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Large particle fluxes and the vertical transport of living carbon in the upper 1500 m of the northeast Pacific Ocean

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Abstract—The distribution, production, and flux of organic carbon were measured at a station in the eastern North Pacific Ocean, off Point Sur, California. Adenosine triphosphate (ATP) and total adenine nucleotide (A_T) concentrations were measured to estimate the contribution of biomass (living) carbon to the total particulate organic pools. Two separate classes of particulate materials were considered: (1) 'suspended' or the relatively abundant, low density, fine-grained particles commonly collected in standard water bottles and (2) sediment trap particles, which represent the larger, relatively rare particles not usually sampled (in a statistical sense) when using standard water bottle collections and which require the use of *in situ* particle traps. The study indicates that living organisms associated with the sedimenting particles can contribute more than 100 times the biomass carbon normally associated with the 'suspended' fraction. The significance of the downward vertical flux of viable organisms associated with large particles may require that we alter our present concepts of the production and cycling of carbon in the open ocean.

INTRODUCTION

THE PELAGIC food web is a complex network of interactions between producers and consumers at all depths. Although the predominant synthesis of reduced carbon in the oceanic ecosystem is derived from photoautotrophic processes occurring within the euphotic zone, recent sediment trap studied by KNAUER, MARTIN and BRULAND (1979) and KNAUER and MARTIN (1981a, b) indicate that significant quantities of particulate organic matter could be collected at all depths sampled. For mesopelagic and bathypelagic organisms, the nutritional value of sinking organics is an important concern because these populations are generally limited by the quantity and nutritional quality of *in situ* food resources.

The most commonly used indices of food quality in pelagic ocean waters are the measurement of particulate organic carbon (POC) and particulate organic nitrogen (PON). For example, GORDON (1970) found that 20% of total POC in the bathypelagic region was enzymatically hydrolyzable, the remaining 80% being refractory and theoretically unsuitable for organisms to utilize. Bulk particulate carbon to nitrogen (C/N) ratios have also been used to estimate the percentage of proteins in the organic material and therefore the presumed food quality. For example, RUSSELL-HUNTER (1970) stated that if the C/N ratio for animal food intake was >17 there was a protein deficiency. A typical

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profile of C/N ratios (GORDON, 1971) indicates that surface particles have a lower ratio (C/N = 7.5), thus a higher presumed nutritive value than bathypelagic particles (C/N = 15).

The use of bulk chemical compositional data (i.e., C/N ratios) may have serious limitations as a measure of food quality. NEWELL (1965), DARNELL (1967), and others (reviewed by FENCHEL and JØRGENSEN, 1977) have suggested that certain refractory particles (i.e., those with C/N ratios >15) may serve as substrates for the growth of heterotrophic microbial populations with low (3 to 5) C/N ratios and consequently high nutritive value. When these surface-coated particles are ingested by detritivores the microorganisms are selectively removed and digested and the original (high C/N ratio) particle is excreted to be recolonized. KNAUER and AYERS (1976) have also emphasized the fact that total C/N ratios may be misleading in terms of estimating potential food quality and stressed the importance of measuring the living microbial carbon component as well.

The measurement of adenosine triphosphate (ATP) has been used extensively in ecological studies as a measure of total biomass carbon ever since its original introduction by HOLM-HANSEN and BOOTH (1966). The rationale, methodology, application, and limitations of such measurements in ecological studies have been reviewed by KARL (1980). One useful application of ATP measurements has been the estimation of biomass carbon relative to the total POC present in various marine ecosystems. Extensive studies (also in KARL, 1980) have revealed that the fraction of living to total organic carbon measured in 'suspended' matter decreases from 30 to 100% in the euphotic zone (0 to 100 m), to 5 to 10% at 200 to 300 m, and eventually to <1% at depths >1500 m. Therefore, it appears that both the concentrations of total POC and PON and the ratio of living carbon to total carbon are very low in 'suspended' oceanic particles beneath the euphotic zone.

Recent *in situ* investigations of surface waters using SCUBA techniques together with particle trap collections from deeper waters have yielded new evidence that the deep sea may not be as nutritionally depauperate as previously believed. For example, rapidly sinking large particles (i.e., >35 μm) may be the major contributors to particle flux in surface waters in addition to serving as nutritive food sources by providing habitats for macro and microorganisms (e.g., see ALLDREDGE, 1972, 1979; HAMNER, MADIN, ALLDREDGE, GILMER and HAMNER, 1975; SILVER, SHANKS and TRENT, 1978; TRENT, SHANKS and SILVER, 1978). ALLDREDGE (1972, 1979) estimated that large aggregates may constitute between 20 and 30% of the total POC while SHANKS and TRENT (1980) concluded that in Monterey Bay, California, surface waters, >80 and 97% of the carbon and nitrogen fluxes, respectively, are from similar aggregates.

Although such large particles may be a major source of food for deep-living organisms, they are relatively rare (1 to 4 aggregates per liter). MCCAVE (1975) pointed out that standard sampling techniques (e.g., water bottles) do not adequately sample such large, fast-sinking particles, but instead collect only the small, slow-sinking background material commonly referred to as particulate organic matter (i.e., 'suspended' POC, PON, ATP, etc.). Thus, the probability of sampling a representative particle spectrum using bottles is small (also see LAL, 1977). Consequently, alternate sampling schemes using sediment traps have been designed to obtain both qualitative and quantitative information on the flux of fast-sinking particles (WIEBE, BOYD and WINGET, 1976; HONJO and ROMAN, 1978; SPENCER, BREWER, FLEER, HONJO, KRISHNASWAMI and NOZAKI, 1978; MÜLLER and SUESS, 1979; KNAUER *et al.*, 1979; ROWE and GARDNER, 1979; HONJO, 1980). However, most of the initial studies used gross chemical investigations of trapped particles in the sense that organic

constituents were assessed as 'bulk' (i.e., total) carbon and nitrogen. Changes that might occur in the quality (i.e., specific chemical composition) were not considered. As ecological questions concerning the production, diagenesis, and decomposition of organic matter in the marine environment become more specific, an evaluation of the individual components of the total organic material flux (e.g., living vs non-living, bacterial carbon vs 'other', etc.) will be required.

