Report on Fouling Panel and Piling Studies

DAARP Settlement Restoration Program

Toxicity Monitoring for the Pacific Herring Spawning Enhancement Project

Andrew N. Cohen

Center for Research on Aquatic Bioinvasions
Richmond, California

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Executive Summary

- Fouling panel studies and piling sampling were conducted at piers at the Port of San Francisco in 2007-2010 to assess the effect of ACZA and creosote treatments and vinyl covering on the surface coverage, biomass and species richness of fouling organisms, as a proxy for impacts of different piling treatments on Pacific herring eggs and as an indicator of the type of substrate available for spawning.

- There were some statistically significant differences between ACZA-treated and vinyl-covered panels in surface cover by some organisms in some deployments, with ACZA-treated panels having greater coverage by barnacles in some cases, and vinyl-covered panels having greater coverage by a bryozoan (*Watersipora*), a tunicate (*Distaplia*), or all organisms in some cases. There were no significant differences in biomass or species richness.

- There were no significant differences in biomass or species richness of attached organisms between older creosote-treated pilings, newer ACZA-treated pilings and vinyl-covered pilings. Species richness of all organisms (attached and mobile) was significantly greater on ACZA-treated pilings than on creosote-treated pilings, but on neither of these was total species richness significantly different from that on vinyl-covered pilings.

- Overall, these studies do not provide support for the hypothesis that ACZA or creosote treatment of pilings interferes with the attachment of organisms or harms the organisms attached to them.

- Similarly, a review of the literature yielded no clear or consistent evidence that ACZA or creosote treatment of pilings interferes with the attachment of organisms or harms the organisms attached to them.

- A review of the two studies that investigated the impact of creosote-treated pilings on Pacific herring eggs spawned on them, which have been cited as demonstrating that creosote pilings have negative impacts on Pacific herring, found that the concentrations of creosote-derived leachate in the test water in these experiments were either much higher (by ≈4-5 orders of magnitude) or possibly much higher than concentrations measured near eight-month-old creosote pilings in a field study. There is thus no evidence that herring eggs spawned on creosote-treated pilings—especially on older creosote-treated pilings—would be negatively affected, or that creosote-treated pilings have an overall impact on Pacific herring populations.

- Four types of studies are recommended to develop the information needed to determine whether creosote-treated pilings—or other piling treatments—might have a negative impact on Pacific herring populations: (1) studies of the proportion of spawned herring eggs on the wood surfaces of pilings, on the organisms attached to pilings, and on other substrates; (2) measurements of the concentrations of leached compounds near treated pilings, including newer and older treated pilings, and pilings

with and without extensive cover by fouling organisms; (3) laboratory experiments exposing artificially spawned Pacific herring eggs to different piling treatments, with leachate concentrations in the test water that are consistent with those measured near treated pilings in the field, with endpoints of hatching success, incidence of developmental abnormalities, or other physiological measures; and (4) field experiments of naturally spawned Pacific herring eggs on test panels with different wood treatments, with endpoints of hatching success, incidence of developmental abnormalities, or other physiological measures.

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Project Overview and Summary

This project consists of two studies conducted at the Port of San Francisco, one involving fouling panels attached to pilings at Pier 45, and other involving the direct sampling of pilings at Piers 1/2 and 45. The objective was to assess the effect of different piling treatments on the diversity, cover and biomass of attached marine organisms (invertebrates and algae). The response of attached organisms was investigated both as a proxy for the response of herring eggs spawned on pilings, and as an indicator of the suitability of pilings for herring spawning (since attached organisms also serve as substrate for spawning).

This section provides a summary of the project, a review of the relevant literature and a discussion of the project's overall implications. The sections that follow (**Part 1. Fouling Panels** and **Part 2. Piling Sampling**) provide the detailed methods, results and discussion for the two studies.

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 and many of the compounds that make up creosote (primarily polycyclic aromatic hydrocarbons (PAHs)) are toxic to the eggs and larvae of fish and invertebrates causing mortality, developmental problems and reduced viability (Poston 2001; Stratus 2006). Two studies have specifically reported toxicity to herring eggs spawned on creosoted timbers (Tasto *et al.* 1996; Vines *et al.* 2000). Wooden pilings are now treated with various water-soluble compounds that are thought to be less harmful to marine life in general; ammoniacal copper zinc arsenate (ACZA) is the compound most commonly use on pilings in marine waters on the Pacific Coast of North America.

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 Plan

The project evaluated the growth of attached marine organisms on fouling panels and pilings that had been given different treatments, 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 was 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. Changes were then made in the plan, modifying and shortening the period of work with fouling panels, and adding the direct sampling of pilings in order to include creosote-treated pilings in the assessment.[1]

Results and Discussion

In general, the results of this study showed that unprotected wood structures are vulnerable to extensive damage from wood-boring marine organisms at the Port of San Francisco; that treatment with ACZA, vinyl spray-coating, or both provide substantial protection to wood; and that there is no great difference in the amount of coverage, biomass or species diversity of organisms that grow on these different treatments or on older creosote-treated pilings. Significant differences were noted between some of these treatments in a few cases (Table 1), but overall there was no consistent pattern suggesting that one treatment or covering was more or less toxic or more or less inhibitory to organism growth than any other. These results are discussed in more detail in **Part 1. Fouling Panels** and **Part 2. Piling Sampling** below.

There has been little study of the effect of piling treatments on the survival or growth of organisms living on or attached to pilings. Of most direct relevance to the Pacific Herring Spawning Enhancement Project, Tasto *et al.* (1996) and Vines *et al.* (2000) investigated the impact of creosote-treated pilings on herring eggs spawned on pilings. These appear to be the only studies that have investigated the effect of any piling treatment on fish eggs spawned on pilings, and Vines *et al.* (2000) is regularly cited in the literature on the impacts of piling treatments.

[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). Both phases of the fouling panel work were covered in a second report: Cohen, A.N. 2010. *Report on Fouling Panel Studies, DAARP Settlement Restoration Program, Toxicity Monitoring for the Pacific Herring Spawning Enhancement Project* (June 10, 2010). The data and analyses from those reports are incorporated in this report.

Table 1. Analyses from the fouling panel and piling studies yielding statistically significant results. In all, 26 analyses of cover, 4 analyses of biomass and 4 analyses of species richness addressing 3 panel treatments in 3 deployments and all deployments of fouling panels; plus 1 analysis of biomass and 3 analyses of species richness addressing 3 piling treatments in 1 sampling were conducted. Significance and differences were determined by ANOVA with *post-hoc* Tukey HSD, or by nonparametric ANOVA (Kruskall-Wallis) with *post-hoc* graphic assessment of differences where data could not be normalized, with $\alpha = 0.05$. Except for the results listed here, no significant differences were found between any treatments. A = ACZA-treated panel or piling, C = creosote-treated piling, V = Vinyl-covered piling, VA = Vinyl-covered ACZA-treated panel, VU = Vinyl-covered untreated panel.

Study	Analysis	p	Significant Differences
Fouling Panels-Deployment B	Cover-Barnacles	0.03	A > VU
Fouling Panels-Deployment C	Cover-Barnacles	0.03	A > VU
Fouling Panels-All	Cover-Barnacles	0.002	A > VU
Fouling Panels-Deployment B	Cover-Watersipora	0.007	VA > A, VA > VU
Fouling Panels-All	Cover-Watersipora	0.03	VA > VU
Fouling Panels-Deployment C	Cover-Distaplia	0.04	VU > A, VU > VA
Fouling Panels-All	Cover-Distaplia	0.02	VU > A, VU > VA
Fouling Panels-Deployment B	Cover-Total Cover	0.001	VA > A, VU > A
Pilings	Species Richness 2	0.03	A > C

Tasto *et al.* (1996) described three experimental approaches tried by CDFG. In the first, two attempts were made to enclose naturally spawned herring eggs within Nitex mesh on old creosote-treated pilings and concrete pilings at Fort Baker Marina in San Francisco Bay, with the intent of observing and comparing the development and hatching success. In the first attempt the edges of the mesh were inadequately secured and larvae escaped; in the second, an “unidentified blight” affected the eggs. No data were reported from these efforts.

In the second experimental approach, four types of panels (wood recently treated with creosote, untreated wood, concrete and recycled plastic) were placed in the water at Sausalito in San Francisco Bay, then collected after herring had spawned on them, transported to the laboratory, and placed in 2-liter beakers with either “static” water conditions (water replaced every 24 hours) or flow-through (20 ml/min) water exchange. The number of eggs that hatched and did not hatch were observed and compared among panel types.

