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VARIABILITY IN RATIOS OF PHYTOPLANKTON CARBON AND RNA TO ATP AND CHLOROPHYLL *A* IN BATCH AND CONTINUOUS CULTURES[1,2]

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ABSTRACT

The feasibility of estimating phytoplankton carbon and RNA concentrations from measurements of ATP and chlorophyll a *(chl* a*) concentrations was studied using chemostat populations of the marine diatom* Thalassiosira weissflogii *(Grunow) Fryxell & Hasle (=* T. fluviatilis *Hustedt). C:ATP and RNA:ATP ratios were studied for six additional marine species in batch culture representing five classes of phytoplankton. Statistical analyses revealed that both the growth rate and the factor limiting growth (NO_3^-, NH_4^+, PO_4^{3-} or light) could alter C:ATP, RNA:ATP, C:chl* a *and RNA:chl* a *ratios by amounts which were large compared to measurement error. An analysis of variance of the batch culture results indicated that both species and the source of inorganic nitrogen (NO_3^- or NH_4^+) had a significant effect on C:ATP and RNA:ATP ratios. Light had less of an influence on C:ATP and RNA:ATP ratios than on C:chl* a *and RNA:chl* a *ratios, and for this reason we feel that phytoplankton C and RNA concentrations can be estimated with greater reliability from ATP than from chl* a *measurements. The range of C:ATP and RNA:ATP values found for* T. weissflogii *under a variety of growth conditions was similar to that for the six additional species grown in batch culture, suggesting that this range of values is indicative of the extremes likely to occur in living cells. Our results and additional data in the literature indicate that phytoplankton C and RNA concentrations can be estimated to within a factor of two by multiplying ATP concentrations by 311 and 35, respectively, in N limited systems, and by 341 and 36, respectively in PO_4^{3-} limited systems.*

Key index words: ATP; carbon; chlorophyll a*; composition; phytoplankton; RNA;* Thalassiosira weissflogii

Measurement of microbial growth rates in the ocean is of increasing interest to biological oceanographers. In oligotrophic areas there is much uncertainty as to whether the low standing stocks of phytoplankton are turning over slowly or rapidly. Various estimates of phytoplankton growth rates in oligotrophic marine areas differ by more than an order of magnitude (Eppley 1980). Direct measures of microbial production include estimates of autotrophic carbon fixation using the ^{14}C method (Steemann-Nielsen 1952, Peterson 1980), and microbial nucleic acid synthesis using tritiated adenine (Karl 1979, 1981, Karl et al. 1981a, b). While these techniques provide measures of microbial production, estimation of growth rates requires simultaneous knowledge of the biomass of the population of living microbes in terms of the parameter whose rate of production is being measured. Unfortunately there is no direct method for determining the concentration of living particulate carbon (C) or nucleic acid (RNA, DNA) in aquatic systems, but indirect methods suggest that at least in oligotrophic marine areas only about one-third of the particulate material in the water column is associated with living organisms

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(Holm-Hansen 1971, Sharp et al. 1980). Since about 90% of the surface area of the ocean falls into the oligotrophic category (Ryther 1969), any method which can estimate living carbon and nucleic acid to better than a factor of roughly three is an improvement over using total particulate carbon or nucleic acid as a measure of biomass over more than half the surface of the earth. Direct measures of living biomass include concentrations of chlorophyll *a* (chl *a*) for autotrophs (Holm-Hansen and Reimann 1978), and particulate ATP for microbes in general (Karl 1980). Measurement of carbon or nucleic acid synthesis can thus be combined with a simultaneous measurement of either chl *a* or ATP to estimate microbial growth rates, provided that one knows the ratio of chl *a* or ATP to carbon or nucleic acid in living cells. Hunter and Laws (1980) reported that C:ATP and C:chl *a* ratios both vary widely in phytoplankton cells, and that the ratio depends on the time of day, factor limiting growth, growth rate and species. A similar study of the variability in the ratio of cellular nucleic acid content to either ATP or chl *a* has not been reported. The present study was undertaken in part to fill this information gap, and in part to gain some quantitative understanding about the accuracy with which microbial growth rates could be estimated from measurements of nucleic acid synthesis rates and ATP or chl *a* concentrations.

