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Impact of bacteria and zooflagellates on the composition of sinking particles: an *in situ* experiment

Gordon T. Taylor[1], David M. Karl[1, 2] & Michael L. Pace[1, 2,*]

[1] Hawaii Institute of Geophysics and [2] Department of Oceanography, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA

ABSTRACT: Multiple sediment trap (MULTITRAP) arrays were deployed at 3 hydrographically and biologically distinct stations in the eastern North Pacific Ocean and used to evaluate experimentally the hypothesis that protozoan grazers are important in the diagenesis of sinking biogenic materials in the ocean. Measurements were made of adenosine triphosphate (ATP) concentrations and the abundances of bacteria and zooflagellates associated with sinking particles collected from *in situ* preserved and 2 sets of unpreserved (live) traps: one containing a selective eukaryote inhibitor (thiram) and the second without thiram which served as a live-control. Particulate organic carbon and nitrogen (POC and PON), NH_4^+, NO_3^-, NO_2^-, dissolved organic nitrogen (DON), diatoms, and fecal pellets were quantified in live-control and live-thiram traps. *In situ* microbial production was estimated by measuring RNA and DNA synthesis in live-control and live-thiram traps pre-charged with ^{3}H-adenine. Trap-collected bacteria and zooflagellates accounted for a low percentage of the total POC (2 to 11 %) but a high percentage of the total microbial biomass (an estimated 25 to $\geq$ 100 % of total ATP). Zooflagellates were the principal protozoans collected and accounted for 2 to 25 % of the total microbial biomass. Synthesis of RNA and DNA was observed at all stations and depths, indicating microbial growth in the live traps. ATP and microscopic analyses, however, independently confirmed that death rates exceeded growth rates on the sedimenting particles. Differences between the live-control traps (coupled microbial assemblage) and live-thiram traps (uncoupled assemblage, grazers inhibited) indicated slower decomposition and nutrient regeneration in the uncoupled system. In general, POC was higher and in the shallowest traps, NH_4^+ was lower, and DON was higher in the live-thiram traps. Zooflagellates appear to strongly influence decomposition and nutrient recycling within the microbial assemblage associated with sedimenting particles.

INTRODUCTION

The sinking of particulate organic matter (POM) is an important pathway for energy and material flux out of the epipelagic zone (Riley 1970, McCave 1975, Eppley et al. 1983, Angel 1984). The widespread use of particle interceptor traps (PITs or sediment traps) has greatly improved our knowledge of flux rates as well as the biological and chemical composition of sedimenting materials (reviewed in Angel 1984). In the uppermost portion of the ocean (50 to 1000 m), POM fluxes typically diminish with depth, indicating losses due to the combined effects of particle fragmentation, solubilization, hydrolysis, microbially mediated remineralization, and ingestion by mesopelagic zone biota (Karl & Knauer 1984). The precise mechanisms regulating the rates of POM flux are, however, poorly understood.

The large, rapidly sinking organic particles consisting mostly of fecal material, zooplankton carcasses and molts, phytoplankton, and testate protozoans, account for the bulk of the vertical flux (Angel 1984) and represent important environments for microbial activity (Iturriaga 1979, Fellows et al. 1981, Ducklow et al. 1982, 1985, Karl et al. 1984). Large amorphous aggregates (marine snow) have concentrations of bacteria and protozoans higher than surrounding seawater (Silver et al. 1978, Pomeroy & Deibel 1980, Silver &

* Present address: Institute of Ecosystems Studies, Mary Flagler Cary Arboretum, Box AB, Millbrook, New York 12545, USA

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Alldredge 1981, Caron et al. 1982). Freshly egested fecal pellets also have high concentrations of bacteria apparently derived from the intestinal tracts of the host organisms or from incompletely digested food (Gowing & Silver 1983, Andrews et al. 1984, Pomeroy et al. 1984). In these microenvironments bacteria are presumed to be decomposers of the POM and the protozoans, primarily zooflagellates and ciliates, represent the primary consumers of bacteria (reviewed in Lee 1980, Taylor 1982). Previous microcosm studies with terrestrial assemblages (Cole et al. 1978, Coleman et al. 1978, Elliot et al. 1979) and coastal aquatic assemblages (Johannes 1965, Fenchel & Harrison 1976, Fenchel 1977) have demonstrated that phagotrophic protozoans accelerate decomposition and enhance rates of inorganic nutrient regeneration. We hypothesized that protozoans associated with sinking particles in the open ocean would have a similar effect. We tested this hypothesis by conducting an *in situ* sediment trap experiment during the VERTEX 5 cruise. At each sampling depth, we assessed: (1) the biomass of microorganisms associated with sinking particles (bacteria and protozoans), (2) *in situ* growth of the attendant microbiota, and (3) rates of POM solubilization and dissolved nutrient regeneration in an unpreserved sediment trap ('live-control trap') and in a replicate trap precharged with the eukaryote inhibitor thiram ('live-thiram trap'). We predicted that the live-thiram traps would have reduced protozoan activity and, therefore, lower rates of particulate organic carbon (POC) and particulate organic nitrogen (PON) mineralization, lower rates of microbial growth, and lower rates of nutrient regeneration relative to the live-control traps. Observations on the *in situ* vertical flux of bacteria, protozoans, algae, fecal pellets, and adenosine triphosphate (ATP) aided in the interpretation of our experiment.

MATERIALS AND METHODS

Sampling locations and experimental design. Free-floating multiple sediment trap (MULTITRAP) systems (Knauer et al. 1979) were deployed during the period 4 to 7 June 1984 from the R/V *Wecoma* at 3 stations in the north Pacific Ocean (Fig. 1). Each MULTITRAP array consisted of 12 individual sediment traps mounted on PVC crosses at each of the 7 sampling depths (Fig. 1). Prior to the cruise, the traps were cleaned with a laboratory detergent and 10% HCl, rinsed with sterile deionized water, and sealed. At sea shortly before deployment, each trap was filled with a specific high-density solution (Table 1), which minimized flushing during recovery and diffusive losses of soluble constituents during the collection period. The 2 live treatments and the *in situ* ATP extraction traps were deployed with a 333 μm Nitex mesh positioned at the base of the baffle (Fig. 1) to exclude large, swimming zooplankters. The 3 MULTITRAP arrays were deployed concurrently and their locations were monitored by Argos satellite telemetry for 13 d (Sta. A) and 21.75 d (Sta. B and C). Upon recovery, each trap was subsampled and processed for the analysis of a variety of parameters (Table 1) as described in detail below.

Microscopy. Microbial (bacterial, algal, and protozoan) biomass was determined by microscopic analysis. Subsamples from both the live-control and live-thiram sediment traps were preserved on board with 2% (v/v, final concentration) borate-buffered formaldehyde for subsequent analysis. Additional subsamples were obtained from sediment trap collectors on the same MULTITRAP array which had been precharged with fixative (see Preserved *[in situ]* treatment; Table 1). Bacterial cell numbers and volume estimates were evaluated by: vigorously shaking each sample on a vortex mixer, staining 1 to 15 ml subsamples (concentration-dependent) with acridine orange (0.05% w/v, final concentration), filtering onto irgalan-black stained 0.2 μm Nuclepore membrane filters, and observing at 1000× with a Zeiss Standard epifluorescent microscope equipped with 100 W mercury lamp (Hobbie et al. 1977, Zimmermann 1977). Since there was non-specific fluorescence of many particles, it was possible to count only bacteria that had been dislodged during sample agitation. However, independent estimates of microbial biomass determined by total ATP concentrations and microscopy were comparable, indicating that our counting procedures were not seriously biased by omitting particle-associated bacteria. We observed that preparations from subsamples that had been homogenized either by macerating in a tissue grinder or by vigorous manual shaking did not yield more bacterial cells relative to the vortex-mixed subsamples. Analysis of replicate subsamples did not improve precision over counting a single subsample (mean coefficient of variation: bacteria, 31% ± 11; zooflagellates, 46% ± 22). Bacterial biomass was estimated from volume measurements and a carbon-to-cell volume relation of 22×10^{-14} g C μm^{-3} (Bratbak & Dundas 1984).

