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DETRITUS-MICROBE INTERACTIONS IN THE MARINE PELAGIC ENVIRONMENT: SELECTED RESULTS FROM THE VERTEX EXPERIMENT

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ABSTRACT

Collections of sinking particulate matter were made using a free-floating sediment trap array deployed at three stations in the north Pacific Ocean as part of the Vertical Transport and Exchange (VERTEX) research program. Each experiment consisted of at least 120 individual sediment traps positioned at 10–12 depths in the mesopelagic zone (100–2,000 m). Our primary objectives were: (1) to obtain information on the magnitude and patterns of the downward vertical flux of selected elements and biochemicals, (2) to evaluate the relationships between microbial biomass and the rates of decomposition of sedimenting organic matter and (3) to measure the metabolic activity of microorganisms associated with particulate detritus. A combination of in situ extraction/preservation techniques (using sediment traps precharged with appropriate chemicals) and unpreserved traps (some precharged with specific growth substrates or radioisotopic tracers) enabled us to evaluate in situ detritus-microbe interactions. Our results indicate the presence of isolated regions of microbial productivity (i.e., "hotspots") with effects large enough to produce a positive rate of change in the downward flux of total organic matter (i.e., an increased flux of organic matter with increasing water depth). It is proposed that this in situ carbon production is the result of chemolithotrophic bacterial activity (i.e., production of organic matter from CO_2) supported by the transport of allochthonous energy in the form of reduced endproducts of anaerobic decomposition of organic matter. A detritus-microbe interaction model is presented and discussed within the context of existing VERTEX data.

Field measurement and elucidation of the rates and mechanisms of organic matter production, recycling and decomposition in the sea are concerns shared by many oceanographers with otherwise unrelated interests. Despite the recognized importance and past record of excellent research achievement in this area, several fundamental ecological concepts remain unresolved. These include: (1) the quantitative significance of the dissolved organic carbon (DOC)-bacterial production pathway, especially in oligotrophic ecosystems, (2) the environmental factors and mechanisms controlling the relative distributions of living (i.e., biomass) versus non-living (i.e., detritus) organic matter and (3) the pathways and rates of exchange of carbon and energy between the epipelagic zone and the deeper portions of the world ocean.

The recent development of novel experimental methods and instrumentation has given us new tools for obtaining reliable information on the pathways and fluxes among the inorganic and organic carbon pools in the sea. One of the more significant contributions in recent years has been the development and successful deployment of particle interceptor traps (i.e., sediment traps) as a means of assessing the absolute downward vertical flux of particulate organic matter at any depth in the ocean. When used in conjunction with steady-state POC and DOC concentrations, the downward vertical flux measurements can be used to estimate the residence time and average rates of change of organic carbon as a function of depth (Knauer et al., 1979; Suess, 1980; Lorenzen et al., 1983; Eppley et al., 1983). Another unique aspect of sediment trap studies is the fact that they provide a mechanism for the collection of rare, fast-sinking particles that are only infrequently sampled using conventional water bottles or in situ pumps. Finally, when

used under the proper set of experimental conditions, sediment traps can be employed as in situ collection-incubation devices for obtaining meaningful biomass and metabolic rate data for microorganisms associated with sinking detrital particles.

In 1979, an interdisciplinary group of scientists sharing a mutual interest in biogeochemical element cycling in the upper "high energy" layers of the ocean (0–2,000 m) was formed. The participants (J. Martin, program co-ordinator, W. Broenkow, K. Bruland, J. Farrington, S. Fowler, D. Karl, G. Knauer, C. Lee, R. Risebrough, M. Silver, L. Small and S. Wakeham) named the research program VERTEX, an acronym for VERtical Transport and EXchange of materials in the upper ocean. This report will summarize selected results from the first four sediment trap experiments (VERTEX I–IV, 1979–1983), and specifically will highlight the research efforts concerning the production and early diagenesis of organic matter in the northeast Pacific Ocean. In this way we hope to emphasize the unique advantages of using sediment traps for measuring detritus transport and for elucidating detritus-microbe interactions.

Materials and Methods

Geographic Location and Oceanographic Conditions for the VERTEX Experiments.—Figure 1 presents a map of the northeast Pacific Ocean showing the location of each of the four VERTEX stations. The VERTEX I study area was located 160 km off Pt. Sur, California (123°45'N, 35°45'W), which was occupied from 17 August to 8 September 1980. The oceanographic conditions were characteristic of late upwelling season, with nearshore surface water temperatures <13°C and chlorophyll *a* concentrations >20 mg m^{-3}. A more detailed data report including vertical profiles of salinity, temperature, oxygen and density, as well as near surface horizontal distributions of chlorophyll fluorescence and geopotential anomaly is presented elsewhere (Broenkow and Green, 1981; Broenkow, 1982). VERTEX experiments II and III were performed at stations off Manzanillo, Mexico (18°N, 108°W, from 26 October to 18 November, 1981; 16°N, 107°W, from 5 to 26 November 1982, for VERTEX II and III, respectively). This location was characterized by relatively warm surface temperatures (>28°C), moderate levels of chlorophyll *a* and a broad (100–800 m) well-developed oxygen minimum layer. VERTEX IV occupied a station in the northeast Pacific gyre (28°N, 155°W) approximately 800 km north of Hawaii. This site was selected as being characteristic of oligotrophic oceanic waters which are considered to represent 90% of the world ocean (Ryther, 1969).

Sediment Trap Design and Deployment.—Sinking particulate materials were intercepted using a free-floating MULTITRAP system described by Knauer et al. (1979), and shown schematically in Figure 2. Typically the MULTITRAP array consisted of 12 individual sediment traps at each of 9–14 depths, two current meters and an inclinometer (Fig. 2). Prior to deployment each trap was cleaned with Micro® and 10% HCl, rinsed thoroughly with sterile deionized water then filled with one of several high density solutions (see below) to prevent advective-diffusive loss of extractants, preservatives or radiotracers during the deployment period, and to eliminate flushing of the traps during recovery.

