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Steady-State Growth and Chemical Composition of the Marine Chlorophyte *Dunaliella tertiolecta* in Nitrogen-Limited Continuous Cultures†

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The marine chlorophyte *Dunaliella tertiolecta* was grown in continuous cultures under NH_4^+-N, NO_2^--N, NO_3^--N, and urea-N limitations. The effect of the nitrogen cell quota (Q_n) on the steady-state growth rate (μ) was the same regardless of the N source. The relationship between μ and Q_n was well described by the Droop equation, but only up to the true maximum growth rate $\hat{\mu}$ (= cell washout rate). The ratio between the minimum cell quota (k_Q) and the maximum cell quota (Q_m) was 0.19. Hence, there is no substitute for determining $\hat{\mu}$ experimentally. That there was no difference in growth response to different N sources suggests that no internal pooling of inorganic nitrogen occurred. Both the carbon (Q_c) and phosphorus (Q_p) cell quotas under N limitation increased with increasing μ in a threshold fashion: virtually no change in either cell quota up to ~0.8 $\hat{\mu}$, followed by a rapid and large increase up to $\hat{\mu}$. In addition, in the region of low μ, there was an increase in Q_p with a decreasing medium N/P ratio of between 15 and 5 (by atoms). The results generally indicate the physiological limits in cellular constituency under N limitation. The usefulness of this information, however, in describing the response of natural populations of marine phytoplankton to transient nutrient exposures on the temporal and spatial microscales that most likely exist is of limited value.

It is well established that the type and degree of nutrient limitation result in dramatic changes in the cellular chemical composition of phytoplankton (25, 27, 28). Attempts to quantify these types of physiological responses have met with various degrees of success. For example, the Droop equation (6), $\mu = \bar{\mu}[1 - (k_Q/Q)]$ (equation 1), in which μ is the specific growth rate (T^{-1}), $\bar{\mu}$ is the specific growth rate at which Q is infinite, Q is the cell quota (mass·[cell]$^{-1}$), or concentration of limiting nutrient per cell, and k_Q is the minimum concentration of limiting nutrient per cell required before growth can proceed, has been used successfully to describe the effects of vitamin B_{12} (6) and PO_4^{3-} (4, 7, 11, 32) limitations on phytoplankton growth rates at the steady state, but for NH_4^+ limitation, restrictions in the applicability of the equation have been identified (12). The major limitation is that there is an upper bound in the cell quota, Q_m, associated with the true maximum growth rate $\hat{\mu}$ (= cell washout rate). Thus, for equation 1 to be valid for a particular limiting nutrient, the ratio k_Q/Q_m must be very small (e.g., $\lesssim$0.1) so that $\hat{\mu} \simeq \bar{\mu}$. For vitamin B_{12} and PO_4^{-3} limitations, $k_Q/Q_m <$ 0.05, but for NH_4^+, it was ~0.2 ($\hat{\mu} \simeq 0.8\ \bar{\mu}$) (12). Thus, in the latter case there was no substitute for experimentally determining $\hat{\mu}$ and Q_m.

To determine whether similar restrictions in the use of equation 1 apply for other potentially available nitrogen sources and to examine the changes in the phosphorus and carbon cell quotas under various degrees of nitrogen limitation, we grew the marine chlorophyte *Dunaliella tertiolecta* in a continuous culture under NH_4^+-N, NO_3^--N, NO_2^--N, and urea-N limitations.

MATERIALS AND METHODS

The continuous-culture apparatus, the culturing protocols, and the experimental analyses were virtually identical to those described previously (12). In summary, a bank of eight 0.5-liter cultures was used, complete with continuous lighting (2,093 J m^{-2} min^{-1} visible), temperature control at 19.2°C, and mixing through magnetic bar stirring and aeration with laboratory-compressed air scrubbed of NH_3 and particulates. The chlorophyte *D. tertiolecta*, clone Dun, was obtained from the collection of R. R. L. Guillard at Woods Hole Oceanographic Institution.