MATERIALS AND METHODS

The diatom *Thalassiosira weissflogii* (Grunow) Fryxell & Hasle (= *T. fluviatilis* Hustedt), obtained from the Food Chain Research Group culture collection at the Scripps Institution of Oceanography (clone *T. fluv.*), was grown at 20° C in a chemostat system identical to that described by Laws and Bannister (1980). Continuous light was provided by a bank of cool white fluorescent lights. Light intensities (400–700 nm) measured at the center of the 1.2 L growth chamber with a Biospherical Instruments model QSL-100 light meter equipped with a 4 π collector were 37 $E \cdot m^{-2} \cdot d^{-1}$ for the nutrient limited studies and ranged as low as 1.7 $E \cdot m^{-2} \cdot d^{-1}$ in the light limited studies. For the NH_4^+ and NO_3^- limited experiments, inorganic P and N concentrations in the nutrient reservior were 50 μM. In the PO_4^{3-} limited system, corresponding NH_4^+ and PO_4^{3-} concentrations were 500 μM and 1.88 μM, respectively, while in the light limited studies reservoir NH_4^+ and PO_4^{3-} concentrations were 500 μM and 50 μM, respectively. During the light limited studies, the cell concentration was kept as a level comparable to that of the nutrient limited studies (ca. 6×10^4 cells$\cdot$mL^{-1}) by small daily adjustments in the rate of addition of new medium. The growth rate was calculated from the rate of change of cell numbers and the pumping rate of the medium. Studies at a given steady state did not begin until the cell concentration, measured on a Celloscope particle counter, had been constant to within ±5% for three days. Growth chamber NH_4^+ and PO_4^{3-} concentrations, measured on a Technicon AutoAnalyzer, were >300 μM and >30 μM, respectively, in the light limited studies. In the nutrient limited studies, limiting nutrient concentrations in the growth chamber were either undetectable or a few tenths micromolar at all growth rates.

Once steady state at a given pumping rate had been established, about 600 mL was withdrawn from the growth chamber and processed as follows. Six 25 mL aliquots were filtered onto precombusted GF/C filters for particulate carbon (PC) and chl *a* analyses. Three of the samples were stored at −20° C and later analyzed for PC on a Hewlett-Packard model 185B CHN analyzer following procedures recommended by Sharp (1974). Three filters were placed in a few milliliters of 100% acetone and stored at −20° C for at least one day to allow for pigment extraction. Chl *a* was measured by the spectrophotometer procedures of Jeffrey (1974) and Jeffrey and Humphrey (1975). A few milliliters of filtrate from the PC samples were used to determine chamber nutrient concentrations following Murphy and Riley (1962), Solórzano (1969) and Wood et al. (1967) for PO_4^{3-}, NH_4^+, and $NO_3^- + NO_2^-$, respectively.

The procedures for cellular nucleotide extractions varied depending upon the nutrient and growth conditions of the cultures. For NO_3^-, NH_4^+ and light limited cells, triplicate 2 mL samples were injected directly into 5 mL of boiling PO_4^{3-} buffer (120 mM, pH 7.4), 0.5 mL at a time. The samples were extracted at 100° C for 5 minutes, diluted to exactly 10 mL with distilled water (final PO_4^{3-} concentration, 60 mM) and stored frozen (−20° C) for nucleotide analyses within one week. For PO_4^{3-} limited cultures the initial concentration of the PO_4^{3-} buffer was increased to 300 mM (final concentration, 150 mM) to repress the anticipated alkaline phosphatase (APase) enzyme activity (Karl and Craven 1980). Comparative extractions using GF/F filter concentrated cells resulted in variable losses of ATP ranging from 0–35% and significantly lower cellular adenylate energy charge (EC_A) ratios. At each sampling period a 2 mL portion of the "cell-free" medium (GF/F filtrate) was extracted as described above to monitor the occurrence of soluble ("cell-free") adenine nucleotides; none was detected in any of the cultures. ATP, ADP and AMP were measured using enzyme systems coupled to the firefly bioluminescence assay (Karl and Holm-Hansen 1978), and EC_A ratios were calculated as described previously (Atkinson and Walton 1967). Standards for preparing calibration curves were diluted in PO_4^{3-} buffer containing 20% (v/v) growth chamber medium to correct for ionic interference caused by the seawater salts.

