The Effects of Slime Film on Barnacle Settlement

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The role of slime film on macrofouling was studied by comparing the ratio of settlement of cyprids to young barnacles on panels with slime films and panels that had been sterilized. Settlement was greater on the panels with slime. The rate of settlement varied significantly during different months and different exposure periods. Similar patterns were found on the sterile as well as the panels with slime. The results were compared to similar observations and studies from different parts of the world.

INTRODUCTION

The effects of slime film on macrofouling has been studied by several authors. Zobell (1939) studied the attachment of barnacle larvae on glass panels and concluded that slime forms a beneficial substrate for their attachment. Slime favors fouling in a variety of ways . . . by enmeshing larvae, by discoloring glazed or bright surfaces, by serving as a food source, by protecting organisms from toxic constituents of paints, and by increasing alkalinity, thus favoring the deposition of calcareous cements. The composition of the primary film varies in different areas. Zobell (1939) found that the primary film in the eastern coastal waters of the United States consists of bacteria. In Australia, Wood (1950) found the biofilm composed of diatoms and algal spores. Daniel (1955) observed in Madras that the film was composed of 38 species of diatoms, 14 species of algae, and a small portion of bacteria. Balasubramanyan and Menon (1963) and Nair and Pillai (1977) also recorded a predominance of bacteria in primary films. American

(Weiss 1948) and Australian (Scheer 1945, Wood 1950) researchers supported the view that a bacterial film forms the primary film. According to Daniel (1955), the primary film is an essential prerequisite for the attachment of barnacles. Nair and Pillai (1977) observed that while a primary film is not an essential prerequisite for barnacle cyprids, a slime surface does favor the attachment of a larger number of settling barnacles. On antifouling surfaces, slime has been found to favor, to interfere with, or to have no effects on fouling forms (WHOI 1952).

METHODS

Panels with slime films and without (sterilized) were immersed at the test site, the north quay of an oil tanker berth at the Cochin harbor, and the number of barnacles attached to the panels were counted. Glass panels, 150 × 100 mm, were used. The panels with slime were prepared by exposing them in a large glass trough containing seawater from the test site for 24 hours under laboratory conditions. The sterile panels were prepared by autoclaving them at 1.05 kg cm^{-2} pressure for one hour. Beginning in March 1991, six panels were immersed each month (except June, July, and August, the monsoon months), the sets were retrieved following 24, 48, and 72 hours, and the attached cyprids and young barnacles microscopically counted. The average values from the two types of panels were then recorded.

RESULTS AND DISCUSSION

The numbers of cyprids and young barnacles attached to the panels are given in figure 1. The settlement ratios indicate that the cyprids settled in larger numbers on the surfaces with slime at all exposures, even though they also settled readily on the sterile panels. The details are given in table 1. Anova (Snedecor and Cochran 1968) was used to determine whether the attachment rate differed during the various months, table 2. It was found not only to vary significantly, monthly, but also during different exposure periods. To separate the months having significantly high settlement, the least significant difference (LSD) at $P < 0.01$ was calculated.

On the panels with slime, while there were no significant differences between March, September, and February, settlement varied significantly between these months and October, November, December, and January. The rate was comparatively high in October, remained uniform for a number of months, and then started a declining trend in February, table 1.

The pattern was almost the same on the sterile panels. During March, April, and May there were no significant differences in the settlement rate,

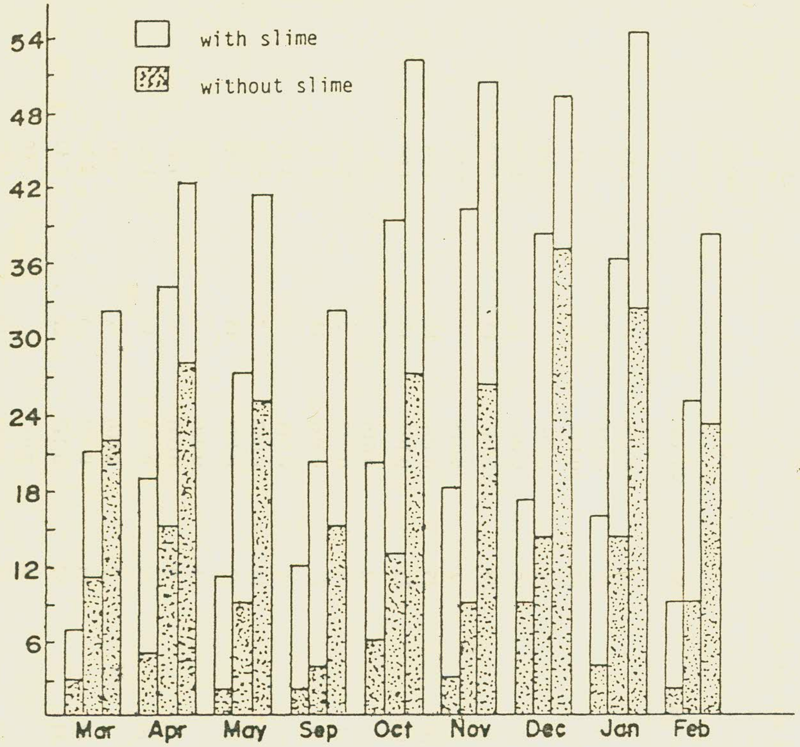

Figure 1. Comparison of barnacle settlement on panels with slime and without slime following 24, 48, and 72 hours of exposure.

