduelles semblent être plus susceptibles d’être déversés que ceux dans les sédiments, bien que la plupart de ceux qui sont dans la fraction aqueuse ont déjà envahi le système. Le nombre d’invertébrés dans les eaux de ballastage résiduelles pourrait être considérablement réduit si les ballasts de ces navires étaient rincés à l’eau salée en haute-mer avant leur entrée dans les Grands Lacs.

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Introduction

Distributions of species change naturally but gradually through time. Human activities have increased both the rate and the spatial scale of this movement far in excess of back-

ground levels owing to deliberate or accidental translocation of organisms (e.g., Hebert and Cristescu 2002). To date, more than 170 nonindigenous species (NIS) are recognized as having established populations in the Laurentian Great Lakes, including ~90 animal species (Ricciardi 2001;

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Holeck et al. 2004). Dispersal mediated by transoceanic commercial shipping has been the dominant vector for these introductions since 1959, after the opening of the St. Lawrence Seaway allowed entrance of large bulk-carrying shipping vessels (Mills et al. 1993; Ricciardi and MacIsaac 2000; Ricciardi 2001). As a result, efforts to reduce the rate of invasions have focussed on ballast water management. Mandatory midocean ballast exchange was implemented for ships entering the Great Lakes in 1993, whereby ships carrying fresh or brackish ballast water from ports outside the system are effectively required to declare "ballast-on-board" (BOB) status and exchange existing ballast water for ocean water during passage (>200 nautical miles from any shore and in waters >2000 m in depth; US Coast Guard 1993). Ballast water exchange is intended to expel any organisms potentially capable of surviving Great Lakes conditions during ballast discharge and to kill individuals remaining in the tanks through osmotic shock from exposure to high-salinity water.

Since ballast exchange was implemented, however, a number of additional species have been reported from the Great Lakes (Holeck et al. 2004). These species are primarily associated with sediments rather than with water (e.g., harpacticoid copepods, rhizopods, and oligochaetes; Horvath et al. 2001; Grigorovich et al. 2003a; Nicholls and MacIsaac 2004). One hypothesis that may explain these patterns is that vessels declaring "no-ballast-on-board" (NOBOB), which are exempt from current ballast management regulations, could be responsible. On average, these vessels constituted ~90% of those entering the system (Colautti et al. 2003) and carry ~60 t of unpumpable residual water (~76%) and sediment (~24%) that may contain viable organisms (Bailey et al. 2003). After unloading cargo within the system, these ships load Great Lakes' water into their ballast tanks. Before exiting the system, they will typically load new cargo for export and expel their ballast water, potentially including organisms associated with the original residual ballast (Colautti et al. 2003). Using a modelling approach, MacIsaac et al. (2002) predicted that NOBOB ships could pose a greater collective risk for invasions than BOB ships undergoing ballast exchange. However, the invasion risk posed by live fauna associated with sediment or residual water from these ships has not yet been quantified. Sediments have, however, been shown to contain viable dormant stages of protists and zooplankton (Hallegraeff and Bolch 1992; Kelly 1993; Bailey et al. 2003). Locke et al. (1991) attempted to sample sediment-associated fauna from ballasted ships by disturbing the sediments with a net. However, this method was deemed inadequate and they suggested tank entry in deballasted ships as the best possible alternative.

Owing to the typically impossible task of eradicating established NIS, it is important to identify high-risk vectors and important invasion pathways from donor regions. Such information is necessary so that risk can be ascertained and methods devised to reduce it. Here, we assess whether residual water and sediments of NOBOB ships provide an invasion risk to the Great Lakes. We examine the identity, abundances, and frequencies of live organisms associated with these residuals in NOBOB ships entering the Great Lakes. We also assess conditions that may provide useful

predictors of species introductions, including high-risk donor regions. We pay particular attention to crustaceans and oligochaetes, as these groups have recent invasion histories in the Great Lakes attributable to ballast water discharge.

Methods

Ballast sediment and residual water were collected from transoceanic ships entering the Great Lakes (i.e., at the first port of call) between 8 March 2001 and 6 December 2002. Living organisms present in ballast tanks at this time have survived the initial, arduous transportation phase of the invasion process (Carlton 1996). These individuals may serve as an inoculum if they survive input of Great Lakes water into the tanks and are subsequently released live at the final port-of-call. We collected samples from 67 tanks in 33 different ships. One ship was sampled on three occasions and three others on two occasions, totaling 38 ship visits. However, on only one occasion was the same tank sampled twice, and visits were separated by over 2 months with a number of ballasting and deballasting events in between. We therefore treat these as separate ship visits in our analyses. Vessels were boarded opportunistically without regard to previous ports of call (i.e., without any predetermined considerations of probable geographical sources of ballast residuals).

Sediment was collected from a subset of 64 tanks from 36 ships. Sediment was collected from at least five areas of each tank in areas of accumulation, usually along longitudinal shell frames away from drainage flows, using sterile scoops and spatulas. Where possible, approximately 5 kg of sediment was collected into a bucket from each tank. After exiting the ship, the sediment was thoroughly stirred and two 500-g subsamples were weighed and preserved in 95% ethanol. The temperature of residual ballast water was recorded at the time of collection (see below) using a Fisher Scientific thermometer and used as a proxy measure of sediment temperature. On return to the laboratory, sediment pore water salinity was measured of the supernatant extracted by centrifugation of a 200-g sediment subsample (3300g for 15 min) using an optical refractometer. Total residual water and sediment loads were estimated using current and historical records of tank residuals from ship crews as well as visual observations made by an experienced shipping vessel consultant (P.T. Jenkins). Time since last ballast tank cleaning and ports where ballast water was last loaded were determined by consultation with ship crews and logs. On one occasion, only one 500-g subsample could be collected from a tank, and for one tank, only one subsample could be examined owing to excessive oil contamination in the sample.

Residual ballast water samples and associated taxa were collected from 64 tanks from 35 ships. Where possible, 50 L of residual water was pumped using a hand bilge, passed through a 30- μ m-mesh net, and the retained material preserved in ethanol. Residual water was collected from a single area, as the water was assumed to be well mixed within each tank. Salinity of residual water was measured using an optical refractometer at the time of collection.

In the laboratory, sediment samples were washed through a 45- μ m-mesh sieve to remove fine sediment. Associated animals were subsequently removed from the remaining sedi-

ment using a Ludox HS40 protocol (Burgess 2001). Organisms from water and sediment samples were enumerated and identified to the lowest level practical using dissecting and compound microscopes. Nematodes from sediment samples were identified from a subset of 16 tanks from 10 ships. For analyses, sediment and water samples were expressed as numbers of organisms per kilogram (wet weight) and organisms per litre, respectively. We acknowledge that expressing sediment organisms relative to sediment wet weight might provide some variability owing to differing substrate characteristics; however, sediments were typically similar in that they were composed of fine sediments, compacted, and collected from areas that were moist but not typically flooded.

