2005

Ridding Ships' Ballast Water of Microorganisms

Fred C. Dobbs
Old Dominion University, fdobbs@odu.edu

Andrew Rogerson

Repository Citation

Dobbs, Fred C. and Rogerson, Andrew, "Ridding Ships' Ballast Water of Microorganisms" (2005). *OEAS Faculty Publications*. 302.
https://digitalcommons.odu.edu/oeas_fac_pubs/302

Original Publication Citation

Dobbs, F. C., & Rogerson, A. (2005). Ridding ships' ballast water of microorganisms. *Environmental Science & Technology, 39*(12), 259A-264A. doi:10.1021/es053300v

Ridding Ships' BALLAST Water *of* Microorganisms

Is it even possible to remove, kill, or "inactivate" all of them—and if so, should we try?

FRED C. DOBBS
OLD DOMINION UNIVERSITY
ANDREW ROGERSON
NOVA SOUTHEASTERN UNIVERSITY

FRED DOBBS

A complex assemblage of microorganisms exists in nearly every aquatic system on earth. In lakes and oceans, every milliliter of water contains about 10^2 protists (single-celled eukaryotes), 10^6 bacteria, and 10^7–10^9 viruses. Therefore, billions of microorganisms inevitably enter ships' ballast tanks during normal operations. It has been argued that microorganisms must certainly be frequent invaders of coastal ecosystems, given the high densities of bacteria and viruses in ballast water—10^8 and 10^9 organisms per liter, respectively (*1*)—their potentially high reproductive rates, broad tolerances to physical conditions, and ability to form resting stages (*2*). The "propagule pressure" of microorganisms contrasts sharply with the mere tens of thousands of mesozooplankton that might be released during ballast discharge (*3*). The phylogenetic diversity of microbes in ballast water is reportedly composed of large, easily recognized forms, such as dinoflagellates, diatoms, ciliates, and foraminifera (*1, 4*). However, the bacterial and viral diversity in ballast water is absolutely unknown. Our understanding of the microbial diversity found in ballast tanks depends on new, sophisticated molecular biological techniques and certainly will increase with more advanced studies (*5, 6*).

Although the overwhelming majority of microorganisms occur naturally and are not harmful to humans, ballast water does include some pathogenic bacteria (*7*) and dinoflagellates (*8*) that represent risks to public health. Their low levels make detection difficult. Moreover, the unpredictable presence of harmful microorganisms and indicator bacteria in ballast tanks and residuals (*9*) may help pathogens, such as *Vibrio cholerae* (*2*), two species of *Pfiesteria* (*10*), and *Aureococcus* (a "brown tide" alga, *11*), spread undetected into fresh and marine waters. Furthermore, biofilms inside tanks could serve as "seed banks" for invasions (*12*).

With these concerns in mind, we address two issues in this paper. First, do technologies exist that can remove, kill, or inactivate microorganisms in ballast water without compromising the structural integrity of ballast-tank walls or their protective coatings, yielding treated water that may be discharged safely and legally into coastal waters?

The second issue is more controversial: Is ballast-water transport of microbes actually a problem? Some microbial ecologists argue that bacteria and most, if not all, protists must already be distributed worldwide, simply because their small size facilitates dispersal. If microorganisms indeed have no biogeography—that is, they are ubiquitous in their distribution—then they cannot be considered "invasive species" and their presence in ships' ballast water is of little concern.


FRED DOBBS

Can we remove or kill microorganisms?

We deliberately use the term "inactivate" in our discussion to extend the phylogenetic reach of the question beyond cellular microorganisms to viruses. Viruses do not replicate independently but instead rely on host cells for propagation of their genetic material. Thus, they are not "living" in any biological sense. Viruses can be inactivated, however, such that they cannot infect host cells. For the sake of simplicity, we shall include "inactivate" whenever we use "kill" in the remainder of this article, although the difference is not merely a semantic one.

