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14. ABSTRACT

The overall goal of our work was to use laboratory and field testing to expedite the down-select process used to develop nontoxic, foul-release coatings and to develop new methodologies to test the performance of foul-release coatings. We have met our deliverables on the project by; 1) assessing novel coatings in both the laboratory and field to contribute to the down-select process, 2) furthering the development of bryozoans as model organisms for testing coating performance in the lab and field, and 3) assessing seasonal fouling dynamics and seasonal variation in environmental parameters in Morro Bay, thereby contributing to the understanding of how fouling varies by season and geographic location.

15. SUBJECT TERMS

laboratory testing of foul-release coatings, field-testing foul-release coatings, environmental parameters and recruitment of fouling organisms, bryozoans as test organisms for foul-release coatings.

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Dean E. Wendt
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CAL POLY
SAN LUIS OBISPO
CORPORATION

April 3, 2015

Defense Technical Information Center
8725 John J. Kingman Road Suite 0944
Fort Belvoir, VA 22060-6218

RE: Award No. N00014-12-1-04074

Dear DTIC Representative,

On behalf of Dr. Dean Wendt, please accept the enclosed Final Technical Report and the Report Documentation Page (SF298) for the above referenced project. Any questions regarding this submission should be directed to me at the following address or telephone number:

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Sponsored Programs Department Bldg. 38 Rm. 102
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I would like to express our sincere appreciation for the opportunity to pursue the goals of this program as they relate to the students and faculty of Cal Poly University and our business and research community.

Best Regards,

Johannah Varland, Grant Analyst
Sponsored Programs

Enclosure

File: 47018

Contract Information

Contract Number	N000141210474
Title of Research	Comprehensive Assessment of Marine Coatings in the Laboratory and Field.
Principal Investigator	Dean E. Wendt
Organization	Cal Poly, San Luis Obispo

Technical Section

Broad Goal

The overall goal of our work is to expedite the down-select process used to develop nontoxic, foul-release coatings through laboratory assays and field testing. We continually work to improve current and develop new methodologies for assessing novel coatings.

Technical Objectives

Objective 1: Our main objective was to provide comprehensive biological assessment of experimental marine coatings through quantitative and qualitative evaluation of coating performance. We used laboratory-based assays, including biological assays of leachates from coatings, larval settlement/attachment assays, critical removal force evaluation, i.e., testing of foul-release efficacy, and challenging marine coatings in a cool-water, temperate marine environment at our static-immersion site in Morro Bay, California.

Objective 2: Our second objective was to continue to improve current and develop new methodologies for coating assessment. We also explored the possibility of developing bryozoans as a new test organism for assessing efficacy of foul-release coatings in the lab and in the field.

Objective 3: Our last objective was to continue to participate in the ongoing intersite calibration study and conduct an analysis to explore the relationship between environmental parameters and the recruitment of fouling species.

Technical Approach

Objective 1: Laboratory and Field Assessments of Coating Performance

We used several approaches to meet our broad objectives of 1) screening emerging coatings for their efficacy as foul-deterrent and foul-release surfaces, and 2) developing new testing methodologies for coating assessment. In general, the approaches we used include:

- Laboratory assessment of coatings including;
    - Biological assays of leachates from coatings
    - Larval settlement/attachment assays
    - Critical removal force evaluation on primary attached or reattached barnacles
- Field assessment of coatings in a cool-water, temperate environment, including:
    - Foul-resistance
    - Critical removal force and waterjet
    - Long-term tests of coating durability

Leachate testing

All coatings were soaked for 3 days in 100 mL of seawater prior to settlement assays. The leachate from coatings was used to conduct assays of survivorship with approximately 100 nauplii larvae of *Artemia* sp. (brine shrimp). The larvae were exposed to the coating leachate and their survival is monitored for 2 days. Survival of larvae in coating leachate was compared to leachate from a glass slide control.

Larval Settlement Assays and Fouling-Resistance

Following the leachate testing, approximately 20-50 barnacle larvae (*Balanus amphitrite)* were "drop assayed" on each of the replicate surfaces. The assays last approximately 48 h; although exact duration depended on the time it took 50% of the larvae to settle in the control conditions; uncoated glass slides and DOW Corning Silastic T2. Stopping the assays after approximately 50% of the larvae have settled in the control condition provided information on antifouling and inductive characteristics of the experimental coatings. At the end of the initial assay period, the numbers of individuals that successfully attached and metamorphosed were counted. Larvae that did not settle by the end of the 48 h period were observed for signs of abnormal behaviour to assess any compromise to normal physiological function. Foul-resistance was estimated by determining the percentage of individuals settled on each coating. Settlement of larvae was ignored on portions of the coupons with coating defects.

The settlement preference assay differed from the drop assay in that larvae were presented with a choice: the experimental coating or the polystyrene surface of the Petri dish in which the coating was submerged. The basic protocol consisted of adding approximately 50 barnacle larvae (*Balanus amphitrite)* in 400 µL of seawater to a Petri dish (100x15mm) containing an experimental or control slide. The duration of the experiment was determined as described above. At the conclusion of the assay, the number of swimming cypris larvae and the number of attached barnacles on the slide and Petri dish were enumerated. The percentage of individuals that settled on the slide and on the Petri dish were calculated. From these data a settlement preference ratio was calculated: the number of settled barnacles per available square millimeter of the experimental coating divided by the number of settled barnacles per available square millimeter of the Petri dish. A settlement preference ratio of 1 indicated no preference

for either surface, whereas a settlement preference ratio less than one indicated a coating that may deter settlement.

