

Naval Surface Warfare Center Carderock Division

West Bethesda, MD 20817-5700

NSWCCD-61-TR-2008/17 October 2008

Survivability, Structures, and Materials Department
Technical Report

Characterization of Fouling at Field Test Sites of the ONR Biofouling Program: Background Information and Results for 2006-2007

by

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE (DD-MM-YYYY) 8-Oct-2008			2. REPORT TYPE Final		3. DATES COVERED (From - To) -	
4. TITLE AND SUBTITLE Characterization of Fouling at Field Test Sites of the ONR Biofouling Program: Background Information and Results for 2006-2007					5a. CONTRACT NUMBER	
					5b. GRANT NUMBER	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) E.R. Holm, D.E. Wendt, L. Brewer, J. Connolly, G. Kowalke, G. Swain, P. Connelly, C. Kavanagh, S. Teo, Lim C.-S., M.G. Hadfield, M. Huggett, and B.T. Nedved					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) Naval Surface Warfare Center Carderock Division Code 617 9500 MacArthur Boulevard West Bethesda, MD 20817-5700					8. PERFORMING ORGANIZATION REPORT NUMBER NSWCCD-61-TR-2008/17	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Sea Warfare and Weapons (Code 33) 875 N. Randolph Street Suite 1425 Arlington, VA 22203-1995					10. SPONSOR/MONITOR'S ACRONYM(S)	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT Spatial and temporal variation in the structure of the fouling community and in the accumulation of fouling organisms needs to be considered in tests of efficacy of experimental hull coatings. The Office of Naval Research Biofouling Program incorporates four field sites, in California, Florida, Hawaii, and Singapore, for testing of experimental coatings. These sites span a range of environmental conditions and fouling communities. The sites provide materials developers within the Biofouling Program with the opportunity for global testing of coating performance, for antifouling, nonfouling and fouling-release materials. In 2005 the Biofouling Program commenced a characterization exercise for the field test sites, in order to develop a baseline description of the local fouling communities and their dynamics, and collect information as to how these communities respond to standard antifouling and fouling-release coatings. The data provide the background necessary for materials scientists to make the most efficient use of these sites, and the context necessary for interpreting test results.						
15. SUBJECT TERMS biofouling, biofouling adhesion, temporal variation, spatial variation, antifouling, fouling-release						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Eric R. Holm	
a. REPORT UNCLASSIFIED	b. ABSTRACT UNCLASSIFIED	c. THIS PAGE UNCLASSIFIED			SAR	116

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Administrative Information

The work described in this report was performed by the test sites of the Office of Naval Research's Biofouling Program. Coordination of the effort, and production of this report, was carried out by the Non-metallic Materials and Engineering Branch (Code 617), Materials Division, of the Survivability, Structures and Materials Department. The work was funded by the Office of Naval Research (Code 332, Dr. S. McElvany).

Acknowledgements

The authors would like to thank the American Stripping Company of Manassass, VA, for preparing and painting the test panels. T. Radakovich (NSWCCD) prepared the PVC surfaces, did touch-up coating of the panels, labelled all the materials, and managed the shipping. Thanks as well to Helen Dickson, Laurel Johnston, Adam Priest, Andrea Alfonso, Meagan Schrandt, Emily Ralston, Sunil Sarangi, Austin Graham, Sarah Rhodes, Sin Tsai-Min, Chen Chia-Lung, and Sherrilynn Kanno for their efforts in data collection and project support at the field sites.

Executive Summary

The approach taken in testing novel strategies for controlling fouling will depend on the means by which that strategy generates its effects on target organisms (for example, antifouling vs. nonfouling vs. fouling-release strategies). Designing and executing different types of tests, and interpreting the resulting data, requires different types of baseline information. Rapid field testing of novel antifouling formulations, for example, may demand an understanding of short-term temporal and spatial variation in the settlement of fouling organisms at the site in order to insure the appropriate level of challenge. Long-duration testing or qualification of a well-developed coating system may not be affected by these considerations, but instead by considerations of time to development of a mature fouling community and the dynamics of that community. Finally, the results derived from testing carried out at a single site must be evaluated in light of the possibility that performance may be influenced by the local environmental conditions or be a function of the particular species making up the fouling community.

Spatial and temporal variation in the structure of the fouling community and in the accumulation of fouling organisms needs to be considered in tests of efficacy of experimental hull coatings. The Office of Naval Research Biofouling Program incorporates four field sites for testing of experimental coatings. These sites span a range of environmental conditions and fouling communities. The sites provide materials developers within the Biofouling Program with the opportunity for global testing of coating performance, for antifouling, nonfouling and fouling-release materials. In 2005 the Biofouling Program commenced a characterization exercise for the field test sites, in order to develop a baseline description of the local fouling communities and their dynamics, and collect information as to how these communities respond to standard antifouling and fouling-release coatings. The data provide the background necessary for materials scientists to make the most efficient use of these sites, and the context necessary for interpreting test results.

Test Sites

The characterization exercise included the four test sites available to principal investigators in the ONR Biofouling Program as of March 2008: Morro Bay, CA; Indian River lagoon, FL; TMSI, Singapore; and Pearl Harbor, HI. Physical and chemical characteristics of the local environment, and composition of the fouling community, vary strongly across the sites. At the Morro Bay site, temperature and salinity fluctuate seasonally from 11.2 - 22.3°C and 13 - 35‰. Salinity fluctuations are similar at the Indian River lagoon site (12 -38‰), but temperature varies over a greater range (8 - 31°C). Salinity and temperature are relatively constant at the Singapore (27 - 30°C, 28-33‰) and Hawaii (24 - 27°C, 34 - 35‰) sites.

Test Coatings

The test coatings employed in the site characterization included an epoxy anticorrosive paint (International Intergard), an ablative antifouling paint that contains a copper biocide

(International BRA 640), and a biocide-free silicone fouling-release paint (International Intersleek 425). Coated panels were inspected at monthly intervals.

Temporal Variation in Recruitment

Monthly estimates of recruitment of fouling organisms to PVC panels provide information as to short-term temporal variation in the intensity of fouling, and reflect to some extent the availability of spores and settling larvae in the water column. Temporal variation in recruitment, and the species of fouling organisms recruiting, differed among the sites. The Indian River lagoon site exhibited strong seasonality, with settlement of barnacles in particular occurring mainly from June to October. A weak seasonal pattern may also be present in recruitment data from Singapore, where fouling was most intense from March to November. In contrast, large numbers of polychaete worms settled from December 2005 to April 2006 in Pearl Harbor, but this pattern could not be confirmed due to generally low recruitment throughout 2007. Macrofouling organisms settled year-round at the Singapore and Hawaii sites. Temporal variation in fouling was very strong at the Morro Bay site, but no seasonal pattern was apparent.

Accumulation of Fouling

The functional groups common to the mature fouling communities developing on the control anticorrosive coating differed across the test sites. Barnacles were typically the dominant macrofouling organism at the Indian River lagoon site, while encrusting bryozoans were dominant at the Morro Bay site. Fouling communities at the Singapore and Pearl Harbor sites were diverse and possessed no clear dominant species in the long term. Despite these differences in community structure, at all sites fouling on the anticorrosive coating reached high levels of coverage (between 70% to 100%) within 60 d to 80 d of immersion, and coverage remained relatively stable thereafter.

Coverage of the fouling-release coating was lower at all of the sites, and varied over time, perhaps due to disturbance or sloughing of larger organisms. The communities on these panels generally differed in structure from those on the anticorrosive panels.

Coverage of fouling on the BRA 640 antifouling coating was always less than 20% (and generally less than 10%) at all the sites save Morro Bay, where after approximately 350 d of immersion heavy accumulations of unidentifiable soft forms and incipient fouling developed. Substantial levels of hard fouling of this coating were only observed at the Pearl Harbor site. The low levels of coverage observed for the antifouling coating relative to the control anticorrosive and fouling-release coatings is probably attributable to the presence of copper biocide in the paint. The results suggest that the fouling communities at all sites were sensitive to this biocide. Presuming similar sensitivity to other antifouling biocides, the rates of accumulation observed indicate that suitable tests for efficacy of an antifouling coating, relative to an inert control coating, can be carried out potentially in as little as 60 d to 80 d of immersion. Distinguishing among highly effective vs. moderately effective antifouling compositions may require longer periods of time to allow for further settlement and development of the encrusting community.

Adhesion of Fouling

The adhesion strength of biofilms and various hard fouling organisms was measured on the fouling-release coating using a waterjet and force gauge, respectively. Adhesion of biofilms was variable over time at all the sites. Initially biofilms were typically very easy to remove at all the sites, but within several months of immersion biofilm adhesion increased substantially, and often microfouling became impossible to remove at the highest pressures attainable with the test device. Temporal variation in adhesion could be due to changes in the physical or biological composition of the biofilm, changes in the surface characteristics of the coating, or changes in the nature of the interaction between biofilm components and the coating surface. All sites supported one to several hard fouling organisms suitable for testing using the ASTM assay or a modification of the assay, including barnacles, tubeworms and oysters. Mean removal stresses for the primary hard fouling organisms evaluated at each site were: Morro Bay, *Balanus crenatus* (barnacle), 0.058 MPa; Indian River lagoon, *Balanus eburneus* (barnacle), 0.042 MPa, *Balanus improvisus* (barnacle), 0.041 MPa; Singapore, *Balanus cirratus* (barnacle), 0.052 MPa, *Balanus reticulatus* (barnacle), 0.059 MPa; Pearl Harbor, *Hydroides elegans* (polychaete tubeworm), 0.094 MPa.

Conclusions

In order for a hull coating to be globally effective, it must be able to prevent the settlement, growth or subsequent adhesion of a diversity of fouling organisms, under a range of environmental conditions. The four field sites participating in the ONR Biofouling Program present different challenges to coatings under evaluation, and thus different testing opportunities to the investigator. Judicious use of these test sites for both short- and long-term evaluations may facilitate the development of new coating systems or approaches to controlling fouling. The current study did not address whether rankings of coating efficacy, in terms of inhibition of fouling or reduction of fouling adhesion, varied among the sites. This question will be addressed by a follow-on study to commence in 2008.

Introduction

In order for a prospective hull coating to be globally effective, it must manifest its protective properties, be they antifouling, nonfouling or fouling-release, against a broad diversity of fouling organisms and under a range of environmental conditions. The structure of fouling communities, in terms of both the species present and their abundance, and the environmental conditions under which they exist, can vary strongly over small (< 1 m) to large (> 100s to 1000s of km) spatial scales. Depending on local environmental conditions (for example, water temperature, salinity, light intensity) particular sites will support different suites of macrofouling species that may exhibit different responses to antifouling and fouling-release coatings. In addition, the rate of accumulation of macrofouling to immersed objects can vary temporally depending on the site. Reproduction of macroalgae and fouling invertebrates in temperate locations changes with season, and within a reproductive season can be variable at time scales from minutes to weeks depending, for example, on the availability of cues inducing spawning or the release of larvae or spores, or factors affecting the production of gametes and the developmental rate of embryos.

Spatial and temporal variation in the structure of the fouling community and in the accumulation of fouling organisms needs to be considered in tests of efficacy of experimental hull coatings. The Office of Naval Research Biofouling Program incorporates four field sites for testing of experimental coatings. These sites span a range of environmental conditions and fouling communities. The sites provide materials developers within the Biofouling Program with the opportunity for global testing of coating performance, for both antifouling and fouling-release materials. In 2005 the Biofouling Program commenced a characterization exercise for the field test sites, in order to develop a baseline description of the local fouling communities and their dynamics, and collect information as to how these communities respond to standard antifouling and fouling-release coatings. The data will provide the background necessary for materials scientists to make the most efficient use of these sites, and the context necessary for interpreting test results.

Materials and Methods

Test Sites

The sites included in the characterization were the four test locations currently (as of March 2008) available to principal investigators in the ONR Biofouling Program for field testing of commercially-available and experimental materials. Physical, chemical and biological characteristics vary strongly across these sites.

California Polytechnic State University-San Luis Obispo (Dr. Dean E. Wendt) – Morro Bay, CA

The test site for California Polytechnic State University, San Luis Obispo, is located near the mouth of the Morro Bay Harbor along the central coast of California (Fig. 1A). The site consists of a 3.66 m x 7.32 m (12' x 24') floating dock (Fig. 1B) with a 2.44 m x 3.66 m (8' x 12') opening in the floor where PVC baskets are submerged. These PVC baskets (Fig. 1C) are the

framework to which the panels are attached. The dock moves with the tidal cycle allowing the panels to remain at a constant depth of about 0.6 m – 0.9 m (2' – 3'), depending on the panel size. The capacity is around 240 panels but this also depends on panel size. The temperature and salinity fluctuates seasonally from 11.2 - 22.3°C and 13 - 35‰. There is a water quality measurement array located on the same pier that monitors several environmental parameters including conductivity, temperature, pressure (converts to depth or tidal state), dissolved oxygen, oxygen saturation, fluorescence, turbidity, nitrate concentrations, and current profile. These data are accessible at www.slosea.org (see links below). Morro Bay's fouling community is very diverse and changes seasonally. Barnacle recruitment is the heaviest in summer to early fall and late winter to spring, and the heaviest fouling of most organisms occurs between spring and fall. The fouling community consists of sponges, tunicates, tubeworms, hydroids, anenomes, tube-dwelling amphipods, arborescent and encrusting bryozoans and several species of barnacles, the most abundant of which is *Balanus crenatus*. The dominant macrofouling species is an invasive encrusting bryozoan *Watersipora subtorquata*. A taxonomic database for species found in Morro Bay can be found at www.slosea.org (see links below).



Figure 1. California Polytechnic State University test site at Morro Bay, California. **A.** Morro Bay. **B.** Floating dock supporting baskets for panels. **C.** Mounting of panels in baskets.

Further information on the environmental conditions at the Morro Bay test site, and organisms found in the benthic communities there, can be found at the following websites:

- 1) Homepage for SLOSEA, the Morro Bay/central California coast ecosystem alliance.

<http://www.slosea.org/>

2) Real-time water quality data for Morro Bay, including water temperature, salinity, state of the tide, chlorophyll concentration, and turbidity.

<http://www.slosea.org/news/dash.php>

3) Database of invertebrates found in Morro Bay, including fouling organisms. Some entries have pictures of the organism in question.

<http://www.slosea.org/taxonomy/invertdata.php>

4) Homepage for the Cal Poly Center for Coastal Marine Sciences.

<http://www.marine.calpoly.edu/>

5) Real-time oceanographic data from the Center for Coastal Marine Sciences pier at Avila, for Avila and the central California coast.

<http://www.marine.calpoly.edu/getwet/>

Florida Institute of Technology (Dr. Geoffrey W. Swain) – Indian River Lagoon, FL

The Florida Institute of Technology's test site is located on the Indian River lagoon approximately 5 km from Sebastian Inlet. The facility includes a floating platform for exposure of panels, which are immersed at a depth of approximately 1 m. The lagoon is a temperate estuary; salinities and temperatures vary strongly on both small and large temporal scales. Water temperature and salinity (measured both on site and at another location approximately 1.5 km to the south) range from 8 - 31°C and from 12 - 38‰. Common fouling species include barnacles (mainly *Balanus eburneus*; *B. improvisus* occasionally occurs in abundance), bivalve mollusks, arborescent and encrusting bryozoans, and sponges.

Predation (by fish) at this site is an important driver of community structure (Swain et al., 1998). In order to exclude predators, frames supporting the panels were caged using 13 mm galvanized steel mesh.

Further information on the organisms that can be found in the waters of the Indian River lagoon, and on the tests carried out at the Florida Institute of Technology Center for Corrosion and Biofouling Control, can be accessed at the following websites:

1) Home page for the Florida Institute of Technology Center for Corrosion and Biofouling Control.

<http://research.fit.edu/ccbc/>

2) Smithsonian field guide to flora and fauna of the Indian River lagoon.

<http://www.sms.si.edu/IRLFieldGuide/index.htm>

3) Species inventory for the Indian River lagoon.

<http://www.sms.si.edu/irlspec/index.htm>

4) Water quality data (not real-time) for the Indian River lagoon. Includes water temperature, turbidity, salinity, pH, and dissolved oxygen concentration.

<http://www.mrcirl.org/water/watch.html>

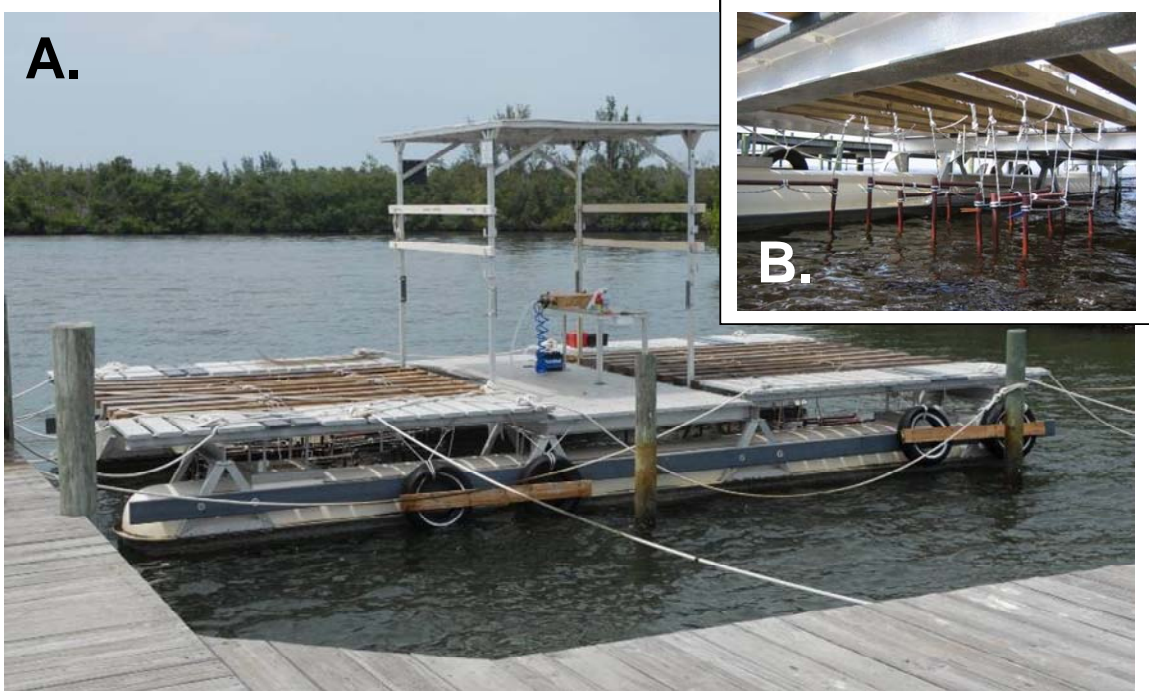


Figure 2. Florida Institute of Technology test site in the Indian River Lagoon, Florida. **A.** The floating platform from which panels are immersed. **B.** View from beneath the platform showing hanging frames to which panels are mounted.

National University of Singapore-Tropical Marine Science Institute (Dr. Serena Teo) – Singapore

The Tropical Marine Science Institute’s static immersion test site is located on the southwestern coast (1° 17’ 40’’N, 103° 45’ 37.6’’E) of the Republic of Singapore, at the Republic of Singapore Yacht Club. The site consists of a raft (Fig. 3) moored at a local marina in the Central Port Area. The raft has four wells, each capable of containing up to 50 panels depending on size. As the site is located in a marina, there is very little wave action. Panels are immersed at a depth of approximately 0.5 m. The environment is typical of tropical southeast Asian coastal seas, characterized by warm sea temperatures with monsoon-driven seasonality patterns, high nutrient levels and productivity, and a rich diversity of organisms. The temperature (27 – 30°C) and salinity (28 - 33‰) of the seawater are relatively constant. Common fouling species include barnacles (for example, *Balanus reticulatus*, *B. cirratus*, *B. amphitrite*), serpulid tubeworms (*Pomatoleios kraussii*, *Hydroides* spp., *Ficopomatus* sp.), sponges, mollusks (for example, *Anomia* sp., *Dendrostrea* spp., vermetid gastropods), colonial ascidians and bryozoans.



Figure 3. Tropical Marine Science test platform at the Republic of Singapore Yacht Club, Singapore.

University of Hawaii-Kewalo Marine Laboratory (Dr. Michael G. Hadfield) – Pearl Harbor, HI

The University of Hawaii's test site is located on the southeast end of Ford Island in Pearl Harbor, Hawaii. The site consists of a number of aluminum pipes attached to pilings on the south side of Pier F-1 ½, to which PVC frames are mounted (Fig. 4). These frames form the support structure for the coated panels being evaluated. The PVC frames do not float, and thus the depth of immersion of panels varies with the state of the tide. The temperature (24 – 27°C) and salinity (34 – 35‰) of seawater at the site are relatively constant. In the absence of (infrequent) nearby ship traffic the location experiences little current or wave action. The fouling community is extremely diverse and exhibits no strong seasonality (Holm et al., 2000). Important fouling organisms include the serpulid tubeworm *Hydroides elegans*, and various species of oysters, barnacles, sponges, colonial and solitary tunicates, and arborescent and encrusting bryozoans.

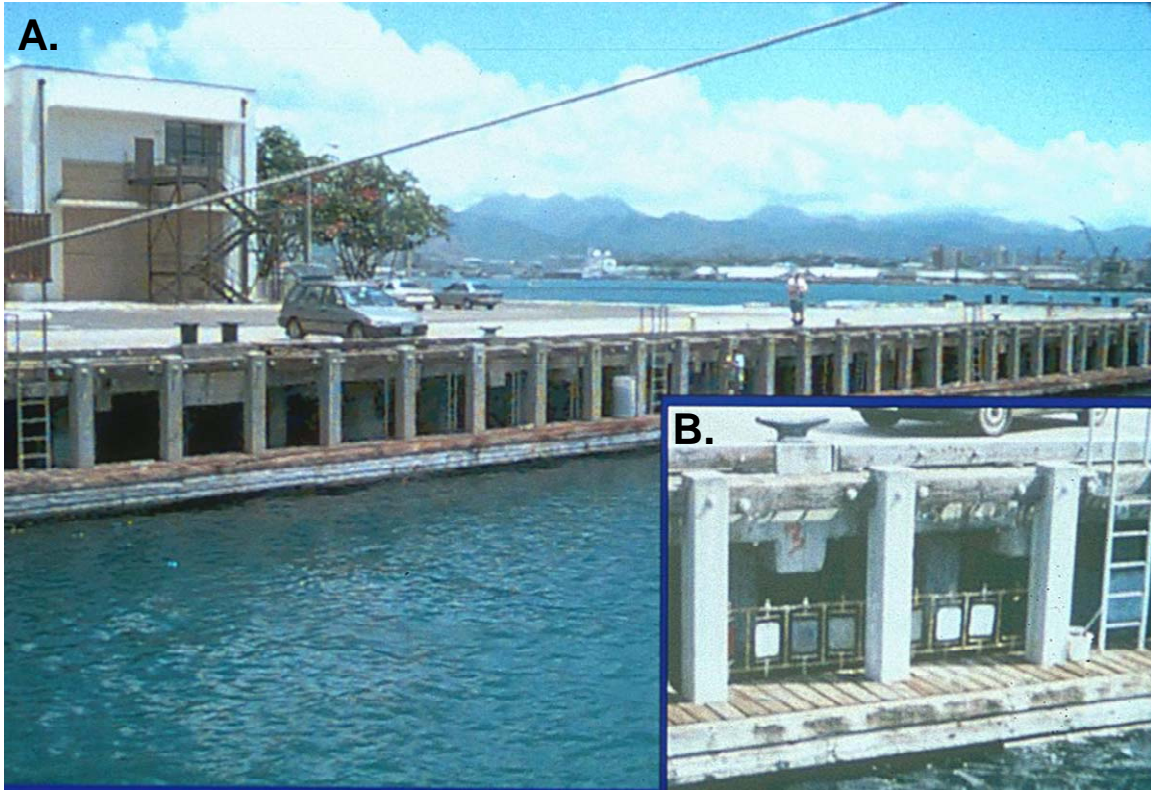


Figure 4. University of Hawaii test site on Ford Island, Pearl Harbor, Hawaii. **A.** Pier F-1 ½. Support frames for panels are mounted along most of the extent of the pier. **B.** Two frames pulled from the water to show arrangement of test panels.

