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Effect of Well-drilling Fluids on the Physiological Status and Microbial Infection of the Reef Building Coral *Montastrea annularis*

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Abstract. The reef building coral, *Montastrea annularis,* was exposed continuously to suspensions of oil and gas well-drilling fluids at concentrations of 0.1, 0.01, and 0.001 μl/ml in flowing seawater at the U.S. Naval Stage I platform (30° 7.5′ N, 85° 46.3′ W). After six weeks of exposure, coral fragments of 12 to 65 cm^2 surface area were broken off, rinsed in seawater, and extracted in a one-phase chloroform-methanol-buffer and returned to the laboratory where the extraction was completed and the phases separated. The lipids were fractionated with silicic acid and by thin layer chromatography. The total, diacylated, and plasmalogen phospholipid content, total lipid acyl linked fatty acids, triglyceride glycerol, triglyceride acyl-linked fatty acids, and wax ester alcohols were measured. The aqueous phase of the lipid extraction was analyzed for amino acid composition. Acyl fatty acid and alcohol composition was measured by capillary gas chromatography. Total phospholipid, triglyceride glycerol, total extractable fatty acids, triglyceride fatty acids and the wax ester fatty alcohols showed no consistent changes with exposure to the drilling fluids. Changes in free amino acid concentrations were detected as well as significant decreases in the recoverable diacyl phospholipid. Significant increases in plasmalogen phospholipids appeared with exposure, suggesting infection by anaerobic fermenting bacteria which can indicate disease. The evidence suggests that biochemical indicators of infection with anaerobic bacteria may be useful as sensitive markers for pollution-induced changes in reef building corals and for monitoring the health of coral reefs.

Oil and gas well-drilling fluids are designed to maintain hydrostatic pressure, bring up cuttings, inhibit corrosion, seal porous substrates, and cool and lubricate drilling bits (Ray 1979). There is a possibility that these materials may reach coral reefs (valuable areas of productivity and habitat in the tropical oceans) and the potential impact needs to be estimated. Unfortunately, they are also vulnerable to damage by natural and human activities. A research program was initiated to devise biochemical measures by which the stress of pollution could be detected in the coral-head microcosm. These assays, if used as monitoring tools, would permit detection of sublethal damage.

Two measures were utilized, both based on an initial lipid extraction. The first used the amount and composition of the endogenous storage lipids as measures of nutritional status. The second tested for the initial phases of bacterial infection.

The nutritional status of organisms is a very useful index of physiological condition. In microbes of estuarine detritus and marine sediments, the accumulation of the uniquely prokaryotic storage lipid, poly beta-hydroxybutyrate (PHB), during conditions of nutrient stress is a valuable tool (Nickels *et al.* 1979; Findlay and White 1983). The relative proportions of biomass measured as the extractable phospholipid compared to the storage lipid, triglyceride (Gehron and White 1982), and wax esters (Sargent *et al.* 1976) are useful in defining the nutritional status of eukaryotic micro-

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biota (fungi, protozoa, metazoa). With these measurements, it was possible to establish that amphipods grazing estuarine detritus in laboratory microcosms and in the field maintained the same nutritional status, which was not the case when they were starved (Smith *et al.* 1982). Many corals exist as a symbiosis between cnidarian cells and dinoflagellate zooxanthellae. The cnidarian cells distort the normal light-driven synthesis of fatty acids by the algae, which results in the synthesis of the unusual saturated fatty acids stored in triglycerides and waxes of the coral cells (Patton *et al.* 1979). This control by the cnidarian cells on the fatty acid synthesis by zooxanthellae could be sensitive to disruptions and result in shifts in either the total content of triglyceride, the wax ester, or in the fatty acid composition of these storage lipids.

The phospholipid content of cells is an excellent assay for the cellular biomass, and phospholipid content correlates with other measurements of biomass (White *et al.* 1979a; 1979b; 1979c). Lipid extraction not only provides a measure of the biomass and of the nutritional status by the level of PHB, waxes, and triglycerides, but it also provides a quantitative recovery of the free nucleotides and amino acids of the cytoplasm (Davis and White 1980). Thus, the free amino acid pools of corals can be measured and the concentration of free amino acids in invertebrates has been related to physiological stress (Kasschau 1975).