For RNA determinations, triplicate subsamples ranging from 75 to 150 mL of cells were concentrated onto a GF/F filter and stored frozen for subsequent analyses. All samples from a given series of growth rates were processed simultaneously. The samples were extracted in cold (4° C) 5% (w/v) trichloroacetic acid (TCA) for 4 h, homogenized, and cellular RNA collected by centrifugation (2500 × g, 5 min). The pellets were washed successively twice in 5% TCA and twice in 95% EtOH, resuspended in 2 mL of 1 N NaOH and hydrolyzed at 37° C for 1 h. Following hydrolysis, the samples were placed in an ice bath and acidified with an HCl-TCA mixture to yield a final pH of ca. 1 and final concentration of ca. 5% TCA. After 15 min the samples were centrifuged and 1 ml of the supernatant solution was assayed for RNA by the orcinol method (Lin and Schjeide 1969), using purified yeast RNA (Sigma) as the primary standard and AMP as the secondary standard. Studies in our laboratory have shown excellent agreement between RNA estimated in this way and estimates based on uniformly ^{32}P labeled algal cells (of known specific activity).

On completing the chemostat studies, a series of batch culture experiments was set up involving six additional species. The species included *Pavlova* (= *Monochrysis*) *lutheri* (Droop) Green, *Dunaliella tertiolecta* Butcher and *Amphidinium carterae* Hulburt from J. Jordan at the Scripps Institute of Oceanography, *Phaeodactylum tricornutum* Bohlin from W. Thomas at the Scripps Institute of Oceanography, and *Agmenellum quadruplicatum* (Menegh.) Bréb. and *Cylindrotheca* sp. from C. Van Baalan at the University of Texas. The culture system consisted of either autoclaved 2.2 L glass flasks or 1–5 L chemostat growth chambers containing appropriate amounts of sterile filtered (0.22 μm) medium. The growth medium was identical to that used in the chemostat studies, except that the inorganic nitrogen and PO_4^{3-} concentrations

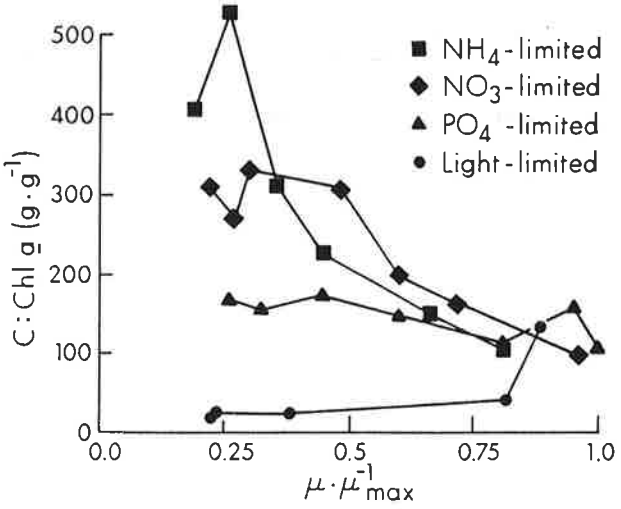


FIG. 1. C:chl *a* ratios in *T. weissflogii* as a function of relative growth rate. $\mu_{max} = 1.45 \cdot d^{-1}$.

