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

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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, Econea®-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.

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

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INTRODUCTION

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

One potential alternative to manual removal of biofouling organisms is the use of antifouling paints. Although antifouling paints were originally developed to prevent biofouling on vessel hulls, the technology is currently used in a multitude of commercial industries and research endeavors. For example, antifouling paints are frequently used by the aquaculture industry because of the tremendous effort required to bring infrastructures on-shore for manual cleaning and maintenance (Simpson, Spadaro & O'Brien, 2013). Additionally, antifouling paints are often used for docks, buoys, transducers, and site indicators. Currently, cuprous oxide is the widely used antifouling biocide. However, there has been mounting evidence illustrating the negative environmental consequences of elevated copper levels on marine organisms (Yebra, Kiil & Dam-Johansen, 2003; Thomas & Brooks, 2010; Guardiola et al., 2012; Qi et al., 2013). In response, paint have developed antifouling paints with lower copper concentrations. Additionally, recent legislative evaluations of copper-based paints have facilitated the emergence and acceptance of Econea®, an organic biocide, as the "ecofriendly" substitute to cuprous oxide. However, there is limited information regarding the toxicity and long-term environmental effects of Econea® (Holman et al., 2011).

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

MATERIAL AND METHODS

Experimental design

To test the efficacy of using antifouling paints as a substitute for manual experimental cage maintenance, a field experiment was conducted to compare the community composition, biomass accumulation, and percent cover of settlement tiles placed inside predator exclusion cages (Fig. 1). The experiment included six levels of a caging treatment: (1) "no-scrub" (no paint and no manual maintenance), (2) "scrub" (no paint, scrubbed clean twice weekly as in traditional manual maintenance), (3) "ecofriendly" paint (blue Hydrocoat Eco Ablative Antifouling Paint ® treatment), (4) "21% Cu_2O" paint (blue "low-copper conc." CPP Ablative Antifouling Paint ® treatment), (5) "40% Cu_2O" paint (blue "medium-copper conc." Horizons Ablative Antifouling

Bottom Paint ® treatment), and (6) "no-cage control" (suspended tile without a cage). Each painted cage received two hand-painted coats of a blue ablative antifouling paint, as instructed by the manufacturer, to ensure consistent thickness and to eliminate any potential experimental artifacts of color. The predator exclusion cages were 21.5 cm (l) x 10.6 cm (w) x 21.5 cm (h) and constructed of Vexar, a pre-galvanized, PVC-covered 0.064 cm metal mesh. The ceramic settlement tiles (11.5 cm (l) x 11.5 cm (w) x 0.6 cm (h)) were suspended within the cage via cable ties threaded through three 31.7 mm^2 drilled holes. Lost surface area due to drill holes was accounted for in percent cover calculations. A 5 cm buffer was maintained between the tile and cage frame to ascertain if antifouling paint ablation, or "wearing off", affected settlement to the tiles without direct contact. Sixty experimental units (n = 10 per treatment level) were deployed in Bayboro Harbor, St. Petersburg, Florida, U.S.A. on July 30th, 2015 (day 0) with a 0.5 meter distance between cages to minimize cross contamination of antifouling paint. Cages were randomly dispersed within a grid design. The experiment concluded six weeks later on September 10th, 2015 (day 42). After tiles were measured and photographed (see below) and cages scrubbed (where applicable according to assigned treatment level) on day 42 of the primary experiment, all cages were returned to the water and left them without additional scrubbing or maintenance for an additional 62 days to qualitatively investigate the longer-term performance of the antifouling paints on the cage materials (i.e., 104 days total).

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

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

Data analysis

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

Statistical analysis

Community composition analysis was completed via nonmetric multidimensional scaling (nMDS) in the statistical software program Primer using the Bray-Curtis (Sorensen) distance matrix. A PERMANOVA and subsequent pairwise comparisons were conducted on the percent cover data to determine whether community composition differed among treatments. Statistical analyses of tile biomass and total percent cover were completed separately using the statistical software program R (version 3.1.1). Tile biomass at the conclusion of the study (i.e., week six) was log transformed (Sokal & Rohlf, 1981), and normality and homogeneity of the variance were confirmed via Shapiro-Wilk normality test and Levene's Test for homogeneity of variance, respectively. Separate one-way analyses of variances (ANOVA, alpha level p = 0.05) were conducted to test whether tile biomass and percent cover differed among treatments at the end of the experiment. Pairwise Tukey HSD post-hoc tests were then conducted on significant main effects to test for differences in tile biomass and percent cover between treatment pairs.

