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Bacterioplankton RNA, DNA, protein content and relationships to rates of thymidine and leucine incorporation

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ABSTRACT: Bacterial macromolecules have often been used successfully as estimates of bacterial biomass in environmental samples. Less is known about the relationship between macromolecular content and rates of growth and activity. The ratio of RNA to DNA in bacteria has long been thought to be proportional to levels of metabolic activity and growth. We have used the nucleic acid fluorochrome thiazole orange combined with specific nuclease digestions to determine RNA:DNA ratios and compared these data to more standard measures of bacterial growth and activity. More than 100 samples from a wide variety of estuarine and marine environments were examined and the average RNA, DNA, and protein content per bacterial cell was found to be 9.44 ± 6.25 fg, 5.75 ± 2.35 fg, and 29.29 ± 11.57 fg, respectively. Initial experiments demonstrated a strong correlation of RNA:DNA ratios with growth rates for a laboratory organism. We then compared RNA:DNA ratios to cell specific rates of ^{3}H-thymidine and ^{14}C-leucine incorporation as estimates of growth rates for naturally occurring bacterioplankton communities in whole water and the <0.8 µm size fraction in estuarine and coastal waters in the Northern Gulf of Mexico. Nucleic acid ratios were generally very low in the natural communities, usually below those determined for late stationary phase pure cultures. No significant or consistent relationship was observed between RNA:DNA ratios and rates of ^{3}H-thymidine and ^{14}C-leucine incorporation in naturally occurring bacterioplankton communities. No relationship could be ascertained whether compared against location or water temperature. Thymidine incorporation rates were found to be completely independent of cellular protein content while leucine incorporation rates were more related to protein content. Our data suggest that while RNA, DNA, and protein content are strongly correlated with bacterial numbers in our environmental samples, the low metabolic activity and the heterogeneous composition of bacterioplankton communities may preclude the use of these parameters as biochemical indicators of activity in the environment.

KEY WORDS: RNA:DNA ratios · Thymidine incorporation · Leucine incorporation

INTRODUCTION

Bacterial biomass has most often been measured with epifluorescence direct counts (Hobbie et al. 1977, Porter & Feig 1980) and occasionally by macromolecular components such as DNA (Paul & Carlson 1984, Paul et al. 1985), protein (Simon & Azam 1989), ATP (Karl 1993), and lipids (Findley et al. 1989). Methods to measure bacterial activity and productivity have not been without controversy and have most often included radiotracer assays using assimilation of ^{3}H-thymidine as an estimator of DNA synthesis (Fuhrman & Azam 1982), ^{3}H-adenine as an estimator of DNA and RNA synthesis (Karl 1982), ^{3}H-uridine to estimate RNA synthesis (LaRock et al. 1979), and ^{14}C-leucine incorporation to estimate protein synthesis (Kirchman

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et al. 1985). Questions about the reliability of the different radiotracer methods has led to the testing of other methods by which bacterial productivity may be estimated. Biochemical indicators of metabolic activity have been more scarce and have included adenylate energy charge (Karl & Holm-Hansen 1978) and ATP:DNA ratios (Jeffrey & Paul 1986a, b). The ratio of RNA to DNA in bacteria has long been thought to be proportional to levels of metabolic activity and growth based on several studies with enteric bacterial strains of *Escherichia coli* and *Salmonella typhimurium* (Neidhardt & Magasanik 1959, Kjeldgaard & Kurland 1963, Maaløe & Kjeldgaard 1966, Rosset et al. 1966) which often used growth rates orders of magnitude faster than those measured in the environment. RNA:DNA ratios have been used as biochemical indicators of growth in a wide variety of marine organisms including phytoplankton (Thoreson et al. 1983, Dortch et al. 1985, Berdalet & Dortch 1991), invertebrates (Sutcliffe 1970), fish (Buckley & Lough 1987, Bulow 1987), and, more recently, bacteria (Mordy & Carlson 1991, Kemp et al. 1993, Kerkof & Ward 1993). Most studies with marine bacteria have analyzed growth under laboratory manipulated conditions (Kemp et al. 1993, Kerkhof & Ward 1993) with few analyses of indigenous bacterial populations (Mordy & Carlson 1991, Lee & Kemp 1994). While RNA:DNA ratios have generated interest in recent years, total protein content and its relationship to rates of bacterial activity and growth has received minimal investigation. We have used the nucleic acid fluorochrome thiazole orange (Berdalet & Dortch 1991) combined with RNAase digestions to determine RNA:DNA ratios in a wide variety of oceanic and estuarine samples and compared these data with more commonly used measures of bacterial activity and productivity, ^{3}H-thymidine and ^{14}C-leucine incorporation. In addition, we have determined protein concentrations in a variety of natural bacterioplankton samples and examined whether protein content is related to bacterial growth rates.