Further information on the organisms (both invertebrates and algae) that can be found in the benthic communities of Hawaii, and on oceanographic characteristics of the waters around Hawaii, can be accessed at the following websites:

1) Homepage for the National Oceanographic Data Center's Hawaii/Pacific Islands Liaison – oceanographic data for Hawaii and other Pacific islands.

<http://ilikai.soest.hawaii.edu/HILO/>

2) Homepage of the Hawaii Biological Survey.

<http://hbs.bishopmuseum.org/hbs1.html>

3) Homepage of the Bishop Museum Invertebrate Zoology department.

<http://www.bishopmuseum.org/research/natsci/invert/invertzoo.html>

4) Homepage of the Bishop Museum Botany department.

<http://www.bishopmuseum.org/research/natsci/botany/botany.html>

5) List of invertebrates of Pearl Harbor, from the Bishop Museum Legacy Project (1996).

<http://www.bishopmuseum.org/research/natsci/invert/nomenclature/phlegacy.html>

6) Searchable checklist of marine invertebrates of Hawaii.

http://www2.bishopmuseum.org/HBS/invert/list_home.htm

7) Kewalo Marine Laboratory's database of reproductive patterns in local marine invertebrates.

<http://www.pbrc.hawaii.edu/db/>

8) Database of invasive algae.

<http://www2.bishopmuseum.org/algae/index.asp>

9) Guidebook of Introduced Marine Species of Hawaii – includes macroalgae and fouling invertebrates.

<http://www2.bishopmuseum.org/HBS/invertguide/index.htm>

10) Dr. Celia Smith's website for Hawaiian reef algae.

<http://www.botany.hawaii.edu/ReefAlgae/default.htm>

Test Coatings

The test coatings employed in the site characterization included an epoxy anticorrosive paint (International Intergard), an ablative antifouling paint that contains a copper biocide (International BRA 640), and a biocide-free silicone fouling-release paint (International Intersleek 425) (Table 1). These coatings are all qualified for use on U.S. Navy vessels. The epoxy coating has no antifouling properties and was used to characterize the rate of accumulation of fouling at each site, the ablative antifouling paint was used to quantify differences among sites in efficacy of a traditional copper-based antifouling coating, and the silicone fouling-release coating allowed us to examine both accumulation of fouling on a non-toxic surface with materials properties different from the epoxy anticorrosive paint, and to measure adhesion strengths of biofilms and 'hard' fouling invertebrates occurring at each site. Coatings were applied (back and front) to 25.4 cm x 30.5 cm (10" x 12") fiberglass (G10) panels according to the specifications of the paint manufacturer.

Table 1. Coating systems used in the test site characterization exercise. DFT = dry film thickness, and represents the target thickness for that particular layer of the system.

Coating Type	Intergard (control)		BRA 640 (biocide, AF)		Intersleek (biocide-free, FR)	
	Paint Name	DFT (mils)	Paint Name	DFT (mils)	Paint Name	DFT (mils)
Anticorrosive 1	INTERGARD FPL274/FPA3 27 epoxy polyamide	5	INTERGARD FPL274/FPA32 7 epoxy polyamide	5	INTERGARD FPL274/FPA327 epoxy polyamide	5
Anticorrosive 2	INTERGARD FPJ034/FPA3 27 epoxy polyamide	5	INTERGARD FPJ034/FPA327 epoxy polyamide	5	INTERGARD FPJ034/FPA327 epoxy polyamide	5
Tie Coat	NA		BRA 642 (Black)	5	INTERSLEEK 381 TIE COAT	5
Top Coat	NA		BRA 640 (Red)	5	INTERSLEEK 425 TOP COAT	6

Each test site was supplied with three replicate panels of the anticorrosive and antifouling coatings, and six replicate panels of the fouling-release coating. One set of three replicate panels of the fouling-release coating was used at each site for measurement of adhesion strength of biofilms and 'hard' foulers, while the other set of replicate panels was used to quantify accumulation of fouling over time.

In addition to the painted test panels (see above), three replicate panels (25.4 cm x 30.5 cm) of uncoated PVC were immersed each month to obtain a record of short-term temporal variation in recruitment of fouling organisms. The panel surfaces were roughened by application of 80 grit garnet sandpaper following a standard protocol. These substrates were retrieved and inspected each month, then replaced with clean PVC panels.

Inspection Procedures

The objectives to be addressed in testing each of the coatings differed with coating type. Inspection procedures for the coatings reflected these varying objectives. All panels, including the PVC panels, were inspected on a monthly basis (conditions permitting). At each inspection

each panel was subjected to a number of procedures (Table 2) depending on the coating type and test objective.

Table 2. Procedures applied to the various coating types at each inspection. Refer to the text for details on visual inspection, water jet and hard fouling adhesion testing.

Inspection Methods	Epoxy	Ablative Antifouling	Silicone Fouling Release A	Silicone Fouling Release B
Visual Inspection - Coverage Determination	X	X	X	
Digital Picture	X	X	X	X
Water Jet				X
Hard Fouling Adhesion				X

Visual Inspection and Coverage Determination

The spatial extent of fouling and damage or wear to the coating were determined by visual inspections from photographs. Photographs were taken with a digital camera of at least 5 megapixel resolution. The camera was positioned and focused such that the face of the panel being inspected filled as much of the image as possible.

Coverage of fouling and damaged areas was quantified from the digital pictures using PhotoGrid software (<http://www.photogrid.netfirms.com/>). Before assessment the digital picture was cropped so that fouling or damage occurring on a 1.27 cm (0.5") wide strip around the edge of the panel was excluded. A file of console 'buttons' (Appendix I) was developed specifically for the project so that all sites were measuring coverage of the same categories of fouling and damage (Table 3, Table 4). Coverage was determined by identifying the organism or type of damage occurring beneath each of 100 stratified random points arranged across the face of the panel. Detailed procedures can be found in Appendix II. The percentage or proportion of the panel face covered by a particular type of fouling or damage was then estimated by dividing the number of points corresponding to the fouling or damage of interest by the total number of points available.

For the purpose of calculating coverage of fouling the total number of points available was often less than 100. This situation commonly arose when points to be evaluated fell over the

mounting points for the panel, over mounting structures (for example, cable ties), or over damaged areas (which are not included in coverage calculations). Such points were subtracted from the 100 points initially available before estimation of coverage. Classes of organisms included in the 'hard' fouling category were encrusting bryozoans, barnacles, polychaete worms with calcareous tubes, molluscs, and all unidentifiable foulers with calcareous structures. 'Soft' foulers included all macroalgae, cnidaria or hydrozoans, arborescent bryozoans, tube-dwelling polychaete worms constructing soft tubes of sediment, sponges, solitary and colonial tunicates, and all unidentifiable foulers lacking calcareous structures. Incipient fouling - newly recruited fouling organisms that are too small to be identified - were also included in the 'soft' fouling category. Assessment software allowed the sites to distinguish four different types of macroalgae (green, red, brown, unidentifiable), but these were combined for the analysis of coverage. Finally, although the protocol and software include a mechanism for quantifying the coverage of biofilms and silt, for the purposes of this report these categories of fouling (microfouling) were considered equivalent to bare space.

The fouling of most interest in any assessment of antifouling efficacy is that which is attached to the primary substratum, that is, directly to the surface of the coating system. Mature, complex fouling communities may feature several layers of fouling organisms making it impossible to determine from a digital photograph the identity of organism(s) attached to the primary substratum. Coverage determined from such communities, however, still provides a valid estimate of the efficacy of the coating, as organisms occurring on secondary substrata (that is, other fouling organisms) cannot occupy more of the panel face than the fouling occurring on the primary substratum (the coating surface).

Table 3. Description of fouling types quantified in the visual inspections.

Fouling Type	Abbr.	Definition
Incipient Fouling	IF	Recently settled and juvenile forms of macrofouling.
Silt	Si	Absorbed organic and inorganic chemicals, trapped silt and detritus and unidentified slimes.
Slime	SI	Diatoms, initial algal germination and low form algae.
Algae, green	MAG	Fully established algae types and larger forms, eg. <i>Ulva sp.</i> and <i>Enteromorpha sp.</i>
Algae, red	MAR	Fully established algae types and larger forms, eg. <i>Ceramium sp.</i>
Algae, brown	MAB	Fully established algae types and larger forms, eg. <i>Ectocarpus sp.</i>
Cnidaria	Cn	Attached branching forms of hydrozoans.
Encrusting bryozoans	EB	Colonial animals forming an encrusting layer over the surface. These layers are generally 1 - 2 mm thick and have a rough texture.
Arborescent bryozoans	Br	Colonial animals forming upright bush- or tree-like colonies rarely exceeding 3 cm in length. They maybe mistaken for plants.
Barnacles	Barn	A hard shelled crustacean that cements itself permanently to a substrate, and is difficult to remove. The outer shell is generally whitish in color and shaped like a truncated cone.
Polychaetes, calcareous	PCal	Worms that may form a hard calcareous tube which becomes cemented to the substrate.
Polychaetes, sedimentary	PSed	Worms that may form a soft sediment tube which becomes cemented to the substrate.
Molluscs	Mol	Animals with two hard shells, hinged along one edge. Typical examples are oysters and mussels.
Sponges	Sp	Soft animals with sponge like texture forming thin surface cover or thicker accumulations. Often brightly colored.
Tunicates	Tun	Soft animals that may be solitary or colonial. Solitary types may reach several centimeters in height and colonial forms tend to form a thin cover over the surface.

Table 4. Description of coating defects quantified in the visual inspections.

Coating Defect	Abbr.	ASTM Number	Definition
Alligatoring	All	na	Small breaks in the coating forming an alligator skin pattern.
Biological Cutting	Bcut	na	The coating is cut by the action of the fouling organism against the surface.
Biological Penetration	Bpen	na	The hold fast of the fouling organism penetrates into coating matrix.
Blistering	Bli	D 714	The formation of liquid or gas filled domes under the coating.
Chalking	Chk	D 659	The formation of friable powder on the surface.
Checking	Chc	D 660	Slight breaks in the surface that do not penetrate the complete coating.
Chipping	Chp	na	Mechanical brittle failure of the coating.
Cracking	Crk	D 661	Breaks that extend through the coating.
Erosion	Ero	D 662	The wearing of a coating by the abrasive action of the water.
Flaking	Flk	D 772	The detachment of coating fragments.
Peeling	Pel	na	The detachment of coating in a continuous sheet.
Rusting	Rst	D 610	The corrosion of the metal substrate.

na = not available

Measurement of Biofouling Adhesion using a Water Jet

Adhesion of biofilms was measured using a water jet. The test apparatus consists of a SCUBA tank containing compressed air and mounting a regulator (so that pressure can be adjusted), connected to a SCUBA tank containing water. The compressed air is used to pressurize the water in the second SCUBA tank. The pressurized stream of water is applied to the panel surface through a nozzle (0.16 cm [1/16"] diameter). The nozzle is equipped with another regulator allowing water pressure to be controlled at the working end of the water jet.

A test comprises cleaning of three 3 cm x 3 cm patches on each face of the coated panels of interest. The SCUBA tank containing water is pressurized to 240 psi. Coverage of biofilm on the patch to be cleaned is assessed visually and recorded. The water jet pressure is then set to 40 psi at the nozzle, and the jet applied to the chosen patch until the maximum amount of fouling that can be removed at that setting is achieved. The jet is applied perpendicular to the coating surface, approximately 5 cm (1") from the panel face. Coverage of remaining biofilm is again assessed visually and recorded. The pressure is then increased to 120 psi at the nozzle and the cycle repeated. The test continues for pressures of 180 and 240 psi, or until all biofilm has been removed from the coating surface. For the Indian River Lagoon site, and initially the Morro Bay

site, coating performance in the water jet test was quantified as the maximum pressure required to completely remove the biofilm. Each patch was assigned to a 'biofilm adhesion category' corresponding to this pressure (1 = 40 psi, 2 = 120 psi, 3 = 180 psi, 4 = 240 psi, 5 = biofilm not removed at 240 psi). For all other sites and sampling periods, ease of removal of biofilm is described using the proportion of the original biofilm coverage remaining after application of the water jet at each pressure.

Measurement of Hard Fouling Adhesion

The method for measuring hard fouling adhesion is based on ASTM D 5618-94, 'Standard Test Method for Measurement of Barnacle Adhesion Strength in Shear' (Anonymous, 1997). Although this procedure was designed for barnacles, with minimal modification it can be used to obtain adhesion strengths (in shear) for tubeworms with calcareous tubes and oysters. Field sites were encouraged to incorporate alternative species into their evaluations if barnacles proved unsuitable or other organisms were readily available.

For the ASTM method, a shear force is applied to the base of an adult fouling organism, using a handheld force gauge, at a rate of approximately 4.5 N s^{-1} . The force at which the organism detaches from the surface is recorded, and removal stress is then calculated by dividing this force by the area of attachment (or basal area) of the organism. If during removal a substantial proportion (> 10%) of the organism's attachment structure remains adhered to the test material, the datum is discarded. Basal area can be measured in the field using calipers or a similar device, or in the field or laboratory using a scanner (for example, Kavanagh et al., 2001).

Results

Recruitment of fouling organisms varied over time at all the sites, and the strength of that variation depended on the particular site. Although levels of coverage of the control anticorrosive panels remained relatively stable after 60 d to 80 d of immersion (depending on the site), the communities resident on these panels appeared to be very dynamic. Due to the limitations of the method used for scoring coverage, this dynamism may reflect any combination of temporally variable recruitment of larvae to the primary substratum, short life spans of adult foulers, and varying patterns of usage of secondary substratum (the surfaces of other fouling organisms). For the fouling-release and antifouling coatings, where coverage was generally much lower, settlement on secondary substratum and overgrowth interactions are probably less important to the temporal variation in community structure.

This section discusses results from each site individually, focusing first on *Temporal Variation in Recruitment* of fouling organisms (which can be used to choose the best times to conduct short-term tests of experimental materials with antifouling or fouling-release properties), rate of *Accumulation of Fouling* to control anticorrosive, fouling-release, and antifouling coatings (which can be used to project the duration of tests designed to quantify antifouling properties), and patterns of *Adhesion of Fouling*, including identities of organisms of interest (which can be used to define a performance baseline for prospective fouling-release coatings, and to understand the range of test organisms, and their adhesion strengths, available at each site).

Morro Bay, California

Temporal Variation in Recruitment

Monthly recruitment of fouling organisms at the Morro Bay site was extremely variable over time (Fig. 5). Recruitment could be relatively high one month and minimal the next. The data set is not currently extensive enough to identify any seasonal patterns, although previous experience suggests that fouling is heaviest between spring and fall. The temporal variation reflected pulses in recruitment of both soft (macroalgae, hydrozoans) and hard (encrusting bryozoans, barnacles) fouling organisms (Fig. 5, Fig. 6). Peaks in recruitment of these species did not correspond to one another (Fig. 6).

Accumulation of Fouling

Panels were immersed on 13 June 2006. The control anticorrosive coating developed approximately 50% cover within 50 d of immersion, and supported approximately 75% cover of macrofouling by September 2006, 84 d after immersion (Fig. 7). Coverage fluctuated between 68% and 100% for the remainder of the trial (Fig. 7). Encrusting bryozoans were the first colonizers of these panels (Fig. 8), but the initial peak in total coverage reflected an abundance of soft fouling including macroalgae, colonial tunicates, hydrozoans and arborescent bryozoans (Fig. 7, Fig. 8). After 112 days of immersion this soft fouling-dominated community was gradually replaced by a community dominated by encrusting bryozoans, that was maintained for approximately 200 d (Fig. 7, Fig. 8). After 300 d of immersion the coverage of encrusting bryozoans began to decline. By the end of the trial in October 2007, coverage was roughly equally distributed among hard and soft fouling organisms (Fig. 7).

Coverage of the fouling-release coating, in contrast, was consistently low, below 20% (Fig. 9). These coatings experienced a sharp increase in coverage after 176 d of immersion, corresponding to abundant recruitment of macroalgae (Fig. 10). Initially the fouling community consisted mainly of colonial tunicates (Fig. 10), but coverage of hard fouling increased with recruitment of barnacles in April 2007, which persisted through the summer months (Fig. 10).

The antifouling coating experienced recruitment of soft fouling (incipient forms, macroalgae) at 139 d and 205 d of immersion (Fig. 11). This fouling did not persist. Between 353 d and 399 d of immersion, however, an unknown soft fouling organism colonized this coating treatment, rapidly developing greater than 95% cover (Fig. 11). This fouling persisted at high levels of coverage until the end of the experiment.

Adhesion of Fouling

Biofilms that formed on the test panels within the first 28 d of immersion adhered poorly, and were completely removed at water jet pressures less than 120 psi (adhesion category 2, Fig. 12). Adhesion strength of biofilm increased sharply thereafter, and after 84 d of immersion on most replicate patches the resident biofilm could not be removed at a pressure of 240 psi (adhesion category 5, Fig. 12). After longer exposure (greater than 300 d of immersion) biofilm adhesion decreased substantially, and except for one trial (427 d immersion) adhesion strengths were relatively low with most fouling being removed at pressures less than 120 psi to 180 psi

(Fig. 13). By the end of the experiment (517 d immersion) approximately 95% of the accumulated biofilm could be removed at a water jet pressure of 40 psi (Fig. 13).

The organism most commonly available for measurement of hard fouling adhesion strength was the barnacle *Balanus crenatus*. Over the course of the experiment 162 measurements of barnacle adhesion strength were taken, resulting in a mean value for removal stress of 0.058 MPa (SE = 0.003).

Monthly Fouling - PVC Panels California

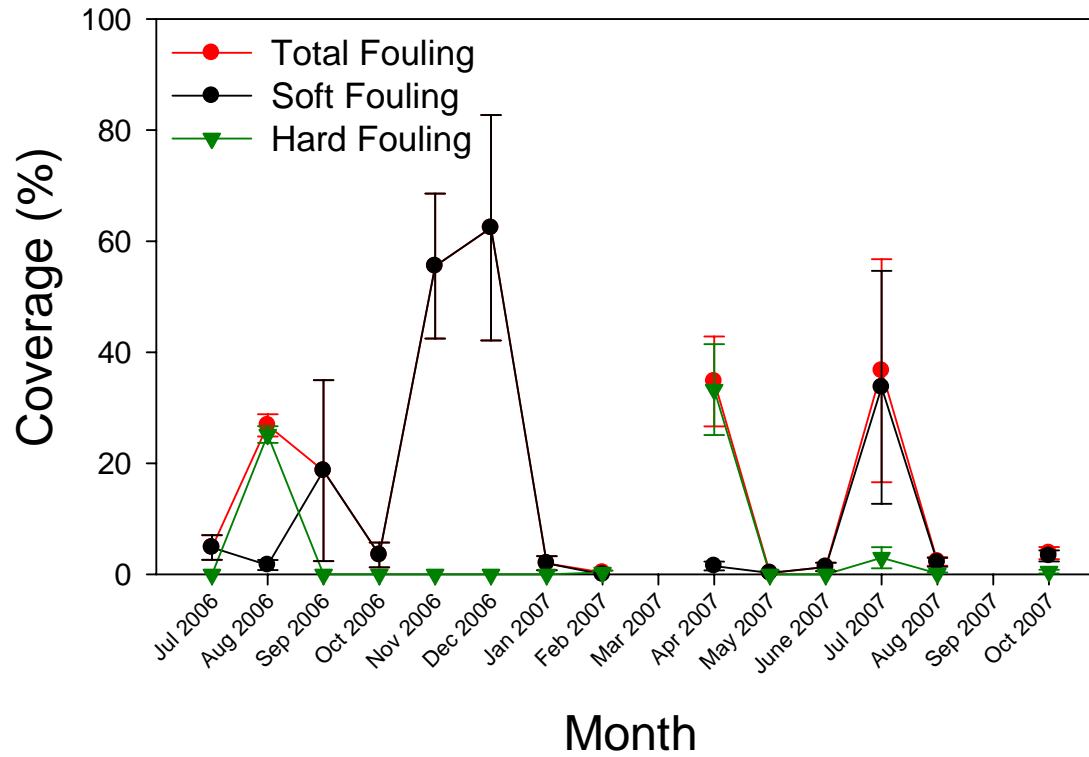


Figure 5. Morro Bay, CA. Recruitment of fouling to PVC panels exposed monthly. Error bars are standard errors.

Monthly Fouling - PVC Panels
California
Focal Species

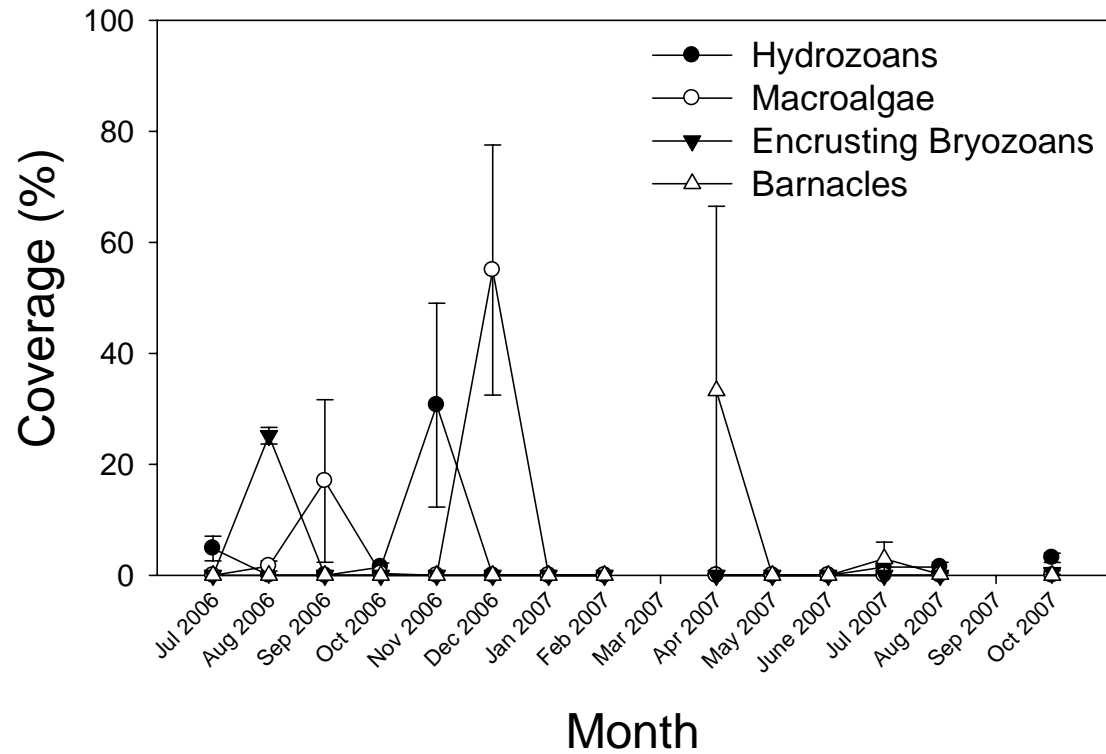


Figure 6. Morro Bay, CA. Recruitment of particular fouling functional groups to PVC panels exposed monthly. Error bars are standard errors.

