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A MODEL SYSTEM FOR THE STUDY OF SUBLETHAL POLLUTION EFFECTS ON --ETC(U)
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A MODEL SYSTEM FOR THE STUDY OF SUBLETHAL POLLUTION EFFECTS ON MARINE ORGANISMS

By

Hugh W. Ducklow and Ralph Mitchell

Technical Report No. 8

July 1978

Division of Applied Sciences
Harvard University • Cambridge, Massachusetts

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER (14) TR- Technical Report No. 8	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) (6) A MODEL SYSTEM FOR THE STUDY OF SUBLETHAL POLLUTION EFFECTS ON MARINE ORGANISMS.	5. TYPE OF REPORT & PERIOD COVERED (9) Interim / Rept.
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) (10) Hugh W. Ducklow, Ralph Mitchell	8. CONTRACT OR GRANT NUMBER(s) (15) N00014-76-C-0262
9. PERFORMING ORGANIZATION NAME AND ADDRESS Division of Applied Sciences, Harvard University, Cambridge, Massachusetts	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS	(11) 12. REPORT DATE July 1978
	13. NUMBER OF PAGES 40
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) (12) 41 p.	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Coral
Microorganisms
Mucins
Pollution
Bacteria

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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Many corals use mucus secretions as a buffer between their tissue surfaces and the environment. In the first part of this report, several coral mucins are characterized as mixtures of proteins, polysaccharides, and lipids. The proteins cont'd →

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20. Abstract continued

CONT

and polysaccharides are partly characterized as to their amino acid or sugar composition. Mucus floc material has been implicated as potentially important food source in the coral reef ecosystem. Using scanning electron microscopy (SEM) the process by which fresh liquid mucus becomes flocculent mucus web material is described.

In the second section, we demonstrate the presence of functioning populations of culturable bacteria in the superficial mucus layers of three coral species. The population levels of bacteria on different corals may be related to differences in coral feeding behavior. *Vibrio alginolytious* is identified as an efficient mucus utilizer. This organism is shown to grow rapidly on coral mucus and to be chemotactically attracted to mucus.

In the final section, an experimental flowing water system is described in which the Red Sea soft coral *Heteroxenia fuscesens* is exposed to sublethal concentrations of crude oil. Such exposures result in significant increases in the population levels of bacteria in the coral mucus. This process may result in the onset of coral diseases. Finally, the microbial analogies between a naturally-occurring and an artificially stimulated coral disease are explored using SEM. It is suggested that *Beggiatoa*, a filamentous, H_2S-utilizing bacterium, may be a characteristic organism in coral diseases.

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ACKNOWLEDGMENTS

We wish to thank the following persons for their assistance during various phases of this work. In Israel: Prof. Lev Fishelson and Yossi Loya, Tel Aviv University; Prof. Yoel Gat, Aharon Nir, D. Mirelman, Weizmann Institute; Prof. I. Chet, Hebrew University Faculty of Agriculture; Dr. Ilan Paperna and Ms. I. Levanon, Eilat Marine Biological Laboratory. Bermuda: Dr. Wolfgang Sterrer and Mr. Foster Brown, Bermuda Biological Station. Barbados: Dr. Finn Sander and Prof. J.B. Lewis, Bellairs Research Institute. Harvard: Mr. Ed. Seling, Museum of Comparative Zoology, Scanning Electron Microscope Laboratory.

INTRODUCTION

In initial studies on the effects of pollutants on tropical corals (18, 19), Mitchell discovered that the addition of various pollutants to aquaria containing the Red Sea brain coral *Platygyra lamellina* rendered the coral vulnerable to invasion by an enriched population of marine bacteria. Addition of antibiotics with the pollutants prevented coral death, indicating that the bacteria and not toxic effects of the pollutant killed the corals. The mucus released by the coral as a response to pollutant stress served as a substrate for bacterial growth in the initial stages of the disease. It was hypothesized that a complex feedback system in which pollution stress, mucus release, bacterial growth, oxygen depection, and toxin production interacted was the cause of coral death. Thus, coral death was seen to be the result of ecological processes rather than either poisoning or specific pathogenicity.

In this report we describe further research into the nature of the interactions between pollutants, corals, mucus, and marine bacteria. In particular, we report detailed studies on the chemistry and ultrastructure of various coral mucins, demonstrations of native microbial populations on the mucus covered surfaces of healthy corals, and further observations on some naturally occurring and artificially-stimulated coral diseases.

Literature on coral biology, reviewed and cited in (7) suggests that coral organisms and coral reef communities may be especially sensitive to environmental perturbations caused by pollution. Corals

are more intimately in contact with the external environment with a larger surface area of unprotected living tissue than most marine organisms. They possess extremely finely attuned tactile and chemical sensory systems. The only buffer between the coral and its environment is the often copious secretions of mucus which bathe the coral surface. We believe that corals make sensitive and useful model systems with which to study pollution stress because of the nature of their relationship with the external environment.

THE BIOLOGY OF CORAL MUCUS

In this part of our study we attempted to characterize the mucus compounds secreted by a variety of commonly-occurring coelenterates from the Gulf of Eilat, Red Sea, Israel. We also examined the appearance of secreted mucus and the process of mucus web formation in the stony coral *Porites astreoides* at Barbados, W.I.

Methods. Individual colonies of different coelenterates were collected from the shallow fringing coral reefs near the Eilat Marine Biological Laboratory. Mucus was sampled from each organism by removing the colony from the water and "milking" it over a collecting vessel. All the mucins were purified by extensive dialysis against distilled water and concentrated by lyophilization into fine brown powders. Various chemical analyses including amino acid analysis, paper chromatography, sugar, lipid, and ash determinations were performed on suitably prepared subsamples of the mucus powder using methods described in (7) and (8).

The process of mucus web formation was followed by observation of pieces of *Porites astreoides* colonies fixed for scanning electron microscopy in 2% buffered glutaraldehyde in seawater. These samples were dried in liquid CO_2 at the critical point and coated with gold-palladium alloy in a sputter coater and examined on an AMR 1000 instrument at the Harvard Museum of Comparative Zoology.

Results. Table 1 lists the organisms from which we collected mucus in Israel, along with information on the amounts of mucus released per sampling. All the mucins are composed of protein, carbohydrate, and lipid moieties, as Table 2 indicates, but the relative contribution of each component to the total mucus varies from organism to organism. However, the nature of the components themselves is relatively similar from species to species. Figure 1 shows that all the organisms except *Cassiopeia* have quite similar proportions of amino acids in their proteins. Table 3 shows that all the mucins contain the same monosaccharides in their carbohydrates, although *Cassiopeia*, *Sarcophyton*, and *Aurelia*.

Several investigators have noted that coral mucus webs are prevalent in coral reef waters, and may be a significant food source in the reef ecosystem (2,5,12,24). Our SEM observations on the web forming coral *Porites astreoides* show that mucus web formation results from the progressive denaturation of liquid mucus as it filters silt and detritus from the surrounding water (8).

