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GREAT LAKES SHIPPING, TRADE, AND AQUATIC INVASIVE SPECIES

Retroactive Evaluation of International Maritime Organization Ballast Water Standards

Prepared for
Committee on the St. Lawrence Seaway:
Options to Eliminate Introduction of Nonindigenous Species into the Great Lakes, Phase 2
Transportation Research Board and Division on Earth and Life Studies

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Since the opening of the St. Lawrence Seaway in 1959, ship ballast water has become the dominant vector for non-indigenous species introduction to the Laurentian Great Lakes. In 2004, the adoption of ballast water management and discharge standards by the International Maritime Organization (IMO) was an important step in reducing the risk of further invasions. Using data reported in the literature on maximum natural densities and maximum salinity tolerance, the efficacy of ballast water exchange (BWE) (IMO Regulation D-1) in reducing invasion risk of six current invaders to the Great Lakes was evaluated. In addition, reported values for maximum natural densities and minimum particle size of these six representative species were used to retroactively evaluate the efficacy of discharge performance standards (IMO Regulation D-2); treatment technologies available to meet these performance standards were also considered. The salinity shock from open ocean water BWE would have minimized the risk of introduction for the Zebra Mussel, the Eurasian Ruffe, and the amphipod *Echinogammarus ischnus*. Raised salinity associated with BWE would have been less effective in preventing the introduction of resting eggs of the Spiny Water Flea *Bythotrephes longimanus*, and all stages of the diatom *Thalassiosira baltica*. A lack of salinity tolerance data on Viral Hemorrhagic Septicemia Virus (VHSV) precluded assessment of the efficacy of BWE in preventing its introduction. Based on maximum natural densities reported in the literature, the number of organisms per volume of ballast water must be reduced by a factor of 100 to 100,000 to meet discharge performance standards. It was not possible to assess the number of VHSV requiring inactivation, since performance standards for viruses are not specified under Regulation D-2. For all species and life stages, with the exception of VHSV, physical removal through media filtration could be used to meet Regulation D-2. All of the representative species would be inactivated using oxidizing biocides. Although such treatment technologies could reduce densities to those required under Regulation D-2, the total number of organisms remaining in a given ballast tank may still be sufficiently high to enable the establishment of viable populations.

INTRODUCTION

Although it has been long recognized that ships' ballast water is a vector for introducing non-native aquatic organisms, it was not until recently that a set of proposed regulations for ballast water discharge standards was promulgated. The issue was formally brought forth to the international community in 1990, and in 1991, the Marine Environmental Protection Committee (MEPC) of the International Maritime Organization (the United Nations entity responsible for shipping and maritime issues) adopted voluntary guidelines for the prevention of the introduction of unwanted organisms, pathogens, and sediment from ballast water. These guidelines, with a few minor modifications, were formally adopted by the IMO in 1993 (International Maritime Organization, 1993), and more comprehensive guidelines calling for the minimization of introductions, precautionary measures, and ballast water exchange were agreed to in 1997 (International Maritime Organization, 1997). The guidelines are important as they call for uniform action by the states and establish internationally acknowledged ballast water management practices. After more than a decade of discussion, negotiation and input from various stakeholders, the *International Convention for the Control and Management of Ships' Ballast Water and Sediments*, which proposes specific ballast water management practices and discharge standards, was adopted by the IMO in February 2004 (International Maritime Organization, 2004).