Accumulation of Fouling Control Anticorrosive Coating California

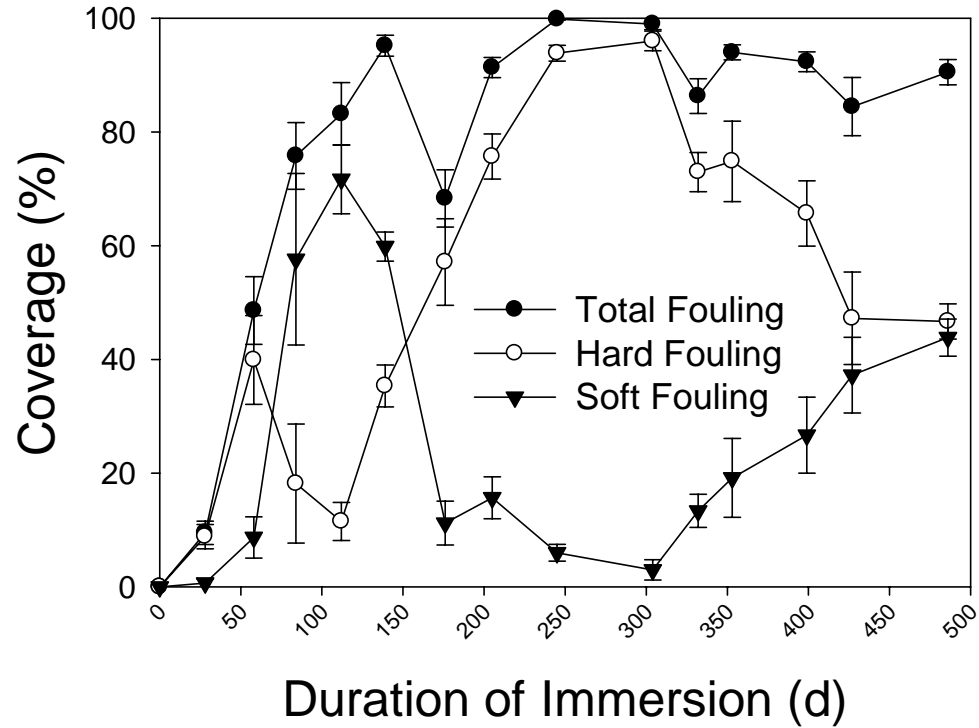


Figure 7. Morro Bay, CA. Accumulation of fouling to the control anticorrosive coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species Control Anticorrosive Coating California

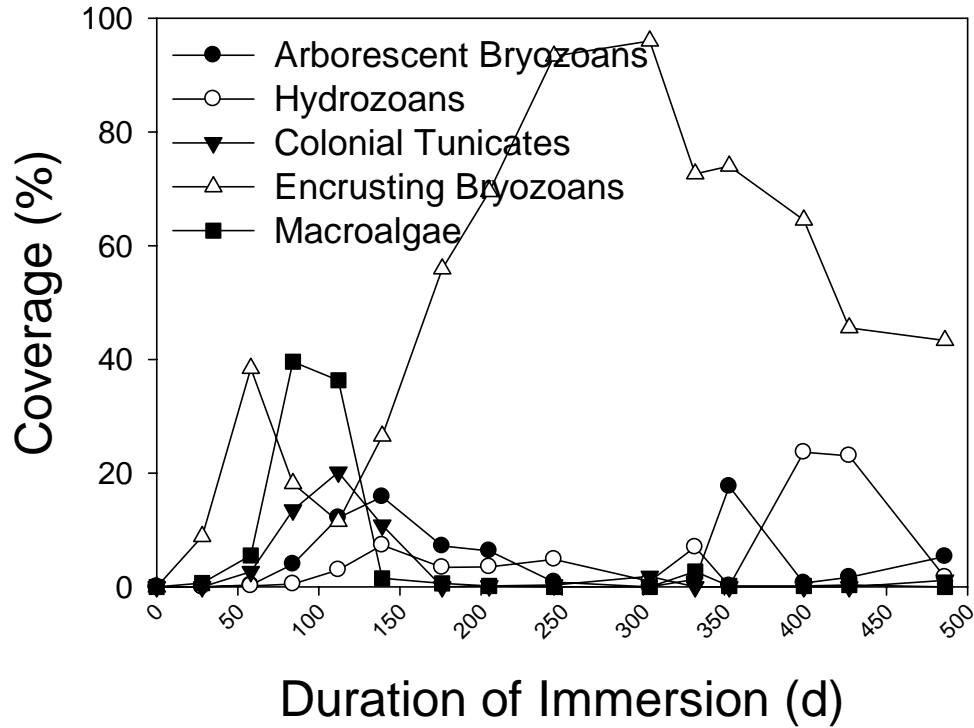


Figure 8. Morro Bay, CA. Accumulation of particular fouling functional groups to the control anticorrosive coating. Error bars omitted for clarity.

Accumulation of Fouling Fouling-release Coating (Intersleek) California

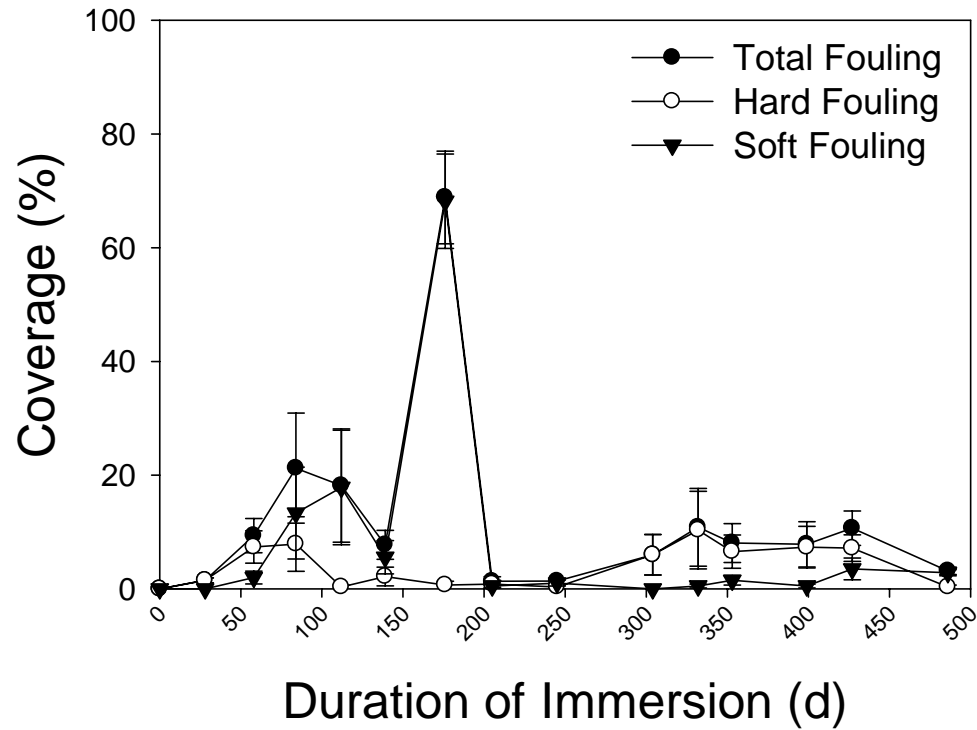


Figure 9. Morro Bay, CA. Accumulation of fouling to the fouling-release coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species Fouling-release Coating (Intersleek) California

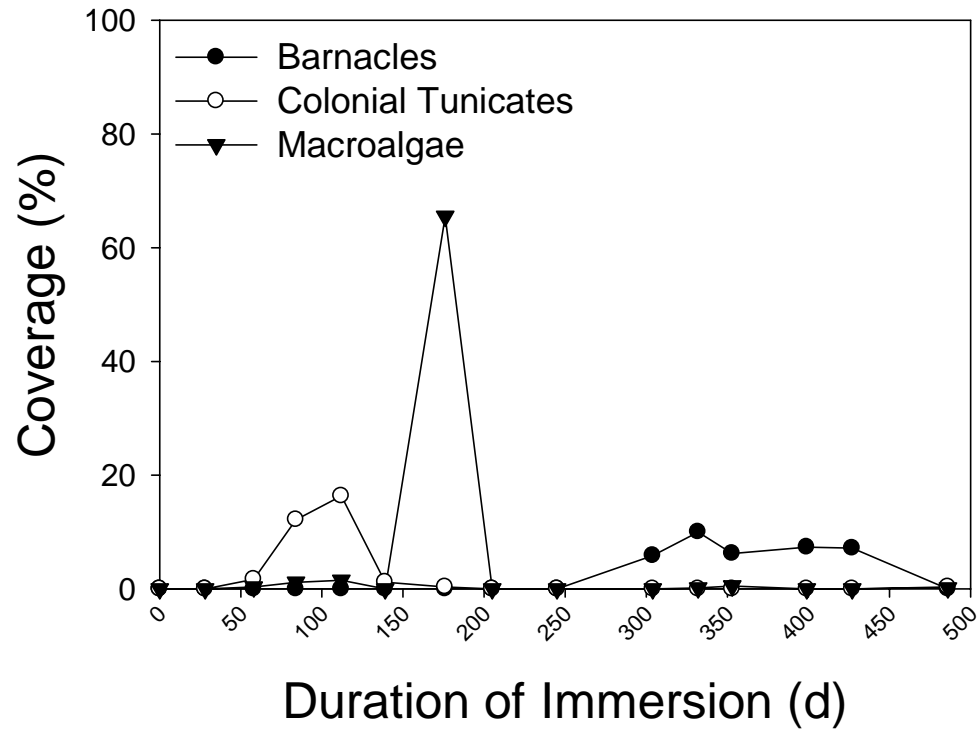


Figure 10. Morro Bay, CA. Accumulation of particular fouling functional groups to the fouling-release coating. Error bars omitted for clarity.

Accumulation of Fouling Antifouling Coating (BRA 640) California

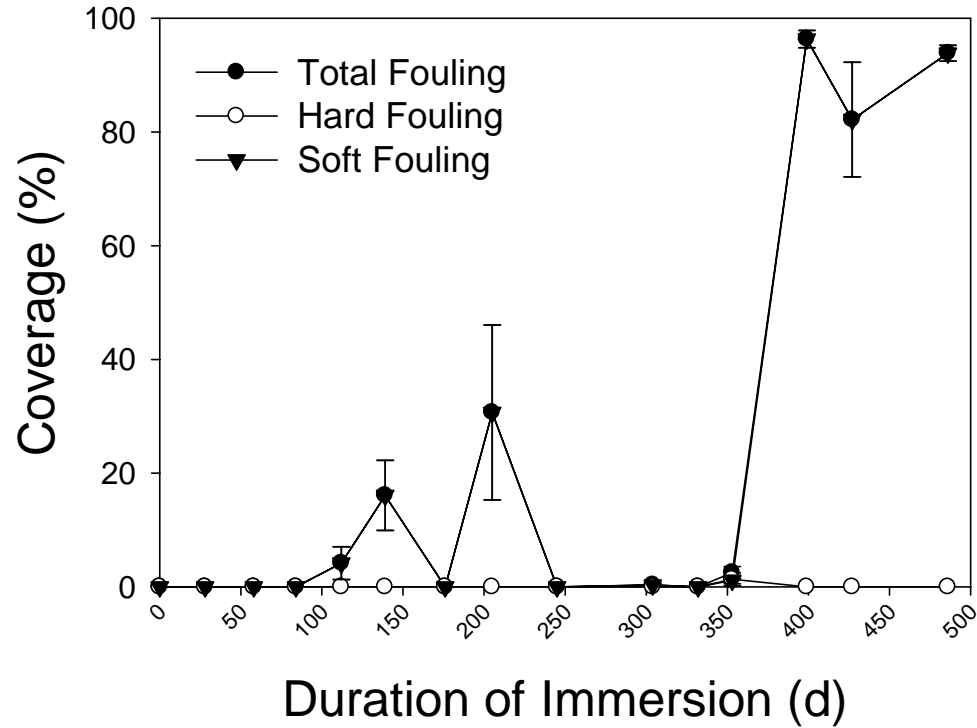


Figure 11. Morro Bay, CA. Accumulation of fouling to the antifouling coating. Error bars are standard errors.

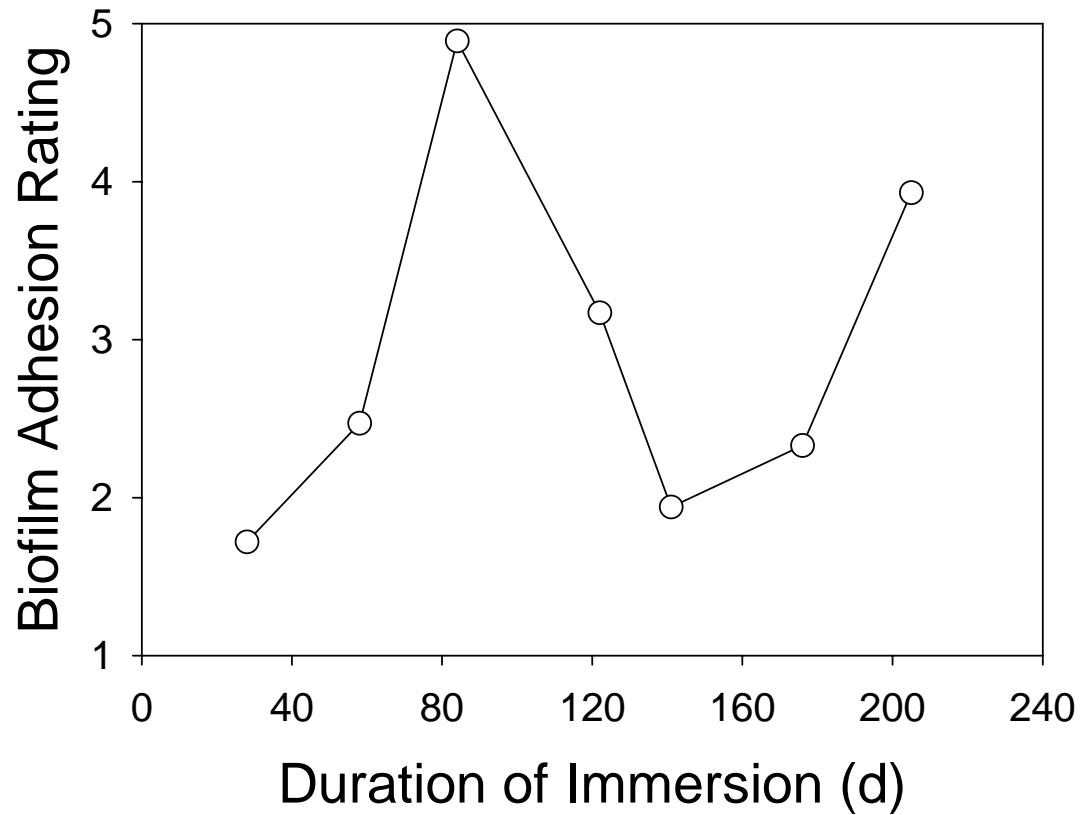


Figure 12. Morro Bay, CA. Adhesion of biofilm to the fouling-release coating over the first 205 days of exposure. Points represent mean ratings. See text for description of adhesion ratings.

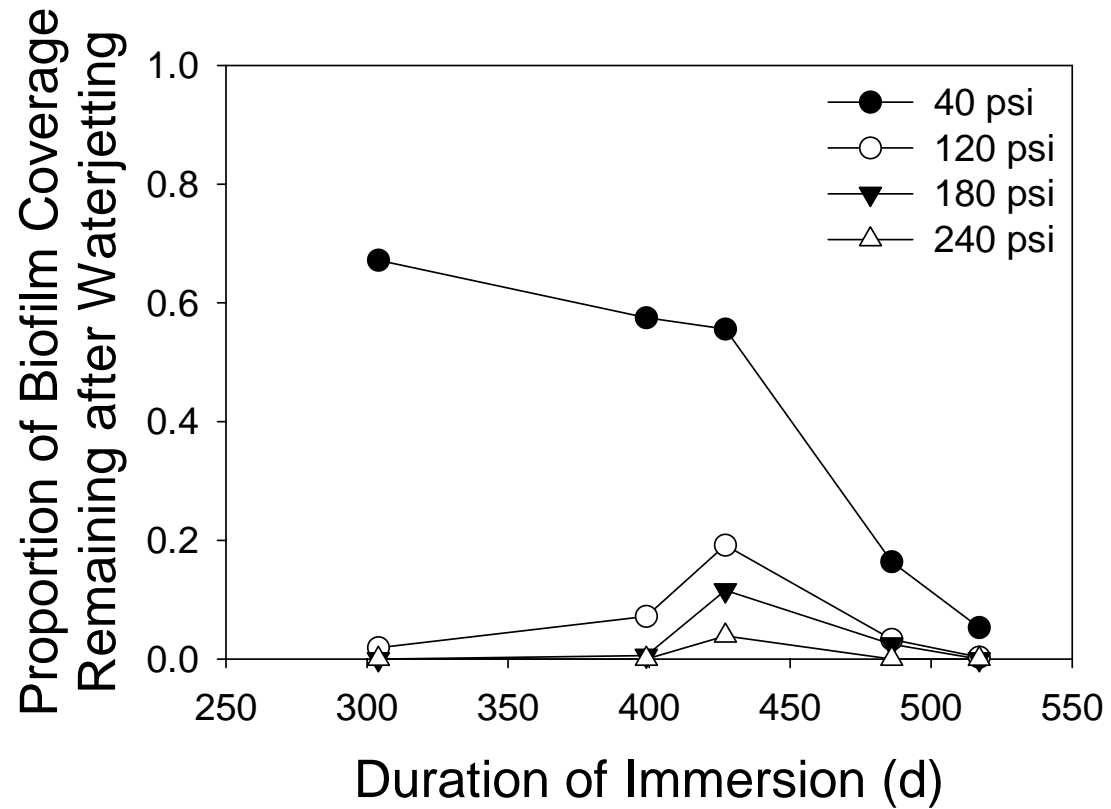


Figure 13. Morro Bay, CA. Adhesion of biofilm to the fouling-release coating, from 300 d of exposure to the end of the end of the experiment. Points represent the mean proportion of biofilm coverage remaining after application of the waterjet. Error bars omitted for clarity.

Indian River Lagoon, Florida

Temporal Variation in Recruitment

Recruitment of fouling organisms in the Indian River lagoon was strongly seasonal. Recruitment was low during the late-fall to early-spring months, but was very high from May to the end of October (Fig. 14). This seasonal pattern was largely driven by heavy recruitment of barnacles during the summer, with some contribution from encrusting bryozoans later in the summer and in the late fall (Fig. 15). Arborescent bryozoans recruited in relatively high abundance from December 2006 to February 2007 (Fig. 15).

Accumulation of Fouling

Panels were immersed on 23 December 2005. Despite being immersed at a period of relatively poor recruitment (Fig. 14), the control anticorrosive coating rapidly accumulated fouling, reaching levels of coverage greater than 85% after only 56 d of immersion (Fig. 16). The resident community consisted strictly of hard fouling organisms (mainly barnacles, Fig. 17) until 143 d of immersion, when soft fouling organisms began to occur at levels between 0% and 10% cover. During the winter months of 2006 – 2007 soft fouling coverage grew, peaking at 54% after 440 d of immersion (Fig. 16). Despite the relative constancy of levels of total coverage, the fouling community was dynamic, showing alternating periods of dominance by barnacles and mollusks (Fig. 17).

Coverage of the fouling-release coating varied strongly throughout the course of the experiment, fluctuating between 20% - 40% cover and 50% - 70% cover (Fig. 18). The composition of the community also alternated between states dominated by hard fouling organisms (0 – 115 d immersion) and soft fouling organisms (381 – 468 d immersion) (Fig. 18). These fluctuations apparently reflected periods of recruitment and growth of barnacles and sponges (Fig. 19).

The antifouling coating supported minimal fouling throughout the majority of the experiment, with a short-lived peak in fouling (8% - 12%) occurring between 411 d and 468 d of immersion (Fig. 20). This peak corresponded to recruitment of bryozoans.

Adhesion of Fouling

For the first 347 d of immersion of the fouling-release panels, biofilms could be removed with the water jet at pressures less than 240 psi, often on the order of 120 psi (adhesion categories 2 – 4, Fig. 21). After 347 d of immersion, however, biofilm adhesion remained high through the remainder of the experiment. From this point on it was not possible to clean the biofilm from some to most of the replicate test patches (adhesion category 5, Fig. 21).

Previously at this site, adhesion strength of hard fouling has been measured for barnacles, oysters and tubeworms (Kavanagh et al., 2001). For this exercise, adhesion strengths were quantified for several species of barnacles. The most abundant species, *Balanus eburneus* and *Balanus improvisus*, yielded removal stresses of 0.042 MPa (SE = 0.0006; n = 711) and 0.041 MPa (SE = 0.002; n = 141), respectively (Fig. 22). *Balanus amphitrite* (n = 9) and *Balanus*

variegatus (n = 8) were also available for measurement. Due to small sample size, mean values of removal stress for these species are not reported here.

