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ANTISLIME COATINGS PART II PRECONDITIONING VALUE OF SLIME FOR BARNACLE ATTACHMENT

G. L. Liberatore, et al

Naval Ship Research and Development Center
Bethesda, Maryland

August 1972

NAVAL SHIP RESEARCH AND DEVELOPMENT CENTER
Bethesda, Md. 20034

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PART II
PRECONDITIONING VALUE OF SLIME FOR BARNACLE ATTACHMENT

by

G. L. Liberatore, E. J. Dyckman,
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MATERIALS DEPARTMENT
Annapolis

RESEARCH AND DEVELOPMENT REPORT

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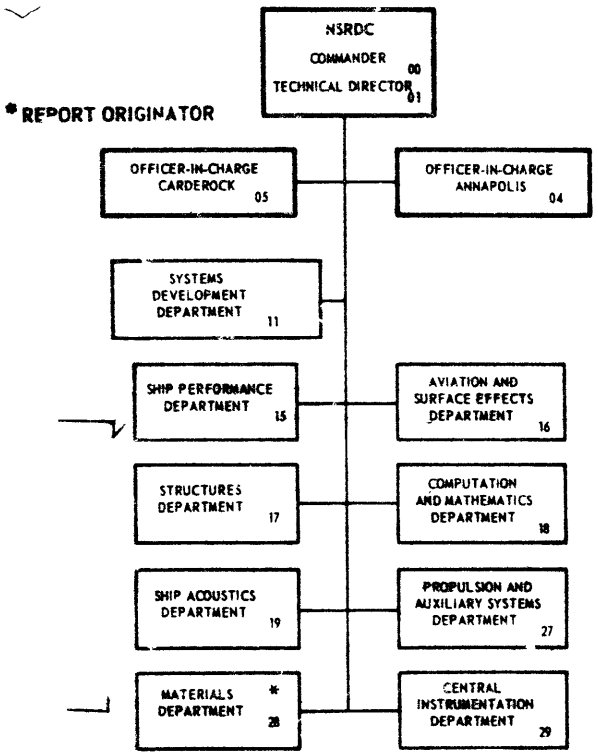

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13 ABSTRACT

Investigations of the relationship between barnacle attachment and the presence of a primary slime film on submerged surfaces has been completed. Using laboratory-reared barnacle cyprids in a statistical settlement survey, it has been determined that the number of barnacle cyprids settling on a slimed surface consistently exceeds by a factor of ten the number that settle on a cleaned control surface. Data describing the slime factor as a naturally occurring surface-preconditioning agent affecting the settlement of other sessile organisms are evaluated.

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Details of illustrations in this document may be better studied on microfiche

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INTRODUCTION

Tighe-Ford[19] estimated that fouling prevention and antifouling maintenance cost the maritime industry over 500 million dollars in 1971. Antifouling coatings and elaborate magnetic, electronic, ultrasonic, and chemical discharge systems have been investigated and represent an indeterminable expenditure of both human and financial resources implemented by Navy and private agencies to ensure the "Foul-Free Hull." Time, however, has proved each system inferior to the age-old biological phenomenon, inherent to marine communities, which affects all submerged surfaces, i.e., fouling. In spite of mechanical, electrical, and chemical aids devised for solving fouling problems, a 5-year antifouling system has not been established. It is, therefore, imperative that a more intimate understanding of the relationships among animals and materials of the ship fouling community be reached. By means of a biological examination of fouling phenomena, we may discover yet-unrealized factors that are significant in the behavior of sessile marine animals in respect to those submerged surfaces upon which they settle. To initiate this effort, we must redefine the time sequence involved in the biotic progression of marine macrofoulants and look more closely at the initial onset of surface preconditioning by bacteria and other microorganisms.

A well known trait of terrestrial bacteria is an affinity for adherence to surfaces (i.e., pebbles). Similarly, we[10] have found this to be an observable feature of marine bacteria in respect to submerged surfaces (i.e., ships hulls, glass coupons, sand, sediments, etc). Before the first bacterial layer is formed, submerged surfaces adsorb dissolved and suspended organic and inorganic matter. However, in less than 1/2 hour, bacteria begin surface colonization, reaching a peak population density within 24 hours after immersion.[10,14] In the water column, the bacterial concentration is lower than on submerged surfaces due to the especially strong periphytic traits of marine bacteria. Zobell[21,22] states that surfaces concentrate organic nutrients by chemisorption which may not only serve as a nutritive source for such bacteria but may also retard diffusion of exoenzymes. Surfaces also serve as resting sites.

Bacteria are attracted and held to a submerged surface by the electrostatic and/or physical forces of that surface.[4,7,9,12,14,16] The energy of this bond is high and varies with surface composition.[3,14] Submerged surfaces which are attractive to bacteria vary in size, shape, and composition: i.e., plastics, metals, wood, glass,[3,10] sand grains, and pebbles.[15,20] Bacterial evolution has progressed toward the production of organisms which have definite hold-fast mechanisms (chemical or physical). The

[19]Superscripts refer to similarly numbered entries in Appendix A.

original variation of bacterial types colonizing a surface may be eliminated within 24 hours and the flora will mainly consist of a few dominant species characteristic of the specific water column.[10,16]

Besides the bacteria itself, slime is also composed of bacterial exudations such as acid polysaccharides and protein polymers. The preconditioning effect these chemicals may have on a surface is to entrap free swimming larvae, change the color of the surface, protect fouling organisms from toxins in the paint, serve as food, increase the surface acidity (thus favoring calcareous depositions), or change the surface's electrical potential.[7,16] The most evident effect of these exudations is to irreversibly attach the bacteria to the surface.[1,4,12,18,22] These bacteria become coated or encapsulated and the slimy envelopes form polymeric bridges to the surface which cannot be removed by purely mechanical methods.[9,14] It is well known that the bacterial slime film in the piping systems of cooling towers cannot be removed by water currents up to 15 knots. The presence of this zooglea[10] or loose slime will result in accelerated bacterial uptake until the entire surface is colonized.[7]

Thus, the slime layer consists of loose slime, entrapped organic detritus and firmly attached bacteria with hold-fast mechanisms. Sliming bacterial species such as the Maraxella, Micrococci, and Vibrios[16] have been isolated. The microorganism which dominates a submerged surface depends not only on the microtopography of that surface but also on the geographical location. In Puget Sound, the first organisms to appear are yeasts while in Australia diatoms are first on a surface, followed by bacteria.[1,16]

Cardarelli[3] found this primary layer on many plastics (fluorocarbons, acrylics, phenolics, and epoxies), glass (rough and polished), mica, metals, salt, and wood. Although the slime may show varying degrees of tenacity to different surfaces, its effects on specific surface materials is pronounced (i.e., microbial corrosion, deterioration, passivation, corrosion from impingement, etc).