TABLE 1

ORGANISMS AND MUCUS SECRETION

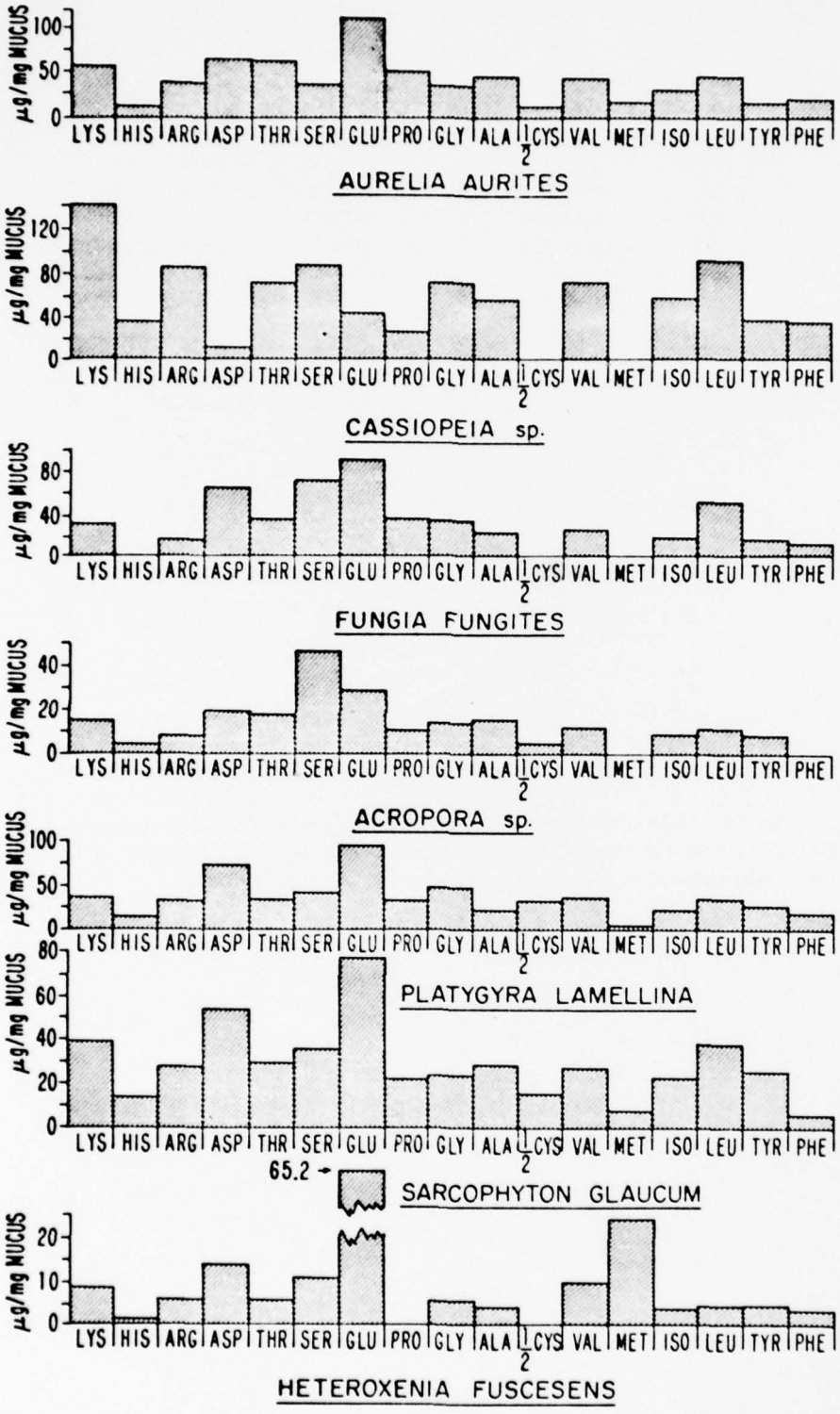
Organism	Diameter of Individuals Sampled	Yield of Dry Pure Mucus from One Sampling of One Individual
Hard Corals (Scleratinia)		
Platygyra lamellina	10 - 15 cm	10 mg
Acropora variabilis	15 cm	3 mg
Fungia fungites	7 - 10 cm	0.4 mg
Soft Corals (Alcyonacea)		
Heteroxenia fuscesens	5 - 10 cm	50 mg
Sarcophyton glaucum	10 - 15 cm	350 mg
Jellyfish (Scyphozoa)		
Aurelia aurita	15 - 20 cm	17 mg
Cassiopeia sp.	15 cm	68 mg

TABLE 2

GROSS COMPOSITION OF COELENTERATE MUCUS

ORGANISM	%PROTEIN	%POLYSACCHARIDE	%LIPID	%ASH	TOTAL %
Platygyra	59	16	0	6	82
Acropora	22	-ND-	-ND-	-ND-	
Fungia	5	2.5	42	60	110
Heteroxenia	17	14	33	24	88
Sarcophyton	40	10	30	7	87
Aurelia	73	5	27	22	127
Cassiopeia	10	2	38	52	102

FIGURE 1



AMINO ACID COMPOSITION: µg/mg lyophilized mucus.

TABLE 3

% SUGAR COMPOSITION OF LYOPHILIZED MUCUS

	HETERO-XENIA	SARCO-PHYTON	AURELIA	PLATY-GYRA	CASSIOPEIA	FUNGIA	ACROPORA
TOTAL HEXOSE	12	10	5	15	1.7	2.5	N.D.
TOTAL PENTOSE	2.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
GLUCOSE	1.2	0.7	0.4	1.1	0.2	+	+
GALACTOSE	1.7	2.1	0.5	2.4	0.4	+	+
GALACTOSAMINE	2.5	+	+	+	+	+	+
GLUCOSAMINE	2.3	+	+	+	+	+	+
FUCOSE	++	-	-	++	-	+	+
ARABINOSE	++	+	+	+	+	+	++

Note: Qualitative indications of sugar presence derived from relative darkness of spots on paper chromatogram. Equal amounts of hydrolysate were developed in all chromatography experiments.

Figure 2 shows a mucus web *in situ* on a colony of *P. astreoides*. These webs grow and collect particulate matter until they are shed into the water by wave action (14). In Figure 3 a microscopic mucus web is shown forming in the oral cavity of an individual polyp. High magnification of this web (Figure 4) shows the netlike structure of the mucus and also shows clay particles trapped in its mesh. As the web grows, the mucus net becomes composed of larger, coarser fibers or cables (Figure 5). This process was originally described in 1906 by Duerden using light microscopy (9).

The chemical filtration and adhesive properties of coral mucus we observed lead one to suppose that microorganisms might naturally be concentrated at coral surfaces. This is discussed in the next section.

