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Research Article

Evaluating efficacy of a ballast water filtration system for reducing spread of aquatic species in freshwater ecosystems

Elizabeta Briski*, Robert Dallas Linley, Jennifer K. Adams and Sarah A. Bailey

Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, 867 Lakeshore Road, Box 5050, Burlington, Ontario, L7R 4A6, Canada

E-mail: elzabriski@yahoo.com (EB), Robert.Linley@dfo-mpo.gc.ca (RDL), jkadams27@gmail.com (JKA), Sarah.Bailey@dfo-mpo.gc.ca (SAB)

**Corresponding author*

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Abstract

Biological invasions by non-indigenous species are considered a leading threat to biodiversity, with prevention being a key management strategy. Consequently, numerous commercial ballast water treatment systems have been, or are being, developed to prevent future aquatic invasions. However, most treatment systems are being designed for the many vessels undertaking long transoceanic voyages in marine waters rather than the relatively few vessels operating on short voyages in freshwater, such as those in the Laurentian Great Lakes. Here we conduct testing of the biological efficacy of a 40 µm ballast water filtration unit through shipboard trials. We test the hypotheses that i) filtration will significantly reduce abundance of zooplankton greater than 50 µm in size but not phytoplankton 10 to 50 µm in size; ii) filtration will reduce zooplankton abundances in ballast water below International Maritime Organization discharge standards, but not those of phytoplankton; and iii) filtration will alter the community composition of zooplankton, non-randomly reducing invasion risk of larger taxa. During the summer of 2012, three shipboard trials were conducted. Ballast water samples were collected using a before-after experimental design. Our study showed that filtration significantly reduced abundance of copepods and cladocerans, but not of juvenile dreissenid veligers and rotifers. Contrary to our expectation, phytoplankton densities were also significantly lower after the treatment. Overall, ballast water treated during our tests would not meet proposed international discharge standards. Filtration altered relative abundance of zooplankton, but did not reduce introduction risk of any taxonomic group due to the small juvenile stages and dormant eggs which passed through the treatment. While we do not rule out filtration as a ballast water treatment option for zooplankton in the future, our tests indicate further development is required for meaningful reduction of invasion risk.

Key words: ballast water treatment, biological invasions, management, non-indigenous species, phytoplankton, zooplankton

Introduction

Biological invasions by non-indigenous species are a global phenomenon considered by many researchers as a leading threat to biodiversity (McGeoch et al. 2010). The most effective way to manage invasive species is to prevent their introduction via vector regulation (Simberloff et al. 2005; Lodge et al. 2006; Hulme 2009). As recent evidence suggests that introduction effort is of vital importance for determining invasion success, reducing the number of individuals being transported to new locations is of primary importance in managing vectors (Von Holle and Simberloff 2005; Colautti et al. 2006; Lockwood et al. 2009; Simberloff 2009). Progress has been made in the management of ballast water by transoceanic ships using saltwater ballast exchange and saltwater flushing when arriving to freshwater ecosystems (Gray et al. 2007; Briski et al. 2010; Bailey et al. 2011); however, vessels operating exclusively in freshwater systems such as those in the Laurentian Great Lakes (i.e. domestic vessels) do not have opportunity to manage ballast water by saltwater exchange. As the large majority (~71%) of ballast water transfers in the Great Lakes are conducted by domestic vessels, and survivorship of zooplankton

on short voyages is high, domestic ballast water is an important mechanism for the spread of non-indigenous species, as well as endemic species with restricted distribution within the Great Lakes (Rup et al. 2010; Briski et al. 2012, 2013). The Great Lakes - St. Lawrence River basin is ecologically divided into multiple watersheds resulting in at least five ecoregions: Superior, Michigan-Huron, Erie, Ontario, and the Lower St. Lawrence (Abell et al. 2000). These ecoregions are characterised by different communities and species endemism, and therefore transport of taxa among the lakes is a concern for resource managers (Briski et al. 2012). A recent study reported transport of 31 species with restricted distributions in domestic ships, of which at least 21 were moved outside of their historical distributions (Briski et al. 2012). Beside the Great Lakes, lakes Ladoga and Onega (Russia), and the Caspian Sea are also freshwater bodies with complex ecoregions and notable invasion histories (Dumont et al. 2004; Kurashov et al. 2012).

