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MODIFICATIONS OF ESTUARINE SEDIMENTARY MICROBIOTA BY EXCLUSION OF EPIBENTHIC PREDATORS

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Abstract: The ability of epibenthic predators (crabs and fishes) to influence biomass and community structure of sedimentary microbiota was investigated in St. George Sound-Apalachicola Bay System, Florida, U.S.A. Replicate areas (4 m^2) of mud-flat sediment were caged in the field to confine and exclude predators. Uncaged areas were used as controls. The microbiota (prokaryotes and microeukaryotes) of the sediments was characterized at Weeks 0, 2, and 6 by measuring concentrations of phospholipid and analyzing fatty acids of the microbial lipids extracted from the sediments. Data were analyzed using analysis of variance and step-wise discriminant analysis. After 2 wk, the microbiota of the predator exclusion treatment was significantly different from that in control and predator inclusion treatments. After 6 wk, these differences became more pronounced. There were no demonstrable caging effects that could account for treatment differences. Results indicated that removal of predators had a profound effect on microbial communities in estuarine sediments. Thus, the top trophic level (epibenthic predators) had an important role in regulating the structure of the lowest trophic level (the microbiota).

INTRODUCTION

Estuarine mud-flat sediments are inhabited by a variety of organisms that are arranged in a complex food web. The base of this food web is the microbiota which includes the bacteria, microalgae, protozoa, fungi, and micrometazoan meiofauna. These microorganisms form a food web among themselves (Fenchel & Jorgensen, 1977; Tietjen, 1979) and are utilized as a food resource by a variety of deposit-feeders such as amphipods, oligochaetes, molluscs, and polychaetes (Hargrave, 1970; Lopez & Levinton, 1978; Fauchald & Jumars, 1979; Fry, 1982). In turn the bioturbation caused by invertebrate feeding activity may alter the chemical and physical characteristics of the sediment habitat and stimulate the growth and activity of the microbiota (Rhoads, 1974; Gerlach, 1978; Yingst & Rhoads, 1979; Morrison & White, 1980; Findlay & White, 1983a; 1983b). Predator exclusion experiments have demonstrated that the biomass and composition of deposit-feeding communities are controlled, at least in

part, by large epibenthic predators (Virnstein, 1977; Petersen, 1979). In this study, predator exclusion and inclusion methodology was used to investigate the role of epibenthic predators (crabs and fish) in regulating the biomass and composition of the microbiota.

A large proportion of the microbes exist in microcolonies of mixed composition that are enmeshed in an extracellular network of polysaccharide which is firmly attached to the sediment particles (Marshall, 1976; Costerton *et al.*, 1981; Moriarty & Hayward, 1982). This strong binding makes enumeration methods that require release of the microbes unreliable and can lead to large errors associated with the subsampling of homogenized sediment samples (Moriarty, 1980; Montagna, 1982). Cultural methods involving isolation and growth of organisms underestimate the microbial biomass (King & White, 1977). Biochemical methods that quantitatively recover and measure cellular components that reflect the biomass and community structure avoid these problems (White, 1983).

Lipids can be quantitatively extracted from the microbiota of sediments and analyzed with a high level of reproducibility. Phospholipids are useful measures of the cellular biomass, because they are found in all membranes, show a rapid loss from non-viable cells in sediments, and are quantitatively extractable from the sediments (White *et al.*, 1979c). The phospholipid content of sediments has been shown to correlate with other measures of microbial biomass and activity (White *et al.*, 1979a; 1979b). Other useful measures of both microbial biomass and community structure are the fatty acids that can be released from the lipids by methanolysis. The fatty acids can be analyzed with great sensitivity and resolution by capillary gas-liquid chromatography (GLC). This type of analysis provides information regarding various component subsets of the community (Bobbie & White, 1980). Pentadecanoic straight chain and branched fatty acids as well as certain monoenoic fatty acid isomers are concentrated in bacteria (Bobbie & White, 1980; Volkman *et al.*, 1980; Parkes & Taylor, 1983). A specific monoenoic fatty acid with the *trans* configuration is found exclusively in the phosphatidyl glycerol of photosynthetic eukaryotes containing photosystem I (Haverkate & Van Deenen, 1965; Nichols, 1970). Therefore this fatty acid is an excellent measure of the algae. Other groups of microeukaryotes can be characterized by their polyenoic fatty acid composition (Erwin, 1973). These methods for characterizing the sedimentary microbial community made it possible to document effects of epibenthic predators in influencing the composition of the mud-flat microbiota.

