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Bryozoan and barnacle settlement in relation to initial surface wettability: A comparison of laboratory and field studies

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SUMMARY: The hypothesis that surface chemistry, specifically initial wettability, can be a determinant in settlement of estuarine macroinvertebrate larvae was tested. Of additional interest was how well laboratory settlement tests would predict results obtained in the field. Initial surface wettability has an effect on settlement of barnacle and bryozoan larvae in the laboratory and in the field. The relationship between wettability and settlement in the laboratory and in the field is opposite for the two species. Barnacles settle in higher percentage on surfaces with higher initial wettability. Bryozoans settle in higher percentage on surfaces with lower initial wettability. At intermediate levels of wettability, both species of larvae settle. Surfaces exposed to sea water did not change wettability measurably in 6 days. Settlement of barnacles and bryozoans was inversely correlated (R = −0.94). These studies demonstrate that surface chemistry can play a significant role in determining the distribution and abundance of barnacles and bryozoans.

Key words: settlement, larval behaviour, wettability, Bryozoans, Cirripedia.

INTRODUCTION

Extensive careful work has been done in the laboratory and in the field on documenting the effects of flow (WALTON-SMITH, 1946; CRISP, 1955; RITTSCHOF *et al.*, 1984; WETHEY, 1986; RITTSCHOF & COSTLOW, 1987b), rugosity (CRISP, 1974), microflora (CRISP & RYLAND, 1960; MIHM *et al.*, 1981; STRATHMANN *et al.*, 1981; KIRCHMAN *et al.*, 1982; WEINER *et al.*, 1985; MAKI *et al.*, 1988), free and adsorbed molecules (YULE & CRISP, 1983; BURKE, 1984, 1986; CRISP, 1984; MORSE, 1984, 1986; STANDING *et al.*, 1984; WALKER & YULE, 1984; YULE & WALKER, 1984, 1985; RITTSCHOF, 1985; RITTSCHOF & BONAVENTURA, 1986; GERHART *et al.*, 1988) on settlement of macroinvertebrate larvae.

The role of surface chemistry in determining settlement of bryozoan and barnacle larvae has been studied in the laboratory (CRISP, 1984; WOOLLACOTT, 1984; MIHM *et al.*, 1981; RITTSCHOF *et al.*, 1984, 1986, 1988; RITTSCHOF & COSTLOW, 1987a,b). The laboratory phenomenon of surface chemistry effects is clearly accepted. However there are conflicting opinions as to the role of the substratum and surface chemistry in determining the distribution and abundance specially of barnacles in nature (WETHEY, 1986). We tested the role of initial surface wettability, in determining the distribution and abundance of barnacles *Balanus amphitrite amphitrite* Darwin and bryozoans *Bugula neritina* Cuvier. Field studies were conducted when larvae of both species were settling. Surfaces were all silane modified glass and were devoid of other surface characteristics known to affect larval settlement. Simultaneous settlement of both kinds of larvae was monitored during a time interval that wettability measures showed little change in surfaces immersed in sea water.

METHODS

Silanization of glass surfaces

The surfaces used are summarized in Table I and presented schematically in figure 1. Laboratory assays and wettability measures were in 23 by 86 mm Wheaton borosilicate glass vials. Field assays were with 7 by 100 mm soda-lime glass rods. Laboratory

TABLE I. — Description of silane treatments

Surface	*Wettability*	*Chemical name*	*Source*
HFIPM	10.9	(HeptaFluoroIsoPropOxy) PropylMehtylDichloroSilane	Petrarch H6900
TMS	20.1	TriMethylChloroSilane	Petrarch T2951
DPS	29.1	DiPhenylDiChloroSilane	Petrarch D5950
ClPrS	31.2	3-ChloroPropylTriMethOxySilane	Petrarch C3300
APS	34.0	AminoPropylTriEthoxySilane	Union Carbide A1100

clean (BAIER, 1973) rods or vials were rendered a standard high surface energy and organically clean by baking at 500 °C for a minimum of 4 hours. Baked glass was stored at 100 °C until use.

