

A practical application of reduced-copper antifouling paint in marine biological research

Andrea S. Jerabek, Kara R. Wall, Christopher D. Stallings

Predator exclusion cages are commonly used to enclose settlement tiles to distinguish between pre- and post-recruitment processes. Biofouling of experimental cages and other field apparatuses can be problematic, and has traditionally been addressed using frequent manual removal (e.g., scrubbing twice per week). However, such intense efforts can be both labor intensive and costly, especially with apparatuses in remote locations, and may also have unintended effects on study results. Recent environmental restrictions and legislative changes have driven the development of less hazardous antifouling products, making antifouling paint a potential alternative option to manual removal. The viability of using these newly developed products as a replacement for the manual cleaning of exclusion cages was experimentally investigated. Six treatment levels were tested, three with and three without antifouling paints. The three antifouling treatments consisted of two reduced-copper paints (21% Cu_2O and 40% Cu_2O) and one copper-free, E-conea®-based paint (considered “ecofriendly”). Antifouling paints were assessed for performance on the cages and whether they elicited local effects on settlement tiles contained within them. The community compositions, biomass, and percent cover of tiles inside cages treated with the copper-based paints were indistinguishable from those inside manually scrubbed cages, while the “ecofriendly” paint resulted in reduced local settlement. The results of this study suggest that the reduced-copper paints tested have the potential to serve as a viable replacement for manual maintenance.

1 **A practical application of reduced-copper antifouling paint**
2 **in marine biological research**

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26 INTRODUCTION

27 Biofouling has historically been an implacable source of contention and frustration for mariners
28 (Woods Hole Oceanographic Institution, 1952). Likewise, biofouling of gear used in field
29 experiments (e.g., predator exclusion cages) can be of concern for scientists. The numerous
30 negative effects of biofouling on study organisms include reduced water flow, decreased oxygen
31 levels, and increased weight and drag of infrastructures (Fitridge et al., 2012). Traditionally,
32 scientific studies have used manual biofouling removal techniques, such as scrubbing and
33 scraping (Smith, Smith & Hunter, 2001; Jompa & McCook, 2002; Burkepile & Hay, 2010;
34 Burkholder et al., 2013). While manual biofouling removal can be effective, it can also result in
35 experimental disturbance, potentially skewing results (Dobrestov, Williams & Thomason, 2014).
36 Manual removal also requires frequent upkeep and monitoring (often at least twice per week).
37 Consequently, it may be neither time- nor cost-effective for scientists to conduct experiments in
38 locations where their infrastructures (e.g., cages, netting, ropes) cannot be maintained on this
39 frequent schedule.

40 One potential alternative to manual removal of biofouling organisms is the use of
41 antifouling paints. Although antifouling paints were originally developed to prevent biofouling
42 on vessel hulls, the technology is currently used in a multitude of commercial industries and
43 research endeavors. For example, antifouling paints are frequently used by the aquaculture
44 industry because of the tremendous effort required to bring infrastructures on-shore for manual
45 cleaning and maintenance (Simpson, Spadaro & O'Brien, 2013). Additionally, antifouling paints
46 are often used for docks, buoys, transducers, and site indicators. Currently, cuprous oxide is the
47 widely used antifouling biocide. However, there has been mounting evidence illustrating the
48 negative environmental consequences of elevated copper levels on marine organisms (Yeber,

49 Kiil & Dam-Johansen, 2003; Thomas & Brooks, 2010; Guardiola et al., 2012; Qi et al., 2013). In
50 response, paint have developed antifouling paints with lower copper concentrations.
51 Additionally, recent legislative evaluations of copper-based paints have facilitated the emergence
52 and acceptance of E-conea®, an organic biocide, as the “ecofriendly” substitute to cuprous oxide.
53 However, there is limited information regarding the toxicity and long-term environmental effects
54 of E-conea® (Holman et al., 2011).

55 This study tested the efficacy of using the newly developed antifouling paints as a viable
56 replacement for manually cleaning experimental apparatuses deployed *in situ*. Specifically, a
57 field experiment was conducted to determine whether two levels of reduced-copper paints, and
58 one copper-free, ecofriendly paint can be used for extended deployments of predator exclusion
59 cages that house settlement tiles. Furthermore, antifouling paints were examined to determine if
60 they elicited local effects on settlement to the tiles.