The nitrogen-limited growth medium consisted of natural seawater obtained from the flowing seawater system at the Environmental Systems Laboratory of

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Woods Hole Oceanographic Institution. The water was first filtered with 1-μm membrane filters and then enriched with either 50, 100, 150, or 450 μg-atoms of the particular nitrogen source (NH_4Cl, $NaNO_2$, $NaNO_3$, CH_4N_2O) liter^{-1}, 10 μg-atoms of NaH_2PO_4 liter^{-1} for N additions of up to 150 μg-atoms·liter^{-1} and 30 μg-atoms·liter^{-1} when the N addition was 450 μg-atoms·liter^{-1}, and trace metals and vitamins in a twofold dilution of *f*-medium (14). In one experiment at the highest dilution rates tested (1.1 to 1.3 day^{-1}), 75 μg-atoms of NH_4^+ liter^{-1} and 15 μg-atoms of PO_4^{3-} liter^{-1} were used. In all of the remaining experiments, the four nitrogen sources were used concurrently, each being fed to duplicate cultures. A desired N/P ratio by atoms of either 5, 10, or 15 was established for a given experiment. Concentrations of the individual nitrogen sources and PO_4^{3-} in the unenriched seawater were typically <0.5 to 1.0 μg-atoms·liter^{-1} and were included in the final determinations of the added nutrients to the medium. Medium was dispensed to the cultures via a multichannel peristaltic pump (Harvard no. 1203). All tubing was glass, except for small sections of silicone inserted through the pumps.

Chemical analyses for the nutrients present in the medium and culture filtrates were carried out on a Technicon two-channel Autoanalyzer by the procedures of Bendeschneider and Robinson (2) for NO_2^-, Wood et al. (35) for NO_3^-, Solorzano (29) for NH_4^+, Newell et al. (22) for urea, and Murphy and Riley (21) for PO_4^{3-}. Particulate carbon and nitrogen were measured on a Perkin-Elmer 240 elemental analyzer. Cells were counted in a Spencer Bright-line hemacytometer. Particulate phosphorus was determined by the difference between influent and effluent inorganic PO_4^{3-}. All culture measurements were made directly on culture samples at the steady state, defined as the time when culture absorbance, measured daily on a Bausch & Lomb Spectronic 88 at 600 nm, did not vary more than ±10% for at least 2 consecutive days. The cultures were not axenic for the reasons cited earlier (11).

The maximum growth rate was estimated both by the cell washout technique (11) and by measurement of the slope of the batch curve representing cell number versus time. Batch experiments were carried out in five replicate cultures after establishing steady-state populations near the washout dilution rate (1.1 to 1.3 day^{-1}), where cell numbers were relatively low (0.1×10^5 to 0.4×10^5 cells ml^{-1}). The feed pump was then stopped, and the culture was immediately enriched with 1,000 μg-atoms of NH_4^+ liter^{-1}, 100 μg-atoms of PO_4^{3-} liter^{-1}, and additional trace metals and vitamins. Cell counts were taken at 3- to 6-h intervals on 2-ml subsamples over a 3-day period. The maximum growth rates were determined from linear-regression analysis of the plots of the natural log of the cell count versus time. In two of the batch experiments, cellular nitrogen, carbon, and phosphorus were measured during three successive sampling periods.

The kinetic coefficients $\bar{\mu}$ and k_Q were determined from regression analyses of the linearized version of equation 1 as follows: $Y = Y_Q[1 - (\mu/\bar{\mu})]$ (equation 2), in which Y is the yield coefficient (Q^{-1}), and Y_Q is the maximum yield coefficient (k_Q^{-1}) (7, 10). The coefficients were calculated considering the data grouped by N source and medium N/P ratio and the ungrouped total data. Ninety-three steady-state experiments were performed from a minimum dilution rate of 0.13 day^{-1} to cell washout.

RESULTS

Maximum growth rate. The highest dilution rate for which a steady-state population could be maintained was 1.33 day^{-1}. This value of μ compared favorably with an average $\hat{\mu}$ of 1.34 ± 0.07 day^{-1} obtained from the batch growth experiments in nutrient-saturated medium (Table 1 and Fig. 1). No lag phase occurred, and the correlation coefficient for each batch curve was >0.99.