METHODS

MATERIALS

Nanograde or glass distilled solvents (Burdick and Jackson, Muskegon, MI, U.S.A.) and freshly distilled chloroform were utilized. Standards and derivatizing agents were purchased from Supelco, Inc. (Bellefonte, PA, U.S.A.), Applied Science Co. (State

College, PA, U.S.A.), Aldrich, Inc. (Milwaukee, WI, U.S.A.) and Sigma Chemical Co., (St. Louis, MO, U.S.A.). Analytical grade formaldehyde was supplied by Mallinckrodt.

STUDY SITE

This study was performed from 13 April to 25 May, 1982, in St. George Sound-Apalachicola Bay System (29°52′N : 84°25′W), located off the northern panhandle of Florida. The sediments were unconsolidated subtidal sandy muds with average depths between 2 and 3 m that were located ≈ 100 m off shore. The tidal excursion averaged 0.7 m with a maximum of 1.2 m so the muds were never exposed. The bottom is flat without rocks or other obstructions. Currents generally average 20 to 40 cm/s, but can rise to over 100 cm/s with strong winds. Compared to other nearby estuaries, the site is characterized by high salinity and relatively low secondary productivity (Ravenel & Thistle, 1981; Livingston, 1982; Federle *et al.*, 1983).

CAGING

Triplicate cages were used to confine and exclude predators from 2 by 2 m areas of sediment. Cages consisted of tubular metal frames covered with plastic netting (OV-3010 plastic mesh, 6 mm openings, Conwed Corp., Minneapolis, MN, U.S.A.) anchored into the sediment with metal flashing. The cages extended above water level and were open on top. Control cages of tubular metal frames lacked netting. Experiments in a nearby estuary established that short-term (2-month) exclusion experiments using large cages with this plastic mesh produced minimal physical or chemical artifacts (Mahoney & Livingston, 1982). Each inclusion cage contained one blue crab (*Callinectes sapidus* Rathbun, 60–70 mm), three croakers (*Micropogonias undulatus* (L.), 50–70 mm) and two spot (*Leiostomus xanthurus*, 50–70 mm). Previous field studies indicated that this was a reasonable density, assortment, and age class of predators for this time period at this site (Livingston, 1982). Samples were taken from three exclosures, three enclosures, and three control cages at 0, 2, and 6 wk. Additional samples (background samples) were taken from randomly selected plots adjacent to the experimental plots.

SAMPLING OF THE MICROBIOTA

The microbiota of the sediment was characterized at 0, 2, and 6 wk after establishment of the cages. Samples were taken from a 2 × 2 m platform placed on top of the cages. The platform contained 100 evenly spaced sampling ports in a grid. Samples were taken with a 2-m polyvinyl tube (3.2 cm diameter) inserted through the sampling ports in a random pattern in the grid. Four samples were taken from the three exclusion cages, the three inclusion cages, the three control sites inside metal frames plus three randomly selected background samples at each of the time 0, 2, and 6 wk intervals. The sampling tube was constructed so the lower 35 cm of the core tube could be detached allowing sampling of the sediment surface (Federle *et al.*, 1983). The top 2 cm of

sediment was recovered and washed through a 500 μm sieve. The material passing through the sieve was preserved with 10% (v/v) aqueous formaldehyde (Federle & White, 1982) and used for the lipid analysis. Macrofauna which were retained on the sieve were saved for further analysis. This prevented their inclusion in the lipid analysis.