MATERIALS AND METHODS

Sampling collection. The location of sampling sites is depicted in Fig 1 Water samples were collected during 2 cruises in the Northern Gulf of Mexico aboard the RV 'Gyre' during 2-6 October 1992 and the OSV 'Peter W. Anderson' during 21-26 May 1993. Surface water samples were collected from coastal waters along the Florida, USA, panhandle during 2 cruises aboard the RV 'Bellows' 11-15 May 1992 and 3-6 May 1993. A 48 h diel study was conducted in Santa Rosa Sound on 29-30 September 1993. Samples were also collected from coastal waters adjacent to Miami, Florida, during 13 and 14 March 1993 while aboard OSV 'Peter W. Anderson' and from coral surface microlayers off Key Largo, Florida, 28 June to 12 July 1992.

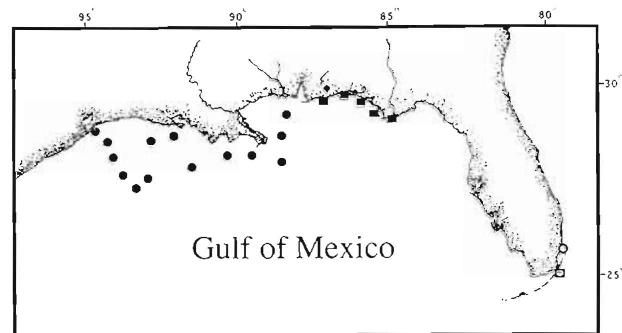

Fig. 1 Locations of water samples collected during a cruise aboard the (●) RV 'Gyre' during 2-6 October 1992 and the OSV 'Peter W. Anderson' 21-26 May 1993, and aboard the (■) RV 'Bellows' 11-15 May 1992 and 3-6 May 1993. (◆) 48 h diel study conducted in Santa Rosa Sound 29-30 September 1993. (o) Samples collected in transects from the Virginia Key sewage outfall aboard the OSV 'Peter W. Anderson' 13 and 14 March 1993. (□) Samples collected from coral surface microlayers off Key Largo, Florida, 28 June to 12 July 1992

Samples for nucleic acid determinations were collected as follows. Whole water samples (sextuplet) of 100 to 500 ml were immediately filtered through 0.22 µm pore-size Millipore (Bedford, MA, USA) GS filters which were then rolled in 2 ml micro-centrifuge tubes (particle side inward) and frozen in dry ice after the addition of 1 ml Tris-Ca^{++} buffer (0.1 M NaCl, 0.1 M Tris, pH 7.5, to which 0.1324 g l^{-1} $CaCl_2 \cdot 2H_2O$ was added after autoclaving; Berdalet & Dortch 1991). To remove organisms larger than bacteria, water was passed through a 10 µm pore-size cartridge prefilter (MSI, Westboro, MA, USA) followed by a 0.8 µm pore-size cartridge filter (Nuclepore, Pleasanton, CA, USA). Samples were then filtered and frozen as described for whole water samples. Samples for protein determinations in the <0.8 µm fraction were collected on 0.22 µm pore-size Millipore GV membranes and all samples were stored on either dry ice or at -70°C until analysis.

Fluorometric quantification of RNA and DNA. Nucleic acids were determined by combining extraction via sonication methods described by Paul & Myers (1982) with the use of the fluorochrome thiazole orange which reacts with both DNA and RNA (Berdalet & Dortch 1991). Thiazole orange (Becton Dickenson, San Jose, CA) was maintained as a 4.33 M stock solution in methanol and added to samples at a final concentration of 3.43 × 10^{-7} M. Samples were allowed to thaw on ice and the filter and Tris-Ca^{++} were transferred to a 20 ml snap cap vial. An additional 2 ml of buffer and Triton x-100 (final concentration 0.05%) were added. Samples were sonicated on