Flagellate cell numbers and volume estimates were also determined by direct microscopy. Protozoans in preserved subsamples (1 to 75 ml) from the live-control, live-thiram and *in situ* preserved sediment traps were counted and sized using the protocol described above for bacteria, except that 0.8 μm Nuclepore membranes were used and both particle-associated and unattached cells were counted under 400× magnification. Unlike preparations of our preserved samples stained with DAPI or proflavine, those stained with

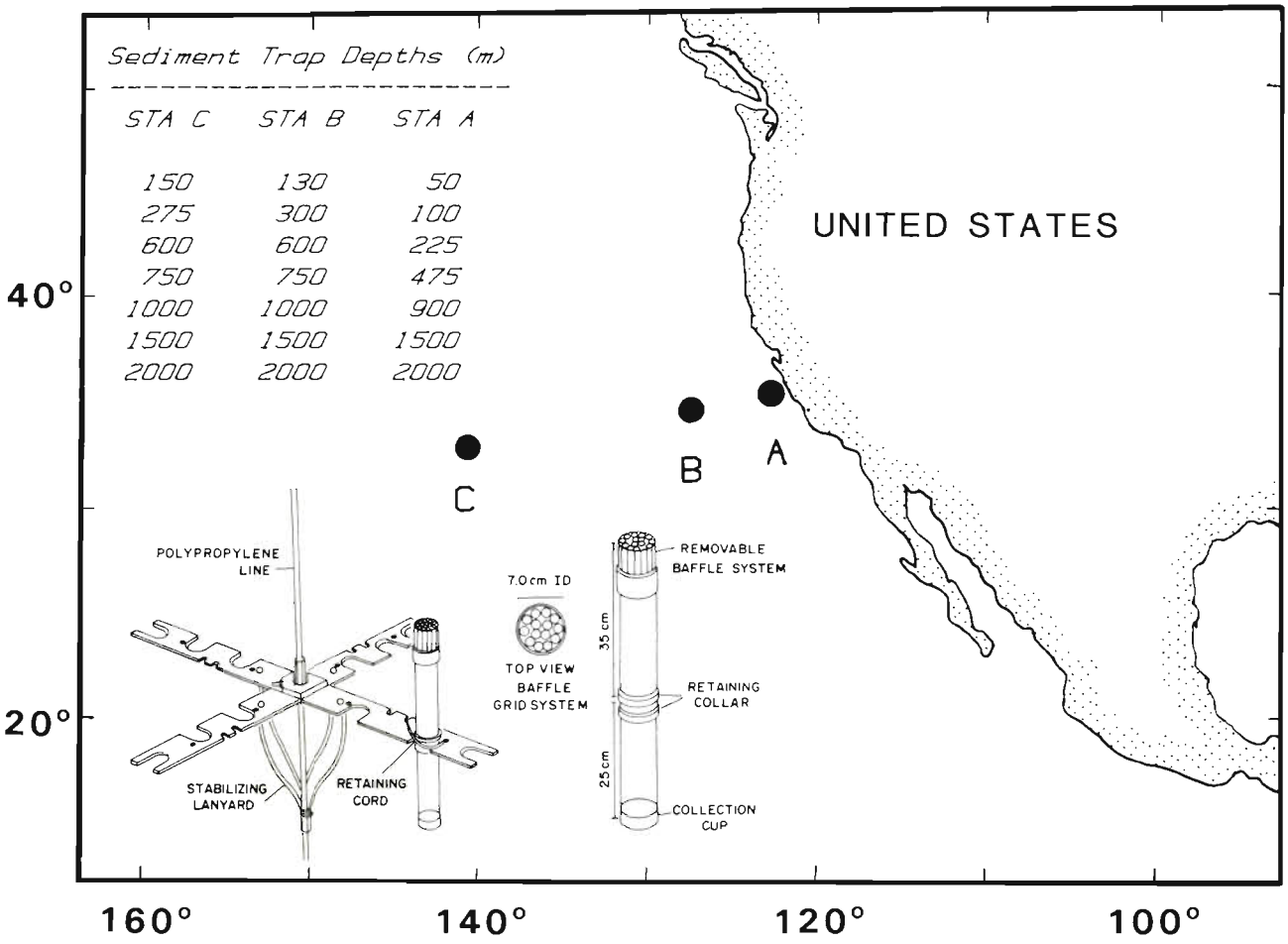

Fig. 1. Map of north Pacific Ocean showing positions of the 3 stations (A: 35°55′ N, 122°34′ W; B: 34°45′ N, 129°54′ W; C: 33°06′ N, 139°34′ W) where our protozoan decomposition experiment was performed, and the sediment trap depths at each station. Lower left: schematic view of one of the MULTITRAP crosses; to its right, expanded view of an individual collector tube (redrawn from Knauer et al. 1979). The 333 µm Nitex screen was positioned at the base of the removable baffle system (see Table 1)

acridine orange consistently yielded high contrast between autofluorescing chloroplasts (orange and red) and cytoplasm (green). Few autofluorescent flagellates (phytoflagellates) were observed in the trap material and only green fluorescing flagellates (zooflagellates) were counted. Although cells were enumerated at 400× magnification and periodically confirmed at 1000×, some small cells were probably overlooked. Reported values are considered to be conservative. Flagellate biomass was estimated by multiplying mean cell volume by 8.8×10^{-14} g C µm^{-3} (Heinbokel 1978).

Diatom frustules and zooplankton fecal pellets were enumerated from the live-control and live-thiram traps using settling chambers (Utermöhl 1931). After vigorous mixing, 10 to 50 ml subsamples were placed in settling chambers, allowed to settle for ≥ 12 h, and diatoms and fecal pellets were enumerated at 100× on a Zeiss inverted microscope. Depending on abundances, either the entire settling area or 10 to 50 fields were counted. Diatom biomass was calculated according to Sicko-Goad et al. (1984) by multiplying mean cell volume by 1.00 g cm^{-3} × 0.25 (dry:wet weight) × 0.10 (carbon:dry ratio). This assumes that all frustules contain live cells and thus represents an upper bound on diatom biomass. Fecal pellet carbon content was estimated by multiplying mean pellet volume by 1.22 g cm^{-3} × 0.11 (dry:wet weight) × 0.20 (carbon:dry ratio) (Urrere & Knauer 1981).