Monthly Fouling - PVC Panels Florida

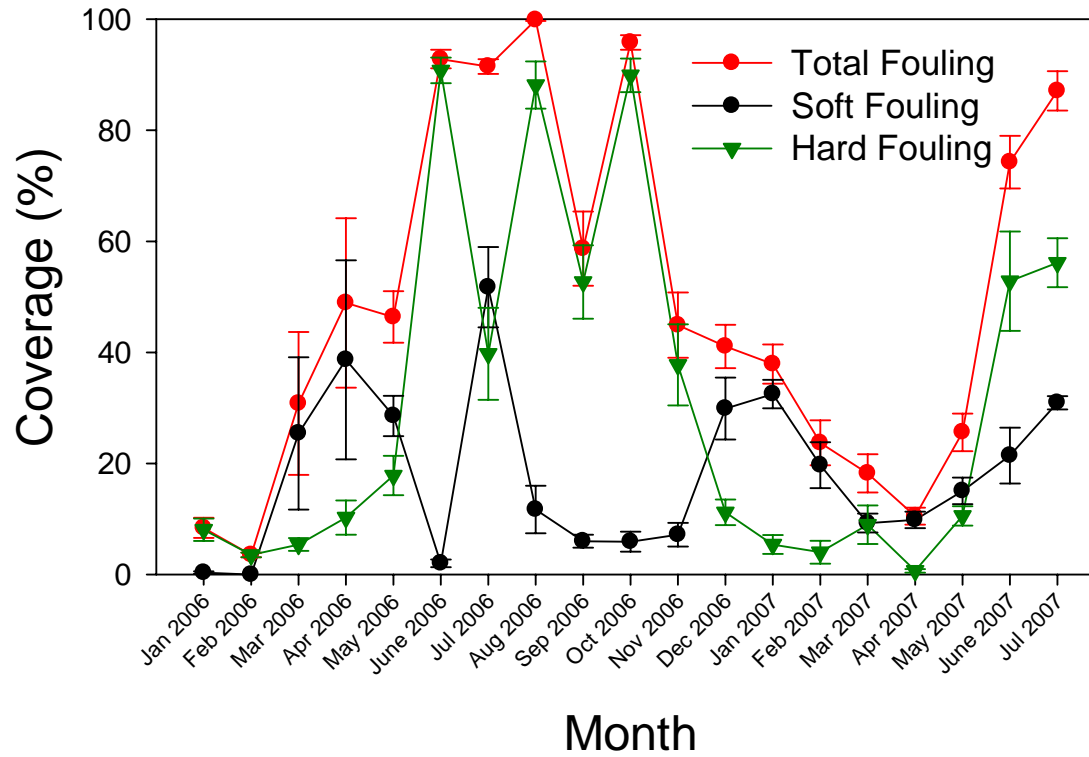


Figure 14. Indian River lagoon, FL. Recruitment of fouling to PVC panels exposed monthly. Error bars are standard errors.

Monthly Fouling - PVC Panels Florida Focal Species

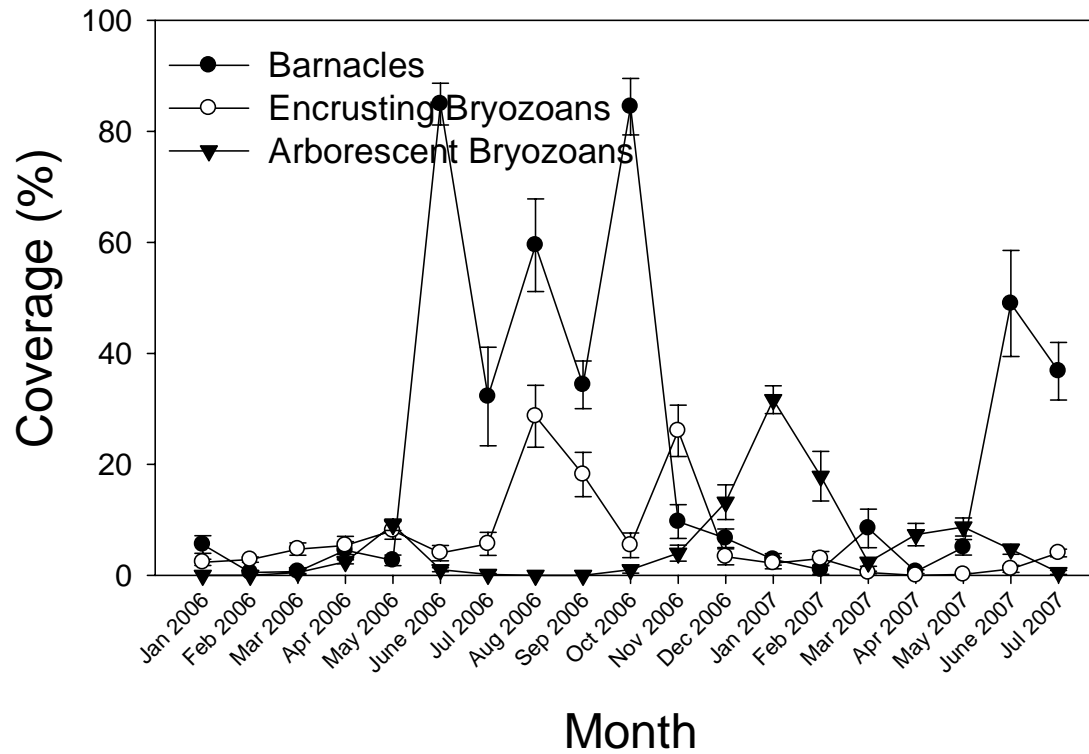


Figure 15. Indian River lagoon, FL. Recruitment of particular fouling functional groups to PVC panels exposed monthly. Error bars are standard errors.

Accumulation of Fouling Control Anticorrosive Coating Florida

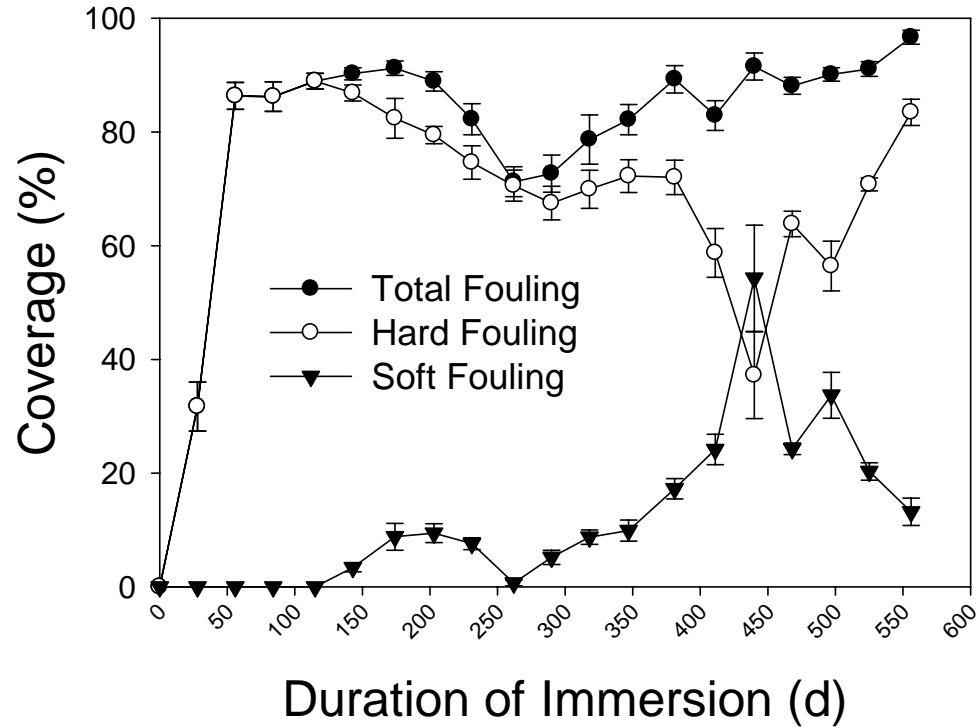


Figure 16. Indian River lagoon, FL. Accumulation of fouling to the control anticorrosive coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species Control Anticorrosive Coating Florida

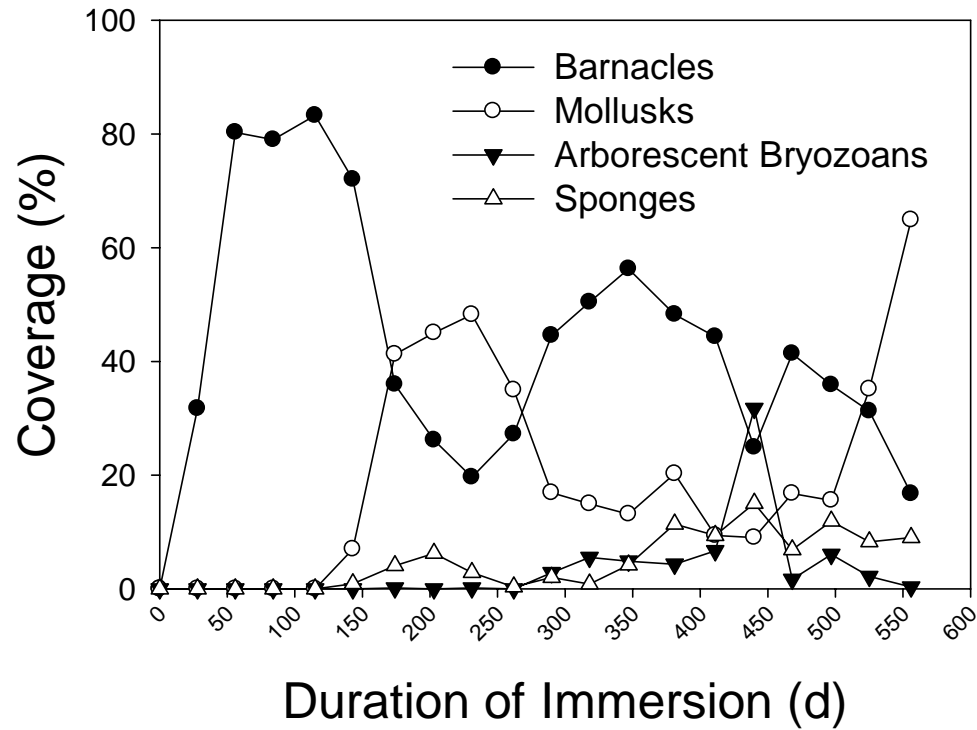


Figure 17. Indian River lagoon, FL. Accumulation of particular fouling functional groups to the control anticorrosive coating. Error bars omitted for clarity.

Accumulation of Fouling Fouling-release Coating (Intersleek) Florida

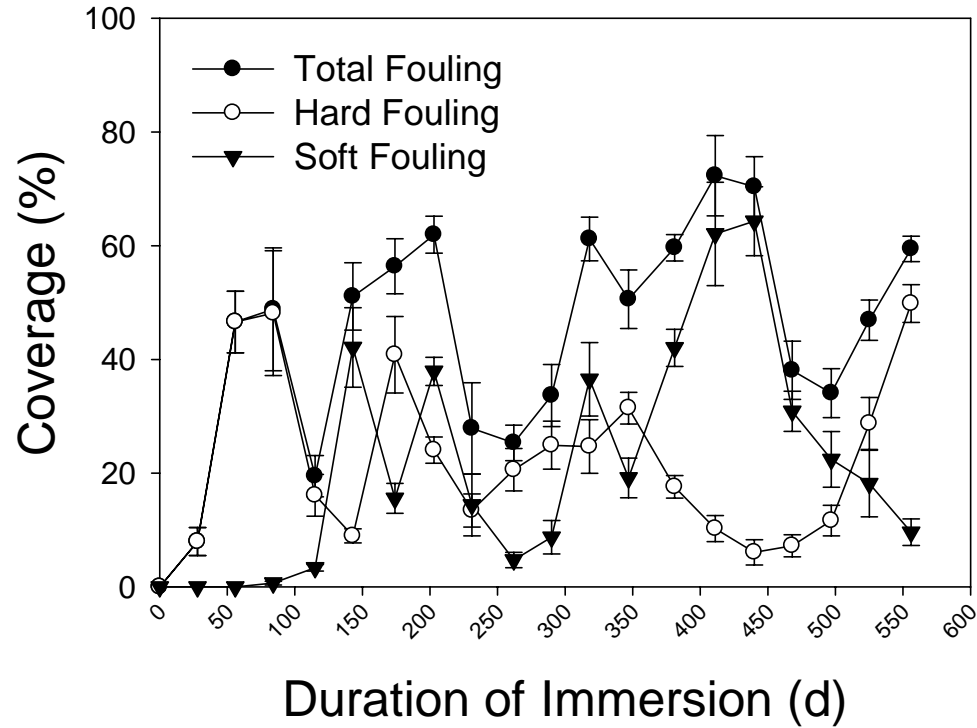


Figure 18. Indian River lagoon, FL. Accumulation of fouling to the fouling-release coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species
 Fouling-release Coating (Intersleek)
 Florida

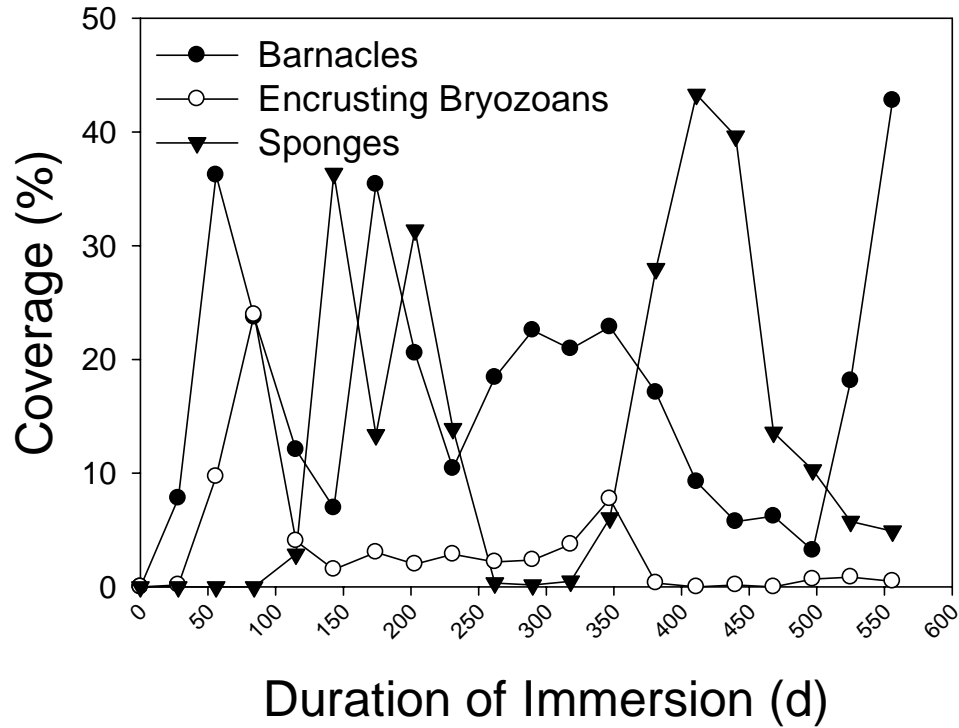


Figure 19. Indian River lagoon, FL. Accumulation of particular fouling functional groups to the fouling-release coating. Error bars omitted for clarity.

Accumulation of Fouling Antifouling Coating (BRA 640) Florida

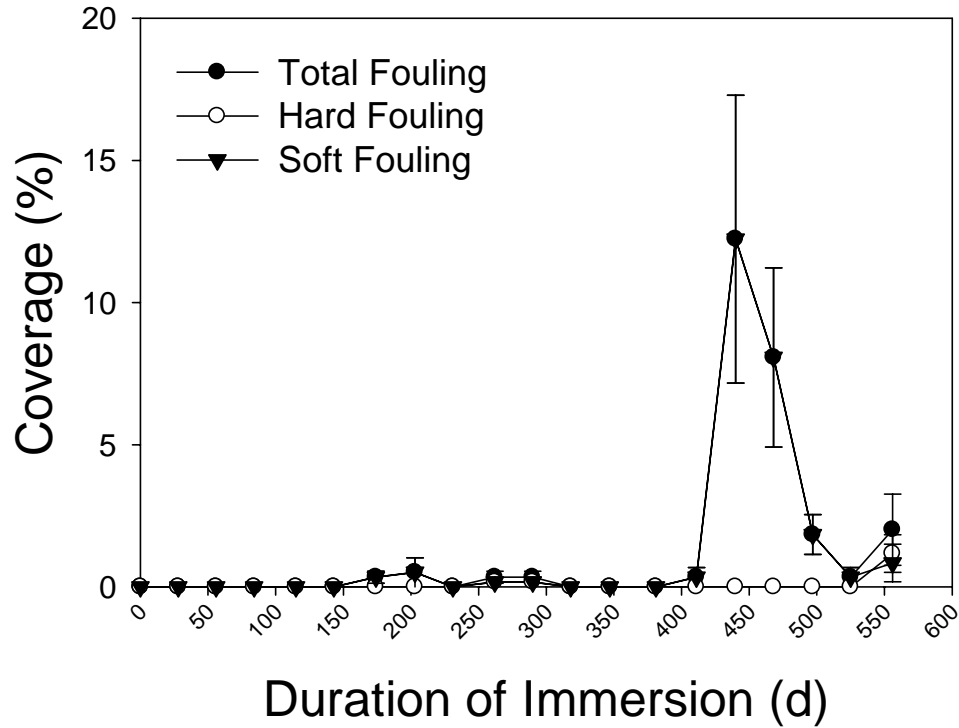


Figure 20. Indian River lagoon, FL. Accumulation of fouling to the antifouling coating. Error bars are standard errors.

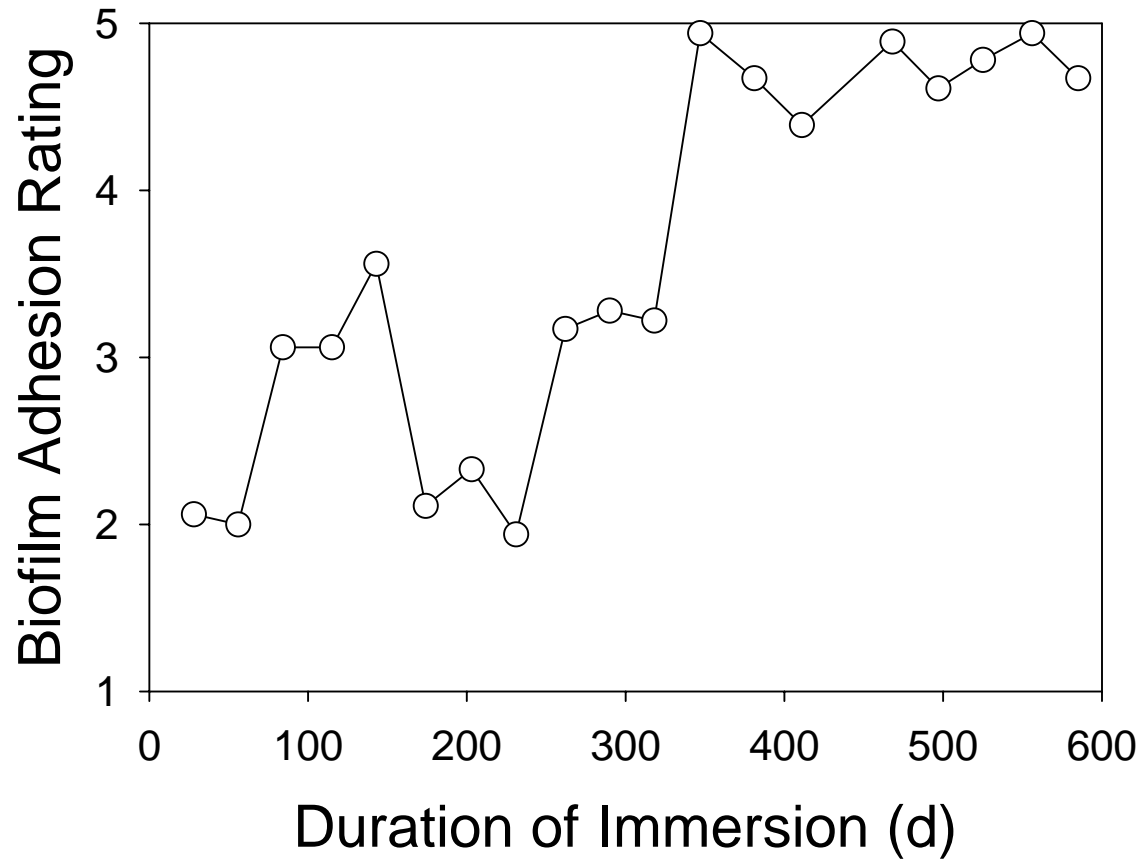


Figure 21. Indian River lagoon, FL. Adhesion of biofilm to the fouling-release coating. Points represent mean ratings. See text for description of the adhesion ratings.

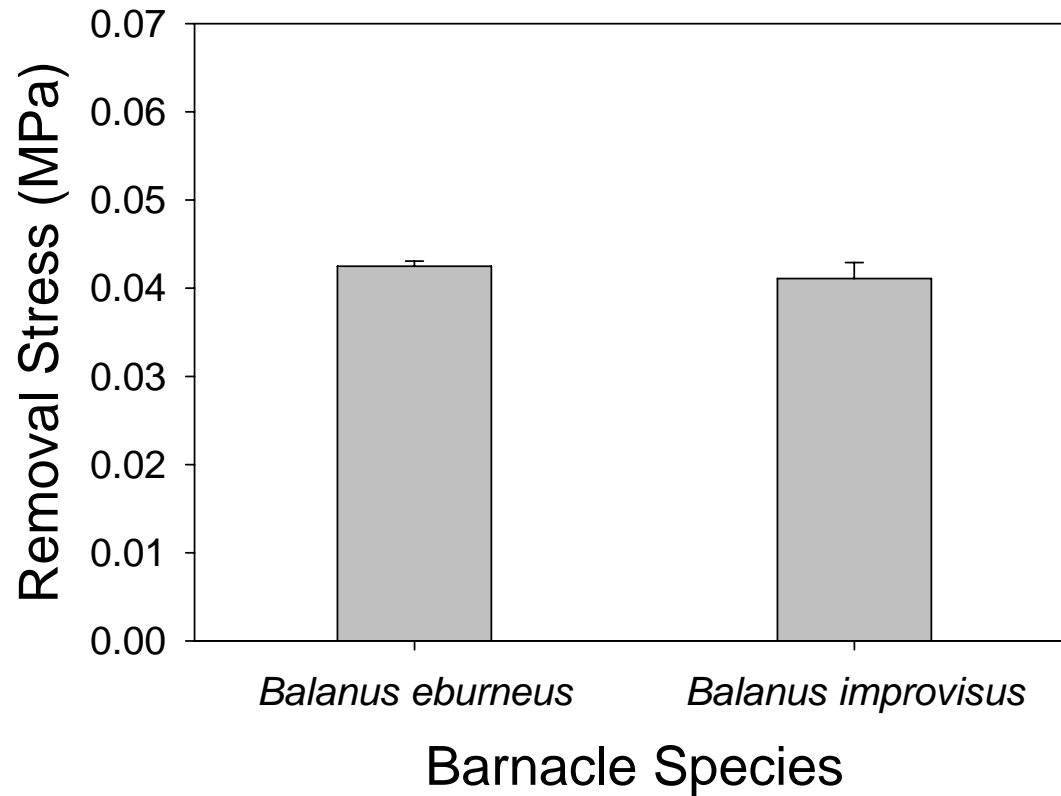


Figure 22. Indian River lagoon, FL. Adhesion of barnacles *Balanus eburneus* and *B. improvisus* to the fouling-release coating. Error bars are standard errors.

Singapore

Temporal Variation in Recruitment

The abundance of newly-recruited fouling organisms at the Singapore site was typically high and, for much of the first year of testing at least, relatively constant (Fig. 23). Coverage of recruits decreased dramatically from December 2005 to January 2006, and also displayed a substantial decline from November 2006 to the end of May 2007 (Fig. 23). At all times, however, coverage was greater than 20%. Recruitment during the late summer months of 2007 was substantially lower than the corresponding months of 2006 (Fig. 23). A large proportion of the organisms contributing to the coverage on the PVC recruitment panels were too small to be identified, and thus classified as incipient fouling. Of those organisms that could be identified, barnacles and tubeworms (with calcareous tubes) were most abundant (Fig. 24). Settlement of tubeworms occurred throughout the year, while barnacles appeared to settle most intensely during the summer and fall (Fig. 24).

