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Ultraplankton growth rates in a subtropical ecosystem *

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Abstract

The ultraplankton (cell diameters $< 3\,\mu$m), which compromises about 70% of the biomass of phytoplankton in subtropical surface waters near Oahu, Hawaii, was isolated for growth rate studies. The specific growth rate (μ) was estimated from the rate of increase of the chlorophyll biomass during incubations in the absence of grazers. This growth rate of the ultraplankton ranged from 0.037 to 0.071 h^{-1} (= 1.3 to 2.5 doublings d^{-1}) during a period when P:B ratios of 5 to 14.5 $\mu g\ C\ \mu g^{-1}$ chl $a\ h^{-1}$ prevailed. The co-occurrence of atypically high P:B ratios and non-limiting ambient nutrient concentrations suggests that the calculated values are higher than those characteristic of such subtropical ecosystems in general. Rates of ammonium uptake and photosynthesis by the $< 3\,\mu$m fraction were also compared to those of larger fractions. Organisms in the $< 3\,\mu$m fraction assimilated NH_4^+ at a rate which was about 75% greater than that of the 3 to 20 μm size fraction. Comparison of μ and P:B data collected over a 2 mo period (November–December, 1980) shows that the correlation between these two rate indices is nonlinear. The predominance of small-celled phytoplankton in oligotrophic waters is explained, in part, by its higher μ, its higher nutrient assimilation rates, and the absence of its loss through sedimentation.

Introduction

In subtropical waters, a well-developed pycnocline restricts upward transport of nutrients into the photic zone. Phytoplankton populations in such areas commonly have a low biomass, grow slowly and exhibit little temporal variability in abundance. In recent years, this paradigm has received scrutiny as a result of skepticism concerning microbial growth rates being slow (Sheldon and Sutcliffe, 1978; Goldman *et al.*, 1979; Jackson, 1980; Peterson, 1980; Sharp *et al.*, 1980; Fitzwater *et al.*, 1982).

The predominance of phytoplankton biomass as very small cells is a distinguishing feature of warm oceanic waters. Recent studies in subtropical Hawaiian waters have shown that 60 to 80% of the phytoplankton biomass occurs in the $< 3\,\mu$m size fraction (Bienfang, 1980; Bienfang and Szyper, 1981; Takahashi and Bienfang, 1983). The present study concerns specific growth rates of the $< 3\,\mu$m fraction, which were measured by a non-isotopic method. The specific growth rate, based on the rate of increase of chlorophyll biomass, was measured by isolating this fraction from large herbivores. The technique assumes (1) that the rates of phytoplankton growth (μ) and herbivorous grazing (g) are closely coupled (i.e., $dN/dt = \mu N - gN \approx 0$, where N is biomass and t is time), and (2) that separation at the 3 μm size interval isolates the $< 3\,\mu$m fraction from herbivorous organisms. The present work focuses on examining various rates of activity (i.e., photosynthesis, ammonium uptake, specific growth rate) of the $< 3\,\mu$m phytoplankton component. The objectives of this study were: (1) to measure specific growth rates for the $< 3\,\mu$m fraction; (2) to examine growth rate response to nutrient (NH_4^+ and PO_4^{-3}) enrichment; (3) to compare the photosynthetic response due to nutrient enrichment of the $< 3\,\mu$m fraction with those of larger (i.e., 3 to 20 μm and $> 20\,\mu$m) fractions; (4) to compare the ammonium uptake rate of the $< 3\,\mu$m fraction to that of the larger (i.e., 3 to 20 μm) nanoplankton component.