When the International Convention for the Control and Management of Ships' Ballast Water and Sediments is ratified, all international ships will be required to meet numeric ballast discharge standards: less than 10 viable organisms ≥ 50 µm per m^3 (nominally "zooplankton"); less than 10 viable organisms ≥ 10 µm to < 50 µm per mL (nominally "phytoplankton"); less than 1 colony forming unit (cfu) of toxicogenic *Vibrio cholerae* (O1 and O139) per 100 mL; less than 250 cfu of *Escherichia coli* per 100 mL; and less than 100 cfu of intestinal Enterococci per 100 mL (IMO 2004). Consequently, numerous commercial ballast water treatment systems have been, or are being, developed (Lloyd's Register 2012). Individual systems typically use a combination of physical and/or mechanical technologies to remove the largest organisms (e.g., filtration) followed by chemical treatment to inactivate smaller organisms and microbes (Tsolaki and Diamadopoulos 2010; Goncalves and Gagnon 2012). In many cases, the treatment systems have been designed with the global market in mind - vessels undertaking trans-oceanic voyages (5 or more days) in temperate marine waters; there have been few technologies developed which can be utilized by the relatively small number of vessels operating on short domestic voyages (3 or fewer days) in freshwater.

Filtration is commonly included as a primary component in ballast water treatment systems to remove larger aquatic taxa and to improve the performance of secondary treatment systems by reducing particulate organic matter (Cangelosi 2002; Tsolaki and Diamadopoulos 2010). Approximately 53% of recent ship-mediated non-indigenous species in the Great Lakes, and all of those with documented negative impacts, are greater than 50 µm in size as adults (Ricciardi 2006; Kelly et al. 2009); therefore, treatment of domestic ballast water transported within the Great Lakes or other freshwater ecosystems for only the largest size category could meaningfully reduce impacts of future non-indigenous species, until such time as full treatment systems are widely available. Here, we test the biological efficacy of a ballast water filtration unit with 40 µm steel candle filter elements through shipboard trials within the Great Lakes. We test three hypotheses: i) filtration will significantly reduce abundance of taxa ≥ 50 µm in size (hereafter zooplankton) but not ≥ 10 µm to < 50 µm in size (hereafter phytoplankton); ii) filtration alone will reduce zooplankton abundances in ballast water below international discharge standards, but not those of phytoplankton; and iii) filtration will alter community composition of zooplankton, non-randomly reducing invasion risk of larger taxa (e.g., copepods and cladocerans) in ballast water.

Methods

Experimental design

The M/V Richelieu is a 729 foot, 35,630 tonne bulk carrier that typically operates in the Great Lakes and the Atlantic coast of North America. The vessel has 27 ballast tanks with a cumulative capacity of 10,478 tonnes; one cargo hold is also used for ballasting operations, having additional ballast water capacity of 5,410 tonnes. The vessel's ballast system includes two pumps, each with a pumping rate approximately 600 m^3/hour; the starboard-side pump, used in our tests, is typically used to fill/empty topside and forepeak tanks. In March 2012, two HYDAC International AutoFilt RFU-5 Automatic back-flushing filter units with 40 µm stainless steel candle filter elements were installed on the vessel, one each for port and starboard ballast lines. The candle filter elements are cylindrical wire mesh tubes that are cleaned during back-flushing cycles that temporarily reverse water flow. In addition, six stainless steel bent elbow pitot tubes were installed as scientific sample collection ports, one upstream of each filter (before treatment), one immediately downstream of each filter (after treatment), and one near the discharge outlet of the vessel, along straight sections of the ballast piping following recommendations

of Richard et al. (2008) and the Great Ship Initiative (GSI 2011a).