The second measure involves demonstration of the initial phases of microbial infection. Black line disease of corals involves a sequence of events that recycle to magnify the injury. An initial injury to the coral induces production of excess viscous mucus, which accumulates. The mucus accumulation stimulates heterotrophic bacteria which create an anaerobic condition by their metabolism. The anaerobic condition permits the growth of fermenting bacteria, which produce short chain fatty acids and hydrogen. These fatty acids and hydrogen together with the sulfate from seawater result in the production of hydrogen sulfide by sulfate-reducing bacteria. The toxic hydrogen sulfide forms a black line of precipitated metallic sulfides and encourages the growth of sulfide-utilizing bacteria. Hydrogen sulfide and other toxic metabolites produced by the bacteria stimulate more mucus production and the cycle is reinforced (Ducklow and Mitchell 1979a; 1979b; Garrett and Ducklow 1975).

Evidence for this cycle may be detected with the recovery of increased levels of plasmalogen type phospholipids in the corals exposed to oil and gas well-drilling fluids. Plasmalogen phospholipids are unique to fermenting anaerobic bacteria in these types of environments (Goldfine and Hagen 1972; Kamio *et al.* 1969).

Materials and Methods

The coral, *Montastrea annularis* (Ellis and Solander), was harvested from Big Pine Key, FL, and was transported by air in oxygenated seawater to tanks of running seawater. The coral samples were housed in glass tanks on the U.S. Navy Stage I platform, 13 km south of Panama City, FL, located at 30° 7.5′ N, 85° 46.3′ W in the Gulf of Mexico. The tanks were filled with seawater drawn from 25 m below the surface, and were illuminated with ambient light. The coral samples were allowed a two week stabilization period prior to exposure. Well-drilling fluids were taken from below the shaker table of an exploratory well in Jay, FL, at approximately weekly intervals and were transported to the Stage for use within two weeks. Drilling-fluid suspensions were made in seawater by continuous stirring, and suspensions with concentrations of 0.1, 0.01, and 0.001 μl/ml were delivered to the tanks containing the coral samples. To better simulate possible drilling-fluid discharges in the field, the coral samples were exposed to four drilling fluids, each for one week, and to a fifth drilling fluid for two weeks. These five drilling fluids contained 84, 132, 96, 200, and 128 μg/L Cr, and <0.0001, <0.06, <0.06, <0.06, 4.8 mg/L of hydrocarbons resembling No. 2 Diesel oil, respectively.

At two-week intervals during the experiment, sections of the coral with 12 to 65 cm^2 surface area were removed, rinsed with seawater and submerged in a one phase chloroform-methanol-aqueous phosphate buffer. Seven coral fragments were analyzed in each treatment. The solution was transferred to a separatory funnel, additional chloroform and water added and, after thorough mixing, the phases were separated (White *et al.* 1979c). The chloroform was filtered through Whatman 2V fluted filter paper (Whatman Inc., Clifton, NJ) to dehydrate the lipid for further analysis. A measured portion of the aqueous phase was recovered for the analysis of the free amino acid pool. The surface area was determined by pressing aluminum foil over the coral surface and then measuring the foil area by its weight.

The total phospholipid was determined, after extraction and digestion with perchloric acid, by colorimetric analysis of the lipid phosphate (White *et al.* 1979c). A portion of the lipids was subjected to mild alkaline hydrolysis, and the aqueous phase analyzed for phosphate to determine the diacylated phospholipid content. The organic phase was recovered, methylated, and purified by thin layer chromatography (TLC). The alkyl fatty acid methyl esters were recovered quantitatively and analyzed by capillary gas-liquid chromatography (GLC). The structural identification was made by determining the quantitative response to catalytic hydrogenation on polar and non-polar columns, by the behavior on silver nitrate impregnated TLC, and by electron impact mass spectral fragmentography (Bobbie and White 1980; Bobbie *et al.* 1981). A second portion of the organic phase of the mild alkaline hydrolysis was subjected to mild acid hydrolysis and partitioned against water. The phosphate recovered in the water phase is the plasmalogen phosphate (White *et al* 1979b).

