# Simultaneous Rates of Ribonucleic Acid and Deoxyribonucleic Acid Syntheses for Estimating Growth and Cell Division of Aquatic Microbial Communities

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A method for measuring rates of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) syntheses using a single radioactive precursor has been devised and tested using bacterial cultures and natural assemblages of marine and freshwater microorganisms. The procedure is based upon the uptake and incorporation of exogenous [³H]adenine into cellular adenosine triphosphate and deoxyadenosine triphosphate pools which serve as the immediate precursors for the adenine incorporated into RNA and DNA, respectively. It is proposed that the DNA/RNA rate ratio is correlated with the specific growth rate of microorganisms and can be used as an index for estimating and comparing the productivities of microbial assemblages in nature. This technique can also be used to detect discontinuous growth and cell division processes which frequently occur in surface plankton populations. The DNA/RNA rate ratios measured in a variety of aquatic ecosystems ranged from 3.3 to 31.8% without significant correlation to total microbial biomass.

The measurement of microbial growth in nature has presented, and continues to present, a challenge to experimental microbial ecologists. In recent years, several new and promising approaches have been devised to estimate rates of macromolecular (ribonucleic acid [RNA], deoxyribonucleic acid, [DNA], and protein) synthesis in aquatic microbial assemblages. All three biomolecules are important in cellular structure and function, are closely coupled to and required for growth and cell division, and are regulated with great precision. Specific conceptual and analytical advantages have been presented for the use of either RNA (12; D. M. Karl, C. D. Winn, and D. C. L. Wong, Mar. Biol., parts I and II, in press), DNA (1, 7, 30; D. J. W. Moriarty and P. C. Pollard, Mar. Ecol. Prog. Ser., in press), or protein (R. L. Cuhel, Ph.D. thesis, Massachusetts Institute of Techology/Woods Hole Oceanographic Institute, Woods Hole, Mass., 1981).

The observed correlations between rates of stable RNA, DNA, and protein syntheses and the rate of cell growth (reviewed in references 15, 19, 22, 25) are so universally applicable among unicellular microorganisms that they appear to be well suited for the analysis of complex communities such as one finds in nature. The results of pioneering studies of macromolecular synthesis in bacteria indicate that the cell quotas of RNA, DNA, protein, and total mass all increase with increasing growth rate (8, 17, 19, 28), and are independent of the chemical composition of the growth medium or nature of the growth-limiting nutrient, only insofar as it affects the growth rate. Furthermore, DNA/RNA and protein/RNA ratios vary in direct proportion to growth rate, whereas protein/DNA ratios remain constant. More recent investigations utilizing refined techniques for the separation and determination of cellular nucleic acids have confirmed the earlier results (2, 5, 23).

Based on the previous studies, it seems reasonable that simultaneous measurements of rates of RNA and DNA syntheses might provide quantitative data on the in situ growth of microbial communities in nature. At the very least, cell growth (increase in mass and synthesis of RNA) and cell division (synthesis of DNA) could be distinguished. An extension of the theoretical considerations and a minor modification of the experimental protocol previously devised for measuring rates of RNA synthesis (12; Karl et al., parts I and II, in press) have been introduced, thus enabling simultaneous measurements of the rates of RNA and DNA syntheses. The present report describes the principles, procedures, and applications of this novel experimental approach, and discusses the advantages and limitations of using DNA/RNA rate ratios for studying microbial growth in aquatic environments.

**Theoretical principles.** The intracellular

precursor of adenine in DNA is deoxyadenosine triphosphate (dATP). Two distinct pathways are known for the biosynthesis of dATP (29); both reaction sequences involve the reduction of a corresponding adenine nucleotide, either at the level of adenosine diphosphate (ADP) (ADP → dADP → dATP) or ATP (ATP → dATP). A summary of the known metabolic pathways is presented in Fig. 1.
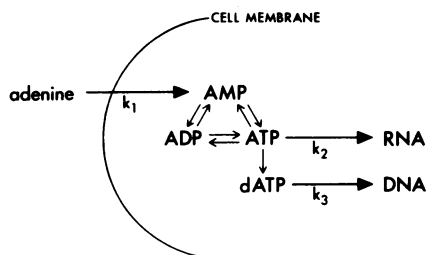
To calculate the absolute rate of DNA synthesis, the specific activity of the intracellular dATP precursor pool must be known. Failure to calculate and correct for differences in the specific radioactivities of the intracellular precursor pools could conceivably lead to serious, albeit unknown, quantitative errors and misinterpretations of ecological data. No previous estimates of DNA synthesis in ecological studies have ever considered precursor specific radioactivities.

When exogenous $[2,^3H]$adenine is assimilated by microorganisms, it is incorporated into a number of adenine-containing derivatives. Due to the numerous interconversions and the extremely rapid turnover of cellular nucleotides, the individual adenine nucleotide pools (i.e., adenosine monophosphate [AMP], ADP, and ATP) rapidly equilibrate to achieve isotopic equilibrium (i.e., specific radioactivity of ATP = specific radioactivity of ADP = specific radioactivity of AMP) over relatively short time periods. Consequently, regardless of the biosyn-

thetic pathway operating for the production of dATP, the specific radioactivity should be equal to that of the parent adenine nucleotide (i.e., either ADP or ATP). Therefore, by measuring the specific radioactivity of any one of the adenine nucleotides, preferably ATP (because of highest intracellular concentration, existing methodology, and minimal analytical interference), the corresponding specific radioactivity of the dATP pool is also accurately known.