Following initial optimization of the filter, it was determined that scientific testing for biological efficacy would be conducted only on the starboard ballast line due to issues regarding access to port-side filter housing. Two parallel scientific sample collection systems were set up in the engine room similar to that described in GSI (2011a) allowing collection of 'before' and 'after' filtration samples; ballast water sampled by each pitot tube was pressure-fed by the vessel's ballast pump through 1'' (2.54 cm) braided clear PVC tubing to a Signet 2551 magmeter sample flow meter, after which the collection system was split to a seep sampler for collection of small volumes of water for phytoplankton analysis and to a conical plankton net with 50 µm (in diagonal) mesh within a wetted sample tub for zooplankton analysis. The seep sampler lines were 1/8'' (0.32 cm) in diameter. Filtered waste water was returned to the vessel's ballast line using a pump connected to the pitot near the vessel's discharge outlet.

During the summer of 2012, three shipboard trials were conducted while the vessel was docked in Quebec City, Quebec (July 6th) and Sarnia, Ontario (July 25th) and at anchor in Thunder Bay, Ontario (July 27th); the ballast water was treated by filtration during uptake as per normal vessel operations. Prior to testing, the filtration unit had been operated only a few times for initial optimization of filter settings; our three tests were conducted on consecutive voyages and the filter was not used in between our tests. For each test, ballast water samples 'before' and 'after' the filtration unit were collected using the scientific sample collection systems described above, at three time points corresponding to the beginning, middle, and end of the vessel's ballast loading process; each sample was collected as a time-averaged representative subsample during a period of 60–75 minutes, when ballast pumping/ filtering rate was approximately 300 m^3/hour (e.g., ballast/ filter operation starts; 60 minutes of sampling occurs; ballast/filter operation continues for 3 hours with no sampling; 60 minutes of sampling occurs; ballast/filter operation continues for 3 hours with no sampling; 60 minutes of sampling occurs; ballast/filter operation ends). At each time point, for each 'before' and 'after' sample, 2000 L of water was passed through the 50 µm mesh net for collection and analysis of zooplankton and 10 L was taken for phytoplankton. During sample collection operational parameters of the filter unit were monitored (e.g., differential pressure, number of back-flush cycles).

Analysis of zooplankton

Each 2000 L time-averaged zooplankton subsample was concentrated to 400 mL volume by conical plankton net with 50 µm mesh. After thorough mixing, 200 mL of concentrated sample was preserved in 80 mL of 95% ethanol for future laboratory analyses and species identification. The second 200 mL subsample was analysed on the vessel approximately half an hour after sample collection to determine the ratio of live:dead individuals and to estimate density of live organisms. The subsample was well mixed and twenty 100 µL subsamples were drawn by pipette for examination using a dissecting microscope and standard movement/response to stimuli techniques. Organisms were enumerated according to broad taxonomic groups, such as copepods, dreissenid veligers, and rotifers.

At the laboratory, the preserved samples were examined under a dissecting microscope in entirety, representative individuals of different taxonomic groups were measured and imaged, and twenty individuals from every taxonomic group per sample separated for taxonomic identification. DNA was extracted from each whole individual following Elphinstone et al. (2003). Fragments of the mitochondrial gene COI were amplified using the universal COI primers LCO1490 and HCO2190 (Folmer et al. 1994). PCR reactions and sequencing protocol followed Briski et al. (2011). Recovered DNA sequences were blasted against those in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the nucleotide blast (default parameters) and compared to those in the Barcode of Life Database (http://www.barcodinglife.org), using the identification engine BOLD-IDS, with the option 'All Barcode Records on BOLD'. Taxonomic identifications were assigned at 96% similarity to reference database, and typically resulted in identifications to the species level.

