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RNA Synthesis as a Measure of Microbial Growth in Aquatic Environments. II. Field Applications

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Abstract

Rates of stable ribonucleic acid (RNA) synthesis have been calculated from time-series measurements of the uptake of ^{3}H-adenine and labeling of the intracellular adenosine triphosphate (ATP) and stable RNA pools for microbial communities from a variety of freshwater and marine ecosystems. Total rates of microbial RNA synthesis ranged from 6.6 pmol to 3.8×10^5 pmol of adenine incorporated into RNA l^{-1} h^{-1}. Most of the variation was attributable to differences in microbial biomass; if total rate data are normalized to living microbial carbon (based on ATP measurements), the variation in specific rates of RNA synthesis is decreased by a factor of 100 to 1 000 for microbial communities from diverse environments. Light/dark experiments indicated that the uptake and incorporation of ^{3}H-adenine are uncoupled from photophosphorylation. The measurement of rates of stable RNA synthesis of microbial assemblages in nature can yield useful information concerning *in situ* growth rates and the response of communities to changes in environmental conditions such as nutrient additions.

Introduction

The measurements of *in situ* microbial activities and growth rates in nature are exceedingly difficult. Approaches suggested as indicators of cellular growth and/or metabolism include the following: kinetics of uptake and assimilation of specific organic radiotracers; measurement of activities of enzymes indicative of growth or metabolism; biochemical indices of gross physiological state; frequency of dividing cells; oxygen respirometry; microcalorimetry and microautoradiography. More recently, the application of nucleotide metabolism and nucleic acid synthesis has been introduced for use in experimental microbial ecology (Karl, 1979; Fuhrman and Azam, 1980); however, extensive field trials have not yet been conducted. Karl *et al.* (1981) described laboratory and field experiments evaluating the assumptions for application of the ^{3}H-adenine technique for estimating rates of stable RNA synthesis. The present paper concerns field applications of our ^{3}H-adenine technique as a means of studying microbial growth in freshwater and marine habitats.

Materials and Methods

Sampling Sites

Freshwater samples were collected from Krauss Pond (on the University of Hawaii at Manoa campus), which is a eutrophic environment dominated by the green alga, *Chlorella* sp. Freshwater samples were also collected from two prawn (*Macrobrachium rosenbergii*) ponds located in Kahuku, Hawaii. The commercial aquaculture ponds differed greatly from the other environments investigated in that they received large daily inputs of organic and inorganic nutrients in the form of supplemental feed.

Coastal seawater samples were collected from Kewalo Basin, Kaneohe Bay on the island of Oahu, Hawaii. Oceanic seawaters were collected at hydrostations off Point Sur, California (36°06.6′N; 122°46.5′W), Ke-ahole Point, Hawaii (19°55′N; 156°10′W) and in the Equatorial Pacific Ocean (0°48′N; 86°09′W) occupied during research cruises aboard the R.V. "Cayuse", R.V. "Kana Keoki", and R.V. "New Horizon".

Sample Collection and Incubation Conditions

Water samples from Krauss Pond, Kahuku prawn ponds and the various coastal marine sampling sites were collected manually in acid-washed polyethylene or Pyrex® glass containers. Oceanic water samples were collected by 1.7, 5 or 10 liter EtOH-rinsed Niskin® bottles

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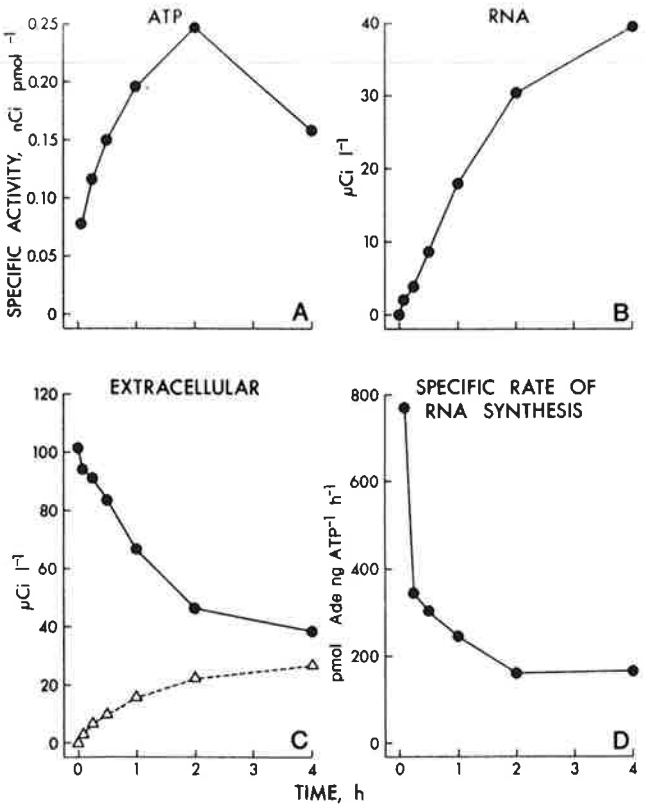