In practice, an environmental sample or cell suspension is incubated with radiolabeled adenine for a predetermined time interval. After this incubation period, the cellular nucleotides are extracted and the mean specific radioactivity of the ATP pool (i.e., nanocuries of ATP per picomole) is determined as described previously (12; Karl et al., part I, in press). Cellular RNA and DNA are extracted, separated, and purified, and the total radioactivity is measured (i.e., nanocuries of RNA or DNA). From these measurements, the actual rate of RNA or DNA synthesis or both can be calculated as picomoles of adenine incorporated into RNA or DNA per unit volume per unit time. Alternatively, relative DNA/RNA synthesis rate ratios can be determined directly from incorporation data (specific radioactivities of precursor pools are equal and consequently cancel out in the ratio calculation), thereby mitigating the necessity for specific radioactivity measurements.

## MATERIALS AND METHODS

**Sample collection, medium composition, and culture conditions.** Freshwater samples from Krauss Pond (on the University of Hawaii at Manoa campus), a eutrophic environment dominated by the green alga, *Chlorella* sp., were collected manually in acid-washed polycarbonate or Pyrex glass bottles. All samples were prefiltered through a 90-μm Nitex screen before incubation. Surface seawater samples (0 to 0.5 m) were collected at a station located in the southeast sector of Kaneohe Bay, Oahu, Hawaii, by manually submerging an 8-liter Pyrex glass carboy. Seawater samples were prefiltered through a 150-μm Nitex screen before incubation.

*Serratia marinorubra* (ZoBell and Feltham) was grown in batch culture on a medium consisting of 1.5 g of peptone, 50 mg of yeast extract, and 25 mg of $FePO_4$ per liter in 80% seawater. All cultures were grown at 25 ($\pm$1)°C on a gyratory shaker table (125 rpm).

**ATP and ADP pool-specific radioactivities.** *S. marinorubra* was grown to mid-logarithmic phase in batch culture (optical density at 560 nm ~ 0.1), and 5 ml of culture was added to a 50-ml flask containing 10 μCi of $[2-^3H]$adenine. After predetermined incubation periods, 200 μl was injected into 5 ml of boiling phosphate buffer (60 mM, pH 7.4) to extract the cellular nucleotides. The specific radioactivities of cellular ATP pools (expressed as nanocuries per picomole)

### I. NTP REDUCTASE PATHWAY (ATP → dATP)



### II. NDP REDUCTASE PATHWAY (ADP → dADP → dATP)
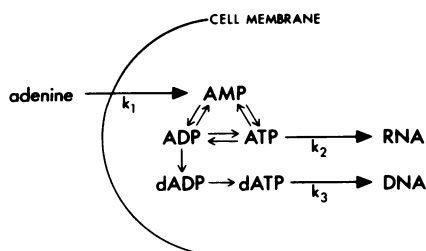


FIG. 1. *Known pathways for the flow of exogenous adenine into RNA and DNA of microorganisms.*

were measured as described previously (Karl et al., part I, in press). The ADP pool-specific radioactivities were calculated in an analogous manner from chemical and radiochemical measurements of total ADP. Chemical ADP was determined by the method of Karl and Holm-Hansen (13), and the radiochemical ADP was determined by thin-layer chromatographic separation, MgCl$_2$ elution, and liquid scintillation spectrometry as described by Karl et al. (part I, in press) for [$^3$H]ATP.

[$^3$H]adenine uptake and metabolism. The uptake and assimilation of [$^3$H]adenine into intermediary metabolites (e.g., [$^3$H]ATP, [$^3$H]ADP, etc.), cellular macromolecules (e.g., [$^3$H]RNA and [$^3$H]DNA) and metabolic by-products (e.g., $^3$H$_2$O) were measured by the procedures devised by Karl and co-workers (12; Karl et al., part I, in press). The frequency of subsampling and general incubation conditions (e.g., concentration of [$^3$H]adenine, light conditions, etc.) varied depending upon the experiment and will be detailed in the following subsections.

Pulse-chase experiment. A pulse-chase experiment measured the relative rates of labeling and metabolic turnover of [$^3$H]RNA and [$^3$H]DNA. The experiment began with the addition of [$^3$H]adenine (5 µCi/ml) to a culture of mid-logarithmic-phase S. marinorubra cells. After a 3-min incubation period, the culture was divided into two equal subsamples, and nonradioactive adenine (Sigma Chemical Co., St. Louis, Mo.) was added to one (10$^{-4}$ M final concentration). Sampling for [$^3$H]RNA, [$^3$H]DNA, and $^3$H$_2$O continued for an additional 12 min.