To tackle these various microbes, we need to evaluate certain treatment technologies—namely, filtration, UV irradiation, and biocides—as well as some proposed approaches. These evaluations are deliberately phrased in general terms, because the peer-reviewed literature on treating ballast water is sparse.

Filtration. Filtration of relatively large volumes of coastal waters through screens with effective mesh sizes as small as 25 µm has been reported (*13*, *14*). Screens of this size will remove cysts of some harmful dinoflagellate species (25–87 µm in diameter) but will not retain more abundant flagellates (2–10 µm), bacteria (0.2–1.0 µm), and viruses (20–200 nm). Some of those microbes are retained on the screen because they are associated with suspended flocs or adhere to the surfaces of biotic and abiotic particles, but the effectiveness of removal depends on the abundance of particulates in the water column. Although filtration can clearly remove ichthyoplankton, invertebrate zooplankton, and the largest phytoplankton and heterotrophic protists, it cannot at present reliably reduce the concentrations of most microorganisms in ballast water.

UV light. Irradiation with UV light is a highly effective, well-understood technology that has been used for decades to reduce or eliminate microorganisms, including viruses, in large volumes of water under high flow conditions. For ballast water, UV light has been successful in the laboratory (*15*) and at larger scales (*14*, *16*). Therefore, without considering the technical problems inherent in operating a UV reactor on a ship, we believe that this approach is a very effective technology for ballast-water treatment.

However, if the treated water stays in the tank, its fate must be considered. Not all treatment vendors appreciate that voraciously grazing protists keep bacterial numbers in check in aquatic systems. Any treatment that kills the heterotrophic protists but does not eliminate all bacteria leaves open the possibility of unchecked, exponential bacterial growth. So-called regrowth has been reported after UV treatments (*14*); this finding implies that if ballast water is treated on uptake, it may also need treatment on discharge.

Biocides. Both oxidizing and nonoxidizing biocides can be very effective against microorganisms, given sufficient concentration and contact time. In particular, oxidizing agents, such as chlorine, bromine, and their multiple compounds, have long been used to decontaminate drinking water and treated sewage. In ships' ballast tanks, ozone has advantages over the halogens. A demonstration project showed killing of heterotrophic bacteria, phytoplankton, and zooplankton (*17*). In laboratory tests, hydrogen peroxide (an oxidizing biocide, *18*) and glutaraldehyde (a nonoxidizing biocide, *19*) were effective against zooplankton and the bacterium *Vibrio fischeri*, respectively.

But at least three other issues need consideration. First, will the chemical accelerate corrosion of metal in tank walls or deteriorate the protective coatings applied to the walls? Second, will the biocide's concentration after treatment be too high for permitted release into coastal waters? Third, if the treatment involves a biologically "active ingredient", will the International Maritime Organization (IMO) preapprove its use, a requirement dictated by the IMO convention currently being considered for ratification? If the answer is "yes" to the first or second question or "no" to the third, then the biocide will be of little worth.

Other proposed technologies. Deoxygenation over the course of several hours or longer certainly will kill most metazoans (*20*); however, it will have little effect on those bacteria and protists with metabolic systems that have evolved to routinely switch between oxic and anoxic environments. Thermal treatments have shown some promise (*21*) but cannot reach the temperatures needed for bulk pasteurization (63–66 °C), much less sterilization (>100 °C), and so they will not kill all bacteria. Electric-pulse techniques have been shown to work in the laboratory (*22*), but we know of no data from larger-scale operations. Plasma techniques, acoustic systems, and magnetic fields have been proposed in the context of ballast-water treatment, but we are unaware of any peer-reviewed data that demonstrate efficacy.

Overall, we conclude that treatment technology has not yet developed to the point where all microorganisms in ballast water either can be removed (even if viruses are excluded from consideration) or can be killed without making the treated water unsuitable for discharge. Although we have not considered here the effects of two treatment methods in series, such as filtration followed by UV irradiation, we believe that the most successful treatments likely will involve concatenated technologies. Even so, complete removal of all biota is unlikely in the near future, even with multiple treatments.