Cellular nitrogen variations. Based on regression analysis of the total N data (equation 2), the nitrogen cell quota (Q_n) varied from 1.3 pg of N·cell^{-1} (k_Q) at $\mu = 0$ to 7.0 pg of N·cell^{-1} (Q_m) at $\hat{\mu}$ (Table 1 and Fig. 2 and 3). Under enriched batch conditions, Q_m was 8.8 pg of N·cell^{-1} (Table 1). The value of $\bar{\mu}$ was 1.66 day^{-1}, so that for continuous growth, $\hat{\mu}/\bar{\mu} = 0.81$. For each of the four N sources considered individually and with the data representing medium N/P ratios of 5 and 15, k_Q, $\bar{\mu}$, and Q_m were virtually identical to the values determined with the combined data (Table 1). The correlation coefficients varied from 0.86 for the combined data to 0.82 to 0.93 for the above groupings of data. Only with the N/P = 10 set of data were the kinetic coefficients significantly different from the other groups (Table 1). Although the correlation coefficient was 0.93, this set of data contained the least number of measurements, i.e., 14.

Cellular carbon and phosphorus variations. Trends in the carbon (Q_c) and phosphorus (Q_p) cell quotas with varying steady state μ were very similar: virtually no effect of μ on either cell quota up to $\mu \simeq 1.1$ day^{-1} (= 0.8 $\hat{\mu}$), followed by a very rapid increase up to $\hat{\mu}$ (Fig. 4 and 5). Moreover, the magnitude of Q_p in the lower-growth-rate region was related to the medium N/P ratio, but appeared to increase to a common value of Q_m at $\hat{\mu}$ (Fig. 5). Virtually all influent PO_4^{3-} was stripped from the medium at all values of μ up to ~1.1 day^{-1} (= 0.8 $\hat{\mu}$), regardless of the medium N/P ratio.

Values of k'_Q and Q'_m (= the cell quotas of nonlimiting nutrients when $\mu = 0$ and $\mu = \hat{\mu}$, respectively) for carbon and phosphorus were estimated by eye from the data shown in Fig. 4 and 5. The values of Q'_m were similar to the respective Q'_m values determined from the batch studies (Table 2).

Cellular carbon/nitrogen ratio. The cellular carbon/nitrogen ratio (by atoms) was affected significantly by μ, decreasing linearly (r

TABLE 1. *Summary of kinetic data for D. tertiolecta grown in nitrogen-limited continuous cultures*[a]

Relationship	Datum points	$\hat{\mu}$ (day^{-1})	$\bar{\mu}$ (day^{-1})	$\hat{\mu}/\bar{\mu}$	k_Q (pg of N·cell^{-1})	Q_m (pg of N·cell^{-1})	C/N$_{min}$	C/N$_{max}$	r[b]
Cell washout		1.33[c]							
Batch curves	55[d]	1.34 ± 0.07				8.8 ± 1.3[e]	6.4 ± 0.8[e]		>0.99
Y vs μ									
Total	93		1.66	0.81	1.3	7.0			0.86
NH_4^+	25		1.62	0.83	1.3	7.5			0.91
NO_2^-	23		1.66	0.81	1.3	6.7			0.82
NO_3^-	23		1.69	0.80	1.3	6.7			0.82
Urea	22		1.69	0.80	1.3	6.6			0.87
N/P = 5	32		1.63	0.82	1.4	7.8			0.88
N/P = 10	14		1.37	0.99	1.1	50.2			0.93
N/P = 15	47		1.69	0.80	1.3	6.4			0.87
C/N vs μ	93		1.85	0.73			5.4	19.8	0.89

[a] Data were obtained from linear-regression analyses of various kinetic expressions described in text.
[b] Correlation coefficient.
[c] Highest dilution rate for which a steady-state population could be maintained.
[d] Total datum points from five batch experiments.
[e] Based on six samples from two batch experiments. Standard deviations included.