During a recent cruise in the eastern North Pacific Ocean a MULTITRAP array (KNAUER *et al.*, 1979) was deployed with individual collectors at five depths ranging from 60 to 1550 m. The experiment was designed to examine the relationships between production, distribution, and sedimentation of living and non-living particulate carbon in the open ocean. It is the purpose of this paper to report our initial findings on the importance of rapidly sinking particles in transporting viable microbial assemblages to depth.

MATERIALS AND METHODS

Sediment trap design and deployment

Particulate materials falling through the water column were collected using a free-floating MULTITRAP system as described by KNAUER *et al.* (1979). The system consisted of 10 to 12 individual cast acrylic collection traps at each of five depths mounted on a polyvinyl chloride (PVC) cross frame. The mouth (0.0039 m^2) of each trap was fitted with a baffle and the bottom section with a polyethylene collecting cup containing a GF/F filter (nominal pore size = 0.7 μm). Prior to deployment, each trap was thoroughly cleaned with Micro® (a laboratory detergent) and 10% HCl, then filled with a high density solution (see below) to prevent flushing. Of the 60 individual traps deployed, 15 were used for nucleotide analyses. The remainder were used for trace metal, chitin, carbon, and nitrogen determinations. Only the nucleotide data are presented in this report.

Two to three traps on each cross were used for *in situ* extraction of cellular nucleotides. The 1550-m trap was never less than 1800 m above the bottom, so that contributions due to sediment resuspension can be ruled out. The array was set at a station approximately 80 km southwest of Point Sur, California (36°06.6'N; 122°46.5'W) and allowed to free-float for 4½ days. Essentially no drift was observed during the deployment.

In situ *nucleotide extraction*

Sedimentary particulate materials were extracted *in situ* for their complement of cellular nucleotides. Each nucleotide collector tube was filled with 2 l of a high density acid–salt solution (0.5 M H_3PO_4, 1.5 M NaCl). The use of phosphoric acid ensures a maximum efficiency of extraction and preservation of *in vivo* nucleotide levels (KARL and CRAVEN, 1980). Sub-samples of the dense solution were processed as blanks. No ATP or A_T was detected in the blanks. Control collector tubes containing the H_3PO_4–NaCl solution and a known concentration of ATP were permanently sealed and deployed with the sample traps to monitor the temporal stability of ATP in the acid–salt medium at *in situ* temperatures and pressures. No hydrolysis of ATP was detected during the experiment (about 5 days); enzymatic hydrolysis of ATP was assumed to be negligible under the experimental conditions (pH < 0.1).

Immediately following recovery of the traps, the dense solutions containing the extracted

cellular nucleotides were drained through a pre-weighed GF/F glass fiber filter positioned at the bottom of each collector tube into acid-washed bottles (because of the fragility of wet GF/F filters we now use Nuclepore® membrane filters). The total volume was recorded and triplicate 7-ml sub-samples were removed, combined with 5 ml of Tris buffer (0.02 M, pH 7.7) plus 2 ml of EDTA solution (0.048 M in Tris buffer) and titrated to pH 7.7 with NaOH. The neutralized cellular nucleotide extracts were stored frozen (−20°C) for shore-based analyses conducted within one week. The residual particulate materials collected on the GF/F filters were subsequently re-extracted by homogenizing in H_3PO_4. No ATP or A_T was detected in these extracts, indicating that the initial *in situ* extraction was complete. The seawater overlying the acid–salt solutions in several collector tubes was also sampled and analyzed for dissolved ATP. No ATP was detected in any of the samples, indicating that the ATP extracted *in situ* was not readily lost to the overlying seawater. This has subsequently been confirmed by monitoring the recovery of radioactive adenine added as an internal standard to determine advective losses (D. KARL, unpublished data).

Shipboard nucleotide extraction

In addition to the nucleotide extractions conducted *in situ*, seawater samples were also collected in acid-washed 5-l Niskin® bottles and processed for particulate nucleotides. Between 150 and 1500 ml were filtered through Reeve Angel® glass fiber filters (984-H) and the concentrated particulate materials extracted by boiling for 5 min in PO_4 buffer (65 mM, pH 7.4). All extracts were analyzed at sea for ATP content and were then stored frozen (−20°C) for additional nucleotide analyses ashore.

Nucleotide measurements

ATP, ADP, and AMP were measured using the firefly bioluminescence coupled reactions described by KARL and HOLM-HANSEN (1978). A_T is defined as the total adenine nucleotide concentration (i.e., ATP molar equivalent of [ATP]+[ADP]+[AMP]) and EC_A as the adenylate energy charge ratio (KARL and HOLM-HANSEN, 1978).

The ATP concentrations of the 1100 and 1550-m trap extracts were at, or slightly below, the detection limits of the assay procedure and the samples were concentrated to obtain more reliable measurements. Ten milliliters of each extract were added to a clean test tube containing 0.5 ml of a slurry consisting of acid-washed activated charcoal and celite (HODSON, HOLM-HANSEN and AZAM, 1976). As adsorption and desorption efficiencies have been shown to be concentration dependent (IRELAND and MILLS, 1966) and because charcoal treatment may catalyze the hydrolysis of ATP (KARL and HOLM-HANSEN, 1978), a series of ATP standards was treated in parallel. The nucleotides were allowed to bind for 5 min and the charcoal was collected by high-speed centrifugation and eluted by two successive rinses of 5 ml each with ammoniacal ethanol (HODSON *et al.*, 1976). The eluates were combined, evaporated to dryness *in vacuo* at 40°C, and reconstituted in 500 µl of distilled water. The samples were assayed as described previously. As neither the binding nor elution efficiencies of ADP and AMP were determined in these latter experiments, A_T and EC_A could not be calculated for the 1100 and 1550-m samples.