We explored whether ballast history parameters could predict high risk ships (i.e., ships that had high abundances of animals). Relationships between the total abundance of sediment organisms and temperature, pore water salinity, total residual sediment, time since last ballasting, and total ballast capacity were analysed using stepwise linear regression using Statistica 6 (StatSoft Inc., Tulsa, Oklahoma). Missing temperature data, not recorded from tanks owing to absence of residual water, were approximated by linear interpolation of values measured from ships sampled before and after that date, as this varied predictably with season. Tanks were excluded from the analysis where environmental conditions (i.e., salinity) did not match the ballast history provided by the crew, giving a final data set containing 58 tanks from 35 ships. Water samples were treated similarly, with tanks removed if environmental variables did not match ballast history or if less than 50 L was collected, resulting in 59 tanks from 33 ships used for analysis. Relationships were examined for these using temperature, residual water salinity, total residual water volume, time since last ballasting, and total ballast capacity as independent variables. For all analyses, abundances and environmental variables for both sediment and water samples were averaged for each ship, since tanks within ships shared common ballast histories. Abundances and environmental data were transformed to improve normality where necessary (usually $\log(x + 1)$). In addition, we investigated the relationship of total abundance of invertebrates to region of ballast origin. Region of ballast origin (i.e., the last port where ballast was loaded into tanks) was assigned to broad oceanic regions: Baltic Sea, Mediterranean and Black seas, North Sea, northwest Pacific Ocean, west-central Atlantic Ocean, Great Lakes, and "other". Median abundances of taxa from each region of ballast origin were examined for trends.

Some organisms in the residual ballast likely do not have the potential to invade owing to physiological constraints (e.g., salinity tolerance). Thus, total organism abundance may overestimate invasion risk posed by live animals in residual ballast. We therefore explored for trends in region of ballast origin using the combined total of freshwater and brackish water rotifers, cladocerans, and copepods per ship. Since copepods living both in fresh water (e.g., *Nitocra hibernica* and *Cyclops strenuus*) and in brackish water (e.g., *Onychocamptus mohammed* and *Schizopera borutzkyi*) have invaded the Great Lakes, this subset of organisms was chosen owing to their well-defined taxonomy and salinity pref-

erences. We did not include species with preferences for higher salinity (i.e., those preferring highly brackish water to marine water).

Propagule supplies

Total propagule supplies of sediment and water animals entering the Great Lakes per year were estimated based on average densities of organisms entering in ships, the average volume of water or sediment components, and the total number of NOBOB ships discharging water in the Great Lakes per year. Data on NOBOB ship activity between 1994 and 2000 were obtained from Colautti et al. (2003). Estimation of propagule supplies for freshwater and brackish water animals were calculated similarly as an indication of actual risk.

To determine whether NOBOB sediments and water pose an invasion risk, we compiled a list of the NIS of copepods and cladocerans found in ballast residuals that are capable of surviving freshwater and brackish water conditions. Taxa were ordered based on frequency and abundance potentially discharged and a comparison made of the relative frequencies and abundances of those that have and have not invaded.

Results

Taxonomic composition of residual sediments

Collected organisms belonged to a broad array of taxonomic groups (Appendix A), although meiofaunal groups dominated numerically. Three of the 35 ships had no taxa present in their sediment samples. Nematodes dominated the overall relative abundances (91%) followed by harpacticoid (5%) and cyclopoid copepods (3%). Nematodes occurred in 91% of ships entering the Great Lakes and harpacticoids 46% and cyclopoids 49%. Based on our samples, these taxa contribute almost 99% of all organisms entering the Great Lakes associated with ballast sediment. Of the remaining taxa, polychaetes were the most abundant and were relatively common (<1% abundance, 23% of ships). Other taxa had low abundances and were recorded from few or single tanks (i.e., <20% of ships).

Nematodes were the most species-rich group, with 48 taxa recorded from the subset of 10 ships. This total includes numerous taxa not reported from the Great Lakes or North America. A total of 35 copepod species were identified from the 33 ships. Twenty harpacticoid species were identified, three of which are native to the Great Lakes: *Bryocamptus zschokkei*, *Canthocamptus staphylinoides*, and *Nitocra spinipes*. Three others are NIS already established in the Great Lakes: *N. hibernica*, *O. mohammed*, and *S. borutzkyi*. Additionally, two are freshwater taxa not known to have populations in the Great Lakes: *Bryocamptus pygmaeus* and *Canthocamptus staphylinus*. Four others are brackish water fauna (*Halectinosoma curticorne*, *Harpacticus uniremis*, *Microarthridian littorale*, and *Schizopera baltica*), while the remaining eight species are typical of more saline conditions. Eleven cyclopoid species were identified, 10 of which are freshwater species. Six of these species are known from the Great Lakes, including *C. strenuus*, a probable invader, which was recorded from two ships. Four species, *Mesocyclops leuckarti*, *Paracyclops fimbriatus*, *Thermocyclops crassus*, and *Thermocyclops oithonoides*, are freshwater spe-

cies that do not have established populations in the Great Lakes. One marine calanoid copepod species and two marine poecilostomatoid copepod species were also recorded. Two epibenthic cladoceran species (*Alona quadrangularis* and *Ilyocryptus sordidus*) were recorded, both of which are cosmopolitan taxa presumably native to the Great Lakes.

Another group with a reported invasion history in the Great Lakes, the oligochaetes, comprised only four species and 0.2% of animals recorded and were present in 14.3% of ships.

Taxonomic composition of residual waters

The taxonomic composition of water fauna differed greatly from that of sediments (Appendix A). There was overlap in occurrence of some taxa, especially where species occur naturally in both habitats (e.g., cyclopoid copepods), while some others were epibenthic species likely sampled incidentally. One ship contained no taxa in the residual water samples, although several additional ships were not sampled owing to an absence of residual ballast water. Copepods formed the most abundant group in residual waters (97.3% of abundance: 66.0% nauplii, 20.4% cyclopoids, and 10.8% harpacticoids, with calanoids and poecilostomatoids comprising the remainder). Rotifers were the next most abundant taxon at 1.2% of total abundance. Remaining taxa collectively made up <1.5% of total abundance.

