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Multidimensional Approach to Invasive Species Prevention

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ABSTRACT: Nonindigenous species (NIS) cause global biotic homogenization and extinctions, with commercial shipping being a leading vector for spread of aquatic NIS. To reduce transport of NIS by ships, regulations requiring ballast water exchange (BWE) have been implemented by numerous countries. BWE appears to effectively reduce risk for freshwater ports, but provides only moderate protection of marine ports. In the near future, ships may be required to undertake ballast water treatment (BWT) to meet numeric performance standards, and BWE may be phased out of use. However, there are concerns that BWT systems may not operate reliably in fresh or turbid water, or both. Consequently, it has been proposed that BWE could be used in combination with BWT to maximize the positive benefits of both management strategies for protection of freshwater ports. We compared the biological efficacy of "BWE plus BWT" against "BWT alone" at a ballast water treatment experimental test facility. Our comparative evaluation showed that even though BWT alone significantly reduced abundances of all tested organism groups except total heterotrophic bacteria, the BWE plus BWT strategy significantly reduced abundances for all groups and furthermore resulted in significantly lower abundances of most groups when compared to BWT alone. Our study clearly demonstrates potential benefits of combining BWE with BWT to reduce invasion risk of freshwater organisms transported in ships' ballast water, and it should be of interest to policy makers and environmental managers.

■ INTRODUCTION

The establishment of nonindigenous species (NIS) has been implicated as the causal mechanism for 20% of animal extinctions globally and a contributor to an additional 34% of animal extinctions.[1] Further, aquatic NIS are a significant and growing contributor to global impoverishment of "red-listed taxa", and are the dominant driver of species homogenization and change in lake ecosystems.[2−4] As a result, environmental managers are under increasing pressure to establish comprehensive management programs to prevent, control and eradicate NIS, with prevention playing a dominant role.[5−7]

Commercial shipping has been identified as a leading vector for aquatic species invasions.[7−9] To reduce the probability of arrival of NIS by ballast water, both Canada and the U.S. have implemented regulations requiring midocean ballast water exchange (BWE) of filled, and saltwater flushing of empty, ballast tanks.[10−12] In theory, BWE and saltwater flushing should reduce abundance and species richness of transported taxa by purging individuals from tanks or, particularly in the case of freshwater species, by killing them with euhaline salt water. Recent scientific research shows BWE and saltwater flushing effectively reduce invasion risk for freshwater systems;[13−15] however, efficacy is mixed for marine ecosystems.[16−20]

An international Ballast Water Management Convention, when ratified, will require ships to replace BWE with ballast water treatment (BWT) using technologies such as filtration, chlorination or deoxygenation that are able to meet a numeric

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performance standard: ships shall discharge less than 10 viable organisms ≥50 μm per m^3; less than 10 viable organisms ≥10 to <50 μm per mL; 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.[21] While the risk of treated ballast water is generally expected to be lower than that of exchanged ballast water, there are concerns that BWT systems may not operate reliably in fresh or turbid water, or that the proposed performance standards are not stringent enough.[22,23]

Invasions are a stochastic process, and while there are numerous established populations of invading species globally, the majority of invasion opportunities are not successful.[24,25] The invasion process consists of a series of stages, including transport, arrival, survival, and establishment, where successful transition between stages depends primarily on three factors: propagule pressure (i.e., number of individuals introduced), physiological tolerance to environmental conditions and integration into the biological community.[25−27] Consequently, the government of Canada proposed combining BWE with BWT to provide best protection of freshwater systems in an effort to maximize the positive benefits of both ballast water management strategies: reduced propagule pressure through BWT and reduced physiological tolerance (i.e., salinity barrier) through open ocean BWE.[28] This combined approach addresses two factors of the invasion process, which will likely be more effective than an approach which focuses on only a single component.[28]

In this study, we conducted a land-based evaluation to compare the effectiveness of simulated "BWE plus BWT" versus "BWT alone", testing the hypothesis that BWE plus BWT more effectively reduces the abundance of live organisms in ballast water than does BWT alone. We end with a brief discussion on management strategies and scientific implications of our results.