Measurement of Sediment Trap ATP Concentrations.—Two separate approaches have been developed to obtain information regarding the biomass of microorganisms associated with sediment trap materials. In the first procedure, the sediment traps are filled with 2.5 liters of an acid-salt solution (0.5 M H_3PO_4 containing 1.5 M NaCl, $\rho = 1.05$ g cm^{-3}). As sinking particles and their resident microbial populations enter the traps, the cells are extracted and the ATP released into the high density solution. The ATP is stable under these conditions for extended periods of time (i.e., weeks; Karl and Craven, 1980). Immediately upon recovery, the contents of the sediment trap were filtered through a 0.8 μm Nuclepore® filter positioned at the base of each trap and a portion of the acid-salt solution was processed for ATP by the methods described previously (Fellows et al., 1981; Karl and Knauer, 1984). The acid insoluble particulate material retained by the filter was processed for RNA using the methods described by Karl and Knauer (1984). The ATP concentrations derived from this procedure are subsequently referred to as "in situ" extracted ATP, and in principle, can be used as an indirect measure of the biomass of microoganisms associated with the sinking particles at the time of collection. The second type of ATP experiment used in the VERTEX program was performed by filling the sediment traps with 2.5 liters of a sterile solution of filtered seawater containing 33.8 g NaCl, 14.26 g $MgCl_2 \cdot 6H_2O$ and 0.93 g KCl liter^{-1} ($\rho = 1.065$ g cm^{-3}) without any addition of poison, preservative or extractant. Under these conditions the biomass of microorganisms associated with the sinking

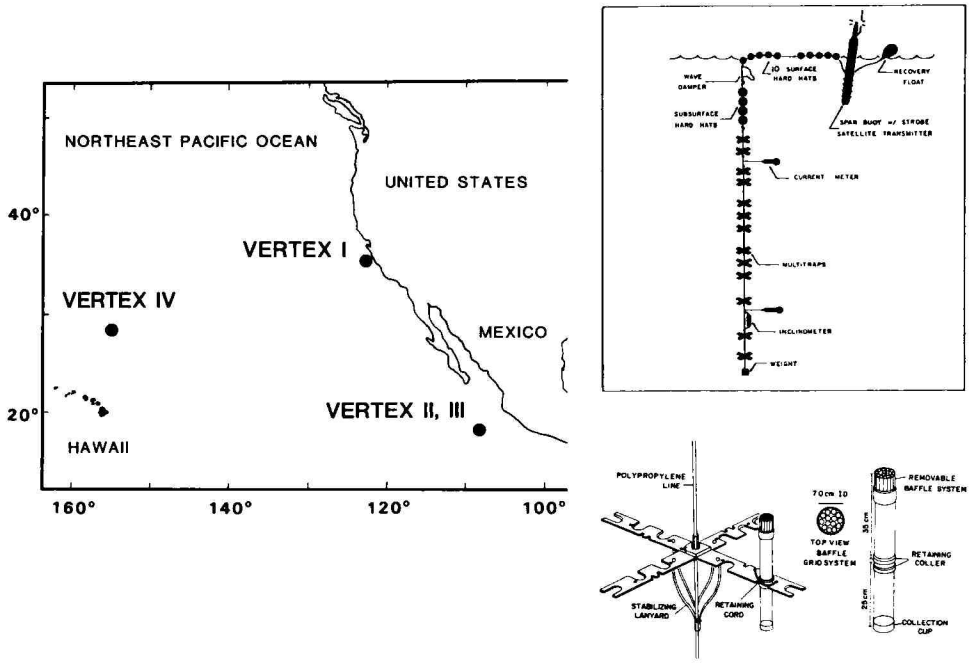


Figure 1. (Left) Map of northeast Pacific Ocean indicating the location of the VERTEX I–IV experiments.

Figure 2. (Right) MULTITRAP array from the VERTEX I experiment (top). The cross at each depth served as the support for 12 individual sediment trap collectors, one of which is shown at the bottom (adapted from Knauer et al., 1979, and Karl and Knauer, 1984).

particulate materials can either increase, decrease or remain the same depending upon the availability of energy sources for net biosynthesis. Following recovery of the traps, a subsample of the collected material was immediately extracted for ATP (Karl and Craven, 1980), and stored frozen for shore-based analysis. We subsequently refer to these ATP values as "shipboard" extracted ATP concentrations so as to distinguish them from the values measured by the in situ technique.

A direct comparison of in situ and shipboard ATP concentrations yields valuable information regarding the metabolic activity and the potential for growth of the microorganism associated with sinking particulate material. In principle, there are three possible results: (1) in situ ATP equals shipboard ATP indicating either that no growth had occurred or that in situ growth was exactly balanced by cell death and lysis, (2) shipboard ATP > in situ ATP, indicating that net growth had occurred during the incubation period presumably at the expense of detrital energy sources and (3) in situ ATP > shipboard ATP indicating that net death had occurred.

Effects of Chemolithotrophic Additions.—During VERTEX III an experiment was performed to evaluate the effects of the addition of potential chemolithotrophic substrates on shipboard ATP concentrations. Duplicate sediment traps were filled with 2.5 liters of a sterile seawater solution containing NaCl, $MgCl_2 \cdot 6H_2O$ and KCl (as above). At each depth, one of the paired traps was also precharged with a chemolithotrophic growth substrate mixture consisting of NH_4Cl, $NaS_2O_3 \cdot 5H_2O$ and $MnCl_2$ to yield final concentrations of 400 μM NH_4^+, 400 μM $S_2O_3^{-2}$ and 100 μM Mn^{+2}. Upon recovery of the sediment traps, subsamples were processed for shipboard ATP as described above.

Sediment Trap Nucleic Acid Synthesis.—In situ rates of RNA and DNA synthesis of microorganisms associated with sinking particles were measured using ^{3}H-adenine (Karl, 1981; Karl and Winn, 1984). Each sediment trap was filled with 2.5 liters of a sterile seawater solution containing NaCl, $MgCl_2 \cdot 6H_2O$, KCl (as above) and 100 μCi ^{3}H-adenine (15 Ci mmol^{-1}; New England Nuclear Co., Boston, MA) liter^{-1}. Upon recovery, subsamples were extracted and processed for ^{3}H-ATP, ^{3}H-RNA and ^{3}H-

DNA (Karl, 1981). For selected calculations, the measured rates of DNA synthesis were extrapolated to rates of carbon production using the conversion factors given in Karl and Winn (1984).

During VERTEX III an experiment was performed to evaluate the effects of oxygen removal on the rates of microbial RNA and DNA synthesis. Three sediment traps were filled with the ^{3}H-adenine/salt solution (as above) and treated as follows: (1) no further treatment (unpreserved control), (2) purged for 15 min with sterile filtered argon to remove dissolved oxygen (purge) and (3) mixed with buffered formalin to yield a final concentration of 1% (preserved control). All three traps were deployed at a depth of 150 m for the duration of the experiment (21 days).

Sediment Trap ^{14}C-Bicarbonate Uptake.—In situ rates of $H^{14}CO_3^-$ uptake were measured by filling sediment traps with 2.5 liters of a sterile seawater solution containing NaCl, $MgCl_2 \cdot 6H_2O$, KCl (as above) and 30 μCi $NaH^{14}CO_3$ (50 mCi mmol^{-1}; New England Nuclear Co., Boston, MA) Following sediment trap recovery, the density solutions were gently mixed and particulate materials from triplicate 100 ml subsamples were collected on GF/F filters, and placed into scintillation vials containing 1 ml of 1% HCl. The samples were evaporated to dryness in vacuo, reconstituted with 1 ml of deionized water, mixed with 10 ml Aquasol® and radioassayed (Packard Model 4060). For calculations of carbon production, we assumed a ΣCO_2 concentration of 2 mM.