The gelatinous bacterially secreted cements provide a physical substrate and/or nutritive source for the normally occurring biotic progression of diatoms, protozoans, hydroids, algae, and barnacles.[12,16,18,22] Although the presence of this primary layer is not an absolute necessity for barnacle attachment, for cyprids can attach to a very clean surface,[7] it has been found to be a preferred condition for many organisms.[6,8,12-14,16,20] The presettling stages of these organisms have the ability to discriminate between slimed and clean surfaces.[5,12,13,16,18,20,22] A strong preference for slimed surfaces has been observed for barnacles, sedentary polychaetes (Serpulids) such as Spirorbis borealis, Ophelia bicornis, etc,[13,15,20] and by all organisms which must attach or be "induced" to metamorphose to the adult form by the presence of nearby surfaces.

There is a hierarchy of stimuli which influence the settling behavior of barnacle cyprids. The cyprid is that stage in the life cycle of the barnacle which is perfectly adapted to investigate a surface and settle on it. It responds to stimuli from the water, such as water current and the presence of food; from the atmosphere, such as light flux, and direction of wind (especially for the littoral and supralittoral species); and from the surface itself, such as texture (rugophilic behavior)[2] and chemical composition. However, all of the stimuli inherent to the surface seem to be mitigated by the primary slime layer. Thus, the presence of this layer is an important event in the settling process. It may entrap the cyprids and make it difficult for them to leave; it provides food for the very young immature barnacles; it may change the color of the surface, making it more attractive; it may absorb various chemicals (which may be responsible for gregariousness); it may reduce the toxic level of antifouling paints (in many cases the actual matrix and/or toxic molecules may be broken down through bacterial degradation), thus decreasing the surface's antifouling effectiveness; and it may affect the tenacity of attachment by alternating the electrostatic properties of a surface or by changing the surface free energy.[7] Notwithstanding the difference of the primary slime layer on submerged surfaces, the same barnacle species will be found attached on all surfaces throughout their distinct geographical sites. The tenacity of the surface-barnacle bond is very high with metals (zinc plates excepted), many plastics, and wood but is low with teflon and low surface-energy elastomers.

Crisp and fellow workers[5,6] found a "tactile chemical sense" in the barnacle cyprids for the recognition of specific molecular groups on a surface. These groups are "releaser" chemicals needed for the cyprid to undergo final metamorphosis and attachment. The relationship between the bacterial slime and these chemicals or between bacterially exuded polymers and specific "releaser" chemicals is not known. Releaser chemicals are arthropodins produced by other barnacles or parts of barnacles (such as the basal plates).[5,6] The cyprids would have to be attracted and induced to stay on a surface before the very thin layer of these kermones could be sensibly detected. Therefore, due to the ubiquitousness and differentiation of the slime layer, the presence of the species-specific arthropodins may be of secondary importance in the attraction of a surface for cyprids. This may explain the common occurrence wherein barnacles of one species settle and attach directly on the shell of totally different species or on the shell of totally different animals.

Many other sedentary organisms need a slimed surface to undergo final metamorphosis and attachment. Organisms which are not sedentary may need the presence of a slimed surface nearby to metamorphose, whether they rest on it temporarily or not. Thus, it is evident (from the scientific literature) that the presence of a primary slime layer on a submerged surface is of the greatest importance in attracting large numbers of fouling organisms and

its impulses to the cyprids supersede impulses from other sources by many orders of magnitude. The fundamental, natural occurrence of marine slime presents novel avenues for solving the fouling problem. Since slime masks the true nature of the surface, it will mediate the original reactions of sessile organisms looking for a site to settle.

This work quantifies the above cited existence and importance of the barnacle-slime interrelationship and represents an effort at this laboratory to reevaluate and advance the state-of-the-art antifouling technology.

MATERIALS AND METHODS

Adult barnacles were obtained from 10- x 12-inch aluminum panels previously exposed in Florida (Miami Marine Research, Inc.) and shipped to the laboratory wrapped in wet newspapers (figure 1). The panels were cleaned by removing most of the gross foulants with a stiff brush and then rinsed quickly with sea water. Balanus eburneus was gently pried off and checked for gravid conditions. Once the basal plate was removed, the mantle membrane was cut with a glass needle to expose the mantle cavity and the two lamellae seated in it (figure 2). These lamellae were placed in a Petri dish with 30-40 ml* sea water and, when sufficient numbers had been collected, were thoroughly rinsed several times with sea water in 60-70 micron plastic sieves. Lamellae were separated in pairs, kept in 300 ml sea water (at room temperature, 20°-22° C), and sieve-washed daily.

Depending on the maturity of the embryos, hatching occurred from 15 minutes to 72 hours following removal from the parent. Being positively phototropic, the first stage nauplii were collected by attracting them to a light source. The healthiest, cleanest, and most viable nauplii, being the fastest swimmers, were quickly drawn up with a Pasteur pipette when they reached the focal point of the light beam. These were placed together in 100 ml of sea water and the numbers per milliliter were estimated by direct count of 1.0 ml aliquots. Following counting, first- and second-stage nauplii were put into 1500 ml beakers (175-200 per container) together with 800 ml sea water and 200 ml of diatom culture. The diatom, Cyclotella nana 3H, was cultured in sea water with the ES enrichment (figure 3).[17] When the diatom cultures reached 1 to 3 x 10^6 diatoms per ml, 200 ml were added to the rearing vessels as described.