The *International Convention for the Control and Management of Ships' Ballast Water and Sediments*, Section D, requires that vessels undergo ballast water exchange (Regulation D-1), or, discharge not more than a specified maximum number of organisms per volume (Regulation D-2), as follows:

- Regulation D-1, Ballast Water Exchange (BWE) Standard: requires at least 95% volumetric exchange in a tank flush-and-fill procedure, or if a pump-through method is used, enough water must be pumped to be equivalent to three times the tank volume. Less than three times would be accepted if the ship demonstrates that at least 95% volumetric exchange is met. (It should be noted that it is extremely difficult to achieve 95% volumetric exchange with less than three times the tank volume exchanged.)
- Regulation D-2, Ballast Water Performance Standard: the ballast water discharge must contain fewer than 10 viable organisms greater than or equal to 50 μm minimum size per m^3, and fewer than 10 viable organisms less than 50 μm and greater than or equal to 10 μm minimum size per ml. In addition, the limits established for the indicator microorganisms are: *Vibrio cholerae* (O1 and O139) less than 1 colony forming unit (cfu) per 100 ml or 1 cfu per g (wet weight) zooplankton; *E. coli* less than 250 cfu per 100 ml and intestinal Enterococci less than 100 cfu per 100 ml.

In the years intervening the initial proposal and final promulgation of the IMO ballast water management standards, ship-ballast mediated invasions of the Great Lakes have continued. A recent report commissioned by the National Academies Committee on the St. Lawrence Seaway Committee addresses this situation (see Kelly, 2007). The main objective of this white paper is to evaluate whether implementation of the IMO ballast water standards retroactively could have prevented the introduction of a representative group of organisms, and to briefly comment on the maximum removal or inactivation achievable using currently available ballast water technologies. To this end, the tasks were to:

1. Select a representative list of aquatic invasive species (AIS) that have been introduced into the Great Lakes via the ballast water of ocean-going vessels since the opening of the St. Lawrence Seaway in 1959. Given public concerns about pathogens, the list was to include the Viral Hemorrhagic Septicemia Virus (VHSV), even though a recent report indicated that this species was most likely introduced with commercial aquaculture operations rather than ship ballast.
2. Characterize the life cycle stages of each organism selected, with special regard to particle size and saltwater tolerance. The characterization was also to include any information available relating to the highest observed densities in natural systems of any of the life stages of the selected AIS.
3. Determine whether each of the organisms, at any of its life cycle stages, would have survived passage through the processes required under the IMO ballast water exchange and performance standards. The determination was to be supplemented by comments about the levels of treatment achievable using currently available technologies.

METHODS

Task 1: Representative List of Aquatic Invasive Species Introduced to the Great Lakes

Six non-indigenous species were selected to represent the diversity of organisms that has been introduced via the ballast water vector to the Great Lakes since 1959, when the current St. Lawrence Seaway system was opened. This diversity, which included a virus, a diatom, two crustaceans, a mollusc and a fish, together with a wide variation in size and life history, ensured that differential species responses to each of the ballast management options were likely. Further details on the species are shown in Appendix 1.

Task 2: Saltwater Tolerance, Size and Highest Observed Densities of the Selected Species in the Environment

In order to retroactively assess the efficacy of IMO Regulations D-1 and D-2, a literature survey was conducted to obtain data on the maximum observed natural density, maximum salinity tolerance (either from experimental or field observations), and minimum reported size for each species and life-stage. It should be noted that since the values obtained for density and salinity tolerance were from single reports, they should be considered as estimates of these parameters for the true ballasted donor population. In some cases, the minimum size was obtained from data that reported a size range of individuals from a population. The results are shown in Table 1.

Task 3: Removal or Inactivation of Propagules after Ballast Water Exchange and Number of Propagules per Volume of Ballast Water that Needs to be Removed or Inactivated to Meet Discharge Standards

Under ballast water management practices of IMO Regulation D-1, freshwater species undergoing ballast water exchange (BWE) should be subject to both a physiological shock due to salinity changes, and a dilution effect due to purging from tanks. The initial evaluation was based on the maximum salinity tolerance/distribution of each species and life-stage reported in Table 1. Species with maximum salinity tolerance values less than that of open ocean water (34 ppt) were assumed incapable of surviving after BWE. Although many of the selected species and life-stages were not expected to survive the salinity change, the density of organisms or propagules surmised to be remaining in a ballast tank after 95% volumetric exchange of ballast water was calculated from the highest observed densities for each species and life-stage reported in Table 1. Some of the organism densities in the literature were reported as number per unit area to reflect their occurrence in the benthos, and several assumptions were made to adjust the densities to those that may be expected in a ballast water tank (i.e., to number per unit volume). These assumptions included the benthic surface area presumed to be affected by ballast water uptake, and the maximum volume of ballast per ocean-going vessel that can enter the Great Lakes (for details, please see Appendix 2). Estimates for survival and propagule reduction after BWE are shown in Table 2.