LIPID ANALYSIS

A flow diagram of the lipid analysis is given in Fig. 1. The lipids were extracted from the sediment with a modified one-phase chloroform–methanol extraction and a portion

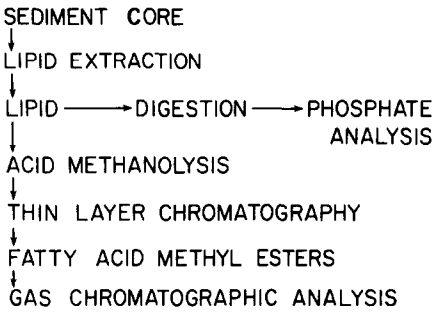

Fig. 1. Diagram of the analysis utilized for the sedimentary microbiota.

was utilized for measurement of the total phospholipid after perchloric acid digestion and colorimetric analysis (White *et al.*, 1979c). The major portion of the lipid was subjected to mild acid methanolysis and the resulting fatty acid methyl esters were purified by thin layer chromatography. In this procedure, the formaldehyde is completely removed from the fatty acid esters (Federle & White, 1982). The fatty acid methyl esters were analyzed by capillary GLC (Bobbie & White, 1980). The assignment of structure was based on chromatographic comparison with authentic standards, the effect of catalytic hydrogenation (White & Cox, 1967), and by mass spectral analysis (Bobbie & White, 1980).

FATTY ACID NOMENCLATURE

Fatty acids are designated as the number of carbon atoms in the chain: the number of double bonds, the position of the unsaturation nearest the methyl (omega, ω) end of the molecule with a *t* indicating *trans* configuration. The prefix Br indicates iso- or anteiso-branching.

STATISTICAL ANALYSIS

All data were standardized to mean zero with a variance of one and first analyzed by a two-way nested analysis of variance (ANOVA) using the BMDP-02V program.

Data from each sampling were then analyzed separately using a one-way ANOVA (BMDP-08V). The data were also analyzed by stepwise discriminant analysis using the SPSS DISCRIMINANT program. Each result was converted to its logarithm prior to analysis. There were no missing cells in these analyses.

RESULTS

EFFECTS OF CAGING ON PHYSICAL AND CHEMICAL VARIABLES

Experiments performed in a nearby estuary for a 3-month period in 1979 have established that use of nylon netting with 6-mm openings with cage size of 2 m^2 had no statistically significant effect on the water temperature, pH, color, turbidity, salinity, dissolved oxygen, or Secchi depth. There was a $<5\%$ decrease in seston settlement into sediment traps and insignificant differences in granulometric characterization, silt-clay fractions, or in current velocity (Mahoney & Livingston, 1982). The experiment was timed to prevent recruitment of predators small enough to pass through the nylon mesh (Mahoney & Livingston, 1982).

ANALYSIS OF VARIANCE

To facilitate statistical analysis, these lipid markers were segregated into five classes of microbial markers based on their taxonomic distribution as reported in the literature. The markers were: total biomass (phospholipid, 16 : 0, 16 : 1ω7, 18 : 2ω6, 18 : 1ω9), bacteria (Br 15 : 0 which includes both anteiso- and iso-branched 15 : 0, 15 : 0), algae (16 : 1ω13 *t*), microeukaryotes (20 : 4ω6, 20 : 5ω3, 20 : 3ω6, 22 : 4ω6, 22 : 6ω3) and a possible bacterial ecotype (18 : 1ω7) which represents the activity of the bacterial anaerobic desaturase activity (Bloch, 1969; Erwin, 1973) and appears to be concentrated in facultative anaerobic bacteria growing aerobically (Parkes & Taylor, 1983). The variables that were grouped together were significantly ($P < 0.001$) correlated with each other (Pearson correlation coefficient). Each measure was standardized to mean zero, variance unity and the combined microbial markers were generated by taking the mean of the standardized measures. Preliminary analysis using a two-way ANOVA showed that most markers exhibited a significant interaction between time and treatment. Hence, each sampling time was analyzed separately using a nested one-way ANOVA in which cores were nested within cages, which were nested within treatments.