Organic Carbon and Nitrogen.—Each collector tube was filled with 2.5 liters with a reagent grade NaCl solution (ρ = 1.05 g cm^{-3}) containing 1% buffered formalin. Upon recovery, the salt solutions were drained through 0.8 μm Nuclepore® filters positioned at the base of the traps and the collected particulate materials rinsed, examined microscopically to remove swimmers (i.e., zooplankton), oven dried, homogenized and analyzed for organic C and N (Knauer et al., 1979; Knauer and Martin, 1981).

Sediment Trap Nutrients.—Concentrations of NO_3^-, NO_2^-, NH_4^+ and HPO_4^{-2} were measured in filtrates (0.2 μm Nuclepore® filter for VERTEX III, or combusted GF/F® filters for VERTEX IV) from unpreserved sediment traps (see above; e.g., ^{3}H-adenine or ^{14}C traps), immediately upon recovery using a shipboard autoanalyzer. During VERTEX III and IV we also measured exchangeable NH_4^+ using the KCl-borate buffer procedure of Stevenson and Cheng (1972). These values were generally <10% of the soluble NH_4^+ concentrations measured in the trap density solutions. No attempt was made to measure fixed NH_4^+ (i.e., NH_4^+ entrapped in interstices of clay minerals), which we considered to be unavailable to microoganisms.

Ancillary Microbiological, Chemical and Hydrographic Measurements.—Primary autotrophic carbon production and total microbial carbon production were measured at each VERTEX station using ^{14}C and ^{3}H-adenine techniques, respectively. Water column nutrient concentrations were measured on filtered (combusted GF/F) Niskin bottle-collected water samples using the shipboard autoanalyzer. Profiles of dissolved oxygen and density (σ_t) were supplied by W. Broenkow (Moss Landing Marine Laboratory). Water column pH was measured using an Orion meter and a quick response combination microelectrode.

Results and Discussion

Presentation of the Detritus-Microbe Interaction Model.—When we began the first VERTEX experiment, there was only a limited data base on the magnitude of the downward vertical fluxes of organic matter in the pelagic environment, and on the average rates of change for carbon flux with increasing water depth (data summarized in Suess, 1980). Consequently, the objective of our initial experiment was to obtain basic information on the transport system, rather than with testing specific a priori hypotheses regarding the mechanisms of biogeochemical cycling of carbon. However since VERTEX I, we have generated and tested several interrelated hypotheses, including: (1) A significant proportion (>40%) of the steady-state rate of total plankton production in the epipelagic zone is derived via heterotrophic assimilation of dissolved organic carbon (i.e., soluble detritus) such that short-term measurements of autotrophic production may underestimate the actual in situ rate of particulate organic carbon production. The potential ecological significance of this soluble detritus-microbe interaction has been thoroughly reviewed in several excellent publications (Pomeroy, 1974; Sieburth, 1976; Williams, 1981). The ratio of total microbial carbon production (bacteria and unicellular algae, as extrapolated from DNA synthesis rate measurements) to

primary carbon production (the uniquely autotrophic portion of the growth of unicellular algae, as extrapolated from the uptake of $H^{14}CO_3^-$) averaged 1.8 for the four VERTEX study areas examined to date. Assuming that both techniques are accurate, these results are difficult to interpret given our current models of phytoplankton production, exudation and bacterial assimilation. Although the ratio of total microbial (primary plus secondary) production to primary production is expected to exceed 1.0 in all oceanic habitats, a reasonable upper estimate of 1.25 is obtained when representative values of phytoplankton exudation and bacterial assimilation efficiencies are assumed. We now believe that onshore-offshore gradients in allochthonous dissolved organic carbon (or some other suitable energy source) might be responsible for this observed secondary carbon production discrepancy. Further experimental details and ecological implications of these data will be presented elsewhere (D. Karl and G. Knauer, in preparation). (2) The chemistry and microbiology of the rapidly sinking sediment trap particles are distinct from the slow sinking particulate organic matter traditionally sampled by conventional water bottles (i.e., suspended particulate organic matter; S-POM). We have tested this hypothesis by comparing the bulk elemental composition of trap materials to background POM and also by measuring the relative rates of microbial activity associated with the two distinct classes of particles. Our results indicate that the C:N ratios of the sediment trap particulates are much lower (5–10 versus 15–30), and that the rates of RNA and DNA synthesis per unit microbial biomass are much higher (by up to an order of magnitude) than for the water bottle S-POM (Karl and Knauer, 1984). In this regard, our data support the conclusions of McCave (1975) and Fellows et al. (1981) regarding the distinction between these two classes of particulate materials in the ocean. (3) The average rate of change for total carbon flux, a value which is equivalent to the combined net effects of physical (solubilization and fragmentation) and biological (decomposition, repackaging and in situ carbon production) processes varies considerably but predictably with depth in the upper 2,000 m of the ocean. Positive rates of change, indicating a new input of particulate organic carbon to the system, are frequently associated with water mass boundaries, pycnoclines and other discontinuity layers located beneath the euphotic zone. (4) Sedimenting detrital materials comprise reduced microenvironments wherein anaerobic decomposition reactions play a major role. The outward diffusion of reduced endproducts (especially NH_4^+) constitutes an inorganic detrital energy source for the in situ growth of chemolithotrophic bacteria in the ocean. To our knowledge, this "detrital electron flux" (Wetzel et al., 1972) has not been previously considered in energy budgets of oceanic ecosystems.

Figure 3 presents a schematic representation of our present conceptualization of oceanic detritus-microbe interactions. The epipelagic zone is the primary site for large organic particle production. The principle carbon pathway is the reduction of CO_2 to CH_2O via photoautotrophic processes with radiant energy providing the energy. A portion of the phytoplankton carbon produced is grazed by microzooplankton (protozoa) populations which serve as the food for larger, and subsequently even larger, consumers. At each trophic transfer there is a loss of carbon and total energy (i.e., 2nd law of thermodynamics). An important aspect of our model, however, is that these losses need not be coupled on either time or space scales. Grazing and metabolic losses at each trophic level also produce detritus, both soluble and particulate, which is also available for further decomposition and remineralization. The total rate of particulate organic matter production (POM, Fig. 3) is equivalent to a value that is derived directly from CO_2 and to that portion which is recycled (i.e., DOM → POM). Although not specifically

