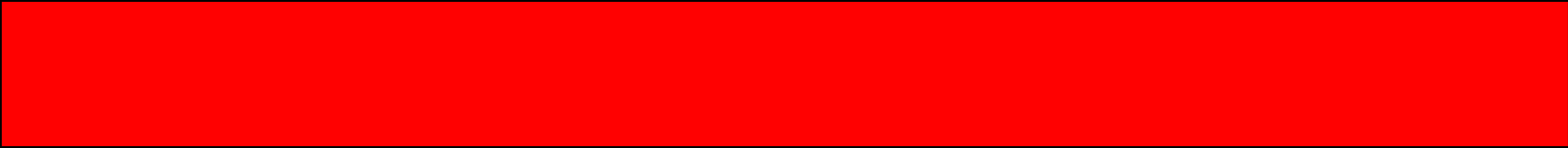


Risk Assessment, Prediction, and Limitation of Transport of Bioinvaders in Biofilms



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The research described here was partially funded by the U.S. National Oceanic and Atmospheric Administration award #NA96RG0483 to the Research Foundation of State University of New York for New York Sea Grant. The views expressed are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies.

Acknowledgments



- Smithsonian Environmental Research Center
 - Dr. Greg Ruiz, Dr. Linda McCann, Safra Altman, Karen Davis
- Old Dominion Univ. (Dept. Ocean, Earth, & Atmos. Sci.)
 - Dr. Fred Dobbs, Dr. Lisa Drake, Jason Williams
- EU Concerted Action Group on Testing Monitoring Systems for Risk Assessment of Harmful Introductions by Ships to European Waters
 - Dr. Stephan Gollasch (Inst. Meereskunde, Kiel, Germany)
- Mr. Jignesh Patel (University at Buffalo)
- New York Sea Grant

Conclusions

- Viable biofilms were formed on all test materials placed in the ballast tanks of the HADERA.
- Biofilms included bacteria and protists of many different species, and biodiversity was different on different test substrata.
- Methylsilicone polymeric coatings supported the least diverse biofilms.
- Biofilms formed on substrata in the ballast water tanks of the HADERA "seeded" secondary biofilms in artificial seawater environments in the laboratory.
- These results indicate that, by coating ballast tanks with nontoxic, corrosion-resistant methylsilicones, transferrable biodiversity of protists can be controlled.

Background

Understanding bioinvasion pathways and international policies for control of aquatic invasive species requires attention to ship-surface biofilms and source ballast water.

During the last few decades,

ballast water discharges have increased throughout the world in most major ports. The probability of successful establishment of self-sustaining populations of exotic species is expected to increase with greater volumes of ballast water and reduced ship transit times.

The first reports appeared by Medcof (1975), followed by Carlton (1985, 1987), Hallegraeff and Bolch (1991) and Subba Rao, et al. (1994). Rosenthal (1980) reviewed the state of knowledge and the risks associated with transplantation to fisheries and aquaculture, particularly indicating that modern aquaculture development in coastal zones is at high risk of disease transfer from ballast water.

Furthermore, it has been noted that the risk of disease transfer to humans may be increased due to exposure to bacteria and viruses carried by protozoans (Adeleke, et al. 1996), perhaps even by protozoans in ballast water.

An overlooked dimension of this problem has been the "interior hull fouling" by ballast biofilms, perhaps equaling or exceeding the transfer potential for nonindigenous species already known for exterior hull biofouling.

The efficient biocidal organotin and copper anti-fouling paints used by commercial and military fleets have reduced the number of fouling organisms on the hulls of present day ships. As alternative paints and technologies (e.g. nontoxic, fouling-release coatings) are inevitably introduced, the likelihood of aquatic nuisance species introduction must be reevaluated.

It is important to recall that biofilm mass is not always directly related to the adhesive strength of fouling to the substratum.

Prior studies have shown that, although hydrophobic, low-energy materials such as methylsilicones may quickly acquire relatively large amounts of fouling, the "cleanability" of the methylsilicone coatings is superior to that of materials that may acquire less mass during a static exposure period.

Objectives

1. Evaluate two biofilm monitoring systems*

- for ease of exposure of model substrata to ballast water
- for development of biofilms under controlled shear stress during the 17-day voyage of a cargo ship between the Mediterranean (Israel) and Chesapeake Bay (U.S.)