The baseline at Week 0 was estimated from three randomly selected 2 by 2 m areas. These areas were not significantly different from each other in any of the 14 lipid measures. This was consistent with previous work at this location (Federle *et al.*, 1983). Fig. 2 shows the mean values of the standardized microbial markers and their standard errors as a function of time and treatment. After 2 wk, control and inclusion treatments declined in biomass, bacteria and 18 : 1ω7, but these declines did not occur in the exclusion treatment. Significant treatment effects existed at this time for total biomass

($P < 0.05$), bacteria ($P < 0.05$) and the fatty acid 18 : 1ω7 ($P < 0.001$). With the exception of microeukaryotes, none of the variables displayed significant among cage differences within the treatments.

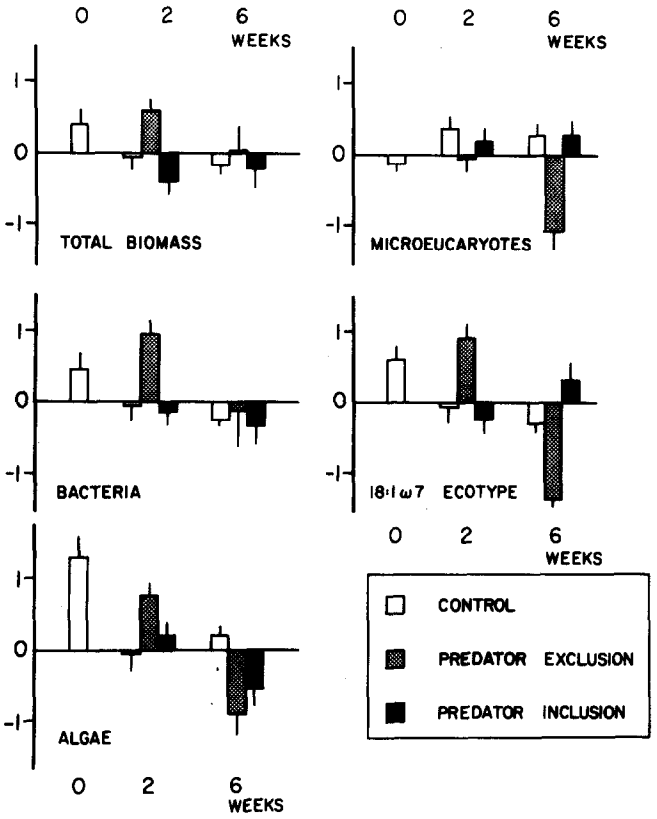

Fig. 2. Standardized values ($X \pm$ SE) for biochemical markers as a function of both time (2 and 6 wk) and treatment (□, control; ▨, predator exclusion; ■, predator inclusion) in replicates analyzed by two-way nested ANOVA: total biomass = phospholipid + 16 : 0 + 16 : 1ω7 + 18 : 2ω6 + 18 : 1ω9; bacteria = iso + anteiso (Br) 15 : 0 + 15 : 0, algae = 16 : 1ω13 *t*; microeukaryotes = 20 : 4ω6 + 20 : 5ω3 + 20 : 3ω6 + 22 : 4ω6 + 22 : 6ω3; a possible bacterial ecotype = 18 : 1ω7.

After 6 wk, biomass and bacteria no longer exhibited treatment effects, but microeukaryotes and 18 : 1ω7 showed significant treatment effects ($P < 0.001$), with the exclusion treatment being lower than the others. No variable displayed significant among cage differences within the treatments. Although algae displayed a significant treatment effect ($P < 0.05$), no pairwise comparisons were significant.

DISCRIMINANT ANALYSIS

Each biochemical marker was used in a stepwise discriminant analysis to determine if predation caused changes in the microbial biomass and community structure, and to identify the variables that were of greatest importance in discriminating among the control, the predator exclusions, and the predator inclusions. The data from each sample time were analyzed separately and together.

When the data from Week 2 were analyzed alone, eight of the 14 biochemical markers contributed to the discrimination of the treatments. Table I lists these variables, the F

TABLE I

Biochemical markers that contribute to the discrimination of samples from control, predator inclusions, and predator exclusions taken after 2 wk.