ice for 40 s using a ca 1.9 cm (0.75 inch) tip on a Tekmar Sonic Disrupter model TM 600 set at 10% max output. Two ml of the buffer was microcentrifuged at 10 000 rpm (8000 × *g*) in an Eppendorf model 5402 centrifuge for 10 min at 4°C and the clarified supernatant divided into 3 subsamples. For total nucleic acid fluorescence, 1 aliquot was brought to a final volume of 2 ml with Tris-Ca^{++} and 1 ml of 1.03 µM thiazole orange working solution added. A second subsample was treated with RNAase (Type III A, Sigma Chemical Co, St. Louis, MO, USA) by adding 50 µl of a 1 mg ml^{-1} stock solution to the sample and incubating at 37°C for 10 min. These samples were then brought to a final volume of 2 ml with Tris-Ca^{++} and 1 ml working solution of thiazole orange added. Fluorescence was determined on a Perkin Elmer LC50 spectrofluorometer with excitation (E_x) and emission (E_m) wavelengths of 511 and 533, respectively. DNA content was determined in a third subsample using the DNA specific fluorochrome Hoechst 33258 (Calbiochem, La Jolla, CA) as previously described (Paul & Myers 1982) using a Turner model 112 filter fluorometer (E_x = 320–390 nm; E_m = 430–490 nm). Unknowns were compared against standard curves generated using calf thymus DNA and *Escherichia coli* tRNA (Paul & Myers 1982, Berdalet & Dortch 1991). Linear regression of typical standard curves resulted in $r^2 \geq 0.99$ (data not shown).

Protein concentrations. Protein content of bacteria size particles was measured using the bicinchoninic acid (BCA) assay (Smith et al. 1985). Bacteria size particles (<0.8 µm) were collected on Millipore type GV membranes (0.22 µm pore size), and the membrane plus particles was stored in a 1.5 ml screw-cap tube with O-ring at -70°C. Protein was extracted by adding 1 ml of 1% sodium dodecyl sulphate (SDS) and heating for 10 min at 100°C. The extract with membrane was centrifuged at 14 000 × *g* for 10 min at room temperature, and an aliquot of the supernatant to be assayed was transferred to a fresh tube. Assay reagents and protocol were according to the Pierce (Rockford, IL, USA) kit #23235. Bovine serum albumin (BSA) diluted in 1% SDS was used as standard. Concentration was calculated from the linear regression equation derived from standard treated as samples (i.e. membranes were added and then tubes were heated). Therefore, standards curves used here accounted for both the filter background and any loss of protein to the filter. The later problem was minimal because Millipore GV membranes are low protein binding. Slopes of standard curves for standards with or without a GV membrane and heating were not significantly different (*t*-test, $p < 0.01$).

^{3}H-thymidine and ^{14}C-leucine incorporation. The method for ^{3}H-thymidine and ^{14}C-leucine incorporation into the total macromolecule fraction has been previously described (Chin-Leo & Kirchman 1988). ^{3}H-thymidine was added to triplicate samples to a final concentration of 10 nM while ^{14}C-leucine was added to a final concentration of 20 nM. All incubations were limited to 30 to 60 min. Bacterial numbers were determined by epifluorescence direct counts of 4',6-diamidino-2-phenylindole (DAPI) stained samples by the method of Porter & Feig (1980).

RNA:DNA in culture. To confirm that the method could detect changes in RNA:DNA ratios in bacteria growing at different rates, *Escherichia coli* JM109 was followed through a typical growth curve and sampled for RNA:DNA ratios. Cells were grown overnight in Luria-Bertani (LB) medium (Sambrook et al. 1989) and a 5% inoculum added to fresh medium. Absorbance at 550 nm was used to monitor cell density. Cells were collected for RNA:DNA ratios at lag, mid-log, late-log, and stationary phases of growth. RNA:DNA ratios were determined as described above.

RESULTS

Fluorometric determination of RNA:DNA ratios

Initial experiments indicated that sonication of the filter containing the cell sample for 40 s using a ca 1.9 cm (0.75 inch) tip on the available sonicator (Tekmar Sonic Disrupter model TM 600 set at 10% max. output) yielded maximal recovery of both RNA and DNA (data not shown). Preliminary experiments indicated that a thiazole orange final concentration of 3.43×10^{-7} M gave linear fluorescence yields with both RNA and DNA standards between 0 and 2 µg. The fluorescence yield between Hoechst 33258 and DNA has been described previously (Paul & Myers 1982), and we did not deviate from the methodology described in that study.

