Report on Fouling Panel Studies
DAARP Settlement Restoration Program
Toxicity Monitoring for the Pacific Herring Spawning Enhancement Project

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Contents

Background

The Cape Mohican oil spill affected aquatic organisms along the San Francisco waterfront, including rocky shore and piling communities. Pacific herring, which spawn on these substrates, were also affected: the substrates were coated with oil only a few weeks before the start of spawning.

Wooden pilings in San Francisco Bay are subject to damage from wood-boring marine mollusks (teredinid bivalves called "shipworms") and crustaceans (limnoriid isopods called "gribbles" and an amphipod *Chelura terebrans*). To deter attack by these organisms and increase the pilings' useful life, pilings have been treated prior to installation with toxic compounds, primarily with creosote in earlier years. Studies, however, have found that creosote is toxic to eggs and larvae of fish and invertebrates causing mortality, developmental problems and reduced viability (Poston 2001; Stratus 2006), and have specifically reported toxicity to herring eggs adhering directly to creosoted timbers (Tasto *et al.* 1996; Vines *et al.* 2000). Wooden pilings are now treated with ACZA (Ammonium-Copper-Zinc-Arsenate) or related compounds, which are thought to be less harmful to marine life.

The Pacific Herring Spawning Habitat Enhancement Project is currently replacing more than 280 creosote-covered pilings with vinyl-coated, ACZA-treated pilings at the Port of San Francisco's Pier 45, where herring have spawned in past years. The vinyl coating is a further protection against wood-borers and is expected to also reduce the exposure of non-target organisms to the toxins in the wood. It was hoped that that the new pilings would provide essentially nontoxic surfaces for herring to spawn on and for a variety of invertebrates (e.g. mussels, anemones, sponges, barnacles, worms) to grow on.

Project Purpose and Phasing

The project evaluated the growth of attached marine organisms on vinyl-coated and ACZA-treated panels, to provide an assessment of the value of treated pilings as habitat for attached organisms and as an indicator of potential toxicity to herring eggs. The overall objective is to determine whether there is a consistent difference in marine growth among treatments that may correspond to differences in toxicity.

The fouling panel experiment was initially designed as a five year project, to be done in two phases. The initial contract covered the first phase, which included the initial deployment and the retrieval and replacement of a portion of the panels after approximately one year, and the analysis and reporting on the retrieved panels.[1] Changes were then made in the plan for the second phase of the experiment, as described below, which is a component of the second (current) contract. An additional component of the second contract, the direct sampling of pilings, will be covered in a later report.

Methods

The project did not attempt to directly assess the effects of ACZA-treatment or vinyl coatings on herring spawning, which conceivably could occur through a variety of direct or indirect pathways, none of which are well-understood. Rather, the project set out to test whether there are significant differences in the amount and/or composition of the marine organisms that settle and grow on pilings with different treatments, as an indicator of possible differences in toxicity. Specifically, the project compared the amount and composition of marine growth on four types of treatments on wood panels mounted on pilings: untreated panels, vinyl-coated panels, uncoated ACZA-treated panels, and vinyl-coated ACZA-treated panels. Since the untreated panels were extensively bored by wood-boring organisms during the period of exposure, so that part of all of each panel was missing when the panel arrays were retrieved, the untreated panels were not included in the statistical analyses.

[1] The first phase of the fouling panel work was reported in: Cohen, A.N. 2009. *Final Report: San Francisco Bay Pacific Herring Habitat Monitoring (CA); 2003-0207-003* (January 23, 2009). The data and analysis from that report are incorporated into the present report.

Figure 1. Port of San Francisco diver Bruce Lanham getting suited up in preparation for panel retrieval.

The experiment was initially designed to run for four years, with retrieval and replacement of some of the panels at approximately 1, 2 and 3 years after initial deployment, and retrieval of all panels at approximately 4 years after initial deployment. After the first year's retrieval the experiment was redesigned so that all panels were retrieved approximately 1.5 years later. As initially planned, several measurements of

the extent of fouling growth were made on the first set of retrieved panels, and assessed as to which were the most effective. A smaller set of measurements was then made on the final set of retrieved panels.

Deployment and retrieval of fouling arrays. In January 2007, we deployed 20 arrays of 9 x 15 cm wood panels rigidly attached to 5 pilings at Pier 45 in San Francisco Bay (at 37° 48.62' N, 122° 25.15' W), with each piling serving as a replicate. Each piling held 4 vertical panel arrays; each array held 4 panels, each of which received a different treatment (untreated; vinyl-coated; uncoated ACZA-treated; and vinyl-coated ACZA-treated) and was randomly assigned to its position in the vertical array. The arrays were positioned on the pilings so that the panels were located between -1.0 m and -1.6 m MLLW. In February 2008, one of the four vertical arrays on each piling was removed and replaced with a new array. In September 2009, all of the panel arrays were removed. There were thus three periods of deployment, referred to in this report as deployments A (Jan. 2007-Feb. 2008), B (Feb. 2008-Sept. 2009) and C (Jan. 2007-Sept. 2009) (Table 1). The Port of San Francisco divers assisted with the manufacture, deployment and retrieval of panel arrays (Figure 1).

Table 1. Deployment periods.

Deployment	Period	Length	Number of Panels
A	1/10/07–2/27/08	413 days	20
B	2/27/08–9/29/09	580 days	20
C	1/10/07–9/29/09	993 days	60

Examination, identification and quantification. During retrievals, a field station was set up on the dock at Pier 45. As each array was removed from its piling it was brought to the field station, photographed, and the lead researcher made tentative identifications and determined the total percent cover and the percent cover of each faunal group (that is, each distinct species or distinct species group) by point estimation using a 66 point grid (these data are reported as "Cover"). Each panel was then removed from its array, labeled and bagged separately in a ziplock bag, transported to the lab, and refrigerated. Over the next two days, all organisms were scraped from the front surface of each panel and weighed to determine total wet biomass (except for untreated panels in Deployments B and C), and for Deployment A, the biomass of each faunal group (reported as "Biomass"). For all of the Deployment A panels and a subset of the Deployment B and C panels, the organisms were examined under a stereo-microscope (40x-100x) by the lead researcher to identify the organisms to the lowest possible taxon, and the number of distinct attached taxa on each panel was recorded (reported as "Species Diversity"). Standard taxonomic references and reference specimens from previous San Francisco Bay taxonomic surveys were used as needed to confirm identifications. For Deployment A, the photograph of each panel taken in the field was overlaid with a drawn 66-point grid and the total percent cover and the percent cover of each faunal group was determined using point estimation (reported as "Photo Cover").