Analysis of phytoplankton

After thorough mixing, one 400 mL subsample was removed from each 10 L time-averaged subsample and processed on-board approximately half an hour after sample collection to examine density of live organisms using fluorescein diacetate as an indicator of viability (GSI 2011b; Adams et al. 2014). Each 400 mL subsample was concentrated using 7 µm (in diagonal) plankton mesh and kept

in 100 mL sample containers. After thorough mixing, a 5 mL subsample of the concentrated sample was transferred to a 20 mL sample container, with 417 µL of fluorescein diacetate stain solution added (Garvey et al. 2007; Reavie et al. 2010). The stain solution was prepared in advance by dissolving 5 mg fluorescein diacetate in 1 mL dimethyl sulfoxide, while the working solution was prepared a few minutes before the subsample was stained by adding 10 µL of stock solution into 0.99 mL of ddH_2O. The subsample was then incubated in the dark for 10 minutes. The 5.5 mL incubated subsample was mixed and 1.1 mL was immediately transferred to a Sedgwick-Rafter cell, covered and observed using epifluorescence microscopy. Single cell entities and cells comprising colonial and filamentous entities were counted; each cell in colonial or filamentous entity was counted separately. Phytoplankton were not identified to any taxonomic level as we were expecting differences in community composition before and after filtration only for taxa ≥ 50 µm.

Statistical analyses

The density counts used in statistical analyses and reported through the manuscript for both zooplankton and phytoplankton are counts of viable individuals. We first tested for differences in ambient plankton densities ('before' samples) within and across sites using two-way multivariate analysis of variance (MANOVA) where taxa (zooplankton and phytoplankton) were dependent variables and sampling sequence (beginning, middle and end) and sampling location (Quebec City, Sarnia, and Thunder Bay) were independent variables. As there was no difference among sampling sequences, they were further treated as independent replicates (Table 1). Two one-way MANOVAs were then conducted to examine the removal efficacy of plankton during ballast uptake. While the abundance (samples 'before' and 'after') was the independent variable in both MANOVAs, plankton type (zooplankton and phytoplankton) and zooplankton taxa (cladocerans, copepods, nauplii, rotifers and dreissenid veligers) were dependent variables in the first and second MANOVA, respectively. All data were log-transformed ($\log_{10}$ (x + 1)) to meet assumptions of parametric tests. The significance level for statistical comparisons was adjusted for multiple pairwise comparisons by Bonferroni-type correction with a family-wise error rate of 0.05. All statistical analyses were conducted using SYSTAT® version 11 (SYSTAT Software 2004).

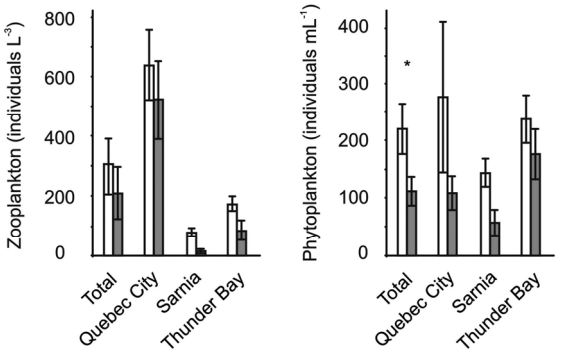

Figure 1. Mean and standard error of total zooplankton and phytoplankton densities, and of individual densities in Quebec City, Sarnia and Thunder Bay samples. White and gray bars represent samples collected 'before' and 'after' the filter, respectively. * denotes significant difference at 0.05 between 'before' and 'after' samples. Note the difference in scale between plots.

Results

The density of ambient plankton ('before' samples) was not variable across sequence samplings (beginning, middle, and end) for either plankton type (zooplankton and phytoplankton; $p > 0.05$) or across sampling locations for phytoplankton (Quebec City, Sarnia and Thunder Bay; $p > 0.05$), but it was variable across sampling locations for zooplankton ($p < 0.05$; Table 1). Average zooplankton and phytoplankton densities in 'before' samples ranged from 72,478 to 868,791 individuals per m^3 and from 88 to 531 cells per mL, respectively (Figure. 1). After filtration, those densities ranged from 17,101 to 787,518 individuals per m^3 and from 24 to 229 cells per mL, respectively (Figure 1). Considering results of the three trials together, phytoplankton density was significantly lower after filtration ($p < 0.05$) while density of zooplankton was not significantly changed ($p > 0.05$) (Figure 1; Table 2). However, when mean densities of 'before' samples were compared to those of 'after' samples for phytoplankton for each location separately (i.e. Quebec City, Sarnia and Thunder Bay), they were not significantly different ($p > 0.05$) (Figure 1). Zooplankton taxa consisted of juvenile dreissenid veligers, rotifers, nauplii, copepods and cladocerans (Table 3; Figure 2).

