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Testing the relative effectiveness of traditional and non-traditional antifouling substrates on barnacle and macroalgae settlement

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Abstract

Due to economic impacts, there is considerable interest in determining effective methods for limiting the attachment of marine invertebrates to submerged materials. We tested the effectiveness of traditional and non-traditional coatings on materials used for boat construction to limit the settlement of barnacles and macroalgae. Substrates included fibreglass, fibreglass coated with wax, aluminium, aluminium coated with antifouling paint, aluminium coated with Vaseline®, and aluminium coated with Vaseline® mixed with cayenne pepper. Tiles of each substrate were attached to frames, placed at two sites in the Intracoastal Waterway near Jacksonville, Florida, and collected after one or two successive months in the field. Barnacles as well as macroalgae showed significantly greater settlement on fibreglass than aluminium. Each type of coating tested reduced settlement relative to controls, with the lowest overall settlement of barnacles being observed on aluminium coated with Vaseline®, both with and without the addition of cayenne pepper.

Key words: Algae, antifouling, barnacles, biofouling, macrofouling

Introduction

Biofouling is the term used for undesired accumulation of organisms; diatoms, algae, barnacles, and others, accumulating on a particular substrate (Costerton et al. 1995). The initial adhesion layer comprised of bacteria and diatoms is referred to as a biofilm and allows for the attachment of macrofouls (Finlay et al. 2010). Biofouling has become an increasing problem in coastal areas in recent years due to increased development bringing more structures and marinas to the coastline that provide submerged, hard surfaces for the attachment of biofouling organisms. The accumulation of organisms has widespread effects in hard bottom communities and can have economical consequences, as it may facilitate erosion of docks, reduce fuel efficiency of waterborne vehicles and can negatively impact fisheries (Champ 1999; Bendick et al. 2010; Schultz et al. 2011). In a study looking at the total cost of fouling on a naval fleet, it was estimated that between $180 and $260 million is spent annually on cleaning hulls, coating ships with antifouling substances, and greater fuel usage due to increased drag (Bendick et al. 2010; Schultz et al. 2011). Because of its economic impacts, there is considerable interest in determining effective methods of limiting the attachment of marine invertebrates and other foulants to the hulls of ships.

The present study concentrated on biofouling by various species of barnacles and macroalgae in the waters of Northeastern Florida. Common barnacles to this area include the ivory barnacle, Amphibalanus eburneus (Gould, 1841) and the striped barnacle Amphibalanus amphitrite (Darwin, 1854), while common algal species include Hypnea volubilis Searles and Gloiocladia atlantica (Searles) R.E. Norris (Hay & Southerland 1988; Searles 1991). Another species of barnacle noted was the recently introduced titan acorn barnacle, Megabalanus cocopoma (Darwin, 1854).

There are many hypotheses as to what makes an effective antifoulant. In order to be effective, attachment of all types of foulants must be limited or prevented all together. There have been many products used to prevent biofouling through chemical
means; these are effective to a point, but all wear off over time (Swain & Schultz 1996). Waxes and other polishes claim to prevent fouling through their hydrophobicity. These tend to be a rather expensive solution, costing around $150 for a 6.5 m vessel. They are not, however, particularly effective and must be replaced at least twice per year to achieve maximum effectiveness (Bendick et al. 2010). Antifouling paints use chemical means to prevent attachment and are much more expensive. For proper coverage of a similar 6.5 m vessel the cost would be at least $500, and the paint must be cleaned and replaced every two or three years (Bendick et al. 2010). Additionally, it is known that there are regional differences in the effectiveness of antifoulants (Swain et al. 2000). Fouling in different regions show adaptations to specific nutrients as well as substrate types in their area, so a chemical or substrate surface that deters biofilm formation in one region may increase affinity in another. Therefore, testing alternatives that are relatively inexpensive yet highly effective is important to determine the most cost-effective methods of reducing biofouling.

The present study focused on testing the relative effectiveness of various substances in reducing biofouling by barnacles and macroalgae on fibreglass and aluminium, two common materials used to build ships. Traditional antifouling products such as fibreglass boat wax and antifouling paint for aluminium were compared to a non-traditional treatment of Vaseline®, alone and mixed with cayenne pepper. The use of cayenne pepper mixed in gel coats and boat paints is fairly popular in the Gulf States, where it is believed the capsaicin from the pepper will discourage fouling. We tested whether these antifouling substances significantly reduced biofouling relative to untreated controls and determined which treatments would be most cost-effective. For the present study, the six substrates were tested in the Intracoastal Waterway (ICW) near Jacksonville, FL. The end goal of this study was to determine which methods tested were the most cost-effective way to prevent fouling.