Two trials using this approach (designated A and B) were conducted using two light spawns in February and March, with either a single panel or no panel of each type used in each trial. For Trial A with flow-through conditions, no results for untreated wood or recycled plastic panels were reported. For Trial A with static conditions, no results were reported for recycled plastic panels, but results for eggs spawned on the polypropylene

line that the panels had been attached to were reported instead. For the Trial B experiments, results were reported for all four panel types. In Trial B, observations of hatching were aborted after three days because of flooding at the laboratory.

Tasto *et al.* (1996) did not test the significance of Trial A's results because of small sample numbers per cell. Computational resources are now available to conduct exact tests of independence and to calculate precise p values, and I used these to further analyze the study's results. These analyses along with the original analytical results for Trials A and B are provided in Tables 2 and 3.

Table 2. Initial statistical analyses of results from Tasto *et al.* (1996). One-tailed Fisher's Exact test for 2x2 table conducted with the calculator at http://www.langsrud.com/fisher.htm for Trial A Flow-through; other analyses for this report conducted with the calculator at http://udel.edu/~mcdonald/statchiind.html. Treatments in the second column are listed in order from highest to lowest hatching success; Co = concrete panel, Cr = creosote-treated wood panel, Li = plastic line, Pl = recycled plastic panel, Un = untreated wood panel. Significance reported as: NS = not significant; * = <0.05; ** = <0.01; *** = <0.001.

Trial	Analysis of	Analysis by	Analysis	p	Significance
Trial A Flow-through	All results (Co,Cr)	Tasto *et al.* (1996)	None conducted	–	–
		This report	Fisher's Exact	0.0528	NS
Trial A Static	All results (Li,Co,Cr,Un)	Tasto *et al.* (1996)	None conducted	–	–
		This report	Fisher's Exact	<0.001	***
Trial B Flow-through	All results (Co,Un,Pl,Cr)	Tasto *et al.* (1996)	Chi-square	<0.05	*
		This report	Chi-square	<0.001	***
Trial B Static	All results (Un,Co,Pl,Cr)	Tasto *et al.* (1996)	Chi-square	<0.05	*
		This report	Chi-square	<0.001	***

In the third experimental approach, herring eggs spawned on kelp were obtained from commercial roe-on-kelp operations at Sausalito, trimmed to pieces approximately 2" square, attached to two types of panels (wood recently treated with creosote and concrete) with small-gauge Nitex mesh with the eggs held in contact with the panel surfaces, and placed in the water at Sausalito for seven days with each panel enclosed in small-gauge nylon mosquito netting. After retrieval and fixing, the eggs were counted and classified as developed (showing either evidence of hatching or the presence of eyed-larvae), or not developed (opaque unhatched eggs or larvae that had not reached the eyed stage). A much larger fraction of the eggs were classified as developed when held in contact with concrete than in contact with creosote-treated wood, an association that statistical tests showed to be significant (re-analysis using chi-square test of independence yielded p <<0.001).

Overall, the experiments in Tasto *et al.* (1996) indicated a negative effect of creosote-treated wood on the development or hatching success of herring eggs that were in contact with the wood, under these test conditions; however, these results were not consistent or consistently strong, differences in handling of the creosote-treated panels

Table 3. *Post-hoc* analyses of results from Tasto *et al.* (1996). Fisher's Exact test conducted using calculator at http://www.langsrud.com/fisher.htm, with one-tailed tests used for comparisons of creosote-treated wood and other panel types (with expected lower hatching success with creosote treatment), and two-tailed tests in other cases. Treatments in the second column are listed in order from highest to lowest hatching success; Co = concrete panel, Cr = creosote-treated wood panel, Li = plastic line, Pl = recycled plastic panel, Un = untreated wood panel. Significance reported as: NS = not significant; * = <0.05; ** = <0.01; *** = <0.001.

Trial	Analysis of	Analysis by	Analysis	p	Significance
Trial A Static	Cr, Un	This report	Fisher's Exact 1-tailed	0.6723	NS
	Co, Cr	This report	Fisher's Exact 1-tailed	0.3177	NS
	Li, Co	This report	Fisher's Exact 2-tailed	<<0.0001	***
Trial B Flow-through	Un, Cr	Tasto *et al.* (1996)	Chi-square	<0.05	*
	Co, Un, Pl	Tasto *et al.* (1996)	Chi-square	>0.05	*
	Un, Cr	This report	Fisher's Exact 1-tailed	0.0001	***
	Co, Cr	This report	Fisher's Exact 1-tailed	0.000009	***
	Pl, Cr	This report	Fisher's Exact 1-tailed	0.0369	*
	Un, Pl	This report	Fisher's Exact 2-tailed	0.1063	NS
Trial B Static	Un, Cr	Tasto *et al.* (1996)	Chi-square	<0.05	*
	Un, Co, Pl	Tasto *et al.* (1996)	Chi-square	<0.05	*
	Un, Cr	This report	Fisher's Exact 1-tailed	0.000013	***
	Co, Cr	This report	Fisher's Exact 1-tailed	0.00008	***
	Pl, Cr	This report	Fisher's Exact 1-tailed	0.2509	NS
	Un, Pl	This report	Fisher's Exact 2-tailed	0.0062	**

may have caused or contributed to the negative effect observed for these panels in Trial B, and the test conditions do not appear to reflect field conditions appropriately. Herring eggs developed poorly or failed to hatched when in contact with creosote-treated wood compared to untreated wood or concrete in Trial B and the roe-on-kelp experiment, but there were no significant differences between creosote-treated wood and untreated wood or concrete in Trial A; and in the static trial of Trial A the percentage of eggs that hatched was slightly greater on creosote-treated wood than on untreated wood (though the difference was not significant).

Hatching success was extremely significantly lower on creosote-treated wood than on untreated wood or concrete in Trial B, but in that trial the eggs on creosote-treated panels were transported to the laboratory inside ziplock bags, while the eggs on other panels were not enclosed in ziplock bags (Tasto *et al.* 1996, at page 4). Transport time to the laboratory was reported as 7 hours, and the total time spent enclosed in ziplock bags was presumably somewhat longer. Thus the eggs on creosote-treated panels may have been exposed to lower oxygen levels than the eggs on the other panels, and were likely also exposed to high concentrations of creosote leachate, during the period between collection in the field and the placement of the panels in test beakers in the laboratory. These differences in handling and transport conditions could be responsible

for the differences in hatching and development between the creosote-treated panels and the other panels.

Finally, the test conditions do not appear to be appropriate for applying these results to field conditions, for two reasons. First, the tests used recently-treated creosote panels, while most or all pilings of potential concern to the Pacific Herring Spawning Habitat Enhancement Project are older pilings, which would be expected to have lower concentrations of creosote compounds near the outer wood surface and lower rates of leaching of creosote compounds (Bestari *et al.* 1998; Poston 2001; Stratus 2006b; Werme *et al.* 2009; Brooks 1994 modeled leaching rates from pilings declining exponentially with the age of the piling, decreasing to 14% of initial values at 20 years, however field observations by Goyette & Brooks 2001 suggest that the actual decline in leaching rates may be greater). Creosote-treated pilings were banned from use in California waters in 1993 (Werme *et al.* 2009) and the creosote pilings at the Fort Baker Marina, the site of Tasto *et al.*'s first experimental approach, were approximately 35 years old at the time of that study (Vines *et al.* 2000).

Second, the test conditions (aside from the transport conditions) exposed the eggs on the creosote-treated panels to water with possibly much higher concentrations of dissolved and total PAHs and other potentially toxic creosote-derived compounds than they would be exposed to if spawned on creosote-treated pilings in the field. This is due to the recent creosote-treatment of the test panels, the relatively high volume of treated wood relative to the volume of water in the test beakers, and the relatively static, contained water conditions in the 2-liter test beakers. The creosote-treated wood in these beakers had a volume of 197 cm^3 and exposed wood surfaces totaling at least 265 cm^2, and were placed in approximately 1,800 cm^3 of water (assuming that wood volume + water volume = 2 liters). Scaling up to field conditions, this would correspond to a rectilinear grid of 30-cm diameter pilings spaced 0.85 m from center to center (if scaled by wood volume), or 2.5 m from center to center (if scaled by exposed surface area). (It's unclear whether scaling by volume or by exposed surface area is more appropriate.) The former density is probably never encountered in the field except over very small areas (*e.g.* within a dolphin), while the latter density can be encountered under wharves. However, piling installations in herring spawning areas such as in San Francisco Bay are typically within water bodies that are free of pilings or other creosote-treated structures over most of their areas, and continuous water movement resulting from the twice daily rise and fall of the tides, wind forcing, river inflows and gravitational circulation, and vessel movements would tend to prevent any substantial build-up of compounds leached from treated pilings in the adjacent water column. In contrast, within the test beakers in the static tests build-up of leachate was allowed to proceed for 24 hours between water changes. In the flow-through tests, a complete water exchange took 90 minutes if the water in the beakers was well-mixed; if not well-mixed, part of the water could be exchanged while part of the water continued to accumulate leachate. The concentration of creosote-derived compounds was not measured in the water in the test beakers, so the possibility of excessive exposure cannot be checked.