■ MATERIALS AND METHODS

Test Facility and Experimental Design. To determine if BWE plus BWT more effectively reduces the abundance of live organisms in ballast water than does BWT alone, in October 2010, we conducted large-scale experimental trials at the Northeast-Midwest Institute's Great Ships Initiative Land-Based Research, Development, Testing, and Evaluation facility in Duluth-Superior Harbor in the Laurentian Great Lakes (i.e., Lake Superior); this facility was purpose-built for intensive testing of BWT systems at operational scales using verified protocols consistent with International Maritime Organization (IMO) testing Guidelines and the U.S. Environmental Protection Agency's Environmental Technology Verification Program.[22,29] Trials were conducted using the IMO Type-approved AlfaWall AB PureBallast 2.0 BWT system, which utilizes filtration (at 40 μm) and a patented advanced oxidation technology (Wallenius AOT) involving ultraviolet radiation and a catalyst on intake, and advanced oxidation technology (no filtration) on discharge. The AlfaWall AB PureBallast 2.0 BWT system was chosen opportunistically for this testing since a unit was already installed at the test facility for standard testing. Synthetic ocean water for simulated BWE was prepared 8−10 hours in advance by slowly mixing Instant Ocean (Spectrum Brands, Inc.; Madison, WI) into potable dechlorinated water to achieve a final salinity of 32 ppt, as measured by a YSI Multiparameter Water Quality Sonde.

Three paired trials of BWE plus BWT and BWT alone were conducted. For each trial, two large holding tanks were filled simultaneously with 100 m^3 of Duluth-Superior Harbor water per tank. Plankton densities were augmented by concentrating natural populations from the harbor and injecting them into the intake flow (Figure 1); the target densities for organisms ≥50 μm

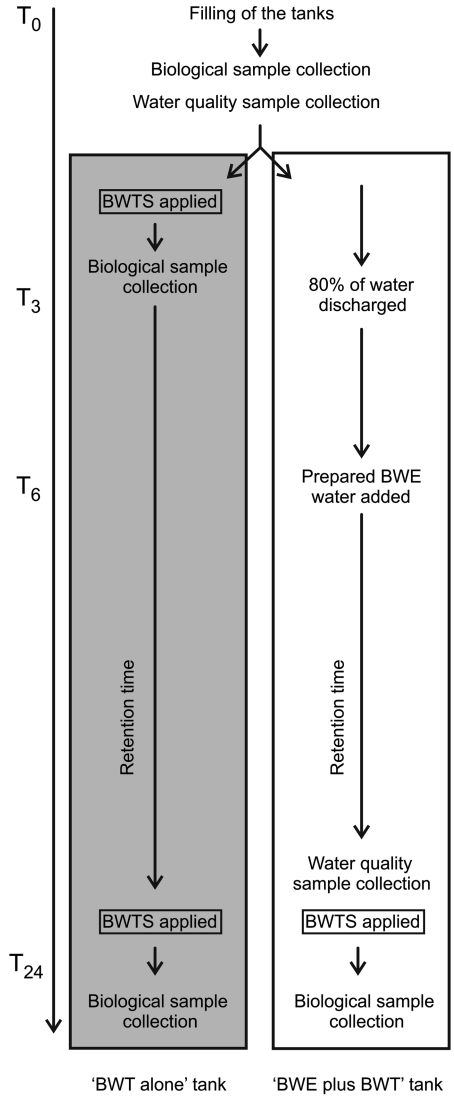

Figure 1. Schematic representation of experimental design comparing effectiveness of simulated ballast water exchange plus ballast water treatment (BWE plus BWT) versus ballast water treatment alone (BWT alone). T_0, T_3, T_6, and T_{24} denote time scale from hour zero to hour 24 (not to scale).

and ≥10 to <50 μm were at least 50 000 individuals per m^3 and 500 cells per mL, respectively. The water directed to the BWT alone tank was treated using the BWT system, whereas that directed to the BWE plus BWT tank was not treated. Immediately after filling, 80 m^3 of water from the untreated tank was discharged to the Duluth-Superior Harbor and replaced with 80 m^3 of 32 ppt synthetic ocean water. These volumes simulated a BWE with 80% volumetric exchange and took approximately six hours to complete; whereas the IMO proposes 95% volumetric exchange for BWE,[21] the lesser proportion was selected to make the experiment conservative, reflecting observations of incomplete BWE in the field. Similarly, the BWT system was not applied during initial intake of the water directed to the BWE plus BWT tank in keeping with a conservative approach. Following 16−24 h retention, first the