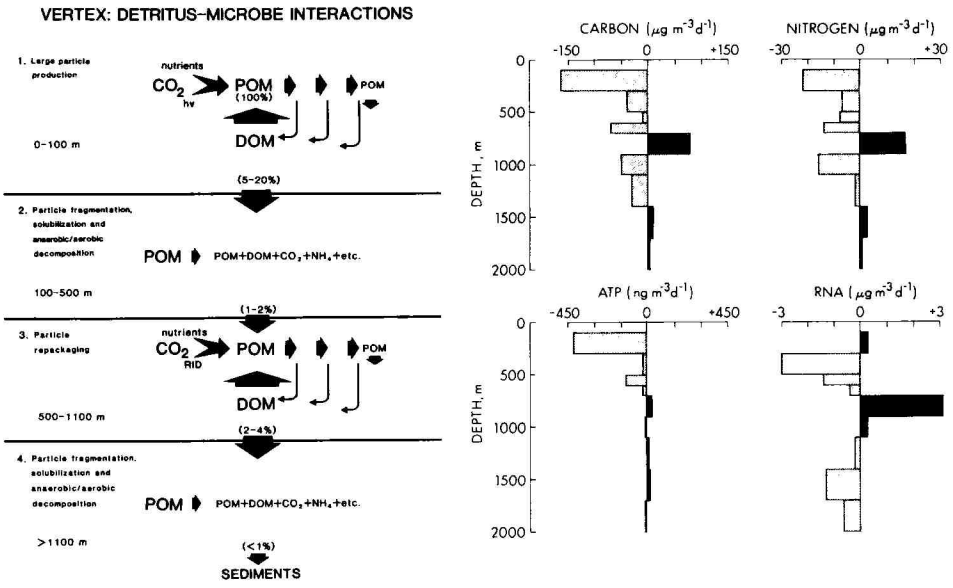


Figure 3. (Left) A generalized detritus-microbe interaction model. POM = particulate organic matter, DOM = dissolved organic matter, RID = reduced inorganic detritus. The arrows indicate pathways for carbon flux, not necessarily drawn in proportion to the actual rates. The values in parentheses are carbon flux values (expressed as a percent of total euphotic zone POM production) typically measured in the VERTEX experiment.

Figure 4. (Right) Average rates of change for the downward vertical flux of organic carbon (POC), organic nitrogen (PON), in situ ATP and RNA from VERTEX I. Negative values indicate a net loss from the sinking particles over the depth interval indicated, and positive values indicate a net gain to the downward vertical flux field. The absolute fluxes for each parameter are given in Table 1.

indicated in this present model there is also a possibility of horizontal advection or upwelling of carbon and energy from allochthonous sources.

A portion of the POM produced in the epipelagic zone is lost by sinking particles. The absolute percentage varies among oceanic habitats but generally ranges between 5 and 20% of the production rate under steady-state conditions. It should be emphasized that these data are derived primarily from carbon flux estimates without any quantitative assessment of energy content. In the upper mesopelagic zone (100–500 m), carbon flux rapidly decreases with increasing water depth. Our model suggests that the decomposition reactions are predominantly anaerobic such that the accumulation of reduced endproducts represents a type of conservation of energy by the sinking detrital ecosystems. Under these conditions carbon flux is uncoupled from energy flux.

Zone 3 (Fig. 3) is dominated by particle repackaging reactions. The principle carbon pathway is the reduction of CO_2 to CH_2O via bacterial chemolithotrophic processes. The energy source is supplied, in part, from the detrital electron flux of reduced inorganic detritus (RID) which become available as the particles start to disintegrate and eventually become well-oxygenated. Coincident with this particle disintegration is the release of large particle-associated materials into the water column to form mid-depth maxima in the concentration versus depth profiles (e.g., PO_4, NH_4^+) and in situ consumption of oxygen which results in a

distinct oxygen minimum zone. The "new" particles are rapidly recycled by metabolically active mesopelagic food webs and the production of DOM supports an active secondary production (actually tertiary production since the fixation of CO_2 in zone 3 must be viewed as secondary production in a thermodynamic sense) by indigenous populations of heterotrophic bacteria. As in the epipelagic particle production zone, the mesopelagic particle repackaging zone may also be fueled, in part, by horizontal transport processes or upwelling of allochthonous reduced carbon, energy or both. One important consequence of this mesopelagic zone particle production is that new carbon is brought into the downward flux system as evidenced by the positive rates of change of carbon flux with increasing depth in this region (i.e., compare percentage POM flux into and out of zone 3 in Fig. 3). The entire process of particle fragmentation, anaerobic decomposition and repackaging may be repeated several times but at an ever decreasing magnitude due to the second law of thermodynamics. Finally, when organic particles arrive at the sediment-seawater interface, a final cycle of chemolithotrophic bacterial production may also occur.

Although our detritus-microbe interaction model (Fig. 3) is guaranteed to eventually represent an oversimplification of the actual processes involved in the production, transport and exchange of carbon and energy in the upper 2,000 m of the ocean we feel that it is a reasonable starting point for future hypotheses regarding the metabolism of mesopelagic habitats. A brief summary of the experimental field data used to formulate this detritus-microbe interaction model is presented in the next section. A more comprehensive discussion of the VERTEX I data has been published elsewhere (Karl and Knauer, in press), and a portion of the VERTEX III results detailing the mesopelagic zone chemolithotrophic production cycle has recently appeared (Karl et al., 1984).

Experimental Data on the Detritus-Microbe Interaction Model.—Before discussing the results of our VERTEX field experiments it may be appropriate to make a few general comments on the use of sediment traps in studies of marine microbial ecology. It is now well documented and accepted that time-integrating particle interceptors (i.e., sediment traps) enable the collection of sinking particulate materials that are not adequately sampled with water bottles, nets or in situ pumps. Unfortunately, in most oceanic habitats and especially at depths exceeding 1,000 m, the sediment traps must be deployed for relatively long periods of time (>1 week) in order to provide both an adequate time frame for the calculation of accurate time-averaged mass fluxes and an adequate sample collection for subsequent chemical and microbiological analyses. A second major limitation is one of sediment trap spacing and replication. In most sediment trap investigations published to date fewer than 10 traps were used per experiment. In the VERTEX program we typically deploy 144 traps (12 depths, 12 traps per depth) on a single array but even that number has proven to be inadequate for a rigorous test of certain vertical transport and exchange hypotheses.