61

62 MATERIAL AND METHODS

63 Experimental design

64 To test the efficacy of using antifouling paints as a substitute for manual experimental cage
65 maintenance, a field experiment was conducted to compare the community composition, biomass
66 accumulation, and percent cover of settlement tiles placed inside predator exclusion cages (Fig.
67 1). The experiment included six levels of a caging treatment: (1) “no-scrub” (no paint and no
68 manual maintenance), (2) “scrub” (no paint, scrubbed clean twice weekly as in traditional
69 manual maintenance), (3) “ecofriendly” paint (blue Hydrocoat Eco Ablative Antifouling Paint ®
70 treatment), (4) “21% Cu₂O” paint (blue “low-copper conc.” CPP Ablative Antifouling Paint ®
71 treatment), (5) “40% Cu₂O” paint (blue “medium-copper conc.” Horizons Ablative Antifouling

72 Bottom Paint ® treatment), and (6) “no-cage control” (suspended tile without a cage). Each
73 painted cage received two hand-painted coats of a blue ablative antifouling paint, as instructed
74 by the manufacturer, to ensure consistent thickness and to eliminate any potential experimental
75 artifacts of color. The predator exclusion cages were 21.5 cm (l) x 10.6 cm (w) x 21.5 cm (h) and
76 constructed of Vexar, a pre-galvanized, PVC-covered 0.064 cm metal mesh. The ceramic
77 settlement tiles (11.5 cm (l) x 11.5 cm (w) x 0.6 cm (h)) were suspended within the cage via
78 cable ties threaded through three 31.7 mm² drilled holes. Lost surface area due to drill holes was
79 accounted for in percent cover calculations. A 5 cm buffer was maintained between the tile and
80 cage frame to ascertain if antifouling paint ablation, or “wearing off”, affected settlement to the
81 tiles without direct contact. Sixty experimental units (n = 10 per treatment level) were deployed
82 in Bayboro Harbor, St. Petersburg, Florida, U.S.A. on July 30th, 2015 (day 0) with a 0.5 meter
83 distance between cages to minimize cross contamination of antifouling paint. Cages were
84 randomly dispersed within a grid design. The experiment concluded six weeks later on
85 September 10th, 2015 (day 42). After tiles were measured and photographed (see below) and
86 cages scrubbed (where applicable according to assigned treatment level) on day 42 of the
87 primary experiment, all cages were returned to the water and left them without additional
88 scrubbing or maintenance for an additional 62 days to qualitatively investigate the longer-term
89 performance of the antifouling paints on the cage materials (i.e., 104 days total).

90 During the primary six-week experiment, tiles were removed from their cages once per
91 week, weighed to measure change in biomass, and photographed for community composition
92 and percent cover analyses. Following data collection, tiles were promptly re-suspended within
93 the cages and returned to the water. Initial tile masses were subtracted from all biomass
94 measurements to obtain a measure of change in biomass. The scrubbed cages were removed

95 from the water for 15 minutes to provide time to be scrubbed with a plastic bristle brush twice
96 per week. To control for the additional time the scrubbed cages were out of the water, all non-
97 scrubbed cages were also removed from the water for 15 minutes. Cages were inspected for
98 performance throughout the duration of the experiment and photographed post-completion.

99

100 **Data analysis**

101 Coral Point Count with Excel extensions (CPCe) version 4.1 software (Kohler & Gill, 2006) was
102 used to analyze tile photographs for community composition. Each photograph was overlaid with
103 60 randomly stratified points. The organisms underneath each point were identified and exported
104 for community composition analysis. Species identification and percent cover was determined
105 for each observed organism. Algae were clumped into a generalized “turf algae” functional
106 group for community composition analysis because photograph resolution was insufficient to
107 distinguish among species. Overall, percent cover was also analyzed using the software ImageJ
108 (Version 1.48V). Image type was changed to 8-bit and image threshold was adjusted. After the
109 adjustment, the fouling organisms were visually distinct from the tile and the area of the fouled
110 organisms could be selected and measured.

111

112 **Statistical analysis**

113 Community composition analysis was completed via nonmetric multidimensional scaling
114 (nMDS) in the statistical software program Primer using the Bray-Curtis (Sorensen) distance
115 matrix. A PERMANOVA and subsequent pairwise comparisons were conducted on the percent
116 cover data to determine whether community composition differed among treatments. Statistical
117 analyses of tile biomass and total percent cover were completed separately using the statistical

118 software program R (version 3.1.1). Tile biomass at the conclusion of the study (i.e., week six)
119 was log transformed (Sokal & Rohlf, 1981), and normality and homogeneity of the variance
120 were confirmed via Shapiro-Wilk normality test and Levene's Test for homogeneity of variance,
121 respectively. Separate one-way analyses of variances (ANOVA, alpha level $p = 0.05$) were
122 conducted to test whether tile biomass and percent cover differed among treatments at the end of
123 the experiment. Pairwise Tukey HSD post-hoc tests were then conducted on significant main
124 effects to test for differences in tile biomass and percent cover between treatment pairs.