RESULTS

Antifouling paint performance

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

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

Community composition on tiles

Over the six weeks of data collection, five different marine organism groups settled on the tiles: barnacles (*Amphibalanus* sp.), tubeworms (*Hydroides* sp.), oysters (*Crassostrea virginica*)(JF Gmelin), mussels (*Perna viridis)*(C Linneaus) and *Geukensia granosissima*)(GB Sowerby III), and various algal species (turf algae). The first organisms to colonize (barnacles, tubeworms, and turf algae) were present by day 7. Oyster recruits appeared on the tiles enclosed by the cages with no-scrub, scrub, and 40% Cu_2O paint treatments by day 28, the ecofriendly and 21% Cu_2O paint treatments by day 35, and the no-cage control by day 42 (Fig. 2). Mussel recruits were observed by day 35, but never accounted for more than 2% cover in any of the treatment levels throughout the experiment. By the end of the experiment, percent cover of barnacles was roughly uniform across treatments ranging from 80% to 100% cover. The ecofriendly painted and no-cage treatments had the largest percent covers of tubeworms and algae settled on the tiles compared to the other treatments. Percent cover of oysters was above 10% only in the no-scrub treatment, remaining below 5% in all other treatments (Fig. 2).

Percent cover was partitioned by species, and analyzed for community composition. By the end of the experiment (day 42), community composition was similar for the scrub, 21% Cu_2O paint, and 40% Cu_2O paint treatments (PERMANOVA, $p > 0.05$; Fig. 3). However, the no-scrub, ecofriendly paint, and no-cage control treatments all formed distinct clusters in multivariate space (PERMANOVA, $p < 0.05$). The no-scrub treatment had the highest percent cover of oysters among all treatments by the end of the experiment (Fig. 2). Additionally, the ecofriendly paint and no-cage control treatments had the highest algal percent cover among all treatments (Fig. 2).

Biomass on tiles

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

Although the additional biomass accumulation on tiles was not quantified after the cages were left unmaintained for the two months following the six-week study, interesting patterns were noted. For the cages with the copper-based paints, additional settlement plus growth of settled organisms resulted in cages that were completely filled, bringing the organisms in contact with the painted frame (Fig. 5). These cages were generally observed to be full of oysters that were dislodged from the tiles. Conversely, the organisms in the ecofriendly painted cages remained constrained to the tiles, with only a limited number of unattached organisms. In both unpainted treatments (i.e., scrub and no-scrub), barnacles were found growing on the tiles and cage frames themselves due to the absence of antifouling paint. Dislodged organisms were observed in both cages of these paint treatments, but in smaller quantities.

Percent cover on tiles

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

DISCUSSION

Using a controlled, six-week field experiment, it was determined that biofouling of cage materials was low for those treated with two levels of copper-based paints (21% Cu_2O and 40% Cu_2O). Importantly, the community composition, biomass accumulation, and percent cover of settlement to tiles inside the cages with copper-based paints were all indistinguishable from that to tiles inside unpainted cages that were scrubbed twice per week. Thus, there were no apparent local effects of the copper-based paints on settlement, suggesting those paints could be a viable substitute for manual scrubbing of cages. In contrast, settlement to tiles inside cages treated with the ecofriendly paint had significantly lower biomass and a different community composition compared to the scrubbed cages. Thus, using the ecofriendly paint as a replacement for manual scrubbing is not recommended.

