

THE EFFECTS OF FEEDING BY THE SAND DOLLAR *MELLITA QUINQUIESPERFORATA* (Leske) ON THE BENTHIC MICROBIAL COMMUNITY

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Abstract: Sediments in which a sand dollar, *Mellita quinquiesperforata* (Leske) (Echinodermata), had fed, were analyzed by multiple biochemical methods to determine which members of the microbial community were utilized as food and the effects of the feeding disturbance on microbial activity. Included in the study were both field and microcosm experiments. Feeding by *M. quinquiesperforata* increased the zone of oxidized sediment, and decreased several lipid components of the microbiota and the number of vital staining foraminifera without significantly changing the total chlorophyll *a*, the proportion of lipid components, or the rate of acetate incorporation into lipid. These findings, in agreement with literature reports concerning sediment grain size selection and gut content analysis, demonstrate that *M. quinquiesperforata* selectively feeds on microeucaryotes and the bacteria attached to the silt and clay fraction of the sediments. Comparison of the field and microcosm experiments illustrates not only the value of microcosm experiments, but a need for careful evaluation of the changes induced by bringing sediments into the laboratory and how these changes will affect the process under study.

INTRODUCTION

The benthic microbial community is an important component of the estuarine ecosystem. This community is responsible for degrading refractory polymers (Bobbie *et al.*, 1978; Federle & Vestal, 1980) and concentrating dilute nutrients (Gosselink & Kirby, 1974). Also it is the primary food source within the detrital food web (Fenchel & Jorgensen, 1977). Invertebrate deposit-feeders in turn have a marked effect on sediment structure (Rhoads, 1974), and influence microbial activity by altering the size of detrital particles, agitating the sediments, and regenerating mineral nutrients (Fenchel, 1977). Studies of deposit-feeding invertebrates have begun to examine the interactions between detritivores and the detrital microbial community.

Newell (1965) showed that upon reingestion of fecal pellets the prosobranch *Hydrobia ulvae* removed the bacteria present. Bacteria and algae were removed from reingested fecal pellets by the amphipod *Hyaella azteca* (Hargrave, 1970, 1976). Fenchel (1970, 1977) has shown that the ingestion of either *Zostera* detritus by the gastropod *Hydrobia*, or *Thalassia* detritus by the amphipod *Parhyalella* decreased the particle size of the detritus and increased both microbial biomass and oxygen utilization.

Analysis of the fecal castings of the sediment-ingesting lugworm *Arenicola marina* showed a decrease in marker fatty acids of the bacterium *Desulfovibrio desulfuricans* (Boon *et al.*, 1978). Fecal mounds of the sediment-ingesting enteropneust *Ptychodera*

bahamensis exhibit a marked decrease in total biomass when compared with surrounding surface sediments (unpubl. data).

Morrison & White (1980), utilizing multiple biochemical methods, have shown that grazing by gammaridean amphipods upon the epifaunal microbial community of estuarine incubated oak leaves increased both the biomass and the metabolic activity of the microbial community. Grazing caused a shift to a community dominated by bacteria. The amphipods were shown to ingest and assimilate radiolabeled bacteria present on the surface of the oak leaves. Smith *et al.* (1982), using similar methods, demonstrated differential grazing with resource partitioning by two sympatric gammaridean amphipods leading to shifts in the community structure of the detrital microbiota.

In this study, the effects of the deposit-feeding sand dollar *Mellita quinquesperforata* (Leske) were analyzed. Sand dollars continuously feed by moving through the sediments. The dorsal surface of the test acts as a sieve selectively collecting the smallest sediment particles, which are then ingested. Analysis showed that 80% of the gut contents were sediment particles 0.062 mm or less in diameter which accounted for only 3% of the total sediments in which the sand dollars had been feeding (Lane, 1977). The test of the sand dollar contains five slits or lunules. Numerous podia (tube feet) are located in these lunules and at the posterior edge of the test. These podia have been observed probing passing grains, accepting some and rejecting others (Ghiold, 1979; Seilacher, 1979). This behavior appears to allow large particles which have been rejected on the basis of size to be resorted on the basis of food quality (Alexander & Ghiold, 1980). The effects of these processes on the benthic microbial biomass, community structure and metabolism were examined. Field and microcosm experiments were conducted and samples were analyzed by multiple biochemical methods (White, 1983). An a posteriori comparison of the field control samples with the laboratory control samples has allowed an evaluation of microcosms as tools for studying natural processes.

METHODS

MATERIALS

Nanograde solvents (Burdick and Jackson, Muskegon, Mich.) and freshly distilled chloroform (Mallinckrodt, St. Louis, Mo.) were used. Derivatizing reagents were purchased from Pierce Chemical Co. (St. Louis, Mo.).