Wood-borer damage was assessed by cutting 15 panels from each deployment in half (5 vinyl-coated untreated, 5 ACZA-treated and 5 vinyl-coated ACZA-treated), and examining the cross-sections to determine the frequency of shipworm bore holes and gribble bore holes (the percentage of panels showing such bore holes) and the occurrence of shipworm bore holes (measured as the number of shipworm borings per cross-section).

The various types of measurements made on the panels are summarized in Table 2. All the data were recorded on Excel spreadsheets (attached as Appendix A).

Table 2. Measurements taken. Deployments (A, B or C) are indicated in the first column. Treatments are: U=untreated; VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated; All=all 4 treatments.

	Cover		**Biomass**		**Photo Cover**		**Species Richness**	**Borer Damage**
	total	by species	total	by species	total	by species		
A	All	All	All	All	All	All	All	VU, A & VA
B	All	All	VU, A & VA	None	None	None	2/5 of VU, A & VA	VU, A & VA
C	All	All	VU, A & VA	None	None	None	5/15 of VU, A & VA	5/15 of VU, A & VA

Analysis. The data were first examined graphically. Statistical analyses were performed in Systat (Version 11). A one-way analysis of variance (ANOVA) was used to examine differences in total Cover, Cover of common faunal groups, total Biomass and Species Diversity for all deployments; and total Photo Cover, Photo Cover of common faunal groups and Biomass of common faunal groups for Deployment A. Statistical significance was considered at $\alpha = 0.05$. Data were analyzed as either raw, log-10, or square root transformed to meet the test assumptions. Normality of error values was verified graphically and by using the Shapiro-Wilk test. Equality of variances was examined by plots of residuals. Significant results were investigated with post-hoc Tukey HSD and corroborated with other post-hoc tests. Data that could not be normalized were assessed by non-parametric ANOVA (Kruskall-Wallis), and significant results were investigated graphically.

Results

In both retrievals, the surfaces of the panels that were vinyl-coated and/or treated with ACZA appeared to be unaffected by wood borers, but the untreated panels were extensively damaged by shipworms that had bored within them and by gribbles that had eroded their surfaces, to the point where many of the panels were nearly or entirely gone, and the remaining wood surfaces had been heavily worked over by gribbles (Fig.

2 to 4). The reworking of these surfaces by gribbles reduced the portion covered by attached organisms and affected the species composition of attached organisms, compared to the panels given other treatments. For this reason, the untreated panels were excluded from the statistical analyses and the graphs discussed below, though the data for the untreated wood panels are included in the data tables.

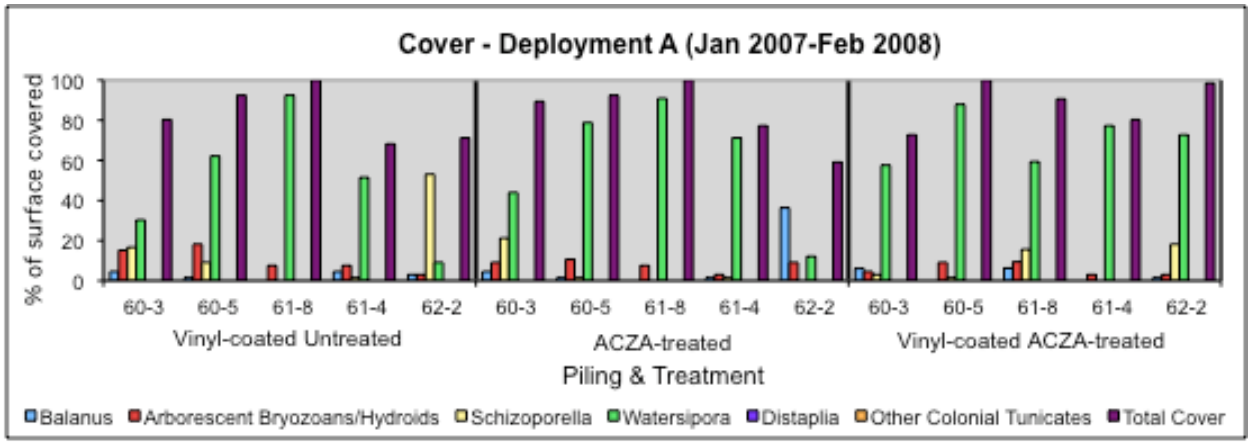
Figure 2. Panel Array 5. The panel treatments are, from left to right, uncoated wood, vinyl-coated ACZA-treated wood, vinyl-coated wood, and uncoated ACZA-treated wood. Only a sliver of the uncoated wood panel remains, projecting in either direction from the stainless-steel screw that held the panel in place.

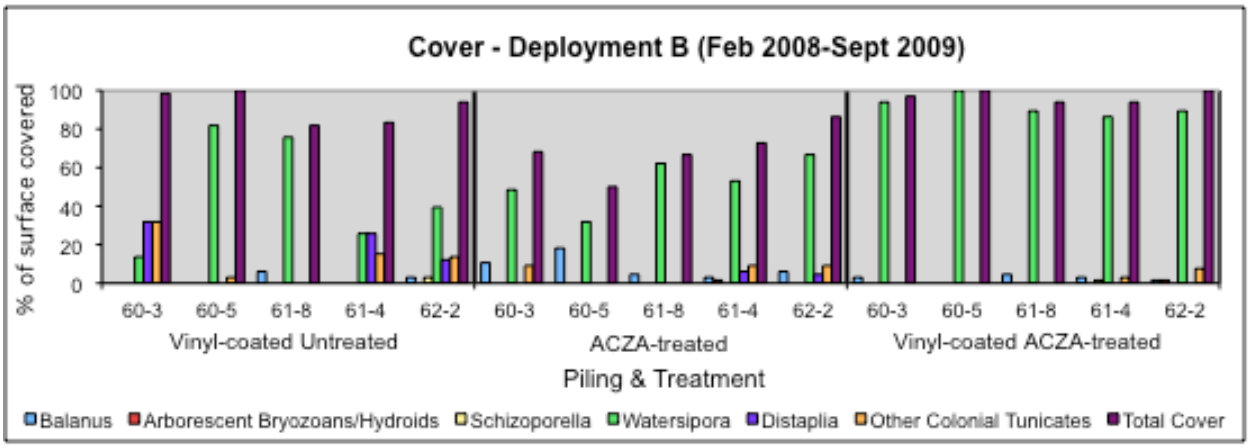
Figure 3. Panel Array 1. Panel treatments from left to right are uncoated ACZA-treated wood, vinyl-coated ACZA-treated wood, uncoated wood, and vinyl-coated wood. Most of the uncoated wood panel remains in place, but the surface has been eroded by minute wood-boring isopods called gribbles, exposing the much larger shipworm borings in some places and the white calcareous tubes that line shipworm borings in others.