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

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

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

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

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

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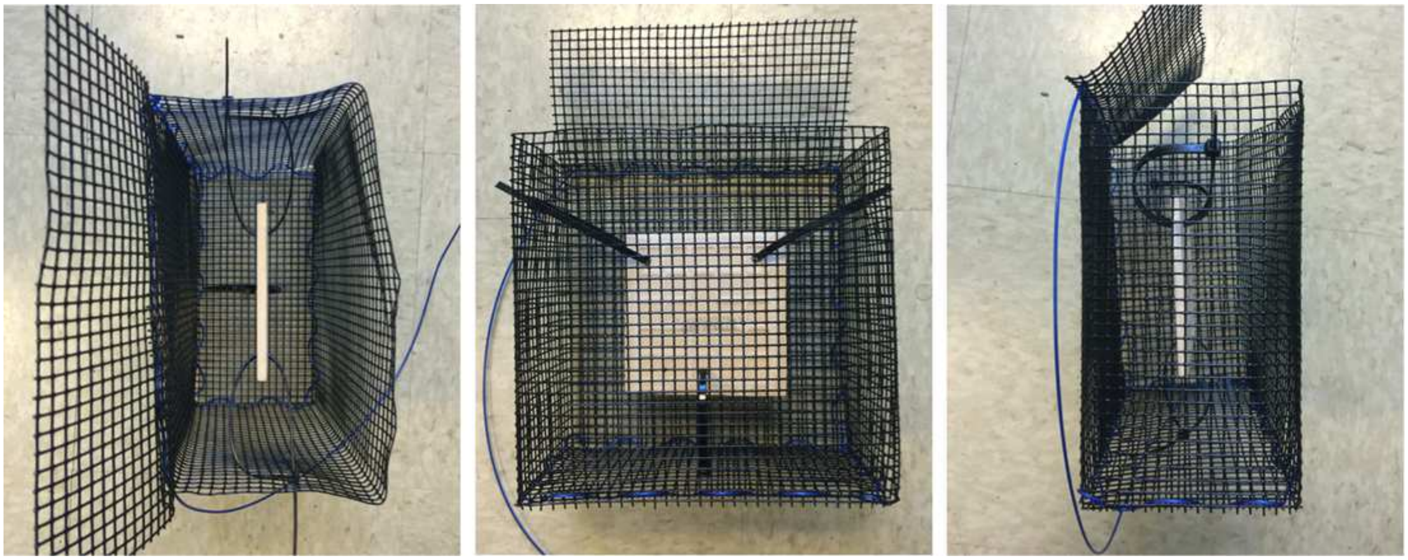
1

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).



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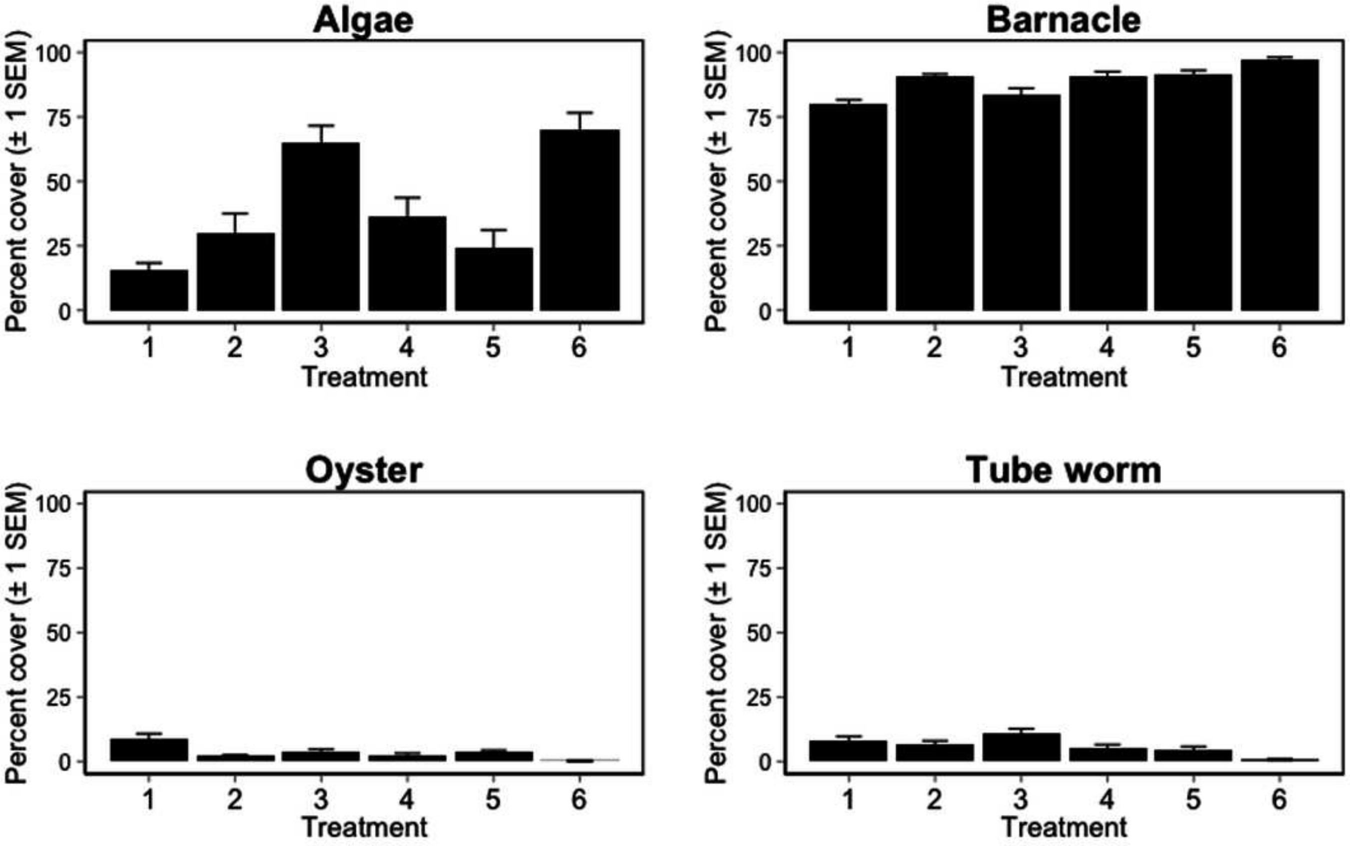
2