Should we try?

Regardless of the ballast-water treatment used, it is inevitable that some living microorganisms will be discharged into receiving waters. Grazing by protists will eliminate some of the discharged microorganisms. Where sunlight is present, UV inactivation will also lower numbers. And dilution often precludes cultivation in large volumes—after all, even the most proficient culturalists cannot grow many otherwise culturable microorganisms when only a few cells are added to an ideal culture medium. We are confident, therefore, that natural attrition processes can significantly reinforce the treatment process as long as the numbers of microbes have been reduced in the water prior to discharge.

Complete removal of all biota is unlikely in the near future, even with multiple treatments.

Therefore, do we need to be concerned about introducing surviving microorganisms into coastal waters after transoceanic or intracoastal transport? In a recent and provocative hypothesis, Finlay says no (*23*). He states that large animals—elephants and tigers, for example—have biogeographies; that is, their distribution is specific or endemic to certain geographical areas. However, free-living microbial species lack a biogeography, Finlay contends; because of their small size, stochastic processes of dispersal will effectively distribute them in a cosmopolitan manner. If they are distributed ubiquitously, then microorganisms cannot be considered invaders, and concern about their presence, at least with respect to invasion biology, is misguided. We must emphasize that Finlay's argument for ubiquitous distribution applies only to free-living microbes. Microorganisms that have an obligate symbiosis with another organism will necessarily have a biogeography if their symbiont has one.

Finlay's hypothesis runs counter to many traditional views about the biogeographic distribution of microbial species and hence the diversity of microorganisms in general. In essence, he contends that the global number of microbial species (as exemplified by protists) is much lower than current estimates because of their ubiquity. He argues that because microbes are locally abundant, relative to larger organisms, their rates of migration will be high, especially because many microbes have resistant cyst and spore stages. Consequently, the rate of allopatric speciation is low, so that endemic species are rare, and the proportion of global species found locally must be high (*24*).

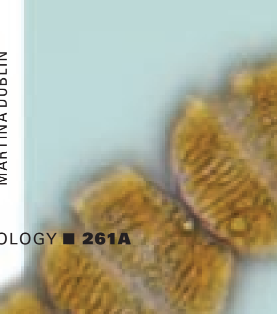
How can these global microbial species be everywhere when we cannot find them everywhere? Finlay et al. argue that this paradox stems from undersampling of habitats and inadequate methods for the detection of rare species (*24*). At the microbial level, rare or cryptic species are simply too elusive to be detected by traditional methods that sample small volumes of water or sediment.

The contention that many microorganisms have cosmopolitan distributions has ramifications for ballast-water treatment standards. If Finlay et al. (*24*) are correct, it should be possible to define a size class that delineates a transition zone between organisms with a cosmopolitan distribution and those with a biogeography. Finlay's other work suggests that this transition zone may range from 1 to 10 mm in size (*23*, *25*).

Note that Finlay's argument is based on a concept in which organisms are classified and identified by their morphology. Although the morphology approach is very reasonable and has been practiced for centuries, it has its drawbacks. Principally, genetic diversity is unaccounted for, except what can be inferred from morphological differences. In the case of bacteria, at least, the presence of numerous genetic variants and their associated phenotypic expressions may justify changing the definition of invasion from considering species to genotypes. On the other hand, if ubiquity of microorganisms is accepted, then ubiquity of strains is also likely, because strains are also locally abundant populations that can be dispersed efficiently. Finlay calls for better information about clonality in microbes (*26*). Clearly, we need to know more about the ubiquity issue before the industry and regulators can act. For now, the idea remains an intriguing theory worthy of more research.

