

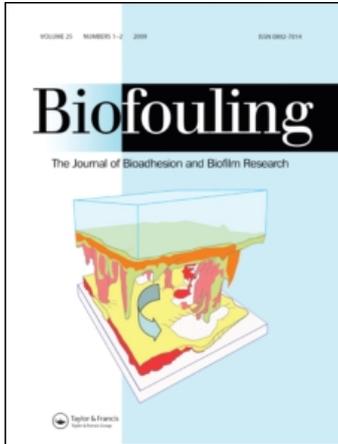
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## Biofouling

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## Variation in Adhesion Strength of *Balanus eburneus*, *Crassostrea virginica* and *Hydroïdes dianthus* to Fouling-release Coatings

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This study compared the shear adhesion strength of barnacles, oysters and tubeworms on eight RTV 11-based silicone fouling-release coatings containing different silicone oil additives. It was found that adhesion strength differed among species and coating types. In most cases, oysters and tubeworms had higher adhesion strengths than barnacles. Barnacle adhesion strength was reduced on all coatings containing oil additives; however, this was not generally true for oysters and tubeworms. The difference in the adhesion strength among the three organisms tested in this study emphasizes the importance of understanding the fundamental interaction between marine invertebrate adhesives and the substratum.

**Keywords:** adhesion strength; barnacle; oyster; tubeworm; silicones; fouling-release marine coatings

### INTRODUCTION

Silicone fouling-release coatings have been developed as an alternative to paints containing biocides for the control of biofouling. They

function by minimizing the adhesion strength of organisms to the substratum and facilitating easy removal of fouling from the surface (Brady & Singer, 2000). The performance of these coatings, however, still does not equal that of current biocide-containing coating systems (Swain, 1999). Silicone coatings tend to be soft and easily damaged, are more difficult to apply than conventional coatings, and are expensive. Therefore, research is underway to better understand the mechanisms of fouling-release and to improve on existing technology.

One method of quantifying the performance of fouling-release coatings is to expose surfaces to biofouling and to measure the shear adhesion strength of hard-fouling organisms that become established (Swain *et al.*, 1992; ASTM D5618, 1994; Swain & Schultz, 1996). A number of investigations have measured the adhesion strength of benthic organisms both to artificial

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substrata (Despain *et al.*, 1972; Becka & Loeb, 1984; Crisp *et al.*, 1985; Ackerman *et al.*, 1992; Swain *et al.*, 1994; Becker, 1993; Swain & Schultz, 1996) and to natural surfaces (Grennon & Walker, 1981; Young & Crisp, 1982; Yule & Walker, 1984; Denny *et al.*, 1985). Results from a few of these studies have reported differences in adhesion strength among species (Crisp *et al.*, 1985; Swain *et al.*, 1992; Becker, 1993). However, data are limited and scientific explanations for these findings have not been made. Possible explanations for the differences in adhesion strength include differences in the chemical and physical properties of the adhesive of the organisms, the morphology of the organism and the properties of the substratum (Crisp, 1973; Nelson, 1995; Brady, 2000). The attachment mechanisms for barnacles (Lindner & Dooley, 1969; Cook, 1970; Saroyan *et al.*, 1970a; 1970b; Walker, 1970; 1972; 1981; Otness & Medcalf, 1972; Barnes & Blackstock, 1976; Cheung *et al.*, 1977; Naldrett, 1993; Naldrett & Kaplan, 1997) and mussels (Waite & Tanzer, 1981; Waite, 1987; 1988; Filpula *et al.*, 1990) are well documented; however, information for other hard-fouling types, including oysters and tubeworms, is limited.

This paper presents shear adhesion strength measurements of representatives of three hard-fouling types (barnacles, oysters and tubeworms) to RTV 11 silicone fouling-release coatings modified by the inclusion of silicone oils. Adhesion strength is discussed with respect to organism size, organism type and coating type.

## MATERIALS AND METHODS

Eight silicone formulations (applied by the General Electric Corporation) were painted on both sides of 254 mm × 305 mm epoxy coated steel panels. The formulations were based on RTV 11, a room temperature vulcanized polydimethylsiloxane coating. Coating 1 was unmodified RTV 11 and acted as a control.

The remaining seven coatings contained different silicone oil additives equal to 10% by weight (Table I). The critical surface tension of the coatings ranged between 21.0 to 24.9 mNm<sup>-1</sup>. Two panels of each coating type were attached to PVC frames in sets of three, and were suspended from a fixed platform approximately 1 m below the water surface in the Indian River Lagoon, Florida. The panels were assigned at random to a frame and a position under the platform. Panels of each coating were suspended within two 1.5 m<sup>3</sup> cages constructed of 25.4 mm mesh to prevent disturbance or removal of attached organisms by fish and crabs (Swain *et al.*, 1998).

The three hard-fouling types present at the test site were *Balanus eburneus* (barnacle), *Crasostrea virginica* (oyster) and *Hydroides dianthus* (tubeworm). While the three species will be referred to generically as barnacles, oysters and tubeworms throughout the text for ease of reading, differences within genera may exist; therefore, the results presented should not be generalized to other species.

The test coatings were initially exposed to fouling in July 1997. Measurements of barnacle shear adhesion strength were taken every two months from September 1997 to September 1998. Oysters and tubeworms were sampled when present, primarily in May and July, 1998.