Figure 4. Close-up of the untreated wood panel from Panel Array 1. Surface removal by gribbles, and the shipworm borings and the white calcareous tubes that line shipworm borings (in a U-curve at upper right corner, near the center, and near the lower left corner) exposed by the gribbles can be clearly seen. Bryozoans and a few empty tests of barnacles are attached to the surface.

The results for Cover, Biomass and Species Richness for Deployments A, B and C are shown in Figures 5, 6 and 7 and in Appendix A, with data summaries in Tables 3 and 4, and the statistical analysis summarized in Tables 5 and 6. We distinguished 29 species attached to the surfaces of the panels, along with ciliates attached to some of the bryozoans, shipworms and gribbles boring in the wood, plus several mobile species (foraminifera, nematodes, polychaetes, gastropods, pycnogonids, ostracods, amphipods, isopods, tanaids and crabs) (Appendix A, Species Diversity Data Tables). The attached organisms included algae, sponges, hydroids, bivalves, barnacles, bryozoans and tunicates. Two sponges were identified, an Atlantic sponge *Halichondria* cf. *loosanoffi*, and in the second retrieval (Deployments B and C) an additional species, the native *Leucilla nuttingi*. The hydroids included at least two species, *Monostaechas quadridens* and one or more species of *Obelia*. Bivalves included bay mussels (either the native *Mytilus trossulus*, the Mediterranean species *M. galloprovincialis*, or hybrids of the two), an Asian mussel *Musculista senhousia*, and the native Olympia oyster *Ostrea lurida*. Nearly all the barnacles were a native species, *Balanus crenatus*, along with a few specimens of an Atlantic species, *Amphibalanus improvisus*. The bryozoans included five encrusting and five arborescent species. *Watersipora* cf. *subtorquata*, an exotic encrusting bryozoan was the most abundant organism, dominating the surface

Figure 5. Total percent cover and percent cover for each common faunal group for all deployments, as determined by direct examination in the field. Replicates are identified by piling (bent and number), and for Deployment C, array number. Numeric data are given in Appendix A.





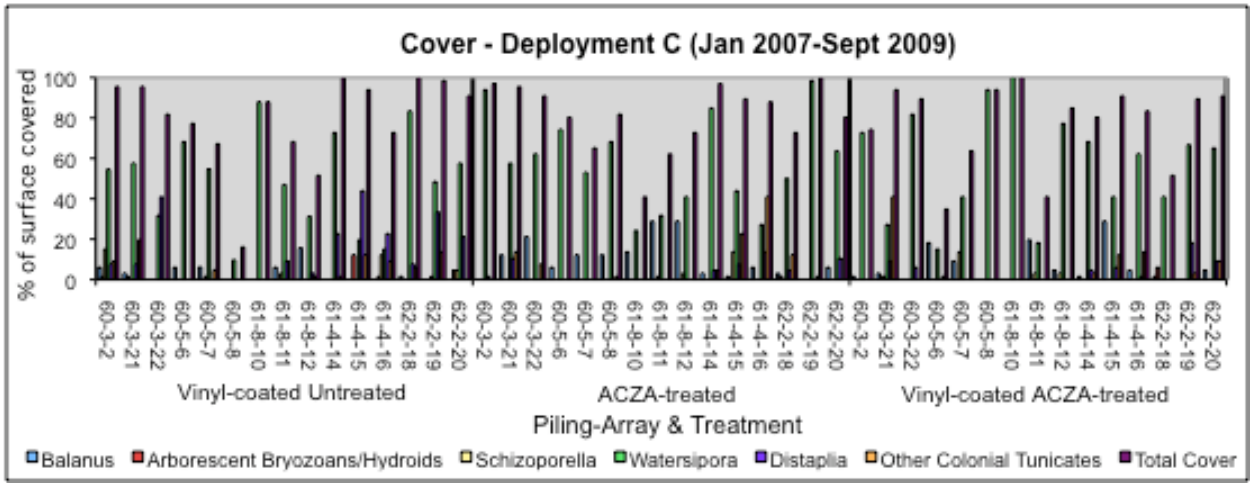


Figure 6. Total wet biomass for all deployments. Replicates are identified by piling (bent and number), and for Deployment C, array number. Numeric data are given in Appendix A.