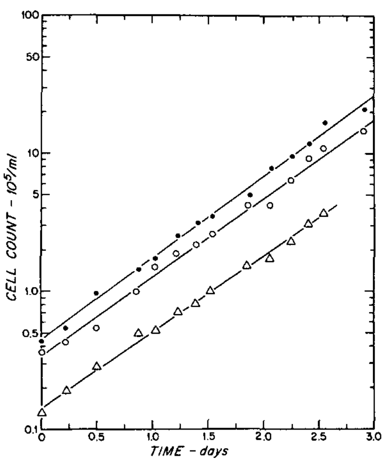

FIG. 1. *Batch growth curve of D. tertiolecta in enriched medium. Inocula were taken from nitrogen-limited steady-state continuous cultures maintained at specific growth rates of 1.1 day^{-1}. Symbols represent three replicate experiments: ●, $\hat{\mu}$ = 1.38 day^{-1}; ○, $\hat{\mu}$ = 1.34 day^{-1}; △, $\hat{\mu}$ = 1.27 day^{-1}. Two other experiments ($\hat{\mu}$ = 1.26 and 1.43 day^{-1}) are not shown for clarity.*

= 0.89) from 19.8 to 5.4 between $0 \leq \mu \leq \hat{\mu}$ (Fig. 6 and Table 1).

DISCUSSION

Effect of nitrogen source on growth rate. The ability of phytoplankton to assimilate preferentially different N sources is a species-specific phenomenon, dependent to a large degree on environmental conditions such as light duration and degree of nitrogen limitation (13, 19). Grant and Turner (13) observed that NO_3^- and NO_2^- uptakes by *D. tertiolecta* were enhanced significantly in the light. Paasche (23), moreover, found that with short (~6 h) photoperiods the growth rate of *D. tertiolecta* was 30% greater when the N source was NH_4^+ rather than NO_3^-, but with continuous light this difference decreased to 10%, irrespective of light intensity. Anitia et al. (1) found a similar slight difference in NO_3^-- and NH_4^+-dependent growth rates in this alga with continuous light and, in addition, observed that NO_2^- and urea were virtually equal to NH_4^+ as N sources. For some species this light dependency for NO_3^- uptake is not apparent (9).

Our results are in general agreement with the above findings. The observed relationship between Q and μ (Fig. 2 and 3) clearly defined conditions of nitrogen limitation, irrespective of the N source (Table 1). Up to ~0.8 $\hat{\mu}$, there was virtually undetectable residual N in all cultures, indicating an extremely low half-saturation coefficient for growth, that is, a very strong and identical dependence of growth rate on each of the four N sources up until just before cell washout.

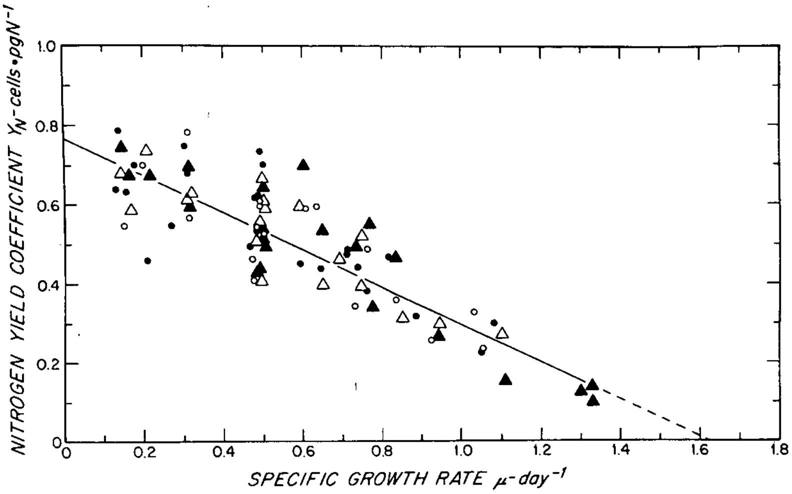

FIG. 2. *Relationship between steady-state nitrogen yield coefficient and specific growth rate for D. tertiolecta maintained in continuous cultures under different limiting nitrogen sources:* ▲, *NH_4^+-N;* △, *urea-N;* ●, *NO_3^--N;* ○, *NO_2^--N. Symbols the same for Fig. 3, 4, and 6.*