Accumulation of Fouling

Panels were immersed on 25 November 2005. In the first 34 d of immersion the control anticorrosive coating developed approximately 60% coverage of fouling (Fig. 25). Fouling accumulated more slowly thereafter. After 147 d of immersion fouling covered approximately 83% of the space available on test panels, and total coverage fluctuated between 78% and 97% until the end of the experiment (Fig. 25). The community attaching to the control coatings consisted mainly of hard fouling organisms (Fig. 25). Polychaete worms (with calcareous tubes) were the dominant hard fouling organisms initially (Fig. 26), but after 117 d mollusks began to obtain more space, and after 224 d barnacles were the most common hard fouler (Fig. 26). Sponges were the most common soft-fouling organism, with coverage ranging between 2% and 15% after 87 d of exposure.

Fouling was less abundant on the fouling-release coating, but did on occasion reach levels comparable to the control anticorrosive coating (74%, 273 d immersion, Fig. 27). Coverage of these surfaces was variable in time, decreasing dramatically to 7% to 8% after 427 d of immersion and increasing again over the next 100 d (Fig. 27). This corresponded to the period of January to March of 2007. The communities present on the Intersleek coating were dominated initially by hard fouling polychaete tubeworms (Fig. 28), but after 224 d of exposure came to represent a mixture of hard and soft fouling organisms including barnacles, mollusks, tubeworms, colonial tunicates, and sponges (Fig. 28).

The antifouling coating reached a maximum coverage of 16% after 427 d of immersion, but typically coverages fluctuated between 1% to 4% (Fig. 29). Fouling of these panels consisted almost completely of newly recruited forms, classified as incipient fouling.

Adhesion of Fouling

Adhesion of biofilms to the fouling-release panels, as determined using the water jet, was initially very poor. After 14 d of immersion, 81% of accumulated biofilm could be removed at a pressure of 40 psi, and no biofilm remained after application of the water jet at 120 psi (Fig. 30). By 87 d of immersion, however, biofilm adhesion had increased such that 120 psi was required

to remove more than 80% of the attached biofilm. As the experiment progressed biofilms became increasingly more difficult to remove, and by 539 d of exposure the complete biofilm could not be removed at maximum water jet pressure (240 psi, Fig. 30). Biofilm adhesion decreased dramatically between 539 d and 729 d of exposure. At the last water jet test more than 99% of biofilm could be removed at 120 psi or less (Fig. 30).

Removal stress was quantified for three species of barnacles, *Balanus amphitrite* (n = 5), *Balanus cirratus* (n = 73), and *Balanus reticulatus* (n = 203). Due to small sample size the results for *Balanus amphitrite* will not be reported here. Removal stress for *Balanus cirratus* was 0.052 MPa (SE = 0.003) and for *Balanus reticulatus* 0.059 MPa (SE = 0.002) (Fig. 31).

Monthly Fouling - PVC Panels Singapore

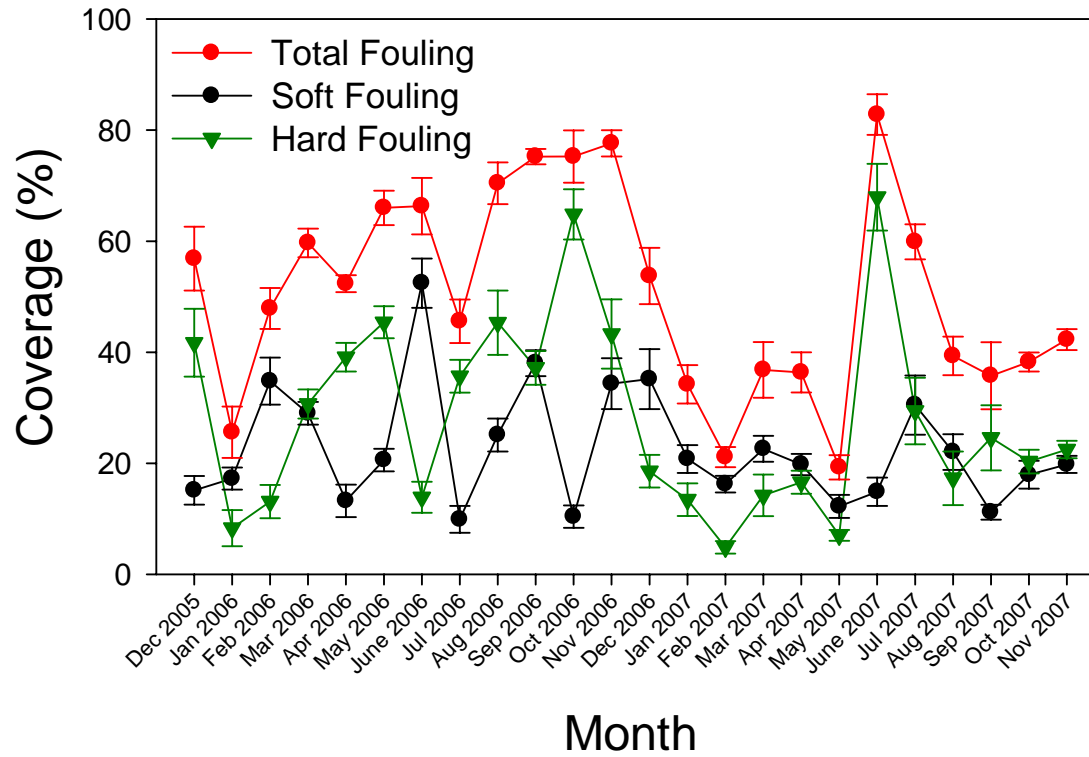


Figure 23. Singapore. Recruitment of fouling to PVC panels exposed monthly. Error bars are standard errors.

Monthly Fouling - PVC Panels Singapore Focal Species

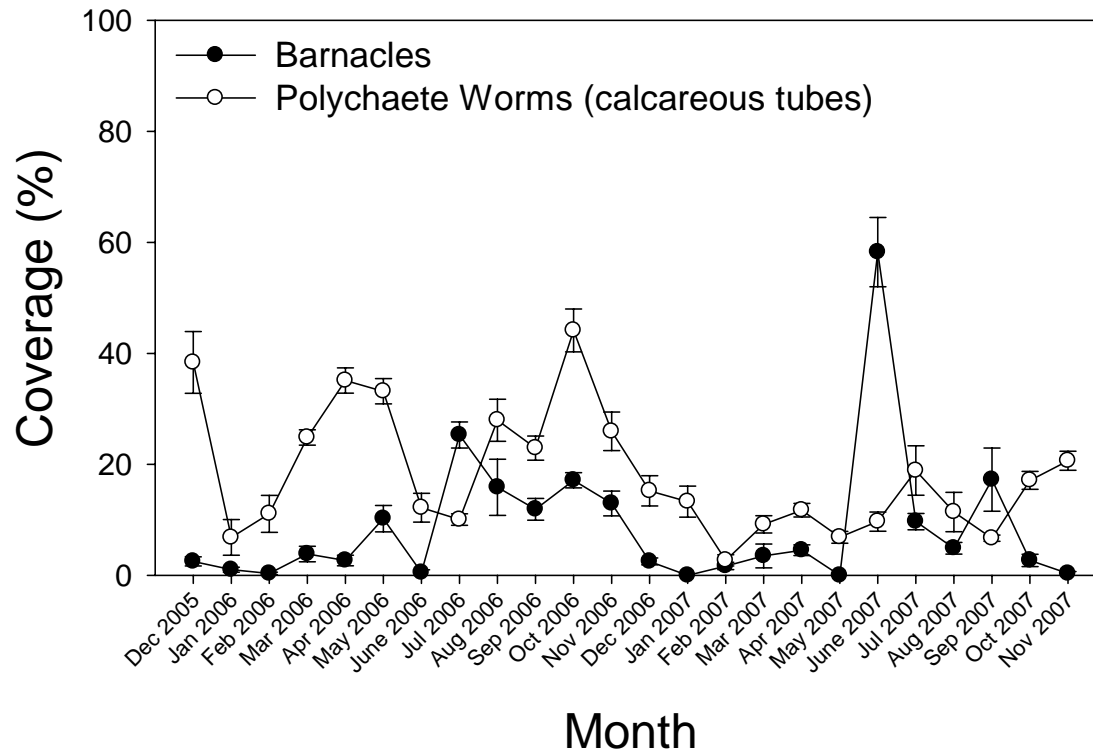


Figure 24. Singapore. Recruitment of particular fouling functional groups to PVC panels exposed monthly. Error bars are standard errors.

Accumulation of Fouling Control Anticorrosive Coating Singapore

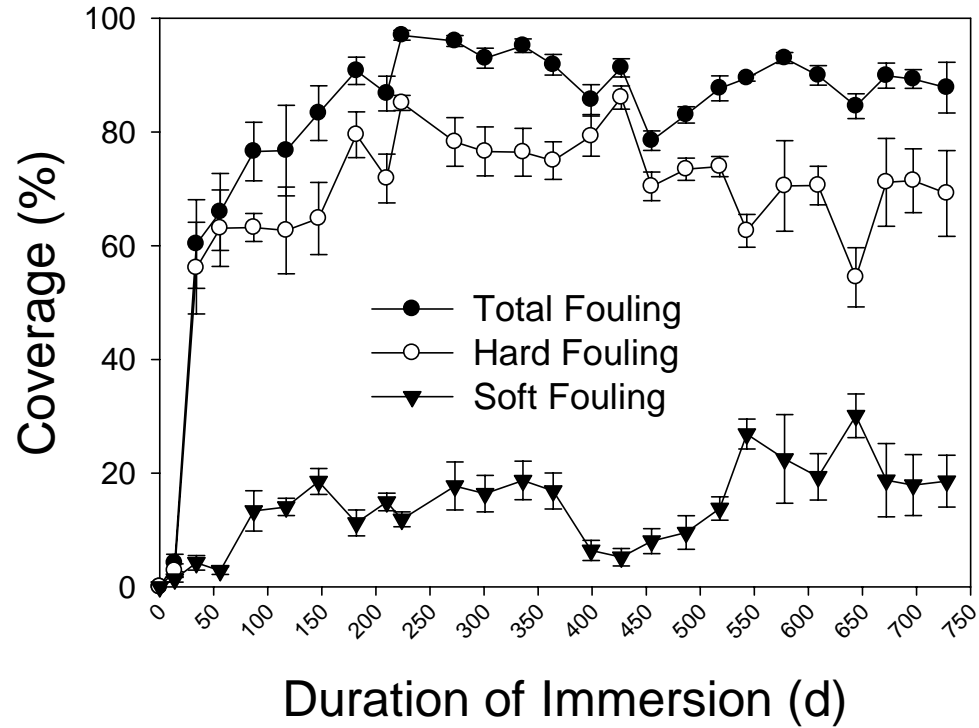


Figure 25. Singapore. Accumulation of fouling to the control anticorrosive coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species Control Anticorrosive Coating Singapore

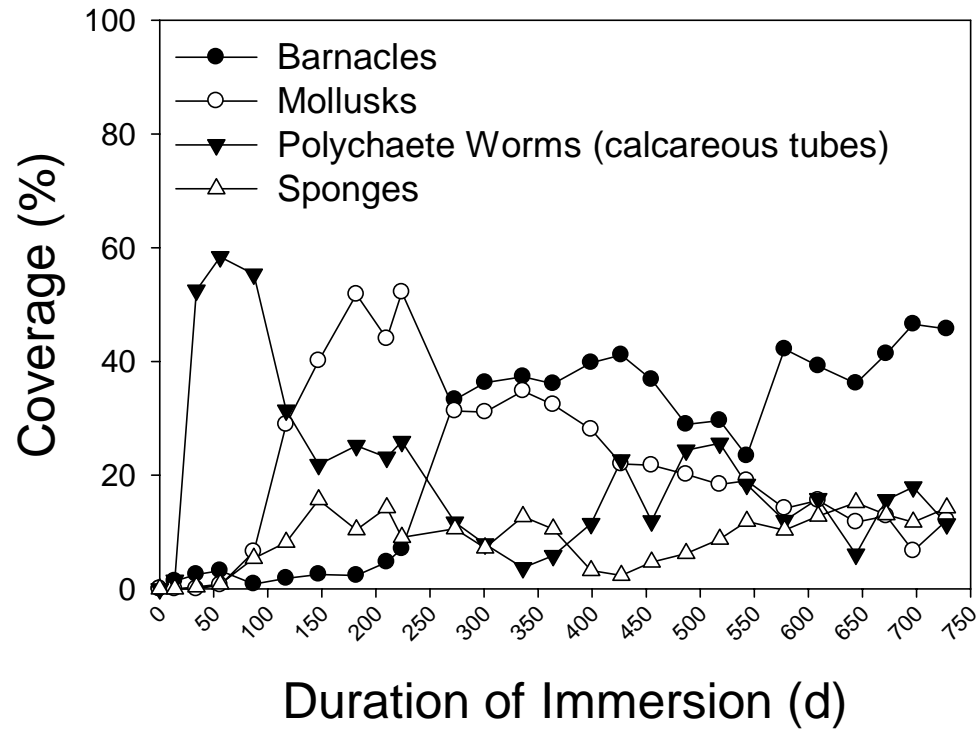


Figure 26. Singapore. Accumulation of particular fouling functional groups to the control anticorrosive coating. Error bars omitted for clarity.

Accumulation of Fouling Fouling-release Coating (Intersleek) Singapore

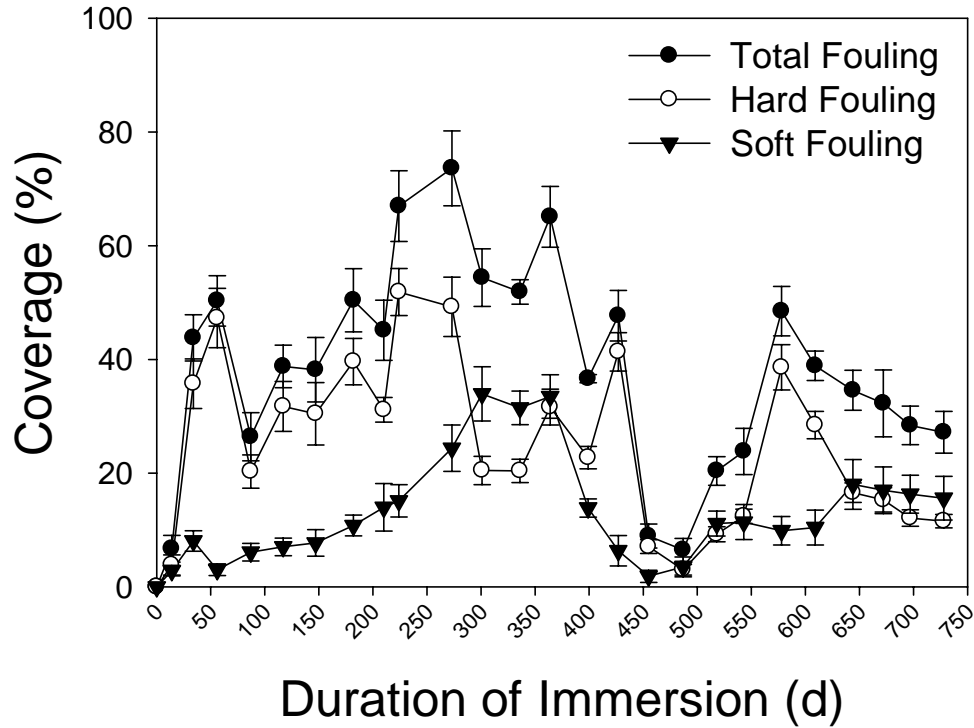


Figure 27. Singapore. Accumulation of fouling to the fouling-release coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species Fouling-release Coating (Intersleek) Singapore

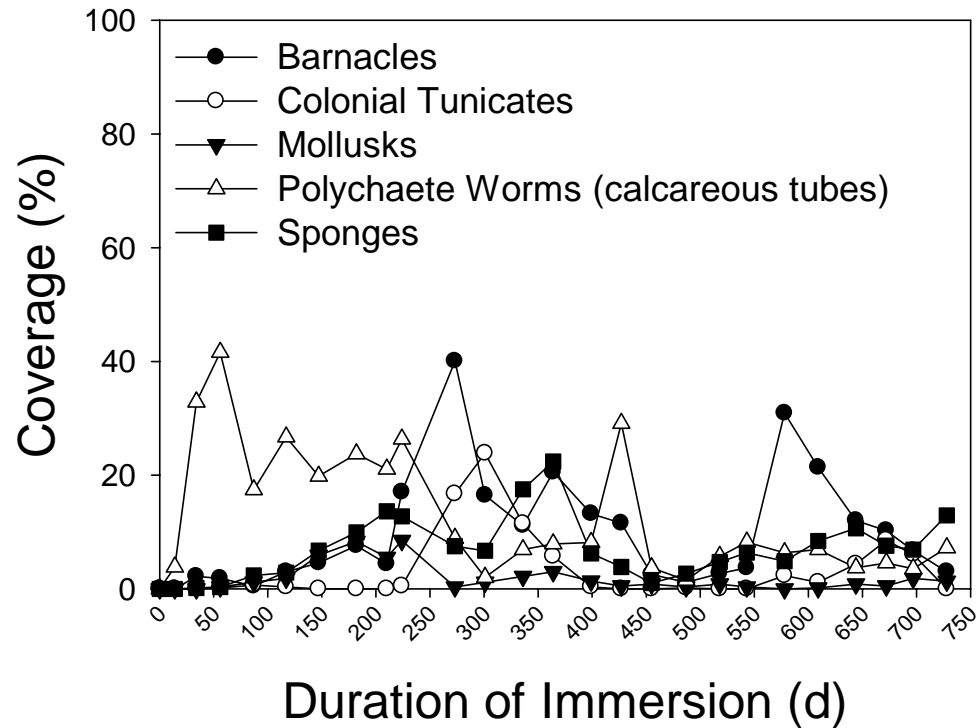


Figure 28. Singapore. Accumulation of particular fouling functional groups to the fouling-release coating. Error bars omitted for clarity.

Accumulation of Fouling Antifouling Coating (BRA 640) Singapore

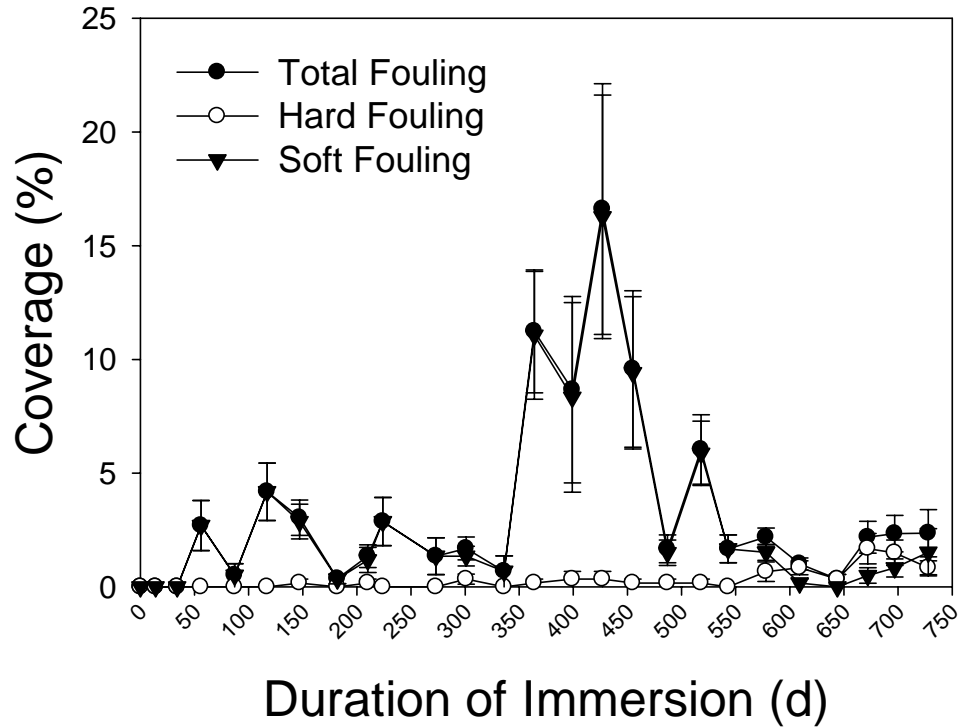


Figure 29. Singapore. Accumulation of fouling to the antifouling coating. Error bars are standard errors.

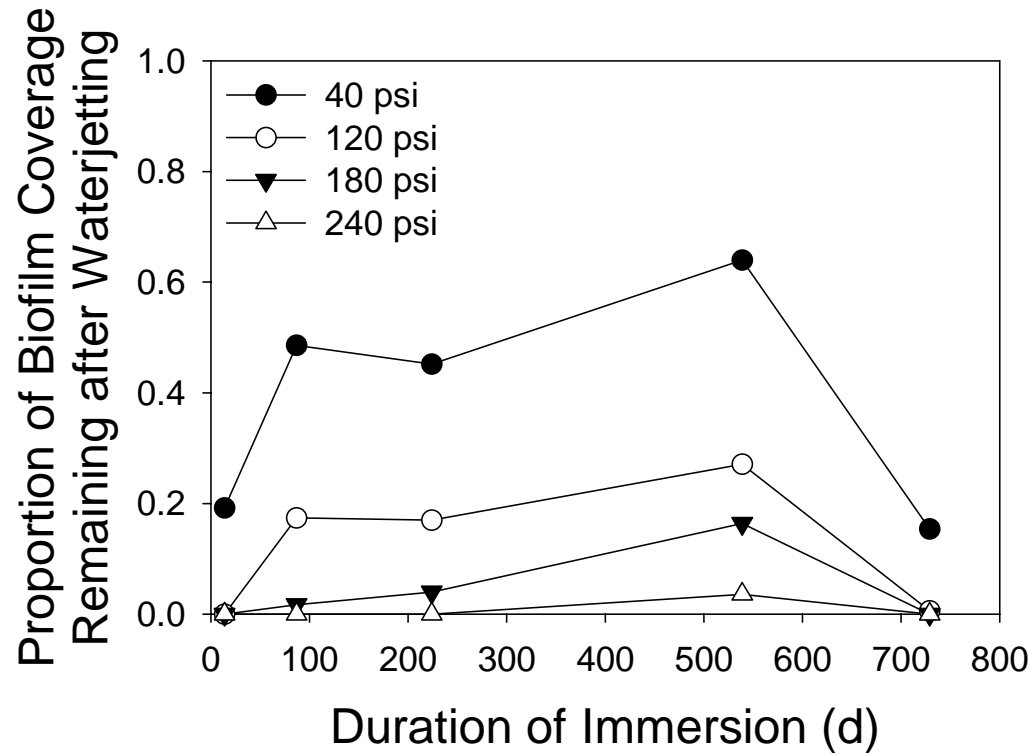


Figure 30. Singapore. Adhesion of biofilm to the fouling-release coating. Points represent the mean proportion of biofilm coverage remaining after application of the waterjet. Error bars omitted for clarity.