Nalidixic acid experiment. Subsamples of a mid-logarithmic-phase S. marinorubra culture were preincubated for 5 min with increasing concentrations of nalidixic acid (Sigma Chemical Co.), an inhibitor of DNA polymerase activity. [$^3$H]adenine was then added to each subsample (final concentration, 5 µCi/ml) and incubated for 15 min on a gyratory shaker table (125 rpm). The cells were extracted, and rates of RNA and DNA syntheses were determined as described previously. .

## RESULTS

Kinetics of labeling cellular ATP and ADP pools. After the addition of [$^3$H]adenine to the medium, the specific activities of both the ATP and ADP pools increased without any detectable lag period (Fig. 2). The initial rates of labeling and maximum specific activity attained in the individual metabolite pools were indistinguishable at all time periods measured (Fig. 2). These data indicate that the ATP and ADP pools are in isotopic equilibrium and substantiate the underlying theoretical principle.

Kinetics of RNA and DNA production in environmental samples. Several experiments were conducted to monitor the rates of [$^3$H]RNA and [$^3$H]DNA productions after the addition of [$^3$H]adenine. In particular, attention was given to the initial period of uptake where kinetic compartmentalization of the ATP pool has been shown previously to affect the measured rates of
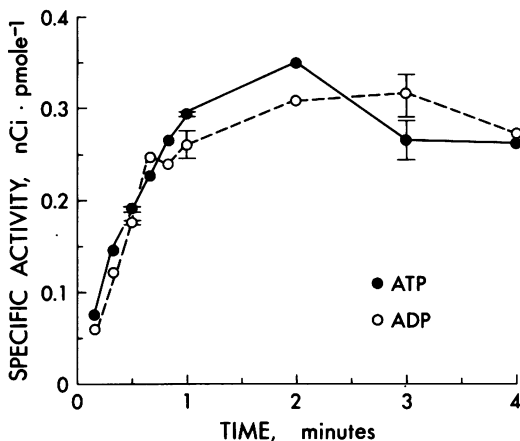


FIG. 2. Kinetics of labeling ATP and ADP pools of S. marinorubra after the addition of [$^3$H]adenine to the culture medium.

RNA synthesis (Karl et al., parts I and II, in press), as well as to incubation periods wherein [$^3$H]adenine had been depleted from the medium. A typical set of data for the metabolism of [$^3$H]adenine by a freshwater microbial assemblage is presented in Fig. 3 and 4. Similar results have been obtained for populations of marine microorganisms.

Karl et al. (parts I and II, in press) have demonstrated that $^3$H$_2$O is produced during [$^3$H]adenine metabolism. Furthermore, the rate of $^3$H$_2$O production was found to be proportional to the assimilation of [$^3$H]adenine from the medium. Figure 3 shows that the rate of [$^3$H]adenine assimilation is constant for approximately 1 h; thereafter $^3$H$_2$O production (and by inference, [$^3$H]adenine assimilation) effectively ceases. Mass balance inventories of radioactivity of this eutrophic freshwater microbial assemblage indicate that, after 2 h, >95% of the exogenous [$^3$H]adenine had been depleted from the sample. The time required to exhaust the [$^3$H]adenine tracer varies substantially among different types of water samples (Karl et al., parts I and II, in press).

Figure 4 presents the production rates for [$^3$H]RNA and [$^3$H]DNA and the corresponding DNA/RNA rate ratios. Consistent with data previously published by this laboratory (12; Karl et al., parts I and II, in press), several kinetic features were evident, including: (i) exogenous [$^3$H]adenine was transported into microbial cells and assimilated into [$^3$H]RNA and [$^3$H]DNA without any detectable lag period; (ii) the rate of [$^3$H]RNA production was linear for approximately 1 h, which was identical to the kinetics of $^3$H$_2$O production (Fig. 3), indicating that [$^3$H]RNA production ceased when [$^3$H]adenine
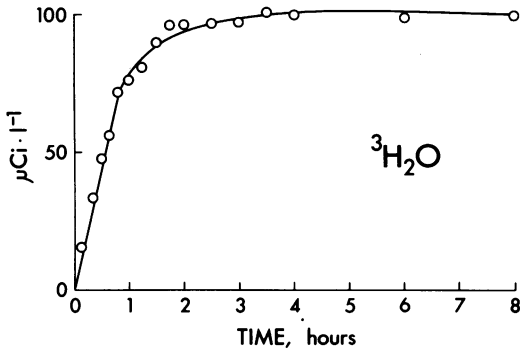
FIG. 3. *Kinetics of $^3H_2O$ production after the addition of [$^3H$]adenine to a freshwater microbial community (Krauss Pond).*
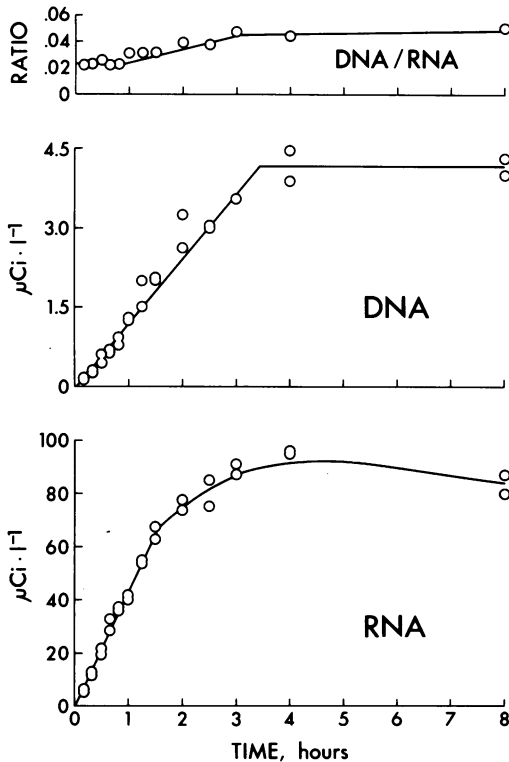