TABLE I Index of identification numbers and silicone oil additives to RTV 11 elastomeric coatings

Coating #	Oil Content
1	None
2	Polydimethyldiphenylsiloxane
3	Dimethylsiloxane-ethylene oxide block copolymer
4	Carbinol terminated polydimethylsiloxane (20% nonsiloxane)
5	Alkylmethylsiloxane
6	Mixture of polydimethyldiphenylsiloxane and dimethylsiloxane-ethylene oxide block copolymer
7	Carbinol functional methylsiloxane
8	Carbinol terminated siloxane (60% nonsiloxane)

The adhesion measurements were based on procedures outlined in ASTM D5618-94. Measurements were made on live organisms with basal areas ranging between 20–250 mm<sup>2</sup>, although oyster bases were as large as 1000 mm<sup>2</sup>. A hand-held force gauge was used to apply a force parallel to the attachment plane of the organism at a rate of approximately 4.5 N s<sup>-1</sup> until it was removed from the surface. Three force-measuring devices of varying range were employed depending on the release characteristics of the coating and the size of the individual. The gauge ranges were 0–9 N, 0–45 N, and 0–90 N with a resolution of 0.05 N, 0.25 N, and 0.5 N, respectively. Gauge accuracy among scales was checked periodically. The force required to detach an organism was recorded, and the individuals collected and taken to the laboratory. The attachment area was determined by scanning the base of the individual and analyzing the images with Jandel Scientific SigmaScan<sup>®</sup> software. Pixel to area determination was based on a three-point calibration, and computations were validated by inclusion of an object of known dimension with each data set analyzed. Adhesive shear strength,  $\tau$ , was calculated by dividing the shear force,  $F$ , required to remove the organism by the surface area,  $A$ , of attachment ( $\tau = F/A$ ).

## RESULTS AND DISCUSSION

A preliminary statistical analysis, using a three way analysis of variance ( $\alpha = 0.01$ ) with coating type, panel side, and replicate as main effects, was performed to determine if adhesion strength differed between sides of a panel or replicates of a coating. Values for adhesion strength of barnacles, from a single date, were drawn from a subset of coatings on which eighteen measurements were taken on each side of both replicates. No significant differences were found between sides of a panel or replicates of the coatings ( $p = 0.598$  and  $0.846$ ,

respectively). Data for further analyses were pooled from all sides representing each coating.

### Variance Described by Force and Area

The adhesion strength of hard-fouling organisms to fouling-release surfaces is determined from the relationship between the contact area and the force required to remove the organism. Polynomial regression analyses ( $\alpha = 0.01$ ) were used to determine the nature of the force to area relationship. Due to departures from assumptions of normality and homogeneity of variance for many of the coatings, data were transformed by the natural logarithm. The results of these analyses revealed a significant, positive linear term for all species on all coating types with coefficients of determination ( $r^2$ ) ranging from 0.289 to 0.767, 0.863 to 0.966 and 0.418 to 0.633 for barnacles, oysters and tubeworms, respectively. In only one case (oysters on unmodified RTV 11) was a higher order term significant, representing 2.0% of the explained variance. The coefficient of determination reflects the amount of variance explained by the force to area relationship. The results indicate that the relationship between removal force and area of attachment is strong for oysters; however, size does not fully describe detachment force for barnacles and tubeworms. Linear regressions of force *vs* area for each organism type on all coatings are graphically displayed as untransformed parameters in Figures 1–3. An intercept of zero was within the 99% confidence interval for all data sets except tubeworms on coatings 3, 4 and 6. Reasons for these exceptions are discussed below (see Size as a Covariate).

While the majority of variance (in the transformed regressions) was described for oysters (mean  $r^2 = 0.904$ ), on average only 53.7% and 58.7% of the variance in detachment force was described by the contact area of the individuals for barnacles and tubeworms, respectively. One reason for the inadequacy of the force to area relationship to completely describe barnacle