Zooplankton nucleotide determinations

To evaluate and correct for the contribution of swimmers (KNAUER *et al.*, 1979) to the apparent gross flux of ATP (and A_T) at each depth interval, measurements were made of the nucleotide content of various zooplankton taxa. Representative members of the

zooplankton community were hand-picked from vertical net hauls (202 μm mesh). Swimmers smaller than 202 μm generally represented <2% of the total swimmer mass. Due to potential nucleotide transitions associated with capture stress (SKJOLDAL and BÅMSTEDT, 1977), only actively swimming individuals were selected for the studies. The data base required for the subsequent calculations of percent contribution of the zooplankton was restricted to those zooplankton extracts that yielded EC_A ratios between 0.75 to 0.9 (i.e., no apparent 'metabolic stress').

The cellular nucleotides were extracted by dropping each organism into a scintillation vial containing 10 ml of the H_3PO_4–NaCl extraction menstruum. The carcasses were allowed to extract at 20°C for 4½ days. The zooplankton remains were then removed and the extracts neutralized as described previously. The particulate materials were rinsed with deionized water and dried to constant weight. Selected samples were combusted for estimates of total organic carbon. Previous zooplankton ATP and carbon studies by TRAGANZA and GRAHAM (1977) reported carbon losses of up to 25% using boiling buffer extraction procedures. Although some loss of cellular carbon undoubtedly occurred during our acid extraction procedure as well, the C/ATP and C/A_T ratios obtained were comparable to published values for marine zooplankton (data summarized by KARL, 1980).

Contribution of swimmers to total nucleotide flux

As various zooplankton species were found in all MULTITRAP collectors, it was necessary to calculate their contribution to the total ATP (and A_T) measured at each depth. The particulate materials from each sample were examined under a dissecting microscope and the remains of the contaminating zooplankton were carefully removed, rinsed with deionized water, and dried to constant weight. Partially decomposed zooplankton and exuviae were not removed (KNAUER *et al.*, 1979). The mean ATP/dry weight and A_T/dry weight values calculated for the selected representative taxa discussed above were used to estimate the total ATP and A_T represented by the dry weight of swimmers collected in each trap. Because copepods were the numerically dominant swimmers collected at all depths (30 to 40% at 1100 m to >95% at 60 m), 'copepod-weighted' ATP/dry weight and A_T/dry weight values were used for each depth. For 60, 210, and 1550 m, the ATP/dry weight and A_T/dry weight values used were 0.292 and 0.437 (wt/wt), respectively. For the samples collected at 385 and 1100 m, the values were 0.334 and 0.50, respectively. The mean C/dry weight ratio measured for the swimmers collected at all depths was 0.39 ± 0.05, representing C/ATP values ranging from 115 to 132, which are in agreement with published values (BALCH, 1972; TRAGANZA and GRAHAM, 1977; SKJOLDAL and BÅMSTEDT, 1977). Net ATP concentrations were determined after subtracting the contribution of the swimmers from the total ATP measured in each collector tube.

Primary production estimates

All primary production measurements were derived using metal-free collection and processing procedures (KNAUER and MARTIN, 1981b). Water samples were inoculated with $H^{14}CO_3^-$ and incubated *in situ* for periods ranging from 4 to 6 h. Primary production was calculated from the equations in STRICKLAND and PARSONS (1972).

Measurement of rates of stable RNA synthesis

Total rates of stable RNA synthesis by the microbial community were determined in the water column using the methods devised by KARL (1979) and modified by KARL, WINN and

WONG (1981). Water samples were collected in specially prepared (KNAUER and MARTIN, 1981b) 5-l Niskin® bottles from 11 depths ranging from 1 to 723 m. For each depth, a Micro® acid-washed 500-ml polycarbonate bottle was filled to within 5 ml of capacity, 50 μCi [2, ^{3}H]-adenine added (in 0.5 ml of sterile seawater), the contents mixed thoroughly, and 1 ml immediately removed for liquid scintillation counting. The bottle was then carefully filled to capacity, capped, and returned to its original depth in the water column. Duplicate samples were prepared for depths of 73, 123, 173, 273, 323, and 473 m. The samples were incubated *in situ* for between 7.5 h (1 m) and 8.33 h (723 m), depending upon their relative position on the line. The incubations were terminated by filtration and processed for total ATP, specific radioactivity of the ATP pool, RNA, and rates of RNA synthesis as described previously (KARL, 1979; KARL *et al.*, 1981).

Ancillary chemical measurements

Organic carbon and organic nitrogen were determined in samples of selected zooplankton, in materials collected from several MULTITRAPS, and in suspended particulate materials collected from the water column, using a Hewlett-Packard Model 185-B CHN analyzer.

Chlorophyll *a* was extracted from 47-mm GF/F filter-concentrated (500 ml) euphotic zone seawater samples as described in STRICKLAND and PARSONS (1972) and measured fluorometrically (Turner, Model 111).

Dissolved oxygen concentrations were determined using the Winkler technique on samples collected in 5-l Niskin® bottles.

RESULTS

Distribution and abundance of organic matter in the water column

Concentration profiles of ATP, A_T, and POC and PON from water column samples taken adjacent to the free-floating MULTITRAP array are presented in Figs 1 and 2. The ATP and A_T distributions represent composite data collected over several days and indicate that both the absolute concentrations of nucleotides in suspended particles and the EC_A ratios are similar to the data for samples collected earlier in the Southern California Bight (KARL and HOLM-HANSEN, 1978).