TABLE 1 Maximum Salinity Tolerance, Density, and Minimum Particle Size Reported for Each Aquatic Invasive Species and Life-Stage. Density Values Are Expressed Per Unit Area or Volume, Reflecting the Use by Species/Life-Stages of Benthic or Pelagic Habitats, Respectively

Aquatic invasive species	Maximum salinity tolerance	Minimum particle size	Maximum natural density
Dreissena polymorpha (Zebra Mussel)			
Adult	18.4 ppt (Karatayev et al., 1998)	Variable	175,000 m^{-2} (Mellina & Rasmussen, 1994)
Veliger	10.0 ppt (Barnard et al., 2003)	50 µm (Ackerman et al., 1994)	>400,000 m^{-3} (Barnard et al., 2003)
Thalassiosira baltica (algae)			
Cell	44 ppt (Hasle, 1978)	15.8 µm (Edlund et al., 2000)	6500 ml^{-1} *Thalassiosira* spp. (Totti, 2003)
Resting cell / cyst	44 ppt (Hasle, 1978)	15.8 µm (Edlund et al., 2000)	6500 ml^{-1} *Thalassiosira* spp. (Totti, 2003)
Echinogammarus ischnus (amphipod)			
Free-living	23 ppt (S. Ellis, pers. comm.)	1.5 mm (Nalepa et al., 2001)	1000 m^{-2} (Krisp & Maier, 2005)
Gymnocephalus cernuus (Eurasian Ruffe)			
Adult	12 ppt (Pethon, 1980)	70 mm (Willemsen, 1977)	1.5 m^{-2} (Savino & Kostich, 2000)
Larvae	Data unavailable	2.5 - 3.2 mm (French and Edsall 1992)	Data unavailable
Egg	>11 ppt (Vetemaa & Saat, 1996)	340 µm (Ogle, 2000)	Data unavailable
Bythotrephes longimanus (Spiny Water Flea)			
Free-living	8 ppt (Grigorovich et al., 1998)	body = 1.1mm + caudal spine = 5.1mm (Grigorovich et al., 1998)	5000 m^{-3} (Palmer et al., 2001)
Resting egg	Data unavailable	197 µm (Jarnagin et al., 2000)	5000 m^{-2} in waters >60 m depth (Yurista, 1997)
Viral Hemorrhagic SepticemiaVirus			
Virion	Data unavailable	Virion minimum = 170 nm x 60 nm (Elsayed et al., 2006).	3.8 x 10^{7} ml^{-1}*** (Drake et al., 2002)

***As data on VHSV natural densities were unavailable, the value shown is the average density of virus-like particles observed in unexchanged ballast tanks (see Drake et al., 2002).

TABLE 2 Estimates of Survival and Propagule Reduction After BWE (IMO Regulation D-1)