Nauplii were washed with sea water in 125 micron sieves daily at which time the rearing vessels were sponge-wiped and rinsed with filtered sea water. The quantity of diatom culture added was

*Abbreviations used in this text are from the GPO Style Manual, 1967, unless otherwise noted.

determined by visually observing the opacity of the stomach contents of the nauplii. Within 10 to 14 days the free swimming nauplii developed to the cyprid stage (figure 4).

The rearing vessels were cleaned daily by sponge wiping and rinsing the sides down to the 200 ml mark. Because the bottom and the sides up to this mark were not cleaned, these areas developed a brown-green slime layer. Daily rinsing of the beakers did not affect the adhesion of the slime layer to the glass surfaces. Two to 3 days after cyprid formation, the vessels were examined and the young, newly attached barnacles were counted.

The slimed and clean surface areas were measured. The slimed surface area was 23.0 in^2 (bottom and 1/2 inch up the side to the 200 ml mark) and the area of the cleaned surface was 42.4 in^2, offering approximately a 2 to 1 ratio of unslimed versus slimed surface.

RESULTS AND DISCUSSION

Although the presence of a slime film on a surface is not absolutely necessary for barnacle attachment, in agreement with previous workers,[6,7,8,12,13,20] we found that the slimed surface attracted and retained many more barnacles than the clean one.

The actual process of attachment did not seem to be affected by the presence of the slime layer. The cyprid walking on the slimed surface, figure 4, showed a scraping motion with its antennules before they attached. These movements were repeated as often as the antennules kept on attaching or moving forward. This "cleaning" motion increased just prior to the secretion of the adhesive and final attachment. The two clear areas become the center of attachment and can be seen in figure 5. Once a site is thus cleared, the cyprids may then be able to recognize the "releaser" chemicals on that surface. It did not seem probable that the natural chemistry of the glass surface could have been sensed by the walking and searching cyprid while the glass was covered with the slime. From the onset, the cyprids were preferentially attracted to the slimed surface, by a factor of nearly 10 (figures 6 to 9), and they could sense and respond to the intimate chemistry of the surface only by removing the slime. Not only did the cyprids settle more on the slimed area, but settlement was quicker, and the associated movements were more frantic. Approximately equal numbers of cyprids touched on both slimed and clean surfaces, but the average number of departures from the clean surfaces was much greater and here the activity during walking was slower and more relaxed.

Favorable barnacle cyprid settlement on slimed surfaces is shown in figures 6 and 7. The number of cyprids settling on the clean surface was 0.41 per square inch (14% out of a total cyprid number of 129) and on the slimed surface 4.9 settled per square inch (87%). These numbers are based on trials during which the walking cyprids could definitively choose between slimed and

cleaned surfaces. Withstanding the fact that a sponged and rinsed surface is not truly "slime-free" (slime begins to form within a few minutes),[10] it is clean in comparison to that area of the vessel which was not scrubbed daily and therefore had accumulated a layer of slime for several days. A series of trials was carried out with 5 to 162 cyprids. The resulting settling rates showed statistically and reproducible significant variations for the clean versus slimed surfaces only during those trials where more than 100 cyprids were used. The positive ratio value indicates that the cyprids did indeed settle more on the slimed surface.

Further proof of the increased number of settlements on the slimed surface as opposed to settlements on clean surface can be seen by comparing the actual to the theoretical numbers of settlement. If it is assumed that the cyprids were given no choice except to settle on the slimed surface, then line 1, figure 9, is the plot. When we compare the actual settlement (with the cyprids given a choice) as in line 2, figure 9, we find this to be 88% of the hypothetical set. Line 3 in figure 9 represents a theoretical settlement number if all the cyprids had settled on the clean surface. The actual set on the clean surface is only 13.4% of the hypothetical set (line 4, figure 9). Therefore, the barnacle cyprids showed an overwhelming preference for slimed surface.

The preference of sessile organisms for slimed surfaces has been shown for the Serpulid polychaete Spirorbis borealis. Knight-Jones[13] found greater attachment on slimed than on clean containers. Figures 10-13 show that settlement on a slimed surface is preferred by a factor of 6 as compared to settlement on a clean surface. We can see in figure 12 that equal numbers of larvae attached on the slimed surface as did not attach to the clean one.

Thus, the percentage attachment of barnacle cyprids and Spirorbis borealis on slimed surfaces overwhelms their percentage attachment on cleaned surfaces by factors of ten and six, respectively (figures 6-12). During experimental trials involving both macrofouling organisms, never less than 60% of the organisms settled on the slimed surfaces whereas never more than 50% settled on the cleaned surfaces (figure 13).

CONCLUSIONS

- Barnacle cyprids settle on a slimed surface 10 times as readily as on a cleaned surface.

- The preconditioning of a surface by bacteria and diatoms overrides all other stimuli to the cyprid in search of a settlement site.

- The polychaete Spirorbis borealis has been found to parallel the barnacle cyprid's affinity for slime conditioning of submerged surfaces as a precursor to settlement; thus, the preconditioning value of the slime layer may be considered to be of a universal nature.

- Successful antifouling procedures must be developed which will eliminate the slime layer, thereby decreasing the frequency of attachment of macrofouling organisms to protected submerged surfaces.