A submersion assay was used for coatings that were extremely hydrophilic or hydrophobic, and therefore the drop assay did not work well. Coatings were applied to the bottom of small glass Petri dishes. A standard volume of filtered seawater (depending on dish size) was added to each dish followed by the addition of 30-60 barnacle cypris larvae (*Balanus amphitrite*). The larvae were allowed to settle until the settlement on the controls was approximately 50% and the percentage of settled larvae was calculated and compared to that of the controls.

Laboratory Rearing of Juvenile Barnacles

Newly metamorphosed juvenile barnacles (*Balanus amphitrite)* were transferred on their respective coatings to growth chambers where they were fed the unicellular alga *Dunaliella tertiolecta* and the diatom *Skeletonema costatum* for two weeks, and then a mixture of *Dunaliella tertiolecta*, *Skeletonema costatum*, and naupliar larvae of *Artemia* sp. Juveniles were maintained in a constant temperature incubator at 25°C on a 12 h light/dark cycle for approximately 2-3 weeks, at which time they were transferred to a seawater tank at 25°C until they achieved a basal plate diameter of 3-5 mm, the minimum size necessary to conduct force gauge tests according to ASTM D 5618.

Critical Removal Stress for Barnacles in Shear

Procedures for critical removal stress followed ASTM D 5618 with the following modifications: 1) The force measuring device was operated by a motorized stand, thus insuring a constant application of force during dislodgement; and 2) dislodgement of barnacles from coatings was performed under water. The apparatus consisted of an IMADA ZP-11 digital force gauge mounted on an IMADA SV-5 motorized stand. The coupons were clamped into a custom-built Plexiglas chamber that allowed complete submersion of coupons during dislodgement tests. Juvenile barnacles were selected for testing based on healthy appearance, normal basal plate morphology and minimum size requirements. Only barnacles occurring at least 5 mm from the edges of the coupon were tested. Other barnacles in close proximity to the test subject were removed if they could potentially interfere with measurements. The basal plate of the barnacle was photographed through the coating using a Canon EOS-10D digital camera mounted on an Olympus SZX12 dissecting microscope and a custom-made jig. The photograph was transferred to a computer and the area was calculated using the digital analysis software ImageJ. After size measurements were taken the slide was clamped into the Plexiglas chamber. The force gauge mounted on the motorized stand was used to apply a shear force to the barnacle's base at a rate of approximately 4.5 N/s (1 lb./s) until the organism detached. Force was applied parallel to the coupon surface. The force required for detachment was noted and observations were made as to the mode of failure. If any portion of the basal plate was left attached to the substratum, the percentage remaining was calculated via digital image analysis. The critical removal stress (N/mm^2) was calculated by dividing the force (F) required to remove the test subject by the area of attachment (A). Results of CRS testing on coatings are shown in Figure 1.

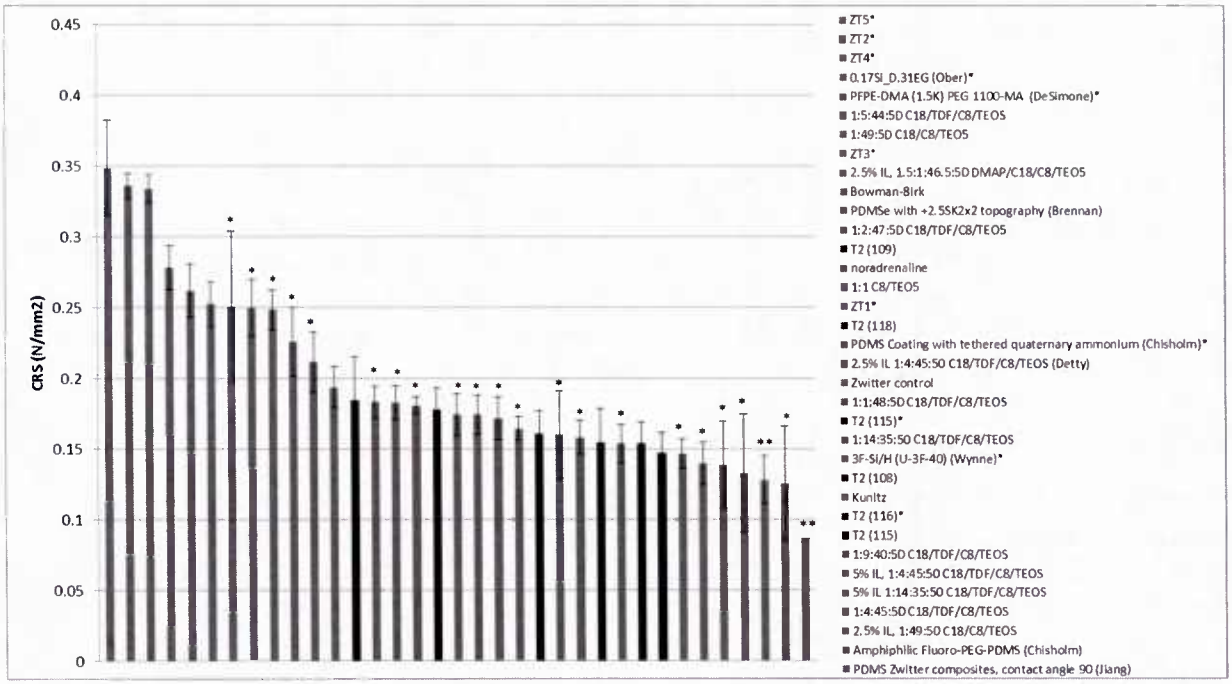

Figure 1. Critical Removal Stress (CRS) of barnacles on the best performing coating types from lab assays. Results presented are from 2008 – 2014. A single asterisk indicates coatings with CRS values not significantly different than their respective T2 controls (black bars). A double asterisk indicates those that were significantly lower than T2 controls. Error bars represent ± one standard error of the mean.