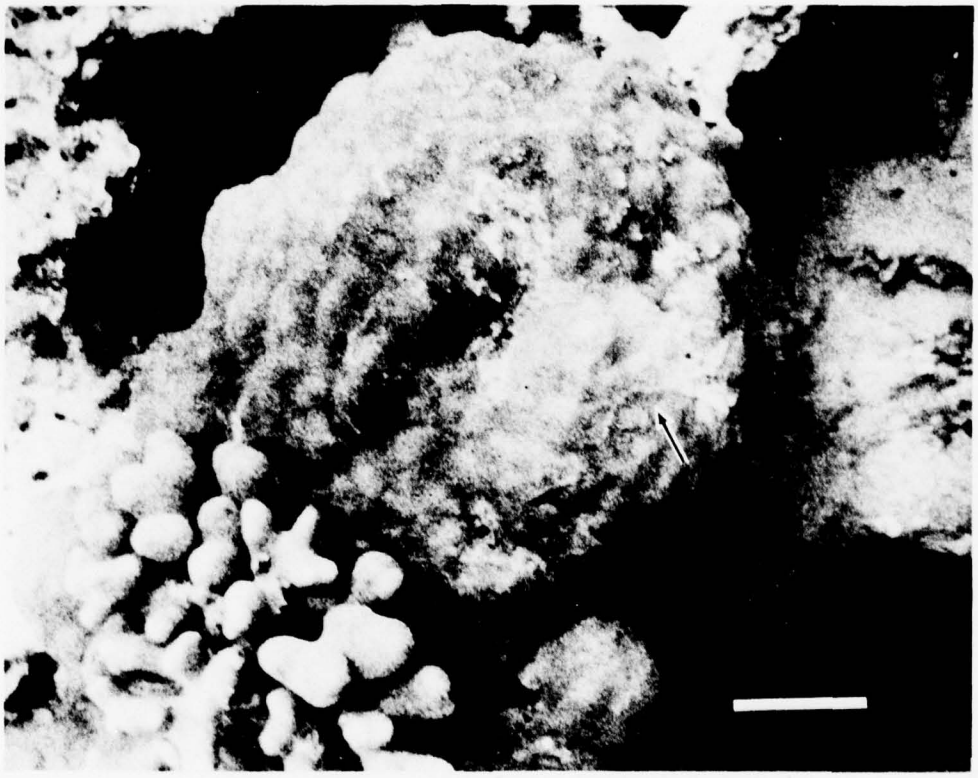


Figure 2. A macroscopic mucus web (arror) shown *in situ* on a colony of *Porites astreoides*. Scale bar is equal to 2.0 cm.

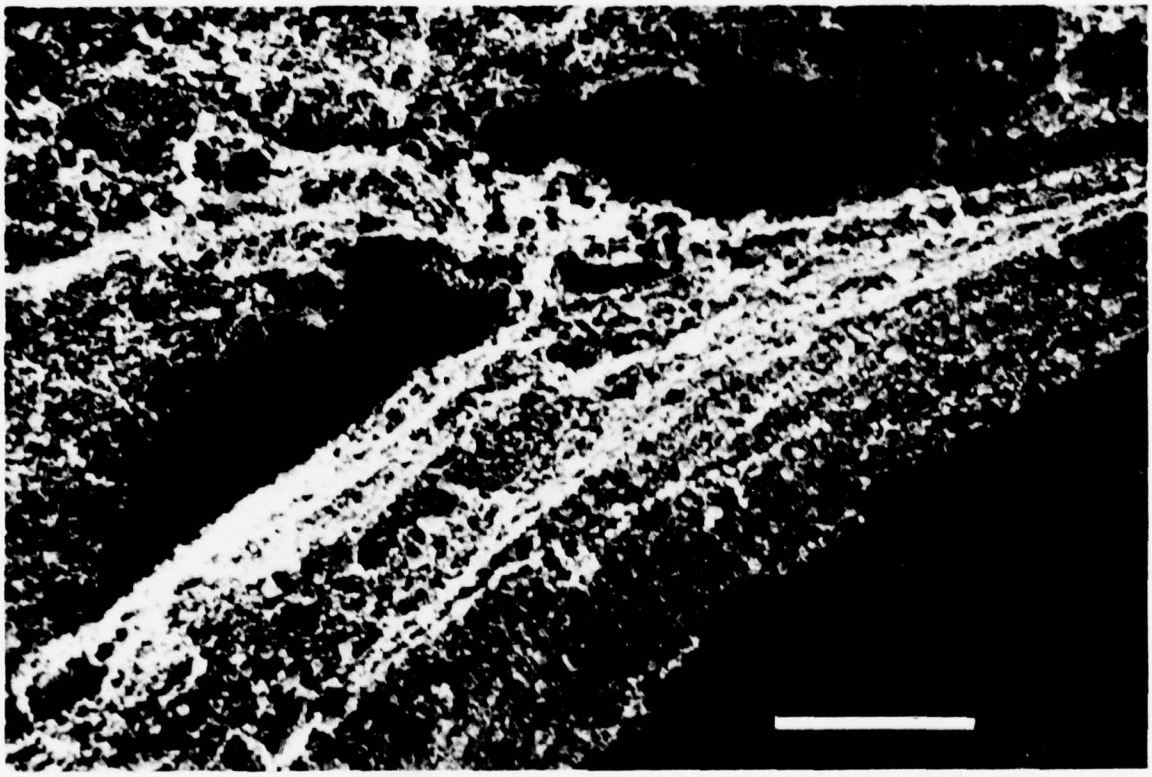


Figure 3. Scanning electron micrograph showing a microscopic mucus web forming on *Porites astreoides*. Scale bar is equal to 10 microns.

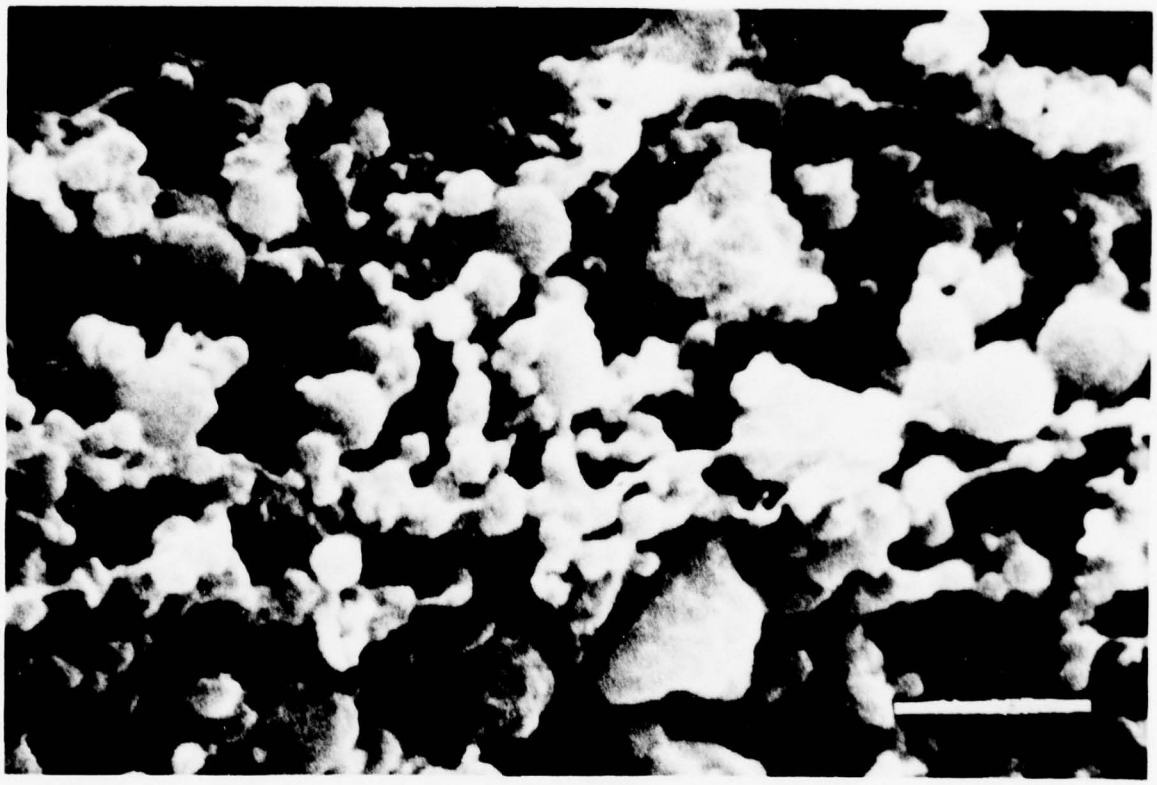


Figure 4. Scanning electron micrograph showing fine structure of a *Porites* mucus web with detrital material. Scale bar is equal to 1 micron.

