

NUTRIENT LIMITATION OF MARINE PHOTOSYNTHESIS

John J. Cullen*, Xiaolong Yang, and Hugh L. MacIntyre

Bigelow Laboratory for Ocean Sciences
McKown Point
West Boothbay Harbor, ME 04575

INTRODUCTION

Guided by insightful presentations at the previous Brookhaven Symposium (Bannister and Laws, 1980; Eppley, 1980; Goldman, 1980) we address here three questions that have challenged oceanographers for decades: 1) Can photosynthetic performance be used to diagnose the nutritional status of phytoplankton? 2) Should nutrients be incorporated into models of oceanic photosynthesis as a function of chlorophyll and light? and 3) How might we assess nutrient limitation of the specific growth rates or standing crop of phytoplankton in the ocean? We find that ambiguities thwart attempts to formulate robust generalizations. Accordingly, when it comes to nutrient limitation of marine photosynthesis, a good paradigm is hard to find.

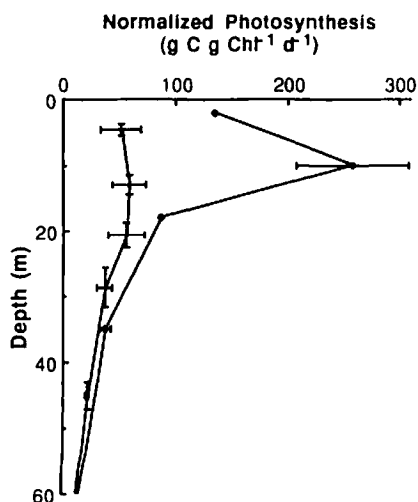
NUTRIENTS AND PHOTOSYNTHETIC PERFORMANCE

The growth and photosynthesis of plants depends on nutrients. It is reasonable to expect, then, that some aspects of photosynthetic performance will reflect the nutritional status of phytoplankton. Here we examine the rate of photosynthesis normalized to chlorophyll *a* (P^b), asking if P^b tells us anything about the nutritional status or specific growth rates of phytoplankton.

Regional Differences in Normalized Photosynthesis

Measurements from different regions of the ocean suggest that P^b may indeed reflect the supply of nutrients to the euphotic zone (Barber and Chavez, in press). Consider a comparison of observations from the central equatorial Pacific and the Gulf of Mexico (Fig. 1). For these two environments, temperature and light conditions were similar, yet normalized photosynthesis ($\text{g C g Chl}^{-1} \text{d}^{-1}$) was much higher over the Texas shelf, where nitrate concentrations were low, but where trace elements, such as iron, were much more abundant than in the equatorial Pacific (cf. Martin et al, 1989). If the

*Address for correspondence: Department of Oceanography, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada



TEXAS SHELF (water depth 70 m)

Surface $[\text{NO}_3] < 0.3 \mu\text{M}$
 Surface Chl = 0.08 mg m^{-3}
 Surface Temperature = 28°C
 1% surface irradiance at 70 m

EQUATORIAL PACIFIC (150°W)

Surface $[\text{NO}_3] > 3 \mu\text{M}$
 Surface Chl $> 0.2 \text{ mg m}^{-3}$
 Surface Temperature = 26°C
 1% surface irradiance at 70 m

Fig. 1. Vertical profiles of normalized photosynthesis ($\text{g C g Chl}^{-1} \text{ d}^{-1}$) from the Texas Shelf (higher values; 4 h simulated *in situ* incubations, hourly rates multiplied by $10 \pm$ range of duplicates; Cullen et al., unpubl.) and from the equatorial Pacific (lower values; 24 h simulated *in situ* incubations \pm s.d. for 6 days; data of R. Barber and F. Chavez presented in Cullen et al., in press).

higher P^B off Texas corresponded to higher specific growth rates of phytoplankton, one might infer that the specific growth rates of the equatorial phytoplankton were limited by nutrient supply, possibly iron. However, specific growth rate, μ , (d^{-1}) is determined by both P^B (expressed as net photosynthesis over 24 h) and the carbon/chlorophyll ratio of phytoplankton (C:Chl):

$$\mu = \frac{P^B}{\text{C:Chl}} \quad (1),$$

so that the relationship between P^B and μ can be determined only if C:Chl is specified. Unfortunately, C:Chl, which is quite variable under physiological control, cannot be measured directly and accurately on natural samples (Eppley, 1972, 1980; but see Redalje and Laws, 1981), so geographical patterns in P^B cannot be related directly to μ unless more information is provided.

