

## Research Article

## Ballast tank biofilms resist water exchange but distribute dominant species

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### Abstract

Shipping in the Laurentian Great Lakes includes numerous “lakers” and a group of international vessels arriving via the St. Lawrence Seaway. It was expected that ballast water exchange techniques, or eventual volumetric ballast water treatment, would eliminate the risks of transfer of ballast tank nuisance aquatic and aerosol organisms to Great Lakes ports and other parts of the world. New and important information is that ballast water exchanges and current ballast water treatments are not effective in destroying biofilms on tank walls and sediments that provide living micro-organisms for new biofilms and bioaerosols. Our Ballast Organic Biofilm (BOB) studies of around-the-world ships associated with multiple ballasting events in the continuous presence of BOB samplers showed that dominant microorganisms are acquired and persist, and can be spontaneously transferred to fresh surfaces and also delivered as bioaerosols from discharged ballast water plumes. This communication uses such studies to infer that a port-based ballast tank water-exchange and cleaning procedure can mitigate such biofilm risks to health and existing biodiversity. A useful site would be the Port of Buffalo, just opposite the Welland Canal entrance for international shipping to the upper Great Lakes.

**Key words:** slime, microorganisms, transfer, contamination, global, cleaning, bioaerosol

### Introduction

Current surveys of shipping in the Laurentian Great Lakes show that approximately 111 ships are usually in transit within or to the Lakes each day, carrying a mean amount of ballast water of 2,335,415 cubic meters from which international efforts are underway to remove any threat of organism re-distributions and possible risks to health (Grigorovich et al. 2003). Assuming that prior-to-Lake-entry underway water exchanges, or port-based or on-board ballast treatments will eventually mitigate these threats, there is still the likelihood of biodiversity limitation and health problems occurring from retained ballast tank biofilms and from sediments not easily exchanged or effectively treated by these methodologies. In the Great Lakes system shared by Canada and the United States, with their large adjacent populations, each time an average-sized ship de-ballasts there are at least (only a 10% biofilm coverage is estimated) 7.4 trillion bacteria still

sequestered within that ship’s biofilm and 100 times that number of virus-like particles (Drake et al. 2007; Drake et al. 2005), and 100 times more than that amount associated with the sediment. With more than 100 ships afloat on any given day, assuming a single event of de-ballasting per ship, 100-fold greater propagule pressure occurs that will eventually lead to biodiversity limitations and health risks if not directly dealt with soon.

We have earlier shown that open-ocean exchange processes, even 80-fold, are incapable of removing bacteria-sized particulates from ballast tank biofilms (Forsberg et al. 2005), but that water spray from ballast plume bubble-breaking can still send concentrated bacteria into the aerosol phase, and be carried with the wind from these plunging water sources (Cipriano and Baier 2003). Others have shown an unusual source of *Vibrio alginolyticus*-related infection: inland Cleveland, Ohio identified a probable ballast tank bioaerosol that contained this nominally seawater-dependent infective organism

(Mukherji et al. 2000). A video film of such transfer of specific biofilm-sequestered organisms to fresh surfaces is available (IUCB 2013).

Using immunofluorescence methods earlier developed for the identification of the dominant oceanic biofilm species (Zambon et al. 1984), importantly including the disease-causing *Vibrio alginolyticus*, we studied the world-wide distribution of ballast tank biofilms to judge their make-ups and possible suppression of biodiversity. This paper reports those results and suggests a U.S. Navy-developed surface-active displacement process (Bernett and Zisman 1966; Baier 1974) to decontaminate ballast tank surfaces (Baier et al. 1982) re-coated with easy-release, corrosion-resistant paints (Wells et al. 1997). The Port of Buffalo on Lake Erie is proposed as the first shore-based ballast biofilm removal and treatment site.

## Methods

The names, positioning and number of every freighter vessel within the St. Lawrence River and the rest of the Great Lakes, including Lake St. Clair, were counted and recorded for the 11<sup>th</sup> of July 2013, as a representative day. Published vessel routes and itineraries from the Great Lakes and Seaway shipping websites were used, taking advantage of each ship's Automatic Identification System (AIS Passage Maps 2013). "Lakers" or local freighters were included in the count as it has been determined that their potential for transporting and spreading microorganisms is even greater than that of international vessels (Briski et al. 2012). Websites were used to find each vessel's type (ex. tanker, bulk carrier, cargo ship) as well as its ballast tank capacity in meters cubed. Average ballast tank sizes for each of these ship types were confirmed by matching up the available ship specifications with the ships' names and, if need be, the maritime mobile service identity. Using mapping websites, six additional daily counts were taken on semi random dates to find numbers of vessels on the Lakes in order to replicate the data and to find a realistic average of all the ships that are on the water at any given time. The numbers for microorganisms within the tanks, sediments, and biofilms of any individual ship as well as all the ships in total were inferred by using previously published data that we had helped to gather (Drake et al. 2007).