FIG. 4. *Kinetics of [$^3H$]RNA and [$^3H$]DNA productions and incubation time-dependent DNA/RNA rate ratios after the addition of [$^3H$]adenine to a freshwater microbial community (Krauss Pond). The $^3H_2O$ production data for this experiment are presented in Fig. 3.*

had been depleted from the medium; and (iii) there was a small but measurable turnover of "stable" RNA as indicated by the slight decrease in total [$^3H$]RNA between 4 and 8 h. In contrast to the kinetics of [$^3H$]RNA production, [$^3H$]-

DNA synthesis was linear for at least 2 h beyond the point where [$^3H$]adenine had been depleted from the medium. These data suggest that the turnover of [$^3H$]RNA may resupply precursors for net [$^3H$]DNA synthesis. Beyond 4 h, [$^3H$]-DNA production ceased and there is no apparent decrease (i.e., turnover) of [$^3H$]DNA during extended incubations.

Due to differences in the kinetics of [$^3H$]RNA and [$^3H$]DNA production, the calculated DNA/RNA rate ratios varied with the duration of the incubation period. Three distinct phases were evident: (i) 0 to 1 h, the ratio was constant at $0.023 \pm 0.003$ ($\bar{X} \pm 1$ standard deviation, $n = 10$); (ii) 1 to 3 h, the ratio increased rapidly from 0.023 to 0.041 in direct proportion to the duration of the incubation; and (iii) beyond 3 h, the ratio increased gradually from 0.041 to 0.050 (Fig. 4). The three portions of this curve represented periods of coordinate [$^3H$]RNA and [$^3H$]DNA production, primarily [$^3H$]DNA production and primarily [$^3H$]RNA turnover, respectively. The calculated DNA/RNA rate ratio is only meaningful during the phase of coordinate [$^3H$]RNA and [$^3H$]DNA production; the increase in the DNA/RNA ratio with extended incubation was due to analytical artifacts resulting from depletion of [$^3H$]adenine from the medium.

The rate at which [$^3H$]adenine is removed from the medium depends upon many variables, including: (i) total biomass and rates of RNA and DNA syntheses, (ii) concentration of reactive nonradioactive molecules present (i.e., the extracellular [$^3H$]adenine-specific radioactivity), and (iii) proportion of de novo synthesis relative to assimilation via salvage pathways. The data presented in Fig. 4 demonstrate the importance of conducting time series measurements, or at least confirming that exogenous [$^3H$]adenine had been present for the duration of the incubation period.

**Kinetics of RNA and DNA productions in bacterial cultures.** From previous experiments conducted in our laboratory, we have found that mid-logarithmic-phase *S. marinorubra* cultures ($5 \times 10^7$ cells per ml; doubling time 35 to 45 min) exhaust a pulse of 5 to 10 $\mu$Ci of [$^3H$]adenine per ml (specific activity; 15.5 Ci/mmol) in less than 10 min. An experiment was performed to evaluate the kinetics of [$^3H$]adenine removal and $^3H$-nucleic acids production during an extended incubation period. The results (Fig. 5) indicate that the turnover of "stable" RNA continues to provide labeled precursors (either [$^3H$]AMP → [$^3H$]ADP → [$^3H$]ATP → [$^3H$]dATP or [$^3H$]-AMP → [$^3H$]ADP → [$^3H$]dADP → [$^3H$]dATP) for net DNA synthesis well beyond the period of time required to exhaust the pulse of [$^3H$]-

adenine from the medium (i.e., the linear phase of [$^3$H]RNA production, 0 to 4 min; Fig. 5). Additionally, the DNA/RNA synthesis rate ratios were constant during the period in which [$^3$H]adenine was present in the medium. These results were similar to data obtained with environmental samples.

**Pulse-chase experiment.** Upon the addition of cold (i.e., nonradioactive) adenine to the medium, the productions of $^3H_2O$ and [$^3$H]RNA immediately ceased, similar to data presented by Karl et al. (part I, in press). In contrast to the immediate cessation of net [$^3$H]RNA accumulation, [$^3$H]DNA production continued at a rate of 50% of the control sample despite the interruption of [$^3$H]adenine uptake (Fig. 6). These data support the hypothesis discussed earlier in which stable RNA turnover continues to supply labeled precursors (as [$^3$H]AMP) for net DNA accumulation, and further suggest that intermediary metabolism of [$^3$H]adenine, rather than turnover of [$^3$H]RNA, is the major source of $^3H_2O$.