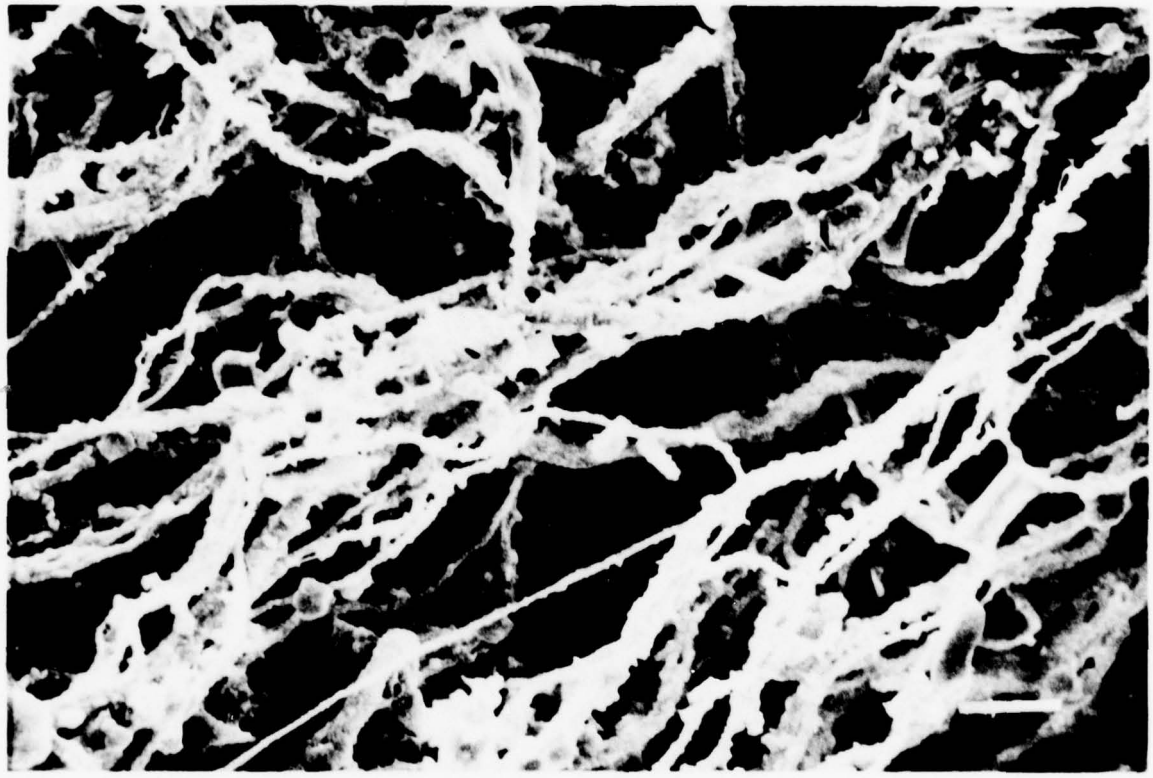


Figure 5. Scanning electron micrograph showing the coarse mucus fibers of a mucus web similar to that shown in Figure 1. Scale bar is equal to 10 cm.

THE CORAL SURFACE AS A MICROBIAL HABITAT

Mucus release by corals is the most conspicuous feature of their surfaces, but close inspection reveals other features of significance to commensal microorganisms. Corals are remarkably well-adapted for grazing on plankton, and for clearing their tissues of sedimented material. For these activities, mucus release works in tandem with directed patterns of ciliary currents, tentacle expansion, and chemically orchestrated feeding behavior (15,16,22). In this section, we describe the architecture of the coral surface and demonstrate the presence of microorganisms on coral surfaces. Some of the adaptations by which certain members of the coral microflora might persists on the corals are also considered.

Methods. For scanning electron microscopy, small branches of *Madracis mirabilis* were fixed, critical-point dried, and sputter-coated as described above.

Three corals were chosen for bacteriological analysis. These were the xeniid soft coral *Heteroxenia fuscescens* at Eilat, the zoanthid soft coral *Palythoa sp.* at Bermuda, and the stony coral *Porites astreoides* at Barbados. Mucus was collected from *Palythoa* and *Heteroxenia* by inverting the colony and collecting the dripping liquid mucus in a sterile petri dish. Mucus webs from *Porites* were collected *in situ* by gently sucking them off the coral heads with sterile syringes. Bacteria were enumerated in the mucus samples by standard spread-plating procedures using sterile seawater dilution tubes and several kinds of nutrient media solidified with 1.5% Difco agar. We used a variety of agar media to

directly enumerate different physiological types of bacteria present in the mucus, which we hoped would yield more data for comparison among the different mucus samples. Seawater collected in the same areas as the corals was also analyzed using the different media, which are described in Table 4. For the chitin, casein, and Tween 80 plates, only colonies showing zones of enzymatic hydrolysis of the plymeric substrates around biochemically active colonies were enumerated. For all the other media, all colonies growing on the plates after 5 day incubations at 25°C were counted.

In order to obtain strains of mucus-degrading bacteria, enrichment cultures utilizing either purified *Heteroxenia* mucus or raw liquid *Heteroxenia* mucus as the sole carbon and energy source were inoculated with raw *Heteroxenia* mucus, and incubated for 48 hours. Serial transfers to new flasks of the seawater-mucus medium were carried out until pure cultures were obtained. The bacteria from these enrichment cultures were identified as *Vibrio alginolyticus* according to the characteristics listed in Bergey's Manual (3).

The behavior of the mucus-associated bacteria obtained in enrichment cultures was examined in the context of the coral surface habitat. The growth rate of *V. alginolyticus* in mucus medium containing 25 mg/50 mℓ (the natural concentration of this mucin) of pure *Heteroxenia* mucus in seawater was determined by inoculating flasks of the medium from an overnite *V. alginolyticus* culture and plating out subsamples at timed intervals. Chemotaxis of *V. alginolyticus* toward *Heteroxenia* mucus was measured using the capillary assay of Adler, already described in detail in previous Technical Reports (20).