Materials and methods

All samples were collected from surface (1 m) waters at a site located ca. 1.5 km off Oahu, Hawaii. The collection site (21°26'N; 157°39'W) is in free communication with the open ocean, and water depth in the area exceeds

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100 m. Experimentation took place in November–December 1980, during a prolonged period of very light winds and extremely calm seas. Samples were collected at about 09.00 hrs and returned (within 30 min) to the laboratory, where size fractionation was conducted at ambient water temperature ($T=25\,°C$). Size separation, at 20 and 3 μm intervals, employed Nitex screen and Nuclepore polycarbonate filters, respectively. All size fractionations were done in subdued light, using only gravity pressure. Particular care was taken to maintain fluid interfaces on both sides of the filters to avoid any exposure of cells to the air. The sample fractions were not concentrated for either the growth rate or photosynthesis studies. All glassware was thoroughly cleaned and rinsed with recently collected seawater prior to use.

Growth rate (μ) measurements were made by isolating the $< 3\,\mu$m fraction and monitoring the rate of change of chlorophyll biomass in the absence of grazers. Samples containing the $< 3\,\mu$m fraction were placed in 4-liter glass containers, covered, and incubated under conditions similar to those of the *in situ* sample origin (25 °C, 250 μE $m^{-2}\,s^{-1}$). Immediately after the isolation and at 2 h intervals thereafter for 8 to 10 h, triplicate 100 ml subsamples were removed in order to describe the time-series increase in chlorophyll biomass. These data were fit to an exponential equation ($B_t = B_0 e^{\mu t}$), where μ, t and B represent the specific growth rate, time, and biomass, respectively; μ values were calculated for the time period over which biomass displayed continual increase.

Chlorophyll samples were filtered (Millipore HA filters), extracted in 90% acetone, and measured with a Turner III fluorometer (Strickland and Parsons, 1972); all samples were corrected for phaeopigments. The coefficients of variation (SD/$\bar{x}$) of triplicate analyses were usually $\leqq 10\%$. Photosynthesis rates were measured in triplicate, by the ^{14}C method (Strickland and Parsons, 1972). After the filtrations, filters (Millipore 18A) were purged of inorganic ^{14}C (Lean and Burnison, 1979), admixed with Aquasol-2 cocktail, and counted in a Searle Delta 300 liquid scintillator. Working ^{14}C activities were standardized for each experiment (Iverson *et al.*, 1976). Samples for photosynthesis were incubated for periods of 4 to 6 h, at 25 °C and 250 μE $m^{-2}\,s^{-1}$; the coefficients of variation of triplicates were $\leqq 10\%$. Nutrient samples were prefiltered through Gelman A/E glass-fiber filters and analyzed in duplicate, in a Technicon Auto Analyzer II (Solórzano, 1969; Strickland and Parsons, 1972).

Results

Fig. 1 shows the increase in chlorophyll concentration during the first experiment, which was designed to measure the specific growth rate of the $< 3\,\mu$m phytoplankton components. Chlorophyll concentration increased continuously throughout the first 10 h of the incubation period (Fig. 1); after this time, chlorophyll concentrations plateaued and remained constant for the next 6 h (not

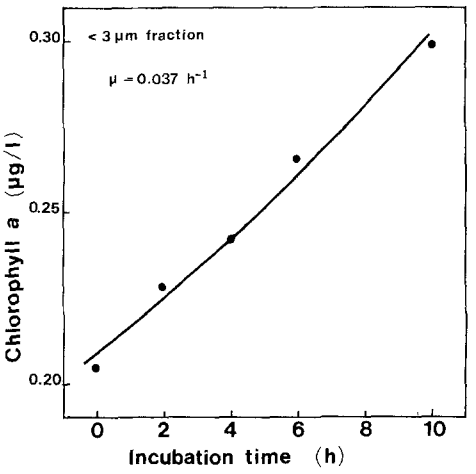

Fig. 1. Time course showing change of chlorophyll biomass in $< 3\,\mu$m fraction after isolation from larger components. Incubation was for 10 h at a light intensity of 250 μE $m^{-2}\,s^{-1}$. Each data point represents mean of triplicate analyses. Curve through the points represents least-squares fit to the exponential equation ($B_t = 0.209\,e^{0.037t}$, $r^2 = 0.99$). Calculated growth rate ($\mu = 0.037\ h^{-1}$) represents a division rate of 1.3 doublings d^{-1}