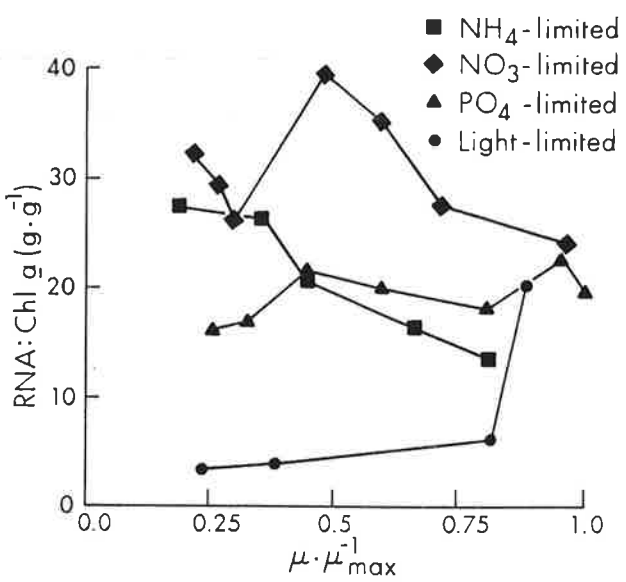


FIG. 2. RNA:chl *a* ratios in *T. weissflogii* as a function of relative growth rate. $\mu_{max} = 1.45 \cdot d^{-1}$.

were set at 500 μM and 50 μM, respectively. The cells were maintained in suspension by bubbling the culture with sterile air and in some cases by additional mixing with a magnetic stirring bar.

When cell counts indicated that the populations were in log phase growth and when the cell concentration was judged to be at a suitable level, concentrations of RNA, ATP and PC were measured following procedures identical to those used in the chemostat studies. At least three analyses were made over a 1–3 day period.

RESULTS AND DISCUSSION

The coefficients of variation (standard deviation: mean) of our triplicate analyses of the chemostat samples averaged 11% for chl *a*, 7% for C, 8% for ATP and 8% for RNA. As a result the coefficients of variation of the ratio of mean values are approximately 11% for RNA:ATP and C:ATP, and 13% for RNA:chl *a* and C:chl *a* (Box et al. 1978). EC_A measurements were reproducible to ±0.02. The results of the chemostat studies of *T. weissflogii* are shown in Figs. 1–5, and the results of the batch culture studies are listed in Table 1.

We were first interested in determining whether ATP or chl *a* was a more useful parameter for estimating phytoplankton C and RNA concentrations. Our criterion for choosing ATP or chl *a* was the variability in ratios of C and RNA to ATP and chl *a* in *T. weissflogii* due to variations in growth rate and the factor limiting growth. We adopted as a null hypothesis that there was no effect of either growth rate or the factor limiting growth on these ratios. To test for effects of the growth rate limiting factor on these ratios, we ran an analysis of covariance (ancova) on the data shown in Figs. 1–4. This test is robust if the dependent variable is constant or a linear function of the independent variable, but is weakened if the underlying relationship is nonlinear. Despite the potential weakness of the test, the ancova revealed a highly significant effect of the growth rate limiting factor on each of the ratios in Figs. 1–4 (F test, $P < 0.005$ in all cases).

Since the ancova revealed that the composition ratios were affected by the growth rate limiting factor, it was illogical to test the general hypothesis that growth rate had no effect on composition ratios. Instead, it was necessary to test the significance of the effects of growth rate on composition ratios for each limiting factor separately. Using the nonparametric Spearman rank correlation coefficient we concluded that growth rates and RNA:ATP ratios were significantly correlated under NO_3^- limitation ($P < 0.02$), that growth rates and RNA:chl *a* ratios were significantly correlated under NH_4^+ and light limitation ($P < 0.001$), that growth rates and C:ATP ratios were significantly correlated under NH_4^+ and PO_4^{3-} limitation ($P < 0.05$), and that growth rates and C:chl *a* ratios were significantly correlated under NH_4^+, NO_3^- and light limitation ($P < 0.04$). Given these results and our estimates of the analytical precision of the composition ratios (i.e. 11–13% coefficients of variation), it appears that most of the scatter in Figs. 1–4 is due to the effects of different growth rates and/or limiting factors, and not to analytical imprecision.