In addition to measuring critical removal stress (CRS) of barnacles reared on the test coatings, we also had the capabilities of measuring CRS of reattached barnacles. Barnacles were grown at the above conditions on Silastic T2 control coatings, removed from those coatings as described above, and then allowed to reattach to novel test coatings. Once removed from T2, barnacles were placed on the desired coating and covered with a fine nylon mesh to secure them in place. The coating (with barnacles held in place by the nylon mesh) were placed in a seawater tank and allowed to reattach for 2 weeks. After two weeks, the barnacles were then removed as described above to measure CRS (Rittschof *et al.* 2008). There were no significant differences in CRS between initially settled barnacles and reattached barnacles ($p = 0.89$). In total, we assessed nine coating formulations and 36 coupons using the barnacle reattachment methods within the ONR program.

Field Assessment

Panels sent to us were exposed in a rack system that was suspended below a static immersion dock. Panels were generally exposed between one to 12 months depending on season and the experiment being conducted. At regular intervals the panels were assayed for percent coverage, water-jet testing, and force gauge removal testing. Percent coverage was calculated by first taking a digital photograph of the panel and

then, using digital image analysis, determining the amount of the panel covered by fouling. Water-jet testing was done as outlined by Swain and Schultz (1996), using the water-jet apparatus described therein. The water-jet was trained on the panel and the entire area of the panel was sprayed, and the pressure was increased incrementally. The type and amount of fouling removed was recorded before testing and after each pressure was applied. Lastly, we used a handheld force gauge to remove barnacles in shear from the face of the panel according to ASTM 5618. The mean force necessary to remove barnacles was compared among coatings using a standard one-way ANOVA (Fig. 2).

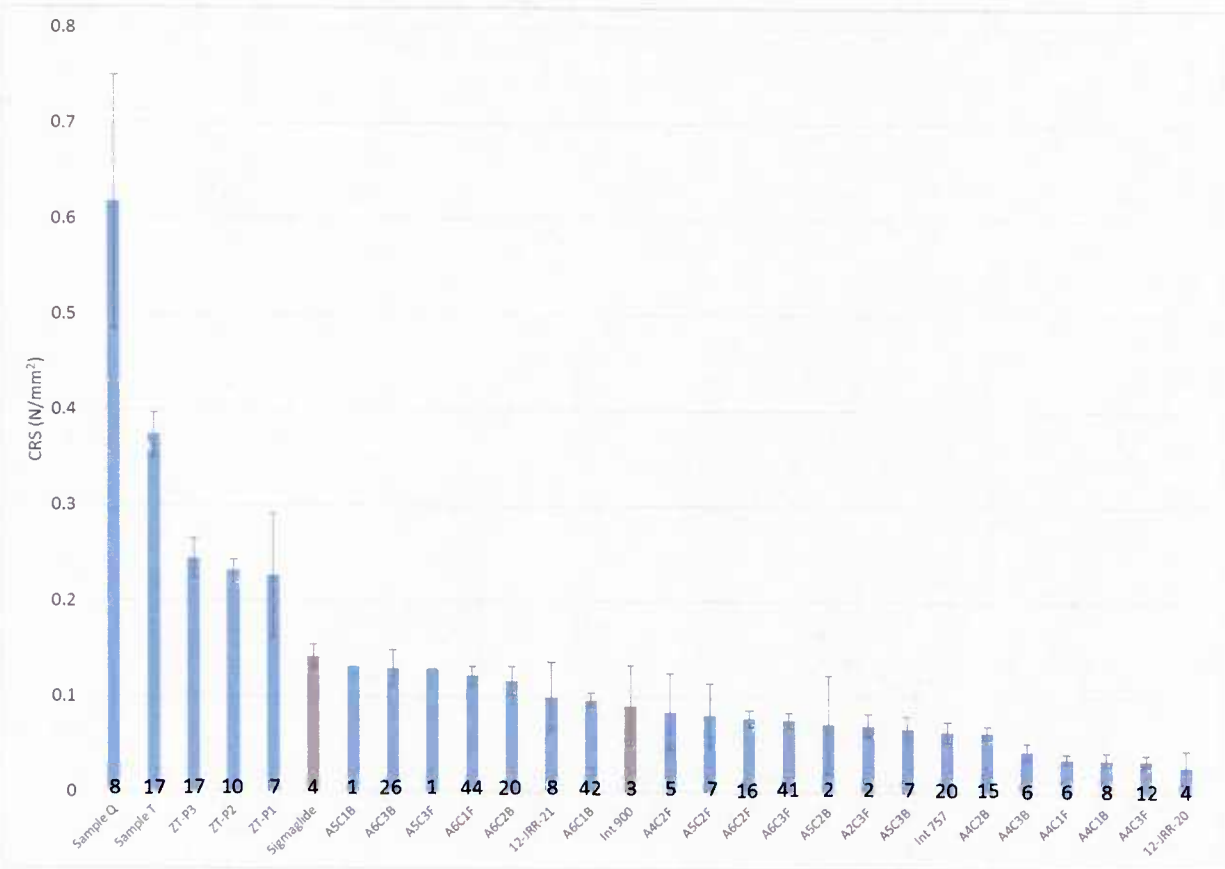

Figure 2. Critical Removal Stress (CRS) of barnacles on the best performing coating types from field tests. Results presented are from 2008 – 2014. Error bars represent ± one standard error of the mean.