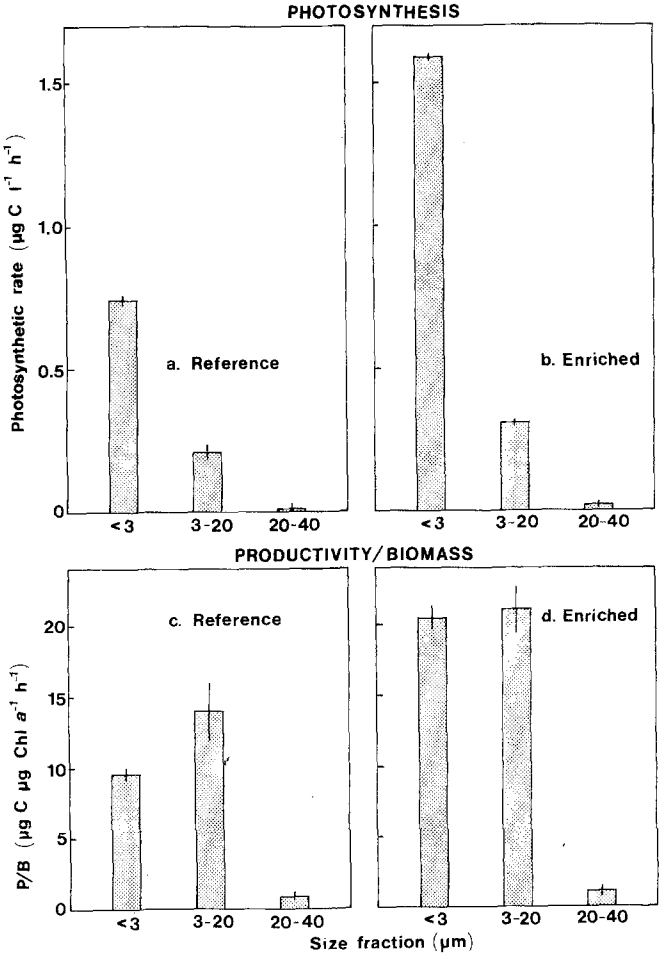

Fig. 2. Photosynthetic response of various size classes of subtropical phytoplankton to nutrient ($NH_4^+ + PO_4^{-3}$) enrichment. Error bars on histograms give the standard deviation about the means of triplicate analyses

illustrated). The initial NH_4^+ concentration in this experiment was 0.16 μM. The specific growth rate, calculated from the rate of change of chlorophyll biomass, during this 10 h period was $\mu = 0.037\ h^{-1}$ (= 1.3 doublings d^{-1}). Microscopic examination showed that small coccoid ($\leqq 1\ \mu m$ diam) and flagellated (< 1 to 3 μm diam) cells were the predominant phytoplankton organisms.

A second experiment examined the response of photosynthesis rates within three size classes (< 3 μm, 3 to 20 μm, 20 to 40 μm) to nutrient ($NH_4^+ + PO_4^{-3}$) addition. The initial nutrient levels for this trial (0.50 μM NO_3^+, 1.40 μM NH_4^+, 0.95 μM PO_4^{-3}) were considerably higher than those in the first experiment, performed 8 d earlier. The enrichment raised ambient nutrient levels to 4.2 μM NH_4^+ and 4.2 μM PO_4^{-3}. Despite the atypically high initial NH_4^+ and PO_4^{-3} concentrations, nutrient additions caused distinct increases in photosynthesis rates (Fig. 2). The stimulation of photosynthesis was more pronounced in the < 3 μm fraction (114%) than in the 3 to 20 μm (50%) or 20 to 40 μm (29%) fractions. In the unenriched (= reference) samples, P:B ratios were 9.51 ± 0.40, 14.01 ± 1.98, and 0.88 ± 0.31 $\mu g\ C\ \mu g^{-1}$ chl $a\ h^{-1}$ for the < 3 μm, 3 to 20 μm, and 20 to 40 μm fractions, respectively; such P:B values are uncharacteristically high for the waters in question. Following the nutrient additions, the P:B ratios of these fractions increased to 20.47 ± 0.84, 21.03 ± 1.59, and 1.13 ± 0.23 $\mu g\ C\ \mu g^{-1}$ chl $a\ h^{-1}$, respectively (Fig. 2 d).