STANDARDS

Lipid standards and chromatographic supplies were acquired from Supelco, Inc. (Bellefonte, Pa.), or Applied Science Laboratories (State College, Pa.). Radioactive nucleotides were supplied by New England Nuclear Corp. (Boston, Mass.).

FIELD EXPERIMENT – 1979

Field experiments were conducted at Bay Mouth Bar, Franklin County, Florida (29° 54' N : 84° 27.5' W) to measure the effect of a single passage of a sand dollar. Sand dollars produce a characteristic disturbance of the sediment surface which is easily detected at low tide. Samples were taken by cores just ahead of and immediately behind sand dollars as they moved through the sediments. Sampling was conducted on 8 October, 1979 at low tide. A total of five matched pairs were taken for each assay performed. A matched pair consisted of the treatment sample taken immediately after the passage of individual sand dollar and the control sample taken just in front of the same sand dollar.

FIELD EXPERIMENT – 1980

The 1980 field experiment repeated the 1979 experiment. Samples were taken on 28 August 1980.

MICROCOSM EXPERIMENT – 1980

A microcosm experiment was undertaken at the Florida State University Marine Laboratory, Turkey Point, Florida to investigate the effects of repeated processing of sediments by several sand dollars. In the summer of 1980, six glass tanks 21.5 × 42 × 26 cm (w × l × h) were filled with 16 cm of sand sediments ($\phi = 2.5$, mean grain size 0.177 mm), collected from Bay Mouth Bar. The sands were sieved through 0.5-mm mesh screens to remove the macrofauna and allowed to equilibrate with unfiltered sea water at a flow rate of 1000 ml/min (11 vol/h) for 2 wk. The sea water used for these experiments averaged 26.5 ‰ salinity, pH 7.35, 28.5 °C, and 3.5 ppm dissolved oxygen. A light intensity of 875 lux was maintained on a 14/10 day/night cycle.

Four sand dollars averaging 10 cm in diameter were added to each of three tanks. Duplicate cores were recovered after 2 wk from each of the six tanks.

SAMPLE HANDLING

Samples for lipid analysis were frozen with dry ice in the field. Samples for Na-acetate-1-¹⁴C and H₂³⁵SO₄ incorporation into cellular lipids were extruded into screw cap centrifuge tubes (50 ml), inoculated in the field, and then transported to the marine laboratory where the incubations were terminated after 4 h. Meiofaunal samples were placed on ice and transported to the marine laboratory for further processing.

Samples extracted for lipid analysis averaged ≈ 50 g wet wt. Samples were obtained by extruding 4.8 cm diameter cores while frozen and removing the top 2 cm.

DEPTH OF THE REDOX DISCONTINUITY

After extruding the frozen cores and before separating the top 2 cm the depth of the redox discontinuity was estimated by observing the depth at which the sediment color changed from light tan to dull gray.

LIPID EXTRACTION

The analytical scheme illustrated in Fig. 1 was utilized. Sediment samples were extracted with a modified one phase chloroform-methanol-water extraction system

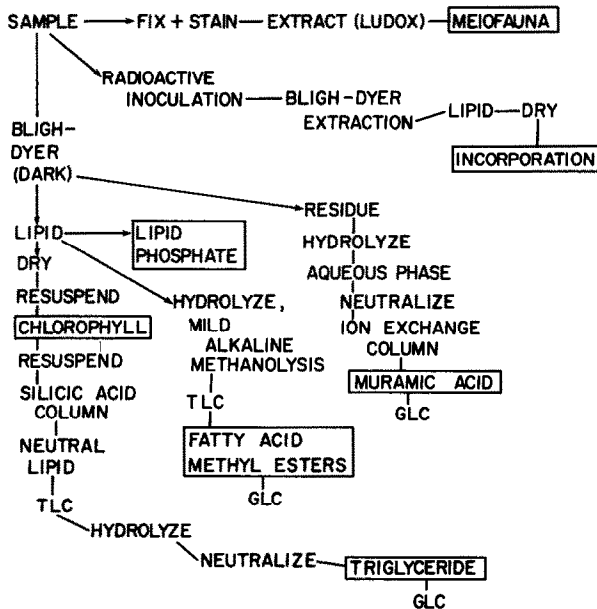


Fig. 1. Diagrammatic representation of analysis scheme.

(1 : 2 : 0.8, vol/vol/vol) (White *et al.*, 1979) in the dark. The monophasic solution was partitioned into an organic and an aqueous phase by the addition of equal volumes of chloroform and water for a final solvent ratio of 1 : 1 : 0.9 (vol/vol/vol). In the dark, the organic phase, which contained the lipid, was recovered and filtered (Whatman 2 V) to remove water. Two aliquots of lipid were removed. One was analyzed for lipid phosphate, and one was kept in the dark for chlorophyll analysis. The remaining chloroform fraction was dried under nitrogen.