The third experimental approach in Tasto *et al.* (1996) has a similar problem of (1) placing the eggs in contact with wood that was recently treated with creosote rather than aged creosote-treated wood that would be more typical of field conditions, and (2) possibly exposing the eggs that were in contact with creosote-treated wood to excessively high concentrations of creosote-derived compounds in the water. In this case the latter concern arises from the combination of using recently-treated wood and confining the eggs in a small space between the wood and a kelp frond, wrapping them in small gauge Nitex mesh, and then enclosing them within a bag of nylon mosquito netting, all of which would tend to trap leachate in the water around the eggs. Again, there was no measurement of creosote-derived compounds in the test water and thus no way to check whether the test conditions caused excessive exposure to these compounds.

In the second study of herring eggs and creosote pilings, Vines *et al.* (2000) conducted two germane experiments. In the first, three groups of naturally spawned herring eggs (eggs that remained attached to pieces of an approximately 40-year-old creosote-treated piling, eggs that were removed from the piling, and eggs removed from a nearby PVC pipe) were collected from the Fort Baker Marina about 2 days before the onset of hatching, transported to the laboratory, and cultured for 2-4 days in water that was changed daily. The results were striking: 96% of the eggs from the PVC pipe hatched with 90% exhibiting normal morphology; only 24% of the eggs removed from the creosote-treated piling hatched, all of which exhibited morphological abnormalities; and none of the eggs that remained attached to pieces of the piling hatched. However, the sample sizes (which were not stated) were too small to analyze statistically. In addition, as in Tasto *et al.* (1996), the test conditions could have caused excessive exposure to creosote-derived compounds in the test water for the eggs attached to pieces of the piling (neither the sizes of these wood pieces nor the volume of water they were placed in was stated), and possibly also excessive exposure of the eggs removed from the piling (depending on the details of handling and transport, which were also not given). There was no measurement of creosote-derived compounds in the test water.

In a second experiment, testes and ovaries were removed from captured Pacific herring, and the eggs were removed and fertilized on glass slides and placed into 200 ml bowls containing seawater, seawater with a wafer of untreated wood, or seawater with a wafer of creosote-treated wood from a 40-year-old piling. Eggs adhered both to the wafers and throughout the bowls. The eggs were incubated with daily water changes until hatching. Hatching success, percentage of larvae with abnormal morphology, heart rate and body movement were assessed. The eggs exposed to creosote-treated wood scored significantly worse than both the eggs exposed to untreated wood and the eggs cultured without wood in nearly all of these assessments.[2]

Again, as in the earlier experiments, test conditions may have exposed the eggs cultivated with creosote-treated wood to levels of creosote-derived compounds in the water that were excessive compared to field conditions. Although the wood was taken

[2] Interestingly, the eggs exposed to wood also had a significantly lower rate of hatching and a significantly higher rate of larval abnormalities than the eggs cultured without wood.

from an old piling, it was cut into thin (1 mm thick) wafers, and thus exposed fresh wood surfaces to the test water and—depending on the depth within the piling from which the wafers were cut—may have contained higher concentrations of creosote or a different, generally more water-soluble composition of creosote compounds than wood at the outer surface of the piling. Scaling up to field conditions, the wafers in test water would correspond to a rectilinear grid of 30-cm diameter pilings spaced 13 m from center to center (if scaled by wood volume), or 1 to 1.3 m from center to center (if scaled by exposed surface area, depending on whether the face of the wood wafer lying on the bottom of the bowl is counted as a surface from which compounds can leach). The relative volume of wood in these experiments was thus small compared to field conditions, but the relative area of exposed surfaces was large compared to typical field conditions. The static test conditions would have allowed the build-up of leached compounds to proceed for 24 hours between water changes.

In this experiment, Vines *et al.* (2000) measured the concentration of creosote in the test water by spectrofluorometric analysis with reference to excitation intensities at 292 nm. Water samples for these measurements were collected daily until the onset of hatching, though it is not stated whether these were collected immediately before water changes or at some other regular time during the daily cycle. The concentration of dissolved creosote compounds in the bowls containing creosote-treated wafers was 1.2 ± 0.3 mg/L (n=15). Unfortunately, this method wasn't used to measure creosote concentrations in the field near pilings. However, in a study in British Columbia, Goyette & Brooks (1998) measured total PAH concentrations[3] of 18-31 ng/L in the water column at 15 cm from pilings that had been freshly treated with creosote and then placed in the water for eight months at a site with very slow currents (average speed of 2.3 cm/s at 2 m depth).[4] Since PAHs typically constitute 80-90% of the compounds in creosote by weight (Stratus 2006b; Werme *et al.* 2009), the concentrations in the test water in Vines *et al.* (2000) were about 30,000-200,000 times the concentrations measured in the field near freshly creosote-treated pilings by Goyette & Brooks (1998), suggesting that the test exposures were indeed excessive.[5]

There are few other studies of the impacts of piling treatments on attached organisms. Studies by J.S. Weis and P. Weis in the 1990s focused primarily on wood treated with CCA (chromated copper arsenate), which is commonly used for pilings on the U.S. Atlantic coast; because Douglas Fir is used for most pilings on the Pacific Coast, and

[3] The sum of 16 EPA priority PAHs, including the most common PAH species in creosote, adsorbed onto polyethylene sheets over 14 days and measured by gas chromatography/mass spectrometry in the Selected Ion Mode (Goyette & Brooks 1998).

[4] For context, the background concentration measured in open water, 91 m distant from the pilings at a right angle to the main current direction, was 13 ng/L. Average total PAH concentrations (estimated as the sum of 25 PAH compounds) in Central San Francisco Bay, which includes the areas where Pacific herring spawn, are 12 ng/L (Oros *et al.* 2007).

[5] Vines *et al.* (2000) also conducted exposed herring eggs to creosote concentrations of 0.003-1.5 mg/l to determine the LC_{50} value for hatching success and the interaction of creosote and salinity. These test concentrations range from ≈100 to ≈300,000 times the concentrations measured in the field by Goyette & Brooks (1998); the LC_{50} value of 0.05 mg/l is around 1,300 to 9,000 times the concentrations measured in the field.

CCA treatment of Douglas Fir is relatively ineffective, CCA is rarely used to treat marine pilings on the Pacific Coast (Stratus 2006a). The Weis studies (summarized in Poston 2001 and Stratus 2006a) showed the following effects:

- Green algae growing on CCA Type C-treated wood accumulated copper, chromium and arsenic above levels in algae growing on rocks;
- Snails fed on such algae died;
- Oysters growing on CCA-treated wood pilings accumulated metals above levels in oysters growing on rocks and had a greater incidence of histopathological lesions;
- Snails fed on such oysters showed reduced growth; and
- Biotic communities on freshly CCA-treated wood had higher metal concentrations and lower diversity than controls, though the diversity differences disappeared after the pilings had been in the water for 2-3 months.

Raftos & Hutchinson (1997) exposed the tunicate *Styela plicata*, which is a common fouling organism on pilings[6], *in vivo* and *in vitro* for up to 9 days to the soluble fraction of creosote at concentrations of 0.001% -10% (corresponding to total PAH concentrations of ≈0.2 µg/l to 2 mg/l), with most of the results were reported for a 0.1-5% creosote solution (≈0.02-1.0 mg/l total PAH concentrations). Some changes in immune responses were recorded at various exposures. Although Raftos & Hutchinson (1997) stated that "the range of doses that were tested in this study include levels that can frequently be found in the environment...PAH levels greater than 10 times the maximum dose used here are reportedly common for harbor waters," this does not appear to be accurate for water column concentrations. For example, the highest median total PAH concentration in water recorded at any sampling site in San Francisco Bay over 1993-2001 is 147 ng/l in the Guadalupe River (Oros *et al.* 2007), which is less than 1/10,000 of the maximum dose in Raftos & Hutchinson (1997).[7] Thus the effects observed in these experiments may have been due to doses that greatly exceeded the concentrations that a tunicate growing on a piling would normally be exposed to.

Goyette & Brooks (1998) grew mussels (*Mytilus* sp.[8]) in cages suspended 0.5 m, 2 m and 10 m downstream of a dolphin constructed from 6 freshly creosote-treated pilings (treated according to industry Best Management Practices and thus designated the

[6] *Styels plicata* is present as an exotic species in southern California harbors.