Two days after the second, a third experiment examined the growth rate response of the < 3 μm fraction to the addition of NH_4^+, PO_4^{-3} and NH_4^+ plus PO_4^{-3}. The calculated increases in biomass for these trials is given in Fig. 3. All treatments displayed systematic chlorophyll increases with time; however, chlorophyll increase showed a plateau after just 4 h in the sample enriched with only NH_4^+ (Fig. 3 c). The initial nutrient levels were again high (e.g. 1.18 μM NH_4^+, 0.95 μM NO_3^-, and 0.91 μM PO_4^{-3}), and enrichments raised the concentrations of NH_4^+ and PO_4^{-3} to 4.21 and 3.41 μM, respectively. The specific growth rates calculated from these trials ranged between 0.047 and 0.067 h^{-1} (= 1.6 to 2.5 doublings d^{-1}). Samples receiving nutrient additions generally did not display increased growth rates. The unenriched reference sample (Fig. 3 a) had a growth rate ($\mu = 0.064\ h^{-1}$) which was considerably (68%) higher than that calculated for samples collected and examined in the first experiment of 10 d earlier ($\mu = 0.037\ h^{-1}$). Ambient nutrient data suggest that non-limiting nutrient conditions prevailed for at least 2 d before this trial.

The nutrient enrichment effects on photosynthesis and growth rate were examined concomitantly, with particular attention to the < 3 μm fraction. Enrichments, which raised concentrations from 1.09 μM NH_4^+ and 0.97 μM PO_4^{-3} to 3.50 μM NH_4^+ and 3.61 μM PO_4^{-3}, failed to cause any increase in either specific growth rates or P:B ratios (Fig. 4). The specific growth rates calculated from the chlorophyll time-series data ranged between 0.058 and 0.071 h^{-1} (= 2.0 to 2.5 doublings d^{-1}), and the P:B values ranged from 14.2 ± 4.3 to 14.5 ± 4.4 $\mu g C\ \mu g^{-1}$ chl h^{-1}. Values