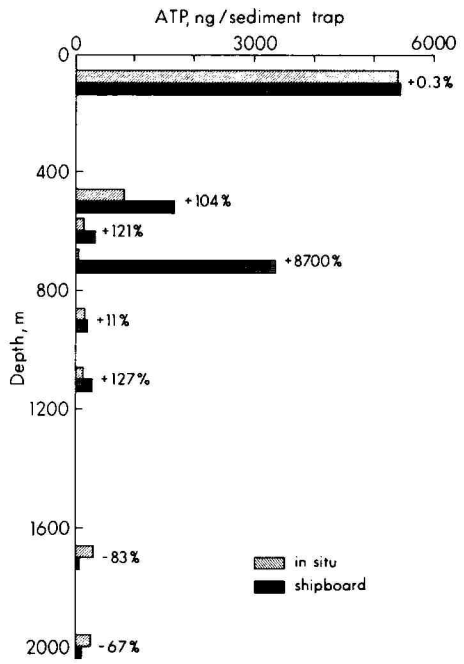
It is possible to obtain meaningful microbiological data from particles collected in sediment traps which have been precharged with preservatives, poisons or extractants. The ecological significance of these data, however, is limited to information on community structure and biomass estimates neither of which can be related to in situ rate processes. If live, microbiological studies are to be performed with sediment trap-collected materials one of two general experimental protocols must be selected. One option is to treat the materials obtained from a sediment trap as an instantaneous collection of particles which upon recovery can be mixed and manipulated experimentally using laboratory techniques and pro-

Table 1. Downward vertical flux of selected chemical and biochemical parameters measured during VERTEX I

Sediment trap depth (m)	POC flux (mg m^{-2} d^{-1})	PON flux (mg m^{-2} d^{-1})	ATP flux (μg m^{-2} d^{-1})	RNA flux (μg m^{-2} d^{-1})
100	64	10	104	878
300	31	5.6	19	929
500	23	4.2	15.9	352
600	22	3.4	3.1	213
700	15	2.0	0.7	169
900	31	5.3	3.6	782
1,100	21	2.1	3.4	843
1,400	12	1.5	3.8	788
1,700	15	2.3	6.4	389
2,000	15	2.3	5.2	221

cedures analogous to those employed with water bottle collected samples. Certain assumptions must then be made regarding the kinetics and extent of microbial growth, or death, which had occurred in the traps during the collection period, prior to sample recovery. The effects of sample mixing (i.e., destruction of microhabitats) and incubation conditions (specifically temperature and pressure) must also be assessed if the data obtained from short-term shipboard laboratory incubations are to be extrapolated to in situ conditions. An alternative to this shipboard experimental approach is to add materials (e.g., growth substrates, specific inhibitors, radiotracers, etc.) directly to the sediment trap solutions prior to deployment which allows one to perform real time in situ metabolic studies of microorganisms attached to sinking particles. This latter approach also has several major limitations and shortcomings. First, only endpoint data are available and there is no opportunity to monitor the kinetics of assimilation, growth or decomposition. Furthermore, it is not obvious what incubation time should be used for rate calculations since there is a continuous input of new biomass as a function of incubation (=deployment) time. Finally it is imperative that the traps are filled with a solution that is more dense than seawater in order to prevent the diffusive-advective loss of the added materials during the in situ experiment. However, the effects of this "high density" solution on microbial growth and viability must also be determined independently. In the VERTEX program we have used all three experimental approaches (i.e., [1] traps precharged with preservatives and/or extractants, [2] unpreserved traps which are treated as instantaneous collections of particles and [3] traps precharged with growth substrates and/or radioisotopes) to obtain information on detritus-microbe interactions.

Table 1 and Figure 4 present data on the downward vertical flux of sediment trap collected particulate organic carbon (T-POC), nitrogen (T-PON), T-RNA and T-ATP, and on the average rates of change for each parameter as a function of water depth during the VERTEX I experiment. In general, the magnitude of the downward vertical flux decreased with increasing water depth which is consistent with the empirical flux model proposed by Suess (1980) for particulate organic carbon in the ocean. However upon closer examination of the data, an apparent anomaly is evident between 700 and 900 m, the depth interval which is coincident with the dissolved oxygen minimum layer. All parameters measured displayed positive rates of change (i.e., an increase in the downward vertical flux with increasing water depth; see Table 1 and Fig. 4) suggesting deep in situ carbon production, horizontal advection of organic matter presumably derived from more productive coastal waters, or both.



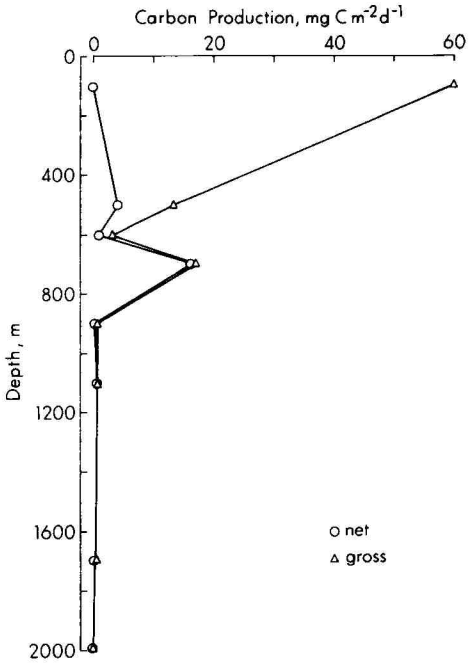


Figure 5. (Left) A comparison of in situ versus shipboard ATP values measured in the sediment traps from the VERTEX I experiment. The values given at the right of each data set indicate the magnitude and direction (i.e., + = increase; − = decrease) of the change in ATP over the duration of the experiment. % change was calculated as: [shipboard ATP − in situ ATP]/[in situ ATP] × 100%.

Figure 6. (Right) A comparison of carbon production as determined by ^{3}H-adenine (Δ) and ATP change (O) for microorganisms associated with sediment trap collected particles measured during VERTEX I.

The absolute magnitude of these mid-depth features were substantial with observed increases (i.e., [flux at 900 m − flux at 700 m]/[flux at 700 m] × 100%) of 100%, 164%, 363% and 414% for T-POC, T-PON, T-RNA and T-ATP, respectively. The large increases in T-ATP and T-RNA mass flux suggest the presence of viable microorganisms in association with the sinking particles. The calculated C:N ratio of 5.6 (by atoms) for this new material supports the hypothesis of in situ production of microbial biomass as the primary mechanism. It is important to emphasize that the observed increase in total carbon flux (16 mg m^{-2} d^{-1}) is equivalent to an in situ mesopelagic production approximately equal to 25% of steady-state flux of organic carbon out of the euphotic zone and 2–3% of the integrated rate of photoautotrophic carbon production in the surface waters.

During VERTEX I we had an opportunity to estimate the in situ rates of microbial carbon production by two independent methods: (1) a comparison of shipboard versus in situ ATP concentrations and (2) by the ^{3}H-adenine technique. Figure 5 summarizes the results of the ATP data. We consider this experimental approach as being equivalent to conducting a microbiological titration of available detrital energy using increases in microbial ATP as the metric. One major limitation of the ATP approach is the requirement for net microbial community growth. For example, even if total ATP levels had remained the same or even

decreased during the in situ incubation period (indicating zero or negative net community growth), this does not necessarily imply that there was no microbial activity of growth. In this regard the value of performing complementary, if not redundant, experiments cannot be overemphasized. Several interesting features were evident, the most distinctive being a very large positive net growth at 700 m (i.e., [shipboard ATP − in situ ATP]/[in situ ATP] × 100% = 8,700%). Although the biomass associated with the sinking particles collected at 700 m was low (as indicated by low in situ ATP concentration, Fig. 5) the potential for supporting net community growth was, by far, greater than anywhere else in the water column. We suggest that these data are indirect evidence for the input of energy-rich detritus into this portion of the mesopelagic zone (see below).