FATTY ACID METHYL ESTERS

The lipid was hydrolyzed in 1 N HCl for 3 h at 100 °C and partitioned against water and chloroform. The lipid phase was dried in a stream of nitrogen and subjected to mild acid methanolysis (methanol : concentrated HCl : chloroform, 10 : 1 : 1, vol/vol/vol) for 1 h at 100 °C, and then partitioned with water and chloroform (Bobbie & White, 1980). The chloroform phase containing the lipids was dried under nitrogen. After redissolving the lipid in chloroform, the lipids were applied to a 250- μ m thick thin-layer silica gel plate (Whatman K6, 40 Å) and fractionated with a solvent of petroleum ether, ethyl

ether and acetic acid (80 : 20 : 1, vol/vol/vol). The band containing the fatty acid methyl esters (R_f 0.56–0.65) was collected, eluted with chloroform and dried under nitrogen.

After redissolving in hexane, the fatty acid methyl esters were separated and identified by gas liquid chromatography using mass spectral analysis where necessary. Fatty acids were designated by number of carbons : numbers of double bonds, ω (omega) position of the double bond closest to the methyl end. Prefixes "i" and "a" refer to iso and anteiso branching.

GAS CHROMATOGRAPHY

The fatty acid methyl esters, to which a methyl nonadecanoate internal standard had been added, were separated on a 50-m glass open tubular capillary column coated with Silar 10C (Applied Science Laboratories, State College, Pa.) using the Varian 3700 gas chromatograph fitted with an autosampler under the conditions described by Bobbie & White (1980).

MASS SPECTROMETRY

Gas chromatography–mass spectrometry was performed using a Hewlett Packard 5995 instrument with an identical capillary column under similar conditions as described by Bobbie & White (1980).

CHLOROPHYLL *a*

A separate aliquot of the lipid fraction, carefully protected from light, was dried in a stream of nitrogen and redissolved in acetone : water (9 : 1, vol/vol) saturated with magnesium carbonate. Absorbance at 665 nm and 750 nm was determined with a Gilford 2400-S recording spectrophotometer in a 1-cm path length cuvette. The sample was acidified with two drops of 6 N HCl and the absorbance at 665 nm and 750 nm was again determined. Chlorophyll *a* and phaeopigment concentrations were then calculated using formulas provided in Strickland & Parsons (1972).

GLYCEROL

The aliquot of lipid previously utilized for chlorophyll *a* analysis was then placed on a silicic acid column and the neutral lipid eluted with chloroform. The neutral lipid was applied to a 250- μ m thick thin-layer silica gel plate (Whatman K6, 40 Å) and fractionated with a solvent of petroleum ether, ethyl ether, and acetic acid (80 : 20 : 1, vol/vol/vol). The band containing the triglycerides was collected, eluted with chloroform and dried under nitrogen.

The lipid was then hydrolyzed in 0.2 N methanolic potassium hydroxide at 40 °C for 15 min. The glycerol produced was partitioned against chloroform : water (1 : 1, vol/vol) and the aqueous phase was then neutralized with 10% acetic acid (vol/vol) and dried under reduced pressure. The glycerol was peracetylated and analyzed by gas-liquid chromatography (Gehron & White, 1982).

LIPID PHOSPHATE

Lipid samples were digested in 23% (vol/vol) perchloric acid for 2 h at 200 °C and the phosphate was determined colorimetrically (White *et al.*, 1979).

MURAMIC ACID

The residue remaining after lipid extraction (Fig. 1) was refluxed in 6 N hydrochloric acid 4.5 h. The bound lipids were extracted by partitioning in chloroform : water (1 : 1, vol/vol). The remaining aqueous phase was neutralized to pH 6.5 with 0.02 N NaOH, centrifuged and the resulting supernatant was eluted through a Dowex 50 (H⁺) cation ion exchange minicolumn (Prefilled Econo-Columns, 100–200 mesh, Bio-Rad Laboratories, Richmond, Calif.) and the muramic acid was analyzed as a peracetylated aldonitrile by gas liquid chromatography (Findlay *et al.*, 1983).

MEIOFAUNA

Meiofauna composition was determined by direct count after extraction with Ludox (De Jonge & Bouwman, 1977). Samples were obtained by extruding 2.4 cm diameter cores and recovering the top 2 cm of sediment. All samples were preserved with 20% ethylalcohol (vol/vol) and stained with Rose Bengal.