TABLE 4

MEDIA USED FOR BACTERIAL RECOVERIES FROM CORAL MUCUS AND REEF SEAWATER

Media Designation	Composition	Target Populations
YP	0.5% proteose peptone 0.3% yeast extract	"total" count: all colony forming bacteria
CAA	0.3% vitamin-free casamino acids	amino acid auxotrophs
C	0.3% vitamin-free casein	proteolytic bacteria
CH	0.3% reprecipitated chitin	polysaccharolytic bacteria
M	75% raw *Heteroxenia* mucus + 25% distilled H_2O	utilizers of coral excretory products and mucus
HX	0.05% pure *Heteroxenia* mucus	mucolytic bacteria
S	0.1% each glucose, galactose, ribose, fucose, mannose	sugar auxotrophs
TCBS	commercial selective medium (BBL, Baltimore, Md.)	vibrios
TW	1% Tween 80	lipolytic bacteria

Results. Scanning electron microscopy reveals the complexity of the coral surface. Figure 6 shows one polyp on a branch of *M. mirabilis* with the tentacles contracted around the oral opening. In Figure 7, a close-up view of the tip of a single tentacle, discharged nematocyst fibers extend across the tissue surface. Finally in Figure 8, undischarged nematocysts, sensory flagella, and cilia are shown. On a healthy coral, all these cellular organs and organelles are in operation, conferring upon this microbial habitat a high degree of structural, chemical and mechanical complexity. Bacterial adaptations and persistance on the coral surface must be considered in the context of the nature of these features.

Some pertinent data on the organisms we sampled for bacterial enumeration, and their localities are tabulated in Table 5. The bacterial content of mucus from each organism and of the surrounding seawater are shown in Table 6. In spite of the notable self-cleaning abilities of corals, the surfaces of all three species possess larger bacterial populations than the surrounding waters. It is obvious that these different corals harbor different-sized populations. *Heteroxenia* maintains consistently low populations in its superficial mucus, while *Palythoa* and *Porites* have larger and more variable populations. These differences may be related to different cleaning and/or feeding mechanisms and behavior in the three species.

Although larger numbers of bacteria are found in the mucus of each coral than in the surrounding water, these data do not show that the populations are active on the coral surface. These bacteria could be concentrated at the coral surface by suspension-filtering activity, but

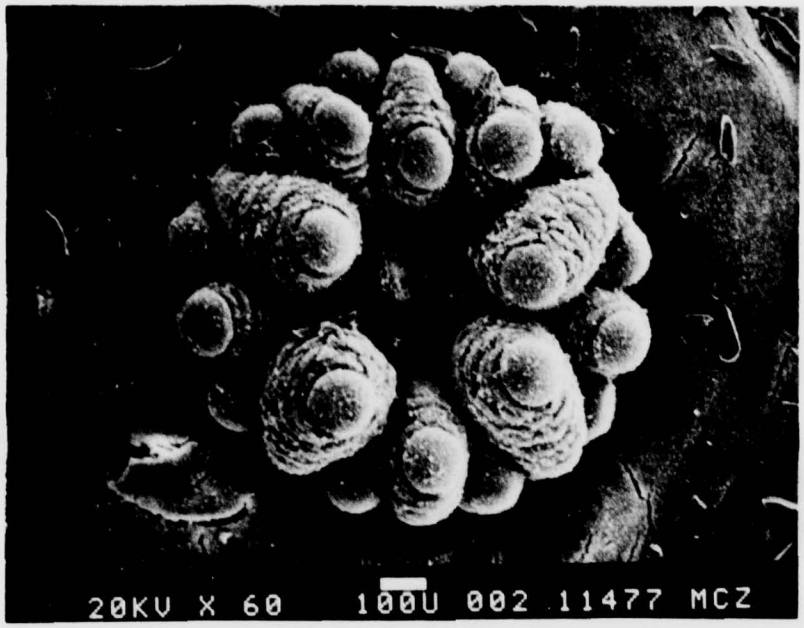


Figure 6. Scanning electron micrograph showing a single poly of the coral *Madracis mirabilis*. Scale bar is equal to 100 microns.

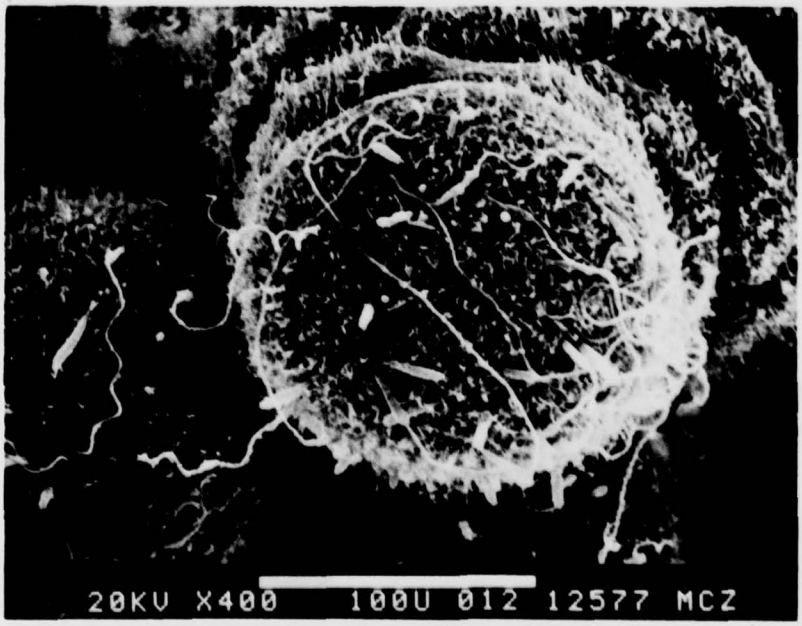


Figure 7. Scanning electron micrograph showing the tip of a tentacle of *M. mirabilis*. The long fibers of several discharged nematocysts can be seen. Scale bar is equal to 100 microns.

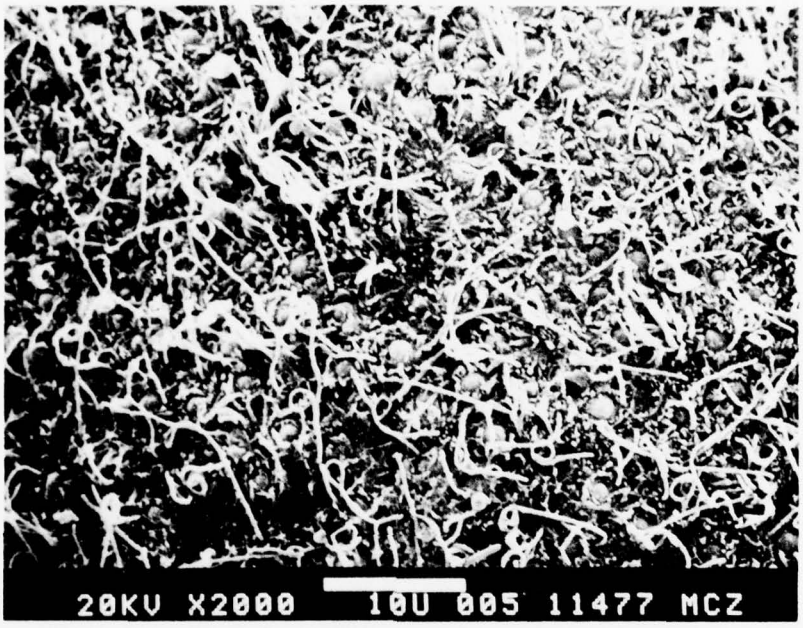


Figure 8. Scanning electron micrograph showing the fine structure of coral tissue of *M. mirabilis*. The tips of nematocysts, and various types of cilia and flagellae can be seen. Scale bar is equal to 10 microns.