Fig. 1. Representative time-series analysis of uptake and metabolism of ^{3}H-adenine by a microbial community from Krauss Pond (eutrophic freshwater pond, Hawaii) (A) Rate of labeling of community ATP pool; (B) rate of incorporation of ^{3}H-adenine into stable RNA; (C) production of ^3H_2O (△) and removal of ^{3}H-adenine from the medium (●); (D) total rate of stable RNA synthesis normalized to ATP biomass

(General Oceanics, Miami, Florida). All experiments commenced within 30 min after collection. The frequency of subsampling and the incubation conditions (e.g. light conditions, temperature, nutrient additions) varied depending upon the experiment and will be detailed in the following subsections. In general, each experiment began with the addition of [2, ^{3}H]-adenine (15 to 25 Ci mmol^{-1}; New England Nuclear Corporation, Boston, Massachusetts) to a final concentration of 0.1 μCi ml^{-1} (4 to 6.7 pmol adenine added ml^{-1}); however, the calculated rate of RNA synthesis is independent of the concentration of ^{3}H-adenine added, and consequently the overall sensitivity of the assay procedure can be increased by the initial addition of a greater amount of radiolabeled precursor.

Measurement of RNA Synthesis

Rates of RNA synthesis were measured according to Karl (1979) and Karl *et al.* (1981), and are expressed as pmol adenine incorporated into RNA l^{-1} h^{-1}. Many of the rate measurements in the present paper have been normalized to the ATP content of the water samples (i.e., pmol adenine incorporated into RNA ng ATP^{-1} h^{-1}). The expression of total production per unit biomass permits more meaningful comparisons of data from different environments and a more direct assessment of the response of the microbial community to environmental conditions *in situ* (see review by Wright, 1978).

Results and Discussion

Kinetics of Labeling Natural Microbial Communities

A typical set of data for incorporation of ^{3}H-adenine into ^{3}H-ATP, ^{3}H-RNA and ^3H_2O is presented for a Krauss Pond community in Fig. 1. The uptake of ^{3}H-adenine and subsequent production of ^{3}H-ATP, ^{3}H-RNA, and ^3H_2O proceed without detectable lag, and increase during the initial period of incubation (0 to 2 h). The specific rates of RNA synthesis are elevated during the initial time periods, presumably due to kinetic compartmentalization of the ATP precursor pool. The cause and effects of ATP pool compartmentalization have been discussed previously by Karl *et al.* (1981). Similar, but not identical, kinetic features were observed with water samples collected from a variety of habitats. The rate and extent of adenine assimilation varied considerably among the samples. In Krauss Pond, exogenous ^{3}H-adenine was completely assimilated in less than 4 h, in Kaneohe Bay less than half of the ^{3}H-adenine was assimilated in 12 h, while at Ke-ahole Point and in the Equatorial Pacific Ocean, respectively, less than 10% of the ^{3}H-adenine was removed in the 5 and 10 h incubations.

Geographical Variations in Rates of Stable RNA Synthesis

Table 1 summarizes rates of stable RNA synthesis for a variety of microbial communities. Total rates of RNA synthesis ranged from 1.5×10^2 to 3.8×10^5 pmol adenine incorporated into RNA l^{-1} h^{-1}, representing greater than a thousand-fold range. Much of the observed variation in RNA synthesis can be explained by variations in the total particulate ATP concentration (i.e., biomass) as evidenced by the significant positive correlation between ATP concentration and rate of RNA synthesis (r = 0.895, $P < 0.05$; Table 1). However, within a particular environment (e.g. Krauss Pond) the agreement between ATP and rate of stable RNA synthesis was not always evident (Table 1), indicating that the standard error of the regression of total rate of RNA synthesis on ATP concentration must be large. For the data presented in Table 1, the 95% confidence limits of the regression analysis was ± 50% at an ATP concentration of approximately 6 000 ng l^{-1}. At higher and lower ATP concentrations, the 95% confidence limits increased to values in excess of ± 400%. Consequently, for the ecosystems analyzed in this study, biomass measurements alone are

Table 1. Representative total and specific rates of stable RNA synthesis for microbial communities in the euphotic zone of fresh and marine waters