RADIOACTIVE INCORPORATION

Rates of incorporation of the isotopes H₂³⁵SO₄ and Na acetate-1-¹⁴C into lipids were measured in separate experiments using 10–20 g wet wt of sediment shaken in 25 ml of filter sterilized sea water with each flask containing 5 μCi of label. Lipid synthetic rates were measured as described by White *et al.* (1977).

STATISTICS

The 1979 and 1980 field experiments were analyzed using a matched pairs *T* statistic (Brown & Hollander, 1977). The outcomes of the two experiments were independent events allowing the significance probabilities to be multiplied. This combined value was then corrected for multiple comparison error using the Bonferroni *t* statistic (Miller, 1966). An overall family error rate of $\alpha = 0.1$ was maintained. This necessitated a statement error rate of $\alpha = 0.005$ or less before an individual parameter was considered significant.

The microcosm experiment was analyzed by analysis of variance utilizing a groups-within-treatments hierarchical design. The data were tested for both treatment and group effects. If the groups (tanks) showed no significant effects at the $\alpha = 0.25$ level the group sum of squares and the error sum of squares were pooled and the data analyzed as a completely randomized one-factor design (Myers, 1979). As with the field experiment, the Bonferroni *t* statistic was utilized to control the family error rate. The

family error rate was a posteriori raised to $\alpha = 0.1$ allowing parameters with a statement error rate of $\alpha = 0.005$ or less to be considered significant.

The field control samples and the microcosm control samples were compared by first randomly choosing one sample from each microcosm and then applying the Wilcoxon two-sample rank sum test (Brown & Hollander, 1977). Due to the low power of the test (maximum $\alpha = 0.02$) and the a posteriori nature of the investigation no correction for family error was made.

RESULTS

EFFECTS OF SAND DOLLAR FEEDING

Sediments

The average depth of the redox discontinuity of Bay Mouth Bar sediments was estimated to be 0.2 cm. A single passage of a sand dollar through these sediments increased this depth to an average of 0.8 cm (Table I). In the microcosm sediments, repeated processing by sand dollars maintained the redox discontinuity at an average depth of 1.77 cm. Control sediments showed no signs of an aerobic zone and H_2S could be detected in the air near the tank.

Microbial biomass

The total microbial biomass can be estimated by measuring total lipid phosphate and total palmitate (Bobbie & White, 1980). Neither measure was significantly affected by processing during the passage of a single *M. quinquesperforata* through Bay Mouth Bar sediments (Tables I and II). In contrast, microcosm sediments where sand dollars had repeatedly fed showed a 45% reduction in community biomass as measured by lipid phosphate when compared to control microcosms (no sand dollars). Lipid palmitate decreased by 54%, although this effect cannot be attributed solely to the presence of sand dollars as there were large differences between control microcosms.

Muramic acid is a specific indicator of procaryotic biomass (Findlay *et al.*, 1983). The fatty acids i 15:0, a 15:0, 15:0, 17:0, D17:0, and 18:1 ω 7 can indicate specific components of the procaryotic community (Bobbie & White, 1980). The passage of a single *M. quinquesperforata* through field sediments tended to decrease the amounts of all these compounds; however, these decreases were not statistically significant (Tables I and II). Microcosm sediments repeatedly processed by feeding sand dollars showed significantly smaller amounts of the bacterial fatty acids i 15:0, a 15:0, 15:0, 17:0, and D17:0. The decreases in the levels of muramic acid and the fatty acid 18:1 ω 7, while substantial, were not significant (Tables I and II).

Eucaryotic biomass can be estimated by measuring triglyceride glycerol and total polyenoic fatty acids. Specific groups within the eucaryotic community can be assayed by measuring the fatty acids: 20:3 ω 6, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3, and 22:6 ω 3 (Bobbie

TABLE I
Effects of sediment processing by *Mellita quinqueperforata* on measures of biomass, activity and bioturbation in field and microcosm experiments.

Test ^a	Field control		Average differences ^b Field experiments		Microcosm control	Microcosm treatment ^c	Significance ^d
	1980	1979	1979	1980			
Lipid phosphate	0.026 (0.007)	-0.002 (0.004)	-0.003 (0.003)	0.037 (0.007)	0.022 (0.002)	ns/**/*	
Lipid glycerol	0.003 (0.001)	-0.004 (0.000)	-0.001 (0.000)	0.008 (0.001)	0.004 (0.001)	***/**/*	
Chlorophyll <i>a</i>	0.144 (0.183)	0.003 (0.071)	-0.072 (0.074)	0.086 (0.059)	0.056 (0.042)	ns/ns/ns	
Muramic acid	0.022 (0.018)	-0.011 (0.010)	-0.010 (0.009)	0.012 (0.007)	0.004 (0.001)	ns/ns/ns	
[¹⁴ C]acetate	3695 (639)	83 (61)	34 (106)	5048 (1793)	2966 (759)	ns/ns/ns	
[³⁵ S]sulfate	2089 (889)	-352 (52)	-111 (455)	1866 (539)	569 (238)	ns/**/*	
Depth of redox discontinuity	0.230 (0.100)	0.75 (0.22)	0.605 (0.12)	0.000 (0.000)	1.77 (0.460)	***/**/*	