Copepods were the most species-rich group, with 5 calanoid, 12 cyclopoid, and 10 harpacticoid taxa recognized. This total includes 13 species already recorded from the Great Lakes, including three established NIS (*Eurytemora affinis*, *S. borutzkyi*, and *C. strenuus*). Ten of the remaining 14 species are marine taxa, which presumably would not survive if introduced into the Great Lakes (e.g., Schallenberg et al. 2003), leaving four freshwater or brackish water species (cyclopoid copepods: *Acanthocyclops venustus*, *Cyclops abyssorum*, *Eucyclops serrulatus*, and *Halicyclops* sp.) that could potentially tolerate conditions in the lakes. At least eight cladoceran species were recorded, of which three are not established in the Great Lakes; one of these, *Daphnia magna*, is a North American species, while the other two, *Daphnia cristata* and *Daphnia atkinsoni*, are European natives. Both of the latter species were recorded as single individuals only, however. *Bosmina maritima* was also recorded, which is a known invader. Seven rotifer species were identified, all of which are native to the Great Lakes. At least three Gammarus species (Amphipoda) were identified, all of which are from European estuarine brackish waters. Small bivalves were recorded on several ships, including *Driessena* veligers. However, these were typically present in low abundance and frequency overall.

Determinants of propagule supply

Total animal abundance in sediments was inversely correlated with pore water salinity, although the explained variance was low (stepwise multiple regression, $r^2 = 0.267$, $p < 0.005$) (Fig. 1). None of the other variables assessed significantly affected animal abundances ($p > 0.05$). Similar results were found for residual water data, with salinity significantly related to total invertebrate abundance (stepwise multiple regression, $r^2 = 0.198$, $p = 0.012$) (Fig. 1). Twenty percent of ships entered the Great Lakes with freshwater resid-

uals (0‰–2‰), 23% with brackish water residuals (3‰–10‰), and 57% with saltwater residuals (>11‰) ($n = 35$).

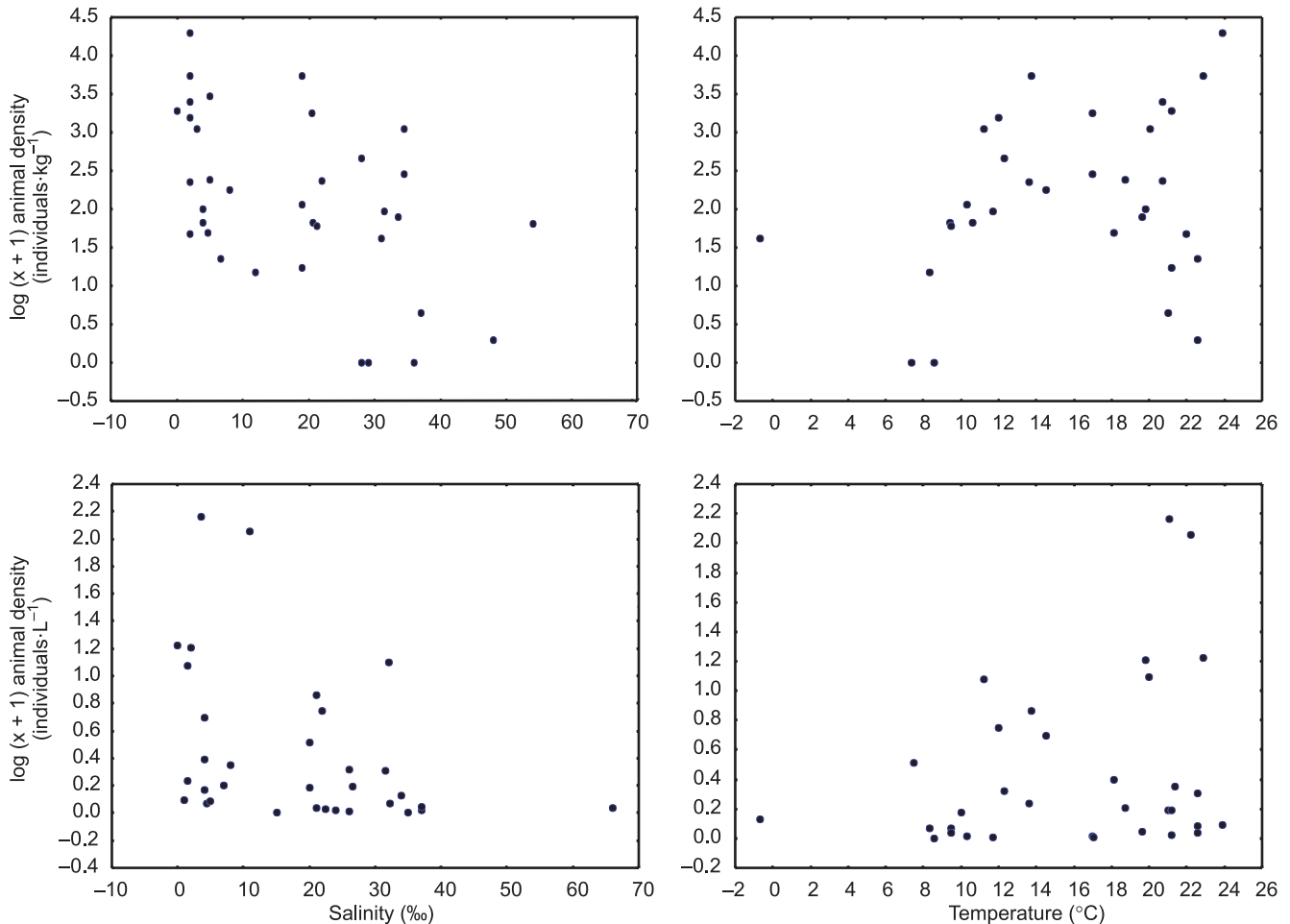
Salinity was broadly related to region of ballast origin (Table 1). Ships that last ballasted at ports in the Great Lakes, North Sea, Baltic Sea, or Mediterranean and Black Sea regions typically had low-salinity residual ballast water and sediment (usually <10‰). No clear relationship existed between total numbers of animals and region of ballast origin, except that areas with medium to high salinities (>20‰) had lower median abundances than those with salinities <20‰. However, examination of freshwater and brackish water animals showed a clear affinity for ships that had most recently loaded ballast at low-salinity ports, with those on the North Sea, Great Lakes, and Baltic Sea having the highest abundances as compared with other regions. This may indicate that freshwater and brackish water taxa are relatively transient and dependent on the last ballast source. Nematodes, on the other hand, may be more or less resident.