Aquatic invasive species	Would species survive BWE based on salinity tolerance?	Density of propagules remaining after BWE based on 95% volumetric exchange
Dreissena polymorpha (Zebra Mussel)		
Adult	No	1.4×10^4 m^{-3**}
Veliger	No	At least 2×10^4 m^{-3}
Thalassiosira baltica (algae)		
Cell	Yes	3.2×10^2 ml^{-1}
Resting cell / cyst	Yes	3.2×10^2 ml^{-1}
Echinogammarus ischnus (amphipod)		
Free-living	No	7.9×10^1 m^{-3**}
Gymnocephalus cernuus (Eurasian Ruffe)		
Adult	No	1.5×10^{-1} m^{-3**}
Larvae	No	Data unavailable
Egg	No	2.4×10^3 m$^{-3**,***}$
Bythotrephes longimanus (Spiny Water Flea)		
Free-living	No	2.5×10^2 m^{-3}
Resting egg	Unknown	4.0×10^2 m^{-3**}
Viral Hemorrhagic Septicemia Virus		
Virion	Yes	1.9×10^6 ml^{-1****}

**Calculations based on reported density per unit area of these benthic life stages, assuming that the benthic surface area affected by ballast water uptake is an area of 3.84×10^4 m^2 and the maximum volume of ballast per ocean-going vessel that can enter the Great Lakes is 24,300 m^3 (Phil Jenkins, personal communication). For details, please see Appendix 2.

***Assumes 50% of adults are females, and each carries the average number of eggs (45,000) (Selgeby & Ogle, 1992).

****Based on 3.8×10^7 ml^{-1} total virus-like particles found in ballast water (Drake et al., 2002).

The calculated number of propagules per volume of ballast water that would have to be removed or inactivated to meet IMO Regulation D-2 was based on the maximum density of organisms expected in a ballast water tank. This was in turn, based on maximum natural densities found in the environment as shown in Table 1. As noted above for densities of organisms with benthic life-stages, a number of assumptions were made to adjust for the volume units specified in the IMO Regulations. The results of these calculations and assumptions made are shown in Table 3 and Appendix 2, respectively.

It should be noted that in the retroactive evaluation of either Regulation D-1 or Regulation D-2, the assumptions made aimed to maximize either the density of organisms theoretically possible after BWE (Regulation D-1), or the number of organisms that need to be inactivated or removed per volume of ballast water to meet discharge performance standards (Regulation D-2).

TABLE 3 Estimates of the Maximum Density of Organisms Present in a Ballast Tank and the Number of Organisms Requiring Removal/Inactivation to Meet Ballast Water Discharge Standards (IMO Regulation D-2).

Aquatic invasive species	Maximum density presumed in ballast water tank	Ballast water discharge standard	Number of propagules to be removed/inactivated to meet ballast water discharge standard
Dreissena polymorpha			
(Zebra Mussel)			
Adult	2.8×10^{5} m^{-3}**	Less than 10 m^{-3}	5 $\log_{10}$**
Veliger	>4.0×10^{5} m^{-3}	Less than 10 m^{-3}	At least 5 $\log_{10}$
Thalassiosira baltica			
(algae)			
Cell	6.5×10^{3} ml^{-1}	Less than 10 ml^{-1}	3 $\log_{10}$
Resting cell / cyst	6.5×10^{3} ml^{-1}	Less than 10 ml^{-1}	3 $\log_{10}$
Echinogammarus ischnus			
(amphipod)			
Free-living	1.6×10^{3} m^{-3}**	Less than 10 m^{-3}	3 $\log_{10}$**
Gymnocephalus cernuus			
(Eurasian Ruffe)			
Adult	2.4 m^{-3}**	Less than 10 m^{-3}	None – standard met**
Larvae	Data unavailable	Less than 10 m^{-3}	Data unavailable
Egg	5.3×10^{3} m^{-3}**,***	Less than 10 m^{-3}	4 $\log_{10}$**,***
Bythotrephes longimanus			
(Spiny Water Flea)			
Free-living	5.0×10^{3} m^{-3}	Less than 10 m^{-3}	3 $\log_{10}$
Resting egg	7.9×10^{3} m^{-3}**	Less than 10 m^{-3}	3 $\log_{10}$**
Viral Hemorrhagic Septicemia Virus			
Virion	3.8×10^{7} ml^{-1}****	Standard not established	Standard not established

**Calculations based on reported density per unit area of these benthic life stages, assuming that the benthic surface area affected by ballast water uptake is an area of 3.84×10^{4} m^{2} and the maximum volume of ballast per ocean-going vessel that can enter the Great Lakes is 24,300 m^{3} (Phil Jenkins, personal communication). For details, please see Appendix 2.