A second portion of the lipid was quantitatively partitioned into neutral and phospholipid fractions by chromatography on silicic acid (Unisil® 100–200 mesh) (King *et al.* 1977; Gehron and White 1982). The neutral lipids were further fractionated by one dimensional TLC, with petroleum ether-diethylether-acetic acid,

Table 1. Total phospholipid, diacyl-phospholipid, plasmalogen phospholipid, total triglyceride fatty acids, and wax ester alcohols per cm^2 surface area of the coral *Montastrea annularis* exposed continuously for six weeks to oil and gas well drilling fluids

Part I

Dosage	Total phospholipids nmoles/cm^2	Diacyl-phospholipids nmoles/cm^2	% of total	Plasmalogen phospholipids nmoles/cm^2	% of total
0 ml/L	1330 (240)[a]	620 (53)[b]	56 (10)[c]	73 (95)[d]	5.5 (6.1)[e]
0.001 ml/L	1020 (260)	422 (109)	42 (6)	155 (51)	15.1 (2.2)
0.01 ml/L	840 (169)	360 (69)	44 (3)	105 (34)	12.4 (1.2)
0.1 ml/L	790 (305)	346 (117)	45 (4)	126 (56)	15.5 (1.9)

Part II

Dosage	Triglyceride Fatty Acids nmoles/cm^2	Wax Ester Alcohols nmoles/cm^2
0 ml/L	1990 (795)[a]	407 (296)[a]
0.001 ml/L	1700 (625)	296 (272)
0.01 ml/L	1860 (805)	191 (127)
0.1 ml/L	2280 (1220)	422 (290)

[a] Mean and standard deviation in brackets, n = 7; no significant difference in means by ANOVA
[b] Significant ($p < 0.01$) by ANOVA
[c] Significant ($p < 0.01$) by ANOVA of logarithm of percentages
[d] Significant ($p < 0.1$) by ANOVA
[e] Significant ($p < 0.001$) by ANOVA of logarithm of percentages

(80:20:1) and the triglycerides ($R_f = 0.6$) and waxes ($R_f = 0.9$) were recovered. These lipids were deacylated by mild alkaline methanolysis, and the lipid-soluble fatty acid methyl esters were separated from the water-soluble glycerol. The glycerol fraction was peracetylated, and its concentration was determined by glass capillary GLC (Gehron and White 1982). The fatty acid methyl esters from the triglycerides were measured with GLC as described above, and the wax alcohols were acylated with heptafluorobutyric anhydride and separated by GLC (Parker *et al.* 1982).

The free amino acids were recovered from the aqueous portion of the lipid extraction and purified by ion exchange chromatography. The analysis was conducted on a high pressure ion exchange column by fluorimetric detection, after post-column derivatization with *o*-phthalaldehyde (White *et al.* 1980).

The fatty acids and alcohols are designated as the number of carbon atoms in the chain: the number of double bonds, and the position of the double bond nearest the methyl end of the molecule indicated as w. The prefixes, i and a, indicate iso and anteiso branching. The suffixes, cis and trans, indicate the configuration of the double bonds.

Results

Biomass Changes

Table 1 illustrates lipid components recovered per cm^2 of coral surface, after exposure to 0.1, 0.01 and 0.001 µl/ml suspended well-drilling fluids for six weeks. The mean total lipid phosphate, when tested by two way ANOVA with time of exposure vs treatment dosage, showed that the variance rested exclusively within test cell and not in treatments or interactions. By refining the analysis to the diacyl (ester) linked phospholipids instead of the total extractable phospholipids, it was possible to show that a six week exposure significantly decreased the diacylated phospholipid portion of the phospholipid (Table 1). There was a dose-response relationship between the decreased diacylphospholipids and increased concentrations of well-drilling fluids. Logarithm (exposure dosage + 2), with the control considered to receive 0.00 µl/ml well-drilling fluid, versus diacylphospholipid showed a linear decrease ($r = -0.58$, $n = 28$, $P < 0.01$). Significant changes induced by exposure to suspended well drilling fluids were not detected in the total triglyceride glycerol, triglyceride fatty acids, and the total wax ester fatty alcohols. The ratio of triglyceride glycerol to diacyl phospholipid has proven a useful index of nutritional status in several organisms (Gehron and White 1982; Smith *et al.* 1982). This index shows a significant ($P < 0.01$) increase with exposure to well-drilling fluids. The plasmalogen phospholipids measured by the release of phosphate after mild acid hydrolysis showed a significant increase in the corals exposed to well-drilling fluids.