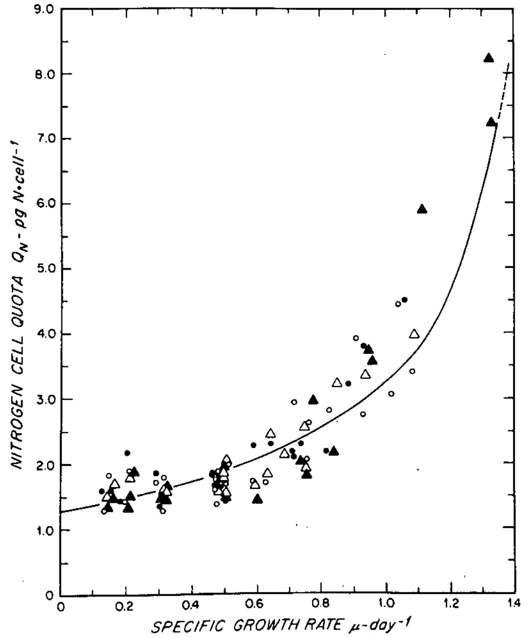

FIG. 3. *Relationship between steady-state nitrogen cell quota and specific growth rate for D. tertiolecta maintained in continuous cultures under different limiting nitrogen sources.*

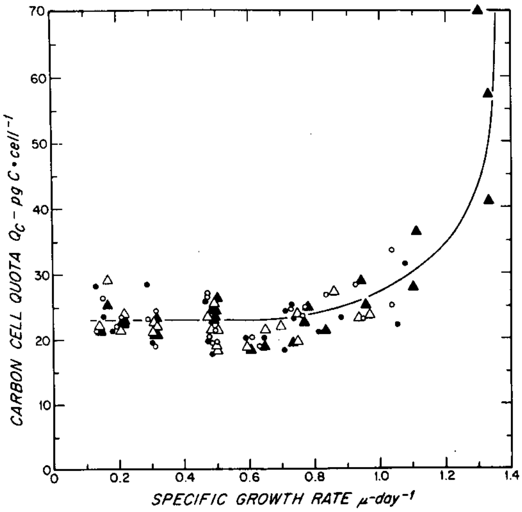

FIG. 4. *Relationship between steady-state carbon cell quota and specific growth rate for D. tertiolecta maintained in continuous cultures under different limiting nitrogen sources.*

It is well established that NH_4^+ is preferentially assimilated when more than one N source is available (20); yet McCarthy et al. (19) demonstrated that under conditions in which NH_4^+ was available, but in short supply (<0.5 to 1.0 μg-atom·liter^{-1}), natural phytoplankton populations could utilize NO_2^-, NO_3^-, and urea along with NH_4^+ in proportion to their respective availability. Similarly, Eppley and Renger (8) found that for the marine diatom *Thalassiosira pseudonana* (clone 13-1) cultured continuously on an N-limited medium containing equal amounts of NH_4^+ and NO_3^-, complete N assimilation occurred at 0.2 and 0.4 $\hat{\mu}$, regions of N limitation. At 0.94 $\hat{\mu}$ (a region of near nutrient saturation), NH_4^+ assimilation was still complete, but NO_3^- uptake had fallen significantly,

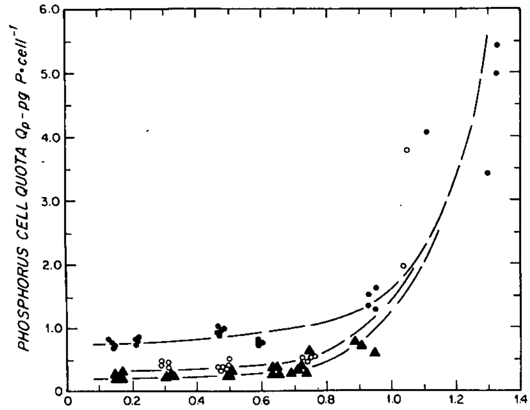

FIG. 5. *Relationship between steady-state phosphorus cell quota and specific growth rate for D. tertiolecta maintained in continuous cultures under different limiting nitrogen sources: ●, medium N/P ratio = 5; ○, medium N/P ratio = 10; ▲, medium N/P ratio = 15.*

so that ~30% of the influent NO_3^- level remained in the culture filtrate.