Propagule supplies transported to the Great Lakes

The average number of NOBOB ships entering the Great Lakes between 1994 and 2000 was 484 (SD = 75), of which 249 (SD = 69) subsequently loaded and then discharged mixed Great Lakes ballast water into the Great Lakes (Colautti et al. 2003). Ships averaged 15 t (= 15 000 kg, range = 0.1–65.0 t) of ballast sediment and 46.8 t (= 46 800 L, range = 0.0–153.0 t) of residual water. Thus, at average animal densities of 1322.5 individuals·kg⁻¹ in ballast sediment, NOBOB ships had an opportunity to discharge 49.5×10^8 individuals into the Great Lakes from sediment per year (Fig. 2). Similarly, the average number of propagules potentially flushed annually from residual water is 12.7×10^7 . Thus, on average, a total of 50.7×10^8 sediment and waterborne animals may have the opportunity for introduction into the Great Lakes each year by NOBOB vessels. For freshwater and brackish rotifers, cladocerans, and copepods, totals of 19.8×10^7 and 27.6×10^6 propagules potentially enter the Great Lakes per year from sediments and residual water, respectively. Some of these taxa may already exist in the Great Lakes or have originated from previous ballasting in the Great Lakes. Thus, propagule supply of nonindigenous freshwater and brackish copepods and cladocerans entering in sediments (see Table 2), including those already invaded, results in 29.1×10^6 entering per year. Excluding those already invaded, the total is reduced to 25.9×10^6 ·year⁻¹. From residual water, the propagule supply of these organisms (including those already invaded) is 15.7×10^5 ·year⁻¹ and, excluding those already invaded, is 20.5×10^4 ·year⁻¹. Thus, most of the nonindigenous propagule supply from the water fraction comprises species that have already invaded, while the sediment fraction contains many that have not yet invaded.

Twenty-one nonindigenous copepod and cladoceran species recorded from residual sediments and water, including those already invaded, were examined based on propagule supplies to the Great Lakes (Table 2). In general, species that enter the Great Lakes more frequently, and are presumably released more frequently, were more likely to have established populations (e.g., *S. borutzkyi* and *E. affinis*) than those that were released less frequently or in smaller numbers (e.g., *D. cristata* and *D. atkinsoni*).

Fig. 1. The $\log(x + 1)$ total abundance of sediment organisms versus pore water salinity and temperature and the $\log(x + 1)$ total abundance of residual water animals versus residual water salinity and temperature. Relationships were also examined for total residual sediment or water volumes, time since last ballasting, and total ballast capacity (not shown), which showed no discernible patterns. Statistically significant relationships were found between pore water salinity and total animal abundance in residual sediments (stepwise multiple regression, $r^2 = 0.267$, $p < 0.005$), and for residual water salinity and total animal abundance in residual waters (stepwise multiple regression, $r^2 = 0.198$, $p = 0.012$). None of the other variables assessed were important in determining animal abundances ($p > 0.05$).



Discussion

Comparison of shipping subvectors

There are several entry mechanisms associated with transoceanic shipping that can introduce NIS to the Great Lakes: fouling of hull and water intake surfaces, ballast water and (or) sediments contained in ships carrying BOB, and residual water and (or) sediments contained in ships declaring NOBOB status; the sediment subvectors can further be subdivided into active or dormant individuals present in sediments. It is difficult to determine the relative importance of ship-related mechanisms, as many established NIS could be introduced via multiple pathways and because there have been few comprehensive studies of these subvectors (Holeck et al. 2004).

The mean density of active animals transported in sediments in this study, $49.5 \times 10^8 \cdot \text{year}^{-1}$, is higher than that of dormant stages from the same sediments, $90.0 \times 10^7 \cdot \text{year}^{-1}$ (cf. Bailey et al. 2005a). However, many of the active and dormant sediment taxa are buried or have adaptations to ensure that they remain in association with sediments, even

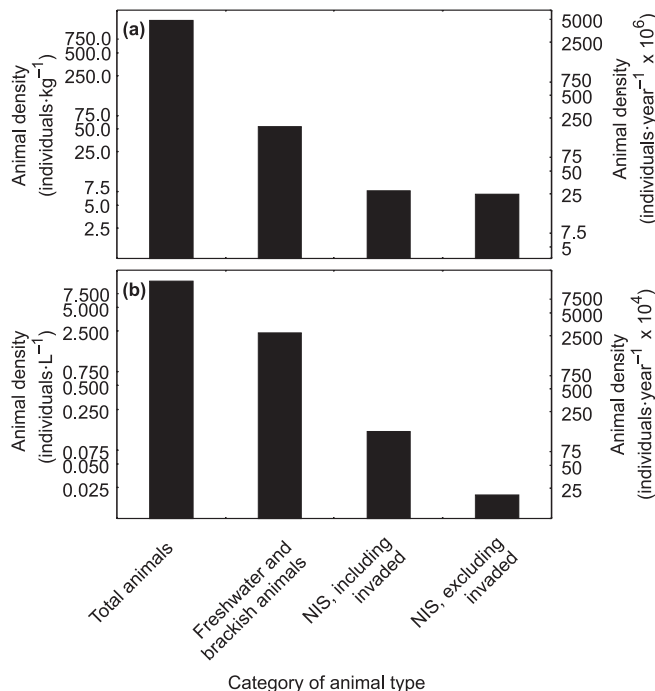
during flow or turbulent conditions, and will have little chance for discharge from ballast tanks. As such, only a proportion of these will enter the lakes with discharged ballast at their final Great Lakes port of call. Risk is likely to vary by taxon, however. For example, nematodes will occur within the sediment and are less likely to be discharged, while more epibenthic taxa (e.g., harpacticoids) may be discharged more readily. This may reflect why most of the common epibenthic nonindigenous organisms found in this study have already invaded the system. In contrast, active invertebrates in residual water are available for discharge at a mean density of $12.7 \times 10^7 \cdot \text{year}^{-1}$. Despite the density of active and dormant taxa in sediments being greater than the number of invertebrates in residual water, planktonic animals probably have greater opportunities for discharge with ballast water (see MacIsaac et al. 2002). Evidence for this comes from the difference between the numbers of freshwater and brackish water NIS that have and have not invaded the system to date; a large proportion of the nonindigenous propagule supply in the water fraction comprises taxa that have already invaded (87%), while only 11% of the

Table 1. Range and median values of salinity, total animals, and freshwater to brackish rotifer, cladoceran, and copepod taxa recorded from sediment and water residuals in ballast tanks of ships entering the Great Lakes.