ATP concentrations and biomass flux. Total microbial biomass flux was determined by measuring the ATP concentrations in sediment traps with 333 µm Nitex screens that had been precharged with H_3PO_4 (Table 1). As particle-associated microorganisms entered the acid-salt high-density solution, their cellular ATP was extracted and preserved (Fellows et al. 1981). ATP is stable under these conditions for periods in excess of 3 mo. Upon recovery, the cell extracts were processed for total ATP as described previously (Fellows et al. 1981). We refer to these determinations as

Table 1. Design of *in situ* protozoan decomposition sediment trap experiment

Treatment	Sediment trap density solution	Analyses performed
Preserved[1] *(in situ)*	Glutaraldehyde (2 %) Sucrose (12 %) $SrCl_2 \cdot 6H_2O$ (90 mmoles l^{-1}) Filtered seawater (800 ml l^{-1}) Distilled H_2O (200 ml l^{-1})	Microscopy 1. Bacteria (counts & volume) 2. Protozoa (counts & volume)
Live-control[2]	NaCl (27.1 g l^{-1}) $MgCl_2 \cdot 6H_2O$ (11.4 g l^{-1}) KCl (0.74 g l^{-1}) ^{3}H-adenine (100 µCi l^{-1}) Filtered seawater[3]	Microscopy (see above) [ATP]: shipboard ^{3}H-adenine assimilation, [POC], [PON], [DON], [NO_3^-], [NO_2^-], [NH_4^+], diatom, fecal pellet counts
Live-thiram[2, 4]	As above for live-control treatment plus thiram (1 mg l^{-1})	As above for live-control treatment
Extracted[2] *(in situ)*	H_3PO_4 (0.5 moles l^{-1}) NaCl (88 g l^{-1}) Distilled H_2O	[ATP]: *in situ*

[1] Preserved treatment samples collected by M. W. Silver & M. M. Gowing, Univ. Calif. Santa Cruz
[2] Sediment traps covered with 333 µm Nitex screen located at base of removable baffle system (see Fig. 1)
[3] Oligotrophic ocean seawater was filtered through 0.22 µm Millipore filter; inorganic salts were then added and solutions autoclaved in polycarbonate bottles. Immediately prior to trap deployment, filter-sterilized ^{3}H-adenine and thiram were added, as indicated
[4] Thiram = tetramethylthiuram disulfide, Aldrich Chemical Co., Milwaukee, Wisconsin

the *in situ* extracted ATP concentrations (or simply, *in situ* ATP).

Total microbial ATP concentrations in the live-control and live-thiram sediment traps were also measured following their recovery (incubation endpoint). Duplicate subsamples (50 to 200 ml) were filtered onto Whatman GF/F filters and immediately extracted in boiling phosphate buffer (60 mM, pH 7.4). The extracts were stored frozen for subsequent analysis as described by Karl et al. (1981). In order to distinguish these determinations from *in situ* extracted ATP concentrations, we subsequently refer to them as the endpoint shipboard extracted ATP concentrations (or simply, shipboard ATP).

The *in situ* and shipboard ATP concentrations were used to calculate microbial growth rate constants (µ, expressed in units of d^{-1}) from the formulations presented in Iseki et al. (1980). This calculation assumes: (1) the live traps at each depth collected the same material as the trap precharged with H_3PO_4, (2) growth processes were exponential functions of time, and (3) the input of particles and attendant microbiota was constant throughout the collection-incubation period. Residual standing stocks of living microorganisms (A, expressed as µg ATP $trap^{-1}$; i.e. shipboard ATP) can be expressed as,

$$dA/dt = F - \mu A \quad (1)$$

where F = total incoming ATP (expressed as µg ATP $trap^{-1}$; i.e. *in situ* ATP); t = collection-incubation time (d). Integrating Eq. 1 with time under initial conditions, A = 0 at t = 0, Eq. 1 becomes,

$$A = F/\mu(1 - e^{-\mu t}) \quad (2)$$

To solve for µ, Eq. 2 was rearranged algebraically and the resulting transcendental formula (Eq. 3) was solved iteratively.

$$\mu = F(1 - e^{-\mu t})/A \quad (3)$$

Growth rate constants, µ, are, therefore, presented as positive values for positive net growth and as negative values for negative net growth, i.e. when assemblage death rates exceed *in situ* production rates.

^{3}H-adenine metabolism. Assimilation of ^{3}H-adenine into microbial ATP, RNA, and DNA and the corresponding production of ^3H_2O were measured in the live-control and live-thiram sediment traps. The sediment traps were precharged with ^{3}H-adenine and upon recovery were processed and analyzed as described previously (Karl & Knauer 1984).

Particulate organic carbon (POC), particulate organic nitrogen (PON), and dissolved nitrogen. POC, PON, and dissolved nitrogen concentrations were measured in the live-control and live-thiram traps after recovery. For POC and PON determinations, subsamples from each trap were filtered onto pre-combusted Whatman GF/F filters and stored frozen for subsequent analysis as described by Sharp (1974). A portion of the filtrate from each sediment trap was analyzed immedi-

ately for NH_4^+, NO_3^-, and NO_2^- with shipboard autoanalyzer procedures (Strickland & Parsons 1972). The remainder of the filtrate was stored frozen for shore-based analysis of dissolved organic nitrogen (DON) by the u.v. oxidation method of Strickland & Parsons (1972).

Efficacy of eukaryotic inhibitor. Before conducting the *in situ* field experiment, the effects of thiram and the live trap density gradient solution on growth of protozoans and bacteria were evaluated in the laboratory. Tests were conducted on a ciliate (*Cyclidium* sp.) and an unidentified zooflagellate, both isolated from sediment trap material from the VERTEX 4 cruise (August 1983), and on a natural assemblage of bacterioplankton (< 0.6 μm filtrates, protozoan-free) collected from Kaneohe Bay, Oahu, Hawaii. A variety of eukaryotic inhibitors were screened by performing dose-response experiments with protozoan growth as the metric. Thiram demonstrated efficacy at a lower concentration than cycloheximide, colchicine, griseofulvin, or neutral red. Comprehensive results and implications of these laboratory investigations will be discussed elsewhere (G. Taylor & M. Pace unpubl.).

Subsamples from enriched suspensions of bacterioplankton in seawater or trap solution were distributed into 24 culture tubes, each containing a single grain of rice. The samples were inoculated with either flagellates or ciliates and incubated at 25 °C in darkness for 24 h. At 24 h, subsamples were fixed in 2% borate-buffered formaldehyde (for flagellates) or Lugol's Iodine solution (for ciliates) for cell counts, and then 1.0 μg ml^{-1} of thiram was added to the replicate zooflagellate and ciliate culture tubes. All samples (thiram, trap solution, and seawater) were incubated for an additional 24 h under identical environmental conditions. Subsamples were preserved and counted. Growth rates ($\mu = \ln N_{48} - \ln N_{24}$) in the 2 treatments were compared to those of the seawater samples. Microscopic observations of live flagellates and ciliates in all treatments were made at 1 and 3 d (zooflagellate) or 6 d (ciliate) after inhibitor addition.

Bacterioplankton were incubated for 3 d with either 1.0 μM glucose and rice grains (heterotrophic potential experiment) or suspensions of stationary phase phytoplankton (growth experiment) in the presence and absence of 4.2 μM thiram to simulate conditions in our field experiment. For heterotrophic potential measurements, 0.05 μCi ml^{-1} of [U-^{14}C]-glucose (346 Ci mol^{-1}; NEC 042X, New England Nuclear Co., Boston, Massachusetts) was added to each treatment. Flasks were incubated on a shaker table at 100 rpm in darkness and after 4 h were subsampled for $^{14}CO_2$ evolution and ^{14}C-incorporation into > 0.2 μm particulates (Wright & Hobbie 1966, Hobbie & Crawford 1969). For growth experiments, subsamples were fixed in 2% (v/v) borate-buffered formaldehyde at 0 and 3 d and cells stained with acridine orange were counted by the epifluorescent microscopic method (Hobbie et al. 1977).