Our results and those above lead to the general conclusion that phytoplankton have adapted to exploit effectively different potentially available N sources in natural waters without appreciable differences in growth rates. This conclusion has important bearing on our understanding of how phytoplankton residing in nutrient-impoverished environments, such as oceanic surface waters, obtain their nitrogen ration. In such waters, nitrogen concentrations are frequently below detectable levels. An hypothesis advanced as to how nitrogen is made available in such situations is that individual cells frequently come into contact with microzones containing high nitrogen concentrations resulting from bacterial degradation of detritus and excretions of secondary marine animals (18). For example, when exposed to saturating NH_4^+ levels for very short periods (5 min), NH_4^+-limited phytoplankton can assimilate this N at a rate considerably greater than their rate of growth (18); thus, a cell need be exposed to high N for only a fraction of its doubling period to fulfill its complete requirement for this nutrient. The question of whether N-limited cells can simultaneously assimilate more than one potentially available N source with high efficiency or readily switch from one source to the other as it might become available on the transient spatial and temporal scales described above has not been answered to date. Nevertheless, based on the available evidence, it would seem that under conditions of total N limitation, the additional energy expenditures required for NO_3^- versus NH_4^+ uptake (with NO_2^- and urea being intermediary) are small enough not to prevent full and rapid exploitation of any available N when total N is in short supply.

Steady-state nitrogen kinetics. Our results confirm the results of Goldman and McCarthy (12) that equation 1 must be used with caution to describe the relationship between the cell quota and the steady-state growth rate for N limitation, regardless of the source of nitrogen: k_Q/Q_m in this study was 0.19, virtually identical to that found for NH_4^+-limited growth of *T. pseudonana* (clone 3H) (12).

Hence, it is imperative that the true maximum growth rate $\hat{\mu}$ be determined experimentally. We found virtually no difference in $\hat{\mu}$ determined by the cell washout technique and by batch growth in enriched medium (Table 1), results similar to those of Toerein and Huang (33). The exact value of $\hat{\mu}$ was difficult to ascertain because the average batch $\hat{\mu}$ of 1.34 day^{-1} was virtually identical to the highest dilution rate for which a steady state could be maintained. Culture artifacts, such as incomplete mixing and wall growth, can lead to apparent $\hat{\mu}$ values determined by the cell washout technique that are greater than those established by batch kinetics (17, 34). We could not detect any wall growth visually, but even slightly undetected wall growth could have led to a slight overestimate of $\hat{\mu}$ by the washout method; yet the similarity in the Q_m values for nitrogen, phosphorus, and carbon determined from the batch and washout data suggests that $\hat{\mu}$ = 1.34 day^{-1} is a reasonable estimate.

We thus conclude that the batch technique is more expedient and precise than the cell washout method for estimating $\hat{\mu}$. With the latter method, $\hat{\mu}$ must be approached slowly by establishing steady-state levels of μ in small incre-

TABLE 2. *Summary of carbon and phosphorus cell quota parameters under nitrogen-limited growth of D. tertiolecta*

Parameter	Growth conditions	k'_Q (pg cell^{-1})	Q'_m (pg cell^{-1})
Q_p			
N/P = 5	Continuous[a]	0.8	5.5
N/P = 10		0.4	5.5
N/P = 15		0.2	5.5
N/P = 10	Batch[b]		5.2 ± 0.7
Q_c	Continuous[a]	23	65
	Batch[b]		59 ± 8

[a] Values estimated by eye from data shown in Fig. 4 and 5.

[b] Based on six datum points from two batch experiments. Standard deviations included.