Effect on Triglyceride Fatty Acid and Wax Ester Alcohol Composition

The acyl fatty acids recovered after mild alkaline hydrolysis of the triglycerides and the fatty alcohols

Table 2. Triglyceride fatty acids and wax ester alcohols recovered from the coral *Montastrea annularis* exposed continuously for six weeks to oil and gas well drilling fluids

Part I				
Fatty Acids[a]	A Control	B 0.001 µl/ml	C 0.01 µl/ml	D 0.1 µl/ml
	% of total			
i 15:0	0.06 (0.06)	0.35 (0.27)	0.12 (0.1)	0.1 (0.08)[b]
a 15:0	0.09 (0.14)	0.27 (0.22)	0.15 (0.16)	0.27 (0.25)
15:0	0.15 (0.12)	0.34 (0.26)	0.19 (0.13)	0.98 (1.59)
16:0	60.9 (9.4)	41.9 (19.9)	57.6 (9.74)	56.8 (11.7)[b]
18:0	13.4 (3.57)	17.3 (4.2)	13.1 (3.34)	13.2 (4.53)
18:1w9	6.75 (4.61)	11.5 (4.2)	6.12 (1.34)	6.73 (1.99)
18:1w7	2.63 (1.68)	3.01 (1.76)	1.64 (0.37)	1.97 (0.36)[b]
18:2w6	0.85 (0.31)	1.70 (0.74)	0.98 (0.44)	1.19 (0.43)[b]
18:3w6	0.82 (0.42)	1.85 (1.15)	0.30 (0.16)	1.03 (0.44)[c]
20:1	1.72 (0.68)	2.35 (0.82)	1.73 (0.38)	2.24 (1.69)[b]
20:3w6	0.73 (0.37)	1.57 (0.68)	0.63 (0.3)	1.15 (0.42)[b]
20:4w6	0.56 (0.28)	1.11 (0.61)	0.67 (0.51)	0.69 (0.24)
20:5w6	0.10 (0.05)	0.23 (0.12)	0.15 (0.14)	0.28 (0.19)
24:0	0.06 (0.01)	0.04 (0.01)	0.1 (0.1)	0.04 (0.01)
22:5w3	0.06 (0.04)	0.1 (0.05)	0.05 (0.01)	0.05 (0.01)
22:6w3	0.26 (0.30)	0.90 (0.51)	0.06 (0.03)	0.41 (0.32)[c]
Part II				
Wax Ester Alcohols[a]	A control	B 0.001 µl/ml	C 0.01 µl/ml	D 0.1 µl/ml
	% of total			
11:0	0.03 (0.01)	0.03 (0.03)	0.02 (0.01)	0.03 (0.01)[c]
12:0	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.04 (0.04)
13:0	0.01 (0.00)	0.00	0.01 (0.00)	0.01 (0.00)
A	0.18 (0.06)	0.19 (0.04)	0.15 (0.05)	0.15 (0.05)[b]
14:0	3.81 (1.28)	4.36 (0.72)	2.66 (1.6)	3.15 (0.95)[c]
15:0	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)
B	4.11 (0.2)	3.38 (0.74)	3.95 (0.19)	4.04 (0.19)[b]
16:1 cis	0.11 (0.02)	0.11 (0.02)	0.15 (0.12)	0.12 (0.01)
16:1 trans	1.65 (0.52)	1.62 (0.27)	1.32 (0.12)	1.22 (0.71)
16:0	81.2 (1.6)	81.4 (2.67)	80.9 (3.34)	81.7 (2.05)
C	2.52 (0.23)	2.22 (0.18)	2.48 (0.07)	2.66 (0.2)[c]
17:0	0.04 (0.02)	0.06 (0.01)	0.08 (0.02)	0.06 (0.01)[b]
D	0.22 (0.1)	0.37 (0.29)	0.70 (0.48)	0.25 (0.14)
18:3 cis	0.31 (0.14)	0.31 (0.13)	0.48 (0.28)	0.26 (0.13)
18:1 cis	1.16 (0.27)	1.18 (0.25)	1.62 (0.22)	1.19 (0.43)[c]
18:1 trans	0.05 (0.07)	0.02 (0.01)	0.08 (0.12)	0.03 (0.01)
18:0	1.77 (2.22)	0.76 (0.15)	0.95 (0.11)	1.04 (0.33)[b]
19:0	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.02)[c]
20:0	0.04 (0.02)	0.02 (0.01)	0.03 (0.01)	0.06 (0.06)[b]

[a] Fatty acids and alcohols designated as number of carbon atoms: number of double bonds with the position of the double bond nearest the methyl end of the molecule indicated. The prefixes anteiso (a) and iso (i) stand for a w terminal branching ethyl and methyl group respectively; cis and trans indicate the configuration of the double bonds. Letters indicate positions of alcohols of unknown structures

[b] Indicates significant differences between means expressed as logarithm of the means ($p < 0.05$), n = 7 by ANOVA

[c] Indicates significant difference between means expressed as logarithm ($P < 0.01$), n = 7 by ANOVA

of the wax esters as proportions of the total are given in Table 2. Although there are some significant differences between the means, these differences show no consistent relationship with the exposure to well-drilling fluids.