Statistical analysis. It was not possible to replicate the treatments at each depth, so we could not perform the ideal statistical tests of comparing the treatments within and among depths as well as among stations. Instead, at each depth the live-control and live-thiram traps were compared and the treatment with the higher value of the parameter of interest was assigned a plus and the lower treatment a minus. We then tallied the number of plus and minus scores for each parameter among all depths and stations (n = 21 in most cases). A simple sign test was then performed by calculating the probability that the distribution of plus and minus scores was due to chance, assuming an equal probability for a plus or minus score (Mendenhall 1971). A critical region was calculated by accumulating the exact probabilities of a particular score in both tails of the distribution. We report these probability values and accept $P < 0.1$ as reflecting a significant difference between treatments. The weakness of this test is that it gives equal weight to large and small differences. Because the values for most of the parameters we measured for each treatment converged with depth, the test should be regarded as conservative in detecting differences between the 2 treatments in this experiment.

RESULTS

Effects of thiram and PIT solution on microbial growth and metabolism

Thiram at a concentration of 1 mg l^{-1} (4.2 μM) effectively inhibited zooflagellate and ciliate growth (Table 2). The negative growth rate of the ciliate (Table 2) indicated that some death obviously occurred in the presence of thiram, but we also observed that a portion of the population was still motile after 6 d. Incubation of protozoans in the live-control PIT solution yielded variable results. Zooflagellate growth was partially inhibited (38%) but was still 10 times greater than the live-thiram PIT solution. Ciliate growth in the PIT solution exceeded that measured in seawater. We observed no effect of the PIT solution on protozoan motility.

Growth of ungrazed bacterioplankton was only slightly affected by thiram (Table 2). Incorporation and respiration of D-[U-^{14}C]-glucose were only slightly reduced in the presence of thiram (3 and 9%, respectively). Karl & Knauer (1984) have previously demonstrated that the live PIT solution does not affect bacterial growth and metabolism.

Table 2. Effects of thiram and particle interceptor trap (PIT) solution on microbial growth and metabolic activity

Organism	Parameter	Control	Thiram (1 mg l^{-1})	% Inhibition	PIT solution[a]	% Inhibition
Zooflagellate (clone v4–1)	Growth (μ, d^{-1})	2.200	0.131[b]	94	1.370	38
Ciliate (clone v4–2)	Growth (μ, d^{-1})	0.100	−0.325	100	0.169	[c]
Bacterioplankton	Growth (μ, d^{-1})	1.440	1.540	[c]	NA	NA
	^{14}C-glucose, incorporation (nCi ml^{-1} h^{-1})	6.15	5.99	3	NA	NA
	^{14}C-glucose, respiration (nCi ml^{-1} h^{-1})	6.55	5.96	9	NA	NA

[a] PIT solution: offshore seawater amended with 27.05 NaCl, 11.40 $MgCl_2 \cdot 6H_2O$, and 0.74 KCl g l^{-1}
[b] Thiram in PIT solution
[c] Growth enhanced
NA: Not available

Particulate organic carbon concentrations

The concentrations of POC in both live-control and live-thiram traps declined exponentially with depth at all 3 stations (Fig. 2). Highest concentrations were measured at the inshore station and lowest concentrations at the offshore station. This pattern is consistent with the measured gradient in primary production and total POC flux as measured by *in situ* formaldehyde preserved collections at these same stations and depths (G. Knauer pers. comm.). The POC concentrations measured in the live traps reflect the amount of carbon remaining after incubation of the microbial assemblage associated with the sinking particles. The live-control traps generally had lower POC concentrations than the live-thiram traps ($P = 0.027$). A lower concentration in the live-control treatment implies a higher decomposition rate of POC during the incubation. Inhibition of the protozoans in the live-thiram traps appeared to lower decomposition, and thus lead to a higher observed concentration of POC.

Nitrogen inventory

Nitrogen inventories were established for the live-control and live-thiram traps by measuring the particulate and dissolved constituents (with the exception of dissolved gases). Concentrations of particulate organic nitrogen (PON), nitrate (NO_3^-), and nitrite (NO_2^-) were frequently at or below the limit of analytical detection at Sta. B and C, so we present data for only the nearshore station. At Sta. A, PON and dissolved organic nitrogen (DON) were measurable at all depths, and these species were the dominant component of the total nitrogen inventory in the sediment traps (Table 3). Ammonium (NH_4^+) concentrations were a significant component of the total nitrogen inventory only for the

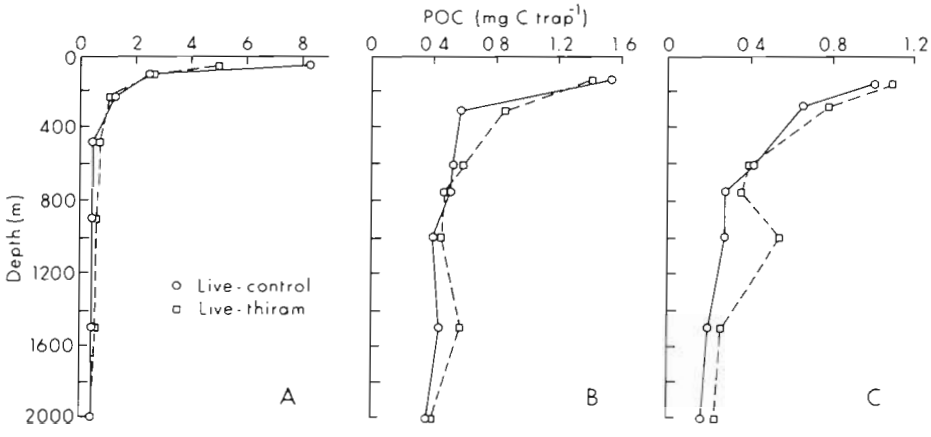

Fig. 2. Vertical profiles of particulate organic carbon (POC) for Sta. A, B, and C collected in live-control (O) and live-thiram (□) traps. Traps deployed for 13 d (A, 4 to 17 Jun 1984) and 21.75 d (B, 5 to 27 Jun 1984; C, 7 to 28 Jun 1984). Means of 3 analytical replicates presented (SD of means smaller than symbols)

Table 3. Nitrogen inventory for live-control and live-thiram traps at Sta. A. All values expressed as μmoles trap^{-1} 13d^{-1}

Depth (m)	PON		NH_4^+		NO_3^-		NO_2^-		DON	
	Control	Thiram	Control	Thiram	Control	Thiram	Control	Thiram	Control	Thiram[1]
50	97.88	47.22	15.54	3.48	0.00	0.25	1.10	0.19	31.29	59.79
100	29.36	27.61	6.19	1.57	0.89	0.00	0.20	0.01	9.15	19.94
225	15.22	11.39	0.43	0.10	2.77	4.88	0.00	0.00	4.16	3.26
475	4.11	5.91	0.00	0.00	5.54	5.35	0.00	0.00	2.23	11.43
900	4.56	4.05	0.00	0.01	2.42	0.00	0.00	0.00	2.93	1.30
1500	3.83	4.84	0.00	0.05	4.91	3.37	0.00	0.01	2.00	1.45
2000	3.05	3.66	0.06	0.08	0.00	0.00	0.00	0.01	1.58	1.12

[1] DON values in thiram traps are corrected for the contribution of thiram (5.51 μmoles DON l^{-1}) measured in blank samples

surface traps (50 and 100 m), while NO_3^- concentrations were significant at intermediate depths (225, 900, and 1500 m).

Substantially higher concentrations of NH_4^+ were observed in the live-control traps, relative to the live-thiram traps, at 50, 100, and 225 m (Table 3). This pattern was also observed consistently at the other 2 stations (data not shown). In contrast to NH_4^+, the concentrations of DON were higher in the live-thiram traps at Sta. A in the 2 shallowest traps (Table 3) and only in the shallowest trap at Sta. B.