[7] It's possible that Raftos & Hutchinson confused sediment concentrations and water column concentrations. Sediment concentrations of total PAHs tend to be much higher on a ppm by weight basis than water column concentrations, and in some harbors may exceed the 2 ppm maximum dose used in Raftos & Hutchinson (1997). For example, Werme *et al.* (2009) reported that average sediment concentrations of total PAHs in Central San Francisco Bay in 2002-2008 were 0.4-3.2 ppm, with a maximum concentration in a single sample of 19 ppm; and that average concentrations in Puget Sound were 0.04-7 ppm, with a maximum sample concentration of 14 ppm.

[8] The authors identified the mussels used as *Mytilus edulis edulis* but did not state where they obtained them. Given our changing understanding of *Mytilus* taxonomy on the Pacific Coast, and without knowledge of where these mussels were obtained (whether from the wild or from an aquaculture operation), the specific identity of the mussels used in these tests is uncertain.

"BMP pilings")[9], 0.5 and 2 m downstream of a dolphin constructed from 6 weathered creosote-treated pilings (obtained from a pier demolition) that were at least 5 years old, and at an open water control site with no pilings within 90 m. PAHs in mussel tissues were initially low at all sites and increased greatly at all sites after 14 days; at that point, tissue concentrations were significantly higher in mussels hanging 0.5 m from the BMP pilings than in mussels further away or at the control site. However, after six months PAH concentrations in mussel tissues were similar at all sites and similar to initial concentrations, and after a year the concentrations in mussels at 0.5 m and 2 m from the BMP pilings were slightly lower than in mussels at the control site. In terms of impacts, the study found:

- No difference in survival among the different sites after 6 and 12 months, and no difference in spawning success or larval development at 6 and 18 months;
- Poorer growth (measured as the increase in shell length after 6 months) nearer the BMP pilings than further away; and poorer growth 0.5 m from the BMP pilings than 0.5 m from the weathered pilings, which in turn showed poorer growth than mussels at the control site; and
- Better mussel condition (measured as the ratio of dry soft tissue weight to shell volume) after 6 months at the BMP and weathered piling sites than at the control site.

The results suggest that there was poorer growth with greater exposure to creosote leached from pilings, but better body condition with greater exposure. The differences in growth may be due to initially higher exposures near pilings, as indicated by the sharp increases in tissue and sediment concentrations over the first 14 days. Goyette & Brooks (1998) reported that "the pilings created a small sheen on the surface of the water during installation. This sheen extended for about a metre around rafted pilings...It is assumed that this rapid increase in PAH concentration [in sediments] was largely due to the pile driving operation, deposition of treated wood debris and presence of treated pilings rafted alongside." Thus any impact on the mussels may have been a result of the piling installation process, rather than the subsequent presence of the pilings.

Goyette & Brooks (1996) noted that mussels or other fouling organisms growing directly on the pilings could have significantly higher exposure to creosote than mussels grown in nearby cages, but the PAH concentrations in mussels collected directly from the BMP pilings approximately 4 years after the start of the experiment were below the detection limit of 20 ng/g (Goyette & Brooks 2001). A dense fouling community developed on the BMP pilings after the first year, which may have slowed the leaching of creosote compounds into the water column (Goyette & Brooks 2001).

Tarakanadha *et al.* (2004) evaluated the effect of a variety of wood treatments on fouling organisms in Krishnapatnam Harbor in India. Biomass, growth and number of individuals were greater on CCA-treated panels than on control panels, and lower on

[9] The target retention was 17 pounds of creosote per cubic foot of wood in the outer 6 cm of the piling, but the actual retention was 27 lb/ft^3 or 158% of the requirement, "further emphasizing the worst case methodology used in this analysis" (Goyette & Brooks 1998).

ACZA-treated panels than on control panels, but no statistical analysis was provided, and the control panels were excluded from the analysis after the first 3 months of the 24 month experiment because of deterioration due to wood-boring organisms.

With the use of vinyl coverings on pilings, data on the effects of plastic structural materials on fouling organisms is of interest. In Tasto *et al.* (1996)'s experiments, Pacific herring eggs had lower hatching rates on plastic panels than on untreated wood or concrete panels, with the difference being highly significant in the static trial but not significant in the flow-through trial (Tables 2 and 3). As with the creosote-treated panels, the test conditions may have resulted in the accumulation of higher concentrations of leached compounds in the test water than would likely be encountered in the field. Weis *et al.* (1992) reported reduced fertilization in sea urchin eggs exposed to leachate from recycled plastic lumber, and identified at least 14 phthalate isomers plus nonyl-phenol in the leachate. Xie *et al.* (1997) also reported phthalates in the leachate from recycled plastic[10] used in a pier in the East River in New York City.

Overall, the evidence from the literature and from the current project does not provide support for the hypothesis that the piling treatments commonly encountered in marine or estuarine waters on the Pacific Coast—old creosote pilings or newly-treated ACZA pilings—harm the organisms growing on or attached to them, or prevent the attachment of organisms, including herring eggs. First, the concentrations of potentially toxic leachate from these pilings measured in the water near pilings appears to be insignificant: creosote compounds have been measured a few ng/l above background levels (Goyette & Brooks 1998), and the concentrations of arsenic, copper and zinc measured in the water under piers containing ACZA-treated wood were not significantly different from concentrations at a reference station (Brooks 2004).[11] These findings are consistent with relatively rapid exchange of water around pilings, which would generally be expected in coastal or estuarine areas. However, there appear to have been few such measurements of leached compounds near pilings and apparently none at the ≈1 mm distance from a piling surface that would be relevant for herring eggs spawned directly on piling surfaces, or the ≈1-10 cm distance that would be relevant for herring eggs spawned on other organisms (such as barnacles, mussels, tunicates or small seaweeds) attached to pilings.

Second, laboratory experiments that have been cited as showing evidence of negative impacts on organisms attached to creosote-treated or ACZA-treated pilings appear to have been conducted with water concentrations of leached compounds that greatly exceed the low concentrations measured near pilings in the field (*e.g.* Tasto *et al.* 1996, Raftos & Hutchinson 1997, Vines *et al.* 2000). It is not possible, with results from such test conditions, to assess the impact that attachment to a treated piling might have in field conditions.[12]

[10] Primarily polyethylene and polyethylene terephthalate.
[11] Similarly, the concentrations of copper, chromium and arsenic in surface water near newly-installed bridge pilings treated with CCA were similar to background concentrations (Stratus 2006a).
[12] Similarly, a review of those studies that show negative impacts of CCA-treated wood (which is commonly used for marine pilings on the Atlantic Coast) on attached organisms (summarized in Poston

Third, field studies do not show consistent, significant impacts from attachment to treated wood or from exposure to leachate in the water column near treated wood. Goyette & Brooks (1998) reported higher PAH concentrations in the short term and poorer growth in mussels near creosote-treated pilings, but also reported better body condition in those mussels. Those results, moreover, may have been due to chemicals released during the pile driving process, when surface sheens of creosote compounds were observed. After 6 months, PAH concentrations in the tissues of mussels near pilings that had been recently treated with creosote were similar to tissue concentrations in mussels at the control site, and after 12 months were slightly lower near the pilings than at the control site. After 4 years, the PAH concentrations in mussels attached to the treated pilings were below detection limits, and a healthy fouling community had developed on the pilings (Goyette & Brooks 2001). Tarakanadha *et al.* (2004) reported poorer growth, lower biomass and fewer individuals in the fouling community on ACZA-treated wood panels than on control (untreated wood) panels, but provided no statistical analyses and the control panels deteriorated so quickly from wood borer attack that comparisons could be made only for the first three months of the experiment.[13] The current study found no significant difference in the biomass or diversity of attached organisms between wood panels treated with ACZA and panels covered with a vinyl coating (over either untreated wood or ACZA-treated wood), between old creosote-treated pilings and vinyl-covered pilings, or between ACZA-treated pilings and vinyl-covered pilings. There were significant differences in the surface coverage of panels by some organisms in some trials, with a bryozoan (*Watersipora*), a tunicate (*Distaplia*) or all organisms covering less of the panel surface on ACZA-treated than on vinyl-coated panels in four cases, and barnacles covering more of the surface on ACZA-treated than vinyl-coated panels in three cases. However, in 19 other analyses there were no significant differences in surface coverage between ACZA-treated and vinyl-coated panels.

Research Recommendations

Laboratory studies have demonstrated that exposure to creosote-treated wood is toxic to the eggs of Pacific herring when the concentration of leachate in the water is sufficiently high (Tasto *et al.* 1996; Vines *et al.* 2000). Those concentrations, however, appear to be either much higher, or possibly much higher, than concentrations encountered in the field, and it is unclear whether herring eggs attached directly to the surfaces of treated pilings—including new or old creosote-treated pilings or new ACZA-treated pilings—or attached to fouling organisms growing on such pilings would experience any toxic effect. I recommend four types of studies needed to support a valid

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2001 and Stratus 2006a) should consider the concentration of CCA-derived compounds in the test water compared to concentrations measured near CCA-treated wood in the field. That assessment has not been done here.