Microbes to worry about

Toxic dinoflagellates can harm aquaculture and human health in several ways, including introducing the human disease paralytic shellfish poisoning (PSP). Before 1970, PSP was unknown in the Southern Hemisphere, but by 1990, cases had been documented throughout Australia, New Zealand, Papua New Guinea, and South Africa; its range also expanded in the Northern Hemisphere (*8*). Did ballast water and its transoceanic transport by ships disseminate PSP, or did local conditions change to allow the proliferation of such a cryptic, indigenous species?



MARTINA DOBLIN

In short, the answer may be ballast water. Dinoflagellate cysts have been reported, sometimes in great abundance, in ballast-tank sediments of ships arriving in Australia, New Zealand, the United States, Canada, Scotland, England, and Wales (*27*). As many as 300 million cysts have been estimated to exist in a single ballast tank (*28*). Hence, dinoflagellate cysts are likely microbial constituents of ballast tanks, especially when sediments have been entrained on ballast uptake.

FRED DOBBS

Moreover, toxic species of dinoflagellates appeared suddenly in Australia in the 1980s, caused PSP, and wreaked severe economic havoc on molluscan shellfisheries (*29*). Although the geographic origin of these dinoflagellates is debated (*30*), they were clearly absent from Australia's coastal phytoplankton before the 1980s. In an independent study, genetic analysis demonstrated that a PSP-causing dinoflagellate, one unreported in the Mediterranean before 1998, had originated in the western Pacific Ocean (*31*). The researchers strongly implicated ballast-water transfer in the dinoflagellate's geographic translocation.

The rapid and large-scale geographic expansion of PSP-causing dinoflagellates is consistent with the argument that these microbes are indeed transported and were not as widely distributed decades ago. Here, the evidence speaks strongly for eliminating (or at least minimizing) the discharge of dinoflagellates from ships' ballast tanks.

V. cholerae is the etiologic agent of human cholera (*32*). Although the bacterium's habitat and distribution were formerly thought to be obligately associated with its human host, we know now that it is a widely distributed aquatic species often found in nearshore environments (*33*).

Ruiz et al. measured *V. cholerae* concentrations of 100 and 1000 cells per liter (for pandemic serotypes O139 and O1, respectively) in ballast water arriving in Chesapeake Bay in the United States (*2*). The motivation for these measurements was, in part, earlier evidence of *V. cholerae* transport via ballast water. In 1991, toxigenic *V. cholerae* O1 was found in oysters and the intestinal contents of fish in Mobile Bay, Ala. (*34*). This strain of *V. cholerae* was genetically indistinguishable from one responsible for a cholera epidemic in South America at the time. When the ballast waters of ships leaving South American countries and arriving in Mobile Bay were later tested, they contained the epidemic-causing strain. This scenario suggests that ballast water was a vector for introducing the epidemic-causing strain to the U.S. Gulf Coast (*7*), although no human health effects were reported.

In a follow-up laboratory study, clinical isolates of *V. cholerae* and one isolated from ballast water were shown to be capable of surviving for several months in ships' ballast tanks, certainly long enough to be transported on any present-day commercial voyage and potentially inoculated into a destination port (*35*). Most environmental isolates of *V. cholerae* are missing the virulence factors characteristic of clinical isolates (*32*), but laboratory studies have demonstrated that they can acquire serological determinants and toxin genes by horizontal (or "lateral") gene transfer (HGT, *36*). (HGT is the exchange of genetic elements among prokaryotes.) HGT has been shown to occur much more frequently than previously thought, with important ramifications for our thinking about the evolutionary forces shaping bacterial communities (*37*). Clearly, implications also exist for ballast-water treatment, particularly with respect to bacteria simultaneously carrying genes for virulence and antibiotic resistance.

One mode in which bacterial HGT occurs is termed conjugation—a process that requires cell-to-cell contact followed by transfer of a plasmid. Plasmids are ubiquitous, self-replicating, extrachromosomal elements. Antibiotic resistance is often carried on plasmids; in some cases, this can include resistance to multiple antibiotics (*38*). Acquired plasmids may also introduce new virulence-factor genes, such as those that encode toxins, into their hosts.