Sample location	ATP concentration ($ng\ l^{-1}$)	Rate of stable RNA synthesis[a]	Specific rate of stable RNA synthesis[b]
Krauss Pond (Feb. 1980)	2 958	1.7×10^5	57.1
Krauss Pond (Feb. 1980)	4 760	7.3×10^4	15.4
Krauss Pond (Sept. 1979)	9 867	1.1×10^5	11.4
Kaneohe Bay (21°26.1'N; 157°46.7'W)	361	2.1×10^3	5.8
Ke-ahole Point (19°55'N; 156°10'W)	53.1	1.5×10^2	2.7
Equatorial Pacific Ocean (0°48'N; 86°09'W)	63.0	2.4×10^2	3.9
Hawaiian Prawn Pond No. 1	21 500	3.8×10^5	17.8
Hawaiin Prawn Pond No. 2	11 600	1.2×10^5	9.9

[a] Data in units of pmol adenine incorporated into RNA per liter of sample per hour
[b] Data in units of pmol adenine incorporated into RNA per ng ATP per hour

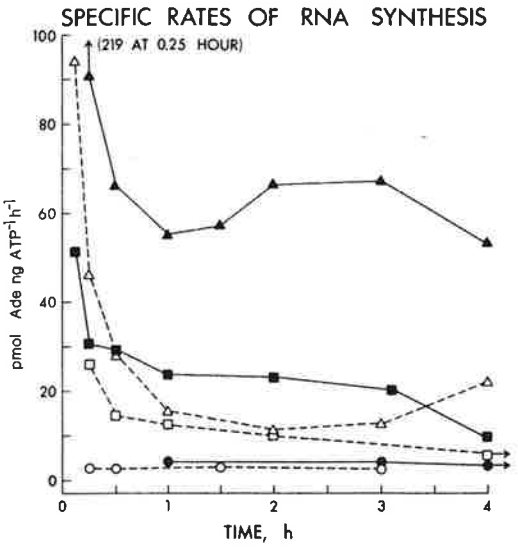


Fig. 2. Representative specific rates of stable RNA synthesis versus incubation period for a variety of freshwater and marine ecosystems. ▲: Krauss Pond (February, 1980); △: Krauss Pond (February, 1980); ■: Krauss Pond (September, 1979); □: Kaneohe Bay, Hawaii; ○: Ke-ahole Point, Hawaii; ●: Equatorial Pacific Ocean. Arrows on Kaneohe Bay, Hawaii and Equatorial Pacific curves indicate that specific rates of RNA synthesis remained constant for at least 28 and 10 h, respectively, the longest time-periods measured

not sufficient to estimate total microbial growth to better than a factor of 0.5 to 5 times the correct value. Furthermore, the specific rates of RNA synthesis presented in Table 1 indicate that the growth per unit microbial biomass is independent of population density. Consequently, changes in the physiological state of the individual components of the total microbial population or alterations in species composition or succession may affect specific rates of RNA synthesis without influencing total biomass.

Fig. 2 presents representative data on specific rates of RNA synthesis for surface waters sampled from a variety of freshwater and marine environments. It is noteworthy that most, but not all, samples exhibited an initial (0 to 1 h) decline in RNA synthesis, as discussed by Karl *et al.* (1981). The two samples with low specific rates of RNA synthesis were not affected by the length of incubation, suggesting that influence of ATP pool compartmentalization may be growth-rate-dependent.

The close correlation between RNA concentration and growth rate (Maaløe and Kjelgaard, 1966; Rhee, 1978), prompted us to determine whether the biomass (ATP) specific rates of RNA synthesis are reasonable estimates for naturally occurring microbial communities. The following are calculations of ATP specific rates of RNA synthesis using hypothetical growth end points of a typical marine bacterium. First, consider a bacterium growing at a doubling time of 0.75 h with an intracellular concentration of RNA equal to 30% of the total cellular dry weight. If we assume that adenine is 25 mol% of the total RNA (Pace, 1973), that carbon equals 50% of total dry weight, and that the C:ATP ratio is 250 (Karl, 1980), then the biomass (ATP) specific rate of RNA synthesis is 144 pmol adenine incorporated into RNA ng ATP^{-1} h^{-1}. Next, consider a bacterium doubling once every 40 h containing 3% RNA (dry weight basis); in this case, the calculated specific rate of RNA synthesis would be 0.27 pmol adenine incorporated into RNA ng ATP^{-1} h^{-1}. Similar calculations are possible using data