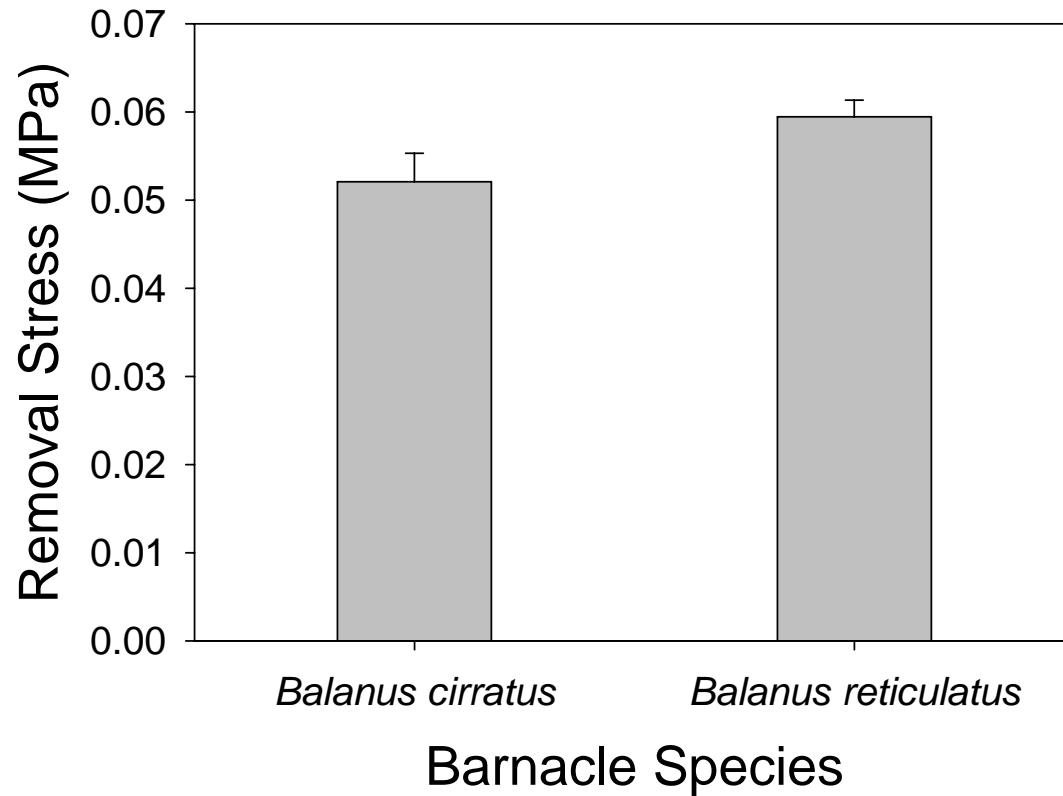


Figure 31. Singapore. Adhesion of barnacles *Balanus cirratus* and *B. reticulatus* to the fouling-release coating. Error bars are standard errors.

Pearl Harbor, Hawaii

Temporal Variation in Recruitment

Recruitment of fouling organisms varied strongly over time, but no seasonal pattern was apparent in the data (Fig. 32). Results, however, suggested the possibility of substantial annual variation, with lower recruitment in the second year of the study (Fig. 32). Most recruitment on monthly timespans was due to colonization of the PVC panels by hard fouling (Fig. 32), in particular tubeworms (Fig. 33). Increased fouling between August and November 2006 was due to a peak in recruitment of macroalgae (Fig. 33). This peak in recruitment was also reflected in the fouling communities present on other test panels at this site (see below).

Accumulation of Fouling

Panels were immersed on 2 December 2005. The control anticorrosive coating reached 70% coverage of fouling within 56 d of immersion. Over the course of the rest of the experiment total coverage varied between 78% and 98% (Fig. 34). Throughout the experiment coverage was mainly composed of hard fouling organisms, although soft fouling organisms did increase their coverage between 110d and 251 d of immersion (Fig. 34). The identity of the important hard fouling organisms changed over time. Tubeworms recruited in abundance to the panels initially, then decreased in cover from 110 d of immersion to the end of the exercise (Fig. 35). Barnacles began to recruit shortly after the tubeworms and maintained coverage between 14% and 38% for approximately 1.5 years (Fig. 35). In general, however, the mature community was diverse and included substantial levels of fouling by mollusks, sponges, and colonial tunicates (Fig. 35).

Except for a period of abundant fouling by macroalgae (which did not persist), coverage of the fouling-release coating increased slowly but steadily (Fig. 36). Again, the important contributors to coverage were hard fouling organisms (Fig. 36), but in contrast to the control coating, after initial colonization by tubeworms encrusting bryozoans became the dominant fouler (Fig. 37). Barnacles never developed coverage greater than 3% on this material, while fouling by mollusks varied between 1% and 7%.

The antifouling coating began to accumulate significant encrustations of organisms after approximately 250 d of immersion (Fig. 38). After this point, mean coverage across all panel faces ranged from 1% to 7% (Fig. 38). Fouling consisted mainly of encrusting bryozoans and a few tubeworms.

Adhesion of Fouling

Adhesion strength of biofilms varied strongly over time. Initially, all of the biofilm resident on the test fouling-release panels could be removed with the water jet at pressures less than 120 psi (Fig. 39). After 100 d of immersion, however, biofilm adhesion increased and pressures of 180 psi to 240 psi were required to remove the fouling. After 200 d of immersion biofilms would occasionally develop that could not be removed at the highest pressures applied (examine results for biofilm remaining after application of the water jet at 240 psi, Fig. 39). Biofilms present on the test panels between approximately 500 – 580 d of immersion were poorly adhered (Fig. 39).

At the Pearl Harbor site, organisms commonly available for measurement of hard fouling adhesion include tubeworms, oysters, and on occasion barnacles (Holm et al., 2000; Holm et al., 2006). For this exercise data were collected from abundant tubeworms *Hydroides elegans* (n = 151) and from a smaller number of barnacles *Balanus amphitrite* (n = 22). Due to small sample size the removal stress for barnacles will not be discussed here. Adhesion strength of tubeworms appears generally to be higher than that of barnacles (Kavanagh et al., 2001; Holm et al., 2006). Mean removal stress for the tubeworms tested in Pearl Harbor was 0.094 MPa (SE = 0.008).

Monthly Fouling - PVC Panels Hawaii

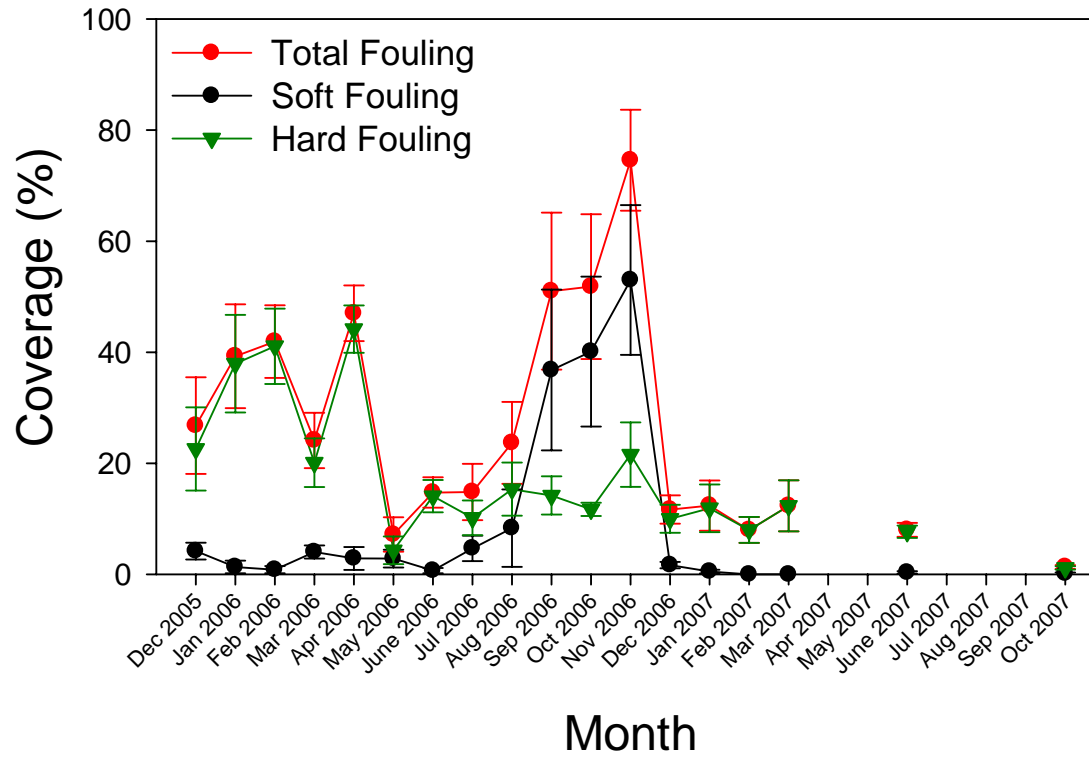


Figure 32. Pearl Harbor, HI. Recruitment of fouling to PVC panels exposed monthly. Error bars are standard errors.

Monthly Fouling - PVC Panels Hawaii Focal Species

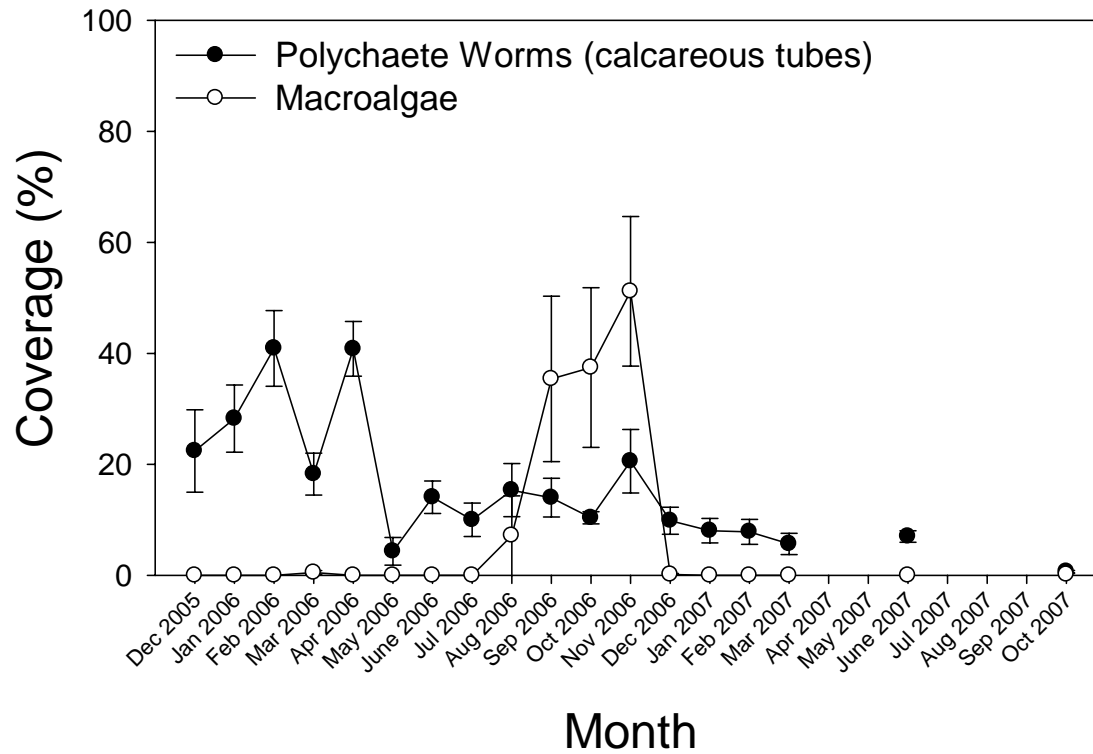


Figure 33. Pearl Harbor, HI. Recruitment of particular fouling functional groups to PVC panels exposed monthly. Error bars are standard errors.

Accumulation of Fouling Control Anticorrosive Coating Hawaii

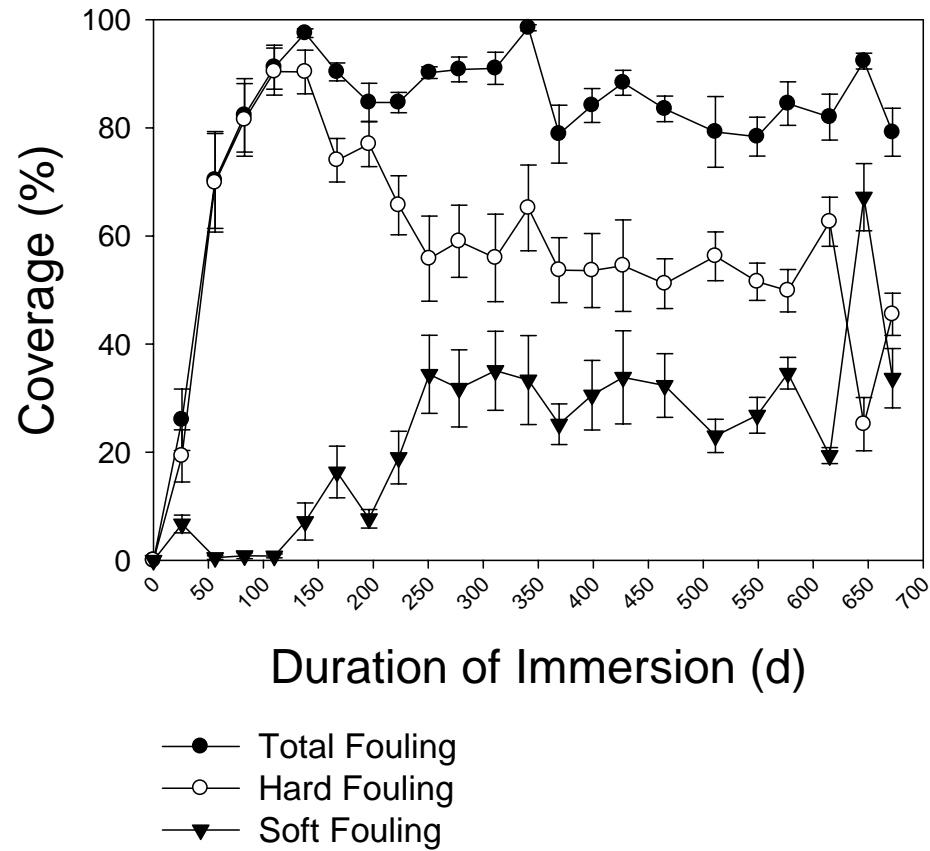


Figure 34. Pearl Harbor, HI. Accumulation of fouling to the control anticorrosive coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species Control Anticorrosive Coating Hawaii

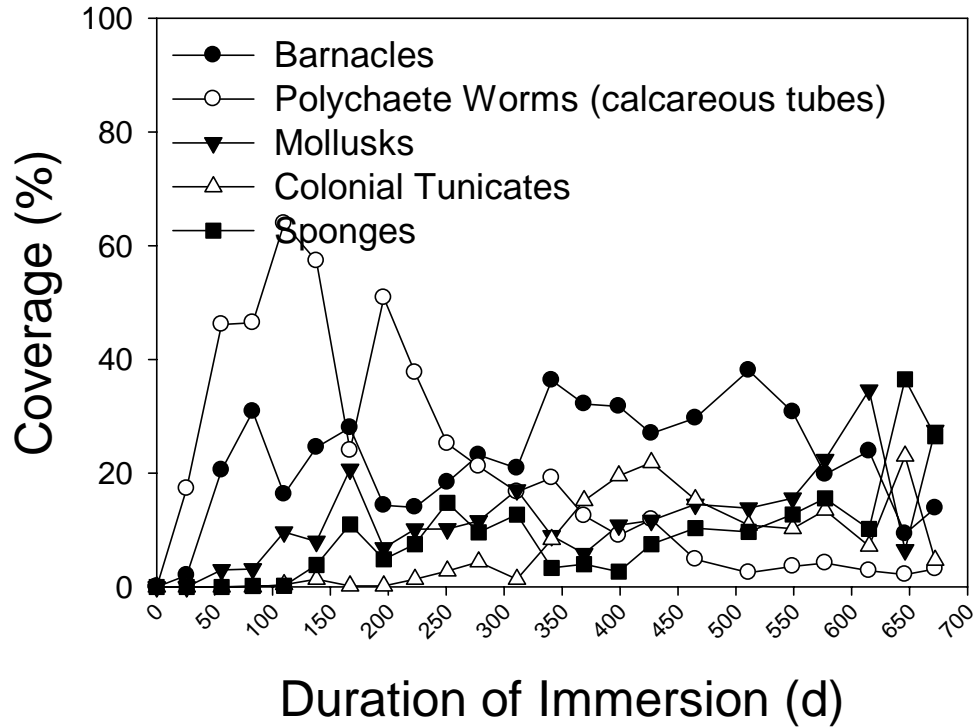


Figure 35. Pearl Harbor, HI. Accumulation of particular fouling functional groups to the control anticorrosive coating. Error bars omitted for clarity.

Accumulation of Fouling Fouling-release Coating (Intersleek) Hawaii

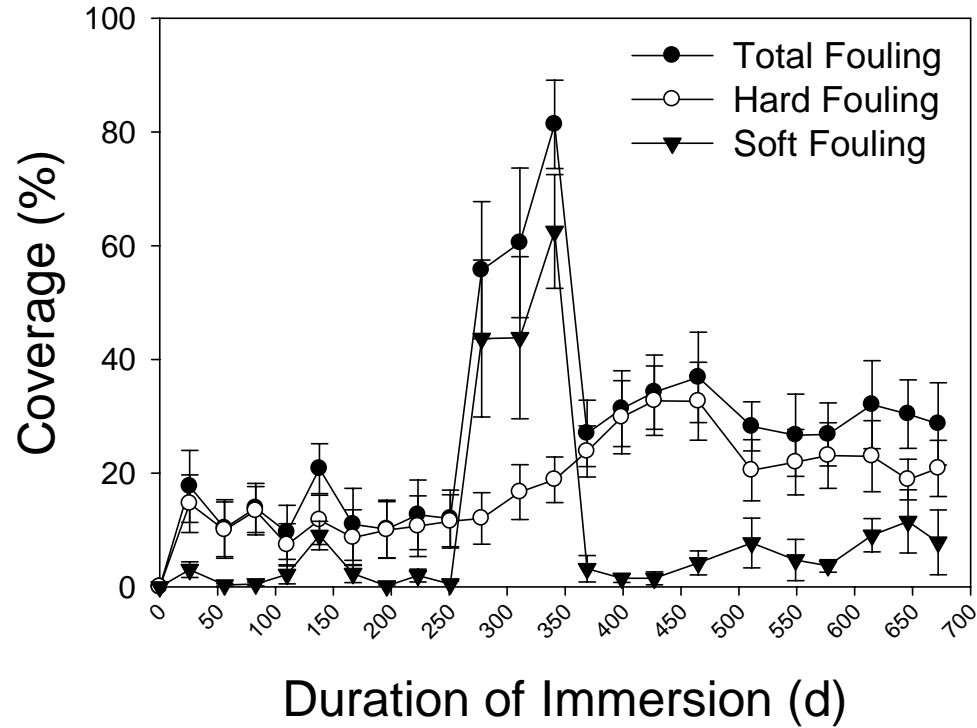


Figure 36. Pearl Harbor, HI. Accumulation of fouling to the fouling-release coating. Error bars are standard errors.

Accumulation of Fouling - Focal Species
 Fouling-release Coating (Intersleek)
 Hawaii

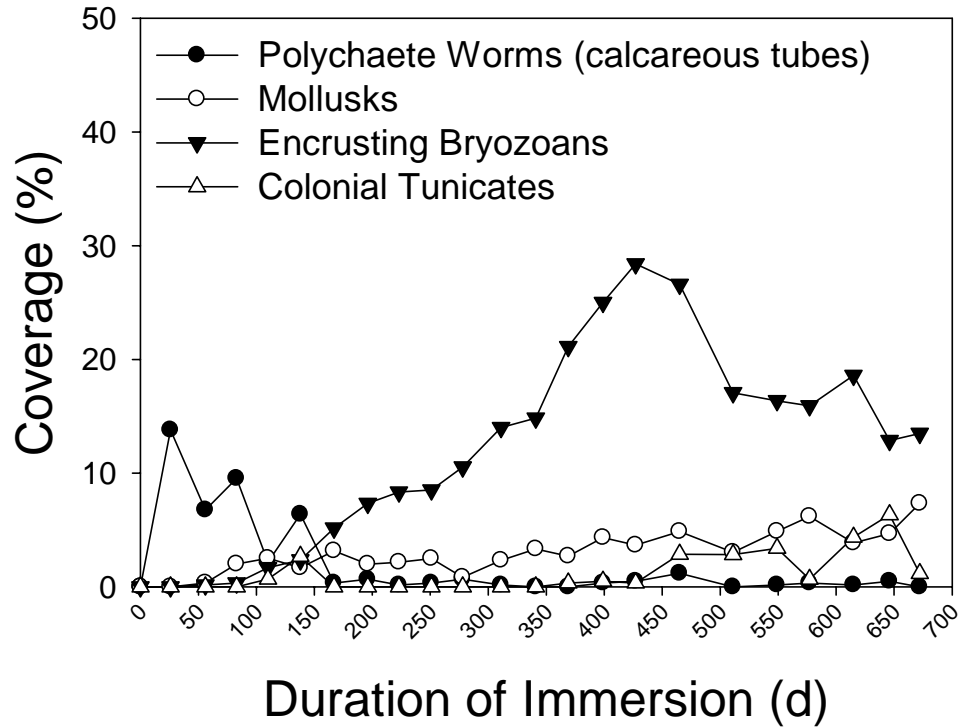


Figure 37. Pearl Harbor, HI. Accumulation of particular fouling functional groups to the fouling-release coating. Error bars omitted for clarity.

Accumulation of Fouling Antifouling Coating (BRA 640) Hawaii

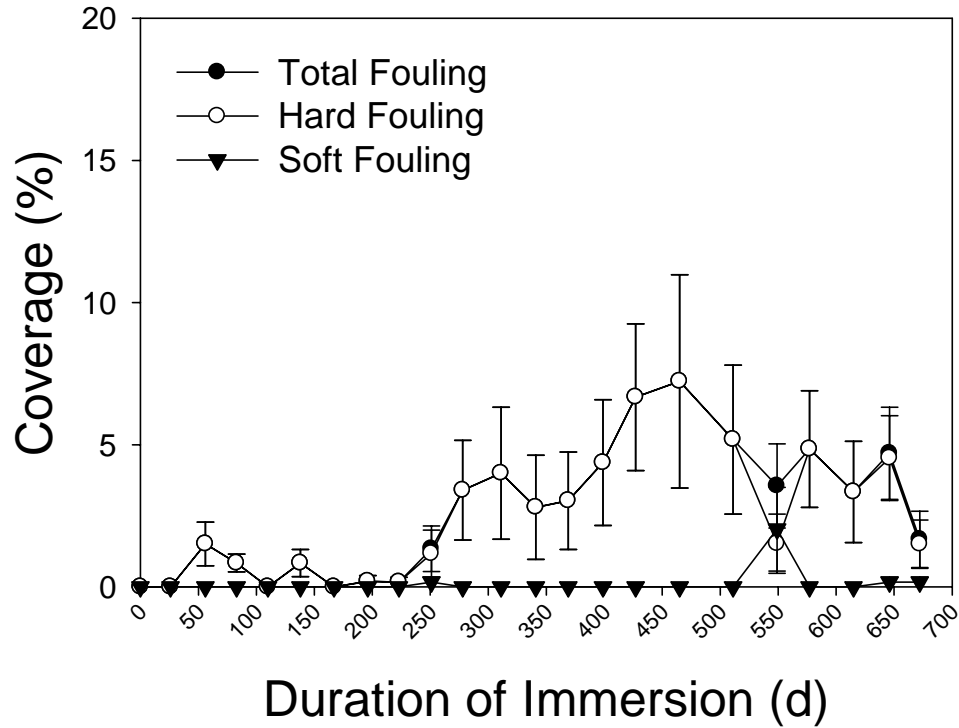


Figure 38. Pearl Harbor, HI. Accumulation of fouling to the antifouling coating. Error bars are standard errors.