Laboratory Models of Nutrient Limitation

Pertinent information on the relationship between P^B and μ comes from models of photosynthesis, nutrition, and growth of phytoplankton. These models, which will not be reviewed here, are based on results from laboratory experiments. The experiments are usually performed either by allowing a culture to deplete a limiting nutrient (nutrient-starved batch culture) or by growing phytoplankton in nutrient-limited continuous culture. The two experimental regimes are fundamentally different, and the differences should be appreciated.

Batch culture. When a batch culture of microalgae runs out of a limiting nutrient, growth is unbalanced (Eppley, 1981) because photosynthesis proceeds, but synthesis of critical cellular constituents is restricted by lack of the limiting nutrient. Biochemical

composition (Strickland et al., 1969; Sakshaug and Holm-Hansen, 1977; Flynn, 1990) and physiological capabilities (Horrigan and McCarthy, 1981; Cleveland and Perry, 1987) change, as does the rate of cell division. Both the assimilation number (P^B at optimal irradiance; Glover, 1980) and photosynthetic efficiency in subsaturating light (Welschmeyer and Lorenzen, 1981; Cleveland and Perry, 1987) decline in what appears to be an unavoidable consequence of nutrient starvation.

Continuous culture. In nutrient-limited continuous culture, physiological regulation culminates in balanced growth of phytoplankton: cellular concentrations of the compounds that require the limiting nutrient decline and storage products (such as carbohydrate or lipid) are accumulated until the amounts of all cellular constituents increase exponentially at the same nutrient-limited rate, averaged over the photocycle (Shuter, 1979; Eppley, 1981). That is, cellular carbon increases until "the growth rate of algal carbon is reduced to match the growth rate allowed by the nutrient supply" (Bannister and Laws, 1980). In response to nitrogen limitation, for example, cellular chlorophyll concentration is regulated so that changes in C:Chl are principally responsible for the variation in growth rate (e.g., Laws and Bannister, 1980). As a result, P^B at growth irradiance can be independent of nutrient-limited growth rate (Bannister and Laws, 1980; Sakshaug et al., 1989; Cullen, 1990).

Thus, the results for nutrient starvation in batch culture are not interchangeable with the results for continuous cultures. When relating experimental results to the ocean, it is useful to consider which laboratory model is likely to apply in a given oceanographic situation (Yentsch et al., 1977).

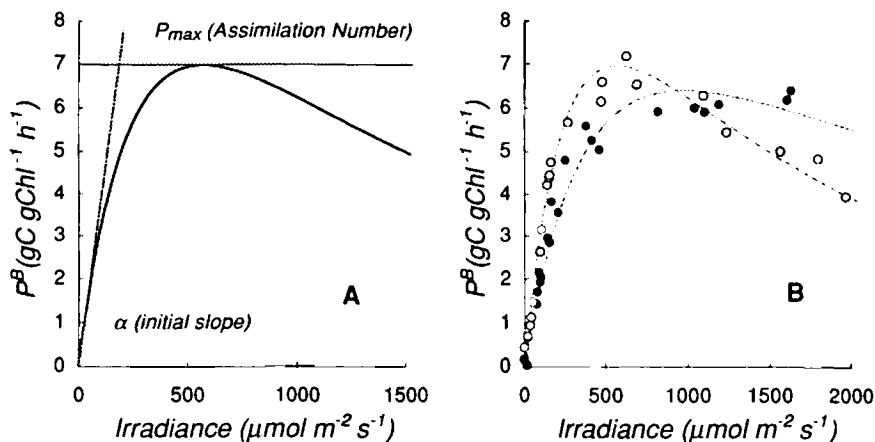


Fig. 2. The relationship between photosynthesis and irradiance. (A) A P versus I curve following the model of Platt et al. (1980), with initial slope α , and maximal rate P_{max} . Susceptibility to photoinhibition is designated by the parameter β . (B) P versus I curves for the diatom *Thalassiosira pseudonana* (clone 3H) grown at $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in nutrient-replete media (filled circles are from semi-continuous cultures, 12 h: 12 h light:dark cycle; $\mu = 0.85 \text{ d}^{-1}$) and in nitrate-limited continuous culture at 0.3 d^{-1} (open circles; Yang et al., see Table 1). In this particular comparison, P_{max} is similar, but the nitrogen-limited culture has a higher α and greater susceptibility to photoinhibition. The generality of this comparison is examined below and found to be lacking.