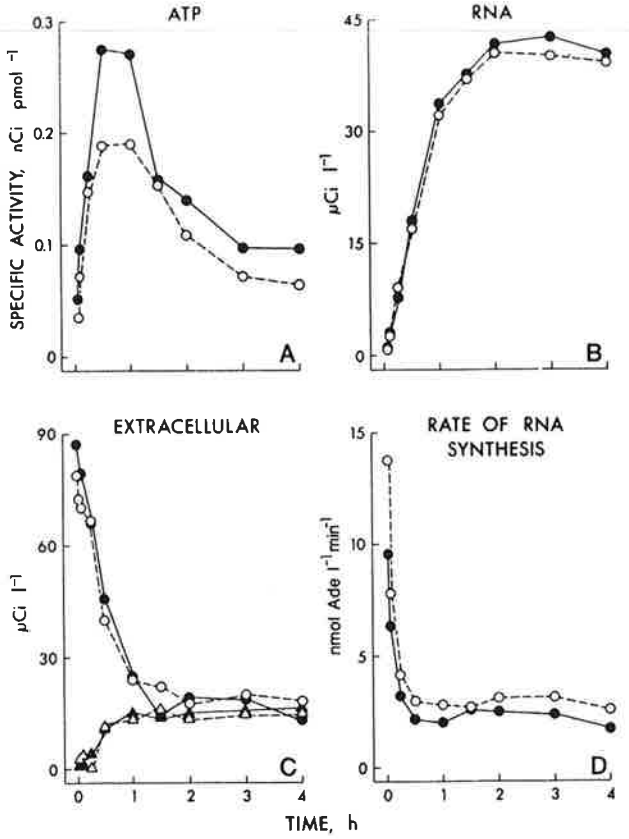


Fig. 3. Representative time-series analysis of uptake and metabolism of ^{3}H-adenine by a Krauss Pond microbial community incubated in the light (○, △) and dark (●, ▲). (A) Rate of labeling of community ATP pool; (B) rate of incorporation of ^{3}H-adenine into stable RNA; (C) △, ▲: production of ^3H_2O, and ○, ●: loss of ^{3}H-adenine from the medium; (D) total rate of stable RNA synthesis over a 4 h incubation period

derived from other microorganisms as well. Such calculations (from extreme conditions) indicate that our estimates of ATP specific rates of RNA synthesis are comparatively reasonable.

Light vs Dark Incubations

In separate experiments, replicate water samples collected from Krauss Pond, Kewalo Basin and the southeast sector of Kaneohe Bay were incubated in the light and in the dark for various time periods, and the total and specific rates of RNA synthesis were measured. All 3 sets of experiments were performed during daylight hours and yielded similar results; data from the Krauss Pond experiment are presented in Fig. 3. The rates of removal of ^{3}H-adenine, the incorporation of ^{3}H-adenine into ^{3}H-RNA and the production of ^3H_2O were indistinguishable between the light and dark treatments. By comparison, the specific activity of the ATP pool attained a lower maximum specific activity in the light, resulting in a detectably lower rate (~10 to 15%) of RNA synthesis in the dark treatment throughout the duration of the experiment.

The role of light energy in the uptake and assimilation of various nutrients and growth substrates has been studied for both photoautotrophic and heterotrophic microorganisms. The results of laboratory studies of N-assimilation of marine phytoplankton in the light and dark have led to conflicting results (Malone *et al.*, 1975). Falkowski and Stone (1975) suggested that ATP generated by cyclic photophosphorylation is required for the translocation of NO_3^- across the cell membrane; however, several laboratory studies have indicated significant rates of NO_3^- uptake (up to 100% of light period values) in the dark for a variety of marine phytoplankton species (Eppley *et al.*, 1971; Bhovichitra and Swift, 1977; Laws and Wong, 1978). In the field, Vincent (1979) reported no significant differences between light and dark methylammonium uptake rates for a lake dominated by diatoms, but there were significant differences in a cyanobacteria-dominated ecosystem.

Our results with ^{3}H-adenine indicate that there were no substantial differences in the rates of RNA synthesis in the light and dark for ecosystems dominated by chlorophytes (Krauss Pond), diatoms (Kaneohe Bay), or microflagellates (Ke-ahole Point and the Equatorial Pacific) throughout the incubation periods investigated (0 to 6 h). The uptake and assimilation of nucleic acid precursors require energy and appear to be controlled by adenylate energy charge (Atkinson and Fall, 1967; Karl, 1979). Therefore, the photoautotrophic populations investigated in this study must be able to regulate their ATP-generating processes (respiration vs photosynthesis) and consequently their intracellular adenylate energy charge, despite these light-dark transitions. Previous studies of adenine nucleotide concentrations in the marine dinoflagellate *Ceratium furca* indicated that the cellular adenylate energy charge was well regulated during alternating light:dark (12 h:12 h) periods and even after 42 h in constant darkness (Weiler and Karl, 1979). Therefore, the uptake of adenine and assimilation into RNA by the mixed (i.e., photoautotrophic and heterotrophic) microbial assemblages of the present study appear to be uncoupled from energy derived directly from photophosphorylation.