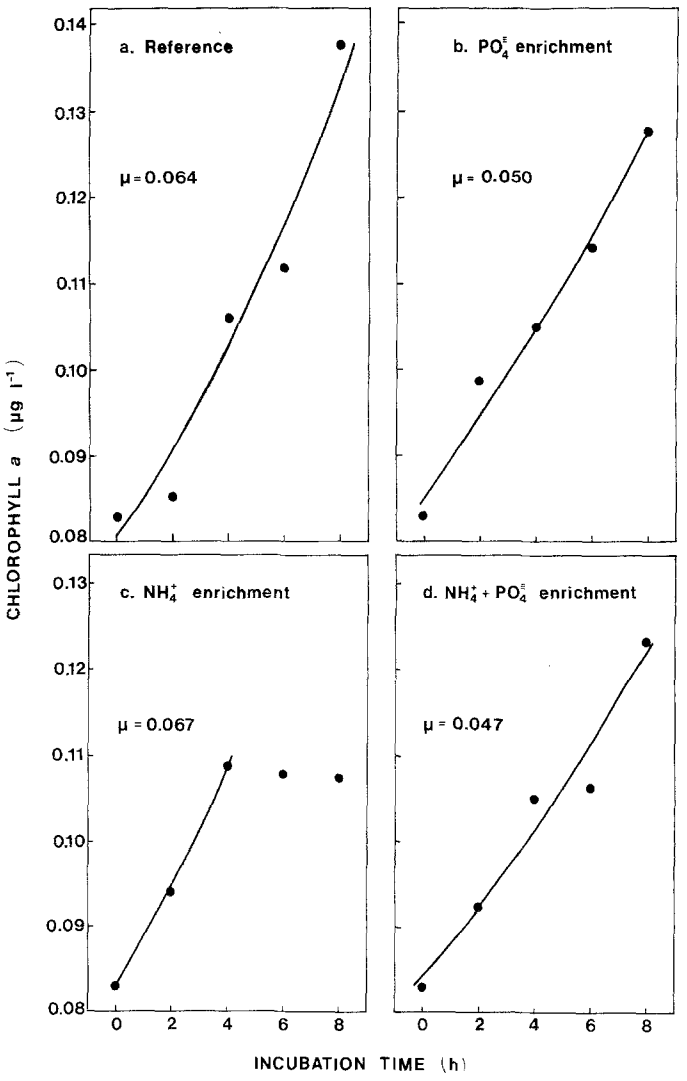

Fig. 3. Time course data showing short-term growth rate response of < 3 μm fraction to nutrient enrichments. Incubations were for 8 h at a light intensity of 250 $\mu E\ m^{-2}\ s^{-1}$. Each data point represents means of triplicate analyses. Curves through the points represent least-squares fits to the exponential equations: (a) $B_t = 0.080\ e^{0.064t}$, $r^2 = 0.94$; (b) $B_t = 0.086\ e^{0.050t}$, $r^2 = 0.97$; (c) $B_t = 0.083\ e^{0.067t}$, $r^2 = 0.99$; (d) $B_t = 0.084\ e^{0.047t}$, $r^2 = 0.96$. Calculated specific growth rates ($\mu = 0.047$ to $0.067\ h^{-1}$) represent division rates of 1.6 to 2.3 doublings d^{-1}

of both rate indices were slightly lower in the enriched samples than in the reference samples.

Ammonium uptake rates of the 0.22 to 3 μm and 3 to 20 μm fractions were compared to determine whether the < 3 μm fraction, which dominated community biomass and photosynthesis, would display higher nutrient assimilation rates. Biomass in the 0.22 to 3 μm and 3 to 20 μm fractions were concentrated to 10.31 and 7.17 μg chlorophyll l^{-1}, respectively. Following the addition of NH_4^+, ammonium disappearance from the medium was followed for several hours (Fig. 5). The 0.22 to 3 μm fraction displayed an uptake rate of 0.026 μM $NH_4^+\ \mu g^{-1}$ chlorophyll $a\ h^{-1}$ (= 0.63 μM $NH_4^+\ \mu g^{-1}$ chlorophyll $a\ d^{-1}$). This