Effect of Free Amino Acids

Of the free amino acids recovered from the aqueous portion of the lipid extraction of the corals, the concentration of aspartic acid showed a significant in-

Table 3. Free amino acids recovered per cm^2 of the coral, *Montastrea annularis*, exposed for six weeks to oil and gas well-drilling fluids

Amino Acid	A Control	B 0.001 μl/ml	C 0.01 μl/ml	D 0.1 μl/ml
	nmoles amino acid/cm^2 coral surface			
Aspartate	143 (48)	170 (53)	97.5 (35.2)	831 (105)[a]
Glutamic acid	896 (909)	367 (203)	277 (97)	128 (28)
Valine	62 (43)	172 (128)	142 (78)	484 (194)

[a] Indicates significant difference between the means ($P < 0.05$) by ANOVA, $n = 7$

crease with exposure to well drilling fluids (Table 3). Glutamic acid showed a decrease and valine an increase with exposure to well-drilling fluids, but the significance was obfuscated by the within sample variance. Free glutamic acid vs the log (exposure dosage + 2) showed a linear decrease with exposure concentration ($r = -0.59$, $n = 28$, $P < 0.01$).

Discussion

Three biochemical measures of the reef building coral, *Montastrea annularis*, the diacyl phospholipids, the free amino acids, and the plasmalogen phospholipids, showed significant sublethal effects that were related to exposure to oil and gas well-drilling fluids. Significant effects with exposure were not detected in the total phospholipids, the total fatty acids (data not shown), the triglyceride glycerol and fatty acids and the wax ester fatty alcohols (Table 1).

The diacyl phospholipids showed a significant decrease that related to the concentration of well-drilling fluids in a dose response manner. Cellular growth is readily detected in the increase in diacyl phospholipids. In the six week period, the unexposed coral ecosystem increased its diacyl phospholipids from 463 ± 95 to 620 ± 53 nmoles/cm^2. Clearly, there was cessation of growth of the coral ecosystem in the presence of well-drilling fluids at the concentrations utilized.

There were also changes in the free amino acids with exposure to well-drilling fluids (Table 3). Kasschau (1975) has shown that changes in free amino acid pools in the cytoplasm of various invertebrates reflects metabolic stresses.

The vinyl ether bond of the plasmalogen phospholipids is resistant to mild alkaline hydrolysis, which quantitatively releases the ester-linked fatty acids (the acyl lipids). The plasmalogens can be recovered and the vinyl ether bond cleaved by mild acid hydrolysis which yields water soluble phosphate fragments and fatty aldehydes that are soluble in the organic phase. In sediments, the phosphate released by mild acid methanolysis of the plasmalogen phospholipids corresponds exactly with the fatty aldehydes recovered from the organic phase (White *et al.* 1979b). Once the exposure to oil and gas well-drilling fluid was initiated, the exposed coral ecosystems showed significant increases in plasmalogen phospholipids when compared to unexposed controls (Table 1). Since plasmalogen phospholipids in the coral head ecosystem are exclusively in the anaerobic fermentative bacteria (Snyder 1972), the increase probably signals the initiation of an infectious syndrome like black line disease.

It is important to emphasize that the exposure utilized in these experiments would be unlikely in the field. The well-drilling fluid discharges would have to be delivered to the coral reef without significant dilution and the continuous exposure would be unlikely as drilling rigs discharge approximately 50% of the time they are on station.

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References

Bobbie, R. J., and D. C. White: Characterization of the benthic community structure by high resolution gas chromatography of fatty acid methyl esters. Appl. Environ. Microbiol. **39,** 1212 (1980).

Bobbie, R. J., J. S. Nickels, G. A. Smith, S. D. Fazio, R. H. Findlay, W. M. Davis, and D. C. White: Effect of light on biomass and community structure of estuarine detrital microbiota. Appl. Environ. Microbiol. **42,** 150 (1981).