Rabbit antisera had already been prepared and fluorescently labeled to form immunofluorescent

reagents for detecting the following benchmark organisms: *Vibrio alginolyticus*, *Comamonas terrigena*, *Pseudomonas putrefaciens*, *Achromobacter spp.* and *Pseudomonas sp.* (Zambon et al. 1984). The reagents were used to identify and quantify these same five species of bacteria in biofilms spontaneously grown within the ballast tanks of four ships, three on trans-oceanic routes and one entering the Great Lakes system.

Non-toxic materials and coatings, with different but well-characterized surface properties, were placed within special ballast organic biofilm (BOB) samplers that were suspended within the ballast tanks (Forsberg et al. 2014a). Additional biofilms were acquired on similar test materials placed at known flow rates and shear stresses within portable biofouling units (Baier and DePalma 1979), circulating ballast waters (Forsberg et al. 2014b). Contact angle (theta) measurements of a series of purified diagnostic fluids on the surfaces of coupons of the test materials were used to determine their Critical Surface Tensions (CST) for correlation with their acquisition of biofilms. Each type of unit provided multiple spontaneously grown biofilms within which the prepared immunofluorescence reagents were easily able to quantitatively label each specific bacterial species. Analyses of the test materials, with and without the acquired biofilms, were also by multiple attenuated internal reflection infrared spectroscopy for determination of chemical compositions, contact angle measurements for determination of surface energies, and scanning electron microscopy plus energy-dispersive x-ray for determination of sedimentary particle types and elemental analyses (Baier 1985).

Some of the ballast water-supplied biofilm coupons were placed, in the dark, into a 30-gallon aquarium of circulating sterile seawater – near the rear wall. A time-lapse video was made by focusing on the opposite front interior wall, originally film-free. A room light was intermittently turned on during short video-recording segments. This was done to determine whether biofilm-located bacteria could generate and export successful propagules to new surface areas while remaining in place.

## Results

When calculating the percentages of various freighter ship types from the count that was taken on the 11<sup>th</sup> of July 2013, it was found that 64.4% percent of the ships in the Lakes were bulk carriers. Their total tank capacities, from the research into each individual ship, averaging

them all together, was 23,800 cubic metres and the average tank size of all ships counted was 21,071 cubic metres. This latter average was used to fill in for the ships that offered no data for their ballast tank capacity. The inferred average was estimated to be 2,335,415 cubic meters. Approximately 5000 square meters, per ship, of surface area was conservatively estimated to be available to form biofilms from this water (Drake et al. 2007), and only a 10% fraction of biofilm coverage was used to estimate the 100 trillion propagule risk per ship.

It was found that materials/coatings with CST values between 20 and 30 dynes/cm, in the so-called “theta surface” range (Baier 2006), had the films with the lowest amount of mass and the least number of protists (Meyer et al. 2000), and were the easiest-released from those materials. Coatings containing methylsilicones were the most optimal in this range, as had also been discovered for zebra mussel fouling (Wells et al. 1997). The coatings with CST values in the range of 20–30 dynes/cm changed normally tightly bound biofilms into looser structures having more clustered particulate matter and more easily detached patches, especially by jet spray techniques. It was confirmed by the infrared spectroscopy and scanning electron microscopy + energy-dispersive x-ray analyses that the background organic layers and particles of these films were of the same general compositions.

All five benchmark bacteria species were nonetheless present on all clean materials and coated materials installed aboard all vessels, confirming their originally found (Zambon et al. 1984) dominance and high persistence over time and in all world-wide maritime environments. Ballast tank biofilms did reveal different associated small particle associations, however, as a result of sediment re-suspension during ballast exchange events and at-sea ship motions. Ballast tank biofilm-coated test pieces later suspended in a dark aquarium of sterile seawater did populate the originally clean glass walls of that aquarium, confirming that biofilm-forming organisms are exported from the original living films and re-deposited to start new biota accumulations on available fresh surfaces. It has not yet been determined if the propagules will join or replace the biota in any pre-existing films (IUCB 2013), thus suppressing biodiversity, but a model ballast tank set-up has been developed to test this prospect with biofilm-coated sedimentary samples from seawater ports placed into fresh water from Lake Erie (work in progress).