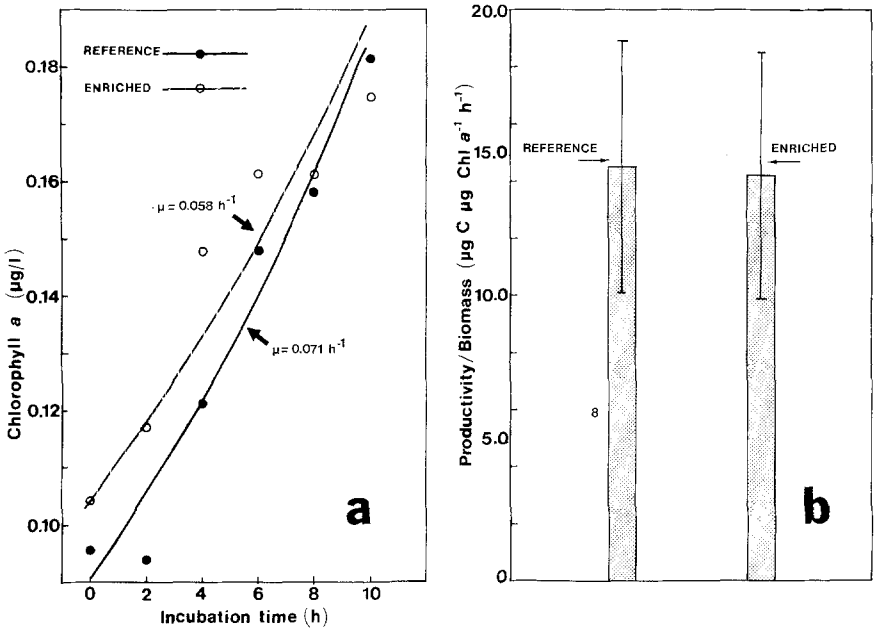

Fig. 4. Comparison of response of $< 3\,\mu$m fraction to nutrient ($NH_4^+ + PO_4^{-3}$) enrichment as indexed by (a) growth rate, and (b) P:B ratios. All incubations were at a light intensity of $250\,\mu E\ m^{-2}\ s^{-1}$. Each data point represents means of triplicate analyses. Curves drawn through the points in (a) represent least-squares fits to the equations $B_t = 0.091\,e^{0.071t}$, $r^2 = 0.95$, and $B_t = 0.105\,e^{0.058t}$, $r^2 = 0.90$ for the reference and enriched samples, respectively. Neither index showed a significant ($P > 0.10$) response to enrichment at the time of analysis

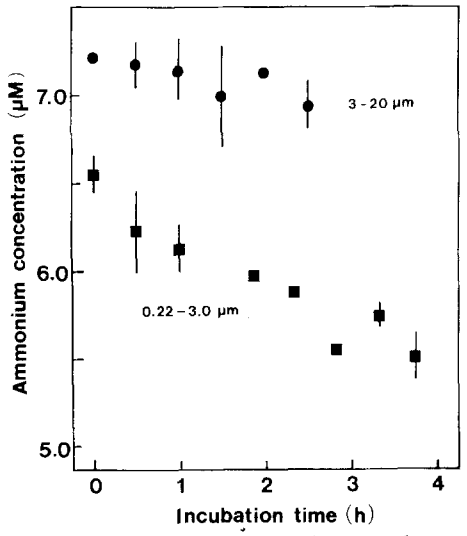

Fig. 5. Comparison of ammonium uptake rates by 0.22 to $3\,\mu$m and 3 to $20\,\mu$m fractions, during incubations of concentrated samples at a light intensity of $250\,\mu E\ m^{-2}\ s^{-1}$. Regression analyses of the data indicate that the 0.22 to $3\,\mu$m fraction displayed an uptake rate ($0.026\,\mu M\ NH_4^+\,\mu g^{-1}$ chlorophyll $a\ h^{-1}$) about 75% greater than that of the larger 3 to $20\,\mu$m fraction ($=0.015\,\mu M\ NH_4^{+4}\,\mu g^{-1}$ chlorophyll $a\ h^{-1}$)

uptake rate was about 75% faster than that calculated for the 3 to $20\,\mu$m fraction ($0.36\,\mu M\ NH_4^+\,\mu g^{-1}$; chlorophyll $a\ d^{-1}$).

Discussion

The predominance of extremely small cells in the phytoplankton biomass represents an important ecological feature of oligotrophic seas. In Hawaiian waters, about 70% of the photoautotrophic biomass, and an even greater share of total primary production, is due to ultraplankton in the $< 3\,\mu$m fraction (Bienfang, 1980; Bienfang and Szyper, 1981; Takahashi and Bienfang, 1983). The findings from the present study indicate that these organisms are *capable* of growing at rapid specific growth rates (i.e., 1.3 to 2.5 doublings d^{-1}).