Table 1. Anova analysis of variance in the settlement rate of barnacles on panels with slime films and without slime films.

Source	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
		Panels with Slime Film		
Total	4956.63	26	–	–
Between months	1036.30	8	129.54	21.84*
Between periods	3825.41	2	1912.71	322.55*
Error	94.92	16	5.93	
		Panels without Slime Film		
Total	2756.00	26	–	–
Between months	342.33	8	42.67	6.18*
Between periods	2304.22	2	1152.11	166.97*
Error	110.45	16	6.90	

* $P < 0.01$

Table 2. Comparison of barnacle settlement on panels with slime and without slime.

Month	Hour	With Slime			Without Slime			Ratio With/Without
		No.	No. cm^{-2}	Rate/Day	No.	No. cm^{-2}	Rate/Day	
March	24	17	0.047		3	0.020		2.33
	48	21	0.140	4	11	0.073	8	1.91
	72	32	0.213	11	22	0.146	11	1.45
April	24	19	0.127		5	0.033		3.80
	48	34	0.227	15	15	0.100	10	2.27
	72	42	0.280	8	28	0.187	13	1.50
May	24	11	0.073		2	0.013		5.50
	48	27	0.180	16	25	0.167	16	1.64
	72	41	0.273	14	25	0.167	16	1.64
September	24	12	0.080		2	0.013		6.00
	48	23	0.153	11	4	0.027	2	5.75
	72	32	0.213	9	15	0.100	11	2.13
October	24	20	0.133		6	0.044		3.33
	48	39	0.260	19	13	0.087	7	3.00
	72	52	0.347	18	27	0.180	14	1.93
November	24	18	0.120		3	0.020		6.00
	48	40	0.267	22	9	0.060	6	4.44
	72	50	0.333	10	26	0.173	17	1.92
December	24	17	0.113		9	0.060		1.89
	48	38	0.253	21	14	0.093	5	2.71
	72	49	0.327	11	37	0.247	22	1.32
January	24	16	0.107		4	0.027		4.00
	48	36	0.240	20	14	0.093	10	2.57
	72	54	0.360	28	32	0.213	18	1.69
February	24	9	0.060		2	0.013		4.50
	48	25	0.167	16	9	0.060	7	2.78
	72	38	0.253	13	23	0.153	14	1.65

but in September it declined, differed significantly from the previous months, and varied significantly in October, November, December, and January. February also showed a slight reduction, table 2. Settlement was not observed during the monsoon months of June, July, and August.

The settlement ratio on both types of panels was high following the initial 24 hours and then decreased at 48 and 72 hours. Even though the number increased steadily, the ratio of panels with slime to the sterilized showed a reduction. In addition, the initial increase from 24 to 48 hours was high on the panels with slime. This increase was found on the sterile panels between 48 and 72 hours. This can be attributed to the slime formation and the settlement that occurs during the first 24 hours on the sterile panels and which aids to accumulate more settlers by 48 hours.

The influences of slime films in attracting more settlers to surfaces were studied by Knight Jones (1951), Wilson (1955), Doochin (1959), Crisp and Medows (1963), Horbund and Freiberger (1970), Disalvo (1971), Nair and Pillai (1977), and several unpublished researchers (WHOI 1952). These authors felt that while the presence of a slime film on a surface is not absolutely necessary for barnacle attachment, it does attract and retain many more barnacles than surfaces without a slime film. In our nine-month study, the total numbers of attached cyprids were significantly greater on the panels with slime film, 0.6 cm^{-2}, than on sterile ones, 0.3 cm^{-2}; 66.9 and 31.1% of the total number settled, respectively. The total settlements on the panels with slime were nearly double that of the sterile panels, which clearly shows that the barnacle cyprids preferentially colonized the slime surfaces. The settlement preference of the sessile serpulid polychaete *Spirorbis borealis* was studied by Knight Jones (1951), who found that there was greater attachment on surfaces with slime than on clean surfaces. Mention is made of the attachments of barnacles and larvae of *Bugula neritina* on slime panels by different workers (WHOI 1952). Even though larger numbers of larvae attached to the slime panels, the slime was not absolutely essential for their attachment. In *B. neritina*, attachment in some cases was fifteen fold. The same observation was made for *Cliona* larvae and *Balanus tintinnabulum*. Coe and Allen (1937) also observed heavy fouling on previously fouled panels. But according to Scheer (1945), barnacle settlement did not seem to be enhanced by slime film. Even though the formation of a primary film is a natural process of accidental impact, the settlement of secondary settlers, such as larval forms of fouling organisms, can be influenced by various factors such as suitability of the substratum, including nutritional sources for the early stage of development.