Thiazole orange fluorescence can be used to determine both DNA and RNA content (Berdalet & Dortch 1991). There was no significant difference between DNA content determined using thiazole orange and Hoechst 33258 ($p < 0.001$). We opted, however, to use Hoechst 33258 for DNA determinations since it would allow direct comparison with earlier studies of DNA content in marine bacterioplankton (Paul & Carlson 1984, Paul et al. 1985). To determine RNA content we opted to use RNAase treatments instead of DNAase treatments because the RNAase reactions were more rapid and did not require the addition of Mg^{++} which appeared to alter fluorescence with thiazole orange. A 10 min incubation was sufficient for complete RNAase digestion and resulted in no loss of

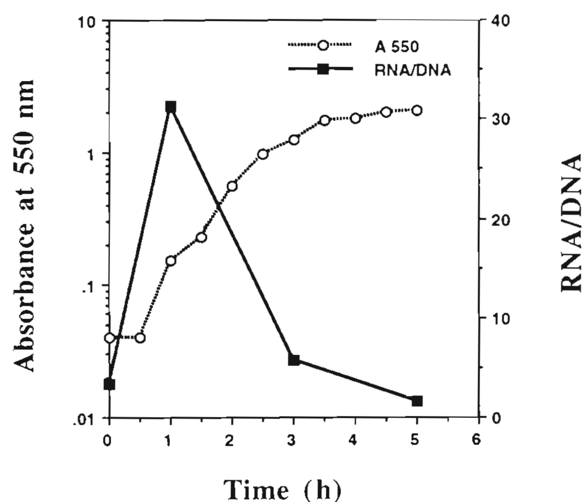

Fig. 2. RNA/DNA ratios of *Escherichia coli* strain JM109 during a growth curve. A sharp increase in the RNA:DNA ratio was seen in mid-log phase followed by a rapid decline toward stationary phase

DNA fluorescence (data not shown). RNA content was determined from the amount of fluorescence lost after RNAase digestion. All samples were conducted in 3 to 6 replicates. The mean coefficient of variation for all samples (n = 180) was 13.61 ± 8.24 for RNA, 7.11 ± 5.72 for DNA, and 17.13 ± 12.77 for RNA:DNA ratios.

RNA:DNA ratios in bacterial culture

RNA:DNA ratios measured in *Escherichia coli* during a growth curve are presented in Fig. 2. The methods were able to detect distinct changes in RNA:DNA ratios in this bacterial culture sampled under widely different growth rates. RNA:DNA ratios were observed to be proportional to growth rate in *E. coli* grown in the lab. A sharp increase was seen in the ratio of RNA:DNA during mid-log phase (maximal growth rate) followed by a rapid decline toward the onset of stationary phase and into late stationary phase.

Macromolecular content and ^{3}H-thymidine and ^{14}C-leucine incorporation rates

Comparison of cellular rates of ^{3}H-thymidine and ^{14}C-leucine incorporation with RNA:DNA ratios in