Davis, W. M., and D. C. White: Fluorometric determination of

adenosine nucleotide derivatives as measures of the microfouling, detrital, and sedimentary microbial biomass and physiological status. Appl. Environ. Microbiol. **40,** 539 (1980).

Ducklow, H. W., and R. Mitchell: Bacterial populations and adaptations in the mucus layer on living corals. Limnol. Oceanogr. **24,** 715 (1979a).

———: Observations on naturally and artificially diseased tropical corals: A scanning electron microscopic study. Microb. Ecol. **5,** 215 (1979b).

Findlay, R. H., and D. C. White: Polymeric beta-hydroxy alkanoates from environmental samples and *Bacillus megaterium*. Appl. Environ. Microbiol. **45,** 71 (1983).

Garrett, P., and H. W. Ducklow: Coral disease in Bermuda. Nature **253,** 349 (1975).

Gehron, M. J., and D. C. White: Quantitative determination of the nutritional status of detrital microbiota and the grazing fauna by triglyceride glycerol analysis. J. Exp. Marine Biol. Ecol. **64,** 145 (1982).

Goldfine, H., and P. O. Hagen: Bacterial plasmalogens. Ether lipids: Chemistry and biology, New York: Academic Press (1972).

Kamio, Y., S. Kanegasaki, and H. Takahashi: Occurrence of plasmalogens in anaerobic bacteria. J. Gen. Microbiol. **15,** 439 (1969).

Kasschau, M. R.: The relationship of free amino acids to salinity changes and temperature-salinity interactions in the mudflat snail *Nassarius obsoletus*. Compar. Biochem. Physiol. **51A,** 301 (1975).

King, J. D., D. C. White, and C. W. Taylor: Use of lipid composition and metabolism to examine structure and functional activity of estuarine detrital microflora. Appl. Environ. Microbiol. **33,** 1177 (1977).

Nickels, J. S., J. D. King, and D. C. White: Poly-beta-hydroxy butyrate metabolism as a measure of unbalanced growth of the estuarine detrital microbiota. Appl. Environ. Microbiol. **37,** 459 (1979).

Parker, J. H., G. A. Smith, H. L. Fredrickson, J. R. Vestal, and D. C. White: Sensitive assay, based on hydroxy-fatty acid from lipoplysaccharide lipid A for gram negative bacteria in sediments. Appl. Environ. Microbiol. **44,** 1170 (1982).

Patton, J. W., S. Abraham, and A. A. Benson: Lipogenesis in the intact coral *Pocillopora capitella* and its isolated zooxanthellae: Evidence for a light-driven cycle between symbiont and host. Mar. Biol. **44,** 235 (1979).

Ray, J. P.: Offshore discharge of drill muds and cuttings. Proc. Symp. on the OCS Frontier Technology, Washington DC: Marine Board, Nat. Res. Council: (Dec. 6, 1979).

Sargent, J. R., R. F. Lee, and J. C. Nevenzel: Marine waxes. Chemistry and biochemistry of natural waxes, New York: Elsevier (1976).

Smith, G. A., J. S. Nickels, W. M. Davis, R. F. Martz, R. H. Findlay, and D. C. White: Perturbations in the biomass, metabolic activity, and community structure of the estuarine detrital microbiota: Resource partitioning in amphipod grazing. J. Exp. Marine Biol. Ecol. **64,** 125 (1982).

Snyder, F: Ether lipids: Chemistry and biology, New York: Academic Press (1972).

White, D. C., R. J. Bobbie, J. S. Herron, J. D. King, and S. J. Morrison: Biochemical measurements of microbial mass and activity. Native aquatic bacteria, enumeration, activity, and ecology. ASTM STP 695. American Society for Testing and Materials, Philadelphia, PA (1979a).

White, D. C., R. J. Bobbie, J. D. King, J. S. Nickels, and P. Amoe: Lipid analysis of sediments for microbial biomass and community structure. Methodology for biomass determinations and microbial activities in sediments. ASTM STP 673. American Society for Testing and Materials, Philadelphia, PA (1979b).

White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie: Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia **40,** 51 (1979c).

White, D. C., R. J. Bobbie, J. S. Nickels, S. D. Fazio, and W. M. Davis: Non-selective biochemical methods for the determination of fungal mass and community structure in estuarine detrital microflora. Bot. Mar. **23,** 239 (1980).

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