The ATP and chlorophyll *a* data were used to estimate total living carbon and phytoplankton carbon, respectively. A first approximation of total living carbon can be obtained by multiplying the ATP values by a factor of approximately 250 ($\pm$100; KARL, 1980). We assume that chlorophyll $a \times 54$ ($\pm$17) is roughly equivalent to total phytoplankton biomass-carbon (EPPLEY, HARRISON, CHISHOLM and STEWART, 1977; $\bar{X}$ of all C/chlorophyll *a* ratios determined by method 6, p. 682). Comparisons can then be made between ATP-derived living carbon (biomass carbon), chlorophyll *a*-derived phytoplankton carbon, and total particulate organic carbon (living + non-living). The results (Table 1) indicate that between 29 and 39% of the total carbon in the euphotic zone (0 to 50 m; 1% light level as determined with a submarine photometer) is living carbon, the majority of which is phytoplankton carbon. This is also reflected in the 0 to 50-m C/N ratios (Fig. 2), which approximate the Redfield ratio of 6.6 (REDFIELD, KETCHUM and RICHARDS, 1963). With increasing water depth, the ratio of living carbon to total POC

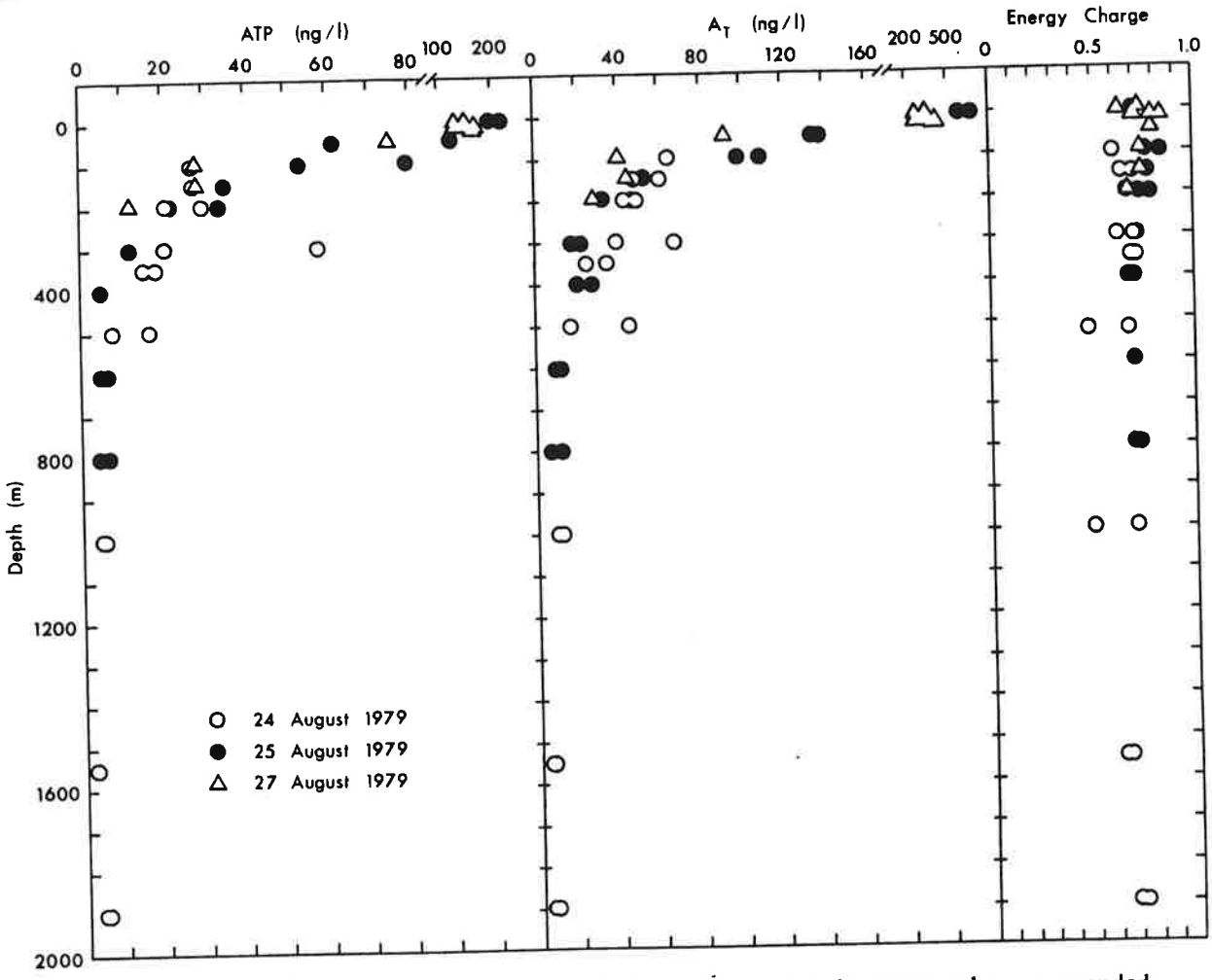


Fig. 1. Vertical distribution of ATP, A_T, and energy charge for the water column suspended particulate materials on three separate days during the period of the MULTITRAP collections.

decreases to a value of $<5\%$ at depths >200 m, which is consistent with data published by HOLM-HANSEN and BOOTH (1966), HOLM-HANSEN (1969), EPPLEY *et al.* (1977), and others.