^a Lipid phosphate, lipid glycerol, chlorophyll *a*, and muramic acid are expressed as $\mu\text{mol/g dry wt}$. Rates of incorporation are given as DPM/g dry wt. Depth of the redox discontinuity is in cm. All data expressed as mean (\pm SD), $n = 6$, except for the 1979 field experiment where $n = 3$.

^b Samples taken from Bay Mouth Bar, Florida. Treatment consists of a single passage of a sand dollar. Differences were calculated by subtracting the control core of a matched pair from the corresponding treatment core.

^c Microcosms containing sediment taken from Bay Mouth Bar. Treatment consists of repeated sediment processing for 2 wk by four sand dollars.

^d First column indicates the combined significance of the field experiments, the second column indicates significance of the comparison of microcosm controls and microcosm treatments and the third column indicates significance of comparison between field controls and laboratory controls. ns, not significant; * $P = 0.05$; ** $P = 0.005$; *** $P < 0.001$.

TABLE II
Effects of sediment processing by *Mellita quinquesperforata* on fatty acid composition of sediments in field and microcosm experiments.

Component ^a	Field control		Average differences ^b Field experiments		Microcosm control	Microcosm treatment ^c	Significance ^d
	1980	1980	1979	1980			
i 15:0	1.29 (0.51)	-0.21 (0.17)	-0.03 (0.06)	-0.21 (0.17)	3.35 (0.66)	2.13 (0.51)	ns/**/*
a 15:0	2.28 (0.58)	-0.34 (0.22)	-0.03 (0.08)	-0.34 (0.22)	2.98 (0.58)	2.28 (0.55)	ns/**/*
15:0	15.47 (7.57)	-4.61 (2.86)	-2.58 (1.38)	-4.61 (2.86)	26.04 (4.42)	9.25 (5.80)	ns/**/*
16:0	38.44 (18.2)	-13.80 (12.1)	-1.60 (2.38)	-13.80 (12.1)	46.64 (7.50)	21.70 (8.80)	ns/ns/ns
17:0	2.84 (1.03)	-1.11 (0.49)	-0.21 (0.13)	-1.11 (0.49)	5.60 (0.89)	2.69 (1.10)	ns/**/*
D17:0	0.21 (0.14)	-0.08 (0.07)	-0.01 (0.03)	-0.08 (0.07)	0.63 (0.15)	0.33 (0.11)	ns/**/*
18:1 ω9	2.47 (0.99)	-0.74 (0.45)	0.06 (0.34)	-0.74 (0.45)	7.06 (1.18)	3.57 (1.57)	ns/ns/*
18:1 ω7	4.45 (1.64)	-1.14 (0.72)	-0.55 (0.38)	-1.14 (0.72)	9.46 (2.35)	4.74 (0.88)	ns/ns/*
20:3 ω6	0.11 (0.04)	-0.04 (0.01)	-0.07 (0.02)	-0.04 (0.01)	0.23 (0.05)	0.13 (0.01)	***/**/*
20:4 ω6	0.58 (0.17)	-0.19 (0.11)	-0.52 (0.19)	-0.19 (0.11)	2.03 (0.80)	1.02 (0.32)	ns/ns/*
20:5 ω3	2.26 (1.08)	-0.56 (0.48)	-0.60 (0.92)	-0.56 (0.48)	2.26 (0.58)	0.94 (0.47)	ns/**/*
22:5 ω3	0.22 (0.17)	-0.13 (0.11)	-0.01 (0.05)	-0.13 (0.11)	nd	nd	ns/ns/*
22:6 ω3	0.20 (0.12)	-0.08 (0.08)	0.01 (0.05)	-0.08 (0.08)	nd	nd	ns/ns/*
Total polyenics	3.37 (1.51)	-1.18 (2.66)	-1.18 (2.66)	-1.18 (2.66)	4.62 (1.40)	2.06 (0.83)	ns/**/ns

^a All fatty acids expressed as nmol/g dry wt. Fatty acids designated as the number of carbon atoms, the number of double bonds, and the position of the unsaturation nearest the ω end of the molecule. The prefixes i, a, and D indicate iso-, anteiso-branching, and the presence of a cyclopropane ring in the chain. All data expressed as mean (± SD), $n = 6$, except for the 1979 field experiment where $n = 3$.