**Nalidixic acid experiments.** The successful application of simultaneous RNA and DNA syntheses rate measurements demands that the separation procedures utilized be free from analytical interferences. Generally, the rates of RNA synthesis exceed the DNA rates by factors ranging from 2 to 50. It is important, therefore, that the chemical isolation of DNA be critically eval-
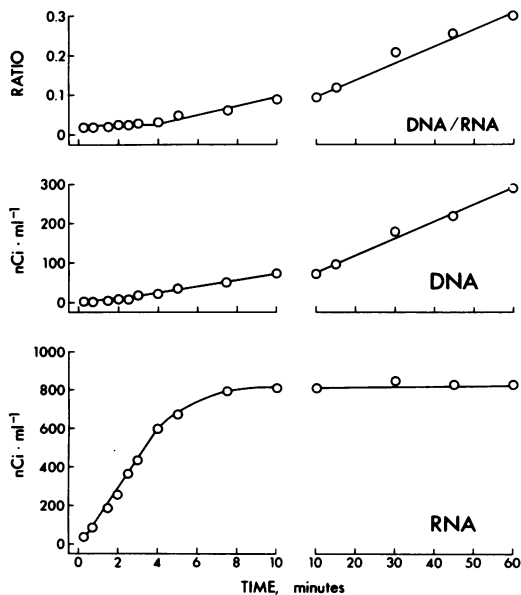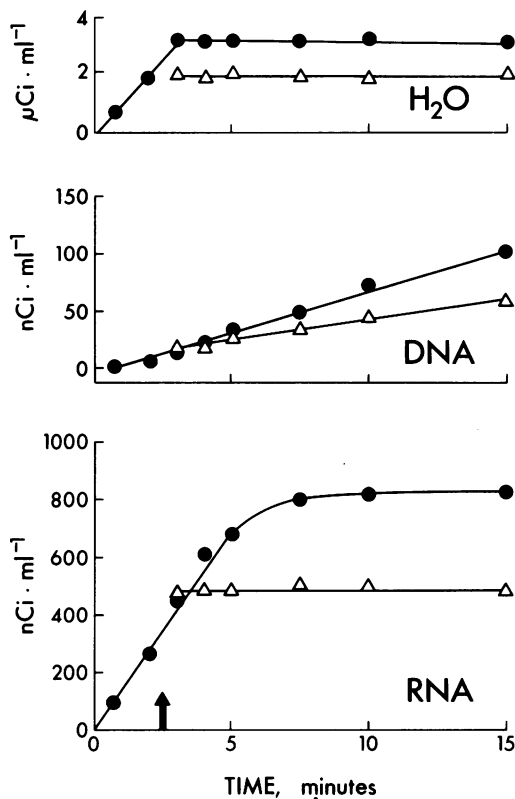


FIG. 6. *Kinetics of [$^3$H]RNA, [$^3$H]DNA, and $^3H_2O$ productions after the addition of [$^3$H]adenine to a mid-logarithmic-phase culture of S. marinorubra. Symbols: △, degree of labeling after a chase of $10^{-4}$ M nonradioactive adenine; ●, the control samples. The [$^3$H]adenine pulse was added at 0 min, and the nonradioactive chase was added at 2.5 min.*

uated and the possibilities of contamination from RNA be eliminated. Nalidixic acid was used to selectively inhibit DNA synthesis in order to determine the effectiveness of the separation procedures used in this study. A summary of the results is presented in Table 1.

The rate of DNA synthesis (expressed as percentage of control) decreased expectedly with increasing nalidixic acid concentration to a maximum inhibition of 95% at 0.2% (wt/vol) nalidixic acid. By comparison, the rate of RNA synthesis was unaffected except at the highest nalidixic acid concentration used (Table 1). Winshell and Rosenkranz (32) reported similar effects of nalidixic acid. Therefore, minimal contamination is indicated in our isolation of [$^3$H]DNA by the higher concentrations of [$^3$H]RNA present.

**Temporal variation in nucleic acid biosyntheses.** Ten liters of Kaneohe Bay surface water was placed in a glass carboy at 7:00 a.m. (local time) and maintained under in situ con-



FIG. 5. *Kinetics of [$^3$H]RNA and [$^3$H]DNA productions and incubation time-dependent DNA/RNA rate ratios after the addition of [$^3$H]adenine to a mid-logarithmic-phase S. marinorubra culture.*

TABLE 1. *Effects of nalidixic acid on RNA and DNA syntheses in S. marinorubra*

| Nalidixic acid concn (%, wt/vol) | RNA synthesis (% of control) | DNA synthesis (% of control) | DNA/RNA (%) |
|---|---|---|---|
| 0.0 | 100 | 100 | 4.5 |
| 0.002 | 103 | 82 | 3.5 |
| 0.01 | 106 | 45 | 1.9 |
| 0.02 | 96 | 23 | 1.1 |
| 0.2 | 76 | 5 | 0.3 |

ditions (i.e., temperature, light) for the duration of the experiment. At predetermined times, 500-ml subsamples were removed and incubated in situ for exactly 1 h with 0.1 $\mu$Ci of [$^3$H]adenine per ml (final concentration), and the rates of RNA and DNA syntheses were determined as described previously (Table 2).