Objective 2: Bryozoans as a new test organism

Measurement of encrusting bryozoan adhesion in the field

The method used for measuring encrusting bryozoan adhesion was adapted from those used on barnacles: ASTM D 5618-94, "Standard Test Method for Measurement of Barnacle Adhesion Strength in Shear" (Anonymous, 1997). A shear force was applied to the circular colony of bryozoans using a hand held force gauge, at the rate of

approximately 4.5 N s-1. The force at which the bryozoan colony detached from the surface was recorded. Colony basal diameters were measured in the field using calipers and were used to calculate the colony area. The critical removal stress (CRS) was then calculated by dividing the force of removal by the surface area of the colony. Encrusting bryozoan colonies that did not remove completely (i.e., the force gauge broke the colony instead of sliding it off of the coating) were not included in calculating CRS. Broken colonies were recorded and used to help evaluate panel efficacy by calculating the percentage of 'plowed' encrusting bryozoan colonies of those colonies on which removal was attempted. Results from field-testing of bryozoans are presented in Figure 3. High CRS values or the presence of colonies that did not slide off the coating indicated that a coating was performing poorly.

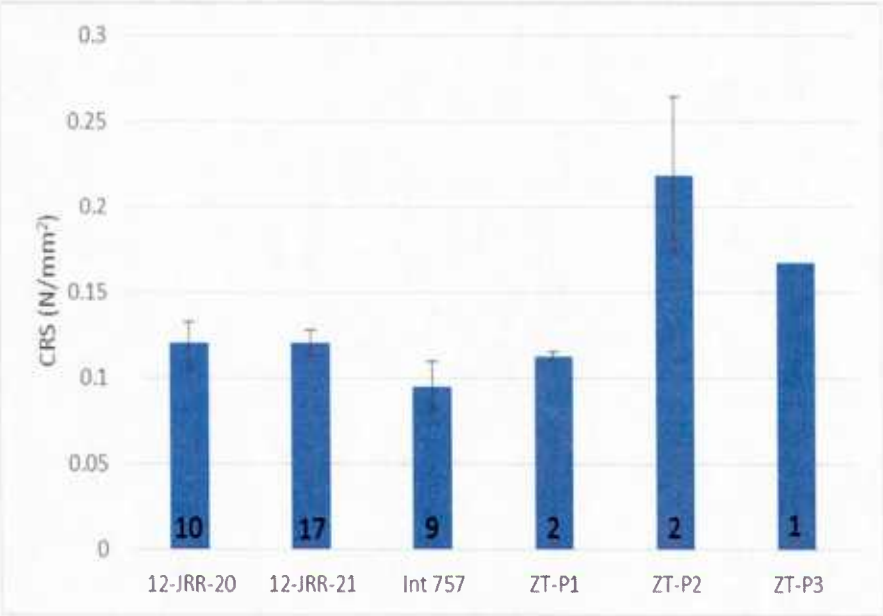

Figure 3. Critical Removal Stress (CRS) of encrusting bryozoans on different coating types from field tests. Results are from 2013. Error bars represent ± one standard error of the mean.

Critical Removal Stress of Bugula neritina in the laboratory

In collaboration with Dr. Nick Aldred at Newcastle University, we began testing using *Bugula neritina* as a model study organism. We collected reproductive *Bugula neritina* from field locations nearby California Polytechnic State University and held them at our laboratory. Using light as a stimulus, we triggered the release of the larvae and then settled the newly released larvae onto glass and Silastic T2 slides. The slides were then shipped to Dr. Nick Aldred to be used in water-jetting experiments. Five glass and seven T2 slides with settled *Bugula* larvae were water-jetted at 20 PSI and 35 PSI respectively. An additional ten T2 slides with settled *Bugula* larvae were water-jetted at 45 PSI only. As some of the *Bugula* had been removed from the glass slides during shipping, glass slides were not water-jetted at 45 PSI.

The results of the water-jetting experiments of settled *Bugula* larvae demonstrate that; 1) the T2 foul-release coating performed better than the glass control surface (Fig. 4) and, 2) that there is a direct correlation between removal pressure and the percentage of individuals removed from foul release surfaces (Fig. 5). Both results suggest that newly settled bryozoans are promising as lab assay organisms for discriminating the performance of new foul-release coatings.