Although it has not been tested, antibiotic resistance or virulence in indigenous *V. cholerae* may be enhanced through horizontal gene transfer from cholera bacteria discharged from ships (*39*). If significant gene transfer could occur, then ballast-water management clearly should become more stringent and targeted at eliminating the discharge of living bacteria. Finally, whether or not a case eventually is made for significant gene transfer, such information will help inform regulators and legislators by providing useful input to risk-assessment models that incorporate microorganisms (*40*).

Proposed standard for discharged ballast water

IMO, the technical organization that sets rules and standards for nations and the global shipping industry, has proposed standards for ballast water. In February 2004, IMO adopted an international convention that is now being considered for ratification by the organization's member states (*41*). A five-section annex to the convention addresses microorganisms in general and some bacteria specifically:

> Ships conducting ballast-water management shall discharge less than 10 viable organisms per cubic meter greater than or equal to 50 µm in minimum dimension and less than 10 viable organisms per mL less than 50 µm in minimum dimension and greater than or equal to 10 µm in minimum dimension; and discharge of the indicator microbes shall not exceed the specified concentrations. The indicator microbes, as a human health standard, include, but are not limited to: a. Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 colony-forming unit (cfu) per 100 mL or less than 1 cfu per 1 gram (wet weight) zooplankton samples; b. *Escherichia coli* less than 250 cfu per 100 mL; c. Intestinal enterococci less than 100 cfu per 100 mL.

As previously mentioned, minimizing and diluting the number of organisms released into coastal waters should help prevent the establishment of nonindigenous species. Yet, it is important to remember that we are still unable to model and predict "harmful algal blooms", despite intense research efforts into the causes. Thus, it would be arrogant and wrong to conclude, on the basis of the scant data available, that a few released protists would not establish an invasive population. Limiting the release of significant numbers of organisms in the size range 10–50 µm should reduce the threat from dinoflagellates and other bloom-forming protists; however, threshold levels need to be determined empirically, and the fate of released cysts must be better understood.

Emerging technologies and management strategies must also consider the microbial system that dominates coastal waters.

The bacterial standard is centered on human-health concerns and borrows criteria from recreational waters, at least in terms of *E. coli* and enterococci. The focus on fecal bacteria is convenient rather than necessary, because ballast water is unlikely to contaminate recreational waters. However, by requiring low numbers of indicator bacteria, the standard also supports a general reduction in total bacterial count, because indicator bacteria cannot be killed nor can their presence be routinely monitored in the ballast tank before treatment. In short, the bacterial standard implicitly acknowledges that not all bacteria will be killed by the treatment, but it requires reduction of the overall abundance of bacteria.

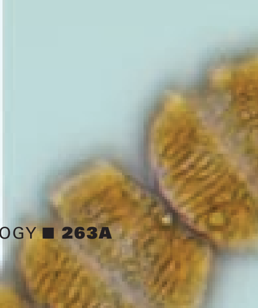
For now, minimizing the release of biota is a wise approach, and future technologies and management strategies must be sensitive to this goal. Emerging technologies must also consider the microbial system that dominates coastal waters. Although we do not have all the answers, we do understand much about the dynamics of such systems. For example, heterotrophic nanoflagellates (2–20 µm) are abundant in seawater (~1000 cells/mL) and ingest bacteria; this makes them major regulators of bacterial abundance. Retaining grazing biota during a long voyage may be wise and could influence decisions on what pretreatments, if any, should be used when uploading ballast water.