Production of living 'background' particulate organic matter

The total production of organic matter was estimated by two independent methods. Primary production was measured in the euphotic zone; the net daily carbon fixation averaged 760 mg C m^{-2}. Primary productivity measurements as routinely conducted do not account for carbon lost from the cells as exudates. Such reduced organic matter, however, is available for assimilation by microheterotrophs into particulate carbon, which may re-enter the marine food web (POMEROY, 1974). Therefore, the rates of stable RNA synthesis were measured in an attempt to estimate total 'suspended' particulate organic carbon production, excluding production associated with rare, rapidly sinking particles (Fig. 3). 'Total' daily RNA synthesis decreased from 1500 pmoles of adenine incorporated into RNA l^{-1} in the top 0 to 75 m of the water column, to a value of 35 pmoles adenine incorporated at 275 m. A subsurface peak was evident at 325 m (162 pmoles adenine incorporated), below which the total rate of RNA synthesis decreased with increasing water depth (Fig. 3a). The specific rates of RNA synthesis (rate per unit ATP-derived biomass) exhibited subsurface peaks at 75 and 325 m. The latter production maximum corresponded

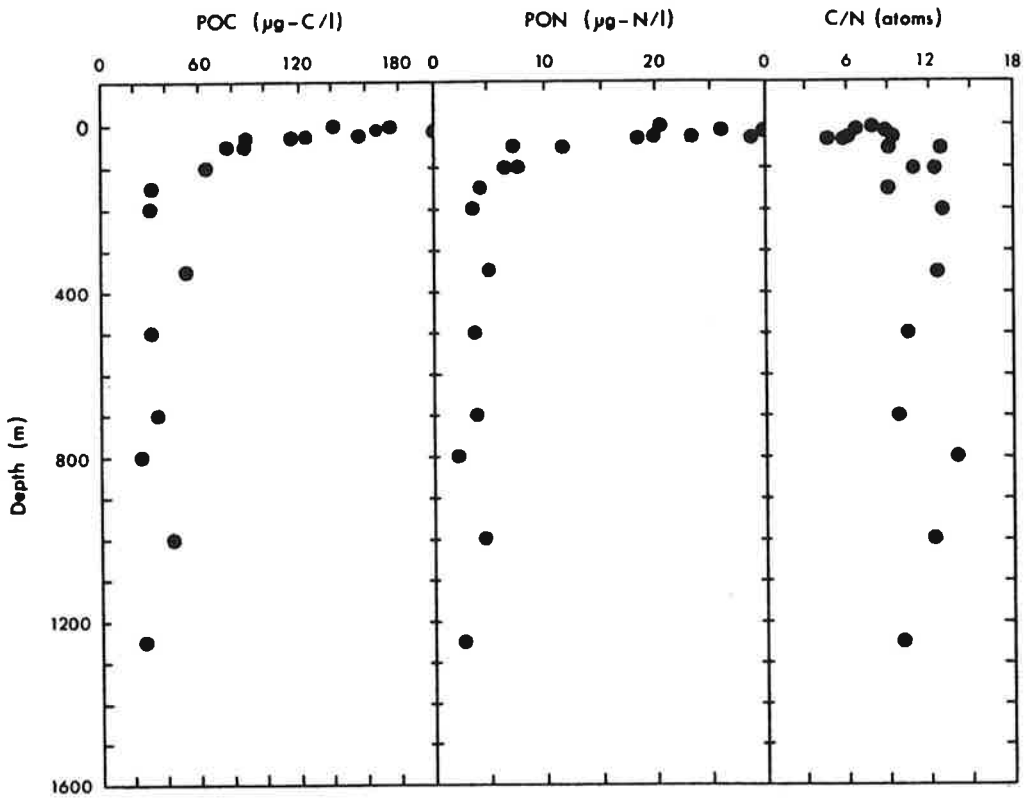


FIG. 2. Particulate organic carbon (POC), nitrogen (PON), and C/N ratios for the water column suspended particulate materials during the period of the MULTITRAP collections.

Table 1. *Biomass carbon, phytoplankton carbon, and particulate organic carbon (POC) derived from 'suspended' particles collected in 5-l Niskin® bottles*

Depth (m)	Total biomass-C* ($\mu g\ C\ l^{-1}$)	Total phytoplankton-C† ($\mu g\ C\ l^{-1}$)	Total POC ($\mu g\ C\ l^{-1}$)	Biomass-C (%) POC	Phytoplankton-C (%) POC
1	51.3	42.4	158	32	27
10	53.3	45.6	183.6	29	25
25	53.3	43.4	139.2	38	31
50	32	6.8	82.2	39	8
100	11	—	66	17	—
150	7.75	—	32	24	—
200	6	—	31	19	—
350	3.5	—	53	7	—
500	2.2	—	32	7	—
700	1.38	—	35	4	—
1000	1.3	—	44	3	—
1250	0.5	—	27	1.8	—

* Based upon the assumption that (ATP) × 250 = biomass carbon (see text for details).
† Based upon the assumption that (chlorophyll *a*) × 54.3 = phytoplankton carbon (see text for details).