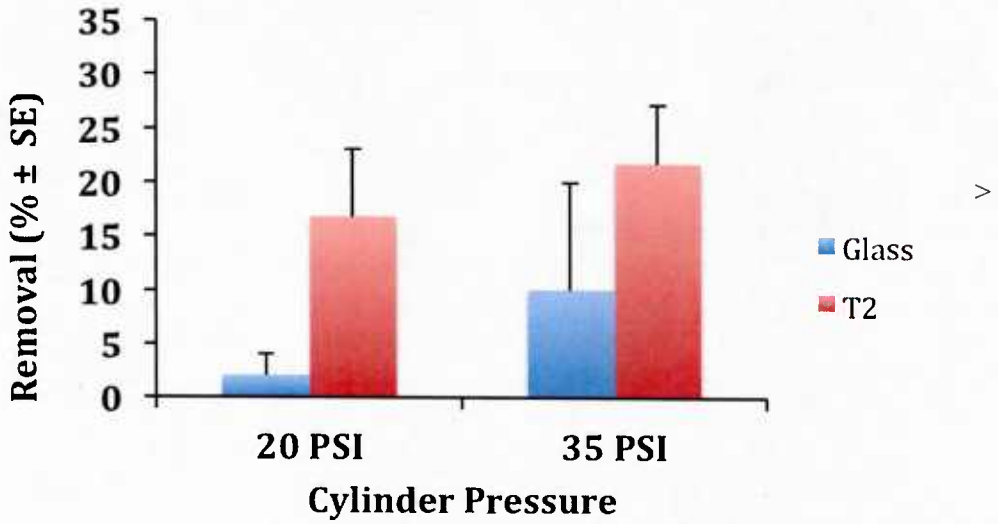

Figure 4. Results from water-jetting experiment on *Bugula neritina* removal from Silastic T2 surfaces (red) and a control glass surface (blue) at 20 PSI and 35 PSI. Results are reported as mean ± SE.

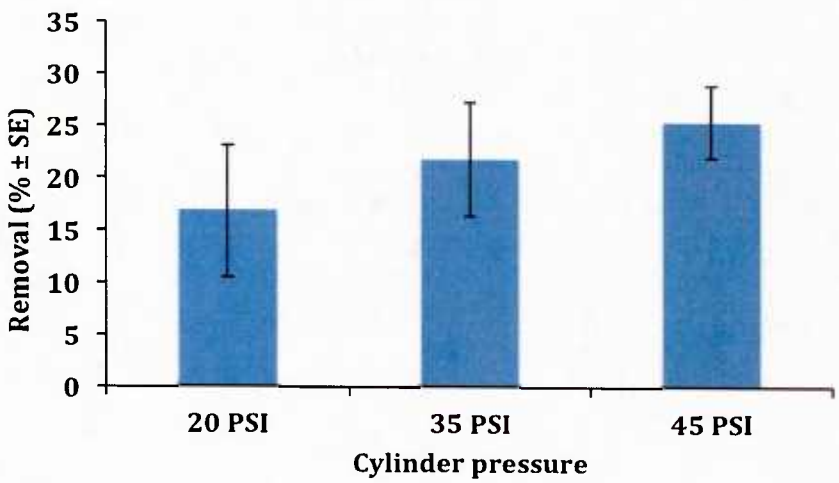

Figure 5. Percent of *Bugula neritina* larvae removed after water-jetting T2 coatings at different cylinder pressures. Results reported as mean ± SE.

Objective 3: Intersite calibration and measuring environmental parameters and recruitment at the Morro Bay field site

Intersite calibration

We worked in collaboration with Eric Holm, Mike Hadfield, Geoff Swain and Serena Teo to provide intersite calibration of field testing. Each site was supplied with three replicate panels (painted back and front) coated with antifouling or foul release coatings. Panels were arranged at random within each block. Consistent with the intersite calibration protocol, we also exposed (3) PVC panels each month to use as a reference of larval recruitment. The anti-fouling coating panels were inspected monthly and fouling was quantified using standard procedures. Anti-fouling panels were replaced without disturbing the fouling community. For foul-release coatings, panels were allowed to accumulate fouling organisms until the hard fouling reached a certain size. At this point the hard foulers were removed using standard ASTM procedures. The PVC panels were assessed for larval recruitment and then replaced with new panels each month. Photographs of panels were taken and the coverage was determined using a 50 point grid generated in the PhotoGrid software. Data analysis and results of this study are centralized with Eric Holm, Naval Surface Warfare Center, Carderock Division.

Measuring seasonal variations in environmental parameters and recruitment

Field-testing consisted of immersing panels off of the Cal Poly static immersion dock in Morro Bay, California. Morro Bay is a cool water, temperate marine environment and thus the fouling community associated with our static test site is different than subtropical and tropical communities. To better track changes in physical parameters at our field site we installed a water quality array that records water quality variables every 15 minutes. The variables recorded by the water quality array included temperature, tide, salinity, water velocity, chlorophyll level, nitrate concentration, and turbidity. These data were uploaded to a server through a telemetry system and were archived for future use. Researchers can access data online or through direct requests (see www.slosea.org). In addition to understanding the physical environment at our site, we have completely characterized the fouling community by doing extensive surveys. All species recorded in our surveys can be accessed through an on-line database (see www.slosea.org). It should be noted that the equipment array, the invertebrate inventory, and the website resource were all funded through external grants outside of the ONR program. Environmental parameters of temperature, salinity, chlorophyll, and nitrate were assessed from 2007-2014 for trends in these parameters (Fig. 6).