ATP concentrations

Surface (100 to 150 m) *in situ* ATP concentrations were 2.5 times higher at Sta. A than at Sta. B and C (Fig. 3). At Sta. A, *in situ* ATP concentrations decreased with depth and approached shipboard ATP concentrations in the upper 225 m, indicating rapid die-off of the sinking microbes. The convergence of *in situ* and shipboard ATP did not occur until 600 m at Sta. B and C. The *in situ* ATP concentrations exceeded shipboard ATP measurements at 20 of 21 sampling sites. Consequently, growth rates of the microbial assemblage, μ, were negative, indicating that microbial death rates exceeded rates of cell division. At Sta. A and C, growth rates were most negative in the surface traps and approached zero with depth (Fig. 4). At Sta. B, growth rates did not vary in a consistent pattern with depth. Concomitantly, living carbon (shipboard ATP × 250 μg C μgATP^{-1}; Karl 1980) to total POC ratios at the base of the euphotic zone were 13, 11, and 7% in live-control traps and decreased 15, 28, and 6-fold over the depth ranges sampled at Sta. A, B, and C, respectively (calculated from Fig. 2 & 3). There were no consistent differences between the live-control traps and the live-thiram traps in terms of either ATP concentrations or community growth rates. Trends in net assemblage growth rates at Sta. B and C between 600 and 1000 m suggest either lower death rates or increased cell division rates in this depth range.

Microbial abundances and biomass

Bacterial biomasses in the *in situ* preserved traps were greatest in the surface waters and decreased with depth (Fig. 5; data given in parentheses). In addition, the biomass of bacteria collected at a given water

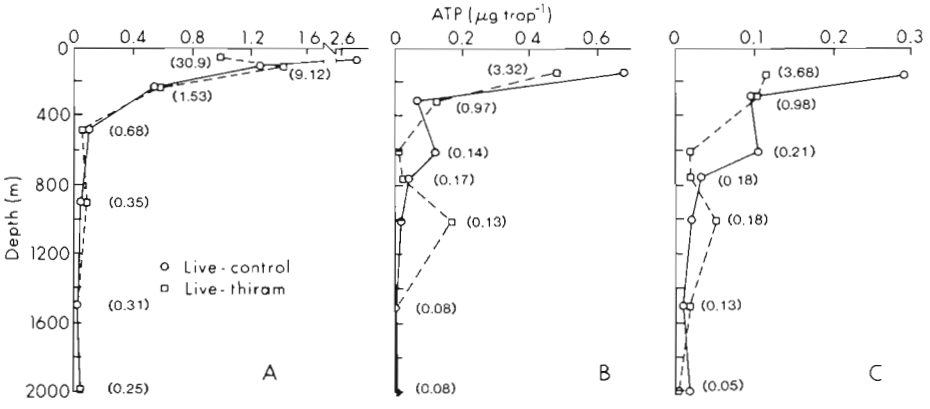

Fig. 3. Vertical profiles of adenosine triphosphate (ATP) remaining in live-control (○) and live-thiram (□) traps after collection-incubation period and extracted shipboard. Values in parentheses are from *in situ* ATP extraction traps and represent input of microbial biomass

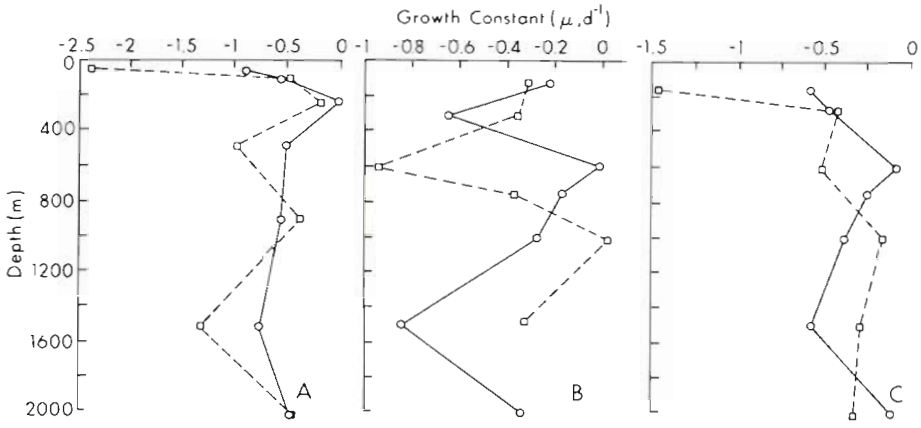

Fig. 4. Vertical profiles of growth rate constants for microbiota associated with particles collected in live-control (○) and live-thiram (□) traps at Sta. A, B, and C. Rates calculated from *in situ* extracted ATP (input) and shipboard extracted ATP (post-incubation) as described above

depth decreased from the nearshore to offshore stations. Total bacterial abundances measured for the *in situ* preserved traps were not statistically different from the live-control traps, which suggests that there was no net growth in the live traps. Bacterial concentrations and mean cell volumes for the live-control traps, however, were greater than for the live-thiram traps ($P = 0.078$), suggesting differential rates of growth and/or death between treatments.

Zooflagellate biomass was greatest in the near surface sediment traps and decreased with increasing water depth (Fig. 6). Zooflagellate biomass was generally greater in the *in situ* preserved traps (Fig. 6; data given in parentheses) than in either the live-control or the live-thiram traps, possibly reflecting sampling differences between the unscreened preserved traps and the screened live traps and/or the death of zooflagellates in the live treatment traps during the collection-incubation period. Zooflagellate biomass was also significantly higher in the live-control traps than in the

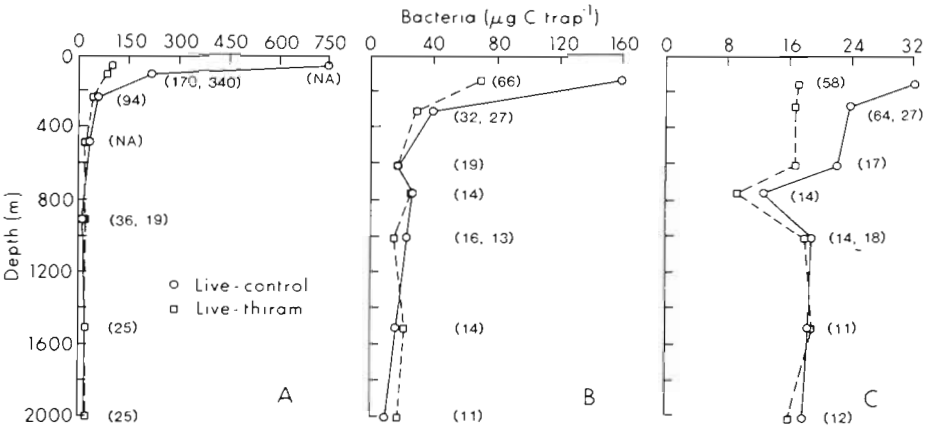

Fig. 5. Vertical profiles of bacterial biomass collected in live-control (○) and live-thiram (□) traps at Sta. A, B, and C. Values in parentheses were calculated from *in situ* preserved traps (glutaraldehyde, M. W. Silver & M. M. Gowing, Univ. Calif. Santa Cruz) that lacked the 333 µm Nitex screens. Values for replicate *in situ* preserved traps deployed at 2 depths at each station are presented. NA = not available

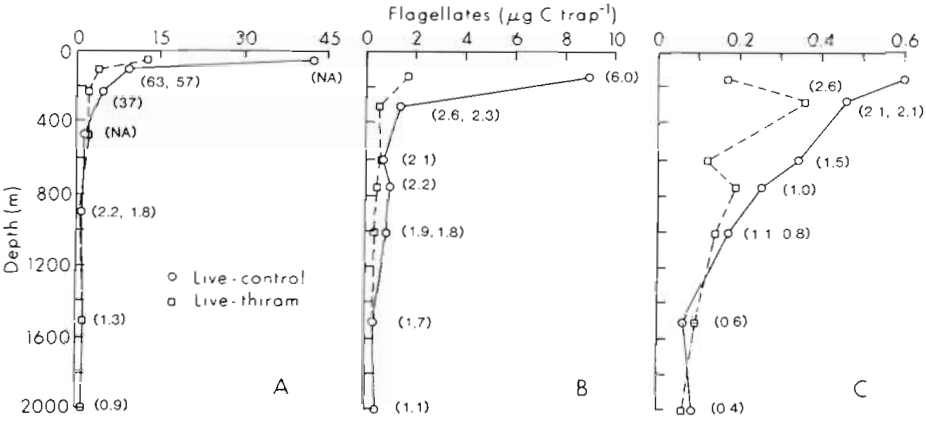

Fig. 6. Same as Fig. 5 for zooflagellates

live-thiram traps (P = 0.027). This difference probably reflects accelerated death as a result of thiram.