[13] Interestingly, CCA-treated panels had better growth, greater biomass and more individuals than did control panels during this period.

assessment of the effect of treated pilings (whether creosote, ACZA or other treatments) on Pacific herring eggs.

(1) Herring egg distribution. Assessments should be made of what proportion of Pacific herring eggs are on average spawned on pilings and what portion are spawned on other substrates; and of those spawned on pilings, what portion of the spawned eggs attach directly in contact with the piling surface and what portion adhere to organisms (including other herring eggs) that are attached to the piling surface, and at what distances from the piling surface.

(2) Leachate concentrations near pilings. Measurements should be made of the concentrations of leached compounds near treated pilings, to understand the range and distribution of concentrations in the water column that herring eggs spawned on pilings are likely to encounter. These should be made at appropriate distances (from ≈1 mm from the piling surface for eggs spawned directly on piling surfaces, to ≈100 mm or more eggs spawned on fouling organisms growing on pilings). Where possible, measurements should be made on newly-treated and installed pilings and on pilings that have been in place in the water for months, years and decades; and also near clean piling surfaces and near pilings with dense fouling cover, to assess the possibility that such cover might reduce the escape of leached chemicals (suggested by Goyette & Brooks 2001).

(3) *Laboratory experiments conducted at appropriate concentrations*. The second experimental approach of Vines *et al.* (2000)—removal and fertilization of herring eggs in the laboratory with exposure to treated wood—should be conducted with the following modifications:

- Pilot tests should be conducted to determine an appropriate mix of source wood, wood volume and surface area, water volume, and water exchange conditions to maintain concentrations of leached chemicals that are similar to those measured in the field. For these experiments, flow-through water exchange at an appropriate rate would probably be preferable to static water conditions with daily changes.
- Concentrations of leached chemicals in the water should be monitored during the experiment.
- To assess the effect of contact with treated wood and of different ages of treated piling, the fertilized eggs should be released onto the uncut, outer surfaces of treated piling wood of different ages. To maintain appropriate water concentrations of chemicals leached from the treated wood, it might be necessary to coat the cut surfaces of treated wood pieces with sealant (epoxy has been used in some experiments), however, in that case a control treatment with sealant should be included in the experiment.

(4) *Field experiments*. A modification of the methods of Tasto *et al.* (1996) would be a useful complement to the laboratory experiments. Mesh of a size capable of retaining eggs and larvae should be placed around herring eggs spawned naturally on panels prepared and placed in the field shortly before spawning. The test surfaces of panels

should consist of the uncut, outer surfaces of treated piling wood of different ages, with untreated wood controls. Concentrations of leached chemicals in the water within the mesh containment should be monitored during the experiment.

Acknowledgments

I am grateful to the Port of San Francisco for assistance with many stages of this project, including: the Port divers—Brent McClain, Kevin Patterson, Bruce Lanham and Jim Werder—for assistance with the manufacture, deployment and retrieval of the test panels and the sampling of pilings; Ozzie Camaano in the Port's sheet metal shop for fabricating the sampling devices; Tom Meisenbach for advice on study design; and Carol Bach for advice and assistance throughout the project. I also want to thank Vicki Lake at the California Department of Fish and Game for help with planning; Anna Weinstein, David Kim and David Cicale for assistance with field and laboratory work; and Aroon Melwani at SFEI for statistical analysis.

Part 1. Fouling Panels

The fouling panel study 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 study's objective was to determine whether there is a consistent difference in marine growth among treatments that may correspond to differences in toxicity.

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 tested 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 or all of each panel was missing when the panel arrays were retrieved, the untreated panels were not included in the statistical analyses.

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 4). The Port of San Francisco divers assisted with the manufacture, deployment and retrieval of panel arrays (Figure 1).

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

Table 4. 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 Richness"). 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 5. All the data were recorded on Excel spreadsheets (attached as Appendix A).

Table 5. 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 Richness 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.

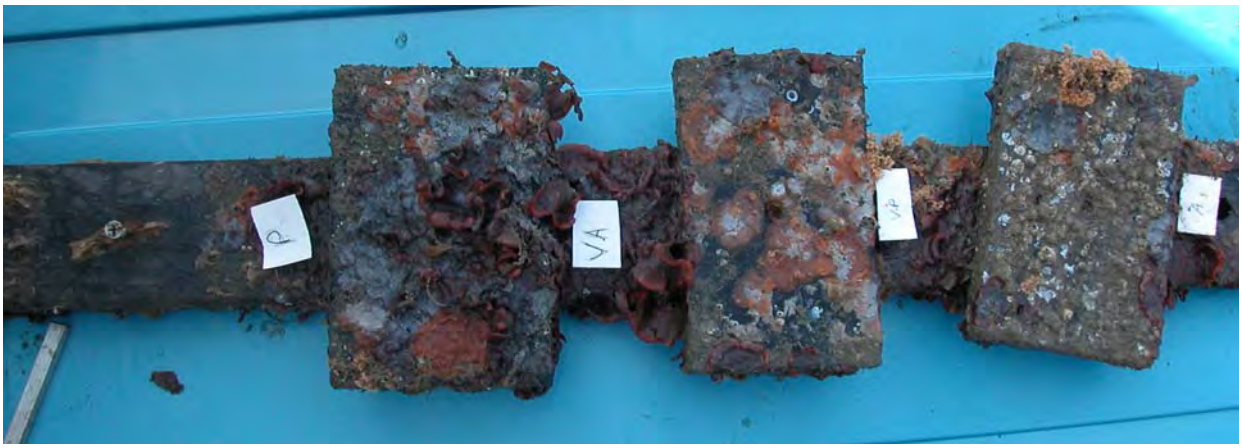
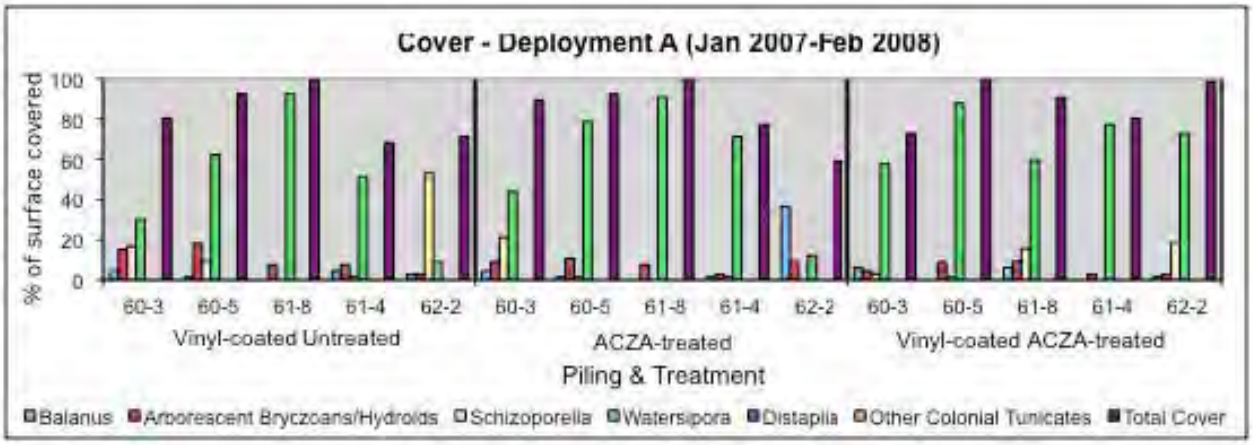


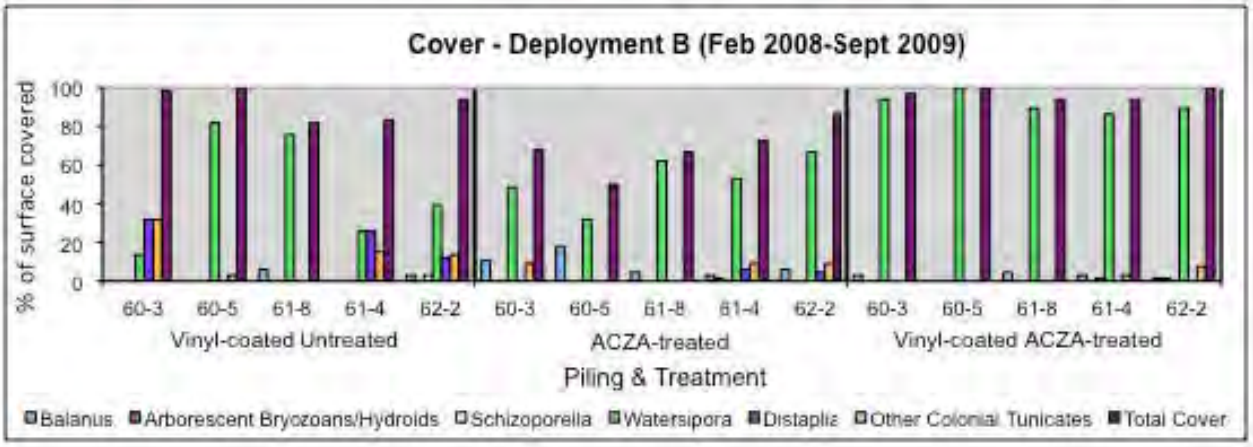
Figure 3. Panel Array 1. Panel treatments from left to right are uncoated ACZA-treated wood, vinyl-coated ACZA-treated wood, uncoated wood, and vinyl-coated wood. Most of the uncoated wood panel remains in place, but the surface has been eroded by minute wood-boring isopods called gribbles, exposing 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 6 and 7, and the statistical analysis summarized in Tables 8 and 9. 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 Richness 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.