These μ values were calculated from measurements of biomass which increased with time in incubated samples. Similar growth rate determinations have been made from increases in ATP (Sheldon and Sutcliffe, 1978) and DNA (Falkowski and Owens, 1982) during incubation. The latter work showed a very good correlation ($r^2 = 0.96$) between growth rate values from time-series analysis and rates based on direct observation of cell *division*. Chlorophyll *a* was chosen as the biomass index in the present trials since it was analytically convenient, and could be related directly to the photoautotrophic components of the sample. With any of these indices, the calculated rates of increase will best reflect rates of cell division when the rate of increase (on a per cell basis) is constant over the incubation period. With the use of chlorophyll *a*, a principal concern is the avoidance of photic conditions which might cause shade-adaptation to occur. The light levels ($250\,\mu E\ m^{-2}\ s^{-1}$) that were used should have prevented any significant light-induced changes in chlorophyll $cell^{-1}$ or chlorophyll C^{-1}; thus, shade adaptation is probably not an important cause of the chlorophyll increases that were observed. The differences in the calculated growth rates measured over the 2 mo period also argue against a pronounced effect of such an artifact.

Our assessment of specific growth rates was made possible by the isolation of the $< 3\,\mu$m phytoplankton from the main herbivorous components of the sample. The measurement of rather high rates of biomass increase in unenriched samples (Figs. 1, 2, 4) is evidence of the degree to which *in situ* grazing pressure controls biomass levels in such waters. The presence of herbivorous organisms in the $< 3\,\mu$m samples would naturally lead to under-estimations of actual growth rates. Microscopic examinations did not reveal any evidence of grazers in these samples, but

difficulty in the detection/identification of small phagoflagellates means that their potential presence cannot be discounted altogether. The consistent increase of chlorophyll during the 10 h incubation periods indicates a sufficiency of nutrients in the cells and surrounding waters to support continued growth during these time periods. The growth rates obtained pertain strictly to only a portion (i.e., $< 3\,\mu$m) of the total phytoplankton population; however, because the $< 3\,\mu$m fraction accounts for 70% of the total biomass, the growth rate calculated for this fraction similarly approximates that of the total assemblage. Data showing the size distribution of P:B ratios (Takahashi and Bienfang, 1983) would suggest that activity rates of the $\leqq 3\,\mu$m fraction are typically greater than those of the larger population components comprising about 30% of the population. If this is valid, the calculated μ values from these experiments probably represent an upper limit to the growth rates of the entire population during these experiments.

The environmental conditions at the time these experiments were performed was marked by a prolonged period of very light winds and extremely calm seas. Dense aggregations of gelatinous herbivores were observed in the surface waters during sample collection, and their presence undoubtedly accounts for the atypically high ambient nutrient levels recorded. These conditions are reflected in the uncharacteristically high rates of primary production and P:B ratios measured. During these trials, P:B ratios were an order of magnitude greater than those commonly measured in Hawaiian waters by one of the investigators (Bienfang, 1981; Bienfang and Szyper, 1981), and approached the maximum value predicted on the basis of photosynthetic turnover times and the number of photosynthetic units (Falkowski, 1981). This observation exemplifies the potential variability of a subtropical planktonic system; however, these atypical conditions preclude direct extrapolation of the measured growth rate values to oceanic waters. The data show, nonetheless, that the $< 3\,\mu$m fraction is capable of sustaining rapid growth rates, and that our incubation procedure can detect such growth when, or if, it occurs.

Both indices of community activity (specific growth rate and P:B ratios) increased over the period of experimentation; these trends suggest the importance of the preconditioning history to the values measured at any given time. Growth rates went from 0.037 h^{-1} (Fig. 1) in the first trial to 0.071 h^{-1} in the trial conducted 3 wk later (Fig. 4); during this same period, the calculated P:B values for the $< 3\,\mu$m fraction increased from 5.04 to 14.5 $\mu g\,C\,\mu g^{-1}$ chlorophyll $a\,h^{-1}$. This pattern of variation with time would suggest that calm conditions, accompanied by intensive grazing over a prolonged period, were required to elicit the phytoplankton activity rates observed. This would seem to place an additional constraint on the conditions required to give such growth rates in nature.