***Assumes 50% of adults are females, and each carries the average number of eggs (45,000) (Selgeby & Ogle, 1992).

****Based on 3.8×10^{7} ml^{-1} total virus-like particles found in ballast water (Drake et al., 2002).

RESULTS AND DISCUSSION

The maximum salinity tolerance, maximum density naturally found in the environment, and minimum size reported in the literature for the six selected aquatic invasive species and their life-stages are shown in Table 1. The maximum salinity tolerance for these species ranged from 8 ppt (freshwater) to 44 ppt (highly saline water), and the minimum size ranged from nm for VHSV to cm for the adult Eurasian Ruffe, *Gymnocephalus cernuus*. This is indicative of the diversity in physiology, size, and taxa of aquatic organisms that have been introduced to the Great Lakes via ballast water of ocean-transiting vessels, and the challenges that need to be faced in order to prevent future invasions.

Species Passage Through the Processes Required Under IMO Regulation D-1

Based on the maximum reported salinity distribution/tolerance, and assuming both fully compliant 95% volumetric BWE and shipping as the primary vector of introduction, it is unlikely that any life-stages of the Zebra Mussel *D. polymorpha*, the amphipod *E. ischnus*, and the Eurasian Ruffe *G. cernuus* would have survived due to physiological saline shock (Table 2). Additional support for this is provided by a recent study that tested the efficacy of open ocean BWE in killing freshwater species intolerant to raised salinity. Gray et al. (2007, *in press*) placed live *E. ischnus* in cage enclosures in the ballast tanks of a transoceanic ship and reported 100% mortality post-BWE but low mortality in unexchanged (freshwater) tanks. While a similar physiological shock would have prevented the introduction of free-living *B. longimanus*, it is unlikely that raised salinity would have inactivated resting eggs. For example, despite a lack of empirical data, *B. longimanus* resting eggs may exhibit similar tolerance to open ocean salinities as reported for the resting eggs of other freshwater zooplankton (see Bailey et al., 2004). Salinity exposure during BWE also would have been ineffective in preventing the introduction of the diatom *T. baltica*. Although a recent molecular survey suggested that the strain of VHSV found in the Great Lakes is of marine origin (DFO, 2006), salinity tolerance data for this organism were unavailable, thus physiological shock induced by open ocean BWE could not be evaluated.

Ballast water exchange under IMO Regulation D-1 would also remove substantial numbers of propagules, but some would still remain, with estimates of up to 10^4 m^{-3} for organisms greater than or equal to 50 μm in size, 10^2 ml^{-1} for organisms less than 50 μm but greater than or equal to 10 μm in size, and 10^6 ml^{-1} for virus-like particles (Table 2). The large-scale experiments of Gray et al. (2007, *in press*), showed that 0 - 4.8 x 10^7 live freshwater zooplankton could still be released in an average discharge volume after BWE. In our evaluation, it is not clear that BWE would have been effective in preventing the introduction of those species and life-stages that are saltwater tolerant. After 95% volumetric exchange, an estimated 320 ml^{-1} of cells or resting stages of the diatom *T. baltica* would be present in ballast tanks. Likewise, an estimated density of 4.0 x 10^2 m^{-3} for *B. longimanus* resting eggs, and approximately 1.9 x 10^6 ml^{-1} of virus-like particles, would remain. For the saltwater tolerant species, determining whether the density estimates following 95% volumetric exchange would have been sufficient for colonization is not straightforward. For example, little is known on the effect of stochastic processes and heterogeneity in abiotic and biotic factors on colonization ability (Minton et al., 2005). Uncertainties regarding establishment probability and the number of propagules required for colonization are further addressed in the following section.