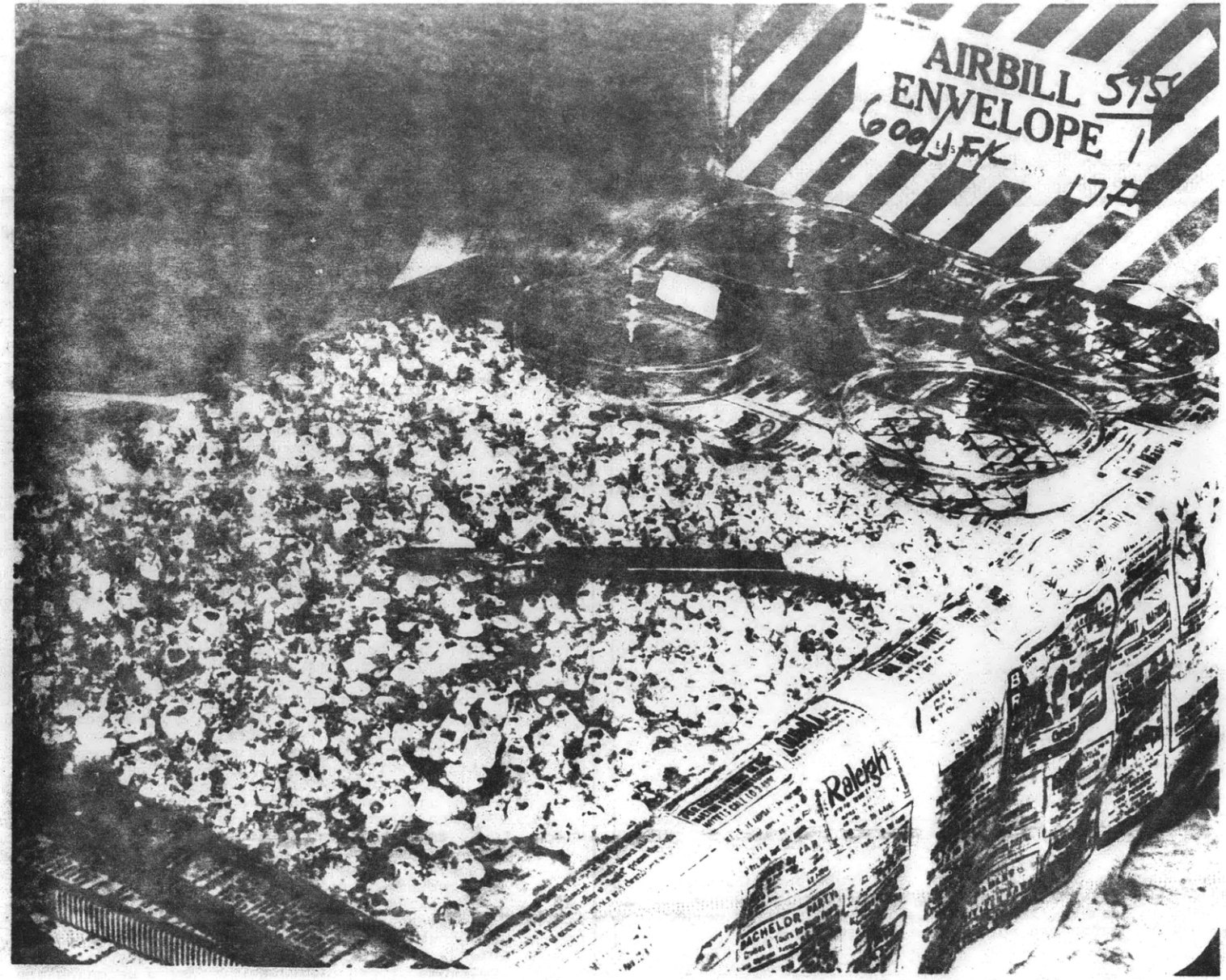

Figure 1 - Aluminum Panel After 6-Months Exposure off Miami, Florida

1. Orifice of shell
2. Lateral compartment of shell
3. Scutum
4. Rostrum or ventral compartment of shell
5. Sheath of rostrum
6. Adductor muscle
7. Cut surface of attachment to the removed part of scutum
8. Muscles
9. Opercular membrane
10. Rostral depressor muscle of scutum
11. Oviduct
12. Ovary
13. Antennules
14. Tergum
15. Carina, or dorsal compartment of shell
16. Sheath of carina
17. 1st., 2nd., and 3rd. cirri
18. Labrum (anterior part of mouth)
19. Basal articulation of 1st. cirrus
20. Aperture of acoustic sac
21. Thorax
22. Carinal depressor muscle of tergum
23. Mantle membrane
24. Basal plate
25. Mantle cavity
26. Prosoma

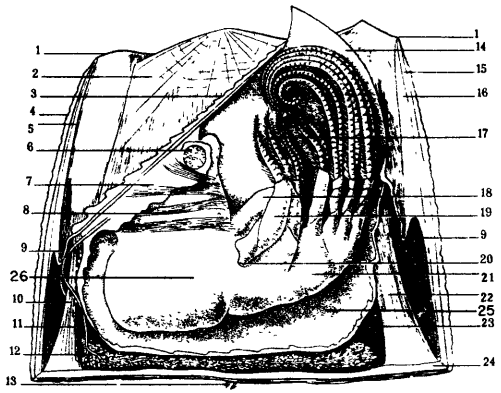

Figure 2
Barnacle Section

H_2O, ml	100.0
Na_2 β-glycerophosphate, mg	50.0
Fe (as EDTA; 1:1 molar), mg[1]	2.5
$NaNO_3$, mg	350.0
P II metals, ml[2]	25.0
Vitamin B_{12}, µg	10.0
Thiamine, mg	0.5
Biotin, µg	5.0
Tris Buffer, mg[3]	500.0

[1] 351 mg $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and Na_2EDTA in 500 ml H_2O. One ml of this is 0.1 mg Fe.

[2] P II metal mix (to make 100 ml). One ml has:

B	0.200 mg x 100 =	20.0 mg x 5.7 =	0.114 mg	H_3BO_3
Fe	0.010 mg x 100 =	1.0 mg x 4.9 =	4.900 mg	$FeCl_3 \cdot 6H_2O$
Mn	0.040 mg x 100 =	4.0 mg x 4.1 =	16.400 mg	$MnSO_4 \cdot 4H_2O$
Zn	0.005 mg x 100 =	0.5 mg x 4.4 =	2.200 mg	$ZnSO_4 \cdot 7H_2O$
Co	0.001 mg x 100 =	0.1 mg x 4.7 =	0.480 mg	$CoSO_4 \cdot 7H_2O$
Na_2EDTA	1.000 mg x 100 =	100 mg =	100 mg	Na_2EDTA

[3] Sigma 7-9 from Sigma Co.