2. Determine whether a fouling "minimum" occurs on methylsilicone materials, as has been observed in other environmental and biomedical scenarios [Baier, et al., 1984; Meyer, et al., 1988; Meyer, et al., 1994; Baier, et al., 1997].

* portable flow cell unit, using parallel-plate flow cells and a flow manifold [submersible pump used to recirculate ballast water]

* portable static unit that contains test plates; this unit is lowered into the ballast tank, where it remains during all water exchanges

Both monitoring systems

were developed by Baier and colleagues to obtain biofilm samples on relevant substrata exposed to natural bodies of water. The on-deck system, which builds upon a parallel-plate flow cell system patented by Baier and DePalma in 1979, has been used frequently by the Buffalo team for monitoring biofilms in the Great Lakes region, the Gulf of Mexico, and on a transAtlantic research expedition, among other sites. The static sampler was designed in response to discussions at the April 1999 EU Concerted Action workshop, and had its first trial run during the voyage of the HADERA in July/August 1999.

Photo on Top: On-deck flow-cell system

Photo Below: Static, in-tank system



Materials, Methods

Test plates of a variety of materials were utilized for the evaluation of the biofilm units during the July/August 1999 voyage of the HADERA. Materials were selected on the basis of their surface energies (*more specifically, their critical surface tensions*) and surface polarities, resistance to corrosion, and adaptability to evaluation by light microscopy.

On-deck flow-cell system

Test plates used in the parallel-plate flow cells in the on-deck unit were 50mm x 20mm x 1mm in size. Materials included polystyrene (“bacterial grade”) [PS], gas-plasma-treated PS (“tissue culture grade”) [gPS], polymethylmethacrylate [PMMA], gas-plasma-treated PMMA [gPMMA], glass [GL], and dimethylsilane on glass [DMS]. Some flow cells were removed on Day 11, prior to the mid-Atlantic ballast exchange. Flow rate was maintained at 350 ml/min.

Days 1-11 only: flow cells containing gPS, gPMMA

Days 11-17 only: flow cells containing PS

Days 1-17: flow cells containing PS, PMMA, GL, DMS

Static, in-tank system

Test plates used in the assembly lowered into the ballast tank were 76mm x 25mm x 1mm in size. Materials included PS, gPS, GL, gGL, glass coated with 3-heptafluoroisopropoxysilane [3H], and metal coupons coated with a methylsilicone epoxy [MSil]. The MSil [tradename “Wearlon”] is a tough, polymeric silicone. All test plates remained in place in the static unit for the 17-day voyage, experiencing all the events of the ballast tank walls.

Analytical Methods

During this first trial of the two units for evaluation of ballast tank biofilm formation, test plate analysis included SEM and light microscopic evaluation of film morphology (e.g. thin v. thick; continuous v. patchy) and whether films contained bacteria and protists. Diversity of the protist population was evaluated by Dr. Hülsmann [additional data in preparation for publication].

Specific analyses of the biofilms used internal reflection infrared spectroscopy, comprehensive contact angle methods, and identification of dominant biofilm microorganisms by immunofluorescence techniques [Zambon, *et al.*, *Appl and Environ Microbio* 48(6):1214-1220, 2984]. The results were published in the M.S. thesis of R.L. Forsberg, State University of New York at Buffalo, 2003.

Summary of Results -1

→ All test substrata in both monitoring units acquired biofilms during the HADERA voyage.

→ The on-deck flow cell unit has these advantages over the static, in-tank unit:

- flow and shear characteristics can be controlled
- simple to exchange one flow cell for another, to capture data for different segments of voyage
- easily sampled for data on planktonic protists, microorganisms, and water chemistry

→ The static, in-tank unit has these advantages over the on-deck unit:

- test plates experience all events of ballast tank walls
- no submersible pump; no need for power source
- small; sturdy; simple to assemble, ship, and deploy

Summary of Results -2

→ **Biofilms on low-energy substrata from flow cells and static unit (17 days) were more patchy in appearance than biofilms on higher-energy substrata.**