# BARNACLE

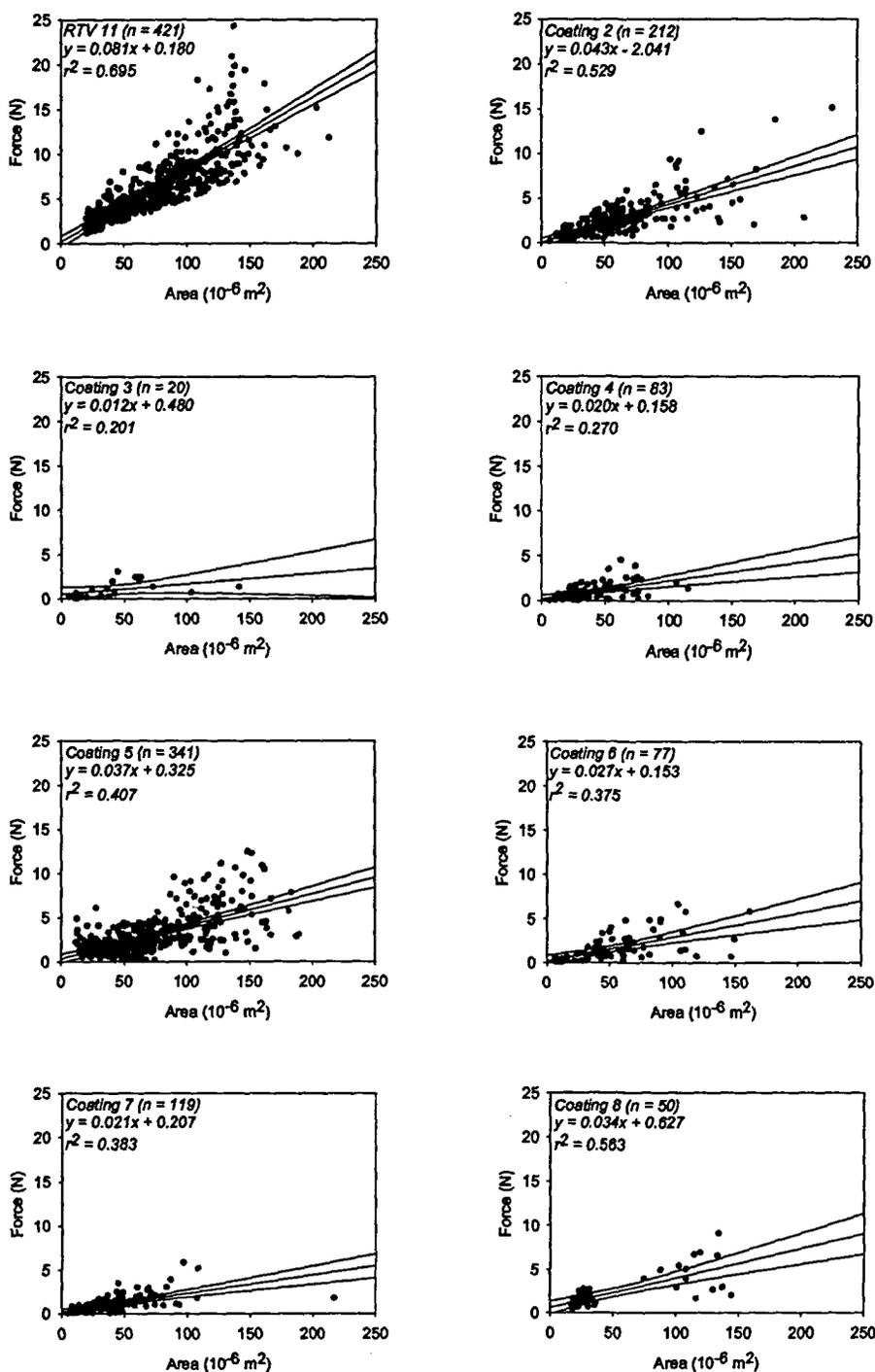


FIGURE 1. Linear regression of force (Newtons) to remove an individual *vs* area of attachment ( $10^{-6} \text{ m}^2$ ) for barnacles on RTV 11 silicone fouling-release coatings. Confidence intervals represent the 99% level. All data met parametric assumptions for the regression analyses when transformed by the natural logarithm. Data are presented in original parameters.