The second approach used to estimate in situ microbial production required the use of sediment traps precharged with ^{3}H-adenine. We consider the results of this technique to be more indicative of gross production since positive values can be derived even though net community growth (as derived from ATP data) may be zero or even negative. The results of this experiment indicated the presence of a substantial subsurface peak in the in situ rates of DNA synthesis at 700 m, which supports the ATP data presented in Figure 5. The rates of DNA synthesis measured for microorganisms associated with the sediment trap-collected materials can be extrapolated to estimates of total carbon production (Karl and Winn, 1984) for comparison to the T-ATP and total T-POC flux data. The extremely high rate of gross microbial carbon production, but low rate of net accumulation observed at 150 m provides indirect evidence for intense recycling and decomposition. This is consistent with the detritus-microbe interaction model presented in Figure 3. At greater depths, gross and net carbon production estimates are comparable (Fig. 6). It is important to note that both of the independent methods used to estimate in situ microbial carbon production indicate a subsurface peak of 16–17 mg C m^{-2} d^{-1} at 700 m, which is remarkably similar to the increase in total T-POC flux measured between 700 and 900 m. These data are consistent with the hypothesis of in situ chemolithotrophic production (Fig. 3) and cannot easily be explained by other mechanisms (Karl and Knauer, 1984).

It was not until VERTEX III that we had an opportunity to conduct a more rigorous test of this in situ chemolithotrophic bacterial production hypothesis. We proposed that detrital energy derived primarily from the anaerobic breakdown of particulate organic detritus (e.g., NH_4^+) was a major chemolithotrophic energy source for this mesopelagic primary production cycle. The data summarized in Figure 7 show the downward vertical fluxes and average rates of change of T-POC, T-PON, T-NH_4^+ and T-NO_2^- with depth. The large decrease in T-POC and T-PON flux between 100 and 150 m indicates an intense rate of decomposition within the warm (>25°C) surface waters. Greater than 50% of the apparent loss of N as PON appears as NH_4^+, again emphasizing the importance of anaerobic decomposition processes in microenvironments of sinking particles. This is further supported by the data presented in Table 2 which indicate that the total rates of microbial RNA and DNA synthesis in the absence of dissolved oxygen (purged), proceed at rates ranging from 80 to 99% of the oxygen sufficient control samples, further supporting the role of anaerobic decomposition processes.

At greater depths the flux of T-NH_4^+ consistently decreased suggesting a loss of T-NH_4^+ from the sinking particles. The flux of T-NH_4^+ thus calculated was 383 and 149 μmol m^{-2} d^{-1} at 150 to 550 m, respectively, indicating a loss of 234 μmol T-NH_4^+ m^{-2} d^{-1} over the depth interval from 150–550 m (Fig. 7). Since dissolved NH_4^+ was undetectable (<0.05 μmol $liter^{-1}$) in this portion of the water column, we suggest that NH_4^+ must have been consumed either in the water

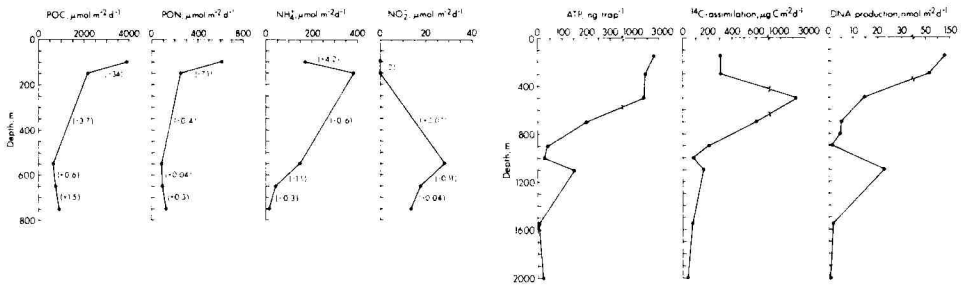


Figure 7. (Left) Downward vertical flux of POC, PON, NH_4^+ and NO_2^- measured during VERTEX III. The numbers in parentheses represent the average rates of change (μmol m^{-3} d^{-1}; see Fig. 4) for each parameter over the depth intervals indicated.

Figure 8. (Right) ATP flux, incorporation of $^{14}CO_2$ and rates of DNA synthesis measured for sediment trap materials collected during VERTEX IV. ^{14}C-assimilation assumes 100% of the cell quota is derived from CO_2 and hence is believed to represent a minimum value.

column or within the sinking particles at a rate equivalent to the measured rate of change with depth (0.6 μmol m^{-3} d^{-1} between 150 and 550 m). Concomitant with this T-NH_4^+ uptake (i.e., $-\Delta NH_4^+/\Delta Z$) is the appearance of NO_2^- in the sediment trap particles (Fig. 7). These data suggest the presence of active bacterial nitrification. As in the VERTEX I experiment, we also observed a positive rate of change for T-POC and T-PON with depth within the region of the water column where nitrification is presumed to be most intense (Fig. 7), although the absolute magnitude of this subsurface increase was not as large.

Nitrifying bacteria of the genera *Nitrosomonas* and *Nitrosococcus* were enumerated using specific immunofluorescence techniques (Ward and Perry, 1980). Nitrifiers were present in high concentrations at all depths where our sediment traps were deployed (data summarized in Karl et al., 1984). Sediment traps precharged with chemolithotrophic substrates, including NH_4^+ (see Materials and

Table 2. Effects of oxygen removal on the rates of microbial RNA and DNA synthesis during VERTEX III for particles collected at 150 m

	Treatment		
	Control	Purge	Formalin
ATP pool specific radioactivity (nCi $pmol^{-1}$)	0.44	0.38	0.00
^{3}H-RNA incorporation (nCi $trap^{-1}$)	2,132 2,096	1,432 1,505	11 9
^{3}H-DNA incorporation (nCi $trap^{-1}$)	334 347	292 289	2 2
Rate of RNA synthesis* (pmol $trap^{-1}$ day^{-1})	229	184	—†
Rate of DNA synthesis* (pmol $trap^{-1}$ day^{-1})	36.9	36.4	—

* Rates are calculated as average incorporation (nCi $trap^{-1}$) divided by endpoint average ATP pool specific radioactivity (nCi $pmol^{-1}$) measured in the sediment traps divided by the mean incubation period.
† Rates cannot be determined since the ATP pool specific radioactivity was 0.00. The minimal incorporation measured in the formalin traps is believed to represent ^{3}H-adenine binding rather than actual RNA and DNA production.