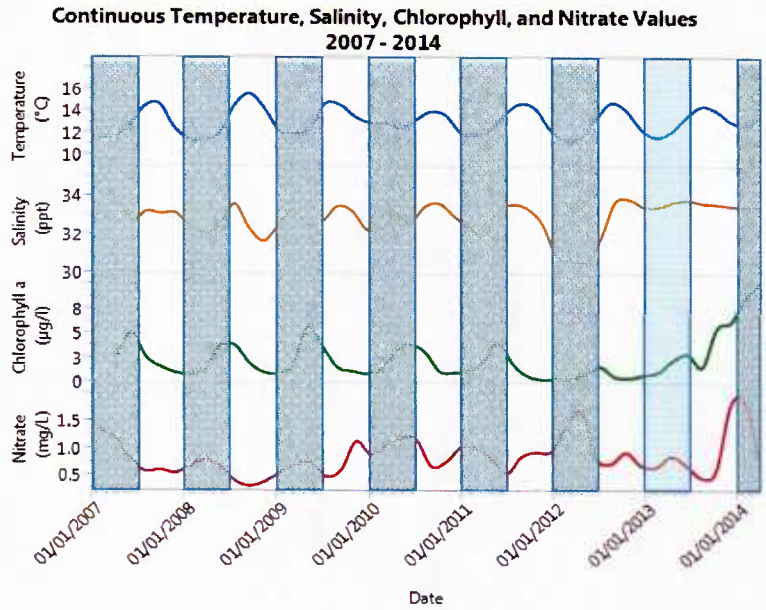

Figure 6. Average annual environmental parameters (temperature, salinity, chlorophyll a, nitrate) at the Cal Poly static immersion dock from 2007-2014. Blue bars indicate the period between January 1 and June 30. Open areas represent the period between July 1 and December 31.

Analyses of environmental parameters demonstrated that at the Morro Bay static immersion field site, temperature peaked during summer months and decreased in winter months. Salinity in general also varied by season with the winter seasons displaying the lowest salinity. However, salinity varied by year with the lowest salinity values corresponding with rainy winters. In the last two years, salinity has not varied much between winter and summer seasons, corresponding with severe drought conditions.

We also assessed mean overall recruitment of all macrofouling organisms and specific groups (barnacles, bryozoans, hydroids) from 2012-2014. The months with the highest overall fouling occurred in July, October and November. February had the lowest overall recruitment (Fig. 7).

The dominant organisms on the panels varied by month with barnacles being the primary fouling organism in all months except February, March and April (Fig 7). Bryozoans and hydroids were the next most common fouling organisms and the percentage of their cover varied by month (Fig. 7). For barnacles, the highest average recruitment occurred in the months of July and October with the lowest recruitment of barnacles between February and April (Fig. 8).

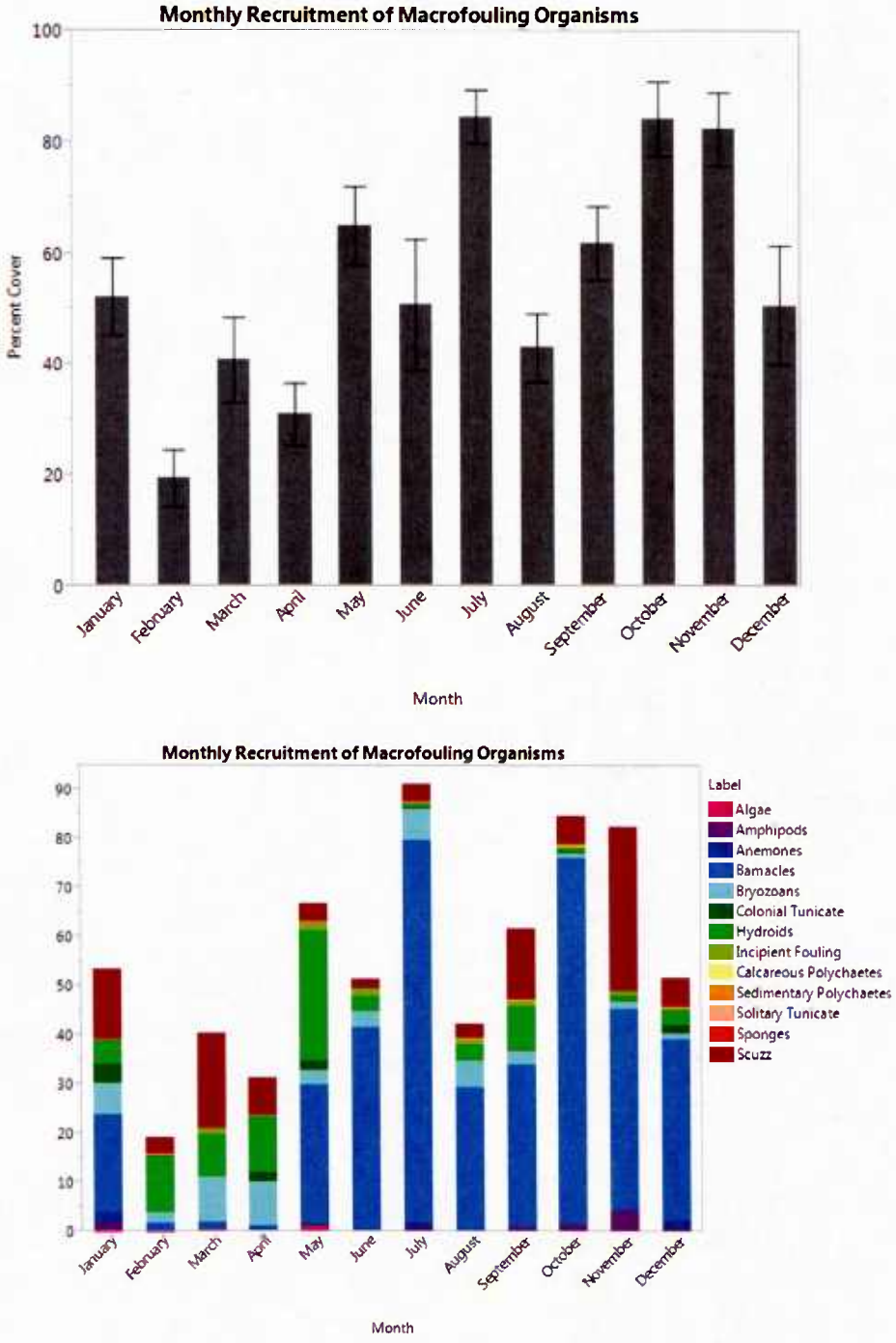

Figure 7. Average monthly recruitment between 2012-2014. (top) all macrofouling organisms and (bottom) by taxonomic classification. Error bars represent ± one standard error of the mean.