Ballast origin	n	Salinity (‰)		Total animals (individuals·kg ⁻¹)		Freshwater – brackish water animals (individuals·kg ⁻¹)	
		Range	Median	Range	Median	Range	Median
Residual sediment							
Great Lakes	1	4.0	4.00	98	98.00	11	11.00
North Sea	15	0.0–28.0	5.00	24.33–5510	465.00	0–905	19.50
Baltic	3	2.0–12.0	8.00	14–2538	178.50	0–157.6	2.00
Mediterranean and Black Sea	3	2.0–29.0	15.50	0–19911	9955.50	0.0	0.00
Northwest Pacific	4	19.0–34.5	24.33	0–1127.5	90.83	0–1.33	0.25
Other	2	19.0–31.5	25.25	16–94.33	55.16	0.0–2.50	1.25
West-central Atlantic	8	4.8–54.0	35.25	0–280.66	45.00	0.0	0.00
Residual water							
Baltic	2	4.0–4.5	4.25	0.17–3.94	2.06	0.08–0.43	0.26
Great Lakes	2	2.0–8.0	5.00	1.26–15.16	8.21	1.24–12	6.62
North Sea	13	0–26.5	7.00	0.09–143.5	1.08	0–40.4	0.10
Mediterranean and Black Sea	2	1–15	8.00	0–0.24	0.12	0	0.00
West-central Atlantic	8	4–66	27.75	0.04–2.25	0.44	0–1.85	0.11
Other	2	22.5–35	28.75	0.01–0.06	0.04	0	0.00
Northwest Pacific	4	0–37	32.15	0.09–11.46	0.11	0–0.15	0.00

Note: Region of ballast origin is ordered by median salinity. Salinity ranges for residual sediments were recorded from the pore water salinity of the sediment.

Fig. 2. Propagule loads transported to the Great Lakes in residual (a) sediment and (b) water of ships that declare “no-ballast-on-board” (NOBOB) status. Animals potentially available for discharge (right scale) are based on 249 NOBOB entries that load and subsequently discharge ballast water in the Great Lakes system per year. Vessels carried an average of 15.0 t of residual water and 46.8 t of residual sediment. NIS, nonindigenous species.



nonindigenous propagule supply in sediments have invaded to date. Thus, despite the sediments containing higher densities of nonindigenous propagules overall, only the most fre-

quently occurring epibenthic species may be able to invade the Great Lakes system. Further, in situ hatching studies suggest that <1% of invertebrate diapausing eggs will hatch and be available for introduction (Bailey et al. 2005b). Therefore, residual ballast water may pose the greatest risk for introduction of invertebrates.

Despite carrying larger volumes of ballast water, ships in BOB status probably present a very low risk of NIS introduction, owing both to the type of water carried (saline) and to the relatively low number of BOBs entering the Great Lakes (Colautti et al. 2003). In addition, hull fouling is probably a minor vector, since freshwater taxa that could survive in the Great Lakes are unlikely to withstand the high salinity encountered during a transoceanic voyage. Although early studies suggested that freshwater taxa may be able to survive incomplete ballast exchange (Locke et al. 1991, 1993), more recent work suggests that ballast exchange is usually very thorough (95%–99%) for ships that exchange ballast through an empty–fill procedure (Ruiz et al. 2004). Thus, presumably only small numbers of euryhaline taxa could exploit these vectors. In terms of ballast sediments, propagule loads of invertebrates (active and dormant stages) should be similar on individual BOB and NOBOB vessels, assuming that they operate in similar trade areas. Again, however, far greater numbers of NOBOB vessels discharge ballast water in the Great Lakes than BOB vessels, and freshwater propagules in BOB ships will be reduced owing to ballast exchange.

Taxonomic composition of residual ballast

Although ballast water is recognized as the dominant vector by which many NIS have entered the Great Lakes, few of the NIS established have ever been collected from ballast water surveys (e.g., *C. strenuus*, *E. affinis*, *Bosmina coregoni*, and *Dreissena veligers*) (e.g., Locke et al. 1991, 1993; Aquatic Sciences, Inc. 1995). We report an additional four

Table 2. Nonindigenous copepod and cladoceran species recorded from residual ballast sediment and water capable of surviving in brackish or freshwater habitats.

	NIS	Sediment		Water		Habitat
		No. of ships	Abundance ($\times 10^5$)	No. of ships	Abundance ($\times 10^4$)	
<i>Schizopera borutzkyi</i>	*	4	13.89	1	0.63	br
<i>Eurytemora affinis</i>	*			4	7.42	fw, br
<i>Canthocamptus staphylinus</i>		3	9.08			fw
<i>Onychocamptus mohammed</i>	*	3	5.30			br
<i>Cyclops strenuus</i>	*	2	10.65	2	126.26	fw
<i>Nitocra hibernica</i>	*	2	2.59			fw
<i>Paracyclops fimbriatus</i>		1	217.96			fw
<i>Schizopera baltica</i>		1	3.20			br
<i>Bryocamptus pygmaeus</i>		1	3.20			fw
<i>Mesocyclops leuckarti</i>		1	2.13			fw
<i>Eucyclops serrulatus</i>				1	12.73	fw
<i>Harpacticus uniremis</i>		1	1.07			br
<i>Thermocyclops crassus</i>		1	1.07			fw
<i>Thermocyclops oithonoides</i>		1	1.07			fw
<i>Acanthocyclops venustus</i>				1	5.31	fw
<i>Bosmina maritima</i>	*			1	1.77	fw
<i>Halicyclops</i> sp.				1	1.77	br
<i>Cyclops abyssorum</i>				1	0.71	fw
<i>Daphnia atkinsoni</i>				1	0.36	fw
<i>Daphnia magna</i>				1	0.36	fw
<i>Daphnia cristata</i>				1	0.36	fw

Note: Abundance is the number of organisms potentially entering the Great Lakes based on average densities in ships that declare "no-ballast-on-board" (NOBOB) status, average volumes of sediments and water in the ballast tanks of the ships, and the number of NOBOB ships discharging ballast into the Great Lakes per year. Taxa are ordered based on frequency and abundance. Asterisks denote nonindigenous species (NIS) already established within the Great Lakes. fw, freshwater taxa; br, brackish water taxa.

known NIS in the Great Lakes that may be linked with ballast water and sediments (three harpacticoid copepod species and *B. maritima*) and provide further records of *E. affinis*, *C. strenuus*, and *Driessena veligers*. Based on these findings, we believe that it is not a coincidence that all of the free-living nonindigenous animal species recorded as newly established populations in the Great Lakes since 1993 are either benthic or epibenthic taxa (e.g., harpacticoids, annelids, and amphipods) or possess diapausing stages (e.g., cladocerans) that may accumulate in sediments (see Grigorovich et al. 2003a). Such organisms are consistent with the NOBOB ship entry vector.