The ratio of zooflagellate cell volume to the total volume of bacteria plus zooflagellates in the *in situ* preserved traps was greater than in the live-control and live-thiram traps at all 3 stations (Table 4: Sta. A – preserved = 25 %, live-control = 10 %, live-thiram = 11 %; Sta. B – 22 %, 8 %, 5 %; Sta. C – 12 %, 3 %, 2 %). There were no consistent trends in relative biomass with depth, but there was a trend toward zooflagellates accounting for a lower percentage of the total biomass along the onshore-offshore gradient.

The ratio of total microbial carbon biomass (bacterial + zooflagellate; Fig. 5 & 6) to total measured POC (Fig. 2) was an average of 5 % and did not vary systematically between stations, depths, or treatments (range = 2 to 11 %). Conversely, the ratios of estimated microbial ATP (bacterial + zooflagellate carbon ÷ 250; Fig. 5 & 6) to total shipboard ATP (Fig. 3) were an average of 80, 89, and 90 % and had ranges of 25 to ≥ 100, 37 to ≥ 100, and 45 to ≥ 100 % at Sta. A, B, and C, respectively. These ratios increased with depth, did not vary systematically between treatments, and were most often ≥ 100 %. These results indicate that most of the POC remaining in the traps was nonliving (≥ 89 %) and the major portion of the biomass measured by ATP could be accounted for by bacteria and zooflagellates.

Table 4. Summary of bacterial and protozoan biomass relations

Depth (m)	Flagellate biovolume proportion (% of total microbial volume)[a]		
	Preserved	Control	Thiram
Station A			
50	NA	11	21
100	39	9	9
225	49	14	9
475	NA	9	19
900	16	11	4
1500	12	9	9
2000	8	9	3
Mean ± SD	25 ± 18	10 ± 2	11 ± 7
Station B			
130	19	11	5
300	17	7	4
600	22	8	8
750	28	8	4
1000	25	7	6
1500	23	5	4
2000	20	8	5
Mean ± SD	22 ± 4	8 ± 2	5 ± 1
Station C			
150	10	4	2
275	12	4	5
600	18	3	2
750	15	4	4
1000	13	2	2
1500	11	1	1
2000	8	1	1
Mean ± SD	12 ± 3	3 ± 1	2 ± 2

NA: Not available
[a] Flagellate cell volume × 100 ÷ (flagellate + bacterial cell volume)

Detrital analysis

Trap material at Sta.A was dominated by a centric diatom of the family Discoideae, which accounted for an estimated 52 to 76 % of the total POC for the traps deployed at 50 m (Table 5). Trap-collected diatoms decreased from 6.2×10^6 to 1.1×10^4 cells trap^{-1} over the depth range examined, and the amounts collected by the 2 live treatments were similar. The conversion factors used to estimate diatom biomass were assumed to be constant, but microscopic observations of cells stained with acridine orange confirmed that cytoplasmic volume decreased with depth. Therefore, the calculated diatom contribution to total POC represents an upper limit, particularly for the deeper traps. Fecal pellet concentrations did not vary with depth, with the exception of higher values at 100 m. The estimated contribution of fecal pellets to total POC was small (< 3 %) throughout the water column. Fecal pellet concentrations in all 7 live-control traps were lower than in the live-thiram traps (P = 0.016), which suggests more decomposition in the presence of active protozoans. The lack of differences in diatom numbers between the treatments is probably due to the refractory nature of the frustules, leading to the expectation that these cells serve as a conservative marker of sinking particulate materials. Diatom frustules and fecal pellets were too few to enumerate at Sta. B and C given the sample volume.

^{3}H-adenine assimilation

^{3}H-adenine was assimilated into RNA and DNA by microorganisms associated with sedimenting particles at all stations and depths (Table 6). This implies that net DNA synthesis, and hence growth, occurred throughout the water column. As expected, the rates of incorporation varied substantially with depth; generally, the highest rates were observed for *in situ* incubation of particles collected at the base of the euphotic zone. Because of undetectable concentrations of ^{3}H-ATP in many samples, specific activities and therefore

Table 5. Summary of diatoms (Discoideae), fecal pellets, and estimated carbon from these sources collected in live-control traps and live-thiram traps at Sta. A after 13d. Diatom carbon estimate based on Sicko-Goad et al. (1984); where C = mean cell volume × 1.00 g cm^{-3} × 0.25 (dry:wet ratio) × 0.10 (carbon:dry ratio). Fecal pellet carbon estimate based on Urrere & Knauer (1981); where C = mean pellet volume × 1.22 g cm^{-3} × 0.11 (dry:wet ratio) × 0.20 (carbon:dry ratio)

Depth (m)	Trap	Diatoms			Fecal pellets		
		No. $trap^{-1}$	µg C $trap^{-1}$	% POC	No. $trap^{-1}$	µg C $trap^{-1}$	% POC
50	Control	6.18×10^6	4274	51.50	1.99×10^3	13	0.15
50	Thiram	5.48×10^6	3792	75.60	5.02×10^3	32	0.64
100	Control	1.33×10^6	918	36.80	8.97×10^3	57	2.29
100	Thiram	1.32×10^6	913	33.80	10.34×10^3	66	2.45
225	Control	1.47×10^5	102	8.09	1.82×10^3	12	0.93
225	Thiram	1.60×10^5	111	10.40	2.66×10^3	17	1.59
475	Control	2.30×10^4	16	3.50	1.66×10^3	11	2.33
475	Thiram	4.20×10^4	29	4.06	1.77×10^3	11	1.59
900	Control	1.99×10^4	14	3.17	1.23×10^3	8	1.82
900	Thiram	1.89×10^4	13	2.25	2.18×10^3	14	2.40
1500	Control	1.44×10^4	10	2.28	1.71×10^3	11	2.50
1500	Thiram	1.50×10^4	10	1.91	2.34×10^3	15	2.74
2000	Control	1.38×10^4	10	2.66	1.21×10^3	8	2.16
2000	Thiram	1.06×10^4	7	1.81	1.55×10^3	10	2.43

Table 6. Assimilation of ^{3}H-adenine and production of ^3H_2O, ^{3}H-RNA and ^{3}H-DNA during *in situ* incubation of sediment trap particles