Table 1. Average, Standard Error (SE) and Percent Difference Abundance of Plankton ≥50 μm per m^3 and ≥10 to <50 μm per mL, in Initial Duluth-Superior Harbor Water, Ballast Water Treatment Alone (BWT Alone)-Initial, BWT Alone-Discharge, and Ballast Water Exchange Plus BWT (BWE Plus BWT)-Discharge Water (n = 3)

ballast water management strategy	≥50 μm		≥10 to <50 μm	
	mean (SE)	% difference from the Duluth-Superior Harbor water	mean (SE)	% difference from the Duluth-Superior Harbor water
Duluth-Superior Harbor water	75,886 (6055)	n/a[a]	766.06 (47.14)	n/a
BWT alone-initial	6729 (1403)[b]	−91.13	278.41 (16.67)	−63.66
BWT alone-discharge	1053 (336)[b]	−98.61	63.68 (13.51)[b]	−91.69
BWE plus BWT-discharge	5.59 (2.37)[b,c]	−99.99	4.73 (2.31)[b,c]	−99.38

[a]n/a Denotes not applicable. [b]Denotes significant difference in density of organisms as compared to initial Duluth-Superior Harbor water. [c]Denotes significant difference in density of organisms between BWE plus BWT-discharge and BWT alone-discharge.

Table 2. Average and Standard Error (SE) of Total Heterotrophic Bacteria, Total Coliform Bacteria, *Escherichia coli* and Enterococci (Most Probable Number (MPN) per 100 mL) in Duluth-Superior Harbor Water, Ballast Water Treatment Alone (BWT Alone)-Initial, BWT Alone-Discharge, and Ballast Water Exchange Plus BWT (BWE Plus BWT)-Discharge Water (n = 3)

ballast water management strategy	total heterotrophic bacteria mean (SE)	total coliform bacteria mean (SE)	*Escherichia coli* mean (SE)	Enterococci mean (SE)
Duluth-Superior Harbor water	24,955.6 (7611.7)	143.2 (14.7)	64.1 (12.3)	26.3 (5.4)
BWT alone-initial	9404.4 (4902.4)	1.9 (0.4)[a]	<1[a]	<1[a]
BWT alone-discharge	31,800.0 (5049.9)[c]	2.2 (1.7)[a]	<1[a]	4.4 (1.4)[a]
BWE plus BWT-discharge	6044.4 (1601.1)[a,b]	68.0 (40.7)[a,b]	<1[a]	<1[a]

[a]Denotes significant difference in density of organisms compared to initial Duluth-Superior Harbor water. [b]Denotes significant difference in density of organisms between BWE plus BWT-discharge and BWT alone-discharge. [c]Denotes significant difference in density of organisms between BWT alone-initial and BWT alone-discharge.

BWT alone and then the BWE plus BWT tank were treated by the BWT system and discharged.

Sample Collection and Enumeration of Live/Dead Organisms. Enumeration of live/dead organisms in three groups (i.e., organisms ≥50 μm, organisms ≥10 to <50 μm in size, and microbes) was undertaken for samples collected at intake before and after BWT, and during discharge after BWT and BWE plus BWT (Figure 1). For analysis of the largest size group, 2−3 m^3 samples were collected at each BWT alone intake sampling event and duplicate 2 m^3 samples at each BWT alone and BWT plus BWE discharge sampling event, which were concentrated through a 50 μm (diagonal dimension) mesh plankton net into 1 L cod ends. The plankton nets were suspended in water within sample tubs to reduce handling effects. Within two hours of sample collection, retained organisms were enumerated using Sedgewick Rafter counting chambers for microzooplankton (e.g., rotifers, copepod nauplii, veligers, etc.) under a compound microscope at 40−100× magnification. For macrozooplankton (e.g., copepods, cladocerans, etc.), a Ward's Counting Wheel was used under a dissecting microscope at 20−30× magnification. Live/dead distinction was made using standard movement and response-to-stimuli techniques.