Propagule Reduction, Available Treatment Technologies Required to Meet IMO Regulation D-2 Discharge Standards, and Establishment Probability

Our retroactive evaluation of Regulation D-2 assumed that the maximum density of propagules carried in a ballast tank reflected maximum reported natural densities; under this assumption, only adult Eurasian Ruffe would meet ballast water discharge standards (Table 3). No other species/life-stage would comply with Regulation D-2 discharge standards as the number of organisms requiring removal or inactivation ranged from 2 $\log_{10}$ to 5 $\log_{10}$ depending on species and life-stage (Table 3). It is difficult to assess compliance for VHSV in its non-host associated form, since a discharge standard for viruses is not specified under Regulation D-2. It should also be noted that in this evaluation, some propagule abundances are likely to be overestimates. For example, it is unlikely that the maximum reported natural density of the resting eggs of *B. longimanus*, which can occur in benthic sediments at depths up to 100 m (e.g., Yurista, 1997), would be loaded at the same density in a ballast tank.

Currently available water treatment technologies, especially in the drinking water treatment field (e.g., screens, media filtration, oxidizing biocides), are capable of handling such reduction in numbers, and do so almost routinely. For shipboard applications, a screen with 200 µm diameter pore size could be consistently run, with minimal operational issues. From the minimum reported size (Table 1), this would remove the free-living stages of the amphipod (*E. ischnus*) and the Spiny Water Flea (*B. longimanus*), and all life-stages of the Eurasian Ruffe (*G. cernuus*). Physical removal of representative organisms and all of their life-stages, with the exception of VHSV, to the levels required to meet discharge standards is attainable by media filtration, though new design parameters will need to be developed for shipboard use. All of the organisms, in all of their life stages, should be inactivated to the numbers required to meet IMO Regulation D-2 standards through the use of oxidizing biocides, although further development will also be necessary. VHSV is reported to be inactivated by exposure to ether, chloroform, glycerol, formalin, sodium hypochlorite, iodophors, ultraviolet irradiation, or heat (56-60°C) (Schering-Plough, 2007). To control the spread of VHSV in aquaculture facilities, fish eggs are usually treated with iodophors, and the hatchery waters treated with ultraviolet irradiation. VHSV has shown to be also inactivated by 540 mg L^{-1} chlorine for 20 minutes, though it is not clear how many log removals of the virus would be inactivated. Typical Ct values (concentration x contact time) for 4 $\log_{10}$ removal of viruses are 4-8 min·mg L^{-1} at environmental pH (6-9) and temperatures (5-15 °C) (US Environmental Protection Agency, 2003). It should be noted that this assumes VHSV in a "free" non-host associated form; higher Ct values would be required to inactivate VHSV-infected fish hosts. In addition, to prevent the introduction of VHSV via infected hosts, other physical treatment technologies such as screens and media filters can be used to remove fish and all of their life-stages.

Since VHSV has a specific sensitivity to high temperatures, and that the other species are also expected to be intolerant to elevated temperatures as they are considered cold-water species, one potential ballast water treatment strategy would be to use heat generated from ships' engines or other shipboard operations to inactivate organisms. This may be a viable option for ocean-going vessels entering into the Great Lakes under No Ballast on Board (NOBOB) status, as they carry smaller volumes of ballast water. Furthermore, thermal treatment would be a feasible choice for vessels undertaking trans-oceanic voyages, where ballast water can be held in tanks for several days at elevated temperatures.