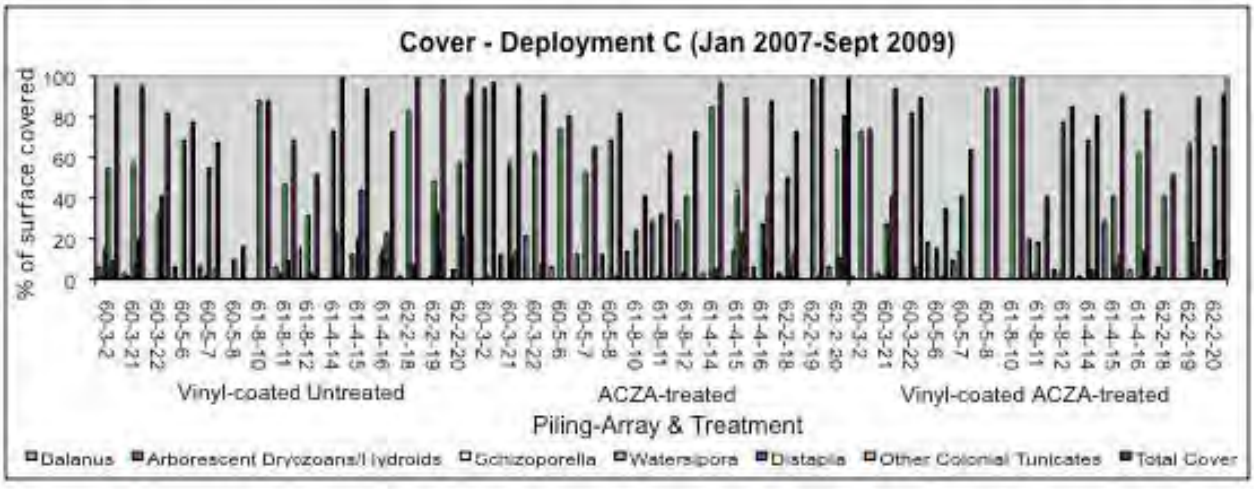


Figure 6. Total wet biomass for all deployments. Replicates are identified by piling (bent and number), and for Deployment C, array number. Numeric data are given in Appendix A.

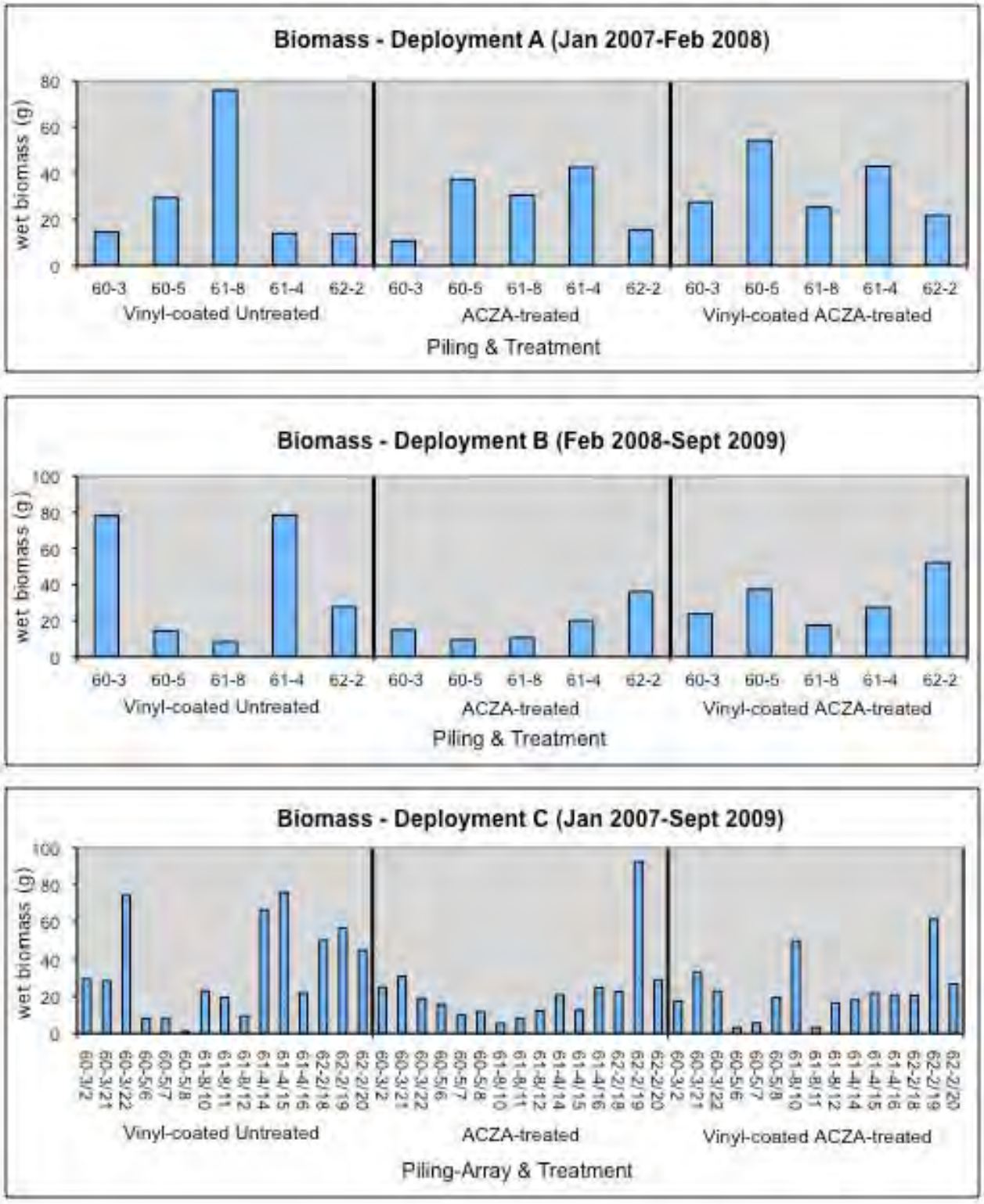
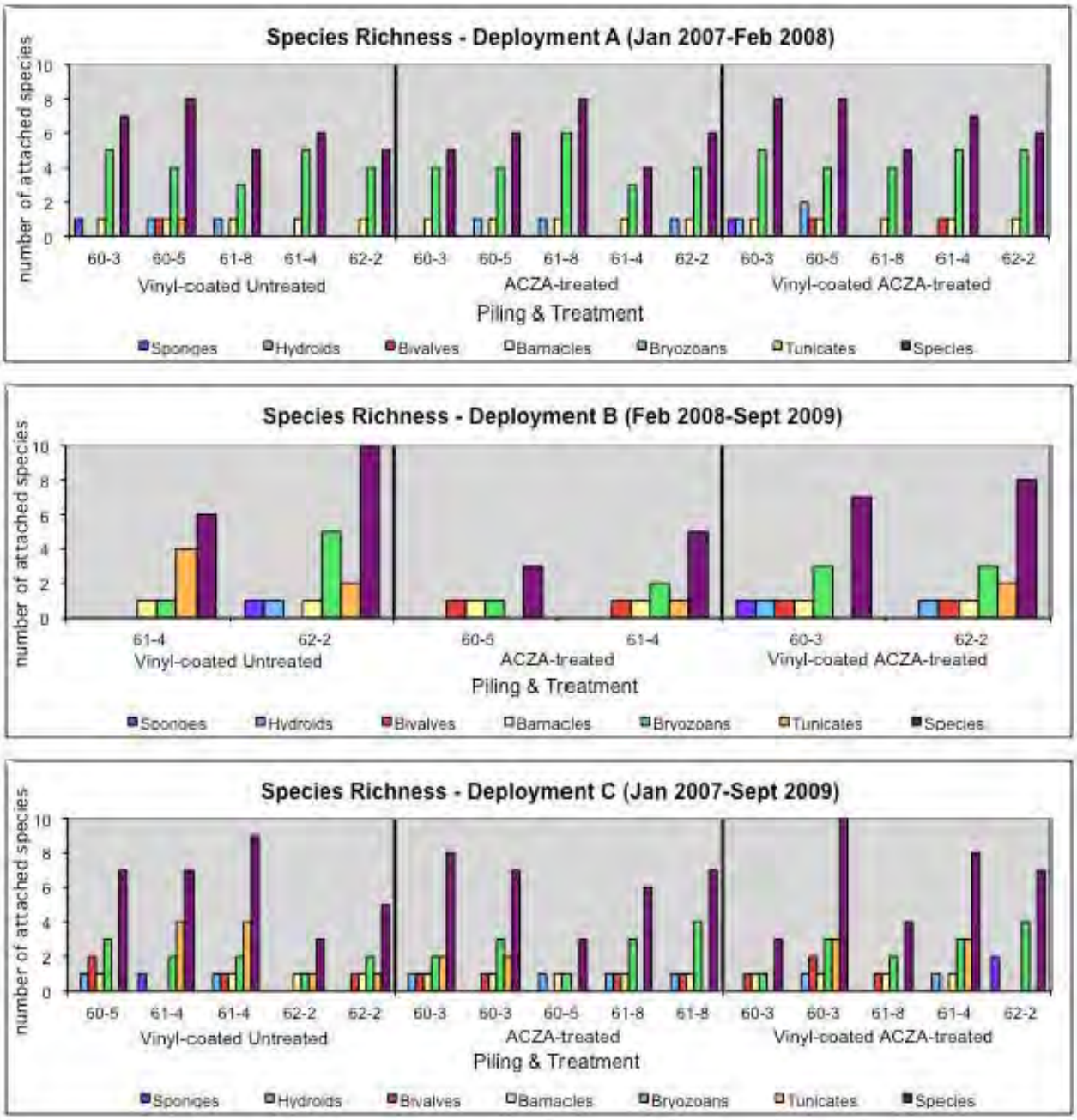
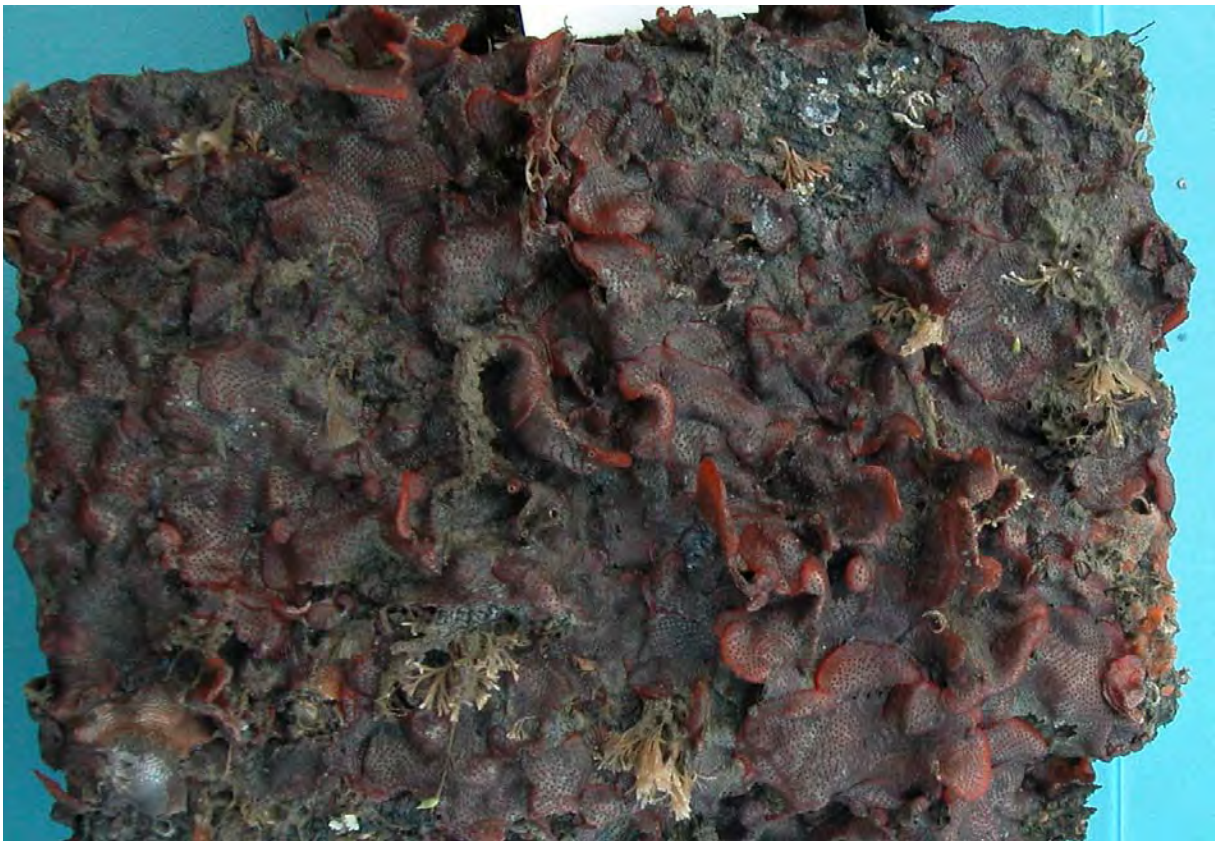


