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# Potential microbial bioinvasions via ships' ballast water, sediment, and biofilm

Lisa A. Drake [a,*], Martina A. Doblin [b], Fred C. Dobbs [c]

[a] Department of Science, US Coast Guard Academy, 27 Mohegan Avenue, New London, CT 06320, USA
[b] University of Technology, Sydney, P.O. Box 123, Broadway New South Wales 2007, Australia
[c] Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, 4600 Elkhorn Avenue, Norfolk, VA 23529, USA

**Abstract**

A prominent vector of aquatic invasive species to coastal regions is the discharge of water, sediments, and biofilm from ships' ballast-water tanks. During eight years of studying ships arriving to the lower Chesapeake Bay, we developed an understanding of the mechanisms by which invasive microorganisms might arrive to the region via ships. Within a given ship, habitats included ballast water, unpumpable water and sediment (collectively known as residuals), and biofilms formed on internal surfaces of ballast-water tanks. We sampled 69 vessels arriving from foreign and domestic ports, largely from Western Europe, the Mediterranean region, and the US East and Gulf coasts. All habitats contained bacteria and viruses. By extrapolating the measured concentration of a microbial metric to the estimated volume of ballast water, biofilm, or residual sediment and water within an average vessel, we calculated the potential total number of microorganisms contained by each habitat, thus creating a hierarchy of risk of delivery. The estimated concentration of microorganisms was greatest in ballast water ≫ sediment and water residuals ≫ biofilms. From these results, it is clear microorganisms may be transported within ships in a variety of ways. Using temperature tolerance as a measure of survivability and the temperature difference between ballast-water samples and the water into which the ballast water was discharged, we estimated 56% of microorganisms could survive in the lower Bay. Extrapolated delivery and survival of microorganisms to the Port of Hampton Roads in lower Chesapeake Bay shows on the order of $10^{20}$ microorganisms ($6.8 \times 10^{19}$ viruses and $3.9 \times 10^{18}$ bacteria cells) are discharged annually to the region.


*Keywords:* Aquatic nuisance species; Bacteria; Invasive species; Management; Viruses

## 1. Why study microorganisms?

In the context of invasive species, the reasons for investigating the transfer of aquatic microorganisms, including viruses, bacteria, protists, and microalgae are threefold: their high densities in natural waters, ability to form resting stages, and potential toxicity or pathogenicity. Aquatic microorganisms are orders of magnitude more abundant than macroorganisms such as copepods and fish: naturally occurring bacteria and viruses are found in concentrations on the order of $10^6$–$10^{11}\ l^{-1}$ (e.g., Ducklow and Shiah, 1993; Proctor, 1997; Fuhrman, 1999; Wommack and Colwell, 2000). Given such high densities, microorganisms are transferred and introduced globally via ships in greater numbers than any other size class of organisms. Nearly all such microorganisms, incidentally, are innocuous to humans. Instead, the viruses infect naturally occurring bacterial and phytoplankton hosts, in which they can cause significant mortality (Fuhrman and Noble, 1996; Suttle, 2005).

Once released, microorganisms are well poised to be invasive species. They are small, a size that facilitates their passive dispersal. They appear to have simpler requirements for survival than do metazoans, based upon their ubiquity in the biosphere, including extreme environments (Deming, 1997). They predominantly reproduce asexually and grow

* Corresponding author. Tel.: +1 401 789 1461.
*E-mail address:* lisa.drake1@verizon.net (L.A. Drake).

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rapidly, factors also contributing to their widespread distribution. Finally, life cycles of many invertebrate metazoans and unicellular organisms such as bacteria, eukaryotic phytoplankton (including toxic dinoflagellates), and other protist species, include resting stages (variously called cysts, spores, auxospores, ephippia, or resting eggs according to taxon) capable of surviving prolonged periods of unfavorable conditions (e.g., Bailey et al., 2003).

These resting stages are typically produced at very low frequency, if at all, under favorable conditions, and at high frequency when environmental conditions deteriorate (e.g., declining nutrient concentrations, shortened photoperiod, or reduced food quality; Blackburn and Parker, 2005). Production of resting stages ensures long-term viability of the population because they are extremely resistant to adverse conditions, including anoxia, exposure to noxious chemicals, freezing, and passage through digestive tracts of fish and waterfowl. Resting eggs of invertebrates and cysts of dinoflagellates are usually negatively buoyant and sink when released or formed. Resting stages may remain viable in sediments in a virtual suspended metabolic state for decades or even centuries (Hairston et al., 1995) and can germinate under a combination of favorable light, temperature, and other environmental conditions (e.g., Kremp and Anderson, 2000; Itakura and Yamaguchi, 2001; Figueroa et al., 2006).