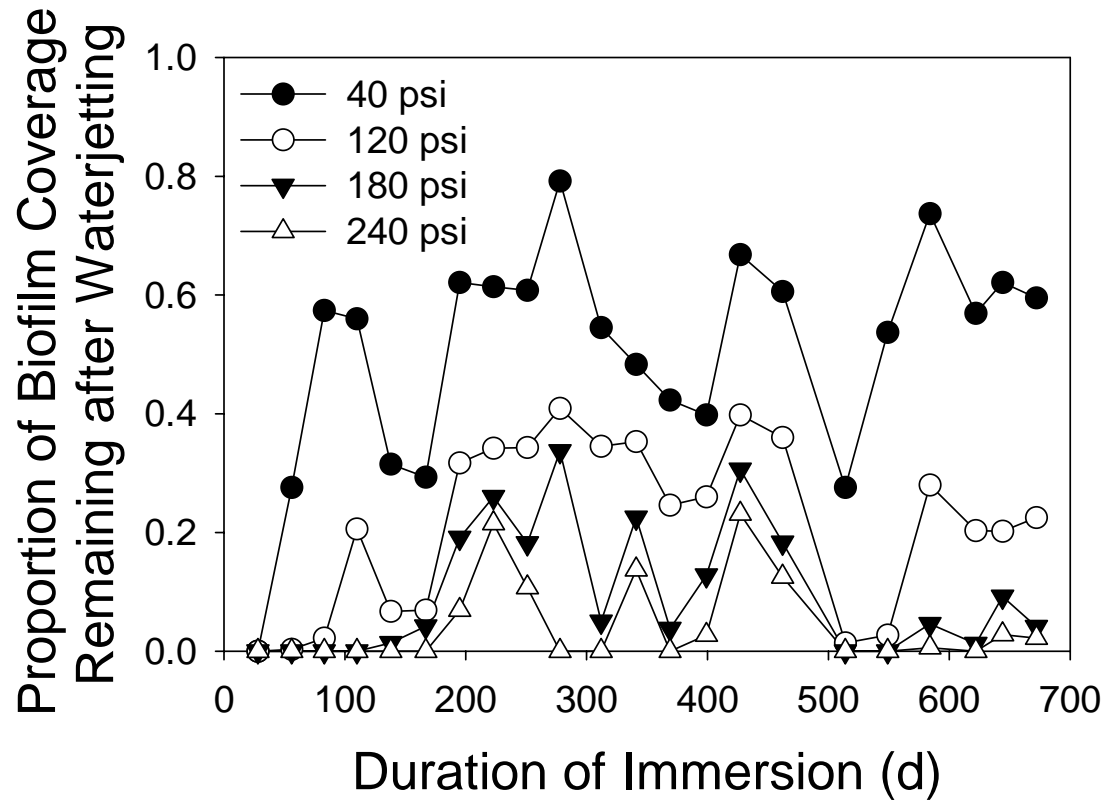


Figure 39. Pearl Harbor, HI. Adhesion of biofilm to the fouling-release coating. Points represent the mean proportion of biofilm coverage remaining after application of the waterjet. Error bars omitted for clarity.

Discussion

The approach taken in testing novel strategies for controlling fouling will depend on the means by which that strategy generates its effects on target organisms (for example, antifouling vs. nonfouling vs. fouling-release strategies). Designing and executing different types of tests, and interpreting the resulting data, requires different types of baseline information. Rapid field testing of novel antifouling formulations, for example, may demand an understanding of short-term temporal and spatial variation in the settlement of fouling organisms at the site, in order to insure the appropriate level of challenge. Long-duration testing or qualification of a well-developed coating system may not be affected by these considerations, but instead by considerations of time to development of a mature fouling community and (if the mode of action of the coating is of interest) the identity of the organisms making up that community. Finally, the results derived from testing carried out at a single site must be evaluated in light of the possibility that performance may be influenced by the local environmental conditions or be a function of the particular species making up the fouling community.

The sites characterized in this research present different challenges to coatings under test, and thus different testing opportunities to the investigator. Environmental conditions (salinity, temperature) varied across the sites and, temporally, within the sites on both short and long time scales. Each site also possessed a characteristic suite of macrofouling organisms that settled with varying intensity depending on the time of year. All sites were able to collect adhesion data from barnacles (with different barnacle species available at each site). However, the fouling communities at each site also included other hard fouling species that present various mechanisms of adhesion that may yield different patterns of adhesion strength across experimental surfaces.

Accumulation of Fouling

The functional groups common to the mature fouling communities developing on the control anticorrosive coating differed across the test sites. The most abundant organisms in the fouling community at the Indian River lagoon, FL, site were barnacles, bivalve mollusks, arborescent bryozoans, and sponges. Depending on the time of year, barnacles were typically the dominant foulers. The community at Morro Bay, CA, was ultimately dominated by encrusting bryozoans, although initially fouling consisted of a mixture of both encrusting and arborescent bryozoans, colonial tunicates, and macroalgae. In contrast to these two locations, the fouling communities in Singapore and Pearl Harbor, HI, were extremely diverse and possessed no clear dominant species in the long term. In Pearl Harbor polychaete worms (tubeworms) settled initially to high levels of coverage but by the end of the experiment the community consisted of sponges, bivalve mollusks, and barnacles with low levels of coverage of tubeworms and colonial tunicates. In Singapore, tubeworms also settled abundantly during the first several months of testing, but as in Hawaii did not maintain their initial high level of coverage. Instead, the apparent mature community included substantial coverage of barnacles, mollusks, and sponges, in addition to tubeworms.

Despite these differences in community structure, and in the time at which the test panels were immersed (November – December 2005 for FL, HI, and Singapore sites, June 2006 for CA site), the level of coverage on the control anticorrosive panels remained relatively stable after 60 d to 80 d of immersion. The fouling communities at each site continued to change in

composition after that time, but the total coverage of fouling remained between 70% to 100% until the end of the experiment.

Rate of accumulation of fouling, and the community that developed, was considerably different for the fouling-release coating. For all the sites total coverage of fouling on this material fluctuated greatly over time. Coverage was typically highest at the Florida and Singapore sites, ranging between 10% and 80%. The resident fouling community in the Indian River lagoon appeared to alternate between periods dominated by barnacles or sponges, while the community in Singapore included five functional groups (barnacles, colonial tunicates, bivalve mollusks, tubeworms, sponges) occurring at roughly similar levels of coverage. In Morro Bay, CA, coverage of fouling on the Intersleek panels was generally low (less than 20%) and consisted mainly of barnacles. At the Hawaii site fouling of the Intersleek panels increased slowly over time except for a brief period of high coverage (approximately 80%) corresponding to abundant attachment of macroalgae. The steady increase in coverage in the background was a function of the slow accumulation of fouling by encrusting bryozoans and bivalve mollusks.

Coverage of fouling on the BRA 640 antifouling coating was always less than 20% (and generally less than 10%) at all the sites save Morro Bay, CA, where after approximately 350 d of immersion heavy accumulations of unidentifiable soft forms and incipient fouling developed. Substantial levels of hard fouling of this coating were only observed at the Pearl Harbor, HI site.

The low levels of coverage observed for the antifouling coating relative to the control anticorrosive and fouling-release coatings can presumably be attributed to the presence of copper biocide in the paint. The results suggest that the fouling communities at all sites are sensitive to some extent to this biocide. Presuming similar sensitivity to other antifouling biocides, the rates of accumulation observed indicate that suitable tests for efficacy of an antifouling coating (or perhaps a nonfouling material), relative to an inert control coating, can be carried out potentially in as little as 60 d to 80 d of immersion. Distinguishing among highly effective vs. moderately effective antifouling compositions may require longer periods of time to allow for further settlement and development of the encrusting community.

The differences observed in fouling community structure and dynamics between the control anticorrosive and fouling-release coatings are more difficult to explain. Variation in community structure may be related to differences in the response of settling larvae to the surfaces of these paints, as well as in patterns of post-settlement mortality. Spores of fouling macroalgae, and larvae of fouling invertebrates, respond (and respond differentially) to surface characteristics such as wettability (for example, Roberts et al., 1990; Gerhart et al., 1992; Callow et al., 2000), which varies between these two coatings. Differences between coatings in post-settlement mortality may also be important. Swain et al. (1998) noted the importance of predation in generating differences in coverage and adhesion strength among various substrata. Depending on the identity of the dominant fouling species, seasonal sloughing can be an important driver of the dynamics of fouling communities (for example, Sutherland and Karlson, 1977). Thus, differences in adhesion strength of fouling between the anticorrosive and fouling-release coatings may influence community structure by affecting the likelihood of removal of organisms by predation or disturbance due to physical forces.

Some types of evaluations or experiments demand only very short periods of immersion. These might include studies of minimum effective release rate or control of release rate of a biocide (for example, Haslbeck et al., 1996), initial tests of a novel biocide, or initial tests of

novel nonfouling or fouling-release materials. In these cases it is important to match the period of immersion of the experimental materials to times when settlement of spores and larvae is likely to be high; that is, when an appropriate biological challenge is present. Monthly records of recruitment of fouling at each site provide the information necessary to plan such tests.

Recruitment of fouling was temporally variable at all of the field sites. At the Singapore site, this variation may not be large enough to affect scheduling of a short-term immersion test. Over approximately two years of observation total coverage on the PVC panels was always over 20%. The focal species making up this coverage, however, did vary considerably, with settlement of barnacles being very low in the months from November to May. Tubeworm larvae appeared to be reasonably abundant year round. In the Indian River lagoon, FL, recruitment of fouling exhibited a strong seasonal pattern, with lower levels of fouling accumulation from November to April. Barnacles at this site settled abundantly (to a mean coverage of approximately 80%) from June to October. In Hawaii, in contrast, the level of fouling on newly-immersed panels was largely driven by the availability of tubeworm larvae, which appeared to be most abundant from December to April, although some (> 10% coverage) settlement of fouling organisms occurred year-round. This site experienced very low levels of recruitment during 2007. One must keep in mind that all the sites may experience *inter*-annual variation in accumulation of fouling that can obscure the *intra*-annual variation described above. We cannot predict when such long-term failures in recruitment might occur. Short-term temporal variation in fouling was particularly strong at the Morro Bay, CA site. No seasonal pattern in settlement of particular functional groups was apparent. Determining appropriate timing of short-term evaluations for this site awaits accumulation of more data or experience.

Table 5 provides a summary of the characteristics of the fouling community at each site.

Adhesion of Fouling

Adhesion of Biofilms

Except for the Morro Bay site, biofilm adhesion followed a pattern of initially being poor, but increasing substantially over the first 100 d of immersion, and varying strongly in time thereafter. At the Indian River Lagoon site, biofilms became largely impossible to remove with the waterjet after 347 d of immersion, but this was not the case at the other sites, where biofilm adhesion continued to vary, and often returned to levels comparable to the initial stages of the experiment. The patterns in adhesion observed could result from variation in the (biological) community structure or physical composition of the biofilm over time, changes in the surface characteristics of the fouling-release coating (for example, Meyer et al., 1988; Nevell et al., 1996), or changes in the nature of the interaction between biofilm components and the coating surface. The waterjet assay cannot distinguish among these possibilities. To some extent this compromises the utility of the assay, as an investigator will not be able to determine if differences in the adhesion strength of biofilms on different materials are due to the characteristics of the materials or the characteristics of the biofilms attached to them. Laboratory assays utilizing single species of bacteria or diatoms (for example, Cassé et al., 2007; Stafslie et al., 2007a; Stafslie et al., 2007b) may be more useful in determining whether experimental fouling-release materials have differential effects on the adhesion of biofilms. Alternatively, the waterjet assay could be coupled with characterization (for example, Shikuma and Hadfield, 2005) of the nature of the biofilm community at the time the assay is conducted.

If the experimenter chooses, however, to take a more holistic view of the assay, wherein 'performance' is a function not just of the adhesion of particular species of microfouling organisms but also of the sources of variation noted above, then the waterjet test may have some utility. In this case, in order to obtain the best results possible, the materials developer and test site should work together to design the necessary experiments, focusing in particular on the type of assay data to be collected (for example, maximum pressure for cleaning vs. biofilm removed at different pressures) and how those data address the questions at hand, and the frequency of calibration of the waterjet device (to minimize experimental error).

Adhesion of Hard Fouling Organisms

All the sites support one to several hard fouling organisms suitable for testing using the ASTM assay or a modification of the assay, including barnacles, oysters, and tubeworms. Previous research (for example, Kavanagh et al., 2001; Holm et al., 2006) suggests that the magnitude of adhesion strength on the same fouling release materials differs among these organisms (typically tubeworms > oysters > barnacles), and also that the individual hard foulers rank fouling-release materials differently. Thus, tests for release of hard fouling that focus only on, for example, barnacles, may fail to identify materials that are effective in reducing the adhesion strength of tubeworms or oysters. As prospective fouling-release coatings will need to be effective worldwide, materials developers should expose their coatings to the broad range of adhesion strategies and adhesive strength patterns expressed by the organisms available at the field sites. If rapid turnaround is desired, experimenters should note the seasonality (if any, see above) in occurrence of the organisms of interest, and plan their tests accordingly.

Table 5 provides a summary of the primary species available for adhesion testing at each site, and the periods of time when they are most available.

Table 5. Summary of characteristics of the fouling community and organisms available for testing of hard fouling adhesion, for each exposure site. See text for additional details.

Site	Dominant Fouling Organism (Long Term)	Dominant Fouling Organism (Short Term)	Ideal Period for Short-term tests	Primary Organism for Hard Fouling Adhesion Test	Notes
Morro Bay, CA	Encrusting bryozoan	None	To be determined (spring - fall)	Barnacle	
Indian River lagoon, FL	Barnacle, Mollusk	Barnacle	June - October (depending on objective)	Barnacle	Intense barnacle fouling during summer months. Have also carried out adhesion tests on oysters, tubeworms.
Singapore	Barnacle, diverse community	Tubeworm, barnacle	March - November (settlement occurs year-round)	Barnacle	
Pearl Harbor, HI	None (diverse community)	Tubeworm	December - April (? , settlement occurs year-round)	Tubeworm	Have also carried out adhesion tests on oysters, barnacles

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Appendix I. Console buttons for use with the PhotoGrid software.

Filename = ButtonsIntersite2.txt

```

11
3,"Coating Defects"
1,"Tunicate"
1,"Mollusc"
1,"Tubeworm"
1,"Barnacle"
1,"Bryozoan"
1,"Cnidaria"
1,"Sponge"
1,"Algae"
1,"Misc"
1,"Bare"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" Chp,,,", "Chipping"
" Crk,,,", "Cracking"
" Ero,,,", "Erosion"
" Flk,,,", "Flaking"
" Pel,,,", "Peeling"
" Rst,,,", "Rusting"
" ,,,", ""
" ,,,", ""
" All,,,", "Allgtr"
" Bcut,,,", "BioCut"
" Bpen,,,", "BioPen"
" Bli,,,", "Blistering"
" Chk,,,", "Chalking"
" Chc,,,", "Checking"
" ,,,", ""
" ,,,", ""
" stun,soft,,", "Solitary"
" ctun,soft,,", "Colonial"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" Mol,hard,,", "Molluscs"
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" ,,,", ""
" Pcal,hard,,", "Clcareous"
" Psed,soft,,", "Sedmntry"
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" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" Barn,hard,,", "Barnacle"
" ,,,", ""
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" ,,,", ""
" ,,,", ""
" ,,,", ""
" EB,hard,,", "Encrsting"
" Br,soft,,", "Arbrscent"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" Cn,soft,,", "Cnidaria"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" Sp,soft,,", "Sponges"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" MAG,soft,,", "GrnAlgae"
" MAR,soft,,", "RedAlgae"
" MAB,soft,,", "BrnAlgae"
" MAU,soft,,", "UnkAlgae"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" Sl,,,,", "Slime"
" Si,,,,", "Silt"
" IF,,,,", "IncFoul"
" Uhard,hard,,", "UnkHard"
" Usoft,soft,,", "UnkSoft"
" ,,,", ""

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" ,,,", ""
" ,,,", ""
" Bare,,,", "Bare"
" NoSubs,,,", "NoSbstrte"
" Ctie,,,", "CableTie"
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
" ,,,", ""
"end list"
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"end list"
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"end list"
"end list"
"end list"
"end list"
"end list"
"end list"
```

Appendix II. Detailed procedures for using PhotoGrid software.

Using PhotoGrid software to determine coverage of fouling for panels

Some tips before starting:

- DO read the help files for the PhotoGrid software. There isn't much in these files, but they do contain some tricks that you will want to know before getting started, and they will help you familiarize yourself with the software.
- DO NOT try to change your preferences while you have a picture opened in the program. This will cause the program to crash.
- DO put all your picture files from one inspection date into a single directory. This will allow you to analyze them all in one session, while minimizing the amount of time you have to move back and forth among directories.
- DO name your picture files something indicating the coating type, panel face (back or front), and inspection date. These file names will end up automatically in the comma-delimited Excel file that will ultimately hold your species counts. If you name the picture files something descriptive, it will save everyone a lot of time.
- DO NOT put any spaces in the file name you choose for the pictures.
- DO crop your images such that the picture seen in the PhotoGrid software does not include the area ½" in from each edge of the panel. The ASTM methodology indicates that fouling occurring within ½" of any edge of the panel should not be counted. PhotoGrid will lay points everywhere within the image. If you crop each panel image such that it represents the interior of the panel **without** the ½" border, you can operate PhotoGrid without having to be concerned about the location of points.

Measuring Coverage

1. Place the **ButtonsIntersite2.txt** file in the 'Species Buttons' folder in the PhotoGrid directory.
2. Open PhotoGrid software.
3. Open the Preferences window from the Options pulldown menu in the menu bar at the top of the program window. For the 'Button Path & File' click the button to the right of the entry box, and choose the appropriate buttons file (ButtonsIntersite2.txt) from the list of files. You can also choose to dump the data files into any directory you wish (I have been using the default directory). In the 'When Exporting Data...' box, click on 'Append CSV file' and check the box next to 'Create One CSV File for all Pictures in Folder.' In the 'Exported CSV File Format' box, click on 'Generic Format.' For the Number Font Size you can enter whatever number works best for you. Realize, however, that larger

numbers will obscure your view of the panel. A size of 20-30 works well (30 is the default). In the box for 'Point Intercept Number Generation', choose 'Stratified Random.' For the colors, feel free to choose whatever colors make it easiest for you to see the cross hairs and numbers. Note that the doubled boxes for crosshairs and circles apparently allows you to mix colors. To change the color, just click on the box with the color. A bright lime green color was very visible on panels supporting a complex fouling community. Apply all your changes, then close the window.

4. From the Options pulldown menu, choose 'Sampling Mode' and make sure that 'Point Intercept' is checked.
5. Open the image to be quantified. You can do this using either the File drop down menu, or by hitting the 'Open' button in the lower left hand corner. Choose the image file you wish to open. Because of the way the automatic file opening procedures work (see below) you will want to choose the first image file in the directory. Once the image is open, you can adjust its brightness using the slider at the bottom. On some computers it may not be possible to adjust contrast.
6. In the box labelled '# Rand Pts' enter 100. Then click on the button labelled 'MakeRandPts'. This will put the desired number of points on the picture.
7. Locate the first point to be scored. It usually can be found on the left side of the picture, towards the top. Highlight the row on the the lefthand scoreboard corresponding to the first point, by clicking on the button labelled '1', or by clicking on the row itself. If you have zoomed in on the photo the frame will shift to the location of the point to be scored. The color of the number will change to red to indicate that you are now scoring that point. Click on the button at the bottom that corresponds to what lies beneath the point (bare space, slime, algae, damage, etc.). The number corresponding to the point you have just scored will change to black (to indicate the point has been scored) and the scoreboard and picture will automatically advance to the next point.
8. After you have scored the desired number of points the program will give you a message saying you have finished ('Pau' in Hawaiian). Hit the 'Export' button and the data will automatically be exported to a comma-delimited Excel file, that will be saved in the directory you designated in the preferences section.
9. After exporting the data, hit the 'Next Pic' button, and the next picture in the directory will be opened by the software.
10. Repeat steps 6 (Make Rand Pts) - 9.

Description of the Buttons

The project developed a buttons file that would incorporate all of the different types of data that we expected to collect, both biological and condition of the coating. Check Tables 3 and 4 for descriptions of these types of data. The buttons are grouped in several categories depending on what they are recording:

Bare:

Bare = free space

NoSubstrate = no substrate beneath point

CableTie = cable tie beneath point (rather than panel)

Misc:

Slime = slime films

Silt = silt accumulations

IncFoul = incipient fouling

UnkHard = unknown 'hard' fouler

UnkSoft = unknown 'soft' fouler

Algae:

GrnAlgae = green algae

RedAlgae = red algae

BrnAlgae = brown algae

UnkAlgae = unknown algae

Sponge:

Sponges = all sponges

Cnidaria:

Cnidaria = all cnidaria – hydrozoans, anemones, corals, etc.

Bryozoan:

Encrusting = encrusting bryozoans

Arbrscent = arborescent bryozoans

Barnacle:

Barnacle = all barnacles

Tubeworm:

Clcareous = polychaete tubeworms with calcareous (hard) tubes

Sedmnlry = polychaete tubeworms with sedimentary (soft) tubes

- Some sites may also have tubedwelling amphipods. A button can be added for that if the sites believe it to be necessary.