Percent cover of macrofouling organisms on settlement tiles.

Percent cover (mean ± 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% Cu_2O, 5) 40% Cu_2O, and 6) no-cage control.



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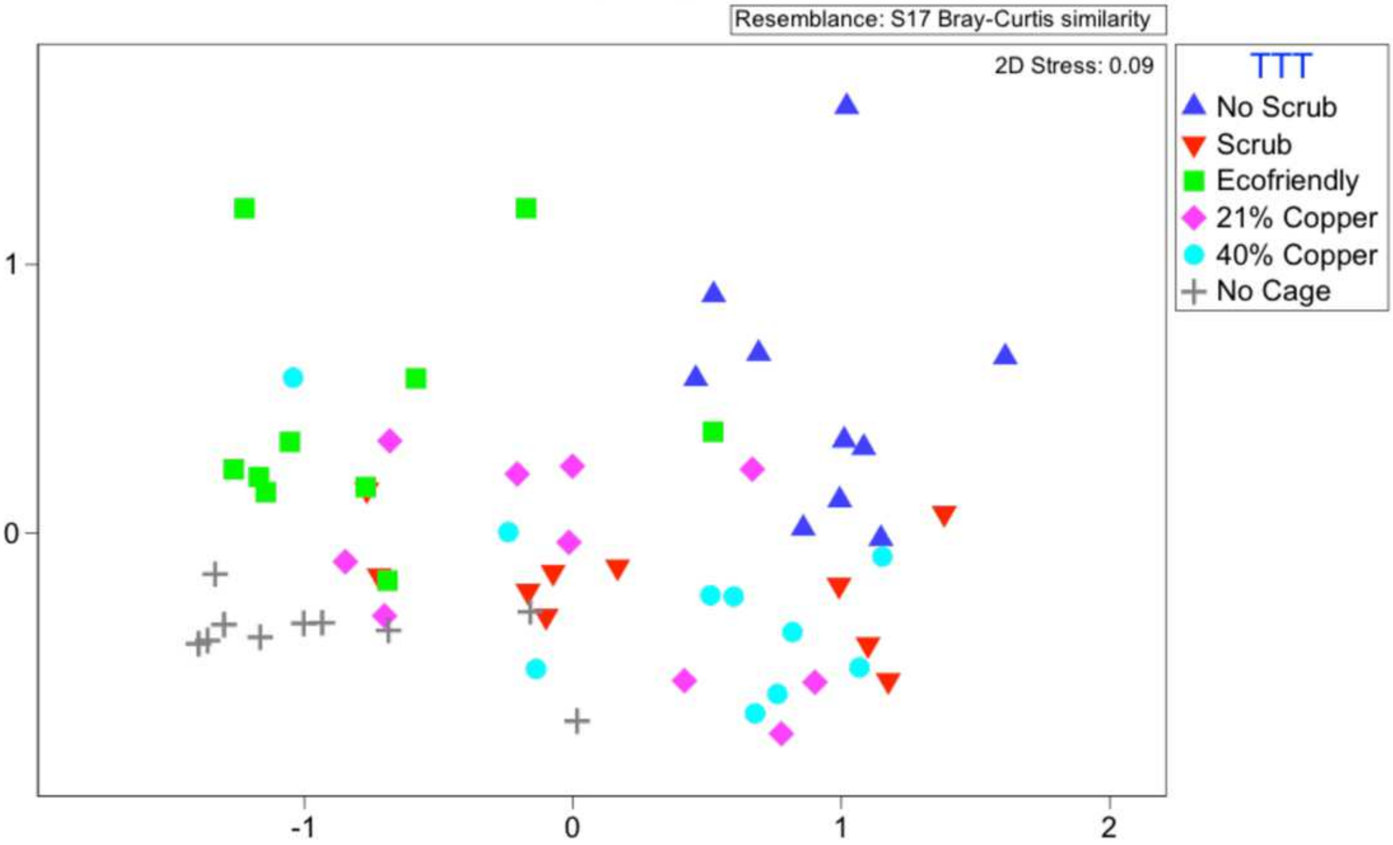
3

Community composition of settlement tiles.