Pathogenic or toxic aquatic bacteria, viruses, protists, and microalgae can have devastating effects on ecosystems and economic resources. There are well-studied pathogen–host systems among many aquatic phyla; for example, viruses terminating algal blooms (e.g., Milligan and Cosper, 1994; Nagasaki et al., 1994; Van Etten et al., 1991; Short and Suttle, 2002), viruses infecting seals (e.g., Osterhaus et al., 1985; Grachev et al., 1989), and protists decimating seagrass beds (e.g., Muehlstein, 1992; Ralph and Short, 2002). Furthermore, the apparent increasing frequency and distribution of toxic microalgal blooms has received much attention in the past two decades (see reviews by Hallegraeff, 1993; CENR, 2000). Given marine pathogens can spread locally much more quickly than terrestrial pathogens (even when instances of obvious human intervention are excluded, McCallum et al., 2003), and considering the relatively fast transport by ships, the threat of global dispersal of aquatic pathogens appears more immediate than the threat of invasion by other groups of organisms.

Thus, by virtue of their abundance, life-history characteristics, and potential pathogenicity or toxicity, microorganisms possess a great capacity to invade and cause detrimental effects in new environments. This paper will explore the extent and potential consequences of bacteria and virus transport within ships.

## 2. Types of habitats within ships

Microorganisms can be found in several locations within a ship—ballast water, residual sediment and water, and biofilms formed on interior tanks surfaces—each of which will be considered separately. Transfer among these habitats has not been fully explored (although see Meyer et al., 2000). We do not consider microorganisms within hull fouling communities in this review.

### *2.1. Ballast water*

Ships' ballast waters are the best investigated of these habitats. Although water has been used regularly as ballast since the 1880s (Carlton, 1985), the transfer of organisms by ballast-water discharge was investigated only sporadically until the late 1980s. The interest in ballast water at that time stemmed largely from the dramatic ecological and economic impacts of introduced species, such as comb jellies (*Mnemiopsis leidyi*) in the Black Sea and zebra mussels (*Dreissena polymorpha*) in the North American Great Lakes (International Maritime Organization, 1999). Much of the work on ballast invaders has been dedicated to studying metazoans (Fofonoff et al., 2003), despite the high densities of naturally occurring microorganisms in aquatic environments. To test the hypothesis that vast quantities of bacteria and viruses are carried in ships' ballast tanks, Ruiz et al. (2000) quantified their abundance in ballast water of vessels arriving to Chesapeake Bay from foreign ports. Indeed, the numbers were high: mean abundances of $8.3 \times 10^8$ bacteria $l^{-1}$ and $7.4 \times 10^9$ virus-like particles (VLPs) $l^{-1}$ were documented.

### *2.2. Sediment and water residuals*

We now know ships declaring no (pumpable) ballast on board may also serve as vectors. Concerns about no ballast on board (NOBOB) invasions have risen from a position of relative obscurity a few years ago to one of the chief environmental concerns in the Great Lakes basin today (e.g., Grigorovich et al., 2003). The potential for NOBOB-mediated invasions lies within tanks' muddy puddles; residuals of sediment and water can contain an assortment of metazoans and microorganisms, including resting stages (e.g., Hallegraeff and Bolch, 1992; Galil and Hülsmann, 1997; Gollasch et al., 1998; Hamer et al., 2000). When NOBOB tanks are later filled with ballast water, the accumulated sediment (and associated biota) may be resuspended and discharged immediately or at subsequent ports of call.

Sediment accumulation can be appreciable, depending on elapsed time since the ship was last dry-docked. For example, double-bottom ballast tanks of a cargo vessel contained up to 30 cm of sediment after only two years of use (Hamer et al., 2000). While circumstances vary from ship to ship, the unpumpable water that remains in most vessels, together with any residual sediment, potentially harbors nonindigenous organisms. A metastudy of 13 European studies recorded 990 species in a combination of ballast water and sediment samples (Gollasch et al., 2002). Furthermore, Kelly (1993) reported Japanese ships visiting the USA carried viable cysts and spores of nonindigenous species after 11–15 days' voyage. Finally, Mac-