125

126 **RESULTS**

127 **Antifouling paint performance**

128 Antifouling paint performance on the cages was consistent for the copper-based paints
129 throughout the experiment. No more than three barnacle recruits were observed to be growing on
130 the cages at the completion of the 42-day study. The ecofriendly paint had a similar antifouling
131 performance with only few barnacles present on the cage frames. However, a thin algal slime
132 was also observed on some of the cages treated with the ecofriendly paint. In contrast, unpainted,
133 no-scrub cages quickly accumulated an algal slime (7-14 days), subsequently providing the
134 foundation for macroalgae, barnacles, and other organisms to settle. The lack of scrubbing on the
135 unpainted, no scrub cages, allowed for the uninterrupted development of fouling communities
136 that likely inhibited flow through to the cages and affected settlement to the tiles. As expected,
137 the unpainted, scrubbed cages remained free of fouling organisms due to the frequent
138 maintenance performed throughout the study. After the additional two months of deployment
139 (following the primary, six-week experiment), there was notable divergence in performance
140 between the copper-based and ecofriendly antifouling paint treatments. Specifically, the copper-

141 based paints had developed a thin algal slime that easily washed off. Conversely, the ecofriendly
142 paint had a dense algal turf on the top of the cages accompanied by algal, barnacle, and hydroid
143 growth on the cage sides and supporting zip ties.

144

145 **Community composition on tiles**

146 Over the six weeks of data collection, five different marine organism groups settled on the tiles:
147 barnacles (*Amphibalanus* sp.), tubeworms (*Hydroides* sp.), oysters (*Crassostrea virginica*)(JF
148 Gmelin), mussels (*Perna viridis*)(C Linnaeus) and *Geukensia granosissima*(GB Sowerby III),
149 and various algal species (turf algae). The first organisms to colonize (barnacles, tubeworms, and
150 turf algae) were present by day 7. Oyster recruits appeared on the tiles enclosed by the cages
151 with no-scrub, scrub, and 40% Cu₂O paint treatments by day 28, the ecofriendly and 21% Cu₂O
152 paint treatments by day 35, and the no-cage control by day 42 (Fig. 2). Mussel recruits were
153 observed by day 35, but never accounted for more than 2% cover in any of the treatment levels
154 throughout the experiment. By the end of the experiment, percent cover of barnacles was roughly
155 uniform across treatments ranging from 80% to 100% cover. The ecofriendly painted and no-
156 cage treatments had the largest percent covers of tubeworms and algae settled on the tiles
157 compared to the other treatments. Percent cover of oysters was above 10% only in the no-scrub
158 treatment, remaining below 5% in all other treatments (Fig. 2).

159 Percent cover was partitioned by species, and analyzed for community composition. By
160 the end of the experiment (day 42), community composition was similar for the scrub, 21%
161 Cu₂O paint, and 40% Cu₂O paint treatments (PERMANOVA, $p > 0.05$; Fig. 3). However, the
162 no-scrub, ecofriendly paint, and no-cage control treatments all formed distinct clusters in
163 multivariate space (PERMANOVA, $p < 0.05$). The no-scrub treatment had the highest percent

164 cover of oysters among all treatments by the end of the experiment (Fig. 2). Additionally, the
165 ecofriendly paint and no-cage control treatments had the highest algal percent cover among all
166 treatments (Fig. 2).

167

168 **Biomass on tiles**

169 By the completion of the experiment (day 42), there were clear patterns in biomass of settled
170 organisms on the tiles (ANOVA, $F_{(5,54)} = 54.474$, $p < 0.001$, Fig. 4). Biomass of tiles from cages
171 that were scrubbed and those coated with either 21% Cu_2O paint or 40% Cu_2O paint were not
172 found to be statistically different from each other (Tukey HSD tests, $p > 0.05$). Overall, these
173 three treatment levels had a mean (SE) accumulated biomass of 480.2g (11.47), which was
174 37.7% greater than tiles enclosed by caged treated with ecofriendly paint ($348.652\text{g} \pm 9.74$),
175 87.6% more than the no-scrub treatment ($255.924\text{g} \pm 7.09$), and 124.1% more than the no-cage
176 treatment ($214.236\text{g} \pm 5.87$; Fig. 4). The ecofriendly paint treatment on average had the next
177 highest biomass accumulation, which was higher than both the no-scrub (Tukey HSD, $p < 0.001$)
178 and no-cage treatments (Tukey HSD, $p < 0.001$). Biomass accumulation to the no-cage tiles was
179 lower than that to tiles inside cages that were not scrubbed (Tukey HSD, $p = 0.005$), presumably
180 due to post-settlement predation on the unprotected tiles (e.g., herbivory, molluscivory).