Note: 2 ml of this medium to 100 ml sea water.

Figure 3
E-S Enrichment for Cyclotella nana

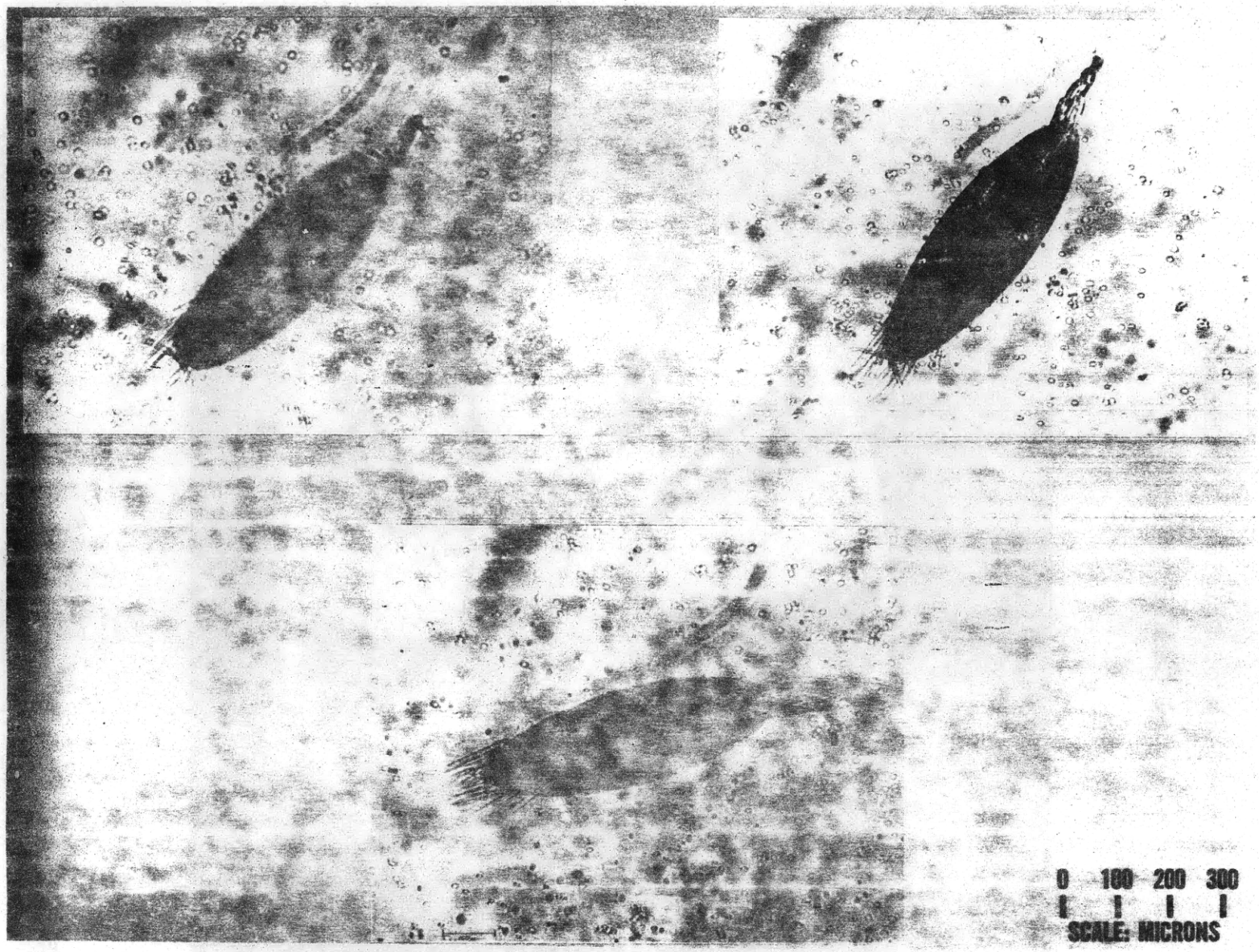

Figure 4 - Barnacle Cyprids Walking on a Surface

A - Points of Original Attachment

B - Radial Canals

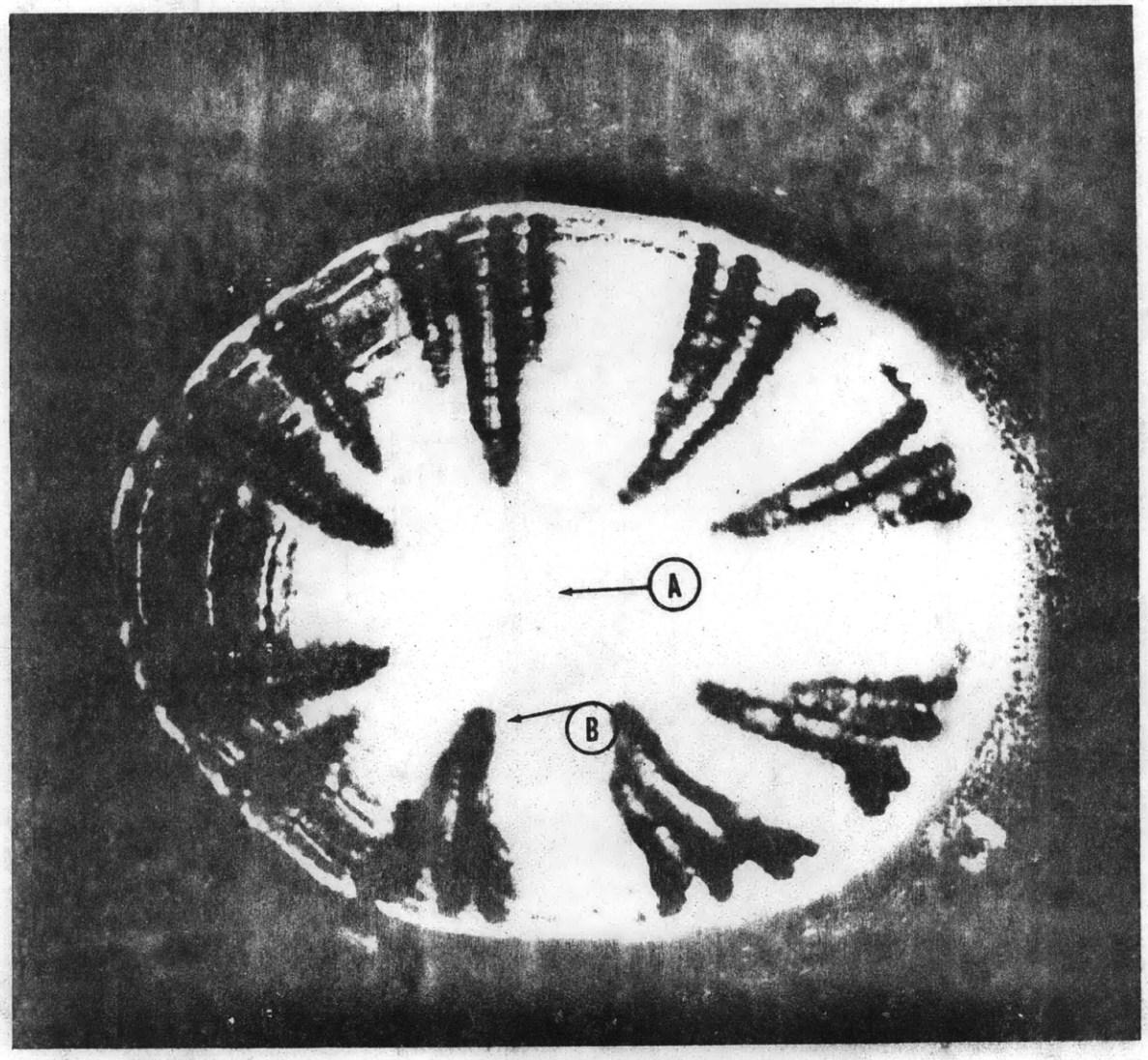

Figure 5
Barnacle Basal Plate with Centers of Attachment

Experiment No.	Total No. of Cyprids	Slimed Surfaces			Clean Surfaces			Ratio Slimed/ Clean
		No. Settled	No. Set/In^2	Set %	No. Settled	No. Set/In^2	Set %	
H7	104	94	4.1	89	10	0.24	11	17.1
H10	112	100	4.4	90	12	0.28	10	15.8
H14	113	103	4.5	91	10	0.24	9	19.0
L3	114	92	4.0	81	22	0.52	19	7.7
H9	114	95	4.2	84	19	0.45	16	9.3
H3	133	121	5.3	91	12	0.28	9	19.0
H4	148	136	5.9	92	12	0.28	9	21.0
H1	161	124	5.4	77	37	0.87	23	6.4
H2	162	139	6.1	86	23	0.54	14	11.2
Average	129	112	4.9	87	17.5	0.41	13	14.1