# OYSTER

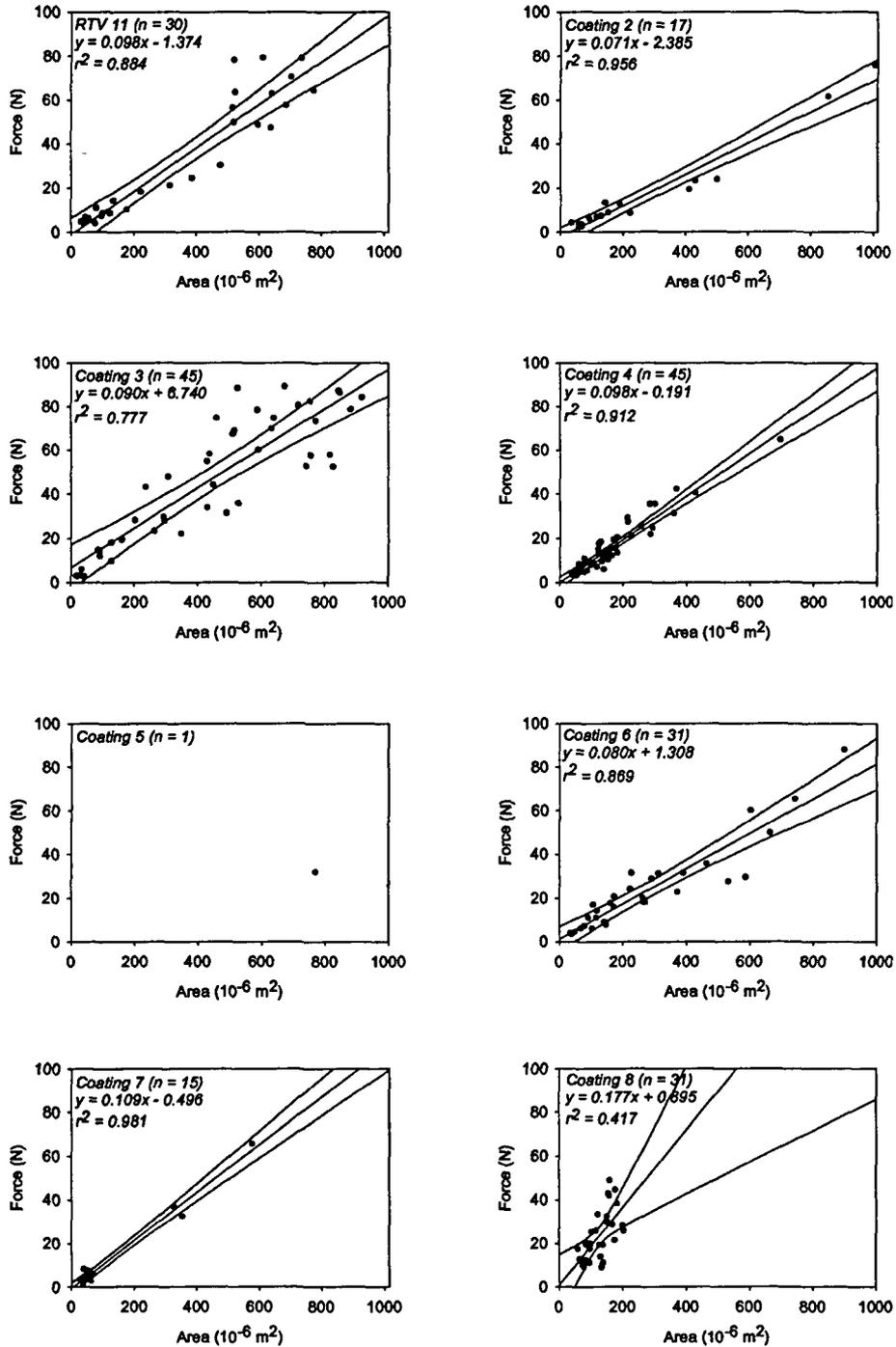


FIGURE 2 Linear regression of force (Newtons) to remove an individual *vs* area of attachment ( $10^{-6} \text{ m}^2$ ) for oysters on RTV 11 silicone fouling-release coatings. Confidence intervals represent the 99% level. All data met parametric assumptions for the regression analyses when transformed by the natural logarithm. Data are presented in original parameters.