Nematodes and harpacticoids numerically dominated the ballast sediment fauna. In freshwater, brackish water, and marine systems also, these groups typically have the greatest abundance (e.g., Smol et al. 1994; Santos et al. 1996; Leeper and Taylor 1998). Free-living freshwater nematodes are a poorly studied taxonomic group in North America and thus contain numerous unreported and undescribed genera and species (Pennak 1978; Poinar 1991). As such, no NIS of nematode has been recognized from the Great Lakes to date. However, nematodes were the most abundant and species-rich taxon in ballast sediments, and the majority of species identified have not yet been recorded from any North American freshwater habitat (cf. Pennak 1978; Poinar 1991). A thorough examination of the nematode fauna of the Great Lakes is warranted.

Three of the five known Great Lakes nonindigenous harpacticoid species were recorded during this study: *S. borutzkyi* (four ships), *O. mohammed* (three ships), and *N. hibernica* (two ships). Harpacticoids have been well represented in the occurrences of new NIS recorded since 1993, when ballast regulations were enacted, with the first recorded occurrences of *Heteropsyllus* sp. (1996), *S. borutzkyi* (1998), and *Nitocra incerta* (1999) since this time (see Grigorovich et al. 2003a). Additionally, *O. mohammed* was first recorded in 1992, after Canadian voluntary ballast exchange regulations were in place. Horvath et al. (2001) argued that the recent timing of discovery of *Heteropsyllus* sp. and *S. borutzkyi*, at least, is not an artefact of study effort for this group, as they dominated the areas where they were collected and had not been encountered previously despite a number of prior sampling efforts over previous years. The presence of these recently reported NIS indicates that NOBOB ships may be an active vector for NIS introductions into the Great Lakes.

The occurrence of two freshwater harpacticoid species not yet recorded from the Great Lakes, *C. staphylinus* (three ships) and *B. pygmaeus* (one ship), illustrates that new species still have opportunities for invasion. Both species have previously been recorded from North American fresh waters (although the former record is questionable) but not from the Great Lakes (Wilson and Yeatman 1959). Species tolerating wide ranges of salinity concentrations, particularly those originating from the Ponto-Caspian region, have also invaded

the Great Lakes (e.g., Ricciardi and MacIsaac 2000). This includes the oligohaline harpacticoid *S. borutzkyi*, native to the Danube River delta of the Black Sea (Horvath et al. 2001), recorded from four ships in the current study. Thus, harpacticoids of brackish water origin, including a Black Sea endemic *H. uniremis* (one ship), may also pose a risk.

Cyclops strenuus, the only cyclopoid species considered an NIS in the Great Lakes (Grigorovich et al. 2003b), was the only cyclopoid that we recorded from more than one ship. Eight other freshwater or brackish water cyclopoids not known from the Great Lakes were also recorded, in all cases from single ships only. Of these, *M. leuckarti* and *P. fimbriatus* have been historically reported from North America, but these older records apparently refer to other species (e.g., *Mesocyclops americanus* and *Paracyclops poppei*; Wilson and Yeatman 1959; Hudson et al. 1998). As all of the other species were recorded from single ships, and in relatively low numbers, their probability of successful establishment may be constrained by propagule supply.

Calanoid copepods are not typically found associated with shallow water or sediments and were therefore found in low abundance (or as single occurrences) in the current study. The only species recorded with moderate frequency was *E. affinis* (four ships), unusual among calanoids as being an epibenthic species (Evans and Stewart 1977). This taxon has also been recorded from ballasted ships entering the Great Lakes (Locke et al. 1991) and has already invaded the system. The risk of invasion by planktonic calanoid copepods may thus have been greatly reduced by midocean exchange in ballasted vessels. Compared with harpacticoids, few propagules of active freshwater calanoid species likely enter the Great Lakes via shipping. However, the egg bank contained in sediments of NOBOB ships may provide opportunities for some species (see Bailey et al. 2003). Three poecilostomatoid species were also recorded and have commonly been recorded on BOB vessels (e.g., Williams et al. 1988; Locke et al. 1991). However, these likely present no risk because of their marine constitution.

Ten species of Cladocera were identified, including three not yet known from the Great Lakes (all *Daphnia* species) and the established invader *B. maritima*. No predatory cladocerans were recorded (e.g., *Cercopagis* or *Bythotrephes*). The entry mechanism for *Cercopagis*, first recorded in 1998 post-implementation of ballast exchange, therefore remains unconfirmed. All of the amphipod species recorded were estuarine European *Gammarus* species. Amphipods have invaded the Great Lakes, including *Echinogammarus ischnus* and *Gammarus tigrinus*, since ballast water exchange was commenced (van Overdijk et al. 2003; Grigorovich et al. 2005), and the presence of species in residual water indicates that further invasions may still occur.

Five oligochaete taxa were recorded from our ballast sediments but were typically in low numbers (comprising only 0.2% of all animals) and on few ships (14.3%). All of the taxa recorded are apparently native to the Great Lakes (Spencer and Hudson 2003). The finding of few oligochaete taxa is interesting given that estimates of the number of oligochaetes that have invaded the Great Lakes system range widely. Spencer and Hudson (2003) recognized three species as suspected NIS, although only one is thought to have entered with shipping as the vector (Mills et al. 1993).

Grigorovich et al. (2003b), in contrast, listed 15 aquatic oligochaete species considered nonindigenous or cryptogenic, although possible vectors were not provided. Unless oligochaetes are able to invade at low inoculum sizes, our findings suggest that oligochaetes are unlikely to enter via NOBOB ships. Polychaetes occurred relatively frequently on ships but are not represented in the NIS component of the Great Lakes fauna. Polychaetes have few representatives in freshwater environments worldwide (Klemm 1985), and those recorded at higher abundances in our study were from high-salinity residuals from ballast derived from coastal waters. These and other marine taxa are likely to be killed with incoming freshwater ballast in NOBOB vessels prior to being purged into the Great Lakes.

The organisms that we collected will not constitute the full complement of species entering the Great Lakes in NOBOB ships, but given the repeated sampling of some taxa, we have confidence that many of the most frequently occurring species were sampled. The proportion of ships from each region that we sampled is similar to that reported by Colautti et al. (2003) for NOBOB entries to the Great Lakes, providing confidence that our samples were from a representative array of ships. However, some less common regions were not represented. For example, Colautti et al. (2003) reported entries by Australian ships, while we did not sample any from this region. In addition, we will not have examined ships from all possible ports within each represented region. Each port is likely to have different environmental conditions, species, and probabilities of ballast uptake of species. This will vary temporally owing to seasonal activity of organisms or conditions that lead to stirring of sediments.