Figure 6
Barnacle (B. eburneus) Cyprid Attachment
on Clean and Slimed Surfaces

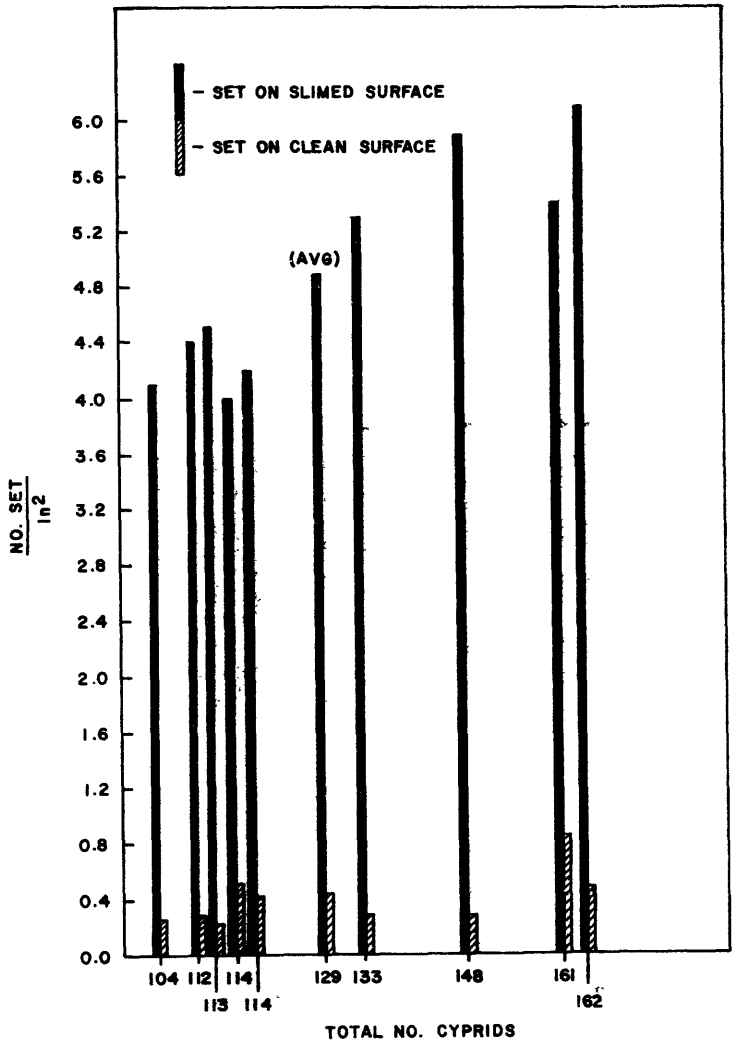

Figure 7
Favorable Barnacle Cyprid Settling on Slimed Surface

Run No.	Total No. of Cyprids	Total Area Available in^2	Theoretical Set/In^2 All Area	Total Slimed Area in^2	Theoretical Set/In^2 on Slimed Area	Total Clean Area in^2	Theoretical Set/In^2 on Clean Area	Theoretical Ratio Slime/Clean
H7	104	65.4	1.50	23.0	4.50	42.4	2.45	1.84
H10	112	65.4	1.70	23.0	4.90	42.4	2.65	1.85
H14	113	65.4	1.72	23.0	4.92	42.4	2.68	1.86
L3	114	65.4	1.74	23.0	4.94	42.4	2.70	1.88
H9	114	65.4	1.74	23.0	4.94	42.4	2.70	1.88
H3	133	65.4	2.05	23.0	5.80	42.4	3.16	1.84
H4	148	65.4	2.25	23.0	6.40	42.4	3.50	1.85
H1	161	65.4	2.48	23.0	7.00	42.4	3.85	1.83
H2	162	65.4	2.50	23.0	7.10	42.4	3.95	1.79
Average	129		1.96		5.61		3.07	1.85