TABLE 5

ORGANISMS AND COLLECTING LOCATIONS

	Eilat, ISRAEL	Holetown, BARBADOS	Hungry Bay, BERMUDA
Latitude	29°32' N	13°12' N	31°17' N
Water temperature	22°C	25°C	26°C
Salinity	43‰	35-36‰	30‰
Collecting depth	1-30 m	5-10 m	0-1 m
Relative reef development	++++	++	+
# coral species at reef	> 100	∿ 40	12
Organism collected	xenid soft coral *Heteroxenia fuscesens*	stony coral *Porites astreoides*	zoanthid *Palythoa*
Liquid mucus production	10-15 colony, mℓ	insoluble mucus webs	1-5
Collecting dates	5/75-9/75	3/76	8/76
Marine Laboratory	Eilat MBL, Hebrew University	Bellairs Research Institute, McGill University	Bermuda Biological Station

TABLE 6

RECOVERIES OF BACTERIA FROM MUCUS AND WATER

Colony forming units/milliliter
Mean ± Std. error
Number of samples in parentheses

	EILAT		BERMUDA		BARBADOS	
Recovery Medium	*Heteroxenia* mucus	Water	*Palythoa* mucus	Water	*Porites* mucus	Water
Total (YP)	1490 ± 120 (6)	350 ± 70 (10)	$8.2 \pm 0.6 \times 10^4$ (3)	510 ± 30 (5)	$3.8 \pm 2.0 \times 10^6$ (4)	1550 ± 640 (4)
Amino Acids (CAA)	1110 ± 250 (6)	520 ± 200 (6)			$3.6 \pm 1.4 \times 10^6$ (5)	1260 ± 410 (4)
Casein (C)	710 ± 220 (7)	90 ± 20 (6)	$4.4 \pm 4.0 \times 10^4$ (2)	260 ± 20 (4)	$2.6 \pm 1.8 \times 10^5$ (5)	100 ± 50 (4)
Chitin (CH)	140 ± 40 (4)	30 ± 10 (4)	570 (1)	120 ± 20 (7)	$1.9 \pm 0.1 \times 10^5$ (2)	35 ± 16 (4)
Sugars (S)	970 ± 180 (6)	170 ± 70 (6)			$1.6 \pm 0.9 \times 10^6$ (5)	25 ± 16 (4)
Raw Mucus (HX)	1080 ± 100 (6)	290 ± 130 (6)				
Vibrios (TCBS)			$1.7 \pm 1.4 \times 10^4$ (3)	140 ± 30 (6)		
Lipids (TW)			$2.6 \pm 2.5 \times 10^4$ (2)	130 ± 10 (6)		

not grow or metabolize there. If this were the case, similar ratios of different types of bacteria would be found in the mucus and water, as the bacteria were passively removed to the coral surface. However, as Table 7 shows, this is not the case. For instance, in *Heteroxenia* mucus, a higher proportion of proteolytic bacteria (Casein medium) are present in the mucus, indicating that these are favored at the coral surface. Conversely, in *Palythoa* mucus, relatively fewer chitinolytic organisms are found, suggesting that the differential growth of nonchitinolytic organisms swamps the chitini-degrading bacteria in this habitat. These relationships indicate that different groups of bacteria are active at the coral surface.

We consistently isolated *Vibrio alginolyticus* from *Heteroxenia* mucus enrichment cultures. This suggests that this organism is efficient at mucus degradation, and may be well adapted to live on the coral surface. We designed several experiments to reveal potential adaptations of *V. alginolyticus* to life on corals. *V. alginolyticus* was found to grow in mucus medium at 25°C with a generation time of 0.75 hr., the same rate at which it grows in conventional rich laboratory media (26). This confirms the ability of *V. alginolyticus* to grow rapidly using coral mucus as a sole energy and carbon source.

Table 8 presents data on the chemotactic attraction of *V. alginolyticus* to *Heteroxenia* mucus. The data clearly indicate that the bacterium is attracted to the mucus, even at one-tenth of its concentration at the coral surface. This suggests that this highly motile organism can use its chemotactic ability to locate coral surfaces or to maintain itself upon them.

TABLE 7

The ratios of numbers of selected bacterial types isolated on different media to total bacterial numbers enumerated on yeast extract-peptone medium.

	EILAT		BERMUDA		BARBODOS	
Ratio	Water	*Heteroxenia* mucus	Water	*Palythoa* mucus	Water	*Porites* mucus
CAA:YP	1.49	0.74			0.81	0.94
C:YP	0.26	0.48	0.51	0.53	0.06	0.07
CH:YP	0.09	0.09	0.24	0.01	0.02	0.05
S:YP	0.49	0.65			0.66	0.42
M:YP	0.77	0.72				
HX:YP	0.77	0.28				
TCBS:YP			0.27	0.21		
TW:YP			0.25	0.31		

TABLE 8

Chemotaxis by *V. alginolyticus* toward *Heteroxenia* mucus culture medium

Expt.	Mucus Concentration	Bacteria in Capillary	Ratio to Control
1	0.5 µg/mℓ	$2.9 \pm 0.6 \times 10^4$	9
1	0.05 µg/mℓ	$9.3 \pm 1.7 \times 10^3$	3
1	0 (control)	$3.2 \pm 0.9 \times 10^3$	1
2	0.5 µg/mℓ	$2.6 \pm 0.2 \times 10^4$	12.3
2	0.05 µg/mℓ	$6.7 \pm 0.3 \times 10^3$	3.2
2	0 (control)	2.1×10^3	1
3	0.5 µg/mℓ	$1.8 \pm 0.3 \times 10^4$	19.7
3	0.05 µg/mℓ	$3.7 \pm 1.0 \times 10^3$	3.9
3	0 (control)	$9.3 \pm 1 \times 10^2$	1

Discussion. In this section we have demonstrated that several corals do possess populations of viable, active bacteria on their mucus-covered tissue surfaces. Further, we show that one bacterial species, *Vibrio alginolyticus* can grow on coral mucus and is attracted to it. Undoubtedly other bacteria possess similar abilities. We suggest that this coral-mucus-bacteria system might serve as an interesting model with which to examine the effects of pollutants on coral-microbial interactions. In the final section of this report we demonstrate some experiments of this type.

INTERACTIONS BETWEEN CRUDE OIL, CORALS, MUCUS, AND BACTERIA USING A MODEL SYSTEM

Since sublethal concentrations of crude oil have already been shown to cause a variety of effects at several levels of biological organization (1,13,17,23), we tested the effects of known sublethal concentrations of crude oil on the *Heteroxenia fuscesens* coral-bacteria model ecosystem described in the previous sections of this report. These experiments not only illustrate the effects of pollutants on coral-bacteria interactions, but also point out some interesting features of the coral-bacterial system itself. In addition, we describe some observations on some naturally occurring and artificially-stimulated coral diseases, which may result from pollution stress.