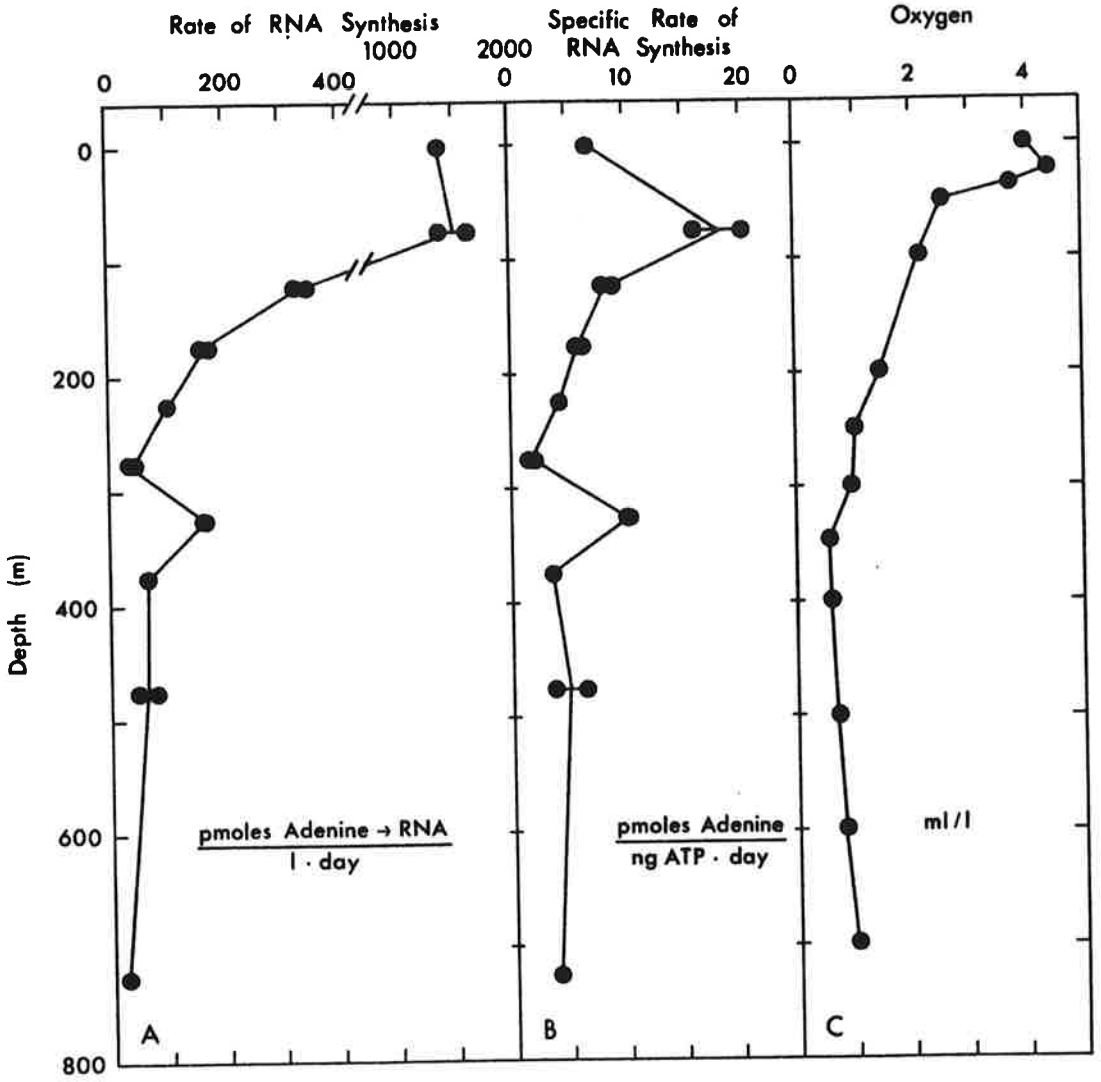


Fig. 3. Rates of RNA synthesis, specific rates of RNA synthesis, and dissolved oxygen concentrations for the water column during the period of the MULTITRAP collections.

to the depth of the oxygen minimum zone. The results support the previously advanced suggestion that subsurface maxima exist in the metabolic activity and growth of heterotrophic microorganisms (KARL, LAROCK, MORSE and STURGES, 1976; KARL, 1979).

Comparison of production estimates

By converting pmoles adenine incorporated into RNA per liter per day to grams [AMP = 347 pg pmol^{-1}] and scaling to total RNA (AMP = 25 mole % RNA) a mean value of 2.1 μg RNA per liter produced daily is derived for 0 to 100 m in the water column. If we assume that RNA ranges from 5 to 15% of the dry weight of phytoplankton cells (D. KARL and E. LAWS, unpublished data) and that organic carbon is 50% of the dry weight, then the average daily production in the upper 100 m is 7 to 21 μg C l^{-1}, based on the measurement of stable RNA synthesis. By comparison, the average daily carbon production derived from ^{14}C measurements is 9 to 22 μg C l^{-1}. As expected, these results indicate the importance of phytoplankton as producers of organic matter in the euphotic zone. It should be emphasized, however, that the production of particulate organic carbon does not cease when net primary production goes to 0 (75 to 100 m), but instead, significant

rates of heterotrophic or secondary production of particulate organic materials continue well into the aphotic zone of the ocean. For example, at the oxygen minimum layer (325 to 350 m; Fig. 3c), the total rate of RNA synthesis is 10% of that in the productive surface layer and the specific rate (rate per unit biomass) is within a factor of two of the surface value (Fig. 3b). These results indicate the presence of a substantial and metabolically active heterotrophic population at the oxygen minimum layer, at least in terms of 'suspended' particles.

Sedimentation of organic matter

Nucleotide concentrations of trapped materials. A summary of the uncorrected nucleotide data derived from the *in situ* extraction of sedimenting particulate materials is presented in Table 2. The total ATP collected in the MULTITRAPS was greatest in the euphotic zone (60 m) samples and decreased gradually with increasing water depth. A similar trend was observed for A_T in the samples measured. Replication between traps at a given depth is often poor for both ATP and A_T, which, in our opinion, is a direct result of the variable contribution of swimmers to the gross adenine nucleotide measurements.

By comparison, the EC_A was constant between individual traps at a given depth. This indicates a consistent co-variation in the relative molar concentrations of ATP, ADP, and AMP for all samples collected at a given depth. On the other hand, significant changes in EC_A were apparent between individual sampling depths. In surface collections, the EC_A ratios were consistently lower (0.58 to 0.62) than they were at greater depths in the water column (e.g., 0.72 to 0.76 at 210 m; 0.75 to 0.77 at 385 m; Table 2). For reasons stated in the Materials and Methods section, A_T and EC_A were not measured for the 1100 and 1550-m samples.

Corrections of ATP and A_T for swimmers. A summary of the data used to correct for the occurrence of trapped zooplankton (i.e., swimmers) in each sample collection is presented in Table 2. The dry weight of swimmers ranged from a minimum of 0.03 mg per trap at 1100 m to a maximum of 19.6 mg per trap at 60 m. When converted to ATP, this represented a contribution of 1.3 to 100% of the total measured ATP concentrations and further emphasizes the importance of correcting the total concentrations for particles that are not truly part of the passive downward vertical flux.

In several instances, the zooplankton captured in the traps appeared to account for 100% of the total ATP (and on two occasions, 100% of A_T). Under these conditions our proposed method for swimmer correction is not precise enough to calculate reliable net nucleotide fluxes (see below). Future experiments of this type would benefit substantially from methods to decrease the numbers of actively swimming zooplankton species entering the gradient solutions.