DPS, TMS, and HFIPM surfaces were prepared by incubating baked glass surfaces in 1.5 % solutions of silane reagents in methylene chloride for 1 hour. Surfaces were rinsed 3 times with methylene chloride then cured for a minimum of 1 hour at 100 °C. APS solution was made with 1 % silanizing reagent in 95 % ethanol. CLPRS solution was made with 2 % silanizing reagent in 95 % ethanol, acidified with 0.2 % v/v glacial acetic acid. APS and CLPRS surfaces were prepared by incubating baked glass surfaces in silanizing solutions for 15 minutes. Surfaces were rinsed 2 times with 95 % ethanol and 1 time with 100 % methanol then cured for 15-30 minutes at 100 °C.

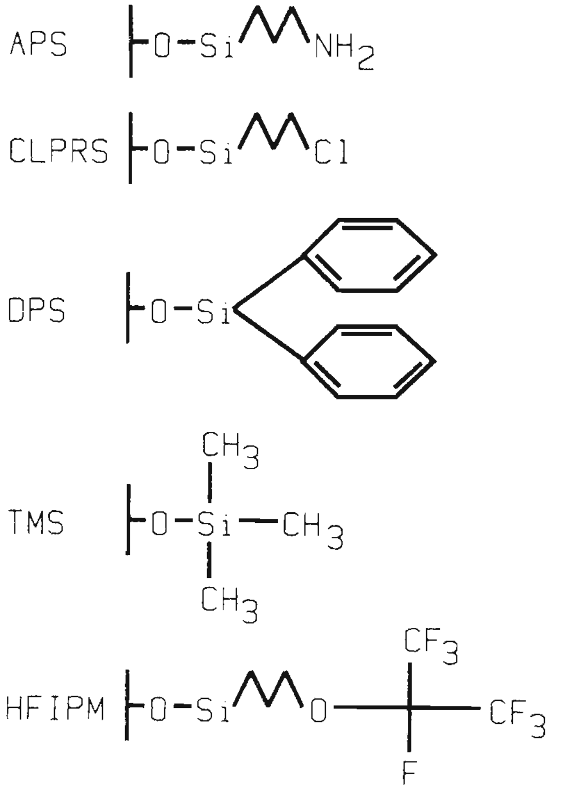

FIG. 1. — A schematic diagram of the result of the five silanization treatments on a glass surface. The abbreviation for each surface treatment is immediately to the left of the effect of that treatment. Clean glass contains hydroxyl groups which can be reacted with the silane reagents to form covalent bonds. The O (oxygen), bonded to the vertical bar (representing the glass surface) and the Si (the silane reagent), is the oxygen that was part of the hydroxyl group on the surface of the glass.

Wettability measurements

Wettability of a surface was determined by measuring the spread of 25 μl drops of a series of solutions of water and methanol. Solutions used were 100, 80, 60, 40, 30, 20, 10, and 0 % HPLC grade water in HPLC grade methanol. Drops were applied to the surface and the drop measured in mm by its longest dimension. Measurements were collected starting with 100 % water and progressed to successively higher concentrations of methanol. When a drop spread of 20 mm or greater was obtained, all succeeding concentrations were assigned a value of 20. Drop spread measurements were reduced to a single number and scaled from 0 to 100 by the following equation:

$$\text{Wettability} = \\ = \left[\left(\frac{8}{1/W100 + 1/W80 + 1/W60 + 1/W40 + 1/W30 + 1/W20 + 1/W10 + 1/W0} - 4\right)/16\right] \times 100$$

where W100 = mm drop spread at 100 % water
W80 = mm drop spread at 80% water in methanol
etc.

8 = Number of solvent concentrations used
4 = minimum possible drop measurement in mm
16 = measurable range in mm.

The scaling results in wettability values that are roughly comparable to the critical surface tensions obtained with a contact angle goniometer (BAIER *et al.*, 1973).

Settlement stage larvae

Laboratory studies were conducted with settlement stage barnacles *Balanus amphitrite* and bryozoans *Bugula neritina*. Barnacle cyprids were mass cultured (RITTSCHOF *et al.*, 1984). Bryozoan larvae were released by light shock the morning of an experiment from colonies collected the previous day.

Laboratory settlement assays

Laboratory settlement assays were single choice substrata assays in glass vials modified by silanization reagents. Barnacle assays were patterned after BRANSCOMB & RITTSCHOF (1984). Settlement stage barnacles of known age were incubated at 28 °C for 22 hours, separated into permanently attached and free larvae and counted.