The rates of RNA synthesis during the daylight period ranged from 1,796 to 2,368 pmol of adenine incorporated into RNA per liter per h with no apparent temporal variability. In contrast to the relatively constant rates of RNA synthesis, the rates of DNA synthesis consistently increased from 28.9 pmol of deoxyadenine incorporated into DNA per liter per h at 7:00 a.m. (7:00 to 8:00 a.m.) to 168 pmol per liter per h at 5:00 p.m. (5:00 to 6:00 p.m.). Consequently the DNA/RNA rate ratio varied by greater than 400% during the daylight hours. These data suggest that the synthesis of DNA (i.e., cell division) is more closely coupled to diurnal processes than is the synthesis of RNA (i.e., cell growth).

The data presented in Fig. 7 indicate the effect of diurnal periodicity in DNA synthesis on the results of a time series experiment. [$^3$H]adenine was added to two 4-liter subsamples of Kaneohe Bay surface water at 7:00 a.m. (local time) and incubated at in situ temperature, one in the light and the other in the dark. Subsamples (500 ml) were withdrawn at predetermined time periods (up to 24 h) and analyzed for [$^3$H]RNA and [$^3$H]DNA. The data indicate that neither RNA nor DNA synthesis (nor the DNA/RNA rate ratio) was adversely affected by the dark incubation conditions. These results are similar to previous light versus dark RNA synthesis experiments conducted with natural microbial communities (Karl et al., parts I and II, in press). The [$^3$H]DNA production was not linear with respect to time but demonstrated an accelerated production during the latter half of the daylight hours, consistent with the data presented in Table 2.

The data reported above suggest that the proposed DNA/RNA rate ratio technique may be used to study discontinuous growth of micro-

bial communities in nature. However, the observed time-varying relative rates of DNA synthesis, and hence DNA/RNA rate ratios, require

TABLE 2. *Temporal patterns of RNA and DNA syntheses in Kaneohe Bay plankton populations*

| Period of incubation | Rate of RNA synthesis (pmol of adenine incorporated into RNA per liter per h) | Rate of DNA synthesis (pmol of deoxyadenine incorporated into DNA per liter per h) | Rate ratio (DNA/RNA, %) |
|---|---|---|---|
| 8:15–9:15 a.m. | 1,796 | 32.2 | 1.8 |
| 9:30–10:30 a.m. | 1,926 | 52.9 | 2.7 |
| 10:45–11:45 a.m. | 2,368 | 76.5 | 3.2 |
| 12:00–1:00 p.m. | 2,029 | 88.3 | 4.4 |
| 1:15–2:15 p.m. | 1,881 | 111.9 | 5.9 |
| 2:30–3:30 p.m. | 2,145 | 115.4 | 5.4 |
| 3:45–4:45 p.m. | 2,073 | 107.8 | 5.2 |
| 5:00–6:00 p.m. | 2,259 | 168.0 | 7.4 |
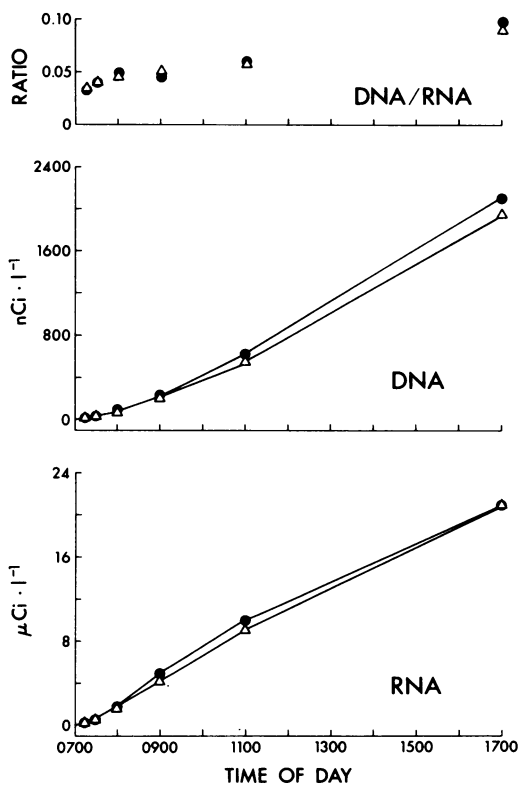


FIG. 7. *Kinetics of [$^3$H]RNA and [$^3$H]DNA productions and calculated DNA/RNA rate ratios for a Kaneohe Bay plankton population after the addition of [$^3$H]adenine. The tracer was added at 7:00 a.m., and subsamples were extracted at the time periods indicated (i.e., variable incubation time). Symbols: △, incubations conducted under in situ light conditions; ●, incubations conducted in the dark. The 24-h DNA/RNA rate ratio was 0.075 in the light and 0.087 in the dark.*

that one also bear in mind the potential significance of these processes whenever conducting short-term (<12-h) investigations.