Methods. Basically the experiments consisted of incubating freshly collected *Heteroxenia* colonies in 10 liter glass tanks which were supplied with rapidly flowing seawater. The average residence time for water in the tanks was approximately five minutes. As a result of research by Cohen and Eisler (4,10) on the toxicity of crude oil to *Heteroxenia* we were able to use sublethal concentrations of crude oil without preliminary experiments. We used 5000 ppm of Agha Jari Iranian crude oil, which was added as a slick to each tank. The siphons draining the tanks worked from the bottom, so the oil was maintained on the water surface throughout the experimental exposure period of 5 days (Figure 9). Mucus sample was taken from each coral at the beginning of the experiment, and each was sampled again at the conclusion, after the oil was removed from the tanks. Control tanks were included in each experiment. The mucus samples were analyzed for bacteria as described previously, using several different media.

Results. In the last section we showed that *Heteroxenia* mucus contained a higher concentration of bacteria than seawater. Figure 10 shows the results of the oil-exposure experiments. Each graph indicates the results of bacterial enumerations for each sample on one of the media described in Table 4. Four types of samples are included in each table: water, fresh mucus (these were described in the last section), and the mucus from corals exposed for five days in the tanks: control mucus, and 5000 ppm crude oil-exposed mucus. The graphs show clearly that (a) incubation of control colonies with no addition of curde oil results in enrichment of the mucus bacteria, and (b) that exposure to the crude

SUBLETHAL EXPOSURE OF CRUDE OIL TO HETEROXENIA FUSCESENS

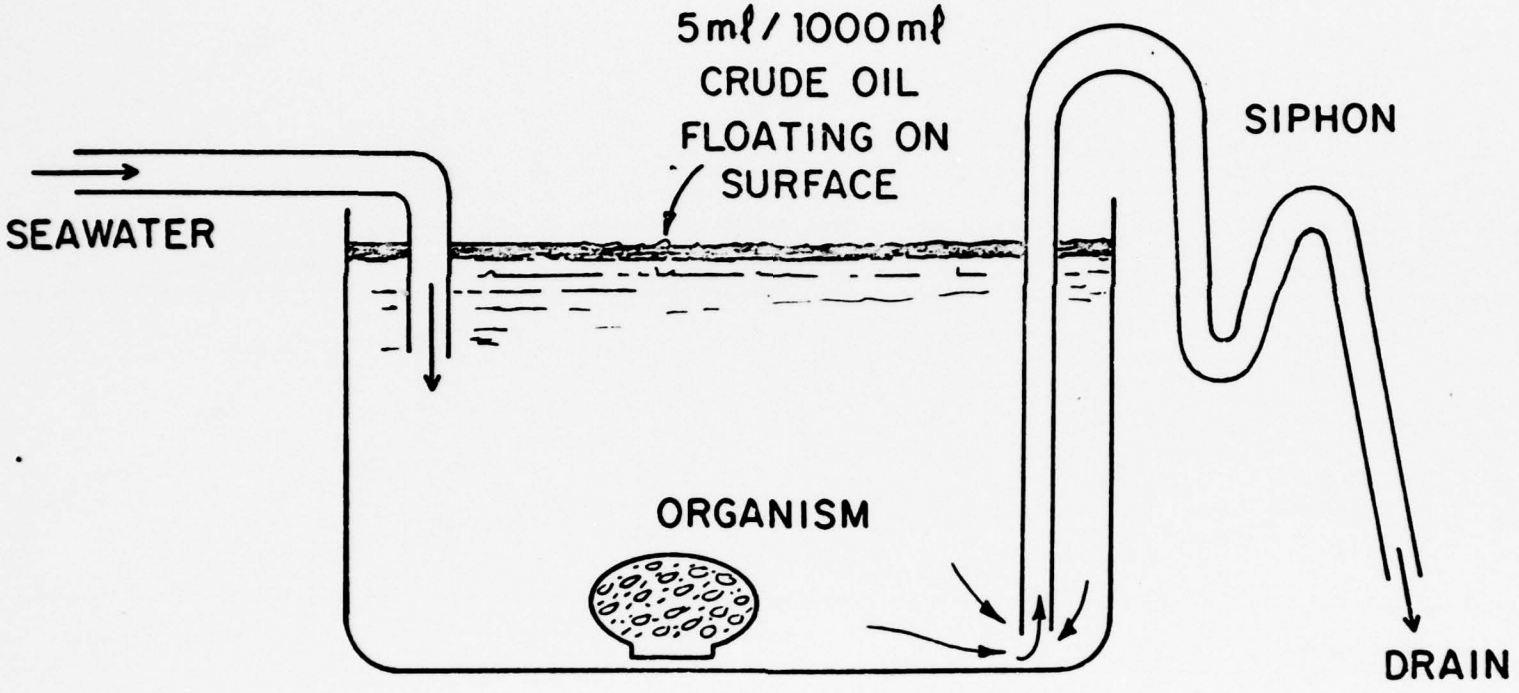


Figure 9

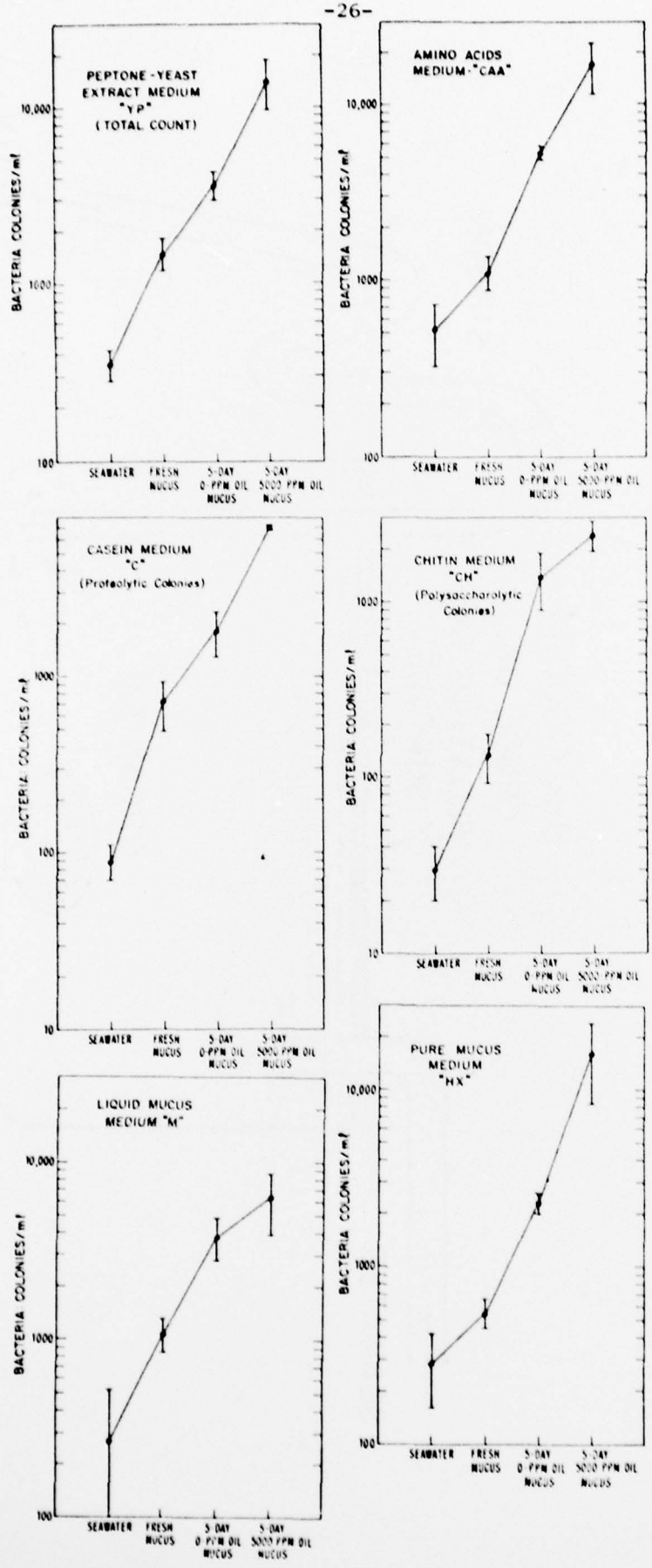


Figure 10. The response of commensal coral bacteria to crude oil.

oil causes a further increase over that of the controls. In all the experiments no *Heteroxenia* colonies died, all recovered their normal behavior, and all survived when replaced in the ocean. Since the rapid flow of water through the tanks precluded the buildup of water populations, the bacterial increases we monitored were due to growth of bacteria in the mucus on the coral heads.