**Materials and methods**

Settlement plates were constructed of 10 cm × 10 cm cut pieces of either fibreglass or aluminium. Some of each substrate were left untreated as controls while others were treated with one of four substances. Treated fibreglass tiles were covered with Collinite 925 fibreglass wax, while treated aluminium plates were either painted with Trilux 33 antifouling paint, covered with a coat of Vaseline®, or covered with a coat of Vaseline® mixed with 0.015 g cayenne pepper per ml. Vaseline® was applied by hand to one side of the substrate in a thin layer (∼2 mm thick) covering the entire surface. Additional fibreglass treatments could not be used due to space constraints on the collectors in order to have sufficient replication.

Five replicates of each substrate were attached to two sheets of plastic mesh (46 cm W × 66 cm H) in a random order. Each sheet was then affixed to opposite sides of a frame made of PVC and the frame attached to one end of a 2.44 m PVC pipe. A settlement collector was then attached via hose clamps to a channel marker at each of two locations within the Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR), including near Fort Matanzas (FM; 29°41′59.9″N, 81°12′0″W) and San Sebastian (SS; 29°48′0″N, 81°17′59.9″W). These sites are located approximately 15 km from each other and were chosen because previous studies showed high settlement of both the introduced barnacle *Megabalanus coccopoma* (Gilg et al. 2010) and the introduced mussel *Perna viridis* (Linnaeus, 1758) (Matthew Gilg, 2013, unpublished data) at these locations. The SS site also corresponds to a permanent collection station for water quality data for the Guana Tolomato Matanzas National Estuarine Reserve, and a second water quality station is within 2 km of the FM site. Both sites show similar patterns of temperature, dissolved oxygen and salinity throughout the year and are quite similar in depth and current velocity. Collectors were deployed at depths such that they remained submerged through all tide cycles.

Substrates were collected and replaced on a monthly basis at FM, from June 2012 to August 2012. To estimate the effectiveness of the treatments for longer duration, the substrates at SS were left in the field for one month, collected, and then replaced with substrates that remained in the field for two consecutive months over the same time period. After collecting, the frames and substrates were allowed to dry for approximately two weeks before analysis. After drying, each substrate was observed under a dissection microscope and barnacles of any species and size were enumerated. A grid forming a total of 25 4 cm² squares was placed over the tiles to ensure accurate counting of barnacles and to determine the percentage of algal coverage. Percentage coverage of algae was determined by dividing the number of squares that contained algal growth by the total number of squares.

After testing for normality of the data, a two-way ANOVA with main effects of time and substrate were conducted to determine if patterns of fouling differed among substrates at both sites, among months at FM or with additional time in the environment at SS. Because all two-way ANOVAs showed a significant
interaction between main effects, each main effect was then tested separately using a one-way ANOVA. Significant results of ANOVA were subsequently analysed using a Tukey’s post-hoc means comparison test. All statistical analyses were performed using SPSS software with an alpha value of 0.05.

Results

Mean numbers of barnacles differed significantly among substrates in all months at Fort Matanzas (Figure 1) and in both one- and two-month deployments at San Sebastian (Figure 2; FM: June \( F = 84.7 \), July \( F = 103.9 \), August \( F = 156.2 \), \( df = 5 \) and \( P < 0.001 \) in all cases; SS: 1 month \( F = 58.5 \), 2 months \( F = 147.6 \), \( df = 5 \) and \( P < 0.001 \) in all cases). In general, greater barnacle settlement was observed on fibreglass substrates than on aluminium substrates and treatment groups reduced settlement relative to controls at both locations, with the exception of aluminium treated with antifouling paint in the two-month test at SS. There was typically no significant difference among any of the antifouling treatments on aluminium; however, in August at FM the aluminium treated with antifouling paint had significantly greater barnacle settlement than aluminium covered with Vaseline® mixed with cayenne pepper. This was the only time cayenne pepper was shown to significantly decrease barnacle settlement compared to other treatments. In all other months the non-traditional treatments on aluminium were just as effective against barnacle fouling as the traditional treatment.

Percentage algal coverage also differed significantly among substrates at both sites and in all time periods examined (Figures 3, 4; FM: June \( F = 167.4 \), July \( F = 522.7 \), August \( F = 2432.9 \), \( df = 5 \) and \( P < 0.001 \) in all cases; SS: 1 month \( F = 330.7 \), 2 months \( F = 1738.1 \), \( df = 5 \) and \( P < 0.001 \) in all cases). Again, greater fouling was observed on fibreglass substrates than on any of the aluminium substrates. The antifouling paint treatment showed significantly greater fouling by macroalgae than either untreated aluminium or aluminium treated with Vaseline® or Vaseline® with cayenne pepper in three of the five tests (three time periods at FM plus two time periods at SS). On the other hand, Vaseline® treatments only showed a significant reduction in algal coverage relative to untreated aluminium in the month of August at FM and after two months in the field at SS.