Nutrient Enrichment Experiment

Surface samples from Ke-ahole Point were incubated in the light at *in situ* water temperatures (27 °C) for exactly 3 h following the addition of either glucose, peptone, or a mixture of inorganic nutrients and vitamins (see Table 2 for concentrations). Rates of RNA synthesis were determined for each treatment (Table 2 and 3). All 3 treatments yielded increases in total ATP concentration (152 to 175%) and stimulation of the total rate of RNA synthesis (120 to 151%) relative to control samples. It is unknown whether the increases in ATP resulted from an increased ATP cell quota (i.e., more ATP cell^{-1}) or from an increase in total biomass. Furthermore, since

Table 2. Effects of nutrient additions on rates of total and specific stable RNA synthesis for microbial community collected from surface waters off Ke-ahole Point, Hawaii (19°55'N; 156°10'W)

Nutrient added	ATP		RNA		ATP specific rate of RNA synthesis (pmol Ade ng ATP^{-1} h^{-1})
	Concentration at end of 3 h incubation (ng l^{-1})	% of control	Rate of synthesis (pmol Ade l^{-1} h^{-1})	% of control	
Control[a]	26.5	–	79	–	3.0
Glucose[b]	42.5	160	95.5	120	2.22
Peptone[c]	46.4	175	114.9	145	2.45
Inorganic nutrient trace metal mixture[d]	40.3	152	119.4	151	2.97

[a] Control treatment received no addition
[b] Final concentration = 1 μg ml^{-1} of sample
[c] Final concentration = 1 μg ml^{-1} of sample
[d] Final concentration equivalent to 4% *f*/2 nutrient medium of Eppley *et al.* (1967)

Table 3. Effects of addition of inorganic nutrients on several biomass, growth and metabolism parameters of an oceanic microbial community collected from the surface waters off Ke-ahole Point, Hawaii (19°55'N; 156°10'W). Data presented are actual values obtained for duplicate determinations throughout

Parameter	Treatment control[a]	Nutrient addition[b]
ATP concentration at end of 3 h incubation (ng l^{-1})	28.8 24.2	40.5 40.0
EC_A ratio[c]	0.89 0.84	0.89 0.85
ATP pool-specific activity (nCi $pmol^{-1}$)	2.68 2.28	2.35 2.43
Rate of stable RNA synthesis (pmol Ade l^{-1} h^{-1})	79.6 78.4	114.2 124.5
Specific rate of stable RNA synthesis (pmol Ade ng ATP^{-1} h^{-1})	2.76 3.24	2.82 3.11

[a] Control treatment received no nutrient addition
[b] Final concentration equivalent to 4% *f*/2 nutrient medium described in Eppley *et al.* (1967)
[c] EC_A = adenylate energy charge, $\frac{[ATP] + 1/2[ADP]}{[ATP + ADP + AMP]}$

these experiments were end-point (3 h) measurements, we have no information concerning the kinetics of the response of the population. A more detailed analysis of the data resulting from the inorganic nutrient addition experiment is presented in Table 3.

The technique of artificial enrichment of naturally occurring communities has been used extensively in experimental microbial ecology. Most studies have concentrated on elucidating the nutrients limiting the growth of phytoplankton populations (Menzel *et al.*, 1963; Thomas, 1969; Glooschenko and Curl, 1971) and, to a lesser extent, the heterotrophic component as well (Wiebe and Bancroft, 1975; Christian *et al.*, 1978). When nutrients are added to cultures of starved or deficient cells, major changes in metabolism and biosynthesis occur. Typically, in such an enrichment, the rate of stable RNA synthesis increases immediately (i.e., no detectable lag) to prepare the cells for accelerated growth (Maaløe and Kjeldgaard, 1966). The immediate dissociation of RNA synthesis from all other cellular activities suggests that nutrient-enrichment bioassay studies employing RNA synthesis as the primary response parameter may be much more meaningful and informative than previous methods of choice. For photoautotrophic nutrient addition experiments, chlorophyll *a* specific rates of RNA synthesis (i.e., rate of RNA synthesis chlorophyll a^{-1}) may be more appropriate, due to the possibility of rapid (but unpredictable) changes in ATP per cell upon the addition of nutrients to nutrient-limited microorganisms (data reviewed by Karl, 1980). Nevertheless, these preliminary data do provide documentation and justification for the use of RNA synthesis rate measurements in studies designed to evaluate organic or inorganic nutrient limitation in aquatic environments.