Discriminating variable[a]	F to remove	Discriminant function coefficient (Z)[b]	
		Function 1	Function 2
1. 20:4ω6	8.0	−1.29	−0.51
2. 18:2ω6	6.1	0.66	0.68
3. Lipid phosphate	5.4	0.62	0.62
4. 16:0	7.5	−0.21	−1.66
5. 20:5ω3	7.0	0.51	−1.42
6. 22:6ω3	3.7	−0.37	−1.10
7. 16:1ω7	3.0	0.30	1.03
8. 22:4ω6	1.7	0.30	0.48

[a] Order of entry into stepwise discriminant analysis. Variables not listed did not contribute to the discrimination.

[b] Standardized coefficient (Z). Those variables with the largest absolute value contribute most to discrimination by an individual discriminant function.

to remove, and their discriminant function coefficients. The order of entry of the variables into the analysis indicates those variables that have the greatest overall discrimination power. Each variable also has associated with it a discriminant function coefficient that relates the weighting of that variable in the mathematical formulation of each discriminant function after the variable has been standardized to mean zero, with standard deviation unity. The variables with the largest highest absolute coefficient have the greatest impact in defining a discriminant function. The F to remove indicates the penalty for removal of the variable from the discriminant function.

Fig. 3 shows a graphical representation of the samples taken from each treatment after 2 wk in relationship to the discriminant variables. Discriminant function 1 differentiated the predator exclusions from the predator inclusions but not from the controls. This function was defined by 18:2ω6 (0.66), lipid phosphate (0.62), and 20:4ω6 (−1.29). Discriminant function 2 differentiated the control samples from the other two treatments. This function was defined by 22:6ω3 (−1.10), 16:1ω7 (1.03),

16 : 0 (– 1.66), and 20 : 5ω3 (– 1.42). By using both discriminant functions 86% of the 2-wk samples could be correctly classified into control, inclusion, and exclusion conditions.

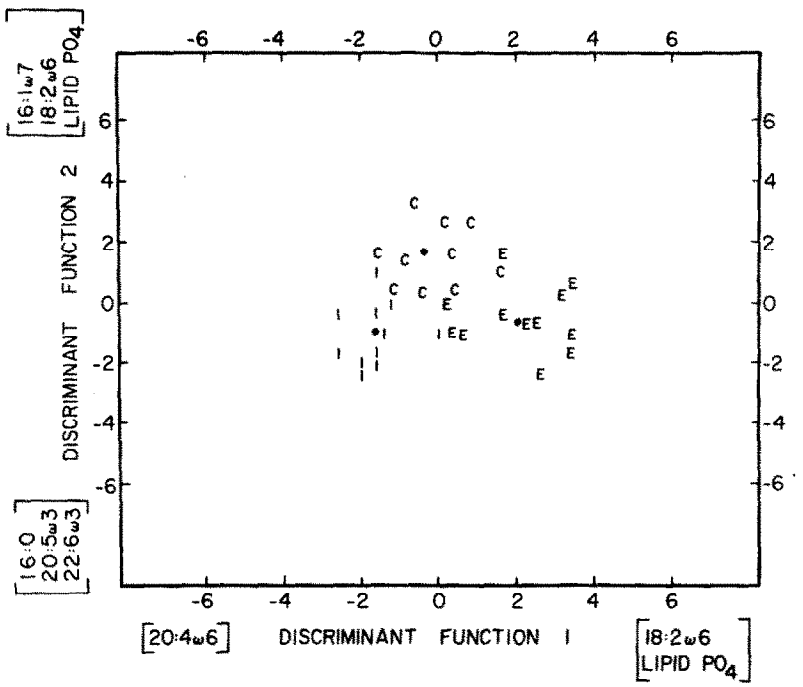

Fig. 3. Discriminant analysis of biochemical measures from sediment taken from the control (C), the predator excluded cages (E), and the predator included cages (I) after 2 wk: ●, centroids of the treatments; the most powerful measures are listed at appropriate places on the axes (see Table I).