For analysis of organisms ≥10 to <50 μm in size, 1 L of water was collected at each intake sampling event while triplicate 1 L samples were collected at each BWT alone and BWT plus BWE discharge sampling event and composited into a single 1 L sample. The samples were analyzed within 1.5 h of sample collection. Samples were concentrated through 10 μm mesh netting to 25 mL final volume. Two mL subsample of the concentrated sample was then transferred to a 5 mL sample container and 5 μL of fluorescein diacetate (FDA) viability stain stock solution added. The subsample was left to incubate in the dark for 5 min before mixing and transferring 1.1 mL to a Sedgwick-Rafter cell for observation using bright field and epifluorescence microscopy at 200× magnification under a compound microscope. Single cell entities and cells comprising colonial and filamentous entities were characterized as alive if cell contents exhibited green fluorescence.[30] All living cells were counted and measured, ensuring that they met size requirements.

Further, 1 L water samples were collected in triplicate at each sampling event for microbial analysis. Samples were transported in an insulated cooler within one hour to the University of Wisconsin-Superior's Lake Superior Research Institute for analysis of total heterotrophic bacteria, total coliforms, *Escherichia coli*, and Enterococci. *V. cholerae* was not tested since it is not present at detectable levels in the Duluth-Superior Harbor (Great Ships Initiative, unpublished data); however, to get insight into the efficacy of the two ballast management strategies against bacteria in general, total heterotrophic bacteria and total coliforms were tested. Viable heterotrophic bacteria were enumerated, and presence and abundance of total coliform bacteria, *E. coli* and Enterococci were checked using standard most probable number (MPN) protocols for the IDEXX system (i.e., IDEXX's SimPlate, IDEXX's patented Defined Substrate Technology and IDEXX's Colilert; GSI 2011).

Additionally, two replicates of 1.125 L of Duluth-Superior Harbor water and two replicates of 1.125 L of the BWE plus BWT-discharge water before the BWT system was applied were collected to check physical and chemical characteristics of water. Total suspended solids (TSS), nonpurgeable organic carbon (NPOC), dissolved organic carbon (DOC), particulate organic carbon (POC), mineral matter (MM), and percent transmittance (% T) were processed following standard procedures.[31]

Statistical Analyses. To examine the effects of the different ballast management strategies, multivariate analysis of variance (MANOVA) was conducted with groups (organisms ≥50 μm, organisms ≥10 to <50 μm, total heterotrophic bacteria, total coliform bacteria, *E. coli*, and Enterococci) as dependent variables and ballast water management strategy (no treatment - Duluth-Superior Harbor water, BWT alone-initial, BWT alone-discharge, and BWE plus BWT-discharge) as independent variable. Data for each group were log-transformed ($\log_{10}$ (x +

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1)) to meet assumptions of parametric tests. Additionally, post hoc Bonferroni tests were applied. Significance levels for statistical comparisons were 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).

RESULTS

Initial plankton densities after augmentation in intake Duluth-Superior Harbor water in the ≥50 μm and ≥10 to <50 μm groups averaged 75 886 individuals per m^3 and 766 cells per mL, respectively (Table 1). The densities of microbes were generally low across samples (Table 2). Average water quality parameters were 2.3 mg/L of TSS, 21.1 mg/L of NPOC, 20.8 mg/L of DOC, 0.3 mg/L of POC, and 2.0 mg/L of MM. Percentage of transmittance was 14.2. Differences in abundances of organisms in all groups following experimental treatment by the different ballast water management strategies were found significant (Table 3).

Table 3. Results of Multivariate Analysis of Variance (MANOVA) Addressing the Effect of Ballast Water Management Strategy (No Treatment - Duluth-Superior Harbor Water, BWT Alone-Initial, BWT Alone-Discharge, and BWE Plus BWT-Discharge) on the Different Groups of Organisms (≥ 50 μm, ≥ 10 μm to <50 μm, Total Heterotrophic Bacteria, Total Coliform Bacteria, *E. coli*, and Enterococci)

variable	df	*F*	*p*
univariate *F* tests			
organisms ≥50 μm	3	400.426	<0.001
organisms ≥10 μm and <50 μm	3	45.324	<0.001
total heterotrophic bacteria	3	29.179	<0.001
total coliform bacteria	3	287.606	<0.001
E. coli	3	815.897	<0.001
enterococci	3	18.029	<0.001
multivariate test			
Wilks' lambda = 0.000	18	59.030	<0.001