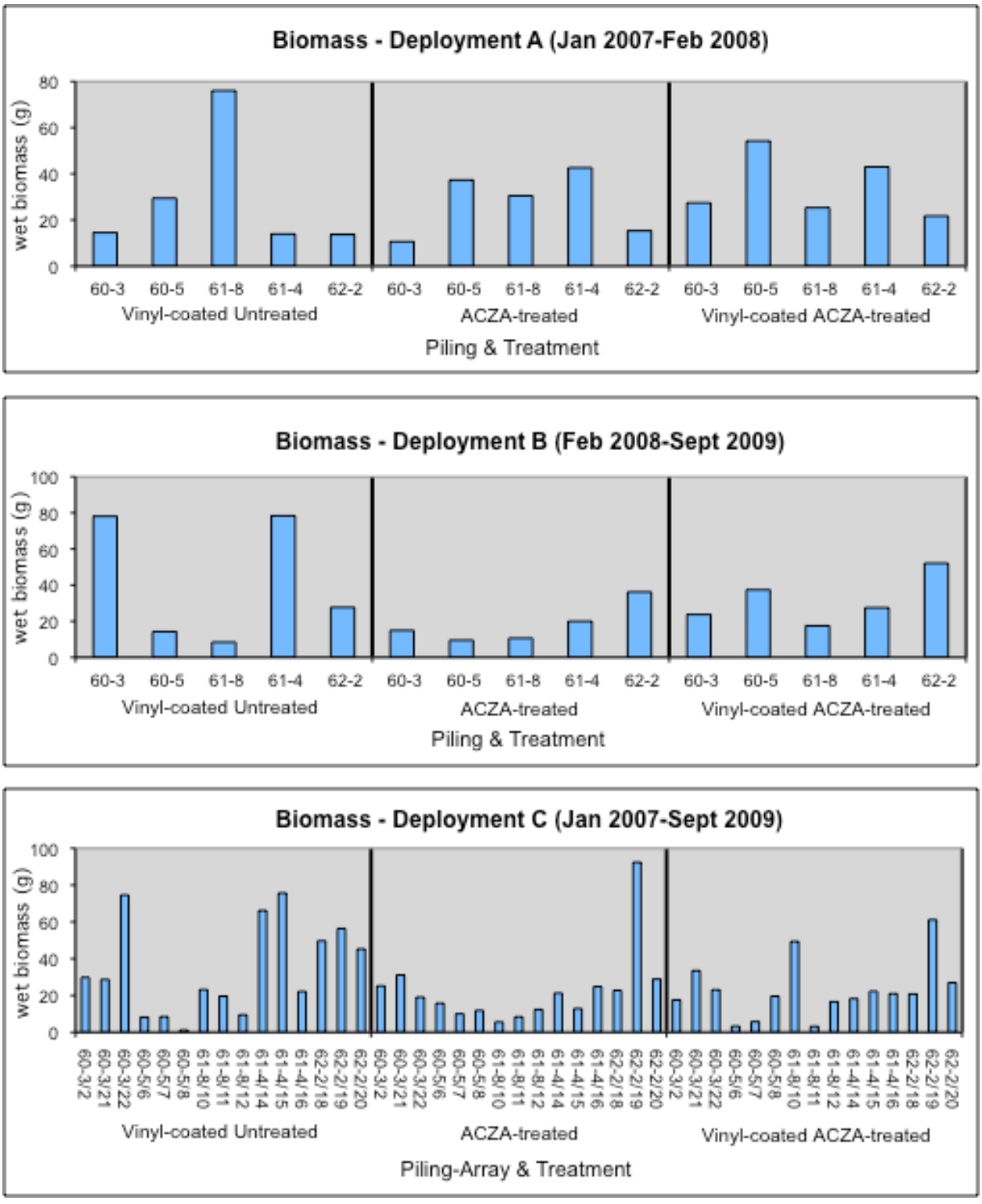
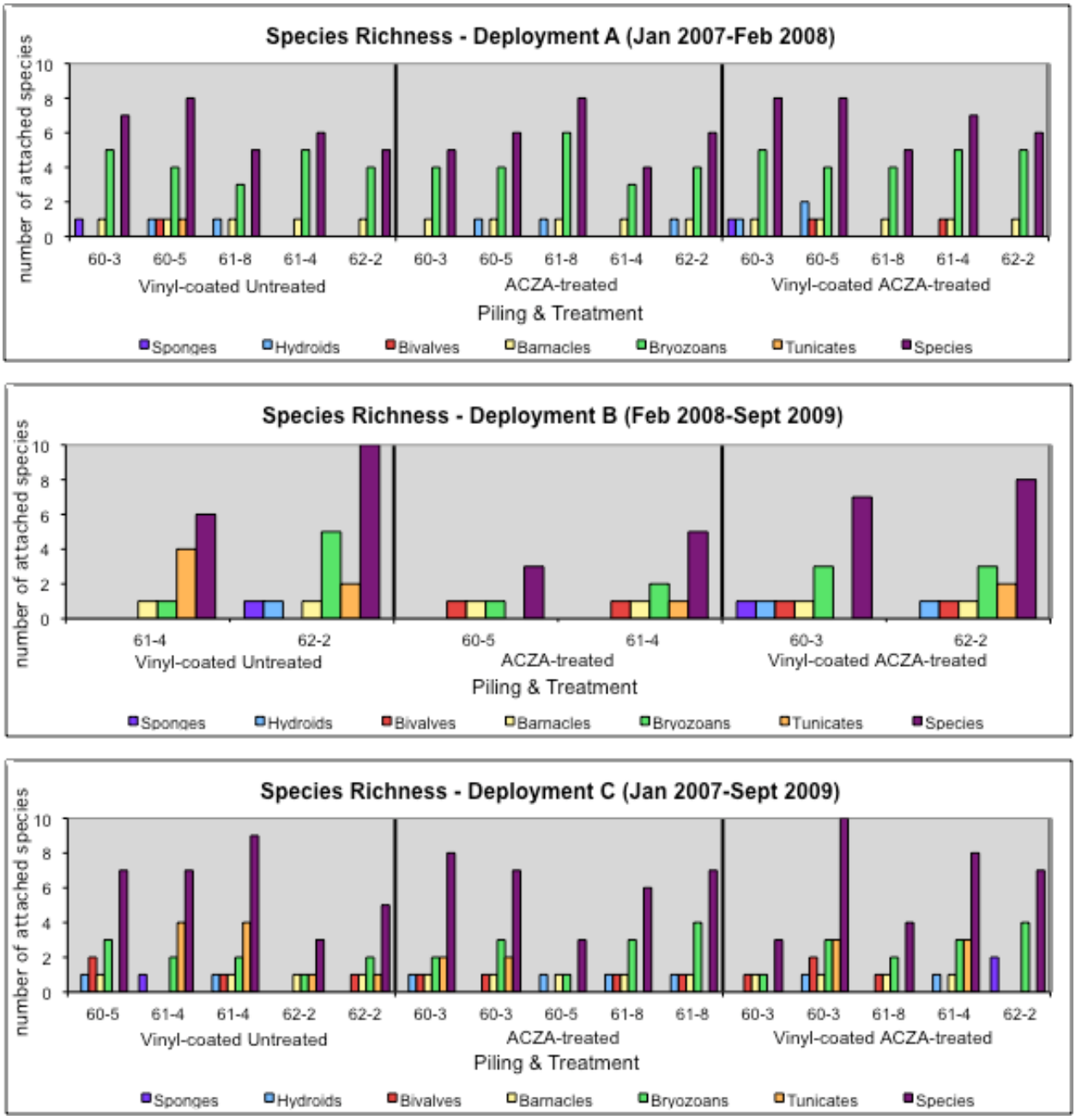
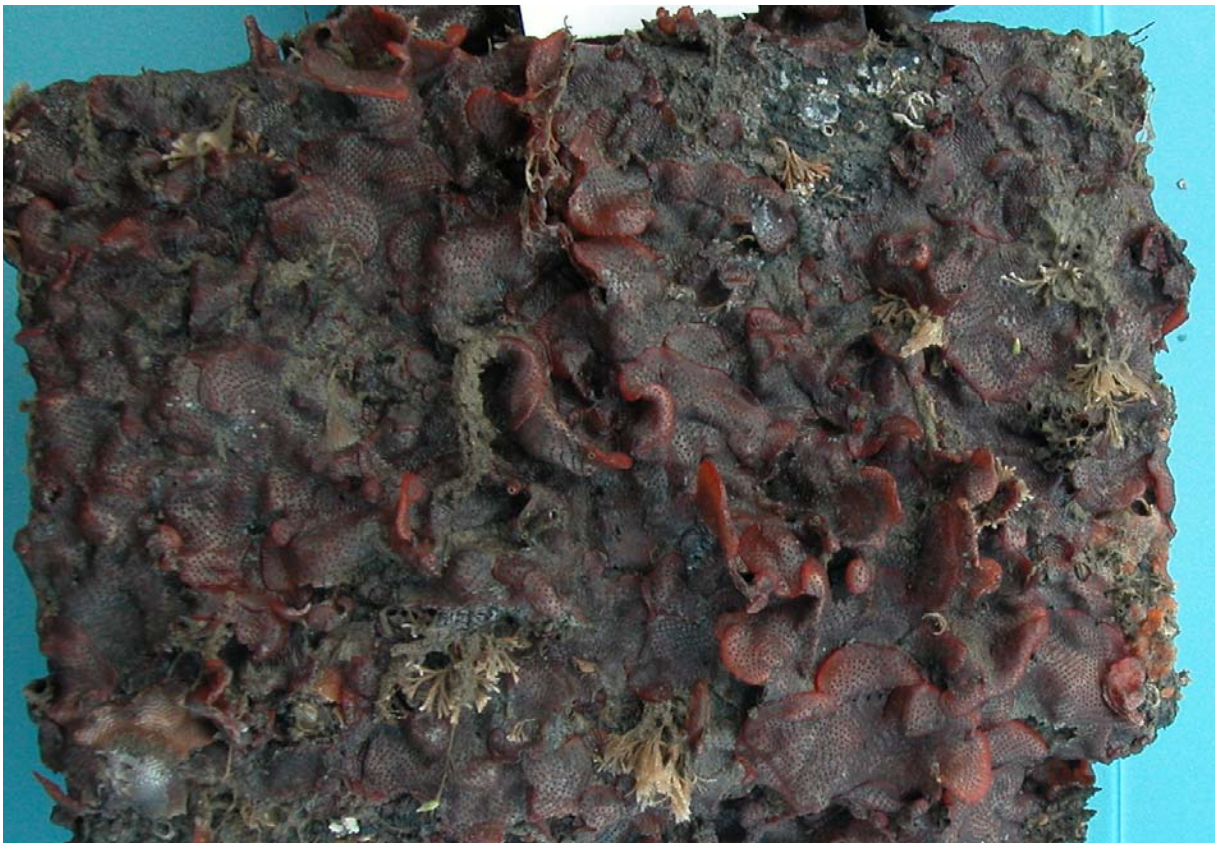