Depth (m)	^{3}H-adenine added (µCi $trap^{-1}$)	^3H_2O (nCi $trap^{-1}$)		^{3}H-RNA (nCi $trap^{-1}$)		^{3}H-DNA (nCi $trap^{-1}$)		^{3}H-RNA:^{3}H-DNA	
		Control	Thiram	Control	Thiram	Control	Thiram	Control	Thiram
Station A									
50	90	39,832	1,691	11,297	799	965	28	12	28
100		26,131	1,575	20,959	1,215	678	25	31	49
225		9,452	1,328	5,634	1,272	633	24	9	53
475		1,650	987	548	811	36	25	15	32
900		754	741	89	50	4	5	25	10
1500		1,051	724	44	29	2	2	21	17
2000		1,140	549	40	32	2	2	22	16
Station B									
130	90	43,920	3,235	12,291	4,077	1,302	788	9	5
300		705	952	322	426	26	63	12	7
600		<50	<50	163	86	5	3	33	29
750		266	513	186	43	15	3	12	15
1000		538	673	60	347	3	22	19	16
1500		<50	<50	37	35	5	6	7	6
2000		<50	<50	39	30	3	4	13	8
Station C									
150	160	9,587	1,646	5,459	384	158	15	35	26
275		3,732	2,770	788	1,859	23	41	34	45
600		<50	<50	99	110	4	5	25	23
750		<50	<50	74	40	3	3	24	13
1000		95	660	38	417	4	37	10	11
1500		410	<50	20	19	1	2	18	13
2000		780	336	17	20	1	1	21	15

rates of total DNA and carbon production could not be calculated for 24 of 42 samples. Estimated microbial carbon production for live-control and live-thiram surface traps were 49.0 and 5.4, 15.4 and 7.5, and 0.4 and 0.1 mg C m^{-2} d^{-1} for Sta. A, B, and C, respectively.

Maxima of 57 (A), 64 (B), and 10% (C) of the radioactivity added as ^{3}H-adenine to the traps were measured in the cumulative ^3H_2O, ^{3}H-RNA, and ^{3}H-DNA pools. ^3H_2O was observed in most sediment traps and, when present, accounted for the bulk of the radiochemical inventory. The ratio of ^3H_2O:^{3}H-RNA for many of the deeper sediment trap microbial assemblages exceeded a value of 10, which is high compared to an average ratio of 1 to 2 for the surface traps (Table 6) and for Niskin bottle collections at all depths (D. Karl unpubl. data). Mean ^{3}H-RNA:^{3}H-DNA ratios were 24, 14, and 22 at Sta. A, B, and C and varied from 5 to 49. This ratio did not vary systematically between treatments or with depth.

DISCUSSION

The reliability of sediment traps as a method of sampling oceanic particles has been previously analyzed (Bloesch & Burns 1980, Dymond et al. 1981, Gardner et al. 1983). Limitations of our individual estimates are discussed as they apply below. The use of 333 µm screens was an integral part of our experimental design in order to keep large zooplankton (i.e. 'swimmers') from entering the sediment traps during the deployment period. The only alternative solution to screening would have been to remove the swimmers manually after sediment trap recovery (Knauer et al. 1979, Knauer & Martin 1981). By following this latter protocol, it would be impossible to separate the impact of the swimmers on the parameters (especially soluble nutrients) measured from the effect of our experimental treatment. For this reason, we elected to use the screening procedure. The screened traps collected less POC and PON and probably fewer larger protozoa, such as radiolarians and foraminiferans, than unscreened traps used at the same stations and depths (G. Knauer & M. M. Gowing pers. comm.). Because the size of particles collected by PIT's decreased with depth (pers. obs.), the bias introduced by screening most likely decreased as a function of depth. However, the screened trap material was qualitatively similar as indicated by comparisons of C:N ratios and microbial community composition.

Results presented by Silver et al. (1984) from 3 previous VERTEX cruises off central California (USA) and Mexico illustrated that sedimenting organic materials are a habitat for ciliated protozoans. Significant results included: the discovery of undescribed species, the measurement of high ciliate flux rates (0.32 to 2.00 × 10^6 cells m^{-2} d^{-1}), measured ciliate to bacteria numerical ratios of 0.06 × 10^{-3} to 1.00 × 10^{-3} (reciprocal of reported data), and estimated ciliate to bacterial biomass ratios of 5 to 158. From these results and from unquantified observations of zooflagellates, they concluded that ciliated and probably flagellated protozoans must be important in the diagenesis of sinking POM. The present study experimentally supports this hypothesis.

We found a large zooflagellate flux from the euphotic zone: 955 × 10^6, 58 × 10^6, and 25 × 10^6 cells m^{-2} d^{-1} (which is equivalent to 1176, 71, and 30 µg C m^{-2} d^{-1}) for the surface traps at Sta. A, B, and C, respectively. However, we observed a reduction in biomass with both water depth and incubation time (i.e. the *in situ* preserved abundances exceeded the live-control abundances). It is interesting that we did not observe any ciliates other than a few tintinnids either by epifluorescence or settling chamber bright-field microscopy techniques in preserved material from the live-control, live-thiram, or *in situ* preserved trap materials. If ciliates were a dominant component of the detrital assemblage, we should have encountered them during microscopic examination of approximately 200 epifluorescent (100, 250, 400, and 1000× magnification) and 30 settling chamber (100 and 250× magnification) preparations. Furthermore, because our direct microscopic estimates of flagellate biomass were generally within a few percent of the total microbial biomass estimated by ATP, it is unlikely that ciliates contributed anything more than negligible biomass.

Examination of microbial assemblages associated with macroaggregates in surface waters of the Sargasso Sea revealed significant quantities of bacteria, flagellates, ciliates, and amoebae (Caron et al. 1982). If their cell numbers are converted to biomass by using reported median cell volumes and conversion factors presented above, mean biomass and % of total microbial biomass are approximately as follows: bacteria, 5.7 µg C ml^{-1} (85%); nanoplankton, 1 µg C ml^{-1} (14.6%); zooflagellates, 0.03 µg C ml^{-1} (0.4%); ciliates, 0.4 ng C ml^{-1} (0.006%). Our trap results correspond closely with these observations.

In the present study, the proportion of zooflagellates (as % of total microbial volume) was observed to vary spatially (with depth at the inshore station, between stations at a given depth), and with particle age (between preserved and live traps) and accounted for between 2 and 25% of the microbial biomass. These observations are consistent with the hypotheses that the total energy of the sedimenting habitat decreases and the POM becomes more refractory as particles sink and age (Ducklow et al. 1985 and references therein). Bacteria associated with particles sinking out of the mixed layer (ca 10 m) in the New York Bight were

growing (by ^{3}H-thymidine and cell count methods) in material collected in traps for 1 d and incubated for a subsequent day (Ducklow et al. 1982). However, growth rates were low relative to the observed vertical fluxes of attached bacteria. Radioassay data reported above indicate that RNA and DNA synthesis, hence growth, occurs at all stations and depths and is enhanced in the oxygen minimum zone. Results from ATP and microscopic analyses, however, strongly indicate that net death and energy loss with depth and time is the overwhelming fate of microbial communities on sedimenting POM. It is possible, although untested by us, that the observed ^{3}H-RNA and ^{3}H-DNA synthesis occurred shortly after the particle-associated microbes entered the traps, cell growth then decreased with incubation time, and a portion of the community actually died. Observed results were not influenced by exhaustion or loss of substrate since 36 to 90 % of the added ^{3}H-adenine remained at the end of the deployments and previous studies have demonstrated minimal diffusive losses from these traps (Karl & Knauer 1984). *In situ* time course experiments are necessary to resolve the dynamics of this system.