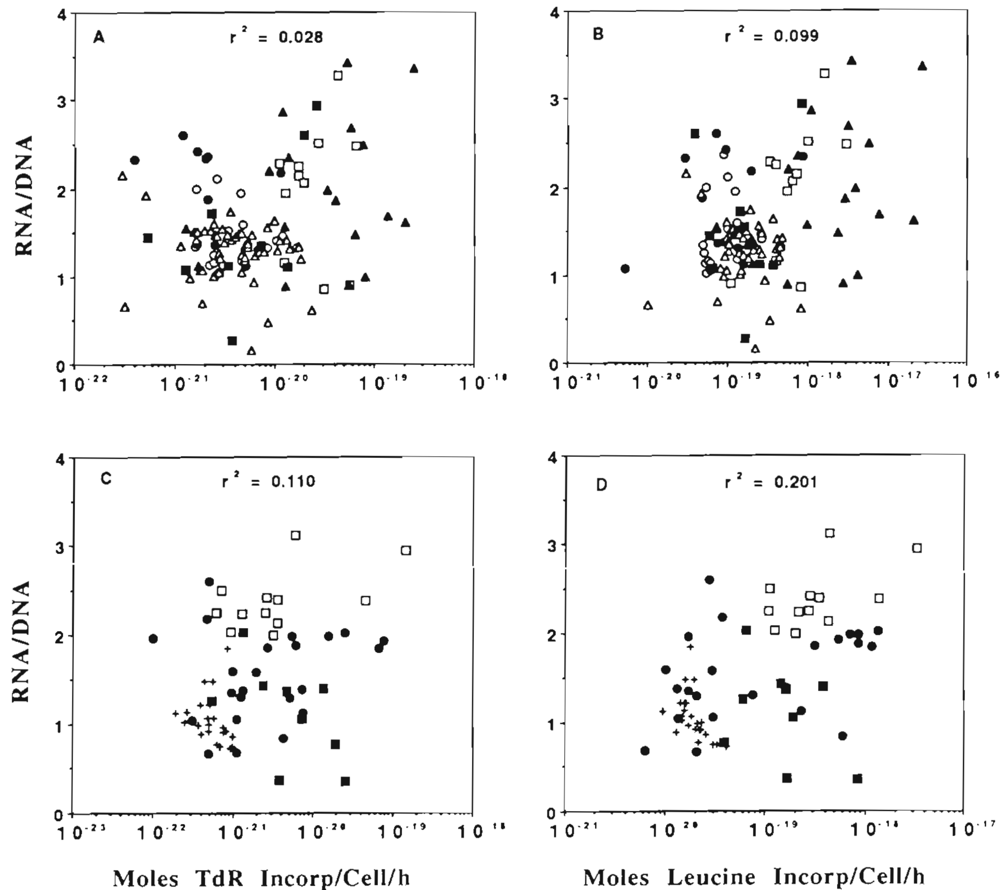

Fig. 3. Comparison of RNA/DNA ratios with ^{3}H-thymidine (TdR) and ^{14}C-leucine incorporation. Symbols represent different sampling locations: (○) RV 'Bellows', May 1992; (●) RV 'Gyre', October 1992; (□) RV 'Bellows', May 1993; (△) Miami, March 1993; (■) OSV 'Peter W. Anderson', Gulf of Mexico, May 1993; (▲) Key Largo, coral Reefs. Cellular rates of (A) ^{3}H-thymidine incorporation and (B) ^{14}C-leucine incorporation in whole water fractions. Cellular rates of (C) ^{3}H-thymidine incorporation and (D) ^{14}C-leucine incorporation in the <0.8 µm fraction. r^2 values were determined by linear correlation

whole water and the <0.8 µm fraction are presented in Fig. 3. A total of 120 whole water and 90 size-fractioned samples were analyzed. The wide variety of environments sampled provided for a large range (greater than 3 orders of magnitude) in rates of thymidine and leucine incorporation. RNA:DNA ratios generally ranged between 0.5 and 3.5 (average 1.54 ± 0.58) for the whole water samples (Fig. 3A, B). No significant relationship was observed between RNA:DNA ratios and either radiotracer method. This absence of correlation was observed when data were grouped together as 1 data set or separated by research cruise location.

In certain environments, significant proportions of the total cellular nucleic acid pool might be due to micro-eukaryotes which have not been documented to incorporate significant amounts of ^{3}H-thymidine and ^{14}C-leucine added at nM concentrations. To ensure that the majority of micro-eukaryotes were removed, water samples were passed through a 0.8 µm pore-size filter. The percentage of the total particulate DNA in the <0.8 µm size fraction ranged from approximately 90% in oligotrophic Gulf of Mexico waters to approximately 20% in some estuarine samples (Jeffrey unpubl. results). Again, thymidine and leucine incorporation rates ranged over several orders of magnitude while RNA:DNA ratios ranged between 0.5 and 3 with an average of 1.42 ± 0.57. Results from the <0.8 µm (presumably bacteria) size fraction are presented in Fig. 3C, D. The correlation coefficients for both thymidine and leucine incorporation and RNA:DNA ratios in the size fractioned samples were again very low (Fig. 3C, D).

Cellular protein content in the <0.8 µm fraction was significantly correlated ($p < 0.05$) with both cellular DNA content ($r^2 = 0.55$) and RNA content ($r^2 = 0.72$) but less correlated to activity measures of ^{3}H-thymidine ($r^2 = 0.08$) and ^{14}C-leucine ($r^2 = 0.49$) incorporation.