A sticky situation

If one considers the bona fide, documented introductions of organisms by ships' ballast waters, the emergent point is that invaders are overwhelmingly macroinvertebrates. With the exception of dinoflagellates (*8*, *31*), no conclusive evidence links ballasting operations to successful invasions by aquatic microorganisms. Nonetheless, it would be simplistic and possibly very wrong to consider that aquatic microbial invasions do not occur via or could not be mediated by ballast water. Unlike many of their invertebrate counterparts, microbial invaders are invisible without a compound microscope, and their presence might only be noticed in spectacular cases, such as red tides or outbreaks of illness. Hence, detection of nonindigenous microorganisms is inherently biased. In that regard, a "smalls rule" has been proposed—the smaller the taxon, the less likely it is to be recognized as introduced and the more likely it is to be considered indigenous (*42*). Thus, tiny species are regarded as native, sometimes despite evidence to the contrary, when they should by default be considered to be cryptogenic (of unknown origin) until proven otherwise.

Unfortunately, the unsatisfactory answer to the question of whether we should try to remove or kill all microbes in ballast water is "maybe". For the vast majority of microorganisms, however, we believe treatment methods aimed at significant in-tank reduction in abundance should reduce the possibility of an exotic introduction. And removing all organisms >1 mm is relatively straightforward with today's filtration technology, at least in pilot-scale and experimental systems. We acknowledge that the rapid range expansion of toxic dinoflagellates and the presence of *V. cholerae* in ships' ballast water present a compelling argument for removing or killing microorganisms known to be problem groups—at least until the global-ubiquity debate has been resolved.

Fred C. Dobbs is an associate professor and graduate program director in the department of ocean, earth, and atmospheric sciences at Old Dominion University. Andrew Rogerson is a professor and the associate dean of the Oceanographic Center at Nova Southeastern University. Address correspondence regarding this article to Dobbs at fdobbs@odu.edu.



Acknowledgments

This work is a result of research sponsored in part by the National Oceanic and Atmospheric Administration (NOAA) Office of Sea Grant, U.S. Department of Commerce, under Grants NA16RG2271 and NA96RG00025 to the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program. In addition, this work was conducted in part under the multi-institutional Great Lakes No Ballast On Board Project funded by the Great Lakes Protection Fund, NOAA, EPA, and the U.S. Coast Guard (www.glerl.noaa.gov/nobob). The project was co-managed by the Cooperative Institute of Limnology and Ecosystems Research and the NOAA Great Lakes Environmental Research Laboratory and sponsored under cooperative agreement NA17RJ1225 from NOAA's Office of Oceanic and Atmospheric Research. We appreciate the constructive comments of three anonymous reviewers.