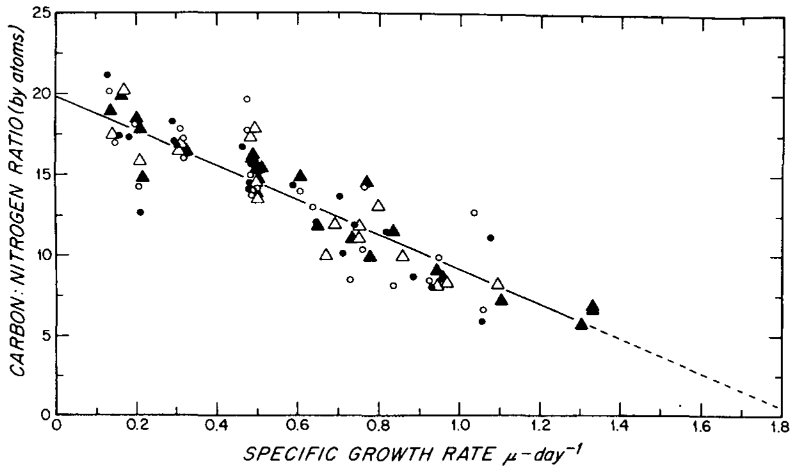

FIG. 6. *Relationship between steady-state cellular carbon-to-nitrogen ratio and specific growth rate for D. tertiolecta maintained in continuous cultures under different limiting nitrogen sources.*

ments until $\hat{\mu}$ is exceeded and washout occurs (30). Slight wall growth and very small daily variations in medium flow rate common with chemostat control can distort the true washout rate (16) and lead to a wide scatter in the data near $\hat{\mu}$, as shown in Fig. 3 through 5. By using an inoculum from a continuous culture maintained near $\hat{\mu}$, we were able to avoid any lag phase in the batch growth curve (Fig. 1). The inoculum clearly was in a physiological state geared for immediate growth at $\hat{\mu}$ because the cell quotas for all of the major cellular constituents were near the respective Q_m values (Table 2).

We could not discern any effect of the N source or the medium N/P ratio between 5 and 15 on the variation in Q with μ under steady-state conditions (Table 1). This would imply that all assimilated N, regardless of the source, was channelled directly into amino acid synthesis under steady-state conditions, i.e., no appreciable internal concentrating of NO_3^- and NO_2^- against a gradient, as has been suggested previously (3). Bienfang (3), in pooling his own data from N-limited continuous cultures of *D. tertiolecta* with those of Caperon and Meyer (5), claimed that the relationship for Q versus μ was hyperbolic for cells grown on NO_3^- or mixtures of NO_3^- and NO_4^+, but was linear when NH_4^+ was the sole N source. We think that this anomaly is because only seven datum points were available for the above NH_4^+ analysis, thereby making it difficult to observe a hyperbolic curve, as we were able to obtain with considerably more data.

It is critical that sufficient data over the entire range of μ be available for determining statistically the various kinetic coefficients (12). As demonstrated by our data analyses (Table 1), distortions in the kinetic coefficients can occur when only a small portion of the data is considered. For example, the N/P = 10 subset of data with 14 points extends only to 0.6 $\hat{\mu}$. Even though the linear fit of these data to equation 2 is excellent (r = 0.93), the slope of the resulting curve is distinctly different from the virtually identical slopes calculated with the other subsets of data (including the N/P subsets of data that bracket this subset of data) and with the entire pool of data (Table 1). Hence, a physiological basis for this difference cannot be advocated.

Carbon cell quota variations. In the growth rate range $0 < \mu < 0.8\ \hat{\mu}$, the carbon cell quota varied slightly from ~23 to 29 pg of C·cell^{-1}, followed by a rapid threefold increase up to ~65 pg of C·cell^{-1} at $\hat{\mu}$ (Table 2 and Fig. 4). This seemingly threshold increase in Q_c above ~0.8 $\hat{\mu}$ (confirmed by the enriched batch data) roughly corresponded to the growth rates at which measurable concentrations of residual nitrogen were first observed in the cultures. The latter effect is a sure indication of the onset of non-nutrient limiting conditions (i.e., μ approaching $\hat{\mu}$) (12). Hence, it appears that as long as N limitation exists, regardless of the nitrogen source and the growth rate, the total cellular level of carbon is relatively fixed, at least for *D. tertiolecta*. In contrast, Rhee (27) observed a constant Q_c under NO_3^- limitation in the freshwater green alga *Scenedesmus* sp.; however, because the highest μ tested in that study was ~0.8 $\hat{\mu}$, the threshold increase in Q_c as $\mu \rightarrow \hat{\mu}$ may have gone unobserved.