Figure 8
Theoretical Settling of Barnacle Cyprids per per Square Inch

① = Theoretical Set on Slimed Surface Only.

② = Actual Set on Slimed Surface.

③ = Theoretical Set on Clean Surface Only.

④ = Actual Set on Clean Surface.

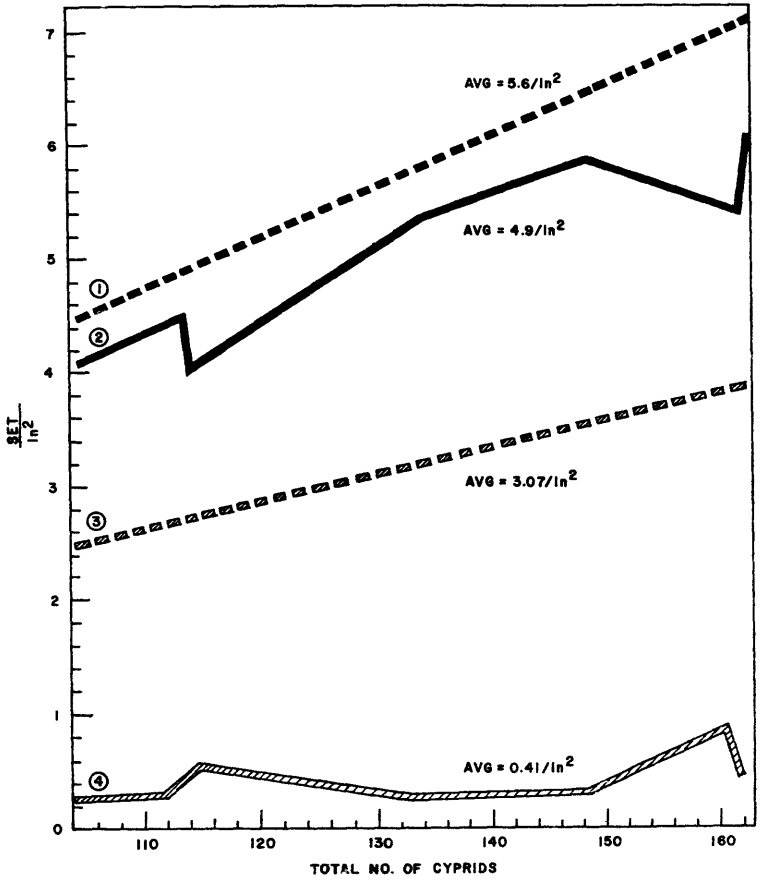

Figure 9
Theoretical and Actual Attachment of Barnacle Cyprids per Square Inch

Run No.	Total No. of Larvae	No. Attached	No. not Attached	Attachment %
		Type of Surface - Slimed		
1	11	11	0	100.0
2	13	12	1	92.0
3	17	16	1	94.0
4	18	14	4	78.0
5	18	16	2	89.0
6	21	21	0	100.0
7	26	21	5	81.0
8	30	30	0	100.0
Totals	154	141	13	
Average	19.2	17.6	1.6	95.0
		Type of Surface - Cleaned		
9	10	3	7	30.0
10	16	0	16	0.0
11	17	0	17	0.0
12	19	9	10	47.0
13	19	4	15	21.0
14	20	0	20	0.0
15	22	1	21	4.5
16	30	6	24	2.0
Totals	153	23	130	
Average	19	2.9	16.2	15.0

Figure 10
Spirorbis borealis
Increased Attachment in Filmed Beakers than in Clean Beakers in 24 Hours[13]