Efficacy of BWT Alone. During intake, immediately after BWT, densities of organisms in the ≥50 μm group were significantly decreased, having values 91.13% lower than initial harbor water ($p < 0.05$) (Table 1). Total coliform bacteria, *E. coli*, and Enterococci were also significantly reduced ($p < 0.05$) (Table 2). Although densities of organisms in the ≥10 to <50 μm group decreased nearly 63.66% of initial harbor water and total heterotrophic bacteria were also reduced, the decreases were not statistically significant ($p > 0.05$) (Tables 1 and 2). After the retention period of 16−24 h, densities of organisms in the ≥50 μm group and the ≥10 to <50 μm group further decreased significantly as compared to both the Duluth-Superior Harbor water and BWT-initial ($p < 0.05$) (Table 1). In contrast, densities of total heterotrophic bacteria and Enterococci increased after retention compared to BWT-initial; however, the increases were not statistically significant ($p > 0.05$) (Table 2).

Efficacy of BWE plus BWT. Average water quality parameters of the BWE plus BWT-discharge water before BWT was applied were 3.9 mg/L of TSS, 5.3 mg/L of NPOC, 5.0 mg/L of DOC, 0.3 mg/L of POC, and 3.7 mg/L of MM. Percentage of transmittance was 67.1%. The 'BWE plus BWT' discharge densities of organisms in all groups were significantly reduced when compared to initial Duluth-Superior Harbor water densities ($p < 0.05$) (Tables 1 and 2). The densities of live organisms in the ≥50 μm and ≥10 to <50 μm groups decreased four and 2 orders of magnitude, respectively, representing a decrease of 99.99 and 99.38% (Table 1). Total heterotrophic bacteria and total coliform bacteria were both reduced 1 order of magnitude, whereas that of *E. coli*, and Enterococci fell below one MPN per 100 mL (Table 2).

Comparison of BWE Plus BWT Versus BWT Alone. Densities of organisms in the ≥50 μm and ≥10 to <50 μm groups, and that of total heterotrophic bacteria were significantly lower after BWE plus BWT than after BWT alone ($p < 0.05$) (Tables 1 and 2). There was no significant difference in efficacy of the two strategies for *E. coli* nor for Enterococci ($p > 0.05$) (Table 2); however, total coliform bacteria densities in the BWE plus BWT discharge samples were significantly higher compared to BWT alone ($p < 0.05$) (Table 2).

DISCUSSION

Our trials provide preliminary data to illustrate potential benefits of combining BWE with BWT to reduce invasion risk by freshwater organisms in ships' ballast water. The BWE plus BWT strategy resulted in significantly lower abundances of individuals in the ≥50 μm and ≥10 to <50 μm groups compared to BWT alone, and only the BWE plus BWT strategy significantly reduced the abundance of total heterotrophic bacteria. Although the aim of our study was not to verify the efficacy of the BWT system, in this particular case, it did not by itself meet the IMO requirements;[21] while these results conflict with earlier reports of 99.999% organism removal,[32] our results are consistent with more extensive trials of this BWT system at the Great Ships Initiative land-based facility earlier in the season.[33] Notably, the discharge from the combined BWE plus BWT strategy tested here met the proposed international performance standard.[21]

The final abundance of live organisms in the ≥50 μm and ≥10 to <50 μm groups after BWE plus BWT was >99% lower than in the initial Duluth-Superior Harbor water. If we assume that there was 80% dilution of organism abundance after BWE, and that the BWT system applied at discharge performed with the same efficacy as it did during the intake of the BWT alone, the expected abundance of organisms in the ≥50 μm and ≥10 to <50 μm groups would be three and 1 order of magnitude higher than our observed results, respectively, indicating that the effect of the combined strategy is more than additive. The lethal effect of osmotic shock during BWE is the most likely explanation for the observed reduction.[13] These results are particularly promising given the fact that our tests conservatively simulated BWE with 80% replacement of water rather than the minimum suggested volume of 95%.[21]

Another explanation for the enhanced performance of BWE plus BWT is that BWE may have facilitated better performance of the BWT system. The poor performance of the BWT system during our tests may be related to the ultraviolet radiation component of the BWT system, which was designed for use in water with significantly greater percent transmittance than was measured in the Duluth-Superior Harbor during our tests. The very low percentage of transmittance (14.2%) encountered during our study is similar to conditions of many fresh and brackish water ports globally, especially those located in river mouths.[33−35] The BWE plus BWT scenario may have mitigated the transmittance challenge through replacement of 80% of the Duluth-Superior Harbor water with artificial salt water having higher transmittance. Unfortunately, from our tests it is not possible to determine whether the same magnitude of benefits of