181 Although the additional biomass accumulation on tiles was not quantified after the cages
182 were left unmaintained for the two months following the six-week study, interesting patterns
183 were noted. For the cages with the copper-based paints, additional settlement plus growth of
184 settled organisms resulted in cages that were completely filled, bringing the organisms in contact
185 with the painted frame (Fig. 5). These cages were generally observed to be full of oysters that
186 were dislodged from the tiles. Conversely, the organisms in the ecofriendly painted cages

187 remained constrained to the tiles, with only a limited number of unattached organisms. In both
188 unpainted treatments (i.e., scrub and no-scrub), barnacles were found growing on the tiles and
189 cage frames themselves due to the absence of antifouling paint. Dislodged organisms were
190 observed in both cages of these paint treatments, but in smaller quantities.

191

192 **Percent cover on tiles**

193 All treatments except the ecofriendly paint and no-cage control averaged 80% cover by day 14
194 and 100% cover by day 21 (Fig. 6). The differences in percent cover of the ecofriendly and no-
195 cage control treatments emerged by day 14 of the study (ANOVA, $F_{(5,54)} = 9.1455$, $p < 0.001$;
196 Fig. 6). At that time, percent cover on tiles inside cages painted with the ecofriendly paint was
197 not different from the no-cage treatment (Tukey HSD, $p = 0.725$), but both were less than all
198 other treatments (Tukey HSD, $p < 0.05$). By day 21, the ecofriendly paint treatment had
199 increased to 100% cover, rendering the no-cage tiles as the only treatment without complete
200 coverage of growth on the tiles (Fig. 6). The significant difference generally persisted through
201 the completion of the experiment (Tukey HSD, $p < 0.05$). However, percent cover on tiles
202 enclosed by unpainted cages that were not scrubbed had decreased by the end of the experiment,
203 and were not significantly different than the no-cage tiles (Tukey HSD, $p = 0.21$).

204

205 **DISCUSSION**

206 Using a controlled, six-week field experiment, it was determined that biofouling of cage
207 materials was low for those treated with two levels of copper-based paints (21% Cu_2O and 40%
208 Cu_2O). Importantly, the community composition, biomass accumulation, and percent cover of
209 settlement to tiles inside the cages with copper-based paints were all indistinguishable from that

210 to tiles inside unpainted cages that were scrubbed twice per week. Thus, there were no apparent
211 local effects of the copper-based paints on settlement, suggesting those paints could be a viable
212 substitute for manual scrubbing of cages. In contrast, settlement to tiles inside cages treated with
213 the ecofriendly paint had significantly lower biomass and a different community composition
214 compared to the scrubbed cages. Thus, using the ecofriendly paint as a replacement for manual
215 scrubbing is not recommended.

216 The different community structures and biomass of the ecofriendly painted cages implies
217 that the paint both altered species composition and decreased overall settlement on the tiles.
218 While traditional copper-based ablative paints contain harsh solvents, Hydrocoat Eco is a water-
219 based ablative paint and free of harsh solvents. It is possible that the water in the ecofriendly
220 antifouling paint caused the biocides to be dispersed differently than those in the copper-based
221 antifouling paints, which may have accounted for the differences in recruitment, growth, and
222 community structure observed. Furthermore, performance of the ecofriendly paint decreased
223 during the additional two-month deployment after the six-week experiment. Indeed, the cages
224 were covered in different levels of turf algae that partially masked the blue paint color. Turf algal
225 growth was greatest on the top of the cages treated with the ecofriendly paint, which potentially
226 obstructed water flow. It is possible that the development of this turf algae may have been
227 partially responsible for the decreased biomass accumulations observed.

228 Although there is continued support and funding going into the development of
229 ecofriendly and alternative paints, the compounds Econeal®, capasicin, and medetomidine,
230 which are collectively referred to as “emerging” biocides, remain relatively unstudied (Thomas
231 & Brooks, 2010; Guardiola et al., 2012; Gee et al., 2013). In 2008, the U.S. Environmental
232 Protection Agency (EPA) highlighted that there were limited data about the newly developed

233 E-conea® and thus information regarding its toxicity and long-term environmental effects were
234 unknown (Holman et al., 2011). Furthermore, there is concern that zinc and other new biocides
235 could create water quality issues (Holman et al., 2011). Despite these concerns, many of the top
236 performing products evaluated by the EPA contained these biocides. Consequently, the results of
237 this study justify further investigation into the ecological ramifications of E-conea® and other
238 copper-free biocides to maintain high water quality standards and reduce the amount of toxic
239 pollutants released into marine ecosystems. Hence, new antifouling technologies should be used
240 with caution before widespread implementation.