Differences observed in ^3H_2O:^{3}H-RNA ratios between surface and deep traps are consistent with the hypothesis that 2 distinct populations may be experiencing different growth rates or at least growth strategies. ^3H_2O production is known to occur as a consequence of ^{3}H-adenine metabolism (Karl et al. 1981); however, the precise mechanisms and metabolic pathways of ^3H_2O production remain unknown. Mineralization of ^{3}H-adenine, metabolic turnover of nucleic acids, and isotope exchange during metabolic conversion of adenine to guanine are all potential pathways. Furthermore, the extremely high ratios of ^{3}H-RNA:^{3}H-DNA for many of the sediment trap samples suggest that the deep populations may be experiencing unbalanced growth since it is unlikely that the RNA:DNA concentration ratio exceeds a value of 10 for any growing microbial population (Karl 1981). Similar results based on ^{3}H-thymidine and ^{3}H-adenine incubations of trap-collected material (1075 m) from the Middle Atlantic Bight are reported in Ducklow et al. (1985). In this regard, microorganisms associated with sediment trap-collected particles appear to be similar to microbial assemblages sampled from surface sediments (Craven & Karl 1985). The addition of thiram to the sediment traps caused a decrease in the production of ^3H_2O, ^{3}H-RNA, and ^{3}H-DNA but only for samples collected in the upper 300 m. Presumably this effect is the result of uncoupling of bacterial metabolism from protozoan grazing pressure.

Thiram has been employed as a selective inhibitor of protozoan activity in soil microbiology (Ramirez & Alexander 1980) and in neritic planktonic assemblages (Newell et al. 1983). Careful experimentation and cautious data interpretation must be exercised when using 'selective' inhibitors for ecological investigations because effects on organisms are likely to vary with trophic mode, cellular organization, ambient nutrient concentrations, and growth rates (Yetka & Wiebe 1974, Iturriaga & Zsolnay 1981, Taylor & Pace unpubl.). Preliminary laboratory results from this study indicate that thiram: (1) effectively blocks flagellate and ciliate growth without immediately killing these organisms, (2) has little effect on short-term (4 h) heterotrophic potential or long-term (3 d) growth of bacterioplankton, and (3) is sufficiently selective when applied to sub-euphotic zone microbial-detrital associations.

The results of field observations suggest that live-thiram traps contained viable zooflagellates, although at reduced levels compared to live-control traps. The possibility that thiram non-selectively depressed bacterial activity in the sediment traps is a potential criticism of our interpretation of the field experiment. Bacterial abundances were consistently lower in the live-thiram traps. Conversely, ATP-derived net growth rates did not vary consistently between treatments. Although production rates of ^3H_2O, ^{3}H-RNA, and ^{3}H-DNA in the shallow ($\leq$ 275 m) live-control traps were consistently greater than in the live-thiram traps, differences between treatments for all depths and stations were statistically insignificant, i.e. varied randomly. It is impossible to evaluate fully the actual effect of thiram on sedimenting bacteria because direct (chemical inhibition) and indirect (thiram-protozoan-bacterial interactions) effects cannot be separated in the field experiment. However, our results suggest that: (1) observed treatment differences in shallow traps (high energy system, large grazer impact) were due to indirect community effects, and (2) the random treatment differences at depth (low energy system, small grazer impact) were a consequence of negligible inhibitory effects on bacteria. The possibility that direct uptake and metabolism of ^{3}H-adenine by zooflagellates accounted for the differences observed between treatments seems unlikely. In a preliminary laboratory experiment with ciliates fed unlabeled, heat-shocked bacteria in the presence of ^{3}H-adenine, uptake and metabolism of ^{3}H-adenine by the ciliates were not evident by radioassay or microautoradiography (unpubl. data). The presence of viable, growth-incompetent zooflagellates in the live-thiram traps suggests that inhibition was incomplete and reported protozoan effects on decomposition and nutrient regeneration are probably conservative.

With few exceptions, POC collected by live-thiram traps was higher than in the live-control traps, suggesting that protozoan grazers accelerate the decomposition of sedimenting POM. Microscopic examination of

fecal pellets at Sta. A confirmed that decomposition of these sinking particles was slower in the uncoupled system than in the coupled system at all depths. Although the amount of POC collected and the zooflagellate proportion of microbial biovolume decreased from Sta. A to C, differences observed between coupled and uncoupled systems suggest that the relative importance of protozoan grazers to decompositional processes increased along this transect. The effects of protozoan grazers on dissolved nutrient production appear to vary. Net production of NH_4^+ was clearly enhanced in live-control traps at the 2 inshore stations, especially in the upper 300 m where microbial metabolism is most rapid. It is difficult to assess NH_4^+ production at the offshore station because the low concentrations observed (1 order of magnitude less than Sta. B) approach the detection limits for NH_4^+. Production of NH_4^+ at this station, however, appeared to be slightly enhanced in the coupled system at all depths except 300 m. We interpret enhanced NH_4^+ production to be the result of protozoan excretion. Additionally, the depth distributions of NH_4^+ and NO_3^- in the traps supports the assertion that sinking POM is a center for bacterial nitrification (Karl & Knauer 1984, Karl et al. 1984).

Although protozoans are known to release a spectrum of dissolved organic compounds (Taylor 1982, Taylor et al. 1985), our results indicate that DON concentrations were greater in the grazer-inhibited systems of the shallow traps where input of nutrients and organisms and NH_4^+ production were greatest. This observation may be explained by at least 2 independent mechanisms: (1) metabolically inhibited protozoans in the live-thiram traps released their cellular pools of DON during the trap incubation period, or (2) sedimenting biogenic particles leach DON which is utilized more rapidly in the coupled system than in the uncoupled system. It is impossible to distinguish between these 2 possibilities in our experiments. However, if one assumes a Redfield C : N ratio for zooflagellates and all zooflagellates lyse upon entry into the 50 and 100 m live-thiram traps at Sta. A, then zooflagellates would contribute only 0.54 to 0.75 μmoles DON $trap^{-1}$. This is clearly insufficient to explain the observed differences of 1.75 to 50.66 μmoles DON $trap^{-1}$.

From preliminary laboratory results and microscopic analysis of field samples, it is apparent that viable but growth-incompetent protozoans were present in the live-thiram traps. Therefore, it seems unlikely that incoming microbes simply lysed upon entry into the traps. The accelerated death of protozoans in the live-thiram traps, which we invoke to explain the diminished regeneration of inorganic nutrients, can account for only a small portion of the observed DON. Reversal of the trend, however, was observed in 3 pairs of traps. It seems likely that cell death and solubilization of detritus (chemically and biologically mediated) both contribute to DON pools to varying degrees and that the differences observed between coupled and uncoupled systems may be functions of the rates of cell death and recycling by bacteria. The coupled bacteria-protozoan community degraded POC and PON, consumed DON, and produced NH_4^+ more actively than the uncoupled system. By our interpretation, the particulate and dissolved organics in the uncoupled systems were higher as a result of a reduction in microbial activity. These observations reflect the increased decomposition occurring in the live-control traps relative to the live-thiram traps. Our results strongly suggest that in sedimenting detrital associations, zooflagellates stimulate regeneration of inorganic nutrients and possibly indirectly stimulate recycling of dissolved organic matter (DON in this case).

The present study has provided clear evidence that decomposition of sedimenting POM in the open ocean is variable and at least partially dependent on phagotrophic zooflagellates. We have also shown that protozoan grazers affect nutrient regeneration, although the mechanisms by which this occurs require further study. The experiments described above are unique because: (1) the design was internally consistent, i.e. all parameters were measured from replicate traps that were subjected to different treatments, (2) multiple traps were positioned in the zone of greatest sedimentation and decomposition (50 to 2000 m), and (3) three stations of divergent productivity and hydrography were investigated. To our knowledge, this represents the first *in situ* experimental study to examine simultaneously the fate of sinking organic matter, their attendant microbes, and the quantitative role of protozoa in decompositional processes.

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