Table 3. Effects of chemolithotrophic additions on the ATP changes observed in sediment trap particles incubated in situ during VERTEX III (adapted from Karl et al., 1984)

Sediment trap depth (m)	Chemolithotrophic additions (+/−)*	In situ ATP (ng trap^{-1})	Shipboard ATP (ng trap^{-1})	ATP change†
100	−	1,760	705 ± 382	−60
	+		1,434 ± 429	−19
150	−	1,310	750 ± 6	−43
	+		556 ± 37	−57
550	−	110	748 ± 94	+580
	+		4,004 ± 543	+3,450
650	−	65	286 ± 106	+340
	+		844 ± 271	+1,198
750	−	220	662 ± 298	+201
	+		1,198 ± 184	+445

* Chemolithotrophic mix consisted of NH_4Cl, $NaS_2O_3 \cdot 5H_2O$ and $MnCl_2$ to yield final concentrations of 400 μM NH_4^+, 400 μM $S_2O_3^{-2}$ and 100 μM Mn^{+2}.
† Calculated as: [shipboard ATP − in situ ATP]/[in situ ATP] × 100%.

Methods), and incubated in situ for 20 days during VERTEX III yielded higher cell densities than the samples without the chemolithotrophic treatments. If we assume a maximum nitrification rate of 1.4 to 2 × 10^{-12} mol NH_4^+ cell^{-1} d^{-1} (Carlucci and Strickland, 1968), the particles collected at 550 m are potentially capable of oxidizing 160–230 μmol NH_4^+ m^{-2} d^{-1}, a value which is in excellent agreement with the measured T-NH_4^+ flux presented in Figure 7.

A comparison of in situ versus shipboard extracted ATP again can be used to ascertain the extent to which net microbial growth had occurred over the duration of the experiment. From the data presented in Table 3, it is evident that the two surface traps (100 and 150 m) exhibited post-incubation decreases in total ATP (i.e., shipboard ATP < in situ ATP) indicating that net microbial death had occurred. However, in that portion of the water column where NH_4^+ was consumed and NO_2^- was produced (i.e., 550, 650, 750 m), there was an increase in total microbial ATP during the incubation period and hence, net carbon production. The greatest increase occurred at 550 m (Table 3). At all three depths where net growth had occurred, we observed an increased biomass yield following the addition of potential chemolithotrophic substrates.

Microbial production associated with the sediment trap materials was also measured in two separate in situ radiotracer experiments. Replicate traps containing ^{3}H-adenine or $H^{14}CO_3^-$ were deployed to obtain independent estimates of total microbial carbon production. We fully realize and appreciate the fact that the $H^{14}CO_3^-$ incorporation data, by themselves, cannot be equated with either chemoautotrophic or chemoheterotrophic carbon production without additional information of the relative proportion of these two metabolic processes. However, in our calculations we have assumed that 100% of the total carbon is derived from CO_2 (i.e., exclusive and obligate chemoautotrophy), hence our microbial carbon production values based on the ^{14}C incorporation data are believed to represent *minimum* estimates of total microbial community growth. If, on the other hand, the $^{14}CO_2$ assimilation was derived exclusively from chemoheterotrophs through anapleurotic reactions, total community carbon production would actually be between 1 and 2 orders of magnitude greater (i.e., heterotrophic CO_2 assimilation is ~1–10% of the total carbon cell quota). The DNA synthesis technique, on the other hand, does not discriminate between autotrophic and hetero-

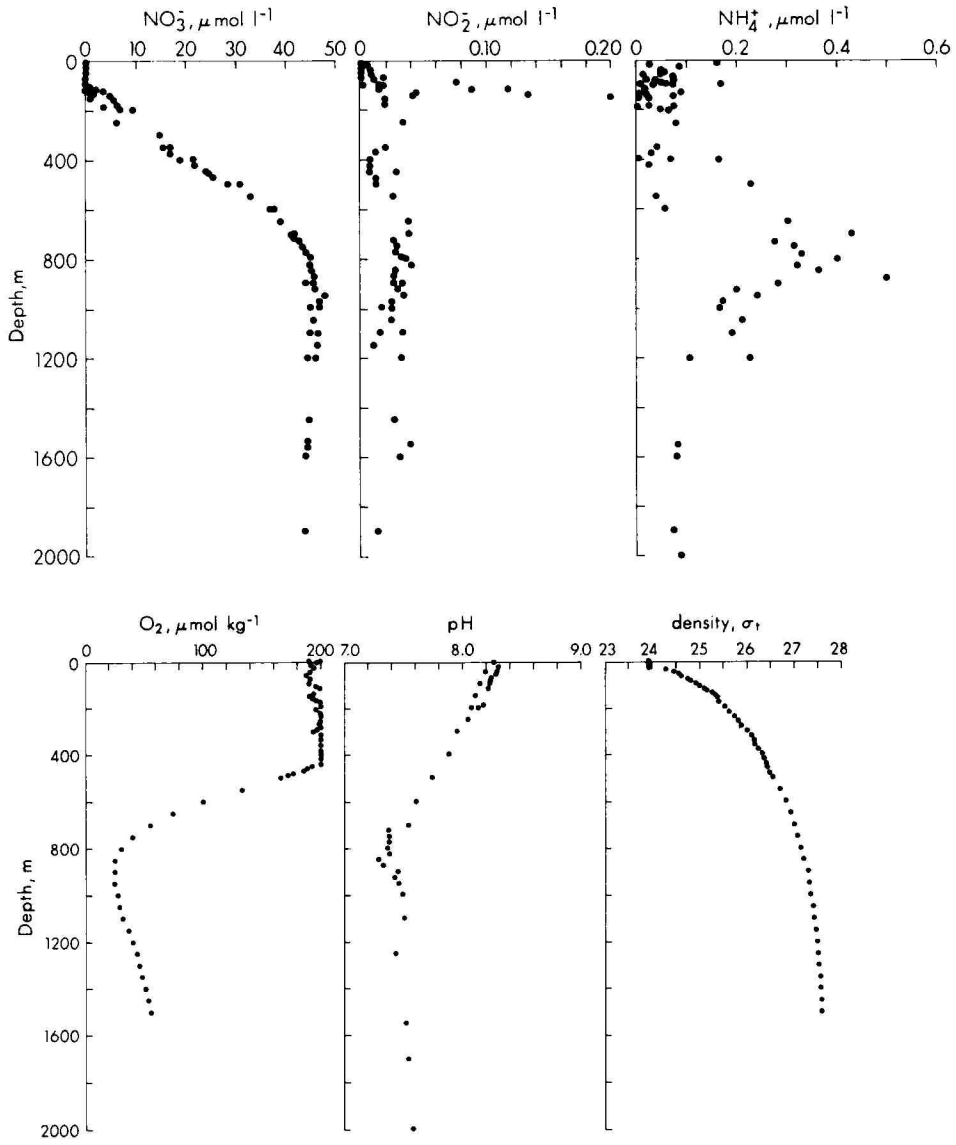


Figure 9. (Upper) Nitrate, nitrite and ammonium concentrations measured in the water column during VERTEX IV.