Figure 7. Total species richness and species richness for major taxonomic groups for all deployments. Replicates are identified by piling (bent and number). Numeric data are given in Appendix A.



coverage on most panels (Fig. 8). Another exotic encrusting species, *Schizoporella* cf. *unicornis*, was also fairly common. In the first retrieval (Deployment A), the arborescent bryozoans were divided into two components, coarse (consisting of the native *Scrupocellaria diegensis* and *Bugula* cf. *californica*) and fine (*Caulibugula* cf. *ciliata* and an unidentified cyclostome) for the purpose of estimating cover; in the later retrieval

Figure 8. A vinyl-coated ACZA-treated panel dominated by the red encrusting bryozoan *Watersipora*. Also visible are white or tan tufts of arborescent bryozoans and, in the lower left corner, a pale orange colony of another encrusting bryozoan, *Schizoporella*. Near the top of the panel, right of center, are a barnacle and some barnacle scars.



these components were not separated. Tunicates were rare in the first retrieval, with only the Asian colonial tunicate *Botrylloides violaceus* observed. Tunicates were both more abundant and more diverse in the second retrieval (Deployments B and C), with the native colonial tunicates *Distaplia occidentalis* and *Diplosoma macdonaldi* and the exotic colonial tunicate *Didemnum vexillum* joining *Botrylloides* in constituting a significant component of the fouling community.

Total cover averaged 81.8% of the panel surface over all deployments and treatments, with a range of 79.0-83.6 for the three treatments (Table 3) (untreated panels are excluded from this analysis), and was not significantly different between treatments when considered over all deployments or for the individual Deployments A and C (Table 5). However, in Deployment B total cover was extremely significantly lower on the ACZA-treated panels than on the vinyl-coated untreated panels and the vinyl-coated ACZA-treated panels ($p < 0.001$; Fig. 5; Tables 5 and 6). There were no significant differences in total biomass or total species richness between treatments over all deployments or for any individual deployment (Fig. 6 and 7; Tables 4 and 5).

Table 3. Percent cover over all deployments. Treatments are: VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated; All=all 3 treatments.