When the data from Week 6 were analyzed, nine variables contributed to the discrimination (Table II). Fig. 4 shows the graphical representation of this analysis. Discriminant function 1 effectively differentiated the predator exclusion samples from the others. Using this discriminant function alone 100% of the exclusion samples were classified correctly. This discrimination function was defined by seven variables with nearly equal coefficients. Discriminant 2 differentiated between the predator inclusions and controls and was defined by four variables with nearly equal weighting. Using both discriminant functions together 97% of the samples could be classified correctly.

When the data from both Weeks 2 and 6 were analyzed together, nine of the 14 biochemical markers contributed to making discriminations among the treatments. Discriminant function 1 which differentiated the predator exclusions from the other treatments utilized 20 : 4ω6 (1.15), Br 15 : 0 (– 0.69), and 18 : 2ω6 (– 0.68). The predator inclusions and controls were differentiated by 15 : 0 (2.01) and Br 15 : 0

TABLE II

Biochemical markers that contribute to the discrimination of samples from control, predator inclusions, and predator exclusions taken after 6 wk.

Discriminating variable[a]	F to remove	Discriminant function coefficient (Z)[b]	
		Function 1	Function 2
1. 18:1ω7	60.5	2.06	−0.60
2. 16:0	11.5	−1.54	0.46
3. 20:5ω3	13.7	1.51	−0.35
4. 18:2ω6	16.7	−2.33	−1.23
5. 16:1ω13 *t*	3.3	−0.13	1.38
6. 22:6ω3	3.7	−0.32	0.84
7. 16:1ω7	6.4	2.16	−0.03
8. Br 15:0	8.9	−2.65	−1.66
9. 15:0	4.0	1.89	−1.32

[a] Order of entry into stepwise discriminant analysis. Variables not listed did not contribute to the discrimination.

[b] Standardized coefficient (Z). Those variables with the largest absolute value contribute most to discrimination by an individual discriminant function.

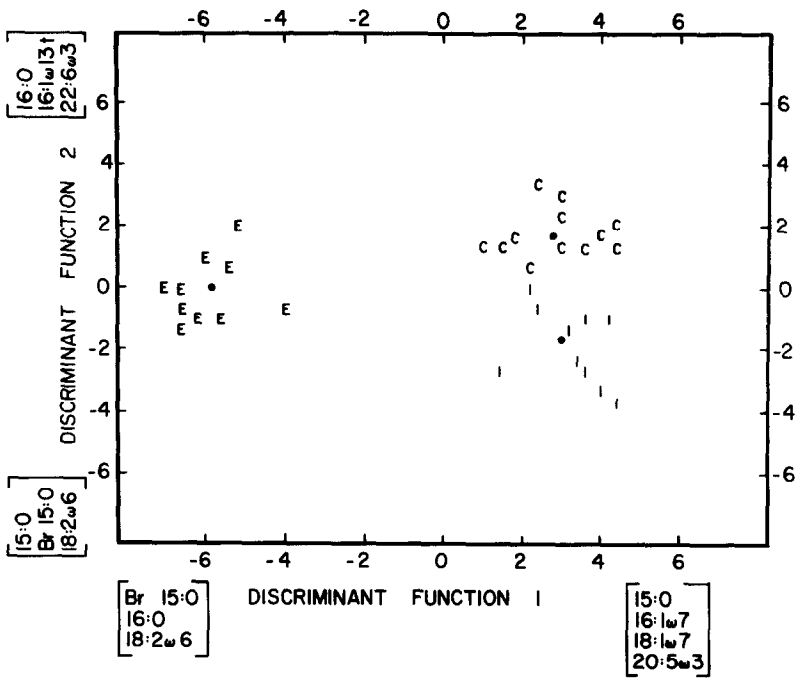

Fig. 4. Discriminant analysis of biochemical measures from sediments after 6-wk exposure as in Fig. 3: see Table II for discriminant variable values.

(−1.55). Using both discriminant functions together 83% of the samples could be classified correctly.