Mollusc:

Molluscs = all molluscs – bivalves and otherwise

Tunicate:

Solitary = solitary (not colonial) tunicates

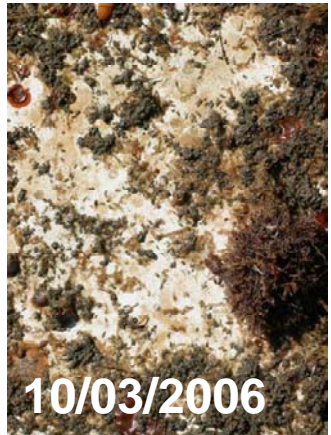
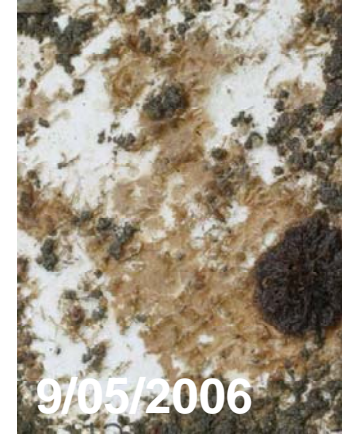
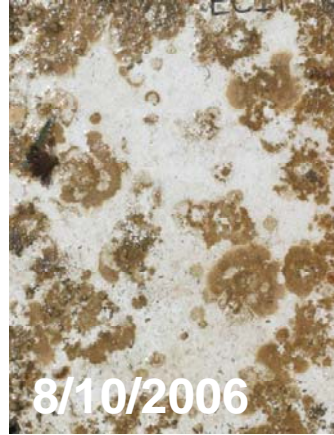
Colonial = colonial or compound tunicates

Coating Defects:

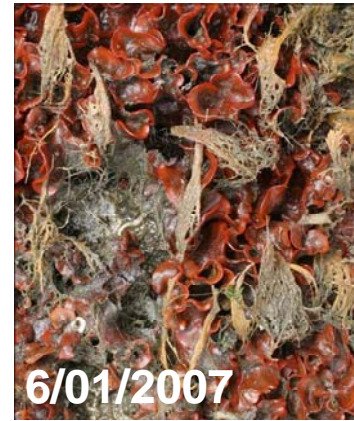
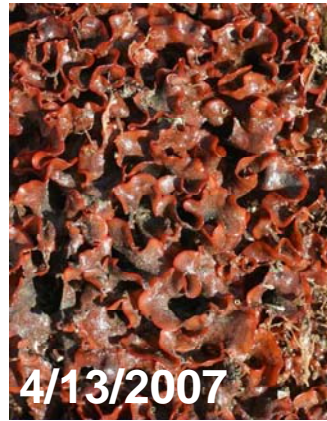
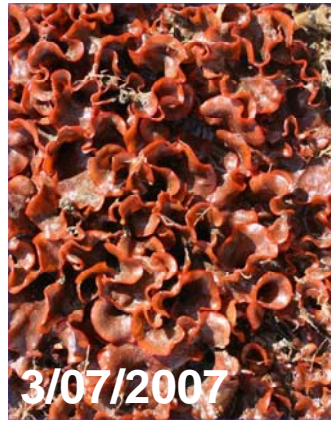
- The buttons (12) correspond to the descriptions listed in Table 4.

Appendix III. Fouling of coated panels immersed in Morro Bay, California.

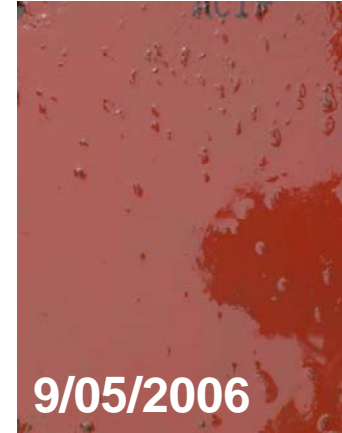
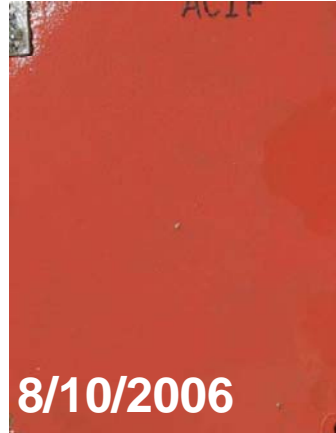
Anticorrosive Coating (control)



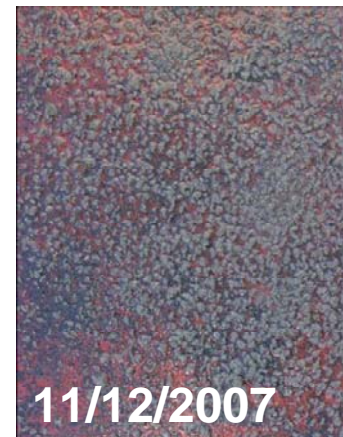
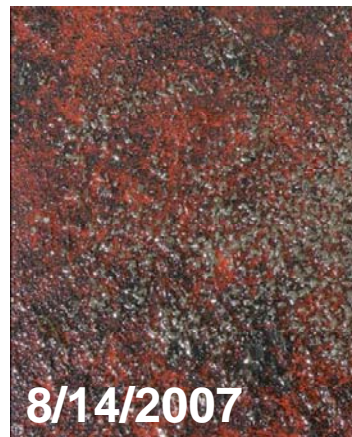
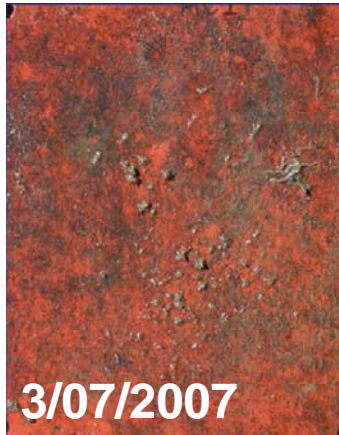
Anticorrosive Coating (continued)



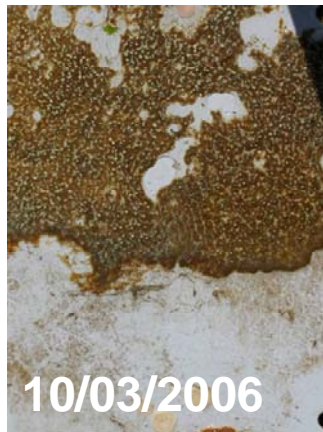
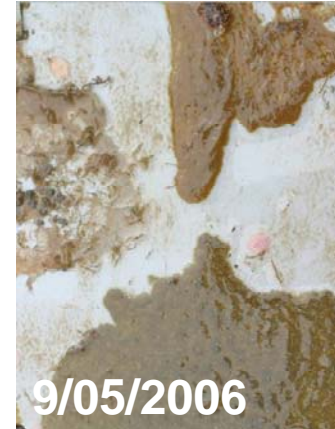
Antifouling Coating



Antifouling Coating (continued)



Fouling-release Coating



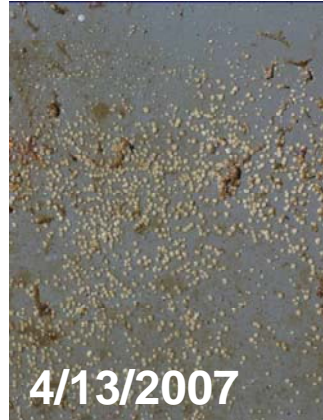
Fouling-release Coating (continued)



Monthly Settlement Panels (PVC)

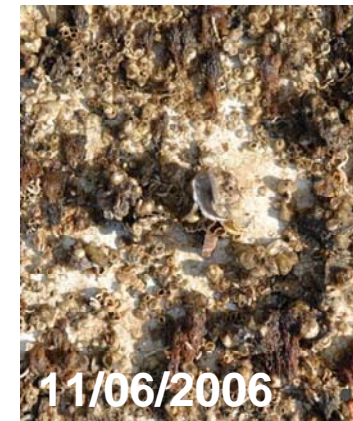
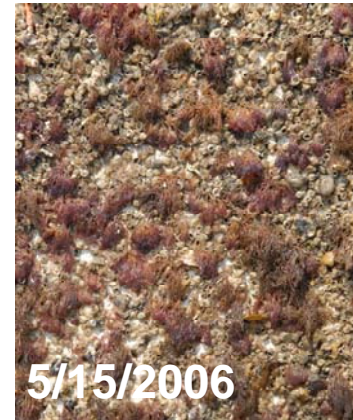


Monthly Settlement Panels (continued)



Appendix IV. Fouling of coated panels immersed in Indian River lagoon, Florida.

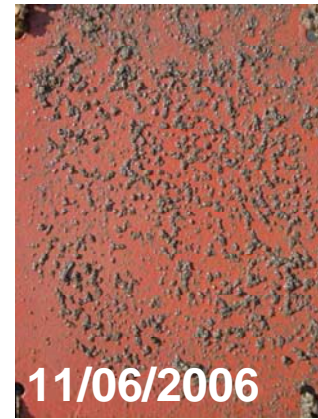
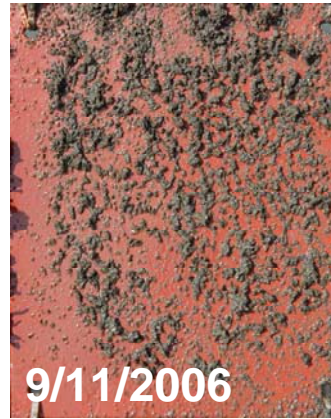
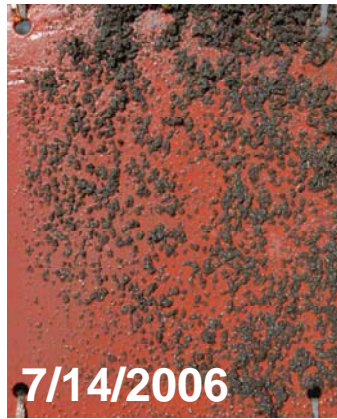
Anticorrosive Coating (control)



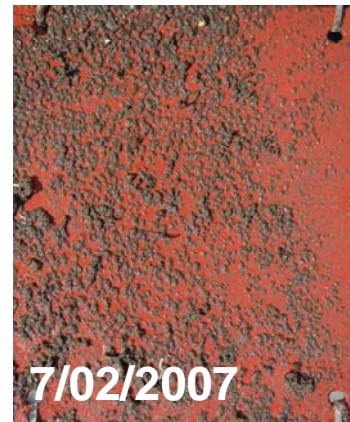
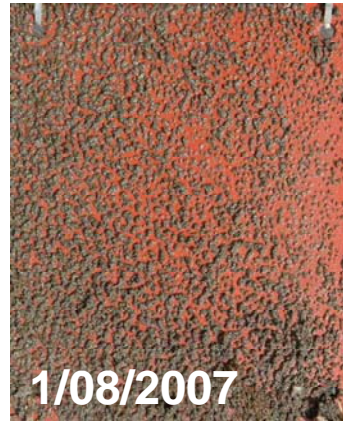
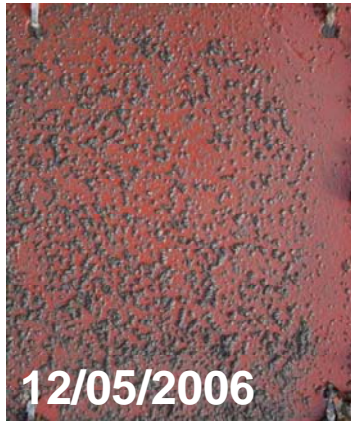
Anticorrosive Coating (continued)



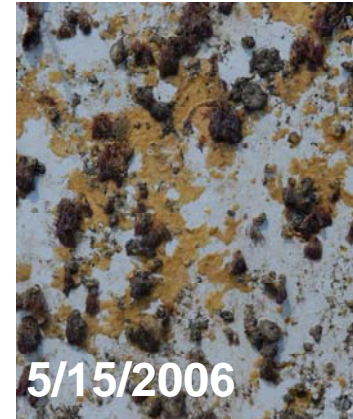
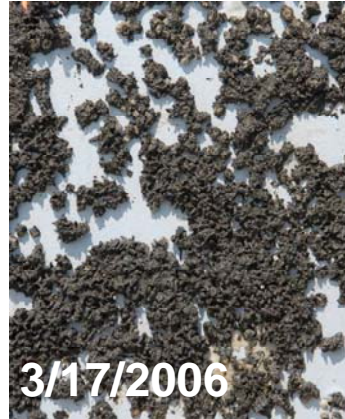
Antifouling Coating



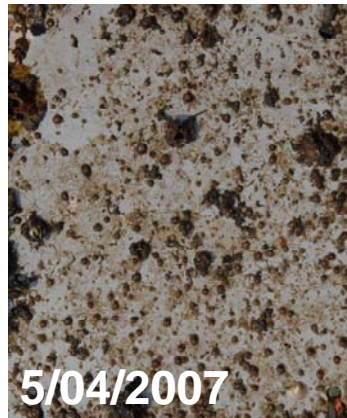
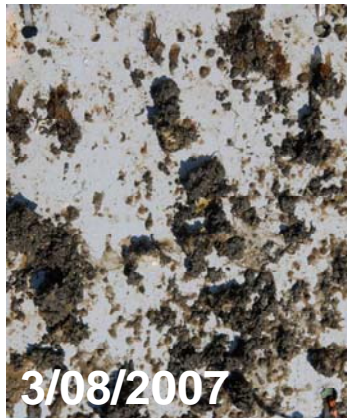
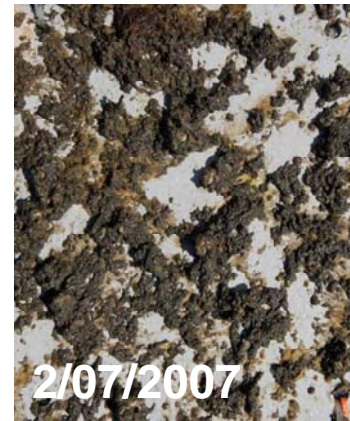
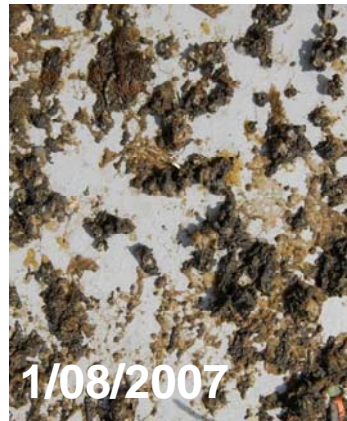
Antifouling Coating (continued)



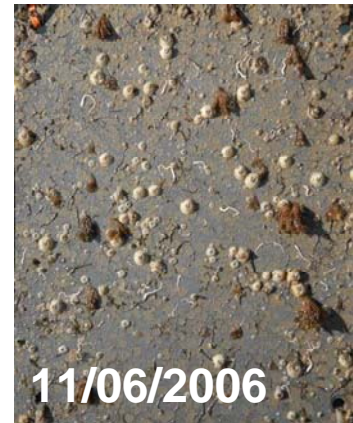
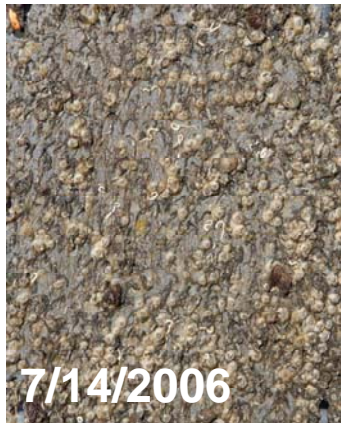
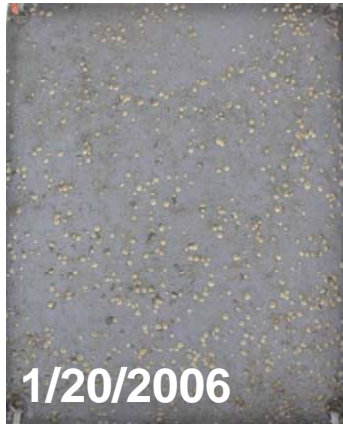
Fouling-release Coating



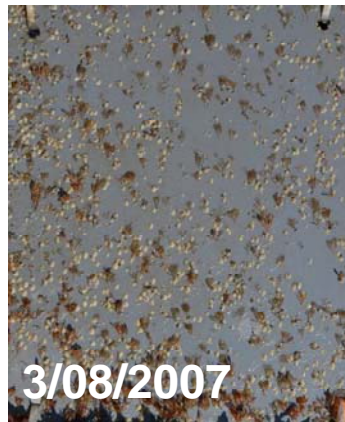
Fouling-release Coating (continued)



Monthly Settlement Panels (PVC)

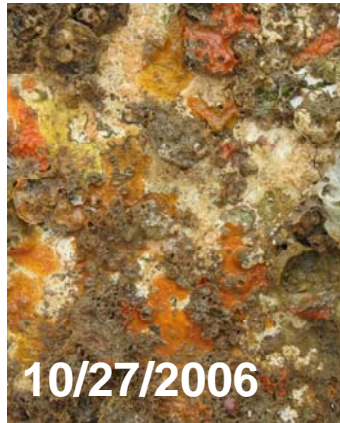
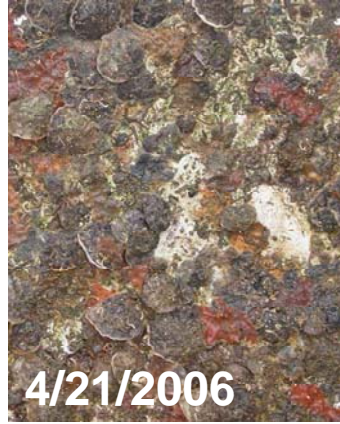


Monthly Settlement Panels (continued)



Appendix V. Fouling of coated panels immersed in Singapore.

Anticorrosive Coating (control)



Anticorrosive Coating (continued)



Antifouling Coating



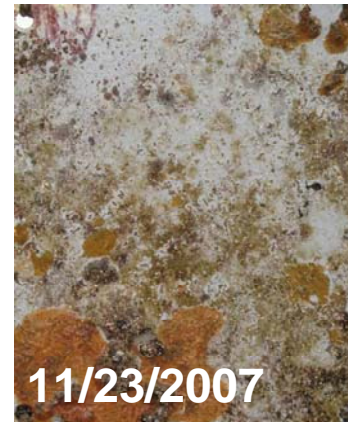
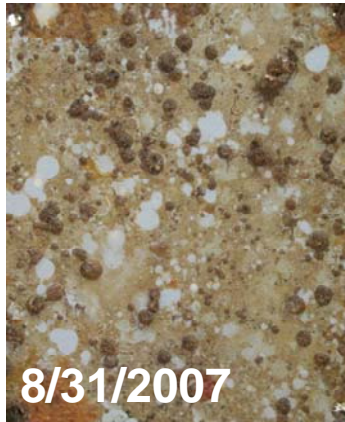
Antifouling Coating (continued)



Fouling-release Coating



Fouling-release Coating (continued)



Monthly Settlement Panels (PVC)

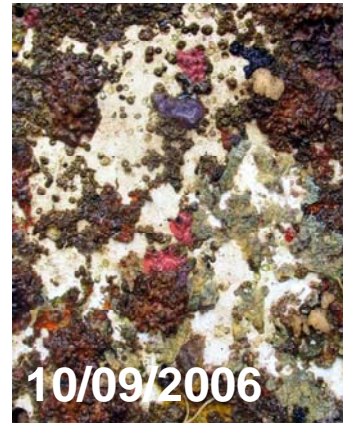
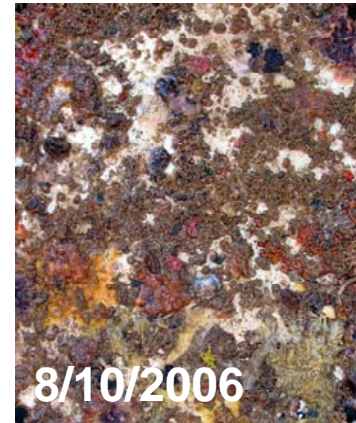
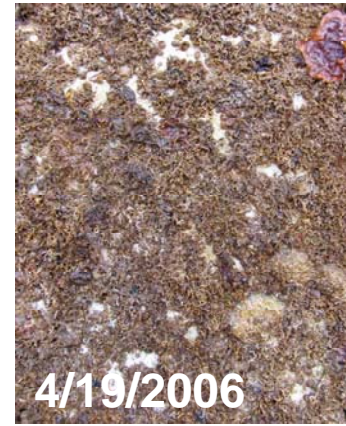
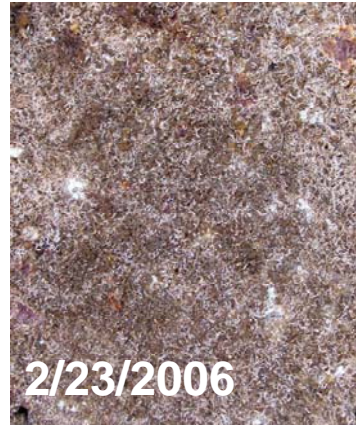


Monthly Settlement Panels (continued)

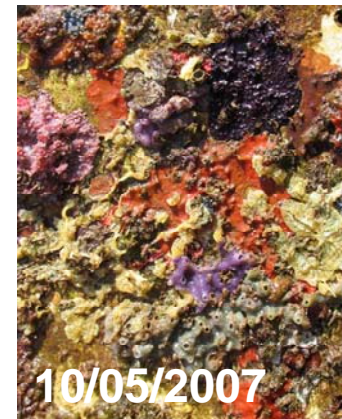
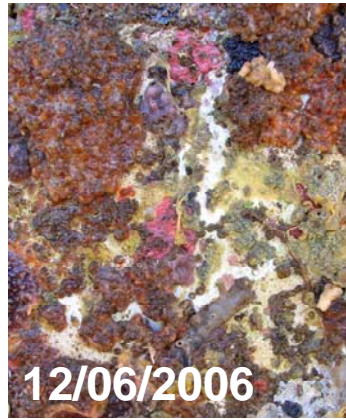


Appendix VI. Fouling of coated panels immersed in Pearl Harbor, Hawaii.

Anticorrosive Coating (control)



Anticorrosive Coating (continued)



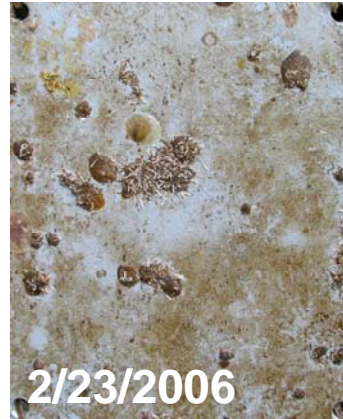
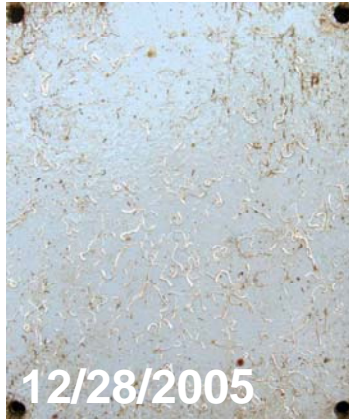
Antifouling Coating



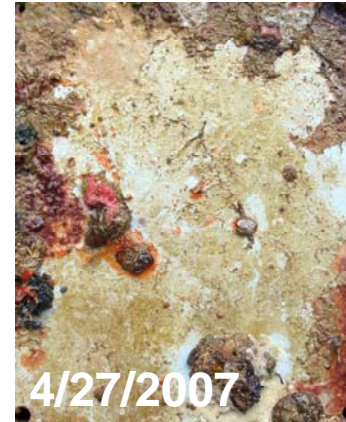
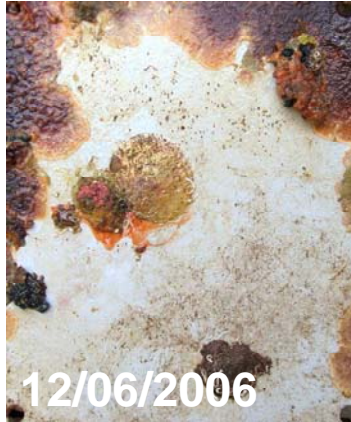
Antifouling Coating (continued)



Fouling-release Coating



Fouling-release Coating (continued)



Monthly Settlement Panels (PVC)



Monthly Settlement Panels (continued)



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