Taxon	Treatment			
	VU	A	VA	All
	% of covered surface			
Sponges	0.9	0.0	0.1	0.3
Hydroids	0.1	0.1	0.0	0.0
Polychaetes	0.1	0.0	0.0	0.0
Barnacles	3.8	14.4	8.1	8.8
Bryozoans	69.3	75.5	83.7	76.2
Arborescent Bryozoans	3.3	2.0	1.9	2.4
Encrusting Bryozoans	65.9	73.4	81.8	73.7
Watersipora	58.9	71.2	78.7	69.6
Schizoporella	6.3	2.0	3.1	3.8
Tunicates	19.4	9.2	6.7	11.7
Distaplia	13.1	3.0	2.4	6.2
Didemnum/Diplosoma	4.0	5.3	2.5	3.9
Botrylloides	2.2	0.9	1.7	1.6
Unidentified Material	6.5	0.8	1.5	2.9
Total Cover (% of panel surface)	82.7	79.0	83.6	81.8

Table 4. Mean values of Total Cover, Total Biomass and Species Richness. Treatments are: VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated; All=all 3 treatments.

Analysis	Deployment	Treatment			
		VU	A	VA	All
Total Cover (% of panel surface)	A	82.4	83.6	88.4	84.8
	B	91.5	68.8	97.0	85.8
	C	79.8	80.9	77.5	79.4
	All	82.7	79.0	83.6	81.8
Total Biomass (g)	A	29.5	27.3	34.3	30.4
	B	41.4	18.2	31.6	30.4
	C	34.6	22.8	22.8	26.8
	All	34.9	22.8	26.9	28.2
Species Richness	A	6.2	5.8	6.8	6.3
	B	8.0	4.0	7.5	6.5
	C	6.2	6.2	6.2	6.2
	All	6.5	5.7	6.7	6.3

Table 5. Statistical analyses. One-way ANOVA conducted in Systat (Version 11), with $\alpha = 0.05$. Deployments (A, B, C or All) are indicated in the first column. NS = not significant; * = <0.05; ** = <0.01; *** = <0.001.

	Analysis	Data Transformation	SS	MS	F	p	Significance
	Cover-Barnacles	square root	1.44	0.72	0.43	0.66	NS
	Cover-arborescent bryozoans	none	22.53	11.27	1.37	0.29	NS
	Cover-Schizoporella	square root	4.82	2.41	0.74	0.50	NS
A	Cover-Watersipora	none	504.4	252.2	0.80	0.47	NS
	Cover-Total Cover	none	35.73	17.87	0.21	0.81	NS
	Biomass-Total Biomass	log-10	0.07	0.03	0.50	0.62	NS
	Species Richness	log-10	0.01	0.01	0.72	0.50	NS
	Cover-Barnacles	log-10	1.02	0.51	4.78	**0.03**	*
	Cover-arborescent bryozoans	non-parametric	–	–	–	0.58	NS
	Cover-Schizoporella	non-parametric	–	–	–	0.58	NS
	Cover-Watersipora	none	5858	2929	7.77	**0.007**	**
B	Cover-Distaplia	log-10	1.67	0.84	3.25	0.07	NS
	Cover-other colonial tunicates	non-parametric	–	–	–	0.16	NS
	Cover-Total Cover	none	2228	1114	13.51	**0.001**	***
	Biomass-Total Biomass	log-10	0.20	0.10	1.21	0.33	NS
	Species Richness	none	19.00	9.50	2.71	0.21	NS
	Cover-Barnacles	log-10	1.80	0.90	4.05	**0.03**	*
	Cover-arborescent bryozoans	non-parametric	–	–	–	0.14	NS
	Cover-Schizoporella	non-parametric	–	–	–	0.47	NS
	Cover-Watersipora	none	768.8	384.4	0.64	0.53	NS
C	Cover-Distaplia	non-parametric	–	–	–	**0.04**	*
	Cover-other colonial tunicates	non-parametric	–	–	–	0.84	NS
	Cover-Total Cover	non-parametric	–	–	–	0.82	NS
	Biomass-Total Biomass	log-10	0.16	0.08	0.62	0.54	NS
	Species Richness	none	0.13	0.07	0.01	0.99	NS
	Cover-Barnacles	log-10	2.53	1.26	6.68	**0.002**	**
	Cover-arborescent bryozoans	non-parametric	–	–	–	0.63	NS
	Cover-Schizoporella	non-parametric	–	–	–	0.28	NS
	Cover-Watersipora	none	4262	2130	3.59	**0.03**	*
All	Cover-Distaplia	non-parametric	–	–	–	**0.02**	*
	Cover-other colonial tunicates	non-parametric	–	–	–	0.45	NS
	Cover-Total Cover	non-parametric	–	–	–	0.29	NS
	Biomass-Total Biomass	log-10	0.17	0.09	0.84	0.44	NS
	Species Richness	none	7.72	3.86	1.10	0.35	NS

Table 6. Mean values for treatments where differences were statistically significant. Different superscripts indicate significant differences based on *post hoc* tests. Treatments are: VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated.

Analysis	Deployment	VU	A	VA	*Post hoc* analysis
Cover-Barnacles	B	2.1^{1}	14.0^{2}	2.5	Tukey
Cover-Barnacles	C	4.5^{1}	14.6^{2}	11.5	Tukey
Cover-Barnacles	All	3.8^{1}	14.4^{2}	8.1	Tukey
Cover-Watersipora	B	52.2^{1}	75.6^{1}	94.7^{2}	Tukey
Cover-Watersipora	All	58.9^{1}	71.2	78.7^{2}	Tukey
Cover-Distaplia	C	16.7^{1}	4.1^{2}	4.1^{2}	graphic analysis
Cover-Distaplia	All	13.1^{1}	3.0^{2}	2.4^{2}	graphic analysis
Cover-Total Cover	B	91.5^{1}	68.8^{2}	97.0^{1}	Tukey

The bryozoan *Watersipora* was by far the most abundant species, accounting for 69.6% of the total cover (with average values ranging from 58.9 to 78.7% for the three treatments) (Fig. 5; Table 3). Barnacles accounted for 8.8% of the total cover over all treatments, ranging from 3.8 to 14.4% for the three treatments. The tunicate *Distaplia* accounted for 6.2% of cover overall, though no colonies were observed on panels from the first deployment, Deployment A. The average values ranged from 2.4 to 13.1% for the three treatments. Each of the other species and distinct species groups accounted for less than 5% of the total cover (Fig. 5; Table 3).