Bryozoan assays (RITTSCHOF *et al.*, 1988) were single choice substrata assays in glass vials modified by silanization reagents. Bryozoan larvae released from colonies by a light shock immediately prior to use were pipetted into vials and incubated at 23 °C for 30 minutes. Several drops of formalin were added to each vial and permanently attached and free larvae were recorded.

Field tests

Field tests were conducted under a floating dock in an area inhabited by both *Balanus amphitrite* and *Bugula neritina*. Twenty-four 7 mm by 100 mm glass rods treated with silanization reagents were used for each of the 5 tests treatments (APS, CIPRS, DPS, TMS and HFIPM) and assigned a position in one of ten replicate 4 by 3 blocks by random numbers table. Rods were fixed in a plastic screen in a rectangular array 10 cm by 100 cm (3 columns by 40 rows). The array was fixed horizontally 25 cm under the surface on a floating dock with the rods projecting down in the water column. The long axis of the array was oriented perpendicular to the tidal flow. Depth of the array was constant as a result of the rise and fall of the dock with the tide. No special care was taken to protect the array from the surface film when it was installed or retrieved. Arrays were deployed and retrieved between 1200 and 1700 hours.

Experiments

Laboratory and field experiments were conducted to determine if there was a relationship between settlement of bryozoan and barnacle larvae and the initial wettability of the surface. Wettabilities spanned a narrow range, natural representatives of which can be found in the field. The most wettable surfaces were slightly above the biocompatible range and the least wettable surfaces were slightly below the biocompatible range (BAIER, 1978). Factors such as surface rugosity were eliminated by using identical surfaces. Rods were chosen to minimize edge effects in field experiments. The nature of the experiments in the laboratory required the surfaces be containers, while those in the field were smooth rods. Previous work has shown no effect of gregariousness on total settlement in the laboratory (BRANSCOMB & RITTSCHOF, 1984; RITTSCHOF *et al.*, 1988). Field data were analyzed in a presence or absence mode to eliminate any gregariousness effect. Frequency analysis was also employed in both series of experiments.

RESULTS

Wettability and exposure of surfaces to sea water

It is known that the wettability of surfaces changes through time as a result of exposure to seawater (BAIER, 1984; MEYER *et al.*, 1988) and that the trend for all inert surfaces exposed to sea water is to move toward a common wettability value. Wettability of HFIPM, CLPRS, and APS surfaces was measured before and after exposure to sea water for 0 to 6 days. The three surfaces changed slightly, moving toward the center of the wettability range after 3 days. By day six, HFIPM had increased from an initial wettability of 11 to a value of 15. CLPRS changed from 30 to 28 in three days and was at a value of 26 on day 6. APS changed from 33 to 28 by day 3. However, the day 6 reading was 30. However, none of these changes was statistically significant (ANOVA for paired comparisons, $P > 0.25$ for each surface; Fig. 2).

Surface wettability and larval settlement in the laboratory

Settlement of barnacle and bryozoan larvae was studied in relation to surface wettability in the la-

FIG. 2. — Wettability measures of APS (triangles), CLPRS (circles), and HFIPM (squares) surfaces exposed to sea water for six days. Published accounts (MEYER *et al.*, 1987) show little effect of exposure to sea water for 1 day, and measurable effects after 3 days. Surfaces toward the middle of the wettability range (20 to 25) change even more slowly in response to sea water exposure.

boratory. Five surfaces, HFIPM, TMS, DPS, CLPRS, and APS were tested with barnacle and bryozoan larvae.

Frequency analysis demonstrated that barnacle settlement varied significantly between surface treatments ($G = 22.2$, d.f. $= 4$, $p < 0.005$). *A posteriori* tests of the frequency data placed TMS, DPS, CLPRS and APS surfaces into one maximally non-significant subset, and HFIPM, DPS and CLPRS into a second. Barnacles settled in relatively high percentage (40 to 47 %) on all except the lowest wettability surface, HFIPM. Settlement on HFIPM was approximately 75 % that of the other surfaces. Linear regression of transformed percent settlement (see methods) on surface wettability showed no significant trend (Fig. 3).

The surface treatments also exerted a significant effect on bryozoan settlement ($G = 482$, d.f. $= 4$, $p < 0.005$). *A posteriori* tests of the settlement frequency data placed HFIPM and DPS into one maximally non-significant subset, and TMS and CLPRS into a second. Linear regression analysis showed a significant inverse trend between surface wettability and bryozoan settlement ($y = -1.4x + 81.6$; regression significant at $P < 0.005$). Bryozoan settlement was highest on the surface with lowest wettability (HFIPM), and lowest on APS, the surface with highest wettability (Fig. 3).