^b Samples taken from Bay Mouth Bar, Florida. Treatment consists of a single passage of a sand dollar. Differences were calculated by subtracting the control core of a matched pair from the corresponding treatment core.

^c Microcosms containing sediment taken from Bay Mouth Bar. Treatment consists of repeated sediment processing for 2 wk by four sand dollars.

^d First column indicates the combined significance of the field experiments, the second column indicates significance of the comparison of microcosm controls and microcosm treatments and the third column indicates significance of comparison between field controls and laboratory controls. ns, not significant; * $P = 0.05$; ** $P = 0.005$; *** $P < 0.001$.

& White, 1980). Bay Mouth Bar sediments processed by a single *M. quinquesperforata* showed significant reductions in triglyceride glycerol (31%) and the fatty acid 20:3 ω 6 (36%). Triglyceride glycerol and the eucaryotic fatty acids 20:3 ω 6 and 20:5 ω 3 significantly decreased in the treatment microcosms. The eucaryotic fatty acids 22:5 ω 3 and 22:6 ω 3 were not detected in any of the microcosms (Table I and II).

Photosynthetic biomass, as measured by chlorophyll *a*, was not significantly affected by *M. quinquesperforata* feeding in either the field or microcosm experiments (Table I).

Meiofauna

The number of vital staining foraminifera declined significantly (50%) with the passage of a single sand dollar. Although the total nematode and harpacticoid levels varied, these findings were not consistent enough to be significant. Microcosm sediments repeatedly processed by *M. quinquesperforata* showed 80% fewer vital staining foraminifera than control sediments. Numbers of nematodes and harpacticoids were not significantly affected (Table III).

Metabolic activities

Effects of sand dollar feeding on metabolic activities were determined by measuring the rate of incorporation of [¹⁴C]acetate into lipid, and [³⁵S]sulfate incorporation into sulfolipid. [¹⁴C]acetate incorporation into lipid has been shown to correlate with microbial mass and activity (White *et al.*, 1979) and [³⁵S]sulfate incorporation into sulfolipid has been associated with eucaryotic mass and activity (White *et al.*, 1980). Predation and the resulting bioturbation by a *M. quinquesperforata* in field sediment did not significantly affect these measures. In the microcosms, the rates of [¹⁴C]acetate incorporation into lipid were similar; however, the rate of [³⁵S]sulfate into sulfolipid was significantly lowered in the presence of sand dollars (Table I).

FIELD CONTROLS VS. MICROCOSM CONTROLS

Sediments

The microcosm control sediments, as previously stated, showed no signs of an aerobic zone. In contrast, an aerobic zone was always present in sediments from Bay Mouth Bar and averaged 0.2 cm in depth (Table I).

Biomass

Of the 28 biomass measures, 16 were significantly different, with 13 of these parameters being greater in the microcosm control tanks. The three measures that were significantly lower in the microcosm controls were the eucaryotic fatty acids 18:3 ω 3, 22:5 ω 3 and 22:6 ω 3. The last two decreased below the level of detectability (Tables I and II).

TABLE III
Effects of sediment processing by *Mellita quinquesperforata* on the number of vital staining meiofauna.

Meiofauna ^a	Field control	Average differences ^b Field experiments			Microcosm control	Microcosm treatment ^c	Significance ^d
		1979	1980	1980			
Nematode spp.	475 (148)	-80 (72)	-198 (98)	382 (263)	284 (156)	ns/ns/ns	
Harpacticoid spp.	52 (11)	-1 (3)	49 (25)	46 (36)	28 (22)	ns/ns/ns	
Foraminifera spp.	91 (24)	-9 (2)	-50 (9)	50 (20)	10 (4)	***/**/ns	

^a Data given as individuals per core. All data expressed as mean (\pm SD), $n = 6$, except for the 1979 field experiment where $n = 3$.

^b Samples taken from Bay Mouth Bar, Florida. Treatment consists of a single passage of a sand dollar. Differences were calculated by subtracting the control core of a matched pair from the corresponding treatment core.

^c Microcosms containing sediment taken from Bay Mouth Bar. Treatment consists of repeated sediment processing for 2 wk by four sand dollars.

^d First column indicates significance of the comparison of field controls and field treatments, the second column indicates significance of the comparison of microcosm controls and microcosm treatments and the third column indicates significance of comparison between field controls and laboratory controls. ns, not significant; * $P = 0.05$; ** $P = 0.005$; *** $P < 0.001$.

Metabolic activity

Neither the rate of [^{14}C]acetate incorporation into lipid nor the rate of [^{35}S]sulfate into sulfolipid showed any significant difference between the laboratory and the field studies (Table I).