RNA, DNA, and protein content in environmental samples

Macromolecular content in bacterial cells in the <0.8 µm size fraction was determined. The average bacterial DNA content for all samples (n = 126) was 5.75 ± 2.35 fg while RNA content averaged 9.44 ± 6.25 fg cell^{-1} (n = 90), and protein content averaged 29.28 ± 11.57 fg cell^{-1} (n = 103). The relationship between DNA l^{-1}, RNA l^{-1}, and protein l^{-1} and bacterial numbers in the <0.8 µm fraction for all locations is presented in Fig. 4. Each macromolecule was significantly correlated with bacterial cell numbers.

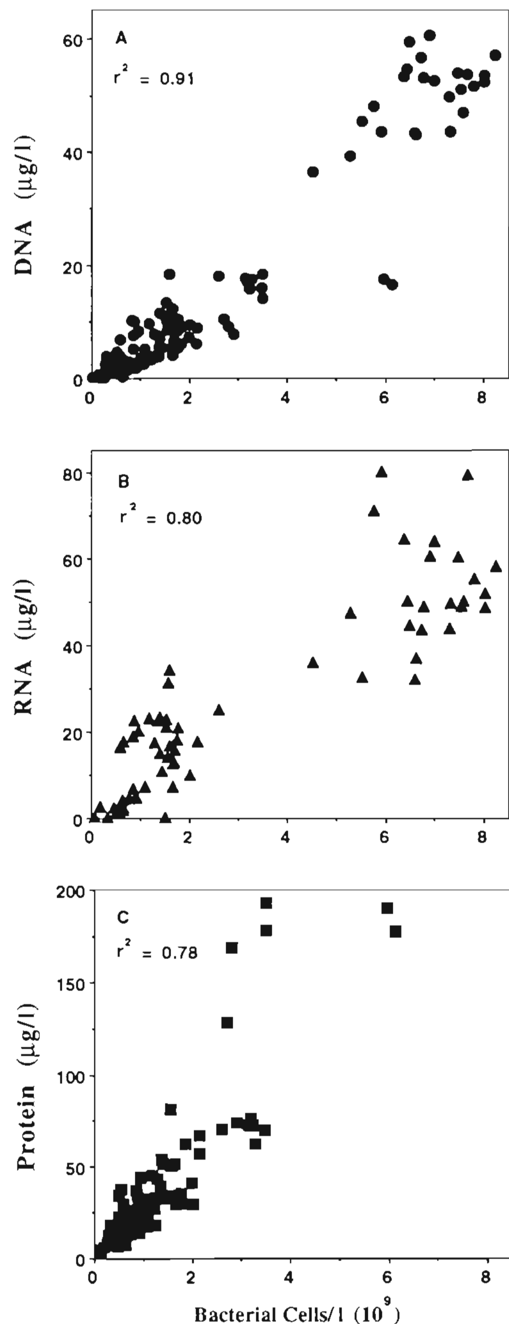

Fig. 4. Bacterial numbers and (A) DNA, (B) RNA, and (C) protein concentrations in the <0.8 µm size fraction

DISCUSSION

Fluorometric quantification of nucleic acids has been conducted for several years in a wide variety of sample types. Hoechst 33258 has been used extensively to quantify bacterial DNA content in marine ecosystems (Paul & Myers 1982, Paul & Carlson 1984, Paul et al. 1985, Boehme et al. 1993). The vast majority of RNA

determinations have been conducted using ethidium bromide as the fluorochrome. In recent years, however, newer fluorochromes, such as thiazole orange (Berdalet & Dortch 1991), which have greater specificity and fluorescence yield have been developed and these fluorochromes should provide for greater sensitivity in RNA quantification. We combined previously existing techniques to extract and quantify both DNA and RNA from sonicated cellular extracts. The method is simple, rapid, and reproducible. Because of the simplicity (i.e sonication and fluorometry), total nucleic acid recovery should be high due to the absence of organic extraction and precipitation steps.