Depth Profiles of Stable RNA Synthesis in the Ocean

Measurements of RNA synthesis were made on water samples collected from various depths at 3 hydrostations in the Pacific Ocean (Table 4). The rates of total RNA

Table 4. Summary of ATP concentrations, ATP pool-specific activities, and total and specific rates of stable RNA synthesis with depth for 3 hydrostations in Pacific Ocean

Depth (m)	ATP concentration (ng l^{-1})	ATP pool-specific activity (nCi $pmol^{-1}$)	Total rate of stable RNA synthesis (pmol Ade l^{-1} h^{-1})	Specific rate of stable RNA synthesis (pmol Ade ng ATP^{-1} h^{-1})
Ke-ahole Point Station (19°55'N; 156°10'W)				
2	39.2	1.5	191.6	4.9
25	36.8	1.06	202.9	5.5
50	23.5	1.08	83.3	3.5
150	20.7	1.05	74.3	3.6
200	13.0	2.12	71.0	5.5
400	4.3	2.99	27.9	6.4
600	6.8	2.63	30.2	4.5
800	10.4	3.10	43.9	4.2
1 000	2.2	5.17	6.6	3.0
Equatorial Pacific station (0°48'N; 86°09'W)				
1	44.4	0.11	334	7.5
20	34.8	0.08	137.6	4.0
50	30.2	0.14	107.6	3.6
70	7.6	0.21	29.6	3.9
200	10.7	0.07	41.1	3.8
400	1.8	0.11	41.9	23.3
600	3.0	0.14	125.4	41.8
800	1.8	0.16	100.2	55.7
1 000	7.3	0.04	242.3	33.2
Point Sur, California station (36°06.6'N; 122°46.5'W)				
1	205	0.65	824	4.0
30	86	0.73	491	5.7
100	38	0.70	102	2.7
150	28	0.97	54	1.9
250	24	1.12	33.6	1.4
300	25	2.10	12.1	0.5
350	16	0.85	55.3	3.5
400	16	1.27	21.7	1.4
500	12	1.40	22.1	1.8
750	5.5	1.13	8.4	1.5

synthesis for the upper 100 m of the water column were greatest at the California hydrostation, although the specific rates were not significantly different. At depths greater than 200 m, the 3 stations differed markedly among themselves both in the biomass (ATP)-specific rates and in the total rates of RNA synthesis. The high specific rates measured at depths from 400 to 1000 m for the Equatorial Pacific Station (23 to 56 pmol adenine incorporated into RNA ng ATP^{-1} h^{-1}) are approximately twice as high as values measured previously in the intermediate depth oxygen minimum of the Columbian Basin of the Caribbean Sea (Karl, 1979). The extent of labeling of the ATP pools (i.e., the specific radioactivity of the ATP pools) varies substantially both as a function of water depth and geographical location. Previous investigators have arbitrarily assumed (1) that the specific radioactivity of the intracellular precursor was identical to that of the added tracer, and (2) that the intracellular specific activities were identical under all environmental conditions. On the contrary, we found that the mean ATP pool specific activities *in situ* were dependent upon: (1) the amount of isotope added, (2) the pool of reactive non-radioactive molecules present (i.e., the extracellular specific activity), (3) the duration of the incubation period, (4) the percentage of actively metabolizing cells, (5) the proportion of *de novo* synthesis to assimilation by salvage pathways, and (6) the degree of RNA recycling (Karl, 1979; Karl *et al.*, 1981). The range of specific radioactivities measured in this study (Figs. 1, 3 and 4; Tables 2 and 4) attests to the need for independent measurements of *in situ* community-precursor-specific radioactivities. Without ATP-pool-specific activity corrections, comparative incorporation rate data may lead to serious quantitative errors and possible misinterpretation of ecological data.

The measurement of mean community-ATP-specific radioactivities to convert precursor assimilation data into rates of RNA synthesis is not without limitation. If the majority of living organisms in any particular environment were either inactive (i.e., not synthesizing RNA) or incapable of adenine transport or assimilation, then the determination of the mean specific activity of

Table 5. Comparisons of ATP concentrations, ATP pool-specific activities, and total and specific rates of stable RNA synthesis for two size fractions of a Krauss Pond (freshwater) microbial community. "Total" refers to particulate materials retained by a Reeve-Angel 984-H microfine glass-fiber filter. Data are means ± 1 SD, as determined from triplicate determinations

Parameter	Treatment	
	> 12 μm	Total
ATP concentration (ng ml^{-1})	10.33 (± 0.58)	15.47 (± 1.77)
ATP pool specific activity (nCi $pmol^{-1}$)	0.036 (± 0.002)	0.048 (± 0.006)
Rate of stable RNA synthesis (pmol Ade ml^{-1} h^{-1})	188 (± 9.77)	432.8 (± 7.72)
Specific rate of stable RNA synthesis (pmol Ade ng ATP^{-1} h^{-1})	18.2 (± 0.61)	28.2 (± 3.21)