Meiofauna

The total abundances of nematodes, harpacticoid copepods, and foraminifera showed no significant difference between field and microcosm sediments (Table III).

Community structure

The community structure of the microbial population was examined by forming the ratios of several of the biochemical measures of biomass (Bobbie & White, 1980). The ratios of $i + a$ 15:0/16:0, total polyenoics/16:0, and lipid phosphate/16:0, measures of the relative importance of procaryotic and eucaryotic organisms within a community,

TABLE IV
Comparison of community structure of field controls and microcosm controls.

Fatty acid ratio	Field controls (SD)	Microcosm controls (SD)	Significance ^a
$i + a$ 15:0/16:0	0.11 (0.04)	0.13 (0.04)	ns
D17:0/16:0	0.006 (0.0002)	0.016 (0.0020)	*
18:1 ω 7/18:1 ω 9	1.81 (0.12)	1.27 (0.33)	*
18:3 ω 6/18:3 ω 3	0.29 (0.11)	5.65 (2.25)	*
Total poly/16:0	0.094 (0.027)	0.107 (0.020)	ns
Total poly ω 6/total poly ω 3	0.28 (0.08)	0.98 (0.21)	*
Lipid phosphate/16:0	0.79 (0.34)	0.82 (0.16)	ns

^a ns, not significant; * $P = 0.05$.

showed little change. The greatest differences were in the ratios 18:3 ω 6/18:3 ω 3 and the total polyenoic fatty acids ω 6/total polyenoic fatty acids ω 3, measures of the relative importance of the two pathways of synthesis for polyenoic fatty acids (Table IV).

DISCUSSION

FOOD OF *M. QUINQUIESPERFORATA*

Analysis of gut contents has shown that *M. quinquiesperforata* ingests diatoms, foraminifera, dinoflagellates, and amorphous organic detritus, as well as the $\phi = 3.5$ and smaller fraction of the sediments (Lane, 1977). Other microeucaryotes present in the sediments may also serve as food (Hummon, 1976; Buzas, 1978). In the field and the microcosm experiments reported here, physical counts of the meiofauna have shown

that *M. quinquesperforata* decreases the number of vital staining foraminifera but not nematodes or harpacticoids. Lipid glycerol and the fatty acid 20:3 ω 6, biochemical measures of eucaryotic organisms, decreased in both experiments. In addition, the fatty acid 20:5 ω 3 and the rate of sulfate incorporation into sulfolipids decreased in the microcosm experiment. Chlorophyll *a* was not affected by sand dollar feeding, supporting Lane's (1977) finding that diatoms pass through the digestive track intact, apparently unharmed. The physical and biochemical evidence, therefore, indicates that *M. quinquesperforata* preys upon non-photosynthetic microeucaryotes.

In the microcosm sediments, the fatty acids 15:0, 15:0, 15:0, 17:0, and D17:0 decreased significantly with sand dollar feeding, indicating that *M. quinquesperforata* ingested bacteria. Processing of Bay Mouth Bar sediment by a sand dollar decreased the measures of procaryotic biomass by $\approx 25\%$; however, these changes were not significant because the variance in microbial biomass of similar sediments can be quite large (Federle & White, 1982). Calculations using the formula $N = (z_{\alpha/2})^2 \times \delta^2 / D^2$ (Brown & Hollander, 1977) where N = the number of matched pairs, $z_{\alpha/2}$ = the upper $\alpha/2$ percentile point of a standard normal distribution, δ = the population standard deviation and D is the average difference between matched pairs indicated an average of 30 samples per experiment would have been necessary to detect changes in microbial biomass as significant at the $\alpha = 0.05$ level.

A preliminary microcosm study of *M. quinquesperforata* feeding utilizing filtered estuarine water showed preferential removal of non-photosynthetic microeucaryotes (White *et al.*, 1980). The use of unfiltered estuarine water, improved sampling technique and a more powerful statistical design in the present microcosm study showed that *M. quinquesperforata* removed bacteria as well as non-photosynthetic microeucaryotes from the sediments and presumably utilized them as a food source.

THE EFFECTS OF SAND DOLLAR BIOTURBATION

Field observations showed that *M. quinquesperforata* moves at ≈ 10 cm/h through Bay Mouth Bar sediments; this rate agrees with those published in the literature (15 cm/h to 1 cm/h; Bell & Frey, 1969; Ghiold, 1979). During feeding, sediment and water are moved downward through the lunules. This movement of the sediments and water effectively increases the depth of the redox discontinuity. Bioturbation has been shown to increase the depth of the redox discontinuity of several other marine sediments (Rhoads, 1974).