241 The lack of local effects from the copper paints contradicts previous research. For
242 example, increased exposure to heavy metal pollution has been shown to alter community
243 composition and decrease native species densities, permitting the dominance of more tolerant,
244 non-indigenous ones (Piola & Johnston, 2007). Conversely, Canning-Clode et al. (2011) found
245 that as exposure to antifouling paint increased, community composition changed and the
246 densities decreased for both native and non-indigenous species. However, it should be noted that
247 Canning-Clode et al. (2011) used paint with much higher copper concentrations (i.e., Interlux®
248 Ultra-Kote 76% cuprous oxide) than what was used in this study. It is possible that the decreased
249 levels of copper in the present study did not surpass organismal tolerances, thus the observed
250 lack of local negative effects. Furthermore, Canning-Clode et al. (2011) exposed the marine
251 communities to various copper concentrations by painting borders with different loads of
252 antifouling paint around the biofouling communities. This direct contact, versus the 5 cm buffer
253 used here, may also explain why community compositions of the copper paint treatments did not
254 differ from the scrub treatment.

255 The copper-based ablative paints were manufactured to last for approximately 12 months
256 and require physical abrasion by water in high vessel velocities or manual removal by methods
257 such as power washing for paint layers to ablate. It is likely that due to the short duration of the
258 study and the location of the experiment (i.e., protected waters in a harbor), the paints did not
259 experience forces strong enough to consistently ablate, thus the observed lack of an effect on the
260 tile within the cage. However, small specs of the copper-based paints were occasionally observed
261 on the tiles, while the ecofriendly paint rarely chipped off the cages. The observed effects on tiles
262 in the no-cage and no-scrub treatments were expected. The distinct community, biomass, and
263 percent cover observed in the no-cage treatment can be attributed to the signs of grazing from
264 local predators such as the common Sheepshead (*Archosargus probatocephalus*) (JJ Walbaum).
265 Additionally, the distinct community cluster and decreasing rate of biomass accumulation in the
266 no-scrub treatment was likely due to biofouling buildup on the cage that subsequently obstructed
267 water flow and settlement on the tile within it. While the no-cage treatment was intended to
268 reflect a common experimental design used to separate pre- and post-settlement processes, the
269 no-scrub treatment illustrated the importance of accounting for biofouling of cage materials.
270 Addressing biofouling using the traditional approach of frequent manual removal will likely
271 continue to be the primary method employed by field scientists, but doing so is not always
272 logistically feasible. The newly developed reduced-copper antifouling paint compositions, such
273 as those used here, have the potential to act as a viable replacement for manual maintenance of
274 infrastructures, such as cages.

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281

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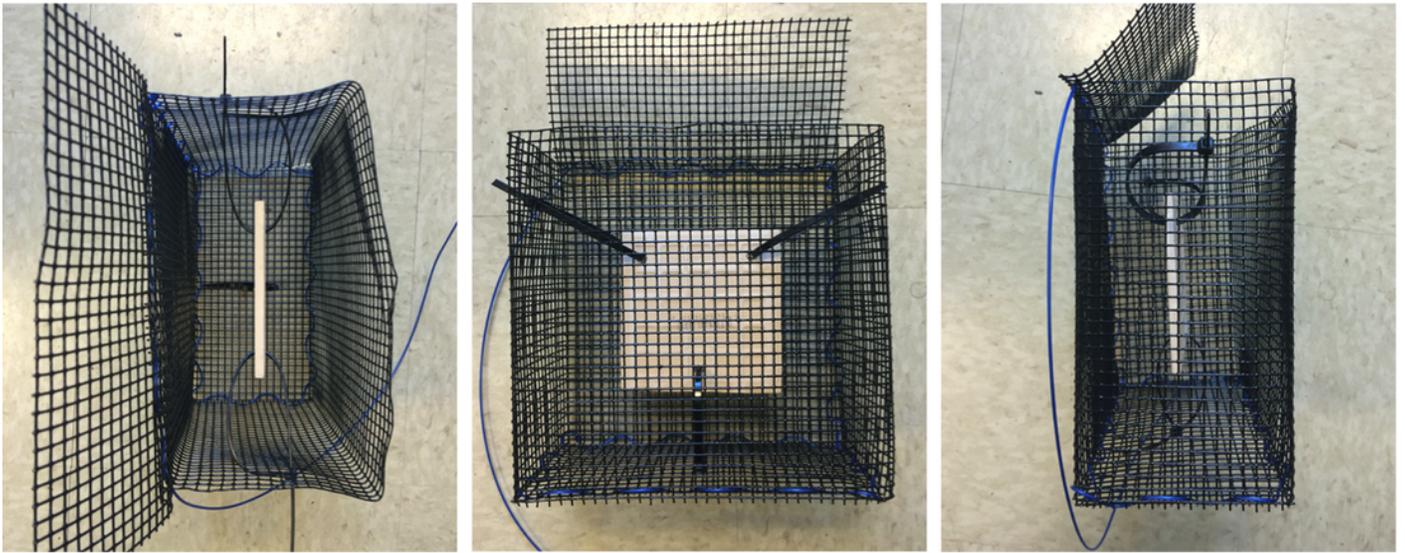
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1

Multiple views of an exclusion cage.