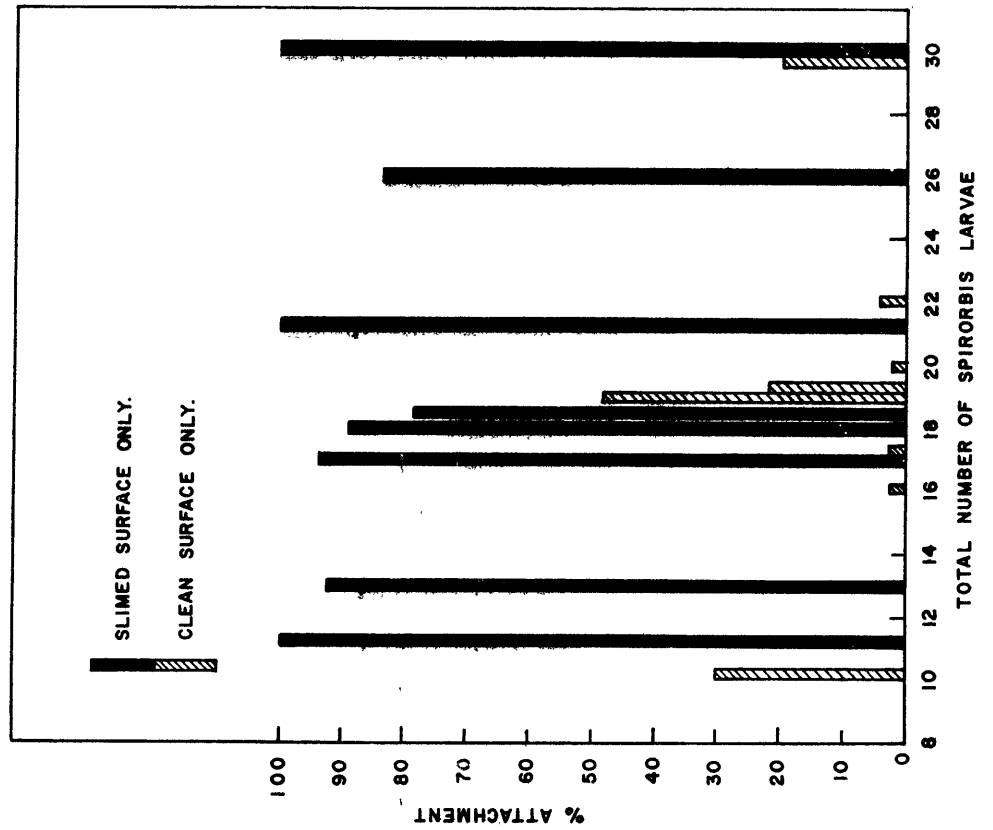

Figure 12
Spirorbis borealis
Percent Attachment on a Slimed and Clean Surface

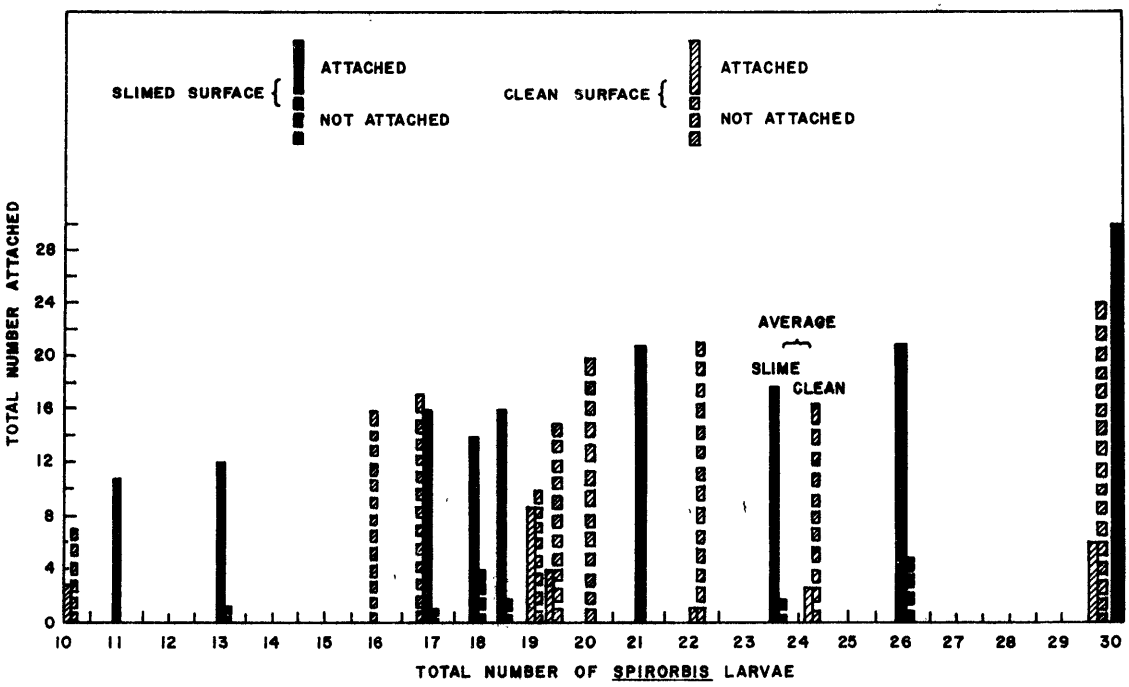

Figure 11
Spirorbis borealis
Greater Attachment on a Slimed Surface Than a Clean Surface

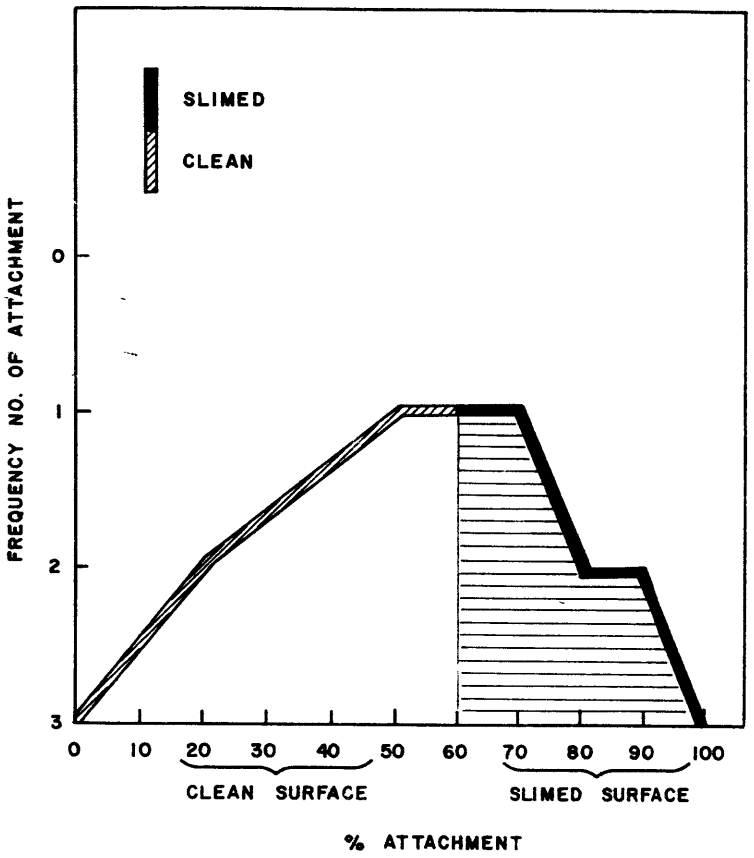

Figure 13
Frequency of Attachment of
Spirorbis on Slimed and Clean Surfaces

APPENDIX A

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