In retrospect, even if available treatment technologies were able to meet ballast water discharge standards, it is not clear whether implementation of Regulation D-2 would have prevented establishment of selected species. Hallegraff (2001) assessed the likelihood of establishment assuming average ship ballasted densities equivalent to that found in the natural environment. For dinoflagellate cysts, the author estimated that 40,000 L^{-1} could be carried in a ballast tank and that a threshold discharge of just 1000 total cysts could pose a high risk of invasion. Assuming a similar threshold cyst or cell density for the diatom *T. baltica*, and a maximum ballast volume of 24,300 m^3 (see Appendix 2), a ship meeting the Regulation D-2 discharge standard of fewer than 10 ml^{-1} could carry from 0 - 2.43 x 10^{11} propagules. Clearly, if a ship discharged only a fraction of its ballast volume, this could result in enough propagules to meet the threshold proposed by Hallegraff (2001).

In addition, many aquatic species (e.g., *B. longimanus*) exhibit parthenogenesis, which allows rapid asexual population growth. Thus, even at low inoculum densities, asexual reproduction may give rise to sufficient numbers which would increase the probability of establishment (Bailey et al., *in review*). In a worst case scenario of ideal survivability conditions and a propagule density of 10 m^{-3}, the authors predicted low to moderate probabilities of establishment (0.04-0.27). Therefore, our retroactive assessment for the species selected suggests a higher risk of establishment for *B. longimanus* due its parthenogenetic nature.

SUMMARY

The Great Lakes has been the recipient of a number of aquatic invasive species that were introduced in the ballast of ocean-going vessels entering the St. Lawrence Seaway. Prior implementation of IMO Regulation D-1 for 95% volumetric exchange of ballast water would likely have prevented the introduction of the majority of the species/life-stages evaluated because of their intolerance to open ocean salinities. For the few salt tolerant species/life-stages that would likely survive BWE with open ocean water, their densities may still be sufficient after 95% volumetric exchange that they may pose a threat of invasion, although their colonization potential is difficult to predict.

Prior implementation of IMO Regulation D-2 would have substantially reduced the number of organisms introduced into the Great Lakes via ballast water. To meet Regulation D-2 discharge standards, the number of organisms per volume of ballast water must be reduced by a factor of 100 to 100,000 depending on the organism and life-history stage. These treatment levels would be achievable by currently available water treatment technologies, although some development (e.g., biocide dose and contact times for egg or cyst stages, new design parameters for media filtration) will be necessary to adapt these technologies to the ballast water treatment arena. In addition, thermal treatment is a viable option for ocean-going vessels entering into the Great Lakes as most, if not all of the species/life-stages evaluated in this paper are considered cold-water species, and would be expected to be inactivated at elevated temperatures. Long trans-oceanic voyage times and relatively small ballast water volumes that require treatment (for those ships in NOBOB status) make thermal treatment a feasible choice.

It should be noted that even if Regulation D-2 discharge standards were met, the abundance of organisms remaining in ballast water might pose a risk of invasion. This may be particularly true for salt-tolerant and parthenogenetic *B. longimanus* and the cyst-producing *T. baltica*, as compared to the remaining species.

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APPENDIX 1

Representative species selected for retroactive assessment of the efficacy of IMO ballast water management and discharge standards. Species were selected to encompass a variety of ecological levels and life-histories, on which additional details are provided.

1. Zebra Mussel, *Dreissena polymorpha*

Adults are benthic and sessile; veliger larvae are pelagic and motile. *D. polymorpha* originates in the Black and Caspian Sea basins, known as the Ponto-Caspian region. *Dreissena* races may display subtle morphological differences such that species identification is only reliable using molecular markers. Therefore, maximum salinity tolerance was based on that reported for any race or sub-species to provide a more robust estimate of the likely efficacy of BWE.

2. Diatom, *Thalassiosira baltica*

Free-living cells are pelagic; resting cysts can occur in the benthos. The Baltic Sea is the most likely origin of *T. baltica*. Although this species has received little attention in the Great Lakes in terms of apparent impact, it provides a good model for testing the efficacy of Regulations D-1 and D-2 on diatoms, a group commonly reported in ship ballast tanks and represented by a large number of cryptogenic species within the Great Lakes (Hallegraeff, 2001; Kelly, 2007).