RNA, DNA, and protein content determined in this study agree with values determined in other studies. We report an average bacterial DNA content of 5.75 fg while Paul and co-workers (Paul & Myers 1982, Paul & Carlson 1984, Paul et al. 1985, Boehme et al. 1993) have consistently reported a bacterial genome size of approximately 5.5 fg DNA cell^{-1}. Our value of average RNA content falls within ranges reported in other studies. Lee & Kemp (1994) recently reported bacterial RNA content between 1.6 and 5.4 fg cell^{-1} while Simon & Azam (1989) calculated RNA contents between 1.9 and 9.5 fg cell^{-1}. Our slightly higher values may be due, in part, to the more direct quantification method that we used. Protein content per cell was similar to that which Simon & Azam (1989) measured for marine bacteria in the biovolume range of 0.05 to 0.4 µm^3 Simon & Azam (1989) used high-performance liquid chromatography (HPLC) determination of amino acid residues from acid hydrolyzed bacteria for quantification of protein content for bacteria growing in seawater cultures. Protein concentration determined by the BCA assay is sensitive to the specific protein used as standard. For determination of protein in phytoplankton samples, for example, using BSA as standard may result in as much as a 30% underestimation of protein content relative to the use ribulose-1,5-diphosphate carboxylase (RuDPCase), an abundant protein in autotrophic organisms (Nguyen & Harvey 1994). Comparable results between the definitive yet labor intensive HPLC determination (Simon & Azam 1989) versus our simple BCA determination of protein in bacterial size particles suggests that there is not a gross underestimation of protein content due to our use of BSA as a standard protein, or that such a bias is within our sample to sample error. We know of no other measure of protein content specifically for marine bacterioplankton with which to compare our results.

Biochemical measures of bacterial activity and growth rate have the advantage of not requiring an incubation period which may introduce bottle effects and changes in community structure. Biochemical measures also do not rely on the ability of the target organisms to take up and incorporate an exogenous substrate (e.g. the presence of thymidine kinase required for thymidine incorporation; Jeffrey & Paul 1990). Interest in determining RNA:DNA ratios in environmental samples has received significant attention in recent years. Much of this attention may be traced to the wide use of 16S rRNA probes and the report by DeLong et al. (1989) demonstrating that both cellular rRNA content and RNA:DNA ratios were significantly correlated with growth rates of *Escherichia coli*. Early reports suggested that taxon specific measures of RNA:DNA ratios are correlated with growth rates. Kerkhof & Ward (1993) reported that RNA content decreased with decreasing growth rate for *Pseudomonas stutzeri* Zobell, even at very long generation times such as those suspected to be found in oligotrophic waters. Kemp et al. (1993) examined 4 marine bacterial isolates at growth rates comparable to those found in the environment. They reported that RNA:DNA ratios were closely related to growth rate for each isolate but that the relationship became less obvious when the individual isolates were combined to create a mixed community. To investigate whether this relationship is maintained in heterogeneous bacterial populations found in the environment, we have determined RNA:DNA in natural assemblages of microorganisms collected from a wide variety of locations in the Northern Gulf of Mexico and along the southeastern coast of the Florida peninsula. These diverse environments ranged from oligotrophic waters to extreme-ly productive coral surface microlayers. Traditional measures of heterotrophic activity ranged over 3 orders of magnitude, from 10^{-22} to 10^{-19} mol cell^{-1} h^{-1} for thymidine incorporation and from 10^{-21} to 10^{-18} mol cell^{-1} h^{-1} for leucine incorporation. These values are equivalent to estimated values for μ ranging from 0.0005 to 0.5 (Hoch & Kirchman 1993). RNA:DNA ratios from these samples varied much less with respect to heterotrophic activity, ranging from approximately 0.5 to 3 with an average of 1.42 ± 0.57 for samples collected from the bacterial size fraction (<0.8 µm). Correlation coefficients between RNA:DNA ratios and rates of thymidine and leucine incorporation demonstrated poor relationships among these parameters (Fig. 3). Further analysis found no distinction between offshore, onshore, and estuarine waters. During the cruise aboard the RV 'Bellows' in May 1992 we followed RNA:DNA ratios during outgoing tidal cycles at 4 estuaries in NW Florida and could not detect a pattern in RNA:DNA ratios even as bacterial productivity doubled.