Table 3 summarizes the ATP concentrations, ATP (and dATP) pool-specific radioactivities, rates of RNA and DNA syntheses, and corresponding DNA/RNA rate ratios for representative freshwater and marine environments. It should be noted that this present technique is suitable for measuring rates of RNA and DNA syntheses in extremely oligotrophic oceanic environments (e.g., Central Pacific Ocean) as well as hypereutrophic freshwaters (e.g., Krauss Pond). The DNA/RNA rate ratios ranged from 3.3 to 31.8% and were not significantly correlated to either total microbial biomass (ATP) or total microbial activity (RNA and DNA syntheses).

## DISCUSSION

Numerous investigations have been conducted on rates of nucleic acids and protein biosyntheses in microorganisms at different growth rates or under various environmental conditions (3, 5, 16, 18, 19, 21, 23, 26–28, 31). It appears well established, therefore, that RNA and DNA syntheses in microbial cells are tightly controlled and well regulated such that the ratio of the rates of RNA and DNA syntheses is positively correlated with the cellular growth rate. Previous investigators have discussed and justified the selective use of either RNA (12;

Karl et al., parts I and II, in press) or DNA (1, 7, 30; Moriarty and Pollard, in press) synthesis to estimate microbial growth in nature. Simultaneous determination of the rates of RNA and DNA syntheses can only boast the advantages attributed to each approach. For reasons to be discussed below, the present approach should yield more meaningful ecological information than previous nucleic acid synthesis methods.

The literature on methodologies in nucleic acid chemistry is extensive (for reviews see references 9, 11, 20); however, the most comprehensive procedures have been developed for the analyses of animal tissues, especially liver, and their unequivocal application to microorganisms without rigorous evaluation is unjustifiable. In the present investigation every effort was made to maximize quantitative isolation, purification, and measurement of [$^3$H]RNA and [$^3$H]DNA. The addition of nonradioactive RNA and DNA to maximize the precipitation of low concentrations of [$^3$H]RNA and [$^3$H]DNA was essential for the quantitative recovery of $^3$H-nucleic acids from most environmental samples. The Schmidt-Thannhauser procedure (as described in references 9, 11, and 20) was employed for the separation of RNA and DNA, and the results of the nalidixic acid experiment (Table 1) indicate that contamination of [$^3$H]DNA by the (generally) much larger [$^3$H]RNA pool is negligible.

In theory, DNA/RNA synthesis rate ratios

TABLE 3. *Summary of ATP concentrations, ATP (and dATP) pool-specific radioactivities, rates of RNA and DNA syntheses, and DNA/RNA rate ratios for selected marine and freshwater environments*

| Sample location | ATP (ng/liter) | ATP (dATP) pool-specific radioactivity (nCi/pmol) | Rate of RNA synthesis (pmol of adenine incorporated into RNA per liter per h) | Rate of DNA synthesis (pmol of deoxy-adenine incorporated into DNA per liter per h) | Rate ratio (DNA/RNA, %) |
|---|---|---|---|---|---|
| Freshwater | | | | | |
| Krauss Pond (3/80) | 14,200 | 0.60 | $3.13 \times 10^4$ | $1.10 \times 10^3$ | 3.5 |
| Krauss Pond (4/80) | 18,600 | 0.39 | $1.10 \times 10^4$ | $3.62 \times 10^2$ | 3.3 |
| Krauss Pond (7/80) | 9,010 | 0.54 | $6.76 \times 10^4$ | $7.57 \times 10^3$ | 11.2 |
| Krauss Pond (4/81) | 5,028 | 0.91 | $4.30 \times 10^4$ | $6.10 \times 10^3$ | 14.2 |
| Marine | | | | | |
| Northeast Pacific Ocean (35° 47·8'N; 123° 39·8'W) | | | | | |
| 1 m | 131 | 1.01 | 76 | 5.5 | 7.2 |
| 20 m | 308 | 1.37 | 163 | 20.5 | 12.6 |
| 50 m | 142 | 0.55 | 92 | 18.0 | 19.6 |
| Central Pacific Ocean (18° 43·8'N; 156° 50·4'W) | | | | | |
| 10 m | 45.8 | 2.30 | 13.3 | 3.85 | 28.9 |
| 50 m | 53.4 | 1.65 | 17.3 | 5.50 | 31.8 |
| 100 m | 39.4 | 1.35 | 11.6 | 2.80 | 24.1 |
| 150 m | 16.9 | 0.60 | 12.6 | 2.15 | 17.8 |
| Kaneohe Bay, Hawaii (21° 26·1'N; 157° 46·7'W) | | | | | |
| 1 m | 418 | 1.45 | 1,435 | 144 | 10.0 |

might be obtained in environmental samples by conducting double-labeling experiments using [$^{14}$C]thymidine and [$^{3}$H]uridine. Unfortunately, uridine labels both RNA and DNA, but to different specific radioactivities (4, 14). Furthermore, there is no evidence that thymidine and uridine are assimilated by all, or even by the same subset of microorganisms. [$^{3}$H]adenine (labeled in position 2) is the only nucleic acid base which unequivocally labels only one base moiety in both DNA and RNA, and to the same specific radioactivity (i.e., specific radioactivity of ATP = specific radioactivity of dATP). The use of a single precursor ([$^{3}$H]adenine) circumvents most of the analytical difficulties and complications encountered with double-labeling experiments. Furthermore, extensive investigations have determined that the majority of unicellular microorganisms assimilate [$^{3}$H]adenine under in situ conditions (Karl et al., parts I and II, in press).