# TUBEWORM

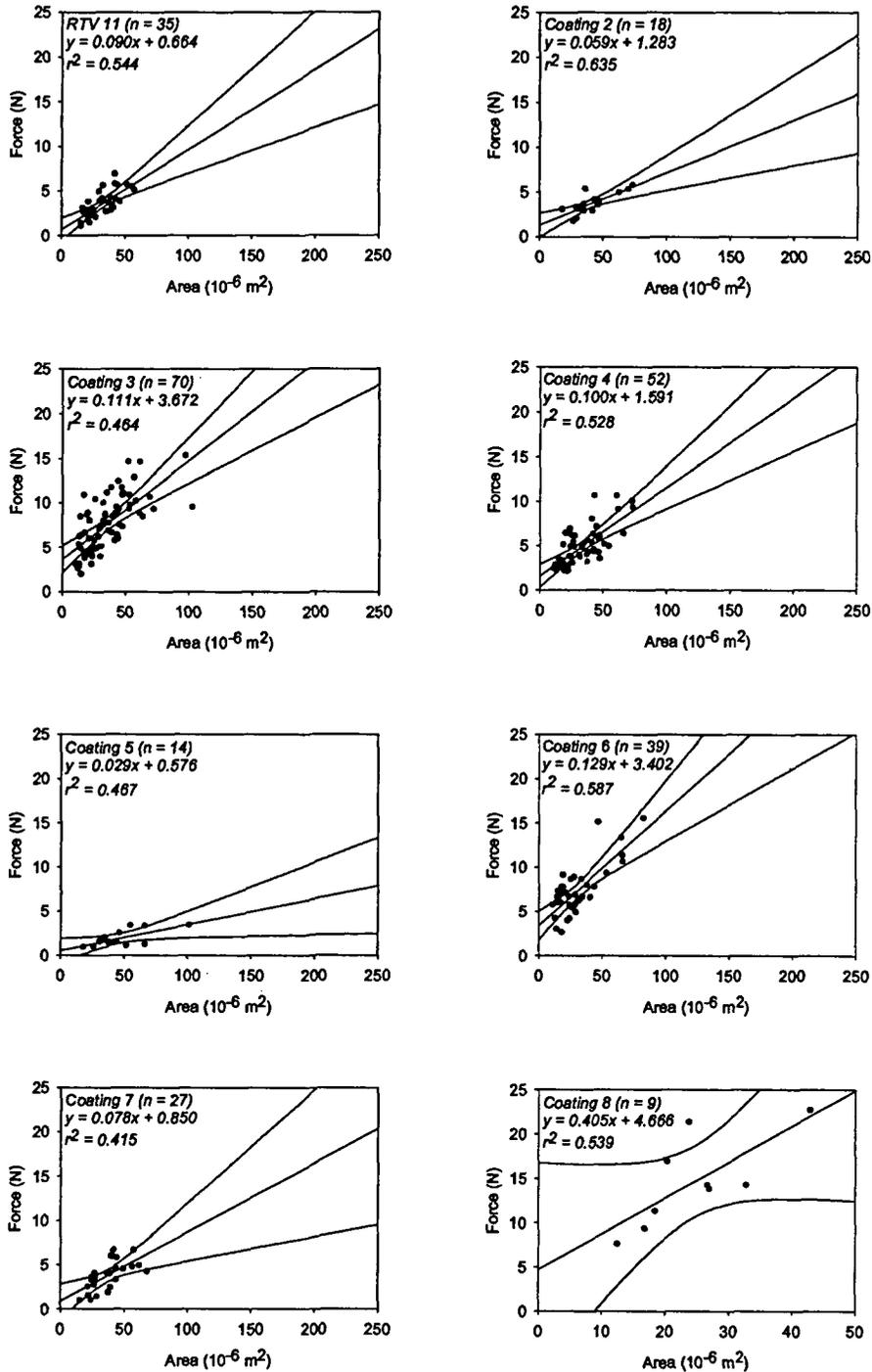


FIGURE 3 Linear regression of force (Newtons) to remove an individual *vs* area of attachment ( $10^{-6} \text{ m}^2$ ) for tubeworms on RTV 11 silicone fouling-release coatings. Confidence intervals represent the 99% level. All data met parametric assumptions for the regression analyses when transformed by the natural logarithm. Data are presented in original parameters.



FIGURE 4 Basal image of *B. eburneus* removed from a RTV 11 coating showing the variability in the adhesive of barnacles on silicones. Bases are approximately 10 mm in diameter.

adhesion strength to silicone may be the variability seen in individual bases. Observations on the condition of the barnacle bases for individuals removed from RTV 11 coatings ranged from thin, hard, transparent films to thick, soft, opaque layers (Figure 4). The results of Berglin and Gatenholm (1999) suggest variability in the locus of failure could also contribute to the variance in adhesive strength. However, electron spectroscopy performed on coatings 1, 2, 4 and 5 (unpublished results) indicated that fracture was interfacial for all surfaces examined. Changes in mode of failure related to properties of the adhesive, variability within the coatings, and imperfectly applied loads may have contributed to scatter in the force measurements of barnacle detachment.

The bases of tubeworms and oysters were consistently found to be hard, solid surfaces. Some of the variation in tubeworm adhesion strength may be due to the morphology of these organisms. The growth form of serpulid tubeworms varies in the number and degree of longitudinal curves of the tube (Figure 5). Therefore, the shear force applied to remove the organism may create uneven stress distributions, and cause shell failure rather than adhesive release. The contribution of localized shell fracture (during tubeworm removal) to the variation seen in adhesion strength remains unknown.

#### Size as a Covariate

The fact that the adhesive strength of organisms may vary with size should not be overlooked. An increase in the adhesion strength of *Balanus balanoides* to slate between metamorphosis and four months growth has been reported

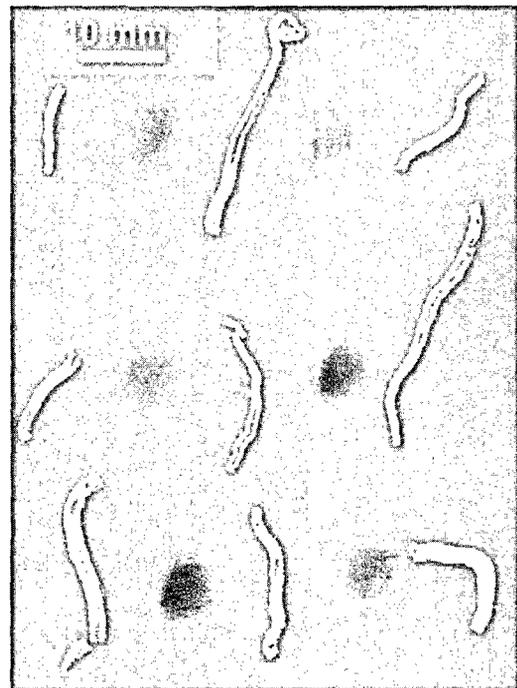


FIGURE 5 Basal image of *H. dianthus* removed from a RTV 11 coating showing the variability in morphology of tubeworms on silicones.

(Yule & Walker, 1984). Denny (1987) found a slight increase in the tenacity of mussels (*Mytilus californianus*) with increased shell length, but questioned its biological significance due to minimal explained variance. Therefore, comparisons of adhesion strength must account for differences due to size. Analysis of covariance was unable to be performed due to failure of tests of parametric assumptions. Instead, linear regressions of each organism type on each coating were used to determine if changes in adhesion strength occurred over the size ranges measured. Data did not require transformation. The significance level for type I error was set at 0.01 for all tests. Slopes deviating from zero infer changes in adhesion strength with size. Intercepts represent the mean adhesion strength.