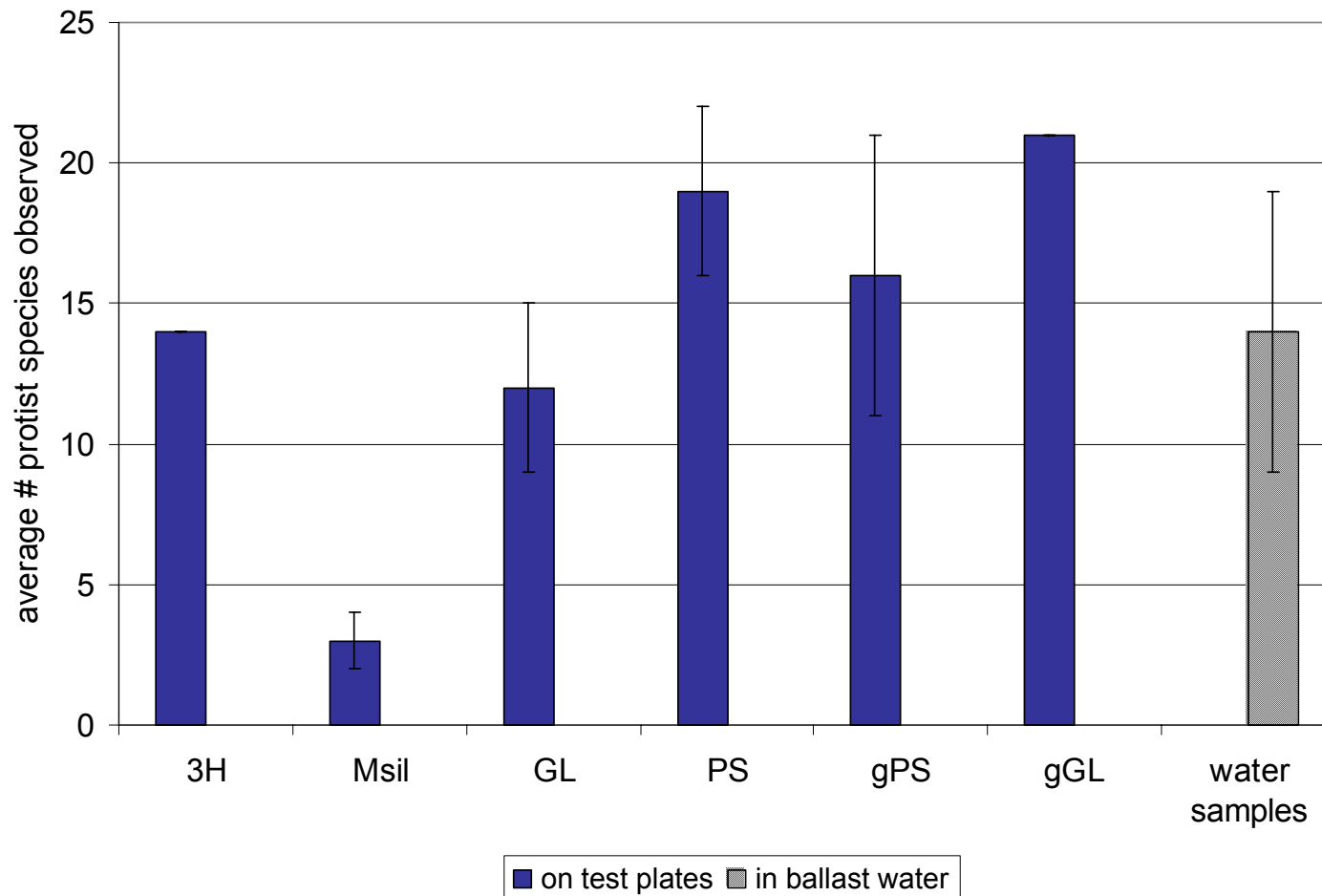
[Light microscopic observations]

→ **Mid-ocean ballast exchange (Day 11) increases the overall biodiversity of biofilms on some higher-energy substrata.** [Light microscopic observations of bacteria and other organisms]

→ **Test plates from static unit retained different populations of protists. Populations in biofilms on the methylsilicone polymer coatings were much less diverse.** [Dr. Hülsmann's analysis of protists; see following figures]