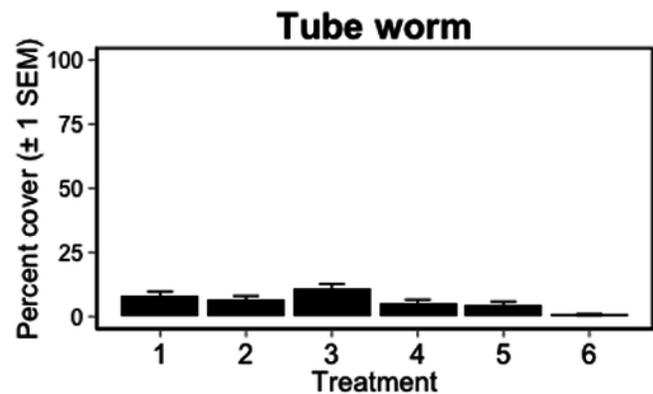
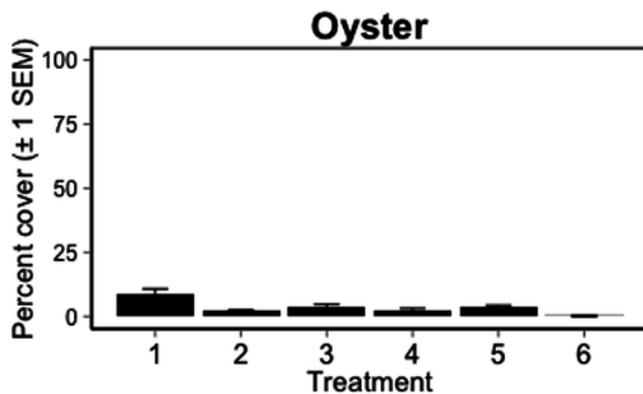
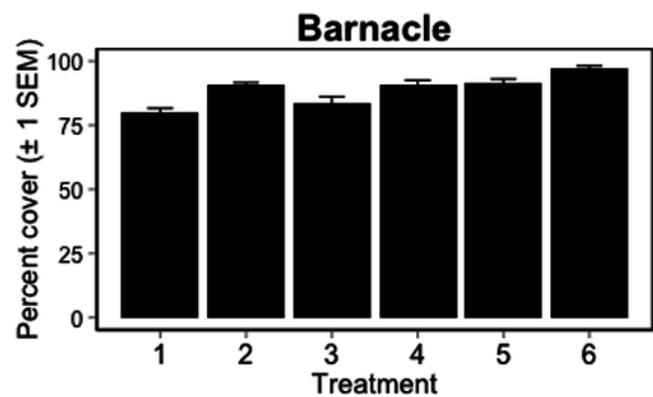
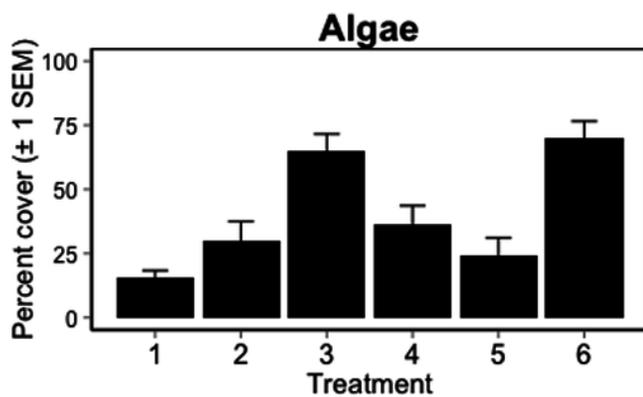
Photographs of an exclusion cage with a suspended settlement tile viewed from the top (left photo), front (center photo), and side (right photo).



2

Percent cover of macrofouling organisms on settlement tiles.