In general, the adhesion strength of the organisms did not vary with their size for the three species (Table II). The slopes of the

regressions did not differ significantly from zero (within 99% confidence intervals), except for oysters on coating 3 ( $p=0.007$ ) and tubeworms on coatings 4 and 6 ( $p<0.001$ ). These three samples had significant negative slopes reflecting decreases in adhesion strength with increased basal area. This precluded these data from being used in further analyses. Tubeworm adhesion strength on coating 3 exhibited heterogeneity of variance and these data were also excluded from further analyses. Comparison of coatings that had no change in adhesion strength with size showed differences in magnitude of adhesion strength among organisms and coatings, shown as the intercept of the regression in Table II. While barnacles (0.09 MPa), oysters (0.09 MPa) and tubeworms (0.13 MPa) exhibited similar adhesion strength on unmodified RTV 11 (coating 1), differences in adhesion strength among species with tubeworms (0.76 MPa) >

TABLE II Linear regression intercept, slope, significance probability value ( $p$ ), coefficient of determination ( $r^2$ ), and sample size ( $n$ ) of the adhesion strength to attachment area relationship for barnacles, oysters and tubeworms for the eight coatings tested in this study

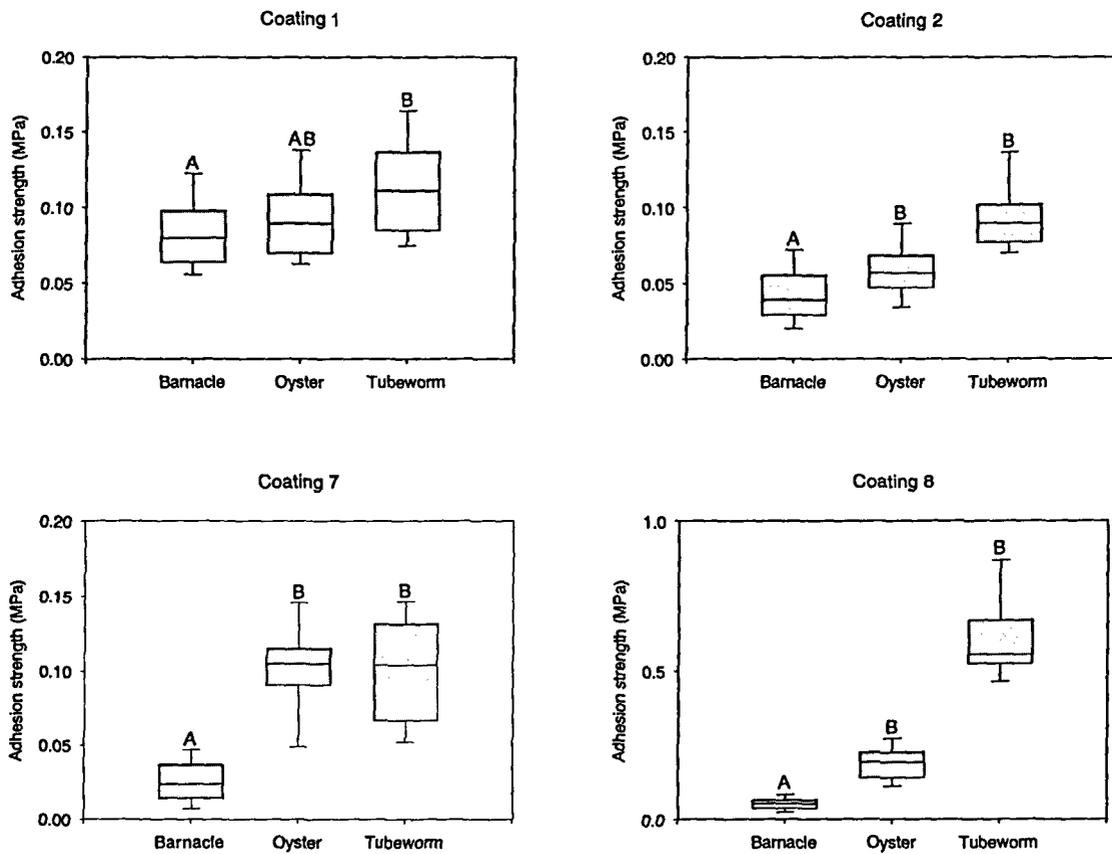
Organism	Coating	Intercept	Slope	$p$	$r^2$	$n$
Barnacle	1	0.091	-0.00007	0.012	0.015	421
	2	0.045	-0.00003	0.396	0.003	212
	3	0.027	-0.00006	0.626	0.013	20
	4	0.025	-0.00003	0.728	0.002	83
	5	0.045	-0.00005	0.096	0.008	341
	6	0.028	+0.00002	0.722	0.002	77
	7	0.027	-0.00002	0.821	< 0.001	119
	8	0.061	-0.00018	0.012	0.124	50
Oyster	1	0.092	0.00000	0.882	< 0.001	30
	2	0.058	0.00000	0.709	< 0.001	17
	3	0.135	-0.00005	0.007	0.159	45
	4	0.093	0.00002	0.594	0.007	45
	5	[0.042]	na	na	na	1
	6	0.100	-0.00003	0.106	0.088	31
	7	0.104	0.00000	0.960	< 0.001	15
	8	0.192	-0.00007	0.829	0.002	31
Tubeworm	1	0.134	-0.00063	0.191	0.051	35
	2	0.128	-0.00082	0.050	0.217	18
	3	[0.337]	Heterogeneous	Variance	-	70
	4	0.216	-0.00167	< 0.001	0.227	52
	5	0.054	-0.00023	0.128	0.210	14
	6	0.389	-0.00373	< 0.001	0.318	39
	7	0.116	-0.00037	0.187	0.066	28
	8	0.760	-0.00598	0.343	0.128	9

oysters (0.19 MPa) > barnacles (0.06 MPa) were seen on coating 8. This trend was investigated further with analysis of variance of the pooled data of each organism type on each of the coatings.