the community ATP pool would be an underestimate of the portion of that total pool which is the actual precursor to the RNA produced during the incubation period. This would yield an overestimate of the true rate of *in situ* RNA synthesis and an incorrect calculation of the biomass (ATP) specific rates of RNA synthesis. Unfortunately, we have no direct measurements of the *in situ* spectrum of metabolic activity or degree of dormancy of microorganisms in natural habitats. Perhaps combined microautoradiographic studies (Meyer-Reil, 1978) or *in vivo* 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) labeling experiments (Zimmermann *et al.*, 1978) will help to resolve the question of the *in situ* metabolic growth status of microbial assemblages in aquatic environments. In theory, the specific activity of the ATP pool under saturating conditions for the uptake of adenine could be used to address questions concerning microbial dormancy and/or ability of microorganisms to assimilate exogenous adenine. If all viable organisms are growing and capable of adenine assimilation, then the specific activity of the ATP pool should be >1 nCi $pmol^{-1}$ using the adenine preparations employed in this study (Karl *et al.*, 1981). If, on the other hand, only a small proportion of the microbial community is undergoing RNA biosynthesis or has the salvage pathways required for adenine transport, then this will be reflected in the estimate of the mean community ATP pool-specific radioactivity. In the present study, we made no attempt to maximize the intracellular specific radioactivities (i.e., by saturating uptake or by incubating for optimum time periods). Nevertheless, in selected marine environments (Table 4) the mean community-ATP-specific radioactivities are equal to or greater than the highest values previously measured in laboratory cultures (Karl *et al.*, 1981). Our results imply that if a portion of the *in situ* microbial population is in fact dormant, its contribution to the total community ATP concentration must be minimal. These results further support the measurement of mean specific radioactivity in environmental studies.

Size Fractionation Experiments

The size distribution of biomass and stable RNA synthesis was evaluated. For Krauss Pond samples we made a post-incubation comparison of RNA synthesis in the size fraction > 12 μm (i.e., retained by 12 μm Nuclepore® filter) and total particulate material (i.e., retained by Reeve Angel microfine glass-fiber filter, 984-H). The results (Table 5) reveal that, even though >65% of the total ATP-biomass is retained by the 12 μm filter, this size fraction accounts for only 43% of the total community rate of RNA synthesis. This is reflected in the differences in specific rates of RNA synthesis (Table 5), and implies that there must exist some fraction of the total biomass (i.e., <12 μm) that maintains a very high specific rate of biosynthesis (40 to 50 pmol adenine incorporated into RNA ng ATP^{-1} h^{-1}).

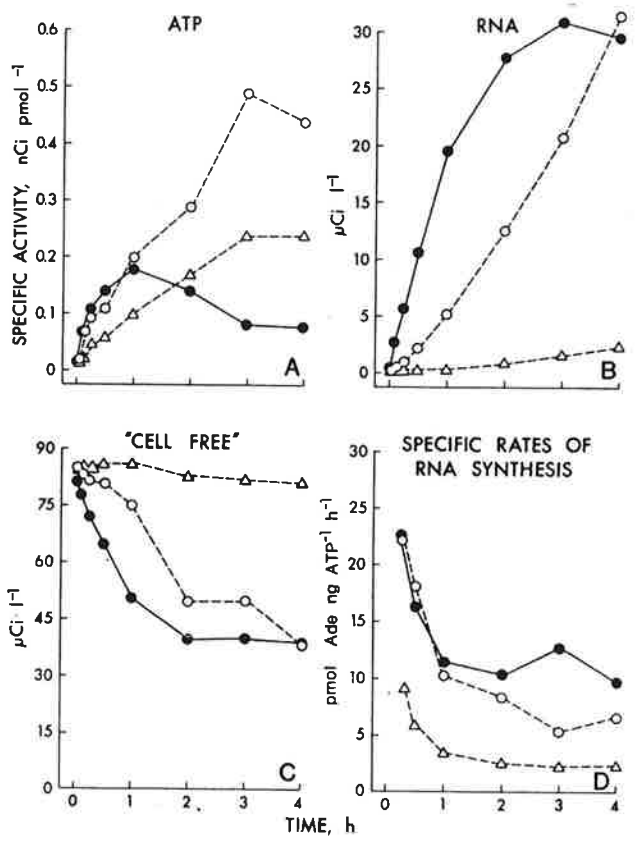
A similar experiment was conducted using coastal seawater (Kewalo Basin, Hawaii) with two procedural differences. First, we compared results from pre-incubation and post-incubation size fractionation. Second, the particulate materials retained by the Nuclepore® filter (>12 μm), were not analyzed as in the previous experiment but rather the particles in the filtrate (<12 μm) were collected onto a microfine glass-fiber filter and processed. The fraction <12 μm was then compared with the total water samples. The results are summarized in Table 6. The size fraction <12μm (expressed as a % of the total) contained 29% of the total RNA synthesis when processed by pre-incubation size fractionation procedures, compared to 68% when the sample was size-fractionated following incubation. Moreover, the specific activities of the ATP pools and the specific rates of RNA synthesis in the individual fractions revealed significant differences between the two procedures.