Figure 7. Total species richness and species richness for major taxonomic groups for all deployments. Replicates are identified by piling (bent and number). Numeric data are given in Appendix A.



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 6) (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 8). 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 8 and 9). 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 7 and 8).

Table 6. 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 7. 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 8. 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 9. 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 8). 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 8).

Differences in cover for some species groups were significant between some treatments in some deployments (Fig. 5; Tables 8 and 9; 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 9). *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 9; 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 10). 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 10. 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 richness 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 species richness were not available for this treatment. For the other three treatments, for all analyses of biomass and species richness, 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[14] for different measurements and deployments (Table 11).[15] 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 11), 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

[14] 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.

[15] 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 11. 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 results 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 8) and apparent in the graph (Fig. 5). However, the other deployments did not produce similar results: the differences between treatments were not significant (Table 8) and the lowest mean value for Total Cover was not on ACZA-treated panels in Deployments A and C (Table 7). 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 9). 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 are 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 12). 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 10).

Table 12. 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

Part 2. Piling Sampling

After the first phase of the fouling panel work was completed, the second phase of the work was redesigned to include a piling sampling component. We had been unable to obtain suitable creosote-treated material to use in manufacturing fouling panels, and a study (funded by the California State Coastal Conservancy) had been initiated to investigate the possible removal of abandoned creosote-treated pilings and other structures from San Francisco Bay (Werme *et al.* 2009). It was thought advisable to conduct some initial assessment into the effect of creosote treatment of pilings on marine life, and so the second phase of work sampled the attached organisms on vinyl-covered pilings, ACZA-treated pilings and creosote-treated pilings, both to check the results of the fouling panel study and include creosote as a treatment.

Methods

The attached organisms growing on ACZA-treated, vinyl-covered and creosote-treated pilings at the Port of San Francisco were sampled and analyzed for statistically-significant differences in biomass or species richness.

Sampling methods. In November 2010, we sampled pilings with the three types of treatments, working from a boat with a scraper-and-sieve sampling device on the end of a pole. The device consisted of a steel box measuring 15 x 10 x 10 cm, open on top, with the bottom consisting of an insert with rows of closely-space perforations 3 mm in diameter. One long edge at the top of the box was sharpened to a scraper edge, and a handle with a socket was attached at a slight angle to the opposite of the box. The device was fabricated with the assistance of the Port of San Francisco's sheet metal shop. Various lengths of wooden poles and a 16' fiberglass extension pole that could be threaded into the socket were used to sample at a roughly constant depth as tide levels changed.

During sampling, the device attached to the end of an appropriate length of pole was lowered to approximately MLLW, the scraper edge pressed firmly against a piling, and the device pulled upward along the piling through a stroke of about 40 cm. Samples were rejected if there was not a smooth, uninterrupted upward pull with contact between the scraper edge and piling felt throughout the stroke. This happened most often with creosote pilings, where fairly commonly the piling was partly eaten way or entirely missing at some point beneath the water surface. At the top of the sampling stroke, the sampling device was pulled away from the piling and brought steadily to the surface, where its contents were emptied into a sealable plastic bag along with an identifying label. The sealed bags containing the samples were placed in insulated coolers and kept cold until they were processed in the laboratory. Qualitative examination of pilings after taking test samples at or near the water surface indicated that the device was equally effective at removing organisms from pilings with all three treatments.