References

(1) Drake, L. A.; et al. Global Redistribution of Bacterioplankton and Virioplankton Communities. *Biol. Invas.* **2001**, *3*, 193–199.
(2) Ruiz, G. M.; et al. Global Spread of Microorganisms by Ships. *Nature* **2000**, *408*, 49–50.
(3) Williamson, M. *Biological Invasions*; Chapman & Hall: London, 1996.
(4) Galil, B. S.; Hülsmann, N. Protist Transport via Ballast Water—Biological Classification of Ballast Tanks by Food Web Interactions. *Eur. J. Protistol.* **1997**, *33*, 244–253.
(5) Aridgides, L. J.; et al. Multiplex PCR Allows Simultaneous Detection of Pathogens in Ships' Ballast Water. *Mar. Pollut. Bull.* **2004**, *48*, 1096–1101.
(6) Joachmistahl, E. L.; et al. Bacteriological Examination of Ballast Water in Singapore Harbour by Flow Cytometry with FISH. *Mar. Pollut. Bull.* **2004**, *49*, 334–343.
(7) McCarthy, S. A.; Khambaty, F. M. International Dissemination of Epidemic *Vibrio cholerae* by Cargo Ship Ballast and Other Nonpotable Waters. *Appl. Environ. Microbiol.* **1994**, *60*, 2597–2601.
(8) Hallegraeff, G. M. Transport of Toxic Dinoflagellates via Ships' Ballast Water: Bioeconomic Risk Assessment and Efficacy of Possible Ballast Water Management Strategies. *Mar. Ecol.: Prog. Ser.* **1998**, *168*, 297–309.
(9) Dobbs, F. C.; et al. Pathogens in Ships' Ballast Water and Sediment Residuals. Presented at the Third International Conference on Marine Bioinvasions, La Jolla, CA, 2003; http://sgnis.org/publicat/dobbdial.htm.
(10) Doblin, M. A.; et al; *Pfiesteria* Species Identified in Ships' Ballast Water and Residuals: A Possible Vector for Introductions to Coastal Areas. In *Harmful Algae*; Steidinger, K. A., et al., Eds.; Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, 2002; pp 317–319.
(11) Doblin, M. A.; et al. Transport of the Harmful Bloom Alga *Aureococcus anophagefferens* by Ocean-Going Ships and Coastal Boats. *Appl. Environ. Microbiol.* **2004**, *70*, 6495–6500.
(12) Drake, L. A.; et al. Potential Invasion of Microorganisms and Pathogens via "Interior Hull Fouling": Biofilms Inside Ballast-Water Tanks. *Biol. Invas.* **2005**, in press.
(13) Parsons, M. G.; Harkins, R. W. The Great Lakes Ballast Technology Demonstration Project Mechanical Testing Program. *Mar. Technol. Soc. J.* **2000**, *37*, 129–140.
(14) Waite, T. D.; et al. Removal of Natural Populations of Marine Plankton by a Large-Scale Ballast Water Treatment System. *Mar. Ecol.: Prog. Ser.* **2003**, *258*, 51–63.
(15) Laroussi, M.; et al. Decontamination of Water by Excimer UV Radiation. *IEEE Trans. Plasma Sci.* **2002**, *30*, 1501–1504.
(16) Sutherland, T. F.; et al. Effect of a Ballast Water Treatment System on Survivorship of Natural Populations of Marine Plankton. *Mar. Ecol.: Prog. Ser.* **2001**, *210*, 139–148.
(17) Herwig, R. P.; et al. Ballast Water Treatment Experiments with Ozone on Board the Oil Tanker S/T Tonsina. Presented at the Third International Conference on Marine Bioinvasions, La Jolla, CA, 2003; http://sgnis.org/publicat/herwperr.htm.
(18) Kuzirian, A. M.; et al. Hydrogen Peroxide: An Effective Treatment for Ballast Water. *Biol. Bull.* **2001**, *201*, 297–299.
(19) Sano, L. L.; et al. Assessing the Potential Efficacy of Glutaraldehyde for Biocide Treatment of Un-ballasted Transoceanic Vessels. *J. Great Lakes Res.* **2003**, *29*, 545–557.
(20) Tamburri, M. N.; Wasson, K.; Matsuda, M. Ballast Water Deoxygenation Can Prevent Aquatic Introductions While Reducing Ship Corrosion. *Biol. Conserv.* **2002**, *103*, 331–341.
(21) Rigby, G. R.; Hallegraeff, G. M.; Sutton, C. Novel Ballast Water Heating Technique Offers Cost-Effective Treatment To Reduce the Risk of Global Transport of Harmful Marine Organisms. *Mar. Ecol.: Prog. Ser.* **1999**, *191*, 289–293.