**Adhesion Strength Analyses**

A comparison of the adhesion strength among species within coatings and among coatings

within species was made where data were available. Two-way analyses of variance to discern differences in adhesion strength for the main factors of coating and species were unable to be performed. Transformation of the data, and removal of outliers using Grubbs method (Sokal & Rohlf, 1985), did not yield homogeneity of variances. Therefore, separate one-way analyses were performed for each main factor ( $\alpha=0.01$ ). When parametric assumptions still



Coating	df	H	p
1	2	27.1	<0.001
2	2	49.9	<0.001
7	2	88.2	<0.001
8	2	67.8	<0.001

FIGURE 6 Adhesion strength (MPa) of species compared within coatings. Boxes reflect median values with 25th and 75th percentiles. Bars represent 10th and 90th percentiles. Statistical results are from Kruskal-Wallis one-way anova on ranks ( $\alpha=0.01$ ). Different letters represent statistically different groupings. H statistic not corrected for ties.

could not be met, a Kruskal-Wallis analysis of variance on ranks was used. Tukey's and Dunn's methods were used for pairwise comparisons of treatments ( $\alpha=0.01$ ).

**Comparisons Among Species within Coatings**

Statistical differences in adhesion strength among species were seen on all coating types (Figures 6 and 7). Barnacles showed consistently lower adhesion strength than tubeworms. Oyster adhesion strength was greater than that of barnacles on coatings with additives, but not on unmodified RTV 11. Oyster and tubeworm adhesion strength did not differ statistically, although an increased tubeworm sample size or parametric testing would likely differentiate adhesion strength to coating 8. The differences in organism adhesion strength reflect a variable response to silicone coatings. This may be due to differing chemical, physical and morphological characteristics of adhesives found among species.

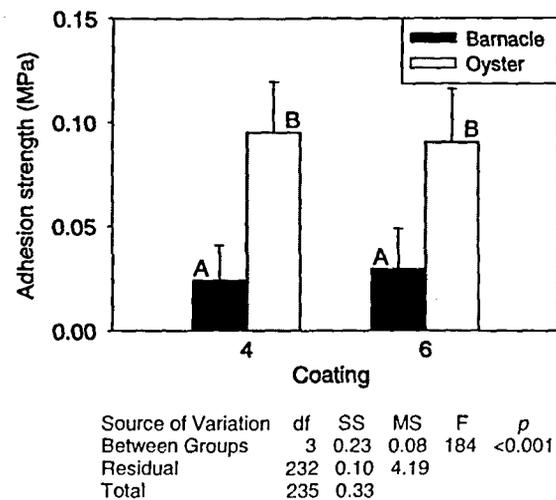
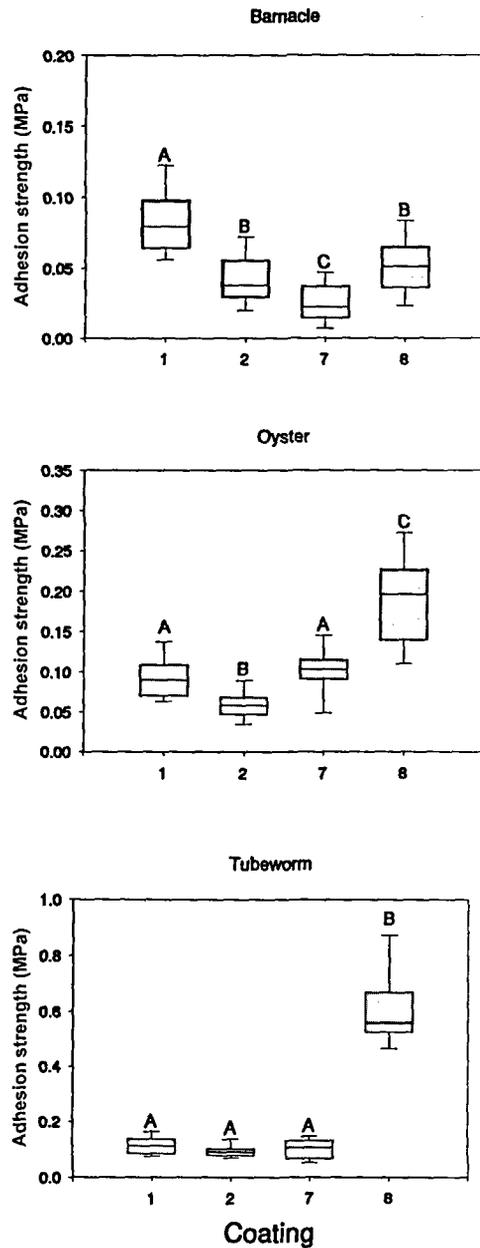


FIGURE 7 Adhesion strength (MPa) of barnacles compared with oysters on coatings 4 and 6. Boxes reflect mean values. Bars represent 1 SD. Statistical results are from a one-way anova followed by Tukey's test ( $\alpha=0.01$ ). Different letters represent statistically different groupings. Anova table values were rounded off.



Organism	df	H	p
Barnacle	3	460.3	<0.001
Oyster	3	53.3	<0.001
Tubeworm	3	27.3	<0.001

FIGURE 8 Adhesion strength (MPa) of coatings compared within species. Boxes reflect median values with 25th and 75th percentiles. Bars represent 10th and 90th percentiles. Statistical results are from Kruskal-Wallis one-way anova on ranks ( $\alpha=0.01$ ). Different letters represent statistically different groupings. H statistic not corrected for ties.