Discussion

ANALYSIS OF VARIANCE

The exclusion of epibenthic predators from the estuarine mud-flat sediments had a profound effect on the microbiota as shown by analysis of variance of grouped measures (Fig. 2). In as little as 2 wk the areas from which predators were excluded showed more biomass, bacteria and bacteria with the anaerobic desaturase pathway (18 : 1ω7) than either the areas with included predators or the controls. After 6 wk the exclusion areas exhibited significantly lower levels of microeukaryotes and the bacterial subset possessing the anaerobic desaturase pathway.

DISCRIMINANT ANALYSIS

Rosswall & Kvillner (1978) have emphasized the value of multivariate analysis in microbial ecology. Sayler *et al.*, (1982) showed the power of discriminant analysis in the examination of the effects of pollution by coal-coking wastewater effluent on sedimentary microbiota. This analysis does not require the organization of measures into subgroups prior to analysis but calculates the measures most effective in predicting which sample points were obtained from the various treatments. The relative spread of points around the centroid of points from a treatment group gives a graphic indication of the variability within the treatment group. The distance between the centroids indicates the degree of difference among treatments and the analysis indicates the degree to which the various individual measures influence the separation between the centroids.

It is clear that both spread about the centroids and distance between centroids was increased with longer exposure (compare Figs. 3 and 4). Even in the 2-wk period it proved possible to distinguish a microbial community inside the cages from which the epibenthic predators were excluded that was enriched in 18 : 2ω6 and depleted in 20 : 4ω6 (Table I, Fig. 3). The community structure after 6 wk showed an even clearer effect of predator exclusion (Fig. 4, discriminant function 1). Again the microbes were enriched in 18 : 2ω6. This fatty acid is common in protozoa, fungi and other microeukaryotes (Erwin, 1973). The microbiota of exclusion cages was also enriched in 16 : 0, the ubiquitous fatty acid found in all microbes, and in Br 15 : 0 which is typical of bacteria. The predator affected sediments were enriched in bacteria of a different ecotype (15 : 0) as well as in bacteria containing the anaerobic desaturation pathway (18 : 1ω7), and facultative anaerobic bacteria growing anaerobically as well as sulfate-reducing bacteria (16 : 1ω7) (Parkes & Taylor, 1983), plus the microeukaryotes (20 : 5ω3). Continuous predation in the predator inclusion cages increased the bacteria containing 15 : 0, Br 15 : 0 and the anaerobic desaturase pathway 18 : 1ω7 as well as microeukaryotes 18 : 2ω6. Intermittent predation in the control cages increased the

photosynthetic microalgae (16 : 1ω13 *t*), the 16 : 0, and the eukaryote fatty acid 22 : 6ω3 (Fig. 4, discriminant 2).

The discriminant analysis of the three treatments shows that there are at least four distinct bacterial classes. One class of bacteria, characterized by 15 : 0, were increased in sediments exposed to predators. Bacteria, characterized by Br 15 : 0, were increased in sediments from which predators were excluded (Fig. 4, discriminant 1). The bacteria with the anaerobic desaturase pathway that form 18 : 1ω7 clearly behave differently from the bacteria containing 15 : 0 and Br 15 : 0 in the predator inclusion cages (Fig. 2). The bacteria that form 16 : 1ω7 are like the ones forming 18 : 1ω7 in being concentrated where there is epibenthic predation (Fig. 4, discriminant 1) but are increased with lesser predation pressure after 2 wk (Fig. 3, discriminant 2). Comparing the bacteria isolated from a multiple-vessel chemostat to marine sediments, Parkes & Taylor (1983) have shown that 18 : 1ω7 is concentrated in facultative heterotrophic bacteria growing aerobically and 16 : 1ω7 is concentrated in both heterotrophic bacteria growing anaerobically and in sulfate-reducing bacteria.