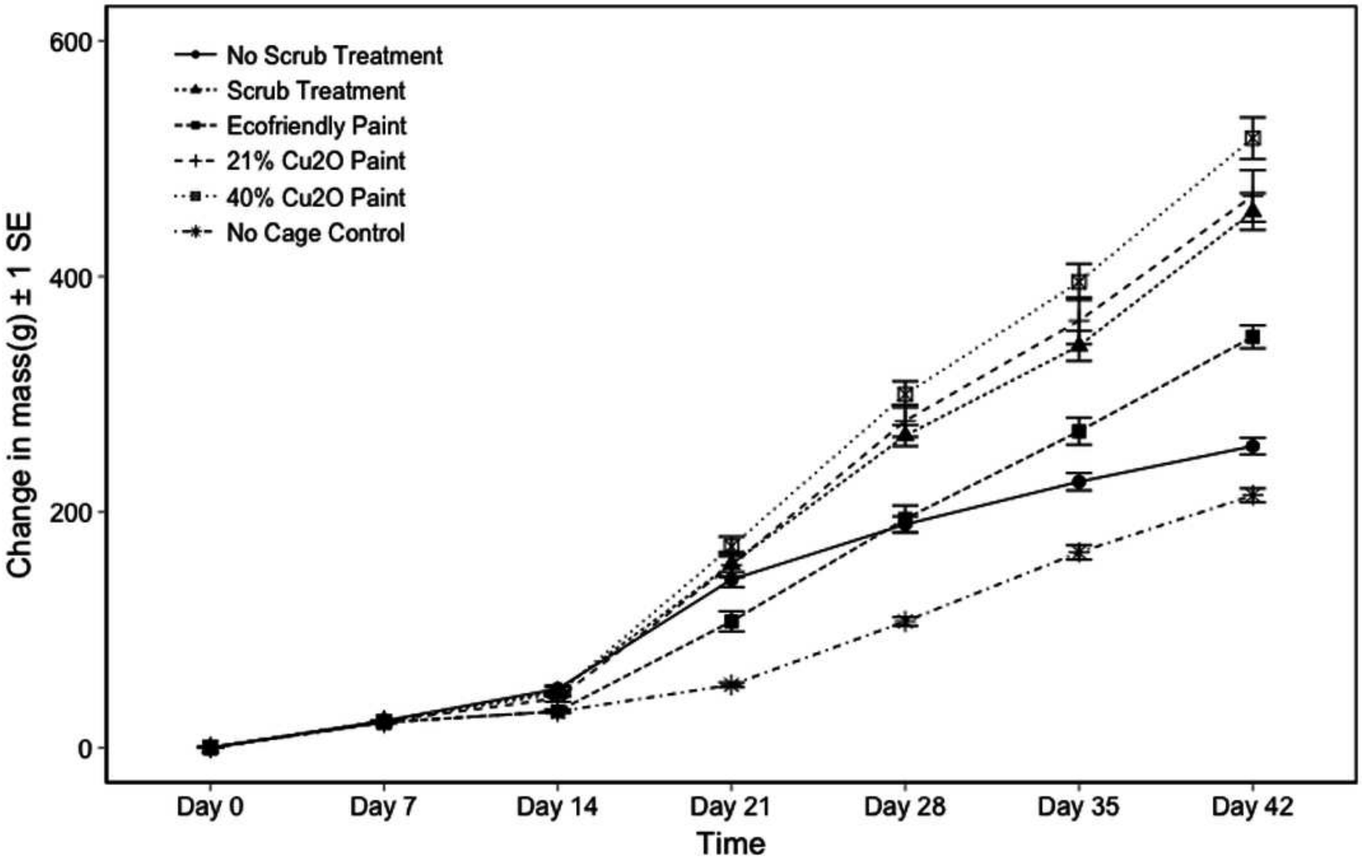
Nonmetric multidimensional scaling (nMDS) of community composition among the six experimental treatments.



4

Settlement tile biomass as a function of treatment and day.

Total biomass accumulation (mean ± 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).

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6

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

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