BWE plus BWT would be observed for tests with a BWT system which performs better on Duluth-Superior harbor water; however, our findings are applicable to a real world application, as ports tend to be turbid, with lower percentage transmittance than open sea or lake water.[33−36] As a result, the BWE plus BWT strategy may be particularly beneficial when there are challenging port water conditions at the point of ballast uptake. In addition, our tests illustrate an additional benefit of a combined approach: BWE would serve as an inherent back-up strategy if the BWT system should fail for any reason.

The variable and nonsignificant results in microbial densities between BWE plus BWT and BWT alone probably stem from the low densities on intake, likely due to the late-season timing of the trials. Voyage characteristics, particularly temperature, and dissolved oxygen, influence bacterial dynamics; whereas increases in dissolved oxygen reduce heterotrophic bacterial abundance, increased water temperature leads to regrowth and increased abundance.[18] Notably, we observed significant regrowth of total heterotrophic bacteria under the BWT alone scenario, and if we assume again that the BWT system applied at discharge performed with the same efficacy as during intake of the BWT alone, the regrowth of total heterotrophic bacteria just before discharge might have been three to four times higher than in initial Duluth-Superior Harbor water. The unpredictable ballast tank environments and fast generation time of microorganisms points out the potential importance of BWT immediately prior to discharge of ballast water in either the BWT alone, or the BWE plus BWT scenario.

Though contrived, our experimental comparison of the two ballast management strategies was also highly controlled, allowing for a more precise comparison than a field trial would afford. Trials using a BWT system which performs well under natural harbor conditions may not lead to significant differences in final discharge abundance between BWE plus BWT and BWT alone scenarios, yet the failure of a BWT system which had already received IMO Type Approval demonstrates the importance of an inherent back-up strategy. The lack of live marine organisms in our synthetic ocean water is suitable for assessing invasion risk to freshwater recipient ports, since oceanic taxa entrained during true BWE are expected to have a low probability of survival when discharged to freshwater receiving systems.[13,15] However, the challenge faced by the BWT system could be more difficult if incoming ocean water has a high abundance of marine taxa. For this reason, it would be beneficial to conduct additional trials using genuine ocean water with resident taxa, ideally under operational conditions at the shipboard scale.

Various BWT technologies developed over the last two decades apply one or more treatment processes, such as filtration, ozonation, ultraviolet radiation, cavitation, chlorination, etc., each of which have inherent advantages and disadvantages regarding biological efficacy, costs, ship and crew safety, power and space requirements, and environmental soundness.[32] Technologies that combine filtration and ultraviolet radiation such as AlfaWall AB PureBallast 2.0 BWT have the advantage of being environmentally friendly and relatively cost-effective. Estimated costs of BWE and BWT are US$0.01−0.02 and US $0.06 per tonne of ballast water, respectively;[32] whereas the combination BWE plus BWT strategy would increase ship operational costs, those costs would still be negligible compared to the costs of delays if ballast water is found noncompliant at the destination port. Due to extra operating and maintenance costs, some vessel crews may choose not to apply BWT before BWE since BWE would subsequently increase organism density after BWT at uptake, however, it may be good practice to utilize BWT at every ballast uptake since foul weather or equipment failure may sometimes preclude BWE without warning.

The invasion process consists of a series of stages, where successful transition between stages depends on the abundance of individuals introduced, the physiological tolerance of introduced individuals to environmental conditions, and integration into the biological community.[25−27] A management strategy using a BWT system alone targets only the first factor, aiming to reduce abundance of discharged individuals, whereas in the case of freshwater systems the combined BWE plus BWT strategy also reduces physiological compatibility of discharged individuals with the recipient environment due to an environmental salinity barrier. Our study clearly demonstrates potential benefits of combining BWE with BWT to reduce invasion risk to freshwater systems, and results presented here should interest policy makers and environmental managers. Beside the shipping vector, this multidimensional approach, resulting in enhanced risk reduction, is applicable for management of a wide variety of invasion vectors.

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Notes

The authors declare no competing financial interest.

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