Table 1. Results of two-way multivariate analysis of variance (MANOVA) where taxa in ambient samples 'before' filtration (zooplankton and phytoplankton) were dependent variables and sampling sequence (beginning, middle and end) and sampling location (Quebec City, Sarnia, Thunder Bay) were independent variables. Significant p-values are presented in bold.

Variable	df	F	p
sampling sequence			
univariate F-tests			
zooplankton	2	0.058	0.944
phytoplankton	2	2.439	0.124
multivariate test			
Wilks' lambda = 0.528	4	1.502	0.248
sampling location			
univariate F-tests			
zooplankton	2	20.559	**< 0.001**
phytoplankton	2	2.308	0.155
multivariate test			
Wilks' lambda = 0.090	4	9.346	**< 0.001**
interaction			
univariate F-tests			
zooplankton	4	0.811	0.549
phytoplankton	4	0.042	0.996
multivariate test			
Wilks' lambda = 0.560	8	0.672	0.710

Table 2. Results of multivariate analysis of variance (MANOVA) where taxa (zooplankton and phytoplankton) were dependent variables and removal efficacy of the filter (sample density 'before' and 'after' filtration unit) was independent variable. Significant p-values are presented in bold.

Variable	df	F	p
univariate *F*-tests			
zooplankton	1	1.992	0.177
phytoplankton	1	6.063	**0.026**
multivariate test			
Wilks' lambda = 0.710	2	3.062	0.077

Table 3. List of taxa identified by DNA barcoding in Quebec City, Sarnia and Thunder Bay.

Higher taxa	Species	Quebec City	Sarnia	Thunder Bay
Mollusks	unidentified mollusks	X	-	-
Dreissenids	*Dreissena polymorpha*	X	X	X
	Dreissena rostriformis bugensis	X	X	-
Rotifers	*Keratella cochlearis*	-	-	X
	Keratella sp.	-	-	X
	Synchaeta sp.	X	-	-
	unidentified rotifers	X	X	-
Copepods	*Eurytemora affinis*	X	X	-
	Leptodiaptomus minutus	-	X	X
	Skistodiaptomus pallidus	-	X	-
	Hemidiaptomus sp.	-	X	-
	Diacyclops sp.	-	-	X
	unidentified cyclopoids	-	X	X
	unidentified calanoids	-	X	X
Cladocerans	*Bosmina liederi*	X	X	-
	Bosmina longirostris	-	X	-
	Eurycercus longirostris	-	X	-
	Sida crystallina	-	X	X
	Holopedium gibberum	-	-	X

Table 4. Results of multivariate analysis of variance (MANOVA) where zooplankton taxa (cladocerans, copepods, nauplii, rotifers, and dreissenid veligers) were dependent variables and removal efficacy of the filter (sample density 'before' and 'after' filtration unit) was independent variable. Significant p-values are presented in bold.