Time did not appear to be a significant factor for either barnacle settlement or algal coverage at FM, as there were no significant differences in either among months (barnacles: \( F = 2.205 \), \( df = 2 \), \( P = 0.116 \); algae: \( F = 2.674 \), \( df = 2 \), \( P = 0.075 \)). The average number of barnacles per tile increased with additional time in the field at SS (\( F = 8.315 \), \( df = 1 \), \( P = 0.006 \)), but there was no significant difference in percentage algal coverage (\( F = 0.777 \), \( df = 1 \), \( P = 0.382 \)).

Discussion

Non-traditional methods were effective at reducing fouling by both barnacles and macroalgae. At both test sites treated aluminium had significantly less accumulation of barnacles and macroalgae than all other materials; however, the Vaseline® treatments typically had the least settlement. Vaseline® treatments were as effective as the antifouling paint in reducing barnacle settlement and in August the cayenne treatment was actually more effective. Furthermore, the Vaseline® treatments were always
more effective than the antifouling paint in reducing algal coverage. Substrates in both Vaseline treatments were typically mostly covered with Vaseline when collected after one month, but the amount of coverage seemed to decrease with time. Still, even after two months in the field most of the substrates would still have significant portions that were covered with Vaseline upon collection.

Vaseline was also the most cost-effective coating, with a total cost of $0.04 per tile. By comparison, the antifouling paint was the most expensive at $0.28 per tile. A Vaseline coating would provide an inexpensive alternative to traditional antifouling coatings. A mid-size boat (6.5 m length, 18.4 m²) can cost approximately $500 to coat properly with antifouling paint, but it would cost a mere $71.40 to coat the same area with Vaseline. The addition of cayenne pepper would bring the cost to $107.10; however, the cayenne addition did not decrease biofouling overall as compared to plain Vaseline. The Vaseline coating would be most effective for boaters leaving their vessels docked for months at a time without use as movement may result in loss of the coating, although this remains untested. Results of the present study suggest Vaseline is an effective deterrent of barnacle and macroalgae fouling for at least two months, but it is unknown how long it would be effective. Antifouling boat paints would likely have longer staying power, but still must be replaced every two or three years to maintain effectiveness (Johnson & Miller 2003).

The fact that Vaseline was so effective at reducing biofouling raises the question of how it is able to deter the settlement and growth of barnacles and algae. Dobretsov & Thomason (2011) found that microfouling is greatly reduced on substrates with low surface energy and hydrophobic qualities. Without an adequate biofilm on the substrata, macrofouling can also be significantly reduced, because the biofilm can provide nutrients and in some cases gives off a chemical signal that promotes settlement and adhesion to the substrata (Zobell & Allen 1935; reviewed by Qian et al. 2007).

At both test sites it was apparent that the hydrophobic materials had less biofoulant build up. Vaseline, a known hydrophobe, had consistently less accumulation of barnacles and algae than all other materials. While we did not measure the size of the barnacles on the plates, in general those found on plates covered with Vaseline tended to be rather small, suggesting that they had settled recently, likely after some of the Vaseline had washed away. The lack of barnacle settlement may simply be the result of reducing the biofilm, but there are also reasons to believe that Vaseline may be having a direct effect on the attachment affinities of the macrofoulants. For example, when barnacles were removed from the untreated fibreglass the first few layers of the substrate were often removed along with the barnacles. The same affect, however, was not observed on the wax-coated fibreglass, suggesting the cement was able to penetrate the fibreglass but not the wax. Barnacles have been shown to change their basal morphology in accordance to substrate thickness and surface characters (Berglin & Gatenholm 2003). The lack of penetration could be due to a response to the hydrophobic properties of the wax.

Still, other studies have found that barnacles actually showed increased attachment to surfaces with low energy (Petrone et al. 2011) and that barnacle larvae prefer hydrophobic surfaces to hydrophilic ones (Bennett et al. 2010; Finlay et al. 2010; Magin et al. 2011). Furthermore, it is known that some barnacles,
such as *Amphibalanus amphitrite*, can change their adhesion mechanisms in response to the substrate they are adhering to (Berglin & Gatenholm 2003; Wendt et al. 2006). Therefore, it would appear that barnacle settlement should be greater on hydrophobic substances, such as Vaseline®, and the decreased settlement we observed is more likely an effect of limited microfouling. However, recent studies analysing the effectiveness of a superhydrophobic substance have shown that these materials reduce fouling of all types, so the question of whether Vaseline® is inhibiting microfoulants, macrofoulants, or both will require additional testing (Genzer & Efimenko 2006; Callow & Callow 2011).

It is also possible that barnacles have greater mortality on Vaseline®-covered surfaces than on the control plates. Mortality was not directly tested in this experiment, but if Vaseline® reduces the strength of attachment to the substrate it is quite possible that there was greater loss of settlers. Additional experiments will be needed to determine if the lack of barnacles on settlement plates treated with Vaseline® is the result of decreased settlement or lower survival after settlement.

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