The effects of sand dollar feeding and burrowing on microbial metabolic activities were not as obvious. Although incorporation of sulfate into sulfolipid decreased, the decrease in eucaryotic biomass is sufficient to account for this result. No change was detected in the rate of [^{14}C]acetate incorporation into lipid. The rate of incorporation of [^{14}C]acetate into lipid has successfully detected changes in microbial activity due to grazing of surfaces (Morrison & White, 1980; Smith *et al.*, 1982) and has been correlated to oxygen utilization (White *et al.*, 1977), a measure that has been found to

increase with grazing pressure (Fenchel, 1970, 1977; Hargrave, 1976). Various reports in the literature (Fenchel & Jorgensen, 1977), suggest that most measures of microbial activity in sediments measure a "heterotrophic potential" rather than reflecting the "in situ" rate of heterotrophic metabolism at the time of sampling. Subsequent studies of the rate of incorporation of [^{14}C]acetate into lipid have shown that shaking the sediment to distribute the label masks the effects of small scale disturbances.

MICROCOSMS AS MODELS FOR NATURAL PROCESSES

Although field control samples and microcosm control samples showed a similar procaryotic-eucaryotic balance, several major differences were evident: the lack of an aerobic zone in the microcosm sediments, a general increase in the biomass in the microcosm samples, and an altered eucaryotic community structure within the microcosm microbiota.

Macrofauna were removed from the sediments used in the microcosms. The resulting decrease in bioturbation, coupled with a lack of physical disturbance, is responsible for the lack of an oxygenated zone in the sediments. This general lack of disturbance also allowed the settling of a layer of silts and clays over the surface of the sediments of the microcosms. In the field any silts and clays that settled to the surface of the sediments are apparently either mixed into the sediments by bioturbation, as was the case in the treatment microcosm, or resuspended by numerous physical disturbances.

This lack of macrofauna could also be responsible for the general increase in biomass. Procaryotic and eucaryotic biomass were higher in the microcosms as evidenced by the increases in lipid phosphate, lipid glycerol, the fatty acids $i + a$ 15:0, and the total polyenoic fatty acids. The greater relative proportion of the fatty acid D17:0, shown to increase in aging bacteria (Knivett & Cullen, 1965; Lechevalier, 1977) indicates that the bacterial population of the microcosm sediments was not turning over as quickly in the field sediments.

Although the microcosm sediments differed from the field sediments in the depth of the redox discontinuity and the number of microorganisms, the general proportion of procaryotic and eucaryotic organisms within the sedimentary biota remained the same. The ratio of $i + a$ 15:0/16:0, a measure of the procaryotic contribution to the community, the ratio of the total polyenoic fatty acids/16:0, a measure of the eucaryotic contribution to the community, and the ratio of lipid phosphate/16:0, a measure sensitive to changes in the procaryotic-eucaryotic balance, all remained unchanged. The major change in community structure occurred within the eucaryotic portion of the community. The ratio of 18:3 ω 6/18:3 ω 3 and of the polyenoic fatty acids ω 6/polyenoic fatty acids ω 3 indicated a shift to the ω 6 series of fatty acids. The phylogenetic distribution of the polyenoic fatty acids is very complex, though several general patterns in the distribution are known. The ω 3 polyenoic fatty acids, with the exception of 22:6 ω 3, are an integral part of photosynthetic membranes of photosynthetic eucaryotic organisms (Erwin, 1973). The ω 6 polyenoic fatty acids, especially those 20 carbons in

length, are common in protozoans and metazoans (Erwin, 1973). Heterotrophic eucaryotes appear to have increased relative to photosynthetic eucaryotes.

The differences between the microbial community present in the microcosm sediments and the microbial community of Bay Mouth Bar sediments have not seriously affected the conclusions of the experiments conducted. The existence of these differences indicates that care should be taken when studying natural processes in microcosms and a thorough assessment of laboratory effects and their influence on the processes under study should be undertaken at the start of any experiment involving microcosms.

In summary the biochemical and physical data show that the sand dollar *M. quinquesperforata* ingests foraminifera, possibly other non-photosynthetic microeucaryotes, and the silt and clay fraction of the sediments, utilizing the attached bacteria. These findings indicate that *M. quinquesperforata* is a selective detritivore utilizing two separate resources present in the sediments. The bioturbation resulting from sand dollar feeding and burrowing increased the depth of the redox discontinuity, but failed to affect the rates of microbial activity as measured in the study. Microcosms proved a useful tool in understanding sand dollar feeding, though analysis of the differences between field and microcosm sediments indicated that careful evaluation of the changes induced by maintaining sediment in the laboratory is needed before a microcosm approach can be applied to an ecological problem.

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