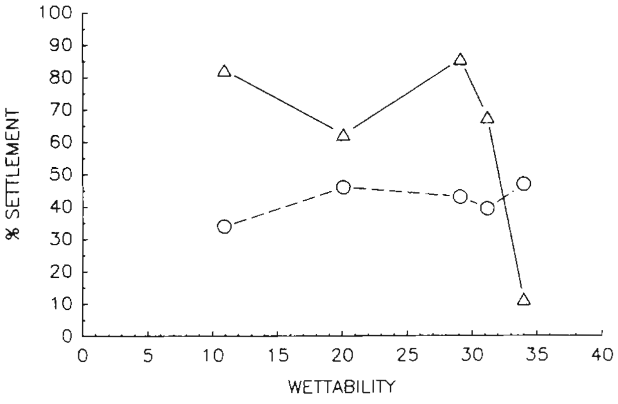

FIG. 3. — Laboratory settlement of barnacles (circles) and bryozoans (triangles) in relation to initial surface wettability. Effects of wettability upon settlement were most obvious at the extremes of the wettability range. In contrast to the field experiments, simultaneous settlement by both barnacles and bryozoans was not possible in these experiments because of differences in the experimental design.

Field settlement of barnacles and bryozoans

At Beaufort, North Carolina, the settlement of barnacles and bryozoans overlaps both temporally and spatially. We studied the simultaneous settlement of these larvae in the field. The 5 surface treatments tested in the laboratory were tested in the field. The field experiment was conducted over three days, from May 22 through May 25, during an interval of bryozoan settlement and the first interval in 1987 in which substantial numbers of barnacle larvae settled.

Highly significant effects of wettability on settlement of barnacles and bryozoans were observed. Frequency analysis (G tests for independence), examining the proportion of replicates of each treatment that contained at least one larva, showed effects of surface treatment upon settlement (for barnacles, $G = 29$, d.f. $= 4$, $P < 0.005$; for bryozoans, $G = 92$, d.f. $= 4$, $P < 0.005$). *A posteriori* tests of the frequency data grouped the surface treatments for barnacles into 4 maximally non-significant subsets: CLPRS, APS, + DPS; CLPRS, TMS, + DPS; HFIPM, TMS, + DPS; and APS + TMS. Similar analysis grouped bryozoan responses to surface treatments into two maximally non-significant subsets, one containing HFIPM and TMS, and the second consisting of DPS, CLPRS, and APS. Barnacle settlement showed a significant linear relationship between initial wettability and settlement ($Fs = 14.6$; d.f. $= 1,3$; $P < 0.05$). Bryozoan settlement (measured as the transformed percentage of replicates in a treatment group with at least one settled larva), showed no significant linear trend with wettability ($Fs = 3.2$; d.f. $= 1,3$; $P > 0.1$; Fig. 4).

Correlation analysis showed that barnacle and bryozoan settlement on the 5 surfaces varied inversely (Pearson's product-moment correlation coefficient $= -0.94$; significant at $P < 0.05$; coefficient of determination $= 0.88$).

Comparison of laboratory and field data

Field and laboratory settlement data for barnacles and bryozoans are similar, in that significant effects of surface treatment are observed. Barnacle settlement assays in the laboratory showed a discontinuous rather than linear trend in wettability and settlement. Barnacle settlement in the field on the same surfaces showed a significant linear relationship between initial wettability and settlement ($Fs = 14.6$; d.f. $= 1,3$; $P < 0.05$). This difference is due mainly to relative increases and decreases in settlement on surfaces at the ends of the wettability range tested.

In contrast to barnacle settlement results, settlement data for bryozoans showed a clear and significant inverse relationship between surface wettability treatment and settlement in the laboratory, but not in the field. The rank order and pattern of settlement on surfaces in the field was similar, but settlement on the higher wettability surfaces was relatively low compared to what would be expected from laboratory data.