Figure 10. (Lower) O_2, pH and density for the water column during VERTEX IV.

trophic carbon production and consequently provides a reliable measure of total microbial carbon production (i.e., autotrophic plus heterotrophic). In the surface sediment traps (i.e., 100 and 150 m), total microbial production (derived from DNA synthesis) greatly exceeds the minimum ^{14}C-derived estimate indicating that the proportion of chemolithotrophic carbon production is relatively small (Table 4). This is consistent with the observed loss of T-POC and production of T-NH_4^+ associated with large sinking particles (Fig. 7). However, between 550 and 750 m, a substantial amount of chemolithotrophic carbon production was detected with estimates ranging up to 90% of the total ^3H-adenine derived mi-

Table 4. In situ microbial production of organic matter and estimates of bacterial chemolithotrophy during VERTEX III (adapted from Karl et al., 1984)

Sediment trap depth (m)	Minimum ^{14}C-derived microbial production* (μg C m^{-3} d^{-1})	Total microbial production† (μg C m^{-3} d^{-1})	% Production by chemolithotrophs
100	100 ± 8	15,900 ± 3,200	<1
150	163 ± 5.4	9,900 ± 750	2
550	425 ± 30	480 ± 83	90
650	69 ± 5.7	1,000 ± 17	7
750	182 ± 57	870 ± 31	21

* Calculation assumes obligate chemolithotrophic CO_2 assimilation.
† Extrapolated from measured rates of DNA synthesis.

crobial production. In this regard, chemolithotrophic carbon production may be a significant, if not dominant, pathway for carbon and energy flow at selected depths in the mesopelagic zone of the ocean.

If our working hypothesis regarding particulate organic matter decomposition, detrital electron flux and bacterial chemosynthesis is correct, then new mesopelagic microbial production (i.e., derived from CO_2 incorporation) should be observed as a ubiquitous feature of all marine environments regardless of latitude, season or distance from continents. In order to test this hypothesis, we performed a sediment trap experiment in the north Pacific gyre (VERTEX IV) where the possibility of horizontal injection of particulate organic matter and/or viable, metabolically active microorganisms derived from allochthonous sources would be expected to be minimal. Figure 8 presents a summary of the microbial biomass and production data obtained for the gyre station. The ^{14}C-assimilation data indicate the presence of a major zone of metabolic activity at 500 m, and a smaller feature centered at 1,100 m. The CO_2 assimilation at 500 m was equivalent to a production of 2.3 mg C m^{-2} d^{-1}, a value which is approximately 10% of the total

Table 5. Nutrient fluxes and average rates of change (in parentheses) versus depth for particles collected during VERTEX IV

Sediment trap depth (m)	Nitrate flux (μmol m^{-2} d^{-1})	Ammonium flux (μmol m^{-2} d^{-1})	Nitrite flux (μmol m^{-2} d^{-1})
50	0.00 (+0.002)*	2,343 (−14.2)	0.57 (+0.18)
150	0.22 (+0.15)	921 (−1.75)	18.4 (+0.018)
300	22.9 (+0.46)	659 (−2.38)	21.2 (−0.54)
500	115 (−0.30)	183 (−0.66)	130 (−0.64)
700	55 (−0.06)	51 (+0.06)	1.16 (−0.001)
900	43 (+3.81)	63 (+0.99)	1.00 (−0.005)
1,000	424 (−2.83)	162 (−0.77)	0.54 (+0.18)
1,100	140 (−0.23)	85 (−0.06)	18.4 (−0.04)
1,550	35 (+0.08)	56 (−0.10)	0.87 (−0.003)
2,000	69	11	0.75

* Average rate of change in units of μmol m^{-3} d^{-1}.

carbon flux out of the euphotic zone. This *minimum* estimate of bacterial carbon production (i.e., chemolithotrophy) is equivalent to (actually slightly exceeds) the *total* carbon production value extrapolated from the DNA synthesis data. These results suggest that the microbial production at this depth is exclusively chemolithotrophic. The estimated contribution of primary carbon production calculated for the second hot spot (1,100 m) is closer to 20% of the total microbial activity, but is nonetheless substantial. The nutrient flux data and average rates of change calculations (Table 5) once again support the in situ chemolithotrophy hypothesis indicating net consumption of NH_4^+ and concomitant NO_2^- production within the proposed nitrification zones. Detailed nutrient concentration profiles (Fig. 9) obtained from Niskin® bottle casts taken on VERTEX IV show evidence of what we consider to be an injection of particle associated NH_4^+ and NO_2^- into the water column between 400–1,100 m. At the present time we do not know whether this represents a permanent loss of detrital energy from the ecosystem, or whether the aphotic planktonic microbial community can make efficient use of these potential growth substrates. However, we suspect the latter. We further hypothesize that the vertical distribution of O_2 and pH (Fig. 10) are also influenced directly by the mesopelagic zone bacterial production which we have documented herein. A previous study in the north Pacific Ocean (Gundersen and Mountain, 1973) has clearly demonstrated that bacterial nitrification (i.e., the production of nitric acid) has a measureable effect on the pH of seawater. Furthermore, the in situ growth of nitrifying bacteria represents a potentially important mechanism for oxygen consumption (2 mol O_2 consumed per mol NH_4^+ oxidized), especially considering the characteristically low cell yields observed for nitrification values which are on the order of 0.03–0.06 mol cell C produced per mol NH_4^+ oxidized (Painter, 1970).

An important outstanding question concerns the mechanisms responsible for positioning the mesopelagic bacterial production layer observed in our field experiments. We hypothesize that the depth of occurrence is ultimately controlled directly by particle density and sinking rates. In this regard, a theoretical accumulation of organic detritus might be invoked at the $\sigma_t = 27.2$ density surface where sinking particulate matter might be expected to attain neutral buoyancy (Fig. 10; Karl et al., 1976). Subsequent in situ particle solubilization may be, in part, responsible for the various chemical signatures we observed in this portion of the water column. Previous models of bacterial consumption of oxygen in the mesopelagic zone of the ocean had always assumed that growth was heterotrophic. From the VERTEX data summarized herein, it is clear that the oxygen minimum zone may be a net exporter, rather than consumer, of organic carbon. This unexpected result may force us to alter our current views regarding the marine organic carbon cycle and especially our concepts of energy flow in mesopelagic food webs.

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