**Comparisons Among Coatings within Species**

Statistical differences in adhesion strength among coatings were seen for each organism

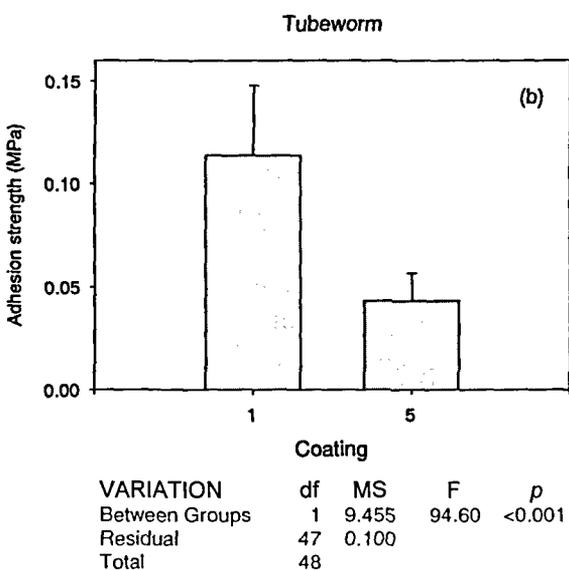
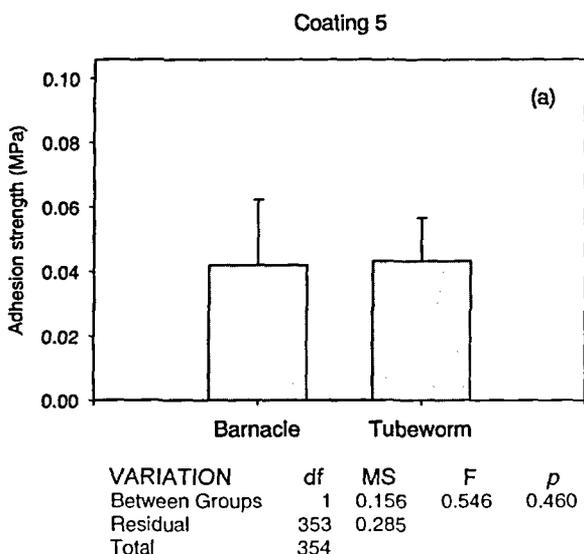


FIGURE 9 (a)= comparison between barnacle and tubeworm adhesion strength (MPa) to modified RTV 11 (coating 5); (b)= comparison of tubeworm adhesion strength (MPa) between unmodified RTV 11 (coating 1) and coating 5. Boxes and error bars represent mean +1SD. One way anova ( $\alpha = 0.01$ ) were performed on  $I_n$  (MPa).

type (Figure 8). Adhesion of barnacles was reduced significantly by the inclusion of oils in RTV 11. Oyster adhesion strength to coatings with different oils varied when compared to unmodified RTV 11. Tubeworm adhesion strength was significantly increased on coating 8 (median = 0.6 MPa) compared to RTV 11 and the other coatings (medians = 0.1 MPa). The results reveal a species by coating interaction, which implies a differential surface response controlled by additive type.

Due to a lack of oyster data on coating 5 ( $n = 1$ ), a separate one way analysis of variance between barnacles and tubeworms was performed. The results revealed no significant difference ( $p = 0.460$ ) between species on this coating (Figure 9a). Tubeworm adhesion strength on coating 5 was compared to unmodified RTV 11. The results showed a significantly reduced tubeworm adhesion ( $p < 0.001$ ) to the modified coating (Figure 9b). These analyses reinforce the previous observation of a coating  $\times$  species interaction.

**CONCLUSIONS**

Significant differences were seen in the shear adhesion strength of hard-fouling types to modified silicone surfaces. In general, it was found that the adhesion strength of *H. dianthus*  $\geq$  *C. virginica*  $\geq$  *B. eburneus*. The contact area of the adhesive represented 90% of the variance seen in the force to remove oysters. However, only half of the variance in force was explained by contact area for barnacles and tubeworms. Change in adhesion strength with size of the organism was not seen on the majority (86%) of the surfaces tested. Silicone oil additives to RTV 11 silicone imparted variable effects on the adhesion strength of the three organism types, indicating a coating  $\times$  species interaction. Barnacle adhesion strength to modified RTV 11 coatings was notably low (ranging from 0.025 to 0.061 MPa), and reduced from unmodified RTV

11 in all cases. Tubeworm and oyster adhesion strength increased, decreased or reflected no change on coatings with oil additives compared to unmodified RTV 11.

The results from this study suggest that further investigation into the fracture behavior of biological adhesives is needed to determine the controlling mechanisms of release. Factors that may contribute to the variability in biological adhesion strength are the chemical and physical properties of the adhesives, the occurrence of discontinuities and inconsistency of composition in the adhesives and the coatings, and variable geometry of interfacial contact. The observed differences in hard-fouling adhesion strength indicate that benthic organisms may inherently differ in ability to stick to a surface, and that ability can be modified by material properties of a coating. It is suggested that a variety of fouling types be used when tests of this kind are part of the selection criteria for potential fouling-release formulations.

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