Variable	df	F	p
univariate *F*-tests			
cladocerans	1	82.674	**< 0.001**
copepods	1	99.974	**< 0.001**
nauplii	1	12.203	**0.003**
rotifers	1	2.079	0.169
dreissenid veliger	1	0.860	0.367
multivariate test			
Wilks' lambda = 0.061	5	36.767	**< 0.001**

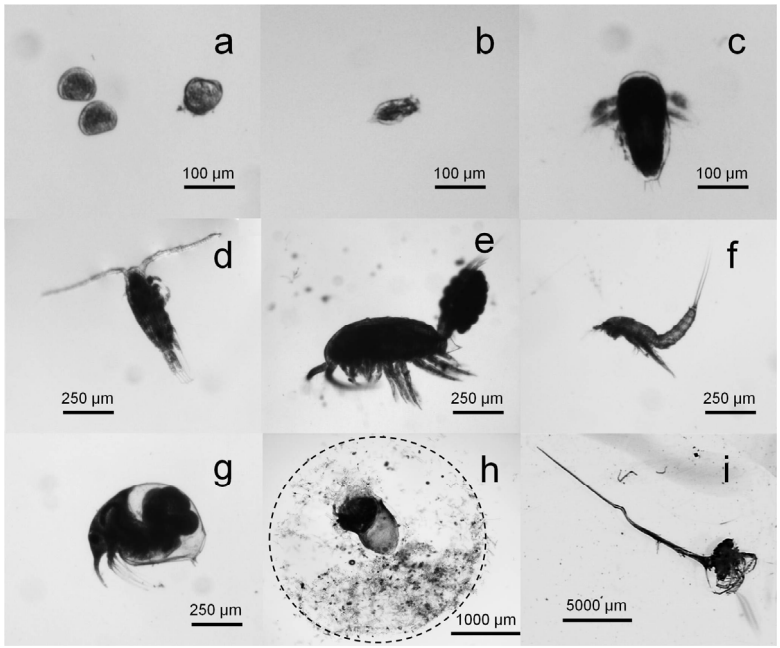

Figure 2. Examples of zooplankton taxa recovered from ballast water: dreissenid veligers (a), rotifers (b), nauplii (c), copepods (d – f), cladocerans (g – i). Scale bars (µm) are included on each image; note differences in scale among plots. The circle on panel h emphasizes total (gelatinous) size of animal. Photomicrographs by Elizabeta Briski.

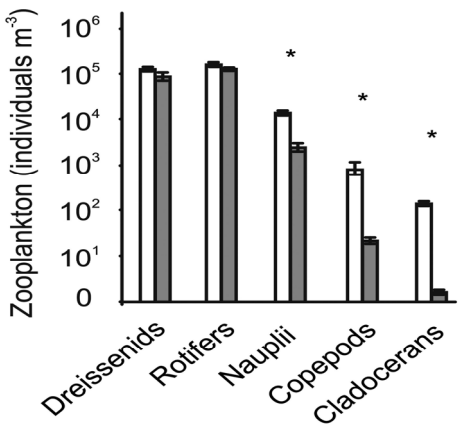

Figure 3. Mean and standard error of diversified taxa in ballast water sampled 'before' (white bars) and 'after' (gray bars) the filter. * denotes significant difference at 0.05 between 'before' and 'after' samples. Y-axis is log-scaled. Dreissenids were recorded only as veliger larvae.

For all three sampling locations, all zooplankton taxa identified in 'before' samples were also recovered in 'after' samples. Smaller taxa were more abundant than larger taxa, consistent with natural community composition in aquatic habitats. Filtration significantly decreased zooplankton abundance for all zooplankton groups ($p < 0.05$) except rotifers and dreissenid veligers, although the proportional reduction was greater for larger taxa (Figure 3; Table 4). Percentage removal of zooplankton in Quebec City, Sarnia and Thunder Bay was 17, 73 and 51%, respectively, while that of phytoplankton was 61, 62 and 26%, respectively.

The rate of filter back-flush cycles was similar in Quebec City and Sarnia at approximately 1.2 back-flushes per hour; however, in Thunder Bay, back-flushing was much more frequent after the first hour of ballast loading, approaching 12–13 back-flushes per hour.