Two mechanisms might explain the remarkable differences in the community structure between the three treatments as illustrated in Fig. 4. Predators could directly affect the microbiota by disturbing the sediments in the act of feeding. Disturbances of anaerobic sediments result in rapid synthesis of the bacterial storage polymer poly-beta-hydroxyalkanoate that can occur without growth (Nickels *et al.*, 1979; Findlay & White, 1983a). Subsequent experiments showed this did not occur in sediments disturbed by epibenthic predator activity (Findlay & White, unpubl. experiments). The release from epibenthic predation in the exclusion cages resulted in increased Br 15 : 0, a fatty acid characteristic of the anaerobic sulfate-reducing consortium found in marine sediments (Parkes & Taylor, 1983). However the activity of epibenthic predators increased both the aerobic (15 : 0, 18 : 1ω7) and anaerobic (16 : 1ω7) bacterial activity (Fig. 4, discriminant 1). With continuous predation (inclusion cages) there was a higher level of both aerobic and anaerobic bacterial activity (15 : 0, Br 15 : 0) than in the control (Fig. 4, discriminant 2). This makes direct aeration of the sediment from the bioturbation activities of the epibenthic predators as the major factor seem unlikely.

The second effect of the epibenthic predators could be in controlling the deposit feeder populations. In this experiment, at Weeks 4, 5, and 6, the number of deposit-feeders was significantly higher in the sediments from which epibenthic predators were excluded (Livingston, unpubl. data). This higher level of deposit-feeders was reflected in an increase in the dominant polychaete *Mediomastus ambiseta* (Hartman). This polychaete is a nonselective deposit-feeder. The dramatic decline in microeukaryotes (nematodes) that are microbial grazers was probably the result of the increased number of these deposit-feeders. The exclusion of epibenthic predators, which resulted in increased deposit-feeding polychaetes, decreased 20 : 4ω6 after 2 wk and 20 : 5ω3 after 6 wk. The major meiofauna of these sediments are nematodes (Findlay & White, 1983b). When nematodes were collected using a density gradient separation with colloidal silica Ludox-TM (De Jonge & Bouwman, 1977), the lipids extracted and the

fatty acids analyzed, a total of 13.4% of the extractable lipid fatty acids were polyenoic with 20 or more carbon atoms (J.A. Nichols & D.C. White, unpubl. data). In the sediments from which the predators were excluded the concentration of 18 : 2ω6 was also significantly higher. This fatty acid is found in high levels in protozoa (Erwin, 1973). The removal of the nematodes by the deposit-feeding polychaetes might have resulted in an increase in bacterial biomass (as reflected in the increased lipid phosphate and 16 : 0). This in turn could have stimulated protozoan grazers.

Caging experiments have been criticized with the contention that the effects result from habitat changes induced by caging itself (Virnstein, 1978; Peterson, 1979; Dayton & Oliver, 1980; Hulberg & Oliver, 1980). For this experiment, the effects of cage size, design, netting, season, period, etc. were the result of extensive experimentation at this site to minimize possible caging effects. In general, the inclusion and control treatments behaved similarly, indicating that the microbiota was responding to the exclusion of predators and not to the cages. In addition, undisturbed and uncaged areas were sampled and found not to be significantly different from either the inclusion or control treatments. The cages themselves did not attract predators as observed by SCUBA.

These experiments illustrate the value of the quantitative analysis of the microbial community structure by the "signature" lipids. With a similar biochemical analysis it was possible to document the partitioning of the detrital microbiota between two sympatric amphipods with different mouthpart morphology (Smith *et al.*, 1982) and to define the selectivity of the deposit feeding sand dollar *Mellita quinquiesperforata* (Findlay & White, 1983b). Currently additional measures of anaerobic bacterial activity are available utilizing the presence of the phospholipid plasmalogens as "signatures" of the anaerobic fermenters (White *et al.*, 1979b) and the diphytanylglycerol ethers of the methane-forming bacteria (Martz *et al.*, 1983). These measures should be helpful in further distinguishing between bioturbation and grazing effects.

In conclusion, a number of studies have shown that predators control the biomass, community composition and diversity of the lower trophic levels in the marine sediment (Kneib & Stiven, 1982). This study indicates that this control extends through the food web to the lowest trophic level in these sediments. Consequently, environmental perturbations that appear to affect directly only the epibenthic predator trophic level may reverberate through the entire food web.

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