Differences in cover for some species groups were significant between some treatments in some deployments (Fig. 5; Tables 5 and 6; Appendix B). Barnacles were the most consistently different, with significantly greater cover on ACZA-treated panels than on vinyl-coated untreated panels in Deployments B and C and in all deployments taken together (Table 6). *Watersipora* cover on vinyl-coated ACZA-treated panels was significantly greater than on vinyl-coated untreated panels and ACZA-treated panels in Deployment B, and greater than on vinyl-coated untreated panels in all deployments taken together. *Distaplia* cover was significantly greater on vinyl-coated untreated panels than on ACZA-treated panels and vinyl-coated ACZA-treated panels in Deployment C and all deployments taken together (Table 6; Appendix B).

As noted earlier, untreated panels were severely damaged and eroded by shipworms and gribbles, to the point where many of the panels were nearly or entirely gone. In the other three treatments, wood-borer damage was assessed by determining the frequency and occurrence of bore holes in panel cross-sections (Table 7). By each measure, the greatest damage was to the vinyl-coated untreated panels, with less damage to the ACZA-treated panels. There was no damage to the vinyl-coated ACZA-treated panels.

Table 7. Damage from shipworms (Teredinidae) and gribbles (Limnoriidae). Five panels per category, 45 panels in all, were sawn in half and the cross sections were examined for the presence of shipworm and gribble bore holes and the number of shipworm boreholes per panel. Treatments are: VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated; All=all 3 treatments.

A. Shipworm Frequency - % of panels				
	VU	A	VA	All
A	40	20	0	20
B	0	0	0	0
C	20	0	0	7
All	20	7	0	9
B. Shipworm Occurrence - number of bore holes/panel				
	VU	A	VA	All
A	2.6	0.4	0	1.0
B	0	0	0	0
C	0.4	0	0	0.13
All	1.0	0.13	0	0.38
C. Gribble Frequency - % of panels				
	VU	A	VA	All
A	0	0	0	0
B	20	20	0	13
C	60	0	0	20
All	27	7	0	11

Discussion

Our expectation was that ACZA-treatment would have an inhibitory effect on the growth of attached organisms, and that the vinyl coating would have a protective effect. Thus, if the vinyl coating was 100% effective, the estimates of percent cover, biomass and species diversity would rank:

$$U = VU = VA > A \qquad \textit{Equation 1}$$

Where:

U = untreated panels
VU = vinyl-coated untreated panels
VA = vinyl-coated, ACZA-treated panels
A = ACZA-treated panels.

If the protective effect of the vinyl was less than 100%, the estimates would rank:

$$U = VU > VA > A \qquad \textit{Equation 2}$$

If the vinyl covering had an inhibitory effect, which we did not expect but needed to test for, then we would find:

$$U > VU \qquad \textit{Equation 3}$$

During the experiment the untreated panels were largely or entirely eaten away by wood-boring shipworms and gribbles, and so meaningful data on percent cover, biomass and diversity were not available for this treatment. For the other three treatments, for all analyses of biomass and species diversity, and most analyses of cover, there was no significant difference between treatments. That is:

$$VU = VA = A \qquad \textit{Equation 4}$$

where "=" means no significant difference. This is an unexpected result, being inconsistent with both Equation 1 and Equation 2. Two possible explanations are: (1) neither ACZA treatment nor vinyl coating has any significant inhibitory effect on the growth of fouling organisms, or (2) both ACZA treatment and vinyl coating have the same level of inhibitory effect, and vinyl coating is also 100% protective, that is, no ACZA gets through the vinyl coating. In either case, since VA = A, the vinyl coating tested does not appear to be preventing or reducing the level of toxic impact on organisms growing on ACZA-treated pilings: either there was no toxic impact on these organisms to start with (explanation 1), or the impact on these organisms from ACZA is blocked but is replaced by an equally toxic impact from the vinyl (explanation 2).

In all, thirty eight distinct ANOVAs were conducted to test for significant differences between treatments[2] for different measurements and deployments (Table 8).[3] The analyses of Total Cover, Total Biomass and Total Species Richness over all deployments, and 8 of the 9 analyses of these measurements over individual deployments, found no significant differences between treatments (Table 8), and thus are inconsistent with the expectations expressed by Equations 1 or 2. There was a significant difference only for Total Cover in Deployment B, with the ACZA-treated panels having less cover than the other treatments (see Fig. 5). This is consistent with Equation 1, suggesting a toxic effect of ACZA and an effective protective effect of the vinyl coating.

The other 26 analyses addressed Cover or Biomass measurements for individual species or species groups. Nineteen of these found no significant difference between treatments, and thus are inconsistent with the expectations expressed by Equations 1 or 2. Three analyses—of barnacle cover for Deployments B and C and for all deployments—found significantly higher barnacle cover on ACZA-treated panels than on vinyl-coated untreated panels, which is contrary to both Equations 1 and 2. Two analyses—of *Watersipora* cover for Deployment B and for all deployments—found

[2] Although 38 statistical analyses were conducted it would not be appropriate to apply a Bonferroni adjustment to the value for statistical significance because the null hypotheses are not independent, rather there is a common null hypothesis of no difference in the fouling community between the three treatments. Instead the set of analyses should be considered in an integrated fashion to assess whether there is persuasive evidence for rejecting the null hypothesis (Motulsky 1995). This is done here.

[3] Five additional ANOVAs were conducted for Photo Cover in Deployment A (for Total Cover, Barnacles, Arborescent Bryozoans, *Schizoporella* and *Watersipora*), but since Photo Cover measures the same parameter as Cover by a different means, and since both the Photo Cover and Cover analyses in Deployment A found no significant differences between treatments, the Photo Cover analyses were considered duplicative and were not included in Table 8.

Table 8. Implications of statistical analyses. Treatments are: VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated. In Implications: > or < means that the measured quantity is significantly greater or less; = means there is no significant difference. Photo Cover was measured for Deployment A but is not listed here since the resuts were the same as for Cover.