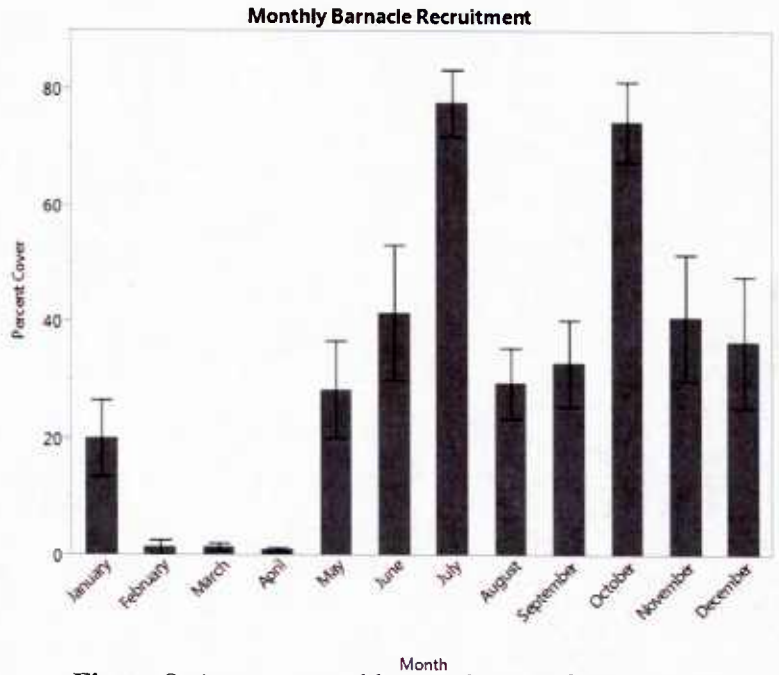

Figure 8. Average monthly recruitment of all barnacles between 2012-2014. Error bars represent ± one standard error of the mean.

In contrast, bryozoan recruitment was highest in March and April (two of the lowest months for barnacle recruitment) and lowest in October, November and December (Fig. 9). Hydroid recruitment varied but was generally highest between February and May (Fig. 10).

We also broke down the type of fouling into three main categories: hard fouling organisms, soft fouling organisms and biofilm. The percent cover of hard fouling organisms was highest in July and October (Fig. 10). This was primarily driven by barnacle recruitment (Fig. 8). The percent cover of soft fouling organisms was highest in March, May and November (Fig. 11).

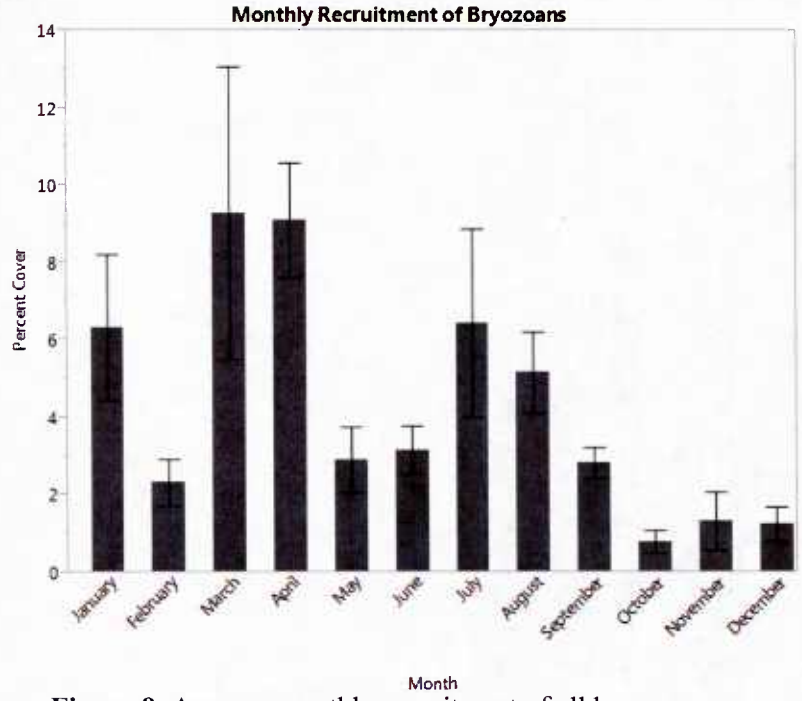

Figure 9. Average monthly recruitment of all bryozoans between 2012-2014. Error bars represent ± one standard error of the mean.