Comparison between barnacle and bryozoan set-

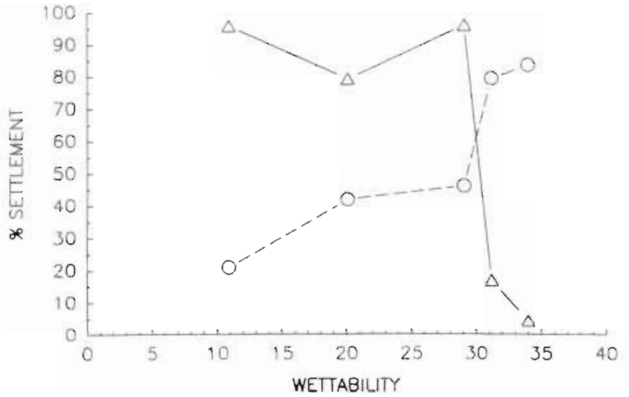

FIG. 4. — Field settlement of barnacles (circles) and bryozoans (triangles) in relation to initial surface wettability. Effects of wettability upon settlement were obvious at the extremes of the wettability range. Simultaneous settlement by barnacles and bryozoans on these surfaces is inversely correlated (Rs = −0.94: coefficient of determination = 88 %). Thus, barnacle and bryozoan larvae responsed very differently to initial surface wettability.

tlement for the field data was possible, since the larvae of both barnacles and bryozoans could settle simultaneously on the same surfaces. Responses in the laboratory were similar in pattern to those observed in the field. The differences observed in the field were underestimated in the laboratory experiments.

DISCUSSION

The purpose of this investigation was to test the hypothesis that surface chemistry, specifically initial wettability, is a determinant in settlement of estuarine macroinvertebrate larvae. Also of interest was how well laboratory settlement tests would predict results obtained in the field. We do not dispute that factors such as surface roughness, color, hydrodynamics, location and specific chemicals are important in determining the settlement distribution of macroinvertebrate larvae. However, in the experiments reported here, larvae settled differentially on smooth, transparent surfaces placed randomly in relation to flow. Surface chemistry, specifically wettability, can be added to the list of determinants of settlement in the field.

Comparison of laboratory and field tests of barnacle and bryozoan settlement in relation to surface wettability yielded striking similarities and dramatic differences. There is no doubt that the phenomena observed in the laboratory also occur in the field, and that surface chemistry can play an important role in determining the distribution of newly-settled larvae of both barnacles and bryozoans.

The relationship between wettability and settlement in the laboratory is opposite for barnacles and bryozoans (MIHM *et al.*, 1981; RITTSCHOF & COSTLOW, 1987b). Over the narrow range of surface wettabilities tested here there is no trend related to wettability for barnacle settlement in the laboratory. Bryozoan settlement in the laboratory is linearly related to wettability in a negative fashion and decreased precipitously at the highest wettability tested (APS). In the field, barnacle settlement was linearly and directly related to wettability. Bryozoan settlement was an inverse and a discontinuous function of wettability.

The differences between laboratory and field data provide a perspective that will improve as additional experiments are conducted. Time is a very important variable. The differences reflect how many times a particular larva comes in contact with a surface, the physiological state of the larva when it comes into contact with a surface (RITTSCHOF *et al.*, 1984) and the nature of the surface itself (BAIER, 1984; MEYER *et al.*, 1988). Using additional field data, laboratory assays can be adjusted to provide more relevant information.

Our findings on the effects of surface chemistry on settlement may also provide a partial explanation to conflicting reports on the effects of bacterial films on macroinvertebrate settlement. If the effect of films is via the resultant altered surface chemistry, then the important determinant is not the presence or absence of the film, but the specifics of the effect of the film on the chemistry of the surface that the larvae encounter. There is evidence (FLETCHER & LOEB, 1979; MIHM *et al.*, 1981; MAKI *et al.*, 1988) that the surface chemistry presented to larvae by an established bacterial film is dependent upon the wettability of the surface as well as the physiological state of the bacteria composing the film. Many of the apparent conflicts in the literature with respect to the effects of microbial films may de resolved when technical advances in wettability measures result in increased investigation in this area.

The field experiment reported here is an internally controlled and contrived look at the effects of surface chemistry in the field. Within fouling communities surface chemistry and wettability are changing continuously as encrustations slough and as community structure changes due to senescence, competition, predation, season, and physical disturbance (SUTHERLAND, 1984). However, on a scale that influences the settlement of individuals, wettability and other aspects of surface chemistry exert their effects.

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