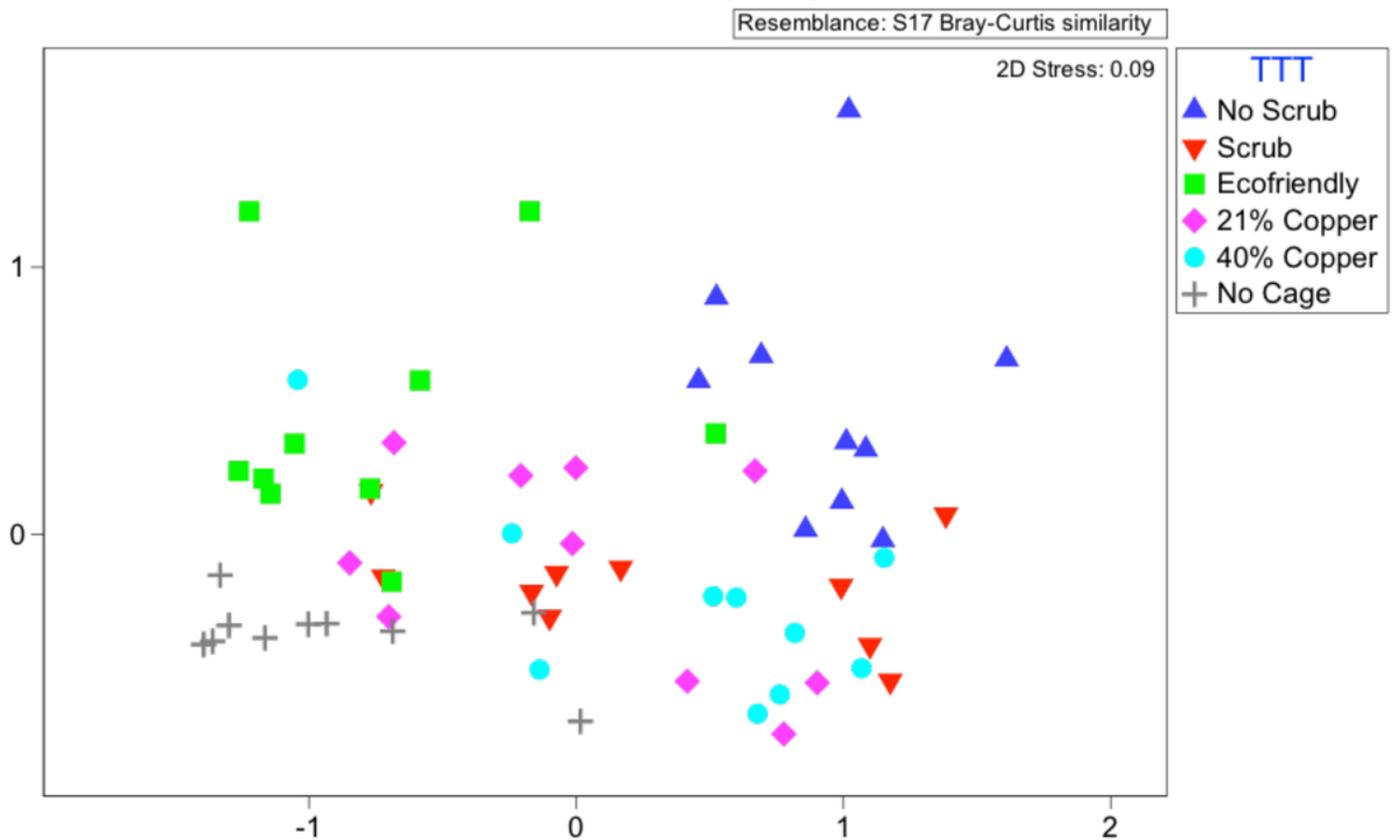
Percent cover (mean \pm 1 SE) of the four primary macrofouling organisms on settlement tiles on day 42 across treatments. Treatment labels are: 1) no-scrub, 2) scrub, 3) ecofriendly, 4) 21% Cu₂O, 5) 40% Cu₂O, and 6) no-cage control.



3

Community composition of settlement tiles.