Figures 1 and 2 show that RNA:chl *a* and C:chl *a* are much different under light and nutrient limited conditions when relative growth rates ($\mu:\mu_{max}$) are less than about 0.7. This observation primarily reflects the large increase in light harvesting pigments required to support algal growth at low light levels (Shuter 1979). RNA:ATP and C:ATP ratios show much less difference between light and nutrient limited conditions (Figs. 3, 4), because ATP

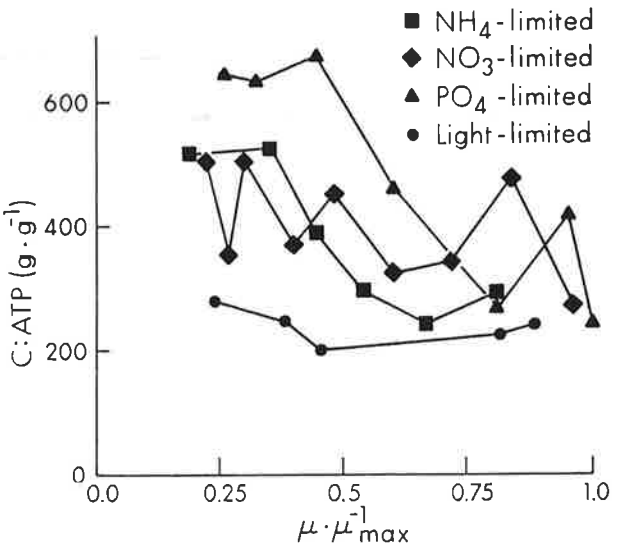


FIG. 3. C:ATP ratios in *T. weissflogii* as a function of relative growth rate. $\mu_{max} = 1.45 \cdot d^{-1}$.

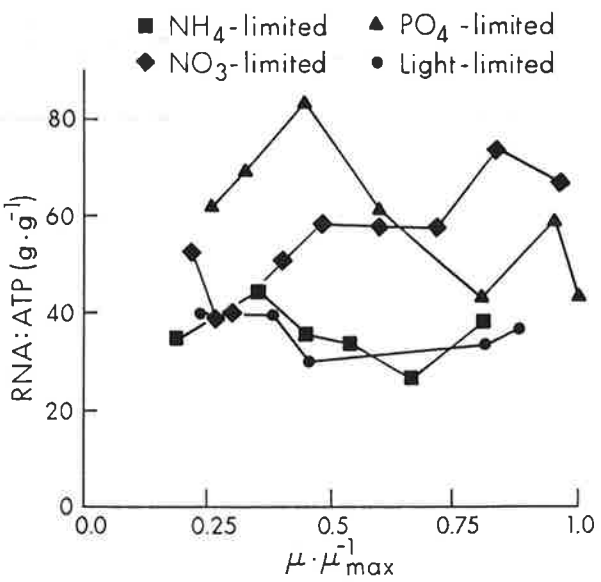


FIG. 4. RNA:ATP ratios in *T. weissflogii* as a function of relative growth rate. $\mu_{max} = 1.45 \cdot d^{-1}$.

is not directly required for light absorption. The difference between the lowest light limited and highest nutrient limited ratios is a factor of 2.8 and 3.4 for RNA:ATP and C:ATP, respectively; corresponding differences for RNA:chl *a* and C:chl *a* are 11.4 and 26.2, respectively. Clearly the variation in RNA:chl *a* and C:chl *a* ratios associated with a transition from nutrient limitation to light limitation could create a serious problem in estimating phytoplankton C or RNA if samples were taken at a series of depths from the surface to the compensation light level in a stratified water column, or if growth rates were to be studied on a seasonal basis in temperate seas or lakes in which the growth rate limiting factor gradually shifted from light to nutrients. Unfortunately an intelligent guess as to whether growth rates are limited by light or nutrients is not always possible (Caperon et al. 1971), and in cases where growth rates are limited by nutrients, composition ratios may still vary as a function of light intensity (Shuter 1979). Thus, we prefer ATP over chl *a* as a means of estimating phytoplankton biomass in terms of RNA or C, at least in cases where contamination of samples by heterotrophs is negligible.