Previous measurements of microbial RNA synthesis in marine and freshwater ecosystems have noted that kinetic compartmentalization of the nucleic acid precursor pools may occasionally affect the calculated rates especially during the initial time periods of labeling (Karl et al., parts I and II, in press). In contrast to this initial time-varying rate of RNA synthesis, the DNA/RNA rate ratio is absolutely constant throughout the entire period of incubation, provided exogenous [$^{3}$H]adenine remains in the medium. This result suggests that the mechanism(s) responsible for ATP pool compartmentalization (kinetic or barrier or both) also affects dATP biosynthesis to the same extent; or, alternatively, our original model of ATP pool compartmentalization to explain the initial labeling kinetics may be in error.

In the present study, time series experiments were conducted wherein the incubation was purposely extended well beyond the time necessary to exhaust the exogenous [$^{3}$H]adenine (Fig. 4 and 5). The results obtained with bacterial batch cultures and environmental samples indicate that turnover of "stable" [$^{3}$H]RNA is sufficient to allow continued production of [$^{3}$H]DNA from nucleotide precursor recycling. The pulse-chase experiment (Fig. 6) supports this model. Therefore, accurate estimates of DNA/RNA rate ratios are only possible for time periods during which exogenous [$^{3}$H]adenine is present in the medium.

The method proposed herein for estimating microbial productivity from DNA/RNA rate ratios eliminates the need to determine precursor-specific radioactivities since both RNA and DNA are synthesized from isotopically equilibrated nucleotide precursor pools. Omission of this measurement will significantly reduce the time required for sample analysis and, additionally, provides a procedure which is amenable for in situ incubation and extraction in specialized marine microbiological investigations (e.g., deep-sea, hydrothermal vents, etc.). However, it should be emphasized that ATP (and dATP) pool-specific radioactivities are still absolutely essential if one desires quantitative estimates of nucleic acid production in nature (i.e., picomoles of RNA or DNA synthesized per liter per hour).

The proposed DNA/RNA rate ratios singly, or used in conjunction with the absolute rates of RNA and DNA syntheses, can yield valuable ecological information concerning the growth rates and productivities of microbial populations in nature. The rate ratio index is a direct measure of the RNA and DNA cell quotas which appear to be a linear function of growth rate, if the possibility of diurnal changes in DNA synthesis is acknowledged. Therefore, direct comparisons can be made among microbial communities of variable composition and biomass. Furthermore, the calculated rates of RNA and DNA syntheses can be used to predict total productivity (primary or secondary or both) or growth rate (doubling time) in nature using an approach which is analogous to that used in phytoplankton ecology, with the relationship: $\mu = 1/P \cdot dP/dt$, where $\mu$ = growth rate (doubling time), $P$ = RNA or DNA concentration of the water sample, and $dP/dt$ = RNA or DNA production rate. Undoubtedly, a portion of the total RNA and DNA may be associated with nonliving particulate materials, thereby underestimating the growth rate of the total microbial community, but surely this interference from detrital nucleic acids is likely to be less than that for other proposed cellular components such as carbon, nitrogen, or phosphorus. In addition, it may be possible analytically to separate "living" from "non-living" nucleic acids in aquatic environments; work is currently underway in my laboratory.

A limitation of this present approach, which is common to all investigations of nucleic acid precursor assimilation, regards the actual base composition of microbial RNA and DNA. Extensive studies have revealed that adenine represents 25.3 ± 1.7 mol% of bacterial RNA (24), whereas the adenine content of DNA is more variable (probable range for representative marine bacteria and unicellular algae is 20 to 35 mol%; 6, 10). Consequently, DNA/RNA rate ratios might be expected to vary between communities or with succession within a given microbial assemblage simply due to differences in the DNA base composition of the dominant and

most actively growing cells. Furthermore, the values calculated for DNA production (grams or moles per liter per hour) would also have the same degree of uncertainty. At the present time, the variability resulting from differences in DNA base composition is considered to be negligible compared with the magnitude of cumulative errors (both systematic and otherwise) and the precision of ecological measurements in general.

In conclusion, a method for the simultaneous measurements of RNA and DNA syntheses in aquatic microbial communities has been devised, evaluated, and tested with natural assemblages of microorganisms. The proposed method is extremely sensitive and operationally simple and straightforward. It appears that this method will be well suited for measuring and comparing microbial productivities in diverse marine and freshwater environments.

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