Sampling was restricted to pilings along the outer edges of the wharves, for two reasons. First, ACZA-treated pilings without vinyl coverings were generally available only as fender pilings on the outer edges of wharves, and we decided to only sample pilings with the other two treatments that were also located along the outer edges of wharves, and thus were exposed to similar light conditions. Pilings located underneath the wharves (which included vinyl-covered and creosote-treated pilings, but no ACZA-treated pilings without vinyl coverings) were in lower light conditions, which would be expected to result in lower algal diversity and biomass, which would also produce different conditions for invertebrates. Other factors, such as the abundance or behavior of fish or other predators, can also differ between sites underneath and along the edges of wharves (Able *et al.* 1998; Duffy-Anderson and Able 1999; Clynick 2007; Nightingale & Simenstad 2001; Cohen 2008). Second, due to the use of a scraper on a pole, sampling pilings underneath the wharves would have been difficult, especially at higher tide levels.

Examination, identification and quantification. In the laboratory, each sample was removed from its plastic bag, any non-organism material was removed (including bits of wood and some hard tarry material in some samples), and the samples were blotter dried and weighed. An initial, visual (by eye with a 10x hand lens) assessment was made of the number of distinct attached taxa in each sample (reported as "Species Richness 1"); these visually distinct taxa were then examined under a stereo-microscope (40x-100x) by the lead researcher to identify the organisms to the lowest possible taxon. Standard taxonomic references and reference specimens from previous San Francisco Bay taxonomic surveys were used as needed to confirm identifications. In a subset of the samples, the entire sample was examined microscopically to determine the number of distinct attached and mobile organisms, to provide two additional (though non-independent) measures of diversity (all organisms reported as "Species Richness 2", and attached organisms only reported as "Species Richness 3").

Statistical analysis. The two sampling sites (Pier 45 and Pier ½) and two types of vinyl covering (flexible and rigid) were tested with Student's t tests for significant differences in biomass or species richness, and combined for further analysis if differences were not significant. Biomass and species richness (the latter at three levels) were then compared across three piling treatments (vinyl-covered, ACZA-treated and creosote-treated) using one-way analysis of variance (ANOVA) or non-parametric ANOVA (Kruskall-Wallis) as appropriate to the data. In these tests, no Bonferroni adjustment was applied to the value for statistical significance because the null hypotheses were not independent; rather the whole set of analyses was considered to assess whether there is persuasive evidence for rejecting the overall null hypothesis of no difference between treatments (Motulsky 1995). Data were analyzed as either raw, log 10, or square root transformed to meet the test assumptions. Statistical significance was considered at $\alpha = 0.05$. Significant differences revealed by these tests were further investigated with *post hoc* assessments (Tukey HSD for ANOVA, and graphical investigation for non-parametric ANOVA).

Results

With the assistance of the Port of San Francisco Divers, we sampled 93 pilings with nearly equal numbers of pilings with each treatment (30-32 per treatment). To obtain enough samples, we sampled pilings in two areas: at Pier 45 (2/3 of the samples), where the fouling panel study had been conducted; and at Pier ½ (1/3 of the samples). Statistical analysis revealed no significant differences in biomass or species richness between the two sites (Tables 13 and 14), and samples from both sites were combined in further analyses.

Table 13. Data summary for piling sampling locations.

Variable	Location	n	Mean (g)	Range (g)
Biomass	Pier 1/2	31	17.5	0.9–66.1
Biomass	Pier 45	62	17.8	0.3–68.7
Species Richness 1	Pier 1/2	31	2.19	1–4
Species Richness 1	Pier 45	62	2.21	1–5

Table 14. Statistical analysis (Student's t test) of piling sampling locations. NS = not significant.

Variable	Data Transformation	t	DF	p	Significance
Biomass	square root	-0.52	49	0.6046	NS
Species Richness 1	log 10	-0.02	61	0.9861	NS

Two types of vinyl coverings were encountered on the pilings sampled, flexible and rigid. Statistical analysis revealed no significant differences in biomass or species richness between the two types of vinyl covering (Tables 15 and 16), and samples from both types of covering were combined in further analyses.

Table 15. Data summary for two types of vinyl covering.

Variable	Vinyl Covering	n	Mean (g)	Range (g)
Biomass	Flexible	19	12.7	0.9-31.6
Biomass	Rigid	11	17.6	6-32.8
Species Richness 1	Flexible	19	1.95	1-4
Species Richness 1	Rigid	11	2.36	1-4

Table 16. Statistical analysis (Student's t test) of two types of vinyl covering. NS = not significant.

Variable	Data Transformation	t	DF	p	Significance
Biomass	square root	1.94	27	0.0628	NS
Species Richness 1	square root	1.23	22	0.2300	NS

Among the 93 samples, 6 ACZA treated-samples and 7 creosote-treated samples had fragments of wood and one creosote-treated sample had some hard lumps of a tarry substance (Appendix C1); these were removed during the initial visual examination of the samples before weighing. There were no significant differences in biomass between the three treatments (Tables 17 and 18) and no significant differences in the number of attached species (Species Richness 1 and 3 in Tables 17 and 18). There was a slightly significant difference in the ANOVA for the total number of species (attached and mobile; Species Richness 2 in Tables 17 and 18). *Post hoc* tests (Tukey HSD) showed this to be due to a significantly greater number of total species on ACZA-treated than on creosote-treated pilings, but no significant difference between either of these treatments and the vinyl-covered pilings. These data are plotted in Appendix D.

Table 17. Data summary for three piling treatments. Species Richness 1 = initial assessment of distinct attached taxa for all samples; Species Richness 2 = assessment of distinct attached and mobile taxa for a subset of samples based on comprehensive microscopic examination; Species Richness 3 = assessment of distinct attached taxa for a subset of samples based on comprehensive microscopic examination.

Variable	Treatment	n	Mean (g)	Range (g)
Biomass	Vinyl-covered	31	14.5	0.9-32.8
	ACZA-treated	30	21.8	2-68.7
	Creosote-treated	32	16.6	0.3-46.4
Species Richness 1	Vinyl-covered	31	2.10	1-4
	ACZA-treated	30	2.35	1-5
	Creosote-treated	32	2.16	1-4
Species Richness 2	Vinyl-covered	9	5.22	2-9
	ACZA-treated	9	5.89	4-10
	Creosote-treated	9	3.56	2-6
Species Richness 3	Vinyl-covered	9	3.67	2-6
	ACZA-treated	9	3.56	1-7
	Creosote-treated	9	2.78	1-4

Table 18. Statistical analysis (ANOVA) of three piling treatments. For Species Richness definitions, ass Table 17 caption. NS = not significant; * = <0.05.

Variable	Data Transformation	SS	MS	F	p	Significance
Biomass	square root	8.96	4.48	1.95	0.1489	NS
Species Richness 1	log 10	0.05	0.03	0.68	0.5077	NS
Species Richness 2	none	26.00	13.00	3.97	0.0325	*
Species Richness 3	none	4.22	2.11	0.94	0.4037	NS

Discussion

Our expectation was that creosote treatment is highly toxic and would have an inhibitory effect on the presence and growth of attached organisms and on the presence of mobile organisms, that ACZA treatment is less toxic to these organisms and would be inhibitory to a lesser degree, and that vinyl covering would have little or no inhibitory effect itself and would protect organisms from the toxic effects of the piling treatments it covered. Thus, we expected that biomass and species richness would rank:

$$V > A > C \qquad \textit{Equation 5}$$

Where:

V = Vinyl-covered pilings
A = ACZA-treated pilings
C = Creosote-treated pilings.

For the analyses of biomass and the species richness of attached organisms, there was no significant difference between treatments. That is:

$$V = A = C \qquad \textit{Equation 6}$$

where "=" means no significant difference. For species richness of all organisms (attached and mobile), ACZA treatment had significantly higher richness than creosote treatment:

$$A > C \qquad \textit{Equation 7}$$

but there was no significant difference between either of these treatments and vinyl covering. The ranking of the mean values were (from Table 17):

Biomass:	A > C > V
Species Richness 1:	A > C > V
Species Richness 2:	A > V > C
Species Richness 3:	V > A > C

Only the results for Species Richness 3 correspond to expectations (Equation 5), and these results were not significant ($p > 0.4$, Table 18). This is an unexpected result.

There a weak indication that creosote treatment is more inhibitory than ACZA treatment (this suggested by the rank order of mean values in all four assessments (A > C), but this difference is only slightly significant and only in one of the four assessments). There is no indication, however, that either ACZA or creosote treatment is more inhibitory than vinyl covering. This could be explained by (1) none of the treatments having a significant inhibitory effect on fouling organisms, (2) both ACZA and creosote treatments having a similar toxic inhibitory effect, and vinyl covering being ineffective at protecting organisms from this effect, or (3) vinyl covering effectively protecting organisms from the toxicity of the treatments it covers, but having its own inhibitory effect.

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