The accuracy with which phytoplankton RNA and C can be estimated from measurements of ATP will depend not only on the growth conditions (Figs. 3, 4), but also on the species composition of the phytoplankton community. Table 1 provides data relevant to this latter problem. An analysis of variance (anova) reveals that there are significant differences in both RNA:ATP and C:ATP ratios between species ($P < 0.02$). The range of measured values was a factor of 4.8 and 3.2 for RNA:ATP and C:ATP, respectively. The anova also revealed a highly significant effect of nitrogen source on both RNA:ATP and C:ATP ratios ($P < 0.0001$). RNA:ATP and C:ATP ratios were higher when NO_3^- was the nitrogen source than when NH_4^- was the nitrogen source. This result is consistent with our NO_3^- and NH_4^+ limited RNA:ATP data for *T. weissflogii*. However, there is little difference in our NO_3^- and NH_4^+ limited C:ATP ratios for *T. weissflogii*.

It is difficult to rationalize in other than qualitative terms the effects of different growth rates, growth limiting factors, and changes in species composition on RNA:ATP and C:ATP ratios. Intuitively one would expect cell quotas of both ATP and RNA to be positively correlated with growth rate, since both substances are associated with growth related processes. Most results confirm the positive correlation between ATP and RNA cell quotas and growth rate (Rhee 1978, Hunter and Laws 1980), but efforts to explain these relationships quantitatively based on theoretical models such as those of Shuter (1979) have not met with success (Laws et al. 1983). Much of this difficulty may well be related to ATP turnover and nonlinear relationships between intracellular concentrations and rate processes (Karl 1980). Similarly, RNA cell quotas may not be correlated in a simple way to rates of cellular protein synthesis (Laws et al. 1983).

One encounters similar problems in explaining the effects of different growth limiting factors on RNA:ATP and C:ATP ratios. Why, for example, are NO_3^- and PO_4^{3-} limited RNA:ATP ratios consistently higher than NH_4^+ and light limited RNA:ATP ratios (Fig. 4 and Table 1)? The uptake and reduction of NO_3^- is an energy-requiring process, but Falkowski and Stone (1975) demonstrated that short-term (ca. 2 h) uptake of NO_3^- and NH_4^+ spikes reduce photosynthetic rates of natural populations

TABLE 1. *Growth conditions and RNA:ATP and C:ATP ratios for various phytoplankton in batch culture. Incident light intensity in all cases was 37 E·m⁻²·d⁻¹. Errors indicated are standard deviations based on replicate (at least triplicate) analyses on cultures over a one to three day period.*

Species	Growth rate (d^{-1})	N source	Temp (°C)	RNA:ATP (g:g)	C:ATP (g:g)
A. quadruplicatum	log phase[a]	NO_3^-	28	34.2 ± 4.2	558 ± 59
A. quadruplicatum	1.3[a]	NH_4^+	28	22.2 ± 3.2	227 ± 31
Cylindrotheca sp.	1.0	NO_3^-	22	30.7 ± 4.8	422 ± 70
Cylindrotheca sp.	1.0	NH_4^+	22	17.4 ± 1.7	173 ± 24
P. lutheri	0.8	NO_3^-	20	76.2 ± 12.2	562 ± 67
P. lutheri	0.8	NH_4^+	20	54.5 ± 6.1	no data
D. tertiolecta	0.9	NO_3^-	29	15.8 ± 2.5	314 ± 30
A. carterae	0.4	NO_3^-	20	25.1 ± 4.7	382 ± 27
P. tricornutum	0.4	NO_3^-	20	27.2 ± 1.7	no data

[a] Growth rates for *A. quadruplicatum* are approximate because of cell clumping.

by comparable amounts. The reduction in photosynthesis, which presumably reflects competition between inorganic carbon and nitrogen for ATP, was in most cases slightly larger during NO_3^- than NH_4^+ uptake, but whether this apparent difference in energy requirements for NO_3^- versus NH_4^+ uptake resulted in lower ATP levels in our NO_3^- limited cells is problematic. Changes in the rates of photophosphorylation and/or respiration could alter ATP turnover rates without changing ATP concentrations. The fact that C:ATP ratios were higher in *Cylindrotheca* sp. and *A. quadruplicatum* when NO_3^- rather than NH_4^+ was the N source (Table 1) suggests that changes in cellular ATP levels largely account for the differences in RNA:ATP levels, but there was virtually no difference in NO_3^- and NH_4^+ limited C:ATP ratios in *T. weissflogii* (Fig. 3). Similarly, Strickland et al. (1969) observed that C:ATP ratios in *Cachonina niei* were 1.6 times higher when the cells were supplied with NO_3^- versus NH_4^+ but that the N source had almost no effect (10% difference) on C:ATP ratios in *Ditylum brightwellii*.