Physical separation of microbial communities by differential filtration techniques presumes: (1) non-overlapping size spectra of individual microbial groups, (2) no association of small cells with larger particulate materials, and (3) a non-destructive filtration procedure. Unfortunately, none of these effects are ever produced exactly (Karl, 1980). Although standard methods involve fractionation of samples after incubation with a specific radiotracer (Berman, 1975; Burnison, 1975; Azam and Hodson, 1977), Wheeler *et al.* (1977) have recently indicated that post-incubation fractionation procedures shift the distribution of labeled particles toward smaller size classes, and our data on the size distribution of rates of RNA synthesis (Table 6) support this. However, our data should not be construed as an endorsement for the use of pre-incubation fractionation methods, as they too may grossly alter the configuration and *in situ* size distribution of microorganisms, but rather as a precautionary warning for the application and interpretation

Table 6. Comparison of pre-incubation and post-incubation procedures on A_T, ATP pool-specific radioactivity, and total and specific rates of stable RNA synthesis of two size classes of a coastal seawater (Kewalo Basin, Hawaii) microbial community. "Total" refers to particulate materials retained by a Reeve-Angel 984-H microfine glass-fiber filter

Parameter	Treatment					
	Pre-incubation fractionation			Post-incubation fractionation		
	< 12 μm	Total	$\frac{<12\ \mu m}{Total}$ (%)	< 12 μm	Total	$\frac{<12\ \mu m}{Total}$ (%)
A_T (ng l^{-1})[a]						
Initial	68.9	102.4		68.9	102.4	
Final[b]	73.1	104.4		81.9	120.8	
X	(71)	(103.4)	68.7	(75.4)	(111.6)	67.6
ATP pool-specific activity (nCi $pmol^{-1}$)	0.90	0.54		0.94	1.18	
Rate of stable RNA synthesis (pmol Ade l^{-1} h^{-1})	2 592	8 805	29	3 514	5 179	68
Specific rate of stable RNA synthesis (pmol Ade ng A_T^{-1} h^{-1})	36.5	85.2		42.9	46.4	

[a] A_T = total adenylate concentration, [ATP + ADP + AMP]
[b] Following a 1 h incubation period



◀ **Fig. 4.** Time-series analysis of uptake and metabolism of ^{3}H-adenine by 3 different size classes of a Krauss Pond microbial community. •, < 90 μm; ○, < 12 μm; ▲, < 3 μm (see "Size Fractionation Experiments" for further details). (A) Rate of labeling of community ATP pool; (B) rate of incorporation of ^{3}H-adenine into stable RNA; (C) removal of ^{3}H-adenine from the medium during a 4 h incubation period; (D) total rate of stable RNA synthesis normalized to ATP biomass

of experiments involving any size fractionation procedures.

A final experiment conducted with Krauss Pond water examined the kinetics of uptake and assimilation of ^{3}H-adenine by various size fractions. A water sample was pre-filtered through a 90 μm nylon mesh (< 90 μm), a 12 μm Nuclepore® filter (< 12 μm) and a 3 μm Nuclepore® filter (< 3μm) to yield 3 distinct fractions. The initial ATP concentrations of the individual fractions were < 90 μm, 5 010 ng l^{-1}; < 12 μm, 1 371 ng l^{-1}; and < 3 μm, 557 ng l^{-1}, representing 100, 27.4 and 11.1% of the total ATP biomass, respectively. All samples were incubated in the light with 0.1 μCi ^{3}H-adenine per ml for pre-determined time periods. The results are presented in Fig. 4. The rate of ^{3}H-adenine assimilation (i.e., production of ^{3}H-RNA, decrease in extracellular ^{3}H-adenine) decreased with decreasing particle size. The specific radioactivities of the individual size fractions varied considerably, again emphasizing the necessity for assessing the extent of precursor pool labeling. The mean total rates and specific rates of RNA synthesis decreased as particle size diminished.

In the present study, particles >12 μm often contributed significantly to the total community rates of stable RNA synthesis, regardless of the methods used to process the samples (Tables 5 and 6, Fig. 4). These results are consistent with the observed ability of phytoplankton to assimilate exogenous ^{3}H-adenine. Somewhat unexpectedly, particles between >12 and <90 μm from selected environments (e.g. Krauss Pond, Fig. 4) maintained higher specific rates of RNA synthesis than the smaller size fractions; this suggests that most heterotrophic microorganisms (i.e., with presumed high specific rates of RNA synthesis) are associated with larger microbial cells or non-living detrital particles. Microscopic examination of acridine-orange-stained samples from Krauss Pond has confirmed the existence of particles heavily colonized by bacteria.

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