Discussion

Filtration is a desirable option for ballast water management since it is an environmentally benign method that relies on physical removal of plankton (as well as their dormant stages, and sediment) rather than extermination by toxic chemicals (Gregg et al. 2009). The filtration unit used in our tests significantly reduced abundances of copepods and cladocerans, but did not effectively remove juvenile dreissenid veligers and rotifers. Ballast water treated during our tests would not meet proposed international discharge standards for either zooplankton or phytoplankton (IMO 2004). We expected that filtration would alter the community composition of zooplankton in ballast water towards smaller taxa such as rotifers, but our study showed that invasion risk was not reduced for even the largest taxa due to smaller juvenile stages which passed through the filter. For example, dreissenid mussels easily passed the filtration unit as small planktonic veliger larvae. In contrast, cladocerans, even though much smaller than adult dreissenids, were more efficiently removed. Linch (1980) reported that neonates of 49 cladoceran species from five different families are bigger than 200 μm, while 25 of them are bigger than 400 μm in size. Taking into account the shape of cladocerans, we suspect that most individuals, even as juvenile stages would not pass through a filtration unit, nor would their dormant eggs (70–150 μm in size (Linch 1980). Thus, we expect only species with large size at all developmental stages could be removed by filtration in entirety.

The low percentage removal of zooplankton in Quebec City can be explained by the very high abundance of juvenile dreissenid veliger larvae and rotifers belonging to the smallest taxonomic groups examined (< 100 μm). The use of 25 μm size mesh could be optimized to reduce or remove such small organisms (Parsons and Harkins 2000, 2002; Cangelosi et al. 2007; Gregg et al. 2009). However, shipboard operations would be adversely affected by reduced flow rates (Parsons and Harkins 2000, 2002). The high flow rate and volume of ballast water that must be treated in a very short timeframe pose a significant technological challenge for shipboard filtration units, and at present only 40–50 μm filtration appears operationally feasible (Gregg et al. 2009). In contrast, the high density of the cladoceran *H. gibberum*, which may reach 3–4 mm in size due to its large gelatinous mantle, led to clogging of the filtration unit in Thunder Bay. As tests in Thunder Bay proceeded, the back-flushing cycles of the filter occurred more frequently, temporarily reversing water flow to clean individual filter candle units, thereby decreasing ballast pumping rates and extending the length of time required to complete the ballasting operation by nearly 30% compared to tests in Quebec City and Sarnia. Under these circumstances, addition of a cyclonic separation unit to remove large particles prior to filtration might be an effective strategy to improve filter performance (Veldhuis et al. 2006; Gregg et al. 2009). Our study showed that even within the ecosystems of the Great Lakes, a great diversity of biological challenge conditions can be encountered. This finding speaks to the importance of equipment validation testing at a variety of locations to ensure global suitability.

The limited removal of taxa larger than 40 μm might be explained by organism plasticity or flexibility, allowing individuals larger than 40 μm to squeeze through the filter openings. Most zooplankton have an exoskeleton which provides stable body shape and physical protection. However, depending on the taxon and the stage of the life cycle, the exoskeleton may become soft or even cast away during the molting process (Ruppert et al. 2004). In this case, we cannot rule out the possibility that organism viability may be adversely impacted; individuals squeezed through filter screening may be alive and respond to standard stimuli techniques or viability stains immediately after passing through the filter, but physical damage could occur followed by death within days or even hours. Delayed treatment responses have been reported for phytoplankton treated by UV irradiation, where the effect of high UV doses is not evident for nearly one day and effects of lower UV doses are delayed up to five days (Stehouwer et al. 2010).

We certainly cannot rule out filtration as a ballast water treatment option for larger organisms (≥ 50 μm) based on three tests of a single filter unit. Filtration has been effectively used to remove large dreissenid mussel veliger larvae (> 200 μm) from high flow cooling systems at power stations and other industrial land-based facilities (Claudi et al. 2008; Murphy et al. 2010). There is therefore a possibility that filtration could be optimized for shipboard use. We caution, however, that significant improvements are needed in order to achieve meaningful reduction in invasion risk. For example, even if 90% removal of zooplankton was achieved during our tests, resulting discharge densities would still be on the order of several thousands to several tens of thousands of individuals per m^3, still in exceedance of IMO discharge standards.

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