## Discussion and conclusions

The problem of residual biofilm- and sediment-contained organisms is not solved, regarding undesired potential ecosystem transfer of aquatic and bioaerosol nuisance species from ballast tanks. To minimize contributions of naval vessels to these problems, the United States (US) Navy has long sought sources for technologies, procedures, concepts, systems, alternative materials, or devices to replace, minimize, process, or otherwise control pollutants present in ballast discharge (Naval Surface Warfare Center 1999). Suppression of any further risk is particularly important for the Laurentian Great Lakes system that has already suffered badly from infestations of the notorious zebra mussels and quagga mussels. These invasions are attributed to ship de-ballasting of these volumetrically suspended organisms whose veligers also travel in ballast tank biofilms along with non-native bacteria (Forsberg et al. 2005). Progress is being made toward removing or killing the actual suspended species in ballast water, but the risk of retained penetration-resistant tank wall- and sediment biofilms has not been adequately addressed.

We have developed and validated surface cleaning and sampling methodologies for first, assessing, and then removing such microfouling (biofilms) of ships (Baier et al. 1982) based on original US Navy technology (Bennett and Zisman 1966). By extrapolating these fundamental and empirical investigations of decades past, we are proposing their translation to the related problems of simply and safely cleaning ballast tank surfaces that have accumulated biological slimes and other fouling deposits. The marginally-stable emulsion-type cleaner proposed would function this way: the dispersed hydrophilic/hydrophobic component of the emulsion would readily penetrate the organic residues, while a water spray carrier would serve to flush away the surface contaminants into a volume phase, easily removed and treated. There would be no flammability issues or health hazards. A monomolecular re-contamination component, adsorbing and replacing the biofilm, could retard secondary fouling and make the next event of removal easier. Alternatively, since the monolayer-coating also dehydrates the cleaned surfaces, the subsequent spray application of a corrosion-preventing, fouling-release paint will be facilitated.

It is an imperative of economic geography that note be taken of Buffalo's prominence as a transportation hub, at the St. Lawrence Seaway entrance to the Upper Great Lakes. Buffalo/Lackawanna, New York, was – until the Seaway

opening in April 1959 – the mandated transfer port for goods arriving to and exported from middle-America, with unexcelled waterborne, rail, and highway resources. Since the Seaway opened, more than 2 billion tons of ship-borne cargo with an estimated value of more than \$300 billion has moved past Buffalo to other locations in the Upper Great Lakes. These same ships have carried aquatic invasive species (AIS) into the Great Lakes, which have now escaped and created havoc across the entire US continent – as exemplified by the notorious travelling zebra mussel and its quagga mussel cousin. Environmental health and safety concerns now require that the main continuing source of such AIS –the foreign ballast waters, their biofilms, and fine sediments transferred across the world oceans to Great Lakes shores – be interrupted by efficient ballast water exchange/treatment and biofilm + sediment removal before ships are allowed to proceed up Lake Erie and inland to the other Great Lakes. The Port of Buffalo has excellent remaining infrastructure, ship berthing, water treatment, rail and road facilities to accomplish this task, and qualified local industry to re-habilitate the needed, now-abandoned “brownfield” properties along the Buffalo/Lackawanna lakefront. What is needed is an economic analysis of how much this cleaning effort costs per vessel. That would support or refute this proposal that the unique geographic location of the Port at Buffalo/Lackawanna should encourage development of such a Ballast Water Exchange/Treatment and Biofilm/Sediment Removal/Tank Spray Painting Facility there. This is a serious matter of costs versus environmental stewardship, awaiting attention.