Hunter and Laws (1980) noted that PO_4^{3-} limited C:ATP ratios are consistently higher than N limited or light limited C:ATP ratios at relative growth rates less than about 0.5. The data in Fig. 3 are consistent with this observation. Furthermore, our PO_4^{3-} limited RNA:ATP data were consistently higher than our N limited and light limited RNA:ATP ratios at relative growth rates less than 0.5. Karl and Craven (1980) observed that in PO_4^{3-} limited systems, alkaline phosphatase activity may be sufficiently great to hydrolyze a large percentage of the ATP if extractions are conducted in boiling Tris buffer, since alkaline phosphatase is stable to boiling at 100° C for at least five minutes (Torriani 1960). Our use of a 150–300 mM PO_4^{3-} buffer in the PO_4^{3-} limited ATP extractions was designed to circumvent this problem, since high PO_4^{3-} concentrations are known to inhibit the in vitro activity of alkaline phosphatase (Karl and Craven 1980). One check of the efficiency of the PO_4^{3-} limited ATP extractions is the EC_A ratios in Fig. 5. Although the PO_4^{3-} limited EC_A ratios are significantly and positively correlated with relative growth rate (Spearman rank correlation, $P < 0.04$), the lowest PO_4^{3-} limited EC_A of 0.75 is little different from the means of the NH_4^+, NO_3^- and light limited EC_A ratios (0.82, 0.81 and 0.84, respectively). Furthermore, addition of ATP spikes to the PO_4^{3-} limited ATP extracts and subsequent monitoring yielded no evidence of alkaline phosphatase activity. We conclude that the high PO_4^{3-} limited C:ATP and RNA:ATP ratios observed at low growth rates are not due to hydrolysis of ATP by alkaline phosphatase.

While our anova indicated that interspecies differences had a statistically significant effect on both RNA:ATP and C:ATP ratios, a single species grown under different conditions or different species within a class of phytoplankton showed almost as much variation in these ratios as was observed over all species and growth conditions. For example, the second highest and second lowest C:ATP ratio in Table 1 were both measured on the same species, *A. quadruplicatum*, in the first case with NO_3^- as the N source and in the second case with NH_4^+ as the N source. The highest and second lowest RNA:ATP ratios reported in this study were both measured on diatoms, PO_4^{3-} limited *T. weissflogii* in the first case and a batch culture of *Cylindrotheca* sp. provided with NH_4^+ as a N source in the second case. We conclude that systematic sampling of a given species or class of phytoplankton over a wide variety of growth conditions gives a very similar picture of the variability of C:ATP and RNA:ATP ratios to that obtained from a systematic screening of many different phytoplankton species grown in log phase batch culture conditions. This observation may reflect the fact that there is a certain range of C:ATP and RNA:ATP ratios within which phytoplankton can grow, and that viable cells will simply not be found with C:ATP or RNA:ATP ratios outside of this range. Evidently it is possible to push a given species close to these limits by appropriate manipulation of growth conditions, and further screening of additional species is therefore not likely to greatly expand the range of observed values.