There are only limited data available with which to compare the RNA:DNA ratios determined in this study. Using the fluorochromes ethidium homodimer and Hoechst 33258, Mordy & Carlson (1991) reported RNA:

DNA ratios very similar to those reported here. They examined RNA:DNA ratios in the <1 µm size fraction in waters collected in a depth profile off the California coast and reported ratios ranging from 1 to 4. It is more difficult to compare values determined in other studies owing to the differences by which RNA:DNA ratios were determined. In general, RNA:DNA ratios measured for cultures grown at environmentally relevant rates (0.01 to 0.001 h^{-1}) were comparable to those measured in the heterogeneous samples reported here, i.e. ratios between 1 and 4 (Kemp et al. 1993, Kerkhof & Ward 1993).

Kemp et al. (1994) reported results comparing thymidine and leucine incorporation rates with RNA:DNA ratios in samples collected from 3 transects near Cape Hatteras, North Carolina, USA. RNA:DNA ratios were determined using oligonucleotide rRNA probes. That study also reported a lack of correlation between bacterial activity and RNA:DNA ratios very similar to that reported here when nucleic acid fluorochromes were used to quantify nucleic acids.

Lee & Kemp (1994) combined multiple rRNA probes and fluorometry to estimate bacterial RNA and DNA content in winter and summer samples collected off of Long Island, New York, USA. They reported an inverse relationship between RNA content and temperature. No consistent relationship was reported, however, between rates of thymidine incorporation and RNA content. We observed no relationships between temperature and macromolecular content ($R^2 = -0.21$). Difference in sample size and locations could contribute to these apparent differences. While our data set was much larger, all samples were collected in subtropical and temperate locations where surface waters were predominately between 20 and 28°C. Hoch & Kirchman (1993) reported that bacterial growth rates in the Delaware Estuary correlated with temperature only at temperatures below 12°C. In locations with small temperature fluctuations, other factors such as community structure and exposure to other stresses such as ultraviolet radiation (Herndl et al. 1993, Jeffrey unpubl. data) may strongly influence bacterial activity.

While RNA:DNA ratios measured do not appear to be related to bacterial activity in the environment (Fig. 3), RNA content was highly correlated with measures of bacterial biomass. The correlation coefficient determined between µg RNA l^{-1} and µg DNA l^{-1} was 0.84 (data not shown) while the correlation between µg RNA l^{-1} and bacterial cells l^{-1} was 0.769 (Fig. 4). This further supports the results that RNA content in bacteria in the environment is relatively constant and does not vary to the magnitude that other measures of microbial activity might.

The results from the environmental samples are in direct contrast with results determined for pure bacterial cultures in laboratory experiments. Early studies with strains of *Escherichia coli* and *Salmonella typhimurium* and more recent studies with marine bacterial isolates have all reported that RNA:DNA ratios are proportional to growth rates (Neidhardt & Magasanik 1959, Kjeldgaard & Kurland 1963, Maaløe & Kjeldgaard 1966, Rosset et al. 1966, Kemp et al. 1993, Kerkhof & Ward 1993) and that bacteria tend to contain a ribosomal complement in direct proportion to growth rate and protein synthesis demands (Maaløe & Kjeldgaard 1966). RNA degradation has been thought to be a virtually universal response to starvation in bacteria, and starving bacteria use RNA as an endogenous metabolism substrate (Dagley & Sykes 1957, Jacobson & Gillespie 1968, Thomas & Batt 1969, Dawes & Large 1970, Boylen & Mulks 1978 among many others). Contrasting results have been reported for marine bacteria. Amy et al. (1983) reported that the RNA content of a marine *Vibrio* sp. increased linearly after the first week of starvation until it was constant after 6 wk. It was postulated that this RNA was nonfunctional during the starvation process but was a reserve waiting for favorable environmental conditions or was present as a result of the absence of cellular regulation.

Kemp et al. (1993) suggested that cellular RNA content was likely to be useful for estimating growth rates for marine bacterial assemblages but that the most success might be had when applying this methodology to the level of individual species. Our results support this statement. Relationships reported for limited sample sizes seem to deteriorate when large samples are analyzed. At the species level, or level at which organisms are clustered via 16S rRNA analysis, measures of RNA:DNA ratios might be indicative of growth rates in the environment. The use of specifically targeted 16S rRNA probes may hold the potential for the most success because of the specificity inherent in the design of these probes. This could prove to be a powerful tool in examining specific activity of members of a heterogeneous microbial community (Lee et al. 1993). We must conclude, however, that, at the population level, the ratio of RNA:DNA cannot be used as an indicator of growth rates or activity in heterogeneous marine bacterioplankton.

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