Determinants of propagule supply and potential invasion risks

Determining the factors that are correlated with high propagule supplies may provide management options to reduce the probability of new invasions. However, few clear relationships were found between organisms and environmental variables associated with ships in this study. A significant decrease in the number of organisms in both sediments and residual water was observed with increasing salinity, although the relationship was weak. In addition, decrease in propagule supplies at higher salinities is likely not caused by salinity per se. Regions of ballast origin show similar trends, with fewer animals generally originating from regions with marine ports and greater numbers from freshwater and brackish water areas. Thus, lower numbers of propagules may enter tanks in saline ports. Regardless, the total propagule supply to the Great Lakes, and most importantly freshwater propagule supply, is lower in ships with saline residuals. Poor correlations between invertebrate abundances and environmental variables, including salinity, likely reflect differences in the number of propagules entering tanks during ballasting operations. Uptake of organisms likely varies from port to port as well as seasonally.

Outside of the possible oligochaete invasions, crustaceans are the only animal group to have invaded the Great Lakes since ballast water exchange was implemented in 1993 (Grigorovich et al. 2003b). We thus listed crustacean species from ballast residuals that could potentially survive in the

Great Lakes based on having freshwater or brackish water tolerances. Most taxa with high occurrence frequencies (i.e., present in two or more ships) have already established in the Great Lakes (e.g., *E. affinis*, *S. borutzkyi*, *O. mohammed*, and *C. strenuus*). This finding is consistent with the propagule pressure hypothesis, with larger or more frequent introductions expected to have higher chances of successful establishment (e.g., Forsyth and Duncan 2001; Lounibos 2002). Such a relationship provides an opportunity to make predictions of which other species may invade this system. Of those species not yet established in the Great Lakes, *C. staphylinus* might have the greatest potential to invade based on the apparent frequency and inoculum sizes, as it is the only species recorded in more than one ship not yet recorded as an invader. Of those taxa recorded on single ships, only *B. maritima* has successfully established. Indeed, this species is planktonic and likely invaded via ballast water of a BOB ship, as it was first recognized prior to implementation of ballast exchange (1988; see De Melo and Hebert 1994).

Not all recent invasions can be linked to NOBOB ships, as some recently established harpacticoid species were not found in our survey (e.g., *N. incerta* and *Heteropsyllus* sp.). These taxa may have entered the Great Lakes in NOBOB ships as rare fauna. Alternatively, we cannot discount invasion through stochastic events of single large inoculation sizes, that donor ports have changed with variation in trade patterns since their introduction, or that conditions and biota have changed within donor regions (sensu Carlton 1996). We also cannot disregard alternative vectors (e.g., Reid and Pinto-Coelho 1994).

Future invasions may occur owing to new opportunities brought about by spread of species to ports involved in Great Lakes trade. One potential example of this is our finding of the harpacticoid *S. borutzkyi*. Prior to discovery in the Great Lakes, *S. borutzkyi* was known only from the Danube River delta of the Black Sea (Montschenko 1967). The most recent sites of ballast uptake for ships carrying this species, however, were at North Sea ports (Antwerp and Ghent, Belgium, Rotterdam, Netherlands, and Bremmen, Germany). A better knowledge of fauna in global ports is required, which will rely on frequent examination of these sites. Alternatively, *S. borutzkyi* may survive well or even be capable of establishing populations within ballast tanks. Survivorship time in ballast tanks of harpacticoids, in particular, may be lengthy. For example, Chu et al. (1997) found that harpacticoids persisted in ballast water for up to a year, and Gollasch et al. (2000) found this group to survive well in ballast tanks relative to other organisms. Supporting this, harpacticoid composition did not always reflect previous port of ballast origin in our study. For example, a single individual of the Black Sea endemic *H. uniremis* was recorded from a ship that last ballasted at a western Atlantic port. Species with adaptations to survive in ballast tanks will presumably be dispersed with greater frequency and abundance and therefore pose a greater invasion risk.

Owing to high abundance and presence of nonindigenous representatives, harpacticoid and cyclopoid copepods, and possibly nematodes, appear to pose the greatest risk of invasion by NOBOB ships, although we cannot disregard stochastic events involving other taxa. The importance of

benthic taxa (e.g., nematodes and harpacticoid copepods) suggests that animals commonly enter ballast tanks with sediment because water either is loaded close to the sediments in some ports or is loaded in turbulent ports (e.g., rivers). Some animals, such as polychaetes and other meroplanktonic taxa, may enter as larvae, which settle once brought in by ballast water. However, these taxa are typically marine and thus should pose little risk to the Great Lakes.

NOBOB ships appear to pose an invasion risk to the Great Lakes, with risk being greatest for animals in residual waters and lower for those buried in ballast sediment. Some recent introductions into the Great Lakes are consistent with a NOBOB ship entry vector. NOBOB ships also may pose a risk to any global system in which ballasting and subsequent deballasting occur without exchange by water of opposing salinity. Currently, structural and operational limitations prevent complete discharge of water and sediments from tanks, and increased efficiency of these systems will likely reduce the supply of propagules. Another option for reducing invasion risk to the Great Lakes by freshwater organisms within residuals is ballasting a lens of salt water into the tanks (i.e., partial ballast exchange). Flushing ballast tanks with small amounts of salt water may aid in dislodging and removing residual sediment from the bottom of the tanks and by decreasing densities of freshwater taxa in ballast residuals (Locke et al. 1993). Moreover, the resultant brackish residual water may adversely affect viability of eggs of freshwater species contained within ballast sediments (Bailey et al. 2003; N. Kanavillil, Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, unpublished data).

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Appendix A

Table A1. Animals recorded in residual sediment and water from ships that declare “no-ballast-on-board” (NOBOB) status entering the Great Lakes.