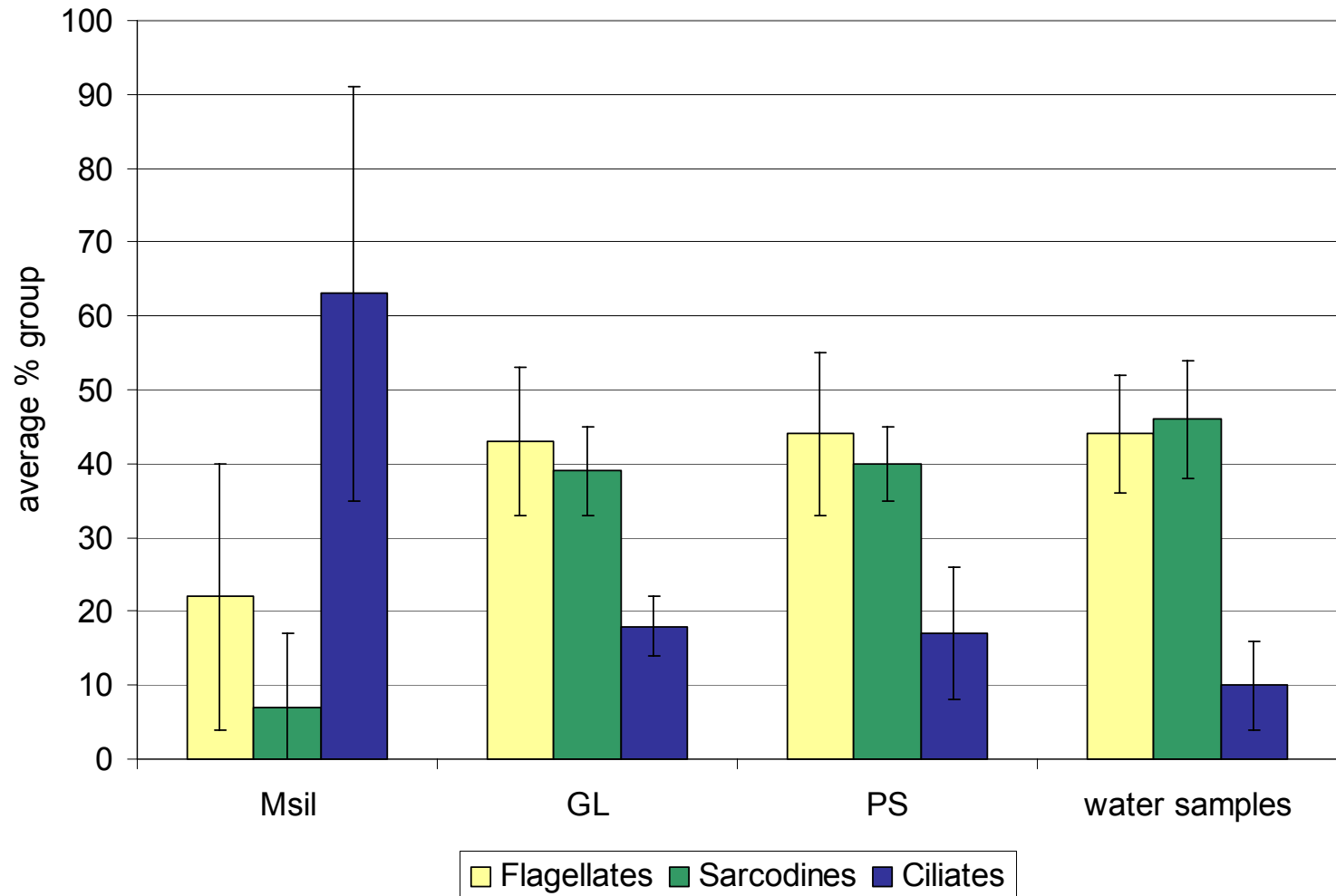
Total Number Protist Species on Test Plates

Least biodiversity is observed on methylsilicone coatings. These are the same types of coatings that produce “minimal fouling adhesion” in other environmental and biomedical systems.



Protist Population Groups on Test Plates

The population distribution on methysilicone coatings is different from that on other substrata.



Follow-up Studies

- Additional static, in-tank units were deployed in ballast tanks of ZIM CHINA and ZIM PACIFIC during roundtrip voyages between Haifa and ports in Spain, Canada, U.S., Korea, and Japan (1999 – 2001).

In these voyages, ballast water was released and taken on several times. Based on observations from the HADERA samples, test plates in ZIM CHINA and ZIM PACIFIC biofilm units included glass [GL], octadecylsilane-coated glass [ODS], and polystyrene [PS].

Results: The lower-energy ODS surfaces retained less biofilm mass, with less diversity of protists, than the higher-energy glass surfaces.

Later studies [Forsberg thesis, 2003] showed all surfaces to be dominated by the same groups of 5 “benchmark” bacteria, indicating that ballast tank biofilm biodiversity has probably been suppressed since the early 1980’s, when the organisms were first noted to also dominate exterior hull biofilms of ships in the open oceans.

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