As indicated above, there was a general correspondence between the $< 3\,\mu$m fractions μ and P:B values measured at various times. Subsequent to these experiments, several growth rate trials identical to those reported here were also performed at sea under conditions more typical of oceanic waters (i.e., winds of about 15 knots, 1 to 2 m seas, low nutrient levels, and P:B values of 1 to 2 $\mu g\,C\,\mu g^{-1}$ chlorophyll $a\,h^{-1}$). None of these experiments showed a discernible increase of chlorophyll during the incubation periods. The implication is that such populations were growing at rates much lower than those studied in the present work, as would be indicated by the large differences in P:B ratios. Correlation of these data (Fig. 6) shows that the paired μ and P:B ratios (at light saturation) acquired at various times are rather well correlated with one another, as would be expected of two indices of specific activity. This co-variation supports the notion that the time-series patterns of chlorophyll increase (Figs. 1, 3, 4) are not artifacts of chromatic adaptation. Consideration of the distribution of points in Fig. 6 also shows that the correlation between μ and P:B is not linear. A fit of the μ and P:B data to an equation of hyperbolic form is represented by the curve drawn through the points (Fig. 6); the fit accounted for 96% of the variance in the data and gave a calculated $\mu = 0.072\,h^{-1}$ for a P:B value of 25. Equivalency between μ and P:B occurs via the C:chl ratio of the organisms. This ratio depends on a complex set of biological interactions; in discussing the limitations of P:B ratios as a growth rate index, Falkowski (1981) points out the responsiveness of the P:B ratio to factors other than growth rate *per se*.

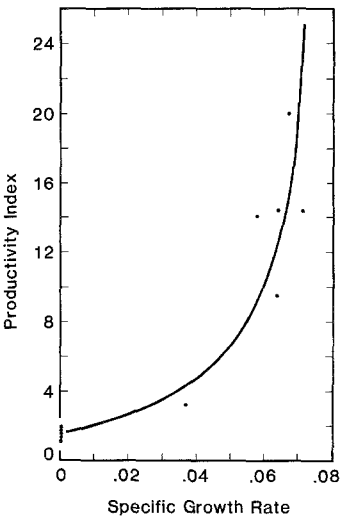

Fig. 6. Correlation between specific growth rate (μ) and productivity index (P:B) (combined data from various collections and times) demonstrates that the relationship between these two growth rate indices is non-linear. Curve through the points describes least-squares fit of the data to an equation of hyperbolic form

The predominance of small-celled phytoplankton in subtropical seas may be related to both the characteristics of the nutrient field and the absence of settling losses of this biomass. The nutrient regime of oligotrophic waters is characterized by low ambient concentrations and numerous (small) nutrient inputs of primarily regenerated origin.

Turpin and Harrison (1980) have shown that the frequency of nutrient additions (i.e., degree of temporal patchiness) can affect the phyletic composition of phytoplankton communities. Results of their competition experiments with heterogeneous assemblages showed that the mean cell diameter decreased as the frequency of nutrient additions increased. Thus, the relative importance of high-frequency nutrient inputs (from *in situ* excretions by grazers) in oligotrophic waters would tend to maintain a predominance of small cells in such populations. Our ammonium uptake results (Fig. 5) showed the $< 3\,\mu$m fraction to be capable of assimilating NH_4^+ at rates which were substantially (75%) greater than the rates of larger (3 to $20\,\mu$m) components. This increased affinity for the acquisition of nitrogen by the $< 3\,\mu$m component is empirical evidence of a competitive advantage in maintaining its predominance. Also, the short-term response of photosynthesis to sudden nutrient availability (Fig. 3) showed that the stimulation of the $< 3\,\mu$m fraction rose to more than twice that of the 3 to $20\,\mu$m fraction. The absence of sedimentation losses reinforces the predominance of the ultraplankton fraction. The importance of this is related to the community growth rate, because when growth (= biomass input) rates are low, sinking losses (= biomass output) constitute a proportionately greater share of net production. If subtropical phytoplankton are truly growing at slow rates, then the absence of sedimentation losses from a sizable portion of the microbial community is a notable feature of this ecosystem which conserves biomass.

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