How accurately can phytoplankton C and RNA concentrations be estimated in the field from measurements of ATP concentrations? The answer will

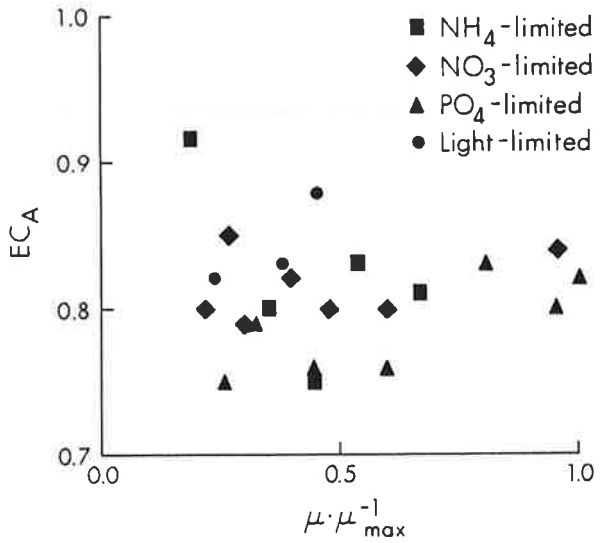


FIG. 5. Adenylate energy charges in *T. weissflogii* as a function of relative growth rate. $\mu_{max} = 1.45 \cdot d^{-1}$.

certainly depend in part on how much one knows about the species composition and growth conditions. In most parts of the ocean, inorganic nitrogen or light intensity are believed to be the principal factors limiting phytoplankton growth (Parsons et al. 1966, Thomas 1969, Ryther and Dunstan 1971), whereas PO_4^{3-} is believed to be the principal growth limiting nutrient in freshwater systems (Schindler 1977). The range of C:ATP ratios reported here under either inorganic N limited or light limited conditions is 173 to 562, a factor of 3.2. We expect that C:ATP ratios in marine phytoplankton rarely fall much outside this range. For example, the mean C:ATP ratio (±one standard deviation) of 30 marine algae studied by Holm-Hansen (1971) was 286 ± 40. We suggest therefore that an assumed C:ATP ratio of about 311 (the geometric mean of 173 and 562) in marine phytoplankton is unlikely to be in error by more than a factor of 311/173 = 562/311 = 1.8. Similarly, we note that under inorganic N or light limited conditions the highest and lowest RNA:ATP ratios reported here were 76.2 and 15.8, respectively. We therefore suggest that an assumed RNA:ATP ratio of $(76.2 \times 15.8)^{1/2} = 35$ in marine phytoplankton is unlikely to be in error by a factor or more than 35/15.8 = 2.2.

Under PO_4^{3-} limited conditions, the highest C:ATP and RNA:ATP ratios found in this study were 673 and 83.3, respectively, values which are less than 20% higher than the corresponding maximum inorganic N or light limited ratios. We conclude that when light intensity and PO_4^{3-} availability are the principal factors limiting growth rates, phytoplankton C concentrations can be estimated to within a factor of $(673/173)^{1/2} = 2.0$ by multiplying ATP concentrations by $(673 \times 173)^{1/2} = 341$, and phytoplankton RNA concentrations can be estimated to within a factor of $(83.3/15.8)^{1/2} = 2.3$ by multiplying ATP concentrations by $(83.3 \times 15.8)^{1/2} = 36$. C:ATP ratios as high as 9500 have been reported in PO_4^{3-} starved batch cultures (Sakshaug and Holm-Hansen 1977). As noted by Karl (1980), these high PO_4^{3-} limited C:ATP ratios may reflect the failure of the investigators to take adequate steps to repress the activity of alkaline phosphatase when performing the ATP extractions. Since this problem has been recognized only recently, there are few reliable PO_4^{3-} limited C:ATP ratios in the literature. Our results indicate that C:ATP and RNA:ATP ratios in PO_4^{3-} limited systems are not likely to be much outside the range of values reported here for inorganic N and light limited populations. However, nutrient limited continuous culture work on additional species would be needed to thoroughly test this hypothesis.

We conclude that if contamination by microheterotrophs is negligible, phytoplankton C and RNA concentrations in nature can be estimated to within about a factor of two by multiplying ATP concentrations by 311 and 35, respectively in N limited systems, and by 341 and 36 respectively in PO_4^{3-} limited systems. This conclusion must be qualified by the realization that there is still considerable uncertainty regarding the physiological status of phytoplankton populations in nature (Eppley 1981), and as noted by McCarthy (1981), "To better interpret field data, we need increased laboratory study with the organisms that are typical in natural assemblages."

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