Young and Mitchell (1972) reported that a microbial population stimulates the development of macrofouling organisms. Crisp (1974), Scheltema (1974), and Kirchman et al. (1982) also suggested that the larvae of many fouling organisms prefer to settle on surfaces coated with a microbial film. Studies have shown that the larval settlement and metamorphosis of the fouling polychaete *Janua brasiliensis* are triggered by specific bacterial films on marine surfaces (Mitchell and Kirchman 1984).

The formation of bacterial biofilms is usually an essential prelude to the development of macrofouling communities. The pioneering bacterial community is followed by diatoms and fungi. The details of the attachment mechanisms and the interrelations of the various constituents are also known. Algal attachment to the surface involves both positive chemoreception and attachment of specific proteins to bacterial polysaccharides (Mitchell and Kirchman 1984).

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LITERATURE CITED

Balasubramanyan, R., and T.R. Menon. 1963. Destruction of boat building timbers by marine organisms in the Port of Cochin. Part I. Raft tests. *J. Mar. Biol. Assoc. India* 5: 294–301.

Coe, W.R., and W.E. Allen. 1937. Growth of sedentary marine organisms on experimental blocks and plates for nine successive years at the pier of the Scripps Institution of Oceanography. *Bull. Scripps Inst. Oceanogr. Univ. Calif.* 4: 101–109.

Crisp, D.J. 1974. Factors influencing the settlement of marine invertebrate larvae. Pages 177–182 in P.T. Grant and A.M. Mackie, eds. *Chemoreception in Marine Organisms*. Academic Press, New York.

Crisp, D.J., and P.S. Medows. 1963. Adsorbed layers: the stimulus to settlement in barnacles. *Proc. R. Soc. Lond. B.* 158: 364–369.

Daniel, A. 1955. Gregarious attraction as a factor influencing the settlement of barnacle cyprids. *J. Madras Univ. B.* 25: 97–101.

Disalvo, L.H. 1971. Evaluation of early steps in the fouling sequence in relation to control of wettable film: film formation on glass surfaces. Pages 1–7 in Interim Report, University of California, Naval Biomedical Research Laboratory.

Doochin, H.D. 1959. The morphology of *Balanus improvisus* Darwin and *Balanus amphitrite niveus* Darwin during initial attachment and metamorphosis. *Bull. Mar. Sci. Gulf Caribb.* 1: 15–22.

Horbund, H.M., and A. Freiberger. 1970. Slime films and their role in marine fouling. *Ocean Eng.* 1: 631–634.

Kirchman, D., S. Graham, D. Reish, and R. Mitchell. 1982. Bacteria induce settlement and metamorphosis of *Janua (Dexiospora) brasiliensis* (Grube). *J. Exp. Mar. Biol. Ecol.* 56: 153–163.

Knight-Jones, E.W. 1951. Gregariousness and some other aspects of the settling behaviour of *Spirorbis*. *J. Mar. Biol. Assoc. U.K.* 30: 201–205.

Mitchell, R., and D. Kirchman. 1984. The microbial ecology of marine surfaces. Pages 49–56 in J.D. Costlow and R.C. Tipper, eds. *Marine Biodeterioration: An Interdisciplinary Study*. Naval Institute Press, Annapolis, Maryland.

Nair, N.U., and A.G.G.K. Pillai. 1977. Observations on the composition of slime film and its influence in the settlement of certain marine fouling organisms in Cochin harbour. Pages 283–286 in *Proceedings of the Symposium on Protection of Materials in the Sea*. Naval Chemical and Metallurgical Laboratory, Bombay, India.

Scheer, B.T. 1945. The development of marine fouling communities. *Biol. Bull.* (Woods Hole) 89: 103–108.

Scheltema, R.S. 1974. Biological interactions determining larval settlement of marine invertebrates. *Thalassia Jugosl.* 10: 263–269.

Snedecor, G.W., and W.G. Cochran. 1968. *Statistical Methods*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Weiss, C.M. 1948. Seasonal and annual variations in the attachment and survival of barnacle cyprids. *Biol. Bull.* 94: 236–242.

WHOI. 1952. *Marine Fouling and Its Prevention* U.S. Naval Institute, Annapolis, Maryland.

Wilson, D.P. 1955. The role of microorganisms in the settlement of *Ophelia bicornis* Savigny. *J. Mar. Biol. Assoc. U.K.* 34: 531–537.

Wood, E.J.F. 1950. Investigations on underwater fouling, the role of bacteria in the early stages of fouling. *Aust. J. Mar. Freshwater Res.* 1: 85–91.

Young, L.Y., and R. Mitchell. 1972. The role of chemotactic responses in primary microbial film formation. In *Proceedings of the Third International Congress on Marine Corrosion and Fouling*. Northwestern University Press, Evanston, Illinois.

Zobell, C.E. 1939. Primary film formation by bacteria and fouling, *Collecting Net* 14: 39–41.