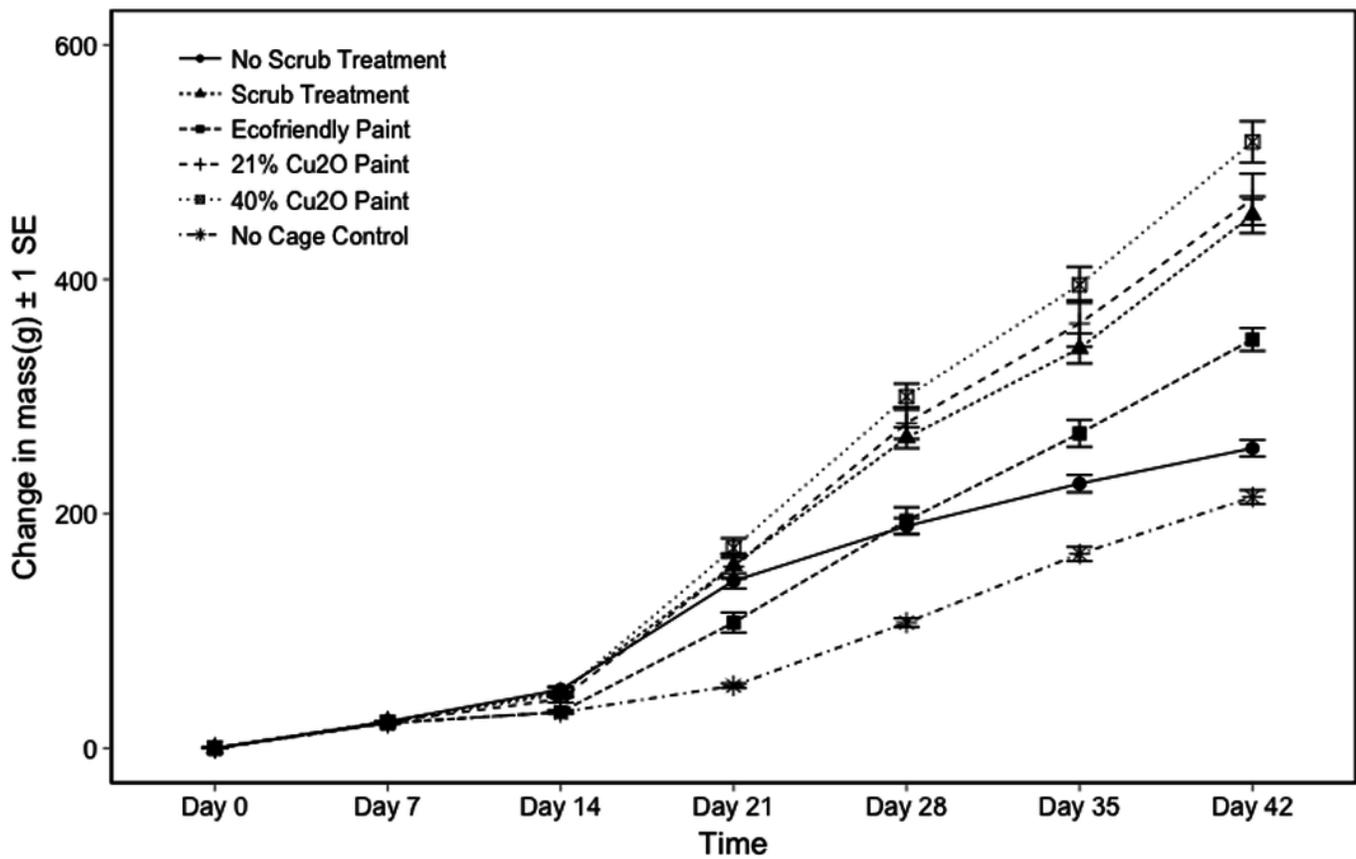
Nonmetric multidimensional scaling (nMDS) of community composition among the six experimental treatments.



4

Settlement tile biomass as a function of treatment and day.

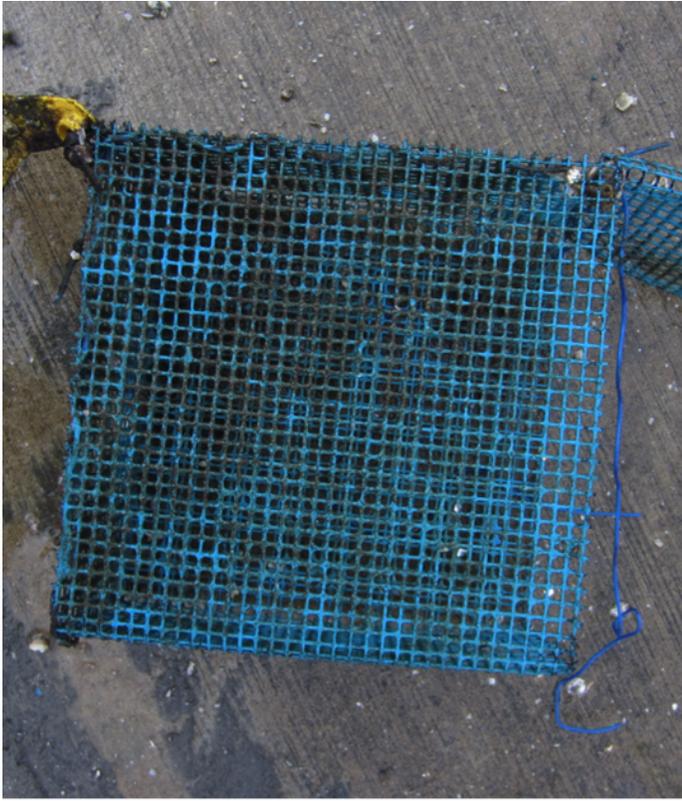
Total biomass accumulation (mean \pm 1 SE) on settlement tiles measured weekly for six weeks across the six experimental treatments.



5

Photographs of post experiment exclusion cages.

Experimental cages after the additional two-month deployment following the primary six-week experiment. Biofouling on cages with copper-based paints remained low (top left photo) while turf algae tended to cover those treated with ecofriendly paints (top right photo). Left unattended, continued settlement to and growth on tiles filled the exclusion cages treated with 21% Cu_2O (bottom left photo) and 40% Cu_2O paints (bottom right photo).



6

Settlement tile percent cover as a function of treatment and day.

Total percent cover (mean \pm 1 SE) on settlement tiles measured weekly for six weeks across the six experimental treatments.