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## References

- Baier R (1974) Process of Dewatering Sewage Sludge and Converting the Same to Useable Product, U.S. Patent No. 3,804,753
- Baier R, DePalma V (1979) Flow Cell and Method for Continuously Monitoring Deposits on Flow Surfaces, U.S. Patent No. 4,175,233
- Baier RE, Meyer AE, Akers CK (1982) Safe and Effective Decontamination of Delicate Surfaces. In: Hall CW (ed), *Biomedical Engineering II: Recent Developments*, Pergamon Press, NY, pages 293–296
- Baier RE (1985) Surface Laboratory: Its Essentials and Fundamentals. *Biomaterials, Medical Devices, and Artificial Organs* 12: 113–126
- Baier RE (2006) Surface behavior of biomaterials: The *theta* surface for biocompatibility. *Journal of Materials Science: Materials in Medicine* 17: 1057–1062, <http://dx.doi.org/10.1007/s10856-006-0444-8>
- Briski E, Wiley CJ, Bailey SA (2012) Role of domestic shipping in the introduction or secondary spread of nonindigenous species: biological invasions within the Laurentian Great Lakes. *Journal of Applied Ecology* 49: 1124–1130, <http://dx.doi.org/10.1111/j.1365-2664.2012.02186.x>
- Bennett MK, Zisman WA (1966) Surface Chemical Displacement of Organic Liquids from Solid Surfaces. *Journal of Physical Chemistry* 70: 1064–1075, <http://dx.doi.org/10.1021/j100876a018>
- Cipriano R, Baier R (2003) Generation of Respirable Bioaerosols in Plumes from Plunging Water: Application to Ballast Water Discharge, in Proceedings, 26th Annual Meeting of The Adhesion Society, Blacksburg, Virginia, pp 456–457
- Drake LA, Meyer AE, Forsberg RL, Baier RE, Doblin MA, Heinemann S, Johnson WP, Koch M, Rublee PA, Dobbs FC (2005) Potential invasion of microorganisms and pathogens via ‘interior hull fouling’: biofilms inside ballast-water tanks. *Biological Invasions* 7: 969–982, <http://dx.doi.org/10.1007/s10530-004-3001-8>
- Drake LA, Doblin MA, Dobbs FC (2007) Potential microbial bioinvasions via ships’ ballast water, sediment, and biofilm. *Marine Pollution Bulletin* 55: 333–341, <http://dx.doi.org/10.1016/j.marpolbul.2006.11.007>
- Forsberg R, Baier R, Meyer A, Doblin M, Strom M (2005) Fine Particle Persistence in Ballast Water Sediments and Ballast Tank Biofilms. Proceedings, 28th Meeting of The Adhesion Society, Blacksburg, Virginia, pp 92–94
- Forsberg RL, Baier RE, Meyer AE (2014a) Chapter 6. Sampling and experiments with biofilms in the environment: Part 2, Sampling from large structures such as ballast tanks. In: Dobretsov S, Thomason JC, Williams DN (eds), *Biofouling Methods*. Wiley-Blackwell (Oxford, UK), in press
- Forsberg RL, Baier RE, Meyer AE (2014b) Chapter 7. Laboratory Experiments and Cultures: Static, constant depth and/or flow cells. In: Dobretsov S, Thomason JC, Williams DN (eds), *Biofouling Methods*. Wiley-Blackwell (Oxford, UK), in press
- Grigorovich IA, Coulatti RI, Mills EL, Holec K, Ballert AG, MacIsaac HJ (2003) Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 740–756, <http://dx.doi.org/10.1139/f03-053>
- IUCB (2000) IUCB video links. <http://www.wings.buffalo.edu/iucb/video.html>
- Meyer AE, Baier R, Hulsmann N, Galil B, Friedmann D, Forsberg R (2000) Risk assessment, prediction, and limitation of transport of bioinvaders in biofilms. Abstracts Book, American Society of Limnology and Oceanography, Aquatic Sciences Meeting, Copenhagen, Denmark
- Mukherji A, Schroeder S, Deyling C, Procop GW (2000) An Unusual Source of *Vibrio alginolyticus*-Associated Otitis. *JAMA Otolaryngology–Head & Neck Surgery* 126: 790–791, <http://dx.doi.org/10.1001/archotol.126.6.790>
- Wells AW, Meyer AE, Matousek JA, Baier RE, Neuhauser EF (1997) Nontoxic Foul-Release Coatings for Zebra Mussel Control. Waterpower '97: Proceedings of the International Conference on Hydropower, American Society of Civil Engineers 1, pp 451–460
- Zambon JJ, Huber PS, Meyer AE, Slots J, Fornalik MS, Baier RE (1984) In Situ Identification of Bacterial Species in Marine Biofouling Films by Using an Immunofluorescence Technique. *Applied and Environmental Microbiology* 48: 1214–1220