Corrected net ATP flux. Table 3 presents data on the net ATP concentrations, downward vertical ATP flux, and a relative comparison between this flux and the suspended particulate ATP concentrations at each of the five depths investigated. The 60, 210, and 385-m values lack precision due to the inaccuracies involved in correcting for the swimmers. However, the duplicate collections at 1100 and 1550 m, where the swimmers were not so important quantitatively, show excellent between-sample agreement (Table 3). The most significant conclusion resulting from this compilation of data is the indication of a relatively high net ATP flux below 385 m, especially when compared to the *in situ* or suspended particulate ATP concentrations. We cannot over-emphasize the importance of

Table 2. Total and swimmer-contributed ATP and A_T in the MULTITRAPS. Total swimmer dry weight, estimated swimmer dry weight/ATP, dry weight/A_T ratios, and measured energy charge (EC_A) values are presented for completeness

Depth (m)	Total ATP (μg/trap)	Total A_T (μg/trap)	EC_A ratio	Swimmer dry wt (mg/trap)	Swimmer dry wt/ATP*	Estimated swimmer ATP (total μg/trap)	Swimmer dry wt/A_T*	Estimated swimmer A_T (total μg/trap)
60	40.7	80.3	0.62	3.98	342	11.6	229	17.4
	67.0	138.4	0.58	19.61	342	57.3	229	85.6
210	13.5	22.8	0.72	6.77	342	19.8	229	29.6
	14.5	23.1	0.76	4.48	342	13.1	229	19.6
	25.9	40.5	0.74	9.21	342	26.9	229	40.2
385	12.4	18.6	0.75	3.64	299	12.2	200	18.2
	7.7	12.1	0.75	1.57	299	5.3	200	7.9
	7.4	11.4	0.77	2.43	299	8.1	200	12.2
1100	8.1	—†	—	0.13	299	0.43	200	0.65
	7.1	—	—	0.03	299	0.09	200	0.14
1550	11.6	—	—	2.00	342	5.85	229	8.7
	6.5	—	—	0.34	342	1.00	229	1.48

* Estimated from fresh net zooplankton ATP, A_T/dry weight relationships and related to weight of swimmers removed from traps (see text for details).
† Value not determined (see text for details).

*Table 3. The relative importance of large-particle ATP (calculated from swimmer-corrected ATP flux) to suspended ATP**

Depth (m)	Trap	Net ATP† (μg/trap)	ATP flux (μg m^{-2} day^{-1})	Suspended ATP (μg m^{-3})	ATP‡ flux/suspended
60	A-1	29.1	1425.1	128	12.9
60	A-2	9.69	552		4.3
210	B-1	—§	—	24	—
210	B-2	1.43	81.5		3.4
210	B-3	—	—		—
385	C-1	0.20	11.4	13.5	0.8
385	C-2	2.42	137.9		10.2
385	C-3	—	—		—
1100	D-1	7.67	437	5	87.4
1100	D-2	7.01	399		80.0
1500	E-1	5.75	328	2	164
1500	E-2	5.50	313		157

* Suspended refers to materials trapped in a standard Niskin® water bottle.
† Corrected for contribution from swimmers as in Table 2.
‡ See text for ATP flux/suspended calculations in terms of assumptions used for dimensional comparison.
§ Estimates could not be made due to insufficient resolution of the swimmer correction.

correcting the total (or gross) ATP fluxes for entrapped swimmers. KNAUER and MARTIN (1981a) discussed this potential source of error with regard to C, N, and Zn fluxes. Failure to remove the remains of these organisms could lead to over-estimates (in some cases, >300%) of the sedimentation of passive particulate materials.

DISCUSSION

The magnitude and vertical distributions of 'suspended' ATP, A_T, and EC_A measured during the course of this experiment (Fig. 1) conform to the data previously reported from eastern North Pacific locations at comparable depths and of comparable productivity (HOLM-HANSEN and BOOTH, 1966; HOLM-HANSEN, 1969; KARL and HOLM-HANSEN, 1978). The values calculated for the fraction of living carbon to total POC and of phytoplankton carbon to living carbon are also consistent with published results (HOLM-HANSEN and BOOTH, 1966; EPPLEY *et al.*, 1977). There are no existing data with which to compare our vertical ATP flux measurements.

Because the present study was concerned with the extraction and measurement of soluble metabolites that are present in all living organisms, the mere removal of zooplankton carcasses from the collected materials in each trap was not adequate to eliminate probable contamination. Instead, a fairly involved and time-consuming set of experiments had to be conducted to provide the data necessary to extrapolate the measured swimmer dry weight to ATP and A_T. The estimate of swimmer-derived adenine nucleotides was subtracted from the total ATP and A_T measured in the individual traps to yield net adenine nucleotide values. The data (Table 2) provide an indication of the magnitude of this correction factor. This potential source of contamination would similarly result in an over-estimate of the fluxes of all materials having a biogenic source component (e.g., C, N, P, specific organic

constituents, trace metals, etc.). The quantitative significance of swimming zooplankton undoubtedly varies in time and space and is likely to be affected by the size and configuration of the sediment traps and possibly by the presence or absence of a poison in the collector.

The net ATP (net ATP = total ATP − zooplankton derived ATP) flux decreased to a value of approximately 10% of the surface value at 385 m (Table 3). The suspended ATP concentrations decreased similarly over this depth interval, suggesting that the downward flux of ATP in the upper 385 m of the water column is proportional to the standing stock of living carbon. At greater depths, the net flux of living carbon increases and remains fairly high (20 to 25% of the surface), even at 1550 m (Table 3). The apparently increased flux is even more surprising if one compares the downward vertical flux data with the 'suspended' ATP concentrations, which continue to decrease with increasing water depth. A similar increased flux has also been reported for organic carbon, organic nitrogen (KNAUER and MARTIN, 1981b), and total number of fecal pellets (URRÉRE and KNAUER, 1981), a situation the authors interpreted to represent *in situ* 'repackaging' of organic materials via feeding activities of zooplankton.