In contrast, the nitrogen cell quota increased over the entire range of μ, although most dramatically when non-nitrogen limiting conditions were approached (Fig. 3) (8, 12, 24, 27, 31). This uncoupling between the rates of carbon and nitrogen assimilation is a common characteristic of N-limited growth and is best observed by the well-established trend of a decreasing C/N ratio with increasing μ (Fig. 6; see references cited above). The degree of uncoupling appears to be species specific. For example, in this study there was a fourfold decrease in the C/N ratio as $\mu \rightarrow \hat{\mu}$, but for NH_4^+-limited *T. pseudonana* (3H) growth, only a twofold decrease in this ratio from ~10 at $\mu = 0$ to <6 at $\hat{\mu}$ was observed (12). Such differences at the biochemical level are impossible to interpret without more detailed experiments. Nevertheless, it would appear that under conditions of N limitation, protein synthesis is limited in *D. tertiolecta* so that carbon is channelled into the formation of storage products, i.e., polyglucans (24). When the growth rate approaches $\hat{\mu}$, protein synthesis completely dominates the metabolic apparatus of the cell (27), giving rise to the rapid increase in Q_c along with increasing Q_n. That this variation in Q_c also seems to be independent of the nitrogen source (Fig. 4) is further evidence, albeit indirect, that at the steady state there is no appreciable accumulation of intracellular inorganic nitrogen ions and that the degree of nitrogen limitation in phytoplankton is manifested in the way that amino acids are processed for various metabolic processes (27).

Phosphorus cell quota variations. The dependence of the phosphorus cell quota on the medium N/P ratio when nitrogen was limiting ($\mu < \sim 0.8\ \hat{\mu}$) (Fig. 4) is identical to the results of Rhee (27). He showed that at a fixed μ (= 0.44 $\hat{\mu}$), Q_p was constant under P limitation (medium N/P ratio > 30) and increased rapidly as the medium N/P ratio was decreased from 30 to 5, with a corresponding increase in the degree of N limitation. Our results extend beyond those of Rhee in showing a rapid increase in Q_p at $\mu > 0.8\ \hat{\mu}$, regardless of the medium N/P ratio in the range of 5 to 15, with convergence at a common maximum Q_p at $\hat{\mu}$ (also confirmed by the enriched batch data); this result led to an almost fourfold increase in the k'_Q/Q'_m ratio for phosphorus as the medium N/P ratio decreased from 15 to 5. As with Q_c, there appeared to be a threshold effect on Q_P when N limitation was diminished as a function of increasing μ. Both Perry (25) and Harrison et al. (15) found a similar trend of increasing Q_p with increasing μ in N-limited continuous cultures of marine diatoms for a fixed medium N/P ratio, although their data were too limited to estimate whether a threshold effect similar to ours occurred. On the other hand, Rhee (26) and Panikov and Pirt (24) found Q_P to be constant with varying μ for N-limited growth of freshwater green algae, but once again, the highest μ tested was $\leq 0.8\ \hat{\mu}$, thus possibly preventing the observation of a threshold increase in Q_p.

In any case, the rapid increase in Q_p near $\hat{\mu}$ is undoubtedly associated with the high cellular protein content and is represented by increased phosphorus in a variety of compounds, particularly nucleotides. At lower growth rates, Q_p decreases, and this phosphorus is stored increasingly in polyphosphates (26). The increased Q_p with a decreasing medium N/P ratio at a fixed low μ most likely also represents increased polyphosphate buildup.

Conclusions. Variations in the chemical composition of phytoplankton, at best, provide limits to the physiological changes that occur as a result of changes in growth rate. One major difference between the steady-state continuous culture and nature is that in the former the growth rate is an independent variable, whereas in nature it is dependent and coupled to available limiting nutrients. The degree of coupling is controlled in a gross fashion by the limiting nutrient cell quota. For nitrogen, as described in this study with N-limited *D. tertiolecta*, the source of nitrogen is not important in establishing the relationship between steady-state nitrogen cell quota and growth rate. However, on the temporal and spatial scales of available sources of nitrogen that marine phytoplankton are exposed to, steady-state information, as collected in this study, does not provide any clue as to the degree of coupling between available nitrogen and cell growth. Only through studies on nutrient pulsing will that type of information become available.

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