3. Amphipod, *Echinogammarus ischnus*

E. ischnus is benthic, free-living and lacks multiple life-stages. Juveniles are produced within a brood pouch, released from adult females, and undergo direct development to maturity. This species is of Ponto-Caspian origin and was reported in ship ballast residual sediment (C. van Overdijk, pers. comm.). Furthermore, a recent study used *E. ischnus* as a model species with which to test the efficacy of mid-ocean BWE (see Gray et al., 2007, in press).

4. Eurasian Ruffe, *Gymnocephalus cernuus*

G. cernuus is a predominantly benthic fish of Eastern European. Adhesive eggs are laid on vegetation or hard substrate, hatch as larvae and undergo a brief or no pelagic phase. Although most larvae settle to benthic feeding, their solitary habitat precludes efficient sampling (Selgeby & Ogle, 1992).

5. Spiny Water Flea, *Bythotrephes longimanus*

B. longimanus occurs as free-living pelagic individuals and reproduces asexual clones from a single female. Sexual diapausing eggs, which are benthic and highly resistant to environmental variation, are also produced. *B. longimanus* is of Ponto-Caspian origin.

6. Viral Hemorrhagic Septicemia Virus (VHSV), a Viral Representative

This viral disease, caused by a rhabdovirus, was previously reported as a natural infection in saltwater finfish but occurs mainly in salmonid hatcheries in coastal North America, Europe and Asia (USDA, 2006). The Great Lakes strain is considered of North American origin since it is closely related to an east coast strain (DFO, 2006). It should be noted that a previous report (see Kelly, 2007) indicated that VHSV was most likely introduced with commercial aquaculture operations rather than ship ballast. However, given public health concerns for introduced diseases, VHSV was chosen as a model to evaluate the efficacy of Regulations D-1 and D-2 on viral pathogens.

APPENDIX 2

The density of some species and life-stages in the literature are expressed as number per unit area, reflecting their occurrence in benthic habitats (Table 1). The IMO regulations, in particular, the discharge standards of D-2, are expressed as number per unit volume. To accommodate this disparity, the protocol below was followed to calculate the number of organisms that would be remaining after BWE (Table 2), and the number of organisms that would need to be removed or inactivated to meet Regulation D-2 (Table 3).

I. Method for Calculating Benthic Surface Area Affected by Ballast Water Uptake

The relationship between flow rate Q, velocity v, and area A, is:

$$Q = A \cdot v$$

where

Q = maximum flow rate into ballast tanks with 2 ballast pumps running; 2500 $m^3 h^{-1}$ (Phil Jenkins, personal communication)

v = velocity required for keeping a particle suspended; 0.010 (range 0.008 – 0.013) cm sec^{-1} for dinoflagellate cysts (Anderson et al., 1985)

A = area; assumes that water taken in as ballast is from a cylinder around the vessel, and is the perimeter of the circle (or benthic surface area) times the height of the cylinder, $2\pi r \cdot h$

Solving for r, or diameter of the benthic surface area affected,

$$r = \frac{Q}{2\pi \cdot h \cdot v}$$

where

h = height of cylinder; assume 10 m, as the maximum draft of a vessel that can be accommodated by the St. Lawrence Seaway system is 8.08 m (Great Lakes St. Lawrence Seaway, 2007)

The benthic surface area affected by ballast water uptake is πr^2, or 3.84 x 10^4 m^2.

II. Method for Calculating the Number of Organisms Per Volume in Ballast Tank

The number of organisms (N) taken up in a ballast tank is:

Number reported per area · Benthic surface area affected by ballast uptake

The number of organisms per volume (N_{vol}) is:

$$\frac{N}{V_{ballast\,tank}}$$

where

$V_{ballast\ tank}$ = Volume of ballast carried by an ocean-going vessel entering the Great Lakes; maximum capacity is 24,300 m^3 (Phil Jenkins, personal communication)