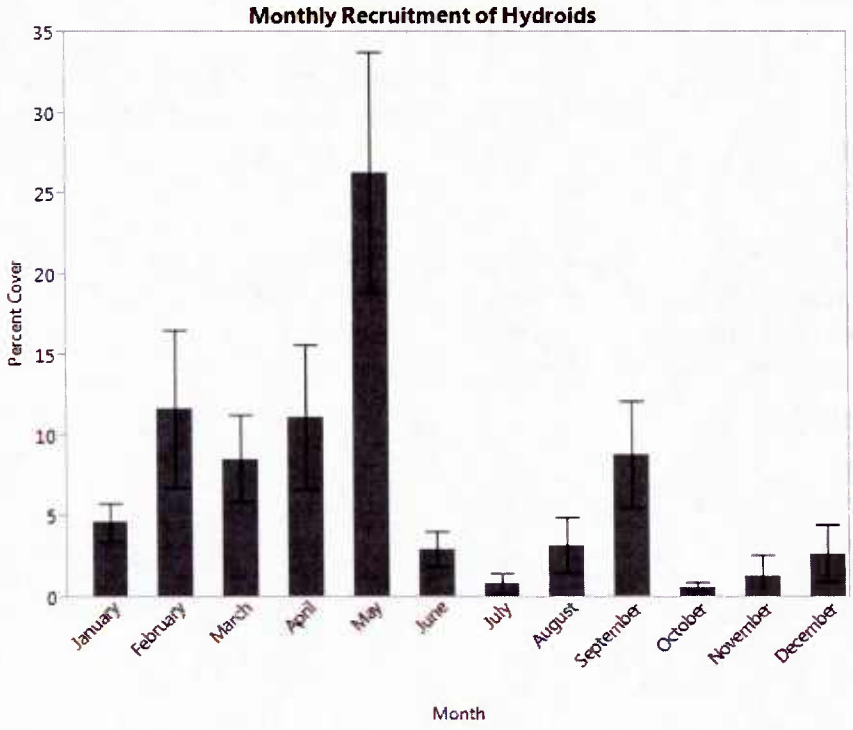

Figure 10. Average monthly recruitment of all hydroids between 2012-2014. Error bars represent ± one standard error of the mean.

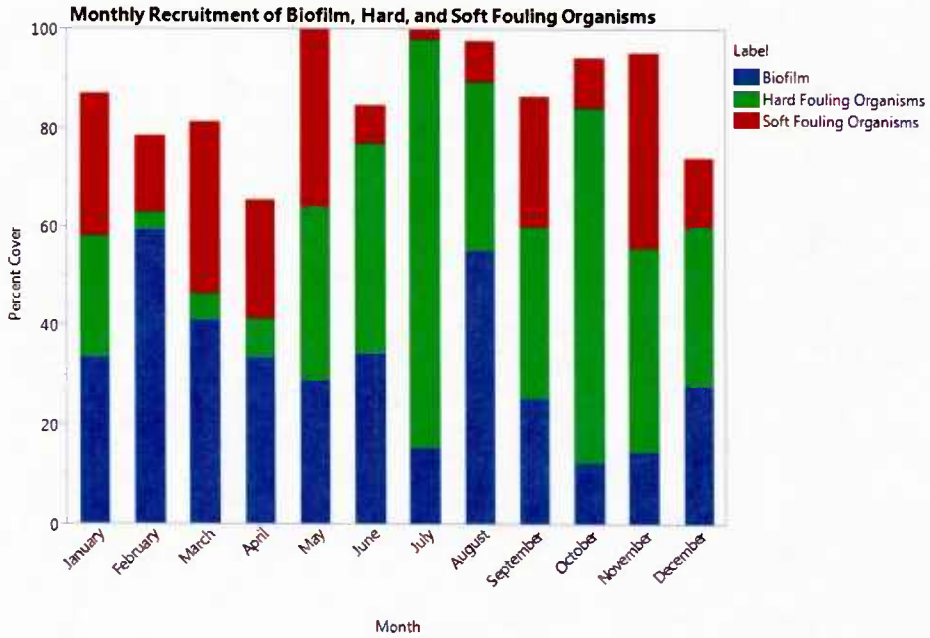

Figure 11. Monthly recruitment of all macrofouling organisms based on fouling type (biofilm, hard fouling, soft fouling) between 2012-2014.

Progress Statement

1. Evaluated efficacy of 784 surfaces totaling 83 coating formulations using laboratory assays;
2. Evaluated efficacy of 473 surfaces totaling 135 different formulations using static field exposure;
3. Produced a total of 52 technical reports for PI's within the program;
4. Used data from water quality array near static immersion test site to categorize fouling trends and seasonal variations of biofouling at our site.
5. In collaboration with Nicholas Aldred and Tony Clare, conducted initial tests using *Bugula neritina* in laboratory assays and demonstrated its potential utility as a test organism
6. Developed field testing of adhesion strength of encrusting byrozoans
7. Evaluated seasonal patterns of environmental parameters from our field site.
8. Over all of the years funded by this grant, we have evaluated over 5,200 coupons using our laboratory assessments and over 1400 test panels immersed at our field site. A summary of these results are included in reports from other principal investigators within the program and are not included here.

Summary Statement

The overall goal of our work was to use laboratory and field testing to expedite the down-select process used to develop nontoxic, foul-release coatings and to develop

new methodologies to test the performance of foul-release coatings. We have met our deliverables on the project by; 1) assessing novel coatings in both the laboratory and field to contribute to the down-select process, 2) furthering the development of bryozoans as model organisms for testing coating performance in the lab and field, and 3) assessing seasonal fouling dynamics and seasonal variation in environmental parameters in Morro Bay, thereby contributing to the understanding of how fouling varies by season and geographic location through the intersite calibration study.

References

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Standard Test Method for Measurement of Barnacle Adhesion Strength in Shear. ASTM, West Conshohocken

Swain, GW, Schultz, MP (1996) The Testing and Evaluation of Non-Toxic Antifouling Coatings. *Biofouling* 10: pp. 187-197