Analysis	Deployment	Implications
Total Cover	A	VU = VA = A
	B	VU = VA > A
	C	VU = VA = A
	All	VU = VA = A
Total Biomass	A	VU = VA = A
	B	VU = VA = A
	C	VU = VA = A
	All	VU = VA = A
Total Species Richness	A	VU = VA = A
	B	VU = VA = A
	C	VU = VA = A
	All	VU = VA = A
Cover - Barnacles	A	VU = VA = A
	B	A > VU
	C	A > VU
	All	A > VU
Biomass - Barnacles	A	VU = VA = A
Cover - arborescent bryozoans	A	VU = VA = A
	B	VU = VA = A
	C	VU = VA = A
	All	VU = VA = A
Biomass - arborescent bryozoans	A	VU = VA = A
Cover - Schizoporella	A	VU = VA = A
	B	VU = VA = A
	C	VU = VA = A
	All	VU = VA = A
Biomass - Schizoporella	A	VU = VA = A
Cover - Watersipora	A	VU = VA = A
	B	VA > VU = A
	C	VU = VA = A
	All	VA > VU
Biomass - Watersipora	A	VU = VA = A
Cover - Distaplia	B	VU = VA = A
	C	VU > VA = A
	All	VU > VA = A
Cover - other colonial tunicates	B	VU = VA = A
	C	VU = VA = A
	All	VU = VA = A

significantly higher cover on vinyl-coated ACZA-treated panels than on vinyl-coated untreated panels, which is also contrary to both Equations 1 and 2; however, one of these, analyzing Deployment B, found significantly higher *Watersipora* cover on ACZA-treated panels with vinyl coating than those without, which is consistent with both Equations 1 and 2, suggesting a protective effect of vinyl coating. Finally two analyses—of *Distaplia* cover for Deployment C and for all deployments—found significantly higher cover on vinyl-coated untreated panels than on panels given the other two treatments, which is partly consistent with both Equations 1 and 2, suggesting a toxic effect of ACZA. In no analysis, however, was the ranking of the three treatments the same as either Equation 1 or 2.

Overall, these analyses do not provide evidence that ACZA has a toxic or inhibitory effect on fouling growth. The most compelling individual statistical finding is that Total Cover in Deployment B was lower on ACZA-treated panels than in other treatments, with the difference being extremely significant (Table 5) and apparent in the graph (Fig. 5). However, the other deployments did not produce similar results: the differences between treatments were not significant (Table 5) and the lowest mean value for Total Cover was not on ACZA-treated panels in Deployments A and C (Table 4). A compelling group of findings is that barnacle cover was highest on ACZA-treated panels in Deployments B and C and over all deployments (and significantly higher than on the vinyl-coated untreated panels) (Table 6). Mean barnacle cover was also highest on ACZA-treated panels in Deployment A, though not significantly so (Appendix A1). The reason for this apparently higher settlement, survival or growth of barnacles on the presumably more toxic ACZA-treated panels is not obvious. There could be an indirect effect via competition—for example, if barnacles are relatively insensitive to ACZA treatment but the treatment reduces settlement of other organisms, then greater settlement of barnacles could result—however, no consistent negative effect on other organisms can be seen in these results.

There three ways in while the extent of fouling growth on different piling treatments may be related to potential effects on herring spawning. First, inhibitory effects on fouling due to the toxicity of piling treatments might indicate the potential for toxic effects on herring eggs spawned on piling surfaces. Second, herring can spawn directly onto fouling organisms and some fouling species, especially those forming large 3-dimensional or arborescent structures, may increase the surface area available for herring eggs to adhere to, so impacts on fouling can affect the amount of available spawning substrate. Third, if piling treatments do have toxic effects on herring eggs, then fouling organisms may have a protective effect, by shielding eggs from direct contact with the surfaces of treated pilings. However, the overall results of this study suggest that ACZA-treatment of pilings has little or no effect on fouling growth (except possibly for some promotion of barnacle species), and thus is expected to have little effect on herring spawning.

Several cautions are in order, however. While these experiments were designed to use fouling growth as a proxy for assessing the toxic effects of piling treatments to herring eggs, it’s possible that herring eggs have different sensitivities to ACZA or vinyl than these fouling organisms do. It's also possible that spawning adult herring could respond

to ACZA-treated or vinyl-coated surfaces in a way that reduces or possibly increases their tendency to spawn on these surfaces, or that the adhesion of herring eggs to the piling surface is affected by biocide or surface treatments. Finally, the presence of fouling organisms may indirectly affect the number and survival of herring eggs on pilings by affecting adult response or egg adhesion, by making eggs more or less visible to predators and more or less easy for predators to remove, by increasing spawning substrate or by shielding eggs from toxic surfaces as discussed above, or by other subtle or indirect effects. Thus, the response of fouling growth to biocide and surface treatments may have more than proxy significance for the success of herring spawning.

In contrast to the data on fouling growth, the data on woodborer occurrence and damage is very consistent with an expected inhibitory effect from both ACZA treatment and vinyl coating. Untreated panels were heavily damaged and largely missing, while panels with either or both AZCA treatment and vinyl coating were not (Table 9). Among the treated panels, which generally showed little or no exterior damage, the frequency and impact of shipworm and gribble borers assessed in via sawn cross-sections was greatest in vinyl-coated untreated panels, less in ACZA-treated panels, and nil in panels that were both ACZA-treated and vinyl coated (Table 7).

Table 9. Mean percent of panel remaining. Treatments are: U=untreated; VU=vinyl-coated untreated; A=ACZA-treated; VA=vinyl-coated ACZA-treated.

Deployment	Treatment			
	U	VU	A	VA
A	42	100	100	100
B	13	100	100	100
C	3	96	100	100
All	13	98	100	100

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References

Motulsky, H. 1995. *Intuitive Biostatistics.* Oxford University Press, New York.

Poston, T. 2001. *Treated Wood Issues Associated with Overwater Structures in Marine and Freshwater Environments*. White paper submitted to Washington Department of Fish and Wildlife, Washington Department of Ecology, and Washington Department of Transportation, Battelle.

Stratus Consulting, Inc. 2006. *Creosote-Treated Wood in Aquatic Environments: Technical Review and Use Recommendations*. Prepared for NOAA Fisheries, Southwest Division, habitat Conservation Division, Santa Rosa, CA.

Taso, R.N., P.T. Phillips, G. Ichikawa, G. Isaac and N. Paszty. 1996. R*eport on Creosote/Pacific Herring Studies in San Francisco Bay for the 1994-95 Spawning Season*. California Department of Fish and Game, Sacramento, CA.

Vines, C.A., T. Robbins, F.J. Griffin and G.N. Cherr. 2000. The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). *Aquatic Toxicology* 51: 225–239.