(22) Abou-Ghazala, A.; et al. Bacterial Decontamination of Water by Means of Pulsed-Corona Discharges. *IEEE Trans. Plasma Sci.* **2002**, *30*, 1449–1453.
(23) Finlay, B. J. Global Dispersal of Free-Living Microbial Eukaryote Species. *Science* **2002**, *296*, 1061–1063.
(24) Finlay, B. J.; Esteban, G. F.; Fenchel, T. Protist Diversity Is Different. *Protist* **2004**, *155*, 15–22.
(25) Finlay, B. J.; Fenchel, T. Cosmopolitan Metapopulations of Free-Living Microbial Eukaryotes. *Protist* **2004**, *135*, 237–244.
(26) Finlay, B. J. Protist Taxonomy: An Ecological Perspective. *Phil. Trans. R. Soc. Lond. B.* **2004**, *359*, 599–610.
(27) Hamer, J. P.; McCollin, T. A.; Lucas, I. A. N. Dinoflagellate Cysts in Ballast Tank Sediments: Between Tank Variability. *Mar. Pollut. Bull.* **2000**, *40*, 731–733.
(28) Hallegraeff, G. M.; Bolch, C. J. Transport of Diatom and Dinoflagellate Resting Spores in Ships' Ballast Water: Implications for Plankton Biogeography and Aquaculture. *J. Plankton Res.* **1992**, *14*, 1067–1084.
(29) Pollard, D. A.; Hutchings, P. A. A Review of Exotic Marine Organisms Introduced to the Australian Region. II. Invertebrates and Algae. *Asian Fish. Sci.* **1990**, *3*, 223–250.
(30) Bolch, C. J. S.; Reynolds, M. J. Species Resolution and Global Distribution of Microreticulate Dinoflagellate Cysts. *J. Plankton Res.* **2002**, *24*, 565–578.
(31) Lilly, E. L.; et al. Paralytic Shellfish Poisoning Toxins in France Linked to a Human-Introduced Strain of *Alexandrium catenella* from the Western Pacific: Evidence from DNA and Toxin Analysis. *J. Plankton Res.* **2002**, *24*, 443–452.
(32) Faruque, S. M.; et al. Epidemiology, Genetics, and Ecology of Toxigenic *Vibrio cholerae*. *Microbiol. Molec. Biol. Rev.* **1998**, *62*, 1301–1314.
(33) Colwell, R. R. Global Climate and Infectious Disease: The Cholera Paradigm. *Science* **1996**, *274*, 2025–2031.
(34) McCarthy, S. A.; et al. Toxigenic *Vibrio cholerae* O1 and Cargo Ships Entering Gulf of Mexico. *Lancet* **1992**, *339*, 624–625.
(35) McCarthy, S. A. Effects of Temperature and Salinity on Survival of Toxigenic *Vibrio cholerae* O1 in Seawater. *Microbial Ecol.* **1996**, *31*, 167–175.
(36) Li, M.; et al. Evidence for the Emergence of Non-O1 and Non-O139 *Vibrio cholerae* Strains with Pathogenic Potential by Exchange of O-Antigen Biosynthesis Regions. *Infect. Immun.* **2002**, *70*, 2441–2453.
(37) van Elsas, J. D.; Bailey, M. J. The Ecology of Transfer of Mobile Genetic Elements. *FEMS Microbiol. Ecol.* **2002**, *42*, 187–197.
(38) Falbo, V.; et al. Antibiotic Resistance Conferred by a Conjugative Plasmid and a Class I Integron in *Vibrio cholerae* O1 El Tor Strains Isolated in Albania and Italy. *Antimicrob. Agents Chemother.* **1999**, *43*, 693–696.
(39) Thomson, F. K., III; Heinemann, S. A.; Dobbs, F. C. Patterns of Antibiotic Resistance in Cholera Bacteria Isolated from Ships' Ballast Water. Presented at the Third International Conference on Marine Bioinvasions, La Jolla, CA, 2003, http://sgnis.org/publicat/thomhein.htm.
(40) MacIsaac, H. J.; Robbins, T. C.; Lewis, M. A. Modeling Ships' Ballast Water as Invasion Threats to the Great Lakes. *Can. J. Fish. Aquat. Sci.* **2002**, *59*, 1245–1256.
(41) International Maritime Organization, www.imo.org.
(42) Wyatt, T.; Carlton, J. T. Phytoplankton Introductions in European Coastal Waters: Why Are so Few Invasions Reported? *CIESM (Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée) Workshop Monographs*, 2002, no. 20, 41–46, www.ciesm.org/publications/Istanbul02.pdf.