This result is interesting for three reasons. First, they demonstrate that sublethal concentrations of crude oil which cause behavioral changes in exposed corals (7) also cause increases in the commensal microflora of the coral surface. While the mechanism of this increase remains undiscovered, it is possible that the bacteria respond to nutrients released by the host during pollution stress. This has been observed in other systems (21,27). These experiments also show that the microflora living on the coral surface are quite sensitively attuned to the physiological state of the coral. Even incubation of the corals in clean, running seawater at normal temperatures without pollutant addition was sufficient to cause increases in the bacterial population. Finally, the experiments show that even in rapidly flowing seawater, the results of bacterial growth on the coral surface can be monitored. The bacteria do not wash off the coral. This suggests that commensal bacteria possess mechanisms for sticking to the coral surface. One of these mechanisms may be the synthesis of multiple peritrichous flagellae and growth into long swarmer cells in response to growth on surfaces, as in *Vibrio alginolyticus* (6,25). These structural modifications would provide increased surface area and multiple points of contact for the bacteria to use in adhering to the surface.

OBSERVATIONS ON CORAL DISEASES

Garrett and Ducklow (11) reported on a naturally occurring coral disease in Bermuda which they postulated was caused by the filamentous gliding bacterium *Beggiatoa*. Here we describe some observations on the relationship between this disease and an artificially-stimulated disease. Subsequent SEM observations of diseased corals collected in Bermuda revealed that the black "disease line" which spreads radially across the coral colony destroying the coral tissue was composed of several filamentous organisms including one resembling cyanobacteria and another resembling *Beggiatoa*. These are shown in Figure 11, which also has several different spiral organisms in view.

We stimulated an artifical disease in zoanthid colonies collected in Bermuda by subjecting them to reduced oxygen concentrations. This resulted in white nets of *Beggiatoa* spreading across the colony (Figure 12). The characteristic *Beggiatoa* morphology is shown in Figure 13. These artificially diseases zoanthids also had several different spiral organisms present in the disease net.

The presence of *Beggiatoa* and unidentified spiral organisms in both the natural and artificial coral diseases, and on different coral species, indicate that these may be characteristic coral pathogens. As yet they have not been directly implicated in the coral disease using Koch's postulates, however. Finally, since *Beggiatoa* colonizes aerobic/anaerobic interfaces, which result from increased heterotrophic activity, we suggest that these coral diseases may result from increased growth of coral commensal bacteria stimulated by exposure to pollutants.

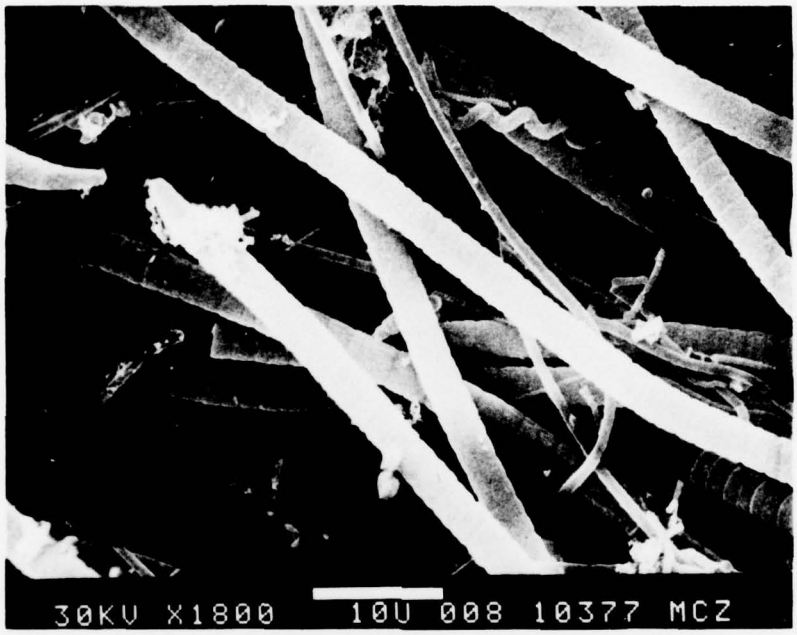


Figure 11. Scanning electron micrograph showing microorganisms in the "disease line" on a colony of *Diploria sp.* from Bermuda. Scale bar is equal to 10 microns.

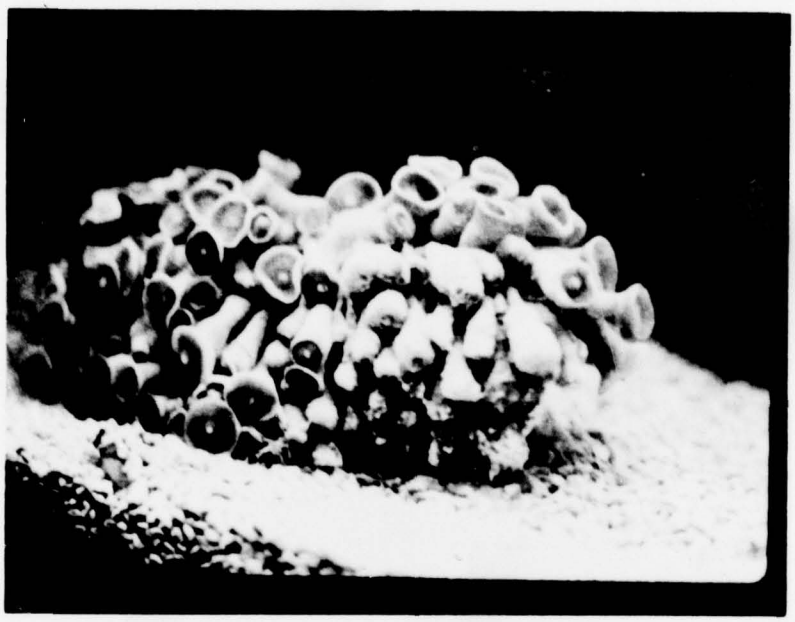


Figure 12. A diseased colony of Bermuda zoanthids (*Palythoa sp.*) in an aquarium showing white nets of *Beggiatoa* covering portions of colony.

Figure 13. Scanning electron micrograph of *Begglatoa* taken from a diseased zoanthid. Note spiral organization. scale bar is equal to 10 microns.

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