	Sediment	Water
Rotifera		
<i>Brachionus angularis</i>		×
<i>Keratella crassa</i>		×
<i>Kellicottia longispina</i>		×
<i>Keratella cochlearis</i>		×
<i>Lecane closterocerca</i>	×	
<i>Lecane hamata</i>	×	
<i>Polyarthra dolichoptera</i>		×
<i>Proales decipiens</i>		×
<i>Synchaeta oblonga</i>		×
Bdelloids	×	×
Copepoda		
Harpacticoida		
<i>Ameira parvula</i>	×	×
<i>Bryocamptus pygmaeus</i>	×	
<i>Bryocamptus zschokkei</i>	×	
<i>Canthocamptus staphylinoides</i>	×	
<i>Canthocamptus staphylinus</i>	×	
<i>Ectinosoma californicum</i>	×	
<i>Halectinosoma curticorne</i>	×	
<i>Harpacticus uniremis</i>	×	
<i>Longipedia minor</i>	×	×
<i>Mesochra pygmaea</i>	×	
<i>Microarthridian littorale</i>	×	
<i>Microsetella norvegica</i>	×	×
<i>Nitocra affinis affinis</i>	×	
<i>Nitocra hibernica</i>	×	
<i>Nitocra lacustris</i>	×	×
<i>Nitocra spinipes</i>	×	×
<i>Nitocra</i> sp.	×	
<i>Onychocamptus mohammed</i>	×	
<i>Schizopera baltica</i>	×	
<i>Schizopera borutzkyi</i>	×	×
<i>Schizopera knabeni</i>	×	×
<i>Tachidius littoralis</i>		×
<i>Tisbe furcata</i>		×
<i>Tisbe gracilis</i>		×
Indeterminate harpacticoids	×	×
Cyclopoida		
<i>Acanthocyclops robustus</i>	×	×
<i>Acanthocyclops venustus</i>		×
<i>Acanthocyclops vernalis</i>		×
<i>Acanthocyclops</i> sp.	×	
<i>Cyclopina litoralis</i>		×
<i>Cyclops abyssorum</i>		×
<i>Cyclops strenuus</i>	×	×
<i>Diacyclops nanus</i>		×
<i>Diacyclops thomasi</i>	×	×
<i>Eucyclops serrulatus</i>		×
<i>Halicyclops</i> sp.	×	×
<i>Mesocyclops americanus</i>	×	
<i>Mesocyclops edax</i>	×	×

Table A1 (continued).

	Sediment	Water
<i>Mesocyclops leukarti</i>	×	
<i>Mesocyclops</i> sp.	×	
<i>Paracyclops chiltoni</i>	×	×
<i>Paracyclops fimbriatus</i>	×	
<i>Thermocyclops crassus</i>	×	
<i>Thermocyclops oithonoides</i>	×	
Indeterminate cyclopoids	×	×
Calanoida		
<i>Calanus</i> sp.		×
<i>Epischura lacustris</i>		×
<i>Eurytemora affinis</i>		×
<i>Leptodiptomus minutus</i>		×
<i>Senecella calanoides</i>		×
<i>Temora longicornis</i>	×	
Poecilostomatoida		
<i>Oncaea media</i>	×	
<i>Oncaea borealis</i>	×	
<i>Saphirella</i> sp.		×
Cladocera		
<i>Alona quadrangularis</i>	×	×
<i>Bosmina lieperi</i>		×
<i>Bosmina maritima</i>		×
<i>Bosmina</i> sp.		×
<i>Chydoris sphaericus</i>		×
<i>Daphnia atkinsoni</i>		×
<i>Daphnia cristata</i>		×
<i>Daphnia magna</i>		×
<i>Daphnia retrocurva</i>		×
<i>Ilyocryptus sordidus</i>	×	
Amphipoda		
<i>Gammarus chevreuxi</i>		×
<i>Gammarus salinus</i>		×
<i>Gammarus zaddachi</i>		×
Indeterminate <i>Gammarus</i> spp.	×	×
Other Arthropoda		
Decapoda juveniles	×	
<i>Crangon crangon</i>		×
Cirripedia juveniles	×	×
Mysida		
<i>Neomysis integer</i>		×
Ostracoda	×	×
Chironomids	×	×
Other Insects	×	
Acarina	×	×
Annelida		
Oligochaeta		
<i>Amphichaeta americana</i>	×	
<i>Pristina</i> sp.	×	×
<i>Vejdovskyella intermedia</i>	×	
Indeterminate tubercifids	×	
Aphanoneura		
<i>Aelosoma</i> sp.	×	
Polychaeta	×	×
Tardigrada	×	×
Mollusca		
Gastropoda	×	×
Fissurellidae		×

Table A1 (concluded).

	Sediment	Water
Bivalvia (including <i>Driessena</i>)	×	×
Hydrozoa	×	×
Bryozoa	×	
Echinodermata, class Crinoidea		×
Nemata		
<i>Acrobeles</i> sp.	×	
? <i>Anonchus</i> sp.	×	
<i>Anoplostoma</i> sp.	×	
<i>Aphanolaimus</i> sp.	×	
<i>Ascolaimus</i> sp.	×	
<i>Axonolaimus</i> sp.	×	
<i>Bathylaimus</i> sp.	×	
<i>Campylaimus</i> sp.	×	
<i>Choriorhabditis</i> sp.	×	
<i>Cobbia</i> sp.	×	
? <i>Criconema</i> sp.	×	
<i>Daptonema</i> sp.	×	
<i>Diploscapter</i> sp.	×	
<i>Ironus</i> sp.	×	
<i>Mesorhabditis</i> sp.	×	
<i>Microlaimus</i> sp.	×	
<i>Molgolaimus</i> sp.	×	
<i>Mononchoides</i> sp.	×	
<i>Oncholaimus oxyuris</i>	×	
cf. <i>Paracytholaimus</i> sp.	×	
<i>Paraphanolaimus</i> sp.	×	
<i>Rhabditis</i> sp.	×	
<i>Sabatieria</i> sp.	×	
<i>Sphaerolaimus</i> sp.	×	
<i>Teratocephalus</i> sp.	×	
<i>Theristus flevensis</i>	×	
<i>Tobrilus</i> sp.	×	
<i>Tripyla</i> sp.	×	
<i>Tripylloides</i> sp.	×	
cf. <i>Viscosia</i> sp.	×	
? <i>Xiphinema</i> sp.	×	
Chromadoridae	×	
<i>Dichromadora</i> sp.	×	
Cyatholaimidae	×	
Diplogasteridae	×	
<i>Diplogasteroides</i> sp.	×	
Dorylaiminae	×	
<i>Dorylaimus</i> sp.	×	
Leptolaimidae	×	
<i>Leptolaimoides</i> sp.	×	
<i>Leptolaimus</i> sp.	×	
Monhysteridae	×	
<i>Diplolaimella</i> sp.	×	
<i>Monhystera</i> sp.	×	
<i>Thalassomonhystera</i> cf. <i>parva</i>	×	
<i>Thalassomonhystera</i> sp.	×	
Plectidae	×	
<i>Plectus</i> sp.	×	

Note: Taxa preceded with a question mark are of uncertain identity.