Previous investigators have attempted to compare carbon fluxes at a particular depth in the water column with estimates of water column net primary production. This assumes that the production of particulate organic matter is restricted to the surface waters and specifically to the process of photosynthesis. Both assumptions are equivocal. As indicated in Fig. 3a, stable RNA synthesis can be detected at depths well below the euphotic zone. This production of particulate organic matter presumably occurs at the expense of dissolved organic nutrients, which are ultimately derived from photosynthetic processes. Exudation, excretion, cell death and lysis, grazing losses, and dissolution of particulate organic materials all contribute to the maintenance of a quasi steady-state dissolved organic carbon (DOC) pool that generally exceeds the concentration of 'suspended' particulate carbon in seawater by a factor of 5 to 50 (MENZEL, 1974). Bacteria and other microheterotrophic organisms can use such soluble nutrients and 'repackage' them into a more concentrated form of particulate carbon, which can then re-enter the marine food web. POMEROY (1974) estimated that the DOC → bacteria → POC route may increase the utilizable yield of organic matter from photosynthesis to a value 30% higher than we estimate from standard primary productivity procedures. The increased flux of ATP at depths below 385 m (Table 3) may be the result of such a microbial 'repackaging'. Several previous studies of bacterial biomass and productivity in the ocean have resolved intermediate depth maxima, usually corresponding to the depth of the oxygen minimum (KARL *et al.*, 1976; KARL, 1979). A similar feature was evident in the present study (Fig. 3a, b). Although the *in situ* activities of heterotrophic microorganisms may be the primary mechanism for the production of particulate ATP beneath the euphotic zone, there must exist some mechanism for supplying reduced carbon to this environment to maintain such heterotrophic activities. Several possible processes include: (1) the accumulation of sinking particulate materials at density discontinuities within the water column (KARL *et al.*, 1976); (2) an input of fecal matter from migrating zooplankton populations (URRÉRE and KNAUER, 1981); and (3) horizontal advective processes (i.e., lateral transport along isopycnals). In all probability it is a combination of the above mechanisms.

To assess the importance of the rapidly sinking trapped particles relative to 'suspended' particles, ATP flux and suspended ATP concentrations must be compared. However, as the sinking rates of the materials collected at each depth are unknown, the flux

(g ATP m^{-2} day^{-1}) and the suspended concentrations (g ATP m^{-3}) cannot be compared directly. For the purpose of this discussion, we will assume that a reasonable sedimentation rate for trap-collected particles at the station we investigated was at least 1 m day^{-1}. The assumption is based upon the settling velocities previously calculated for oceanic particles (SOMAYAJULU and CRAIG, 1976) and is considered to be conservative because many of the particles such as fecal pellets undoubtedly have faster sinking rates (FOWLER and SMALL, 1972; SMALL, FOWLER and ÜNLÜ, 1979).

Using the above sedimentation rate assumptions, the net ATP fluxes (g ATP m^{-2} day^{-1}) can be converted to quasi *in situ* particle concentrations (g ATP m^{-3}) to allow for a dimensional comparison with the measured ATP concentrations (Table 3). The results indicate that, within the surface waters (0 to 385 m), the ATP concentrations associated with the rapidly sinking particles exceed those in the slow sinking 'suspended' materials (on a daily basis) by a factor of between 1.4 and 15.4 (Table 3). Moreover, with increasing water depth, the quantitative role of the sedimenting materials becomes even more significant, with mean flux-derived to 'suspended' concentration ratios of 83.5 and 160.5 at 1100 and 1550 m, respectively. Therefore, the steady-state suspended particulate ATP concentrations at 1550 m are insignificant ($<1\%$) compared to the daily transit of particulate ATP through this environment. If the above assumptions and estimates are reasonably accurate, even on order-of-magnitude scales, our current understanding of the distribution, abundance, and production of microbial biomass carbon in the sea, and especially in mesopelagic and bathypelagic habitats, is incomplete. Even within the euphotic zone, omission of rapidly sinking particles and their associated biota from experiments designed to measure the rates of autotrophic and heterotrophic production may result in errors in the quantitative assessment of these important biological oceanographic processes.

At present, there is a major difference of opinion and equivocal data concerning the effect(s) of particles on microbiological activity and growth in the oceans. Laboratory studies conducted by JANNASCH and PRITCHARD (1972) have convincingly demonstrated a stimulation of metabolism from the addition of organic-free particulate materials to carbon-limited cultures of marine bacteria. It has generally been assumed, therefore, that particles (especially organic detritus) should be the centers of intense metabolic activity in seawater. Several laboratory and field studies have supported this hypothesis (JANNASCH, 1967; PAERL, 1975; POMEROY and JOHANNES, 1968; SEKI, 1972; KNAUER and AYERS, 1976). In contrast to these studies, AZAM and HODSON (1977) presented extensive data from numerous marine environments indicating that $>90\%$ of the total heterotrophic activity (as determined by the assimilation of ^{3}H-labeled organic substrates) is associated with 'free-living' or unattached bacteria (<1.0 μm). The latter results and interpretation may be in error, because the sampling methods used (conventional Niskin® bottle casts) undoubtedly biased the collection of particles in favor of the 'suspended' materials. Microscopic analyses of these background particles reveal amorphous structures of uncertain origin (RILEY, 1970). The absence of microorganisms from certain near-surface oceanic particles (JANNASCH, 1973) and the observation that POC is rapidly produced from DOC when the particles are removed by filtration (CHAVE, 1973), suggest that 'suspended' particles may have an ephemeral existence or short half-life in seawater. Only through the use of sediment traps, which allow for the collection of rapidly sinking oceanic particles, can a complete assessment be made of the role of particulate materials in the total heterotrophic activity in seawater.

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