Nitrogen Limitation and Photosynthetic Performance: Experimental Results

When phytoplankton are grown at steady state in nutrient-limited continuous culture, the growth rate equals the dilution rate, which is under precise experimental control. The influence of nutrient limitation on photosynthesis can then be examined by measuring C:Chl and calculating P^b at growth irradiance by rearranging eq. 1 (e.g., Laws and Bannister, 1980). Also, photosynthesis versus irradiance (P versus I) can be measured directly on subsamples. More sophisticated biochemical or biophysical measurements also can be made (Falkowski, this volume), but the P versus I relationship is examined here because it is relatively easy to measure, and it contains information on photosynthetic efficiency, photosynthetic capacity, and the susceptibility of the alga to photoinhibition (Fig. 2A). Each of these facets of photosynthetic performance might respond independently to nutrient limitation (Fig. 2B). Environmental influences on P versus I of natural phytoplankton have been examined (e.g., Platt and Jassby, 1976; Harrison and Platt, 1980, 1986; Falkowski, 1981; Malone and Neale, 1981), but as the following discussion will show, the effects of nutrient-limitation on P versus I merit review.

Assimilation number. The assimilation number is P^b at light saturation, equivalent to P_{\max} in the P versus I relationship. Assimilation number is perhaps the easiest photosynthetic parameter to use to compare measurements between the laboratory and the field, because it is relatively insensitive to differences in light quality, its calculation does not rely on accurate measurement of irradiance, and it can reasonably be compared to the maximum P^b measured in vertical profiles. The variability of assimilation number has been studied for years (e.g., Curl and Small, 1965; Eppley, 1972; Yentsch et al., 1974; Platt and Jassby, 1976; Glover, 1980; Harrison and Platt, 1980; Falkowski, 1981). Here, we examine one aspect, the relationship between nitrogen-limited growth rate and assimilation number in continuous culture.

Results spanning nearly 20 years are compiled in Fig. 3. The basic experimental design is straightforward, although details differ (Table 1). Simply, cultures were grown at a series of nitrogen-limited growth rates, and photosynthesis was determined experimentally, either by measuring O_2 evolution or the uptake of ^{14}C -bicarbonate. For comparison, we converted published results to plots of P_{\max} ($g\ C\ g\ Chl^{-1}\ h^{-1}$) versus relative growth rate (μ/μ_{\max} ; Goldman, 1980). Also, we included our own results from studies of nitrate-limited growth of the neritic diatom, *Thalassiosira pseudonana* (clone 3H). Our cultures were grown on light:dark cycles, and the diel variation of P versus I was examined.

A pattern can be seen in each experimental series, but patterns differ between experiments. Several studies show that P_{\max} is strongly depressed at low growth rates (Thomas and Dodson, 1972; Glover, 1980; Osborne and Geider, 1986; Kolber et al., 1988; Chalup and Laws, 1990), whereas others indicate that P_{\max} is largely independent of nitrogen-limited growth rate (Eppley and Renger, 1974; Herzig and Falkowski, 1989; Yang et al., Fig. 3H). Experimental conditions and cultured species differed (Table 1), but we find no factor or set of factors that is clearly associated with either result. For example, one might look for a contrast between neritic and oceanic species (Sakshaug et al., 1987). However, both patterns have been observed in oceanic diatoms (Thomas and Dodson, 1972; and Eppley and Renger, 1974), and different patterns have been observed in the same clone of a neritic diatom (Kolber et al., 1988; and Yang et al., unpublished, Fig. 3H). Perhaps the experimental photocycle is important: assimilation number was little affected by nitrogen-limitation in two studies that employed light:dark cycles (Eppley and Renger, 1974; Yang et al., Fig. 3H), but it was strongly a function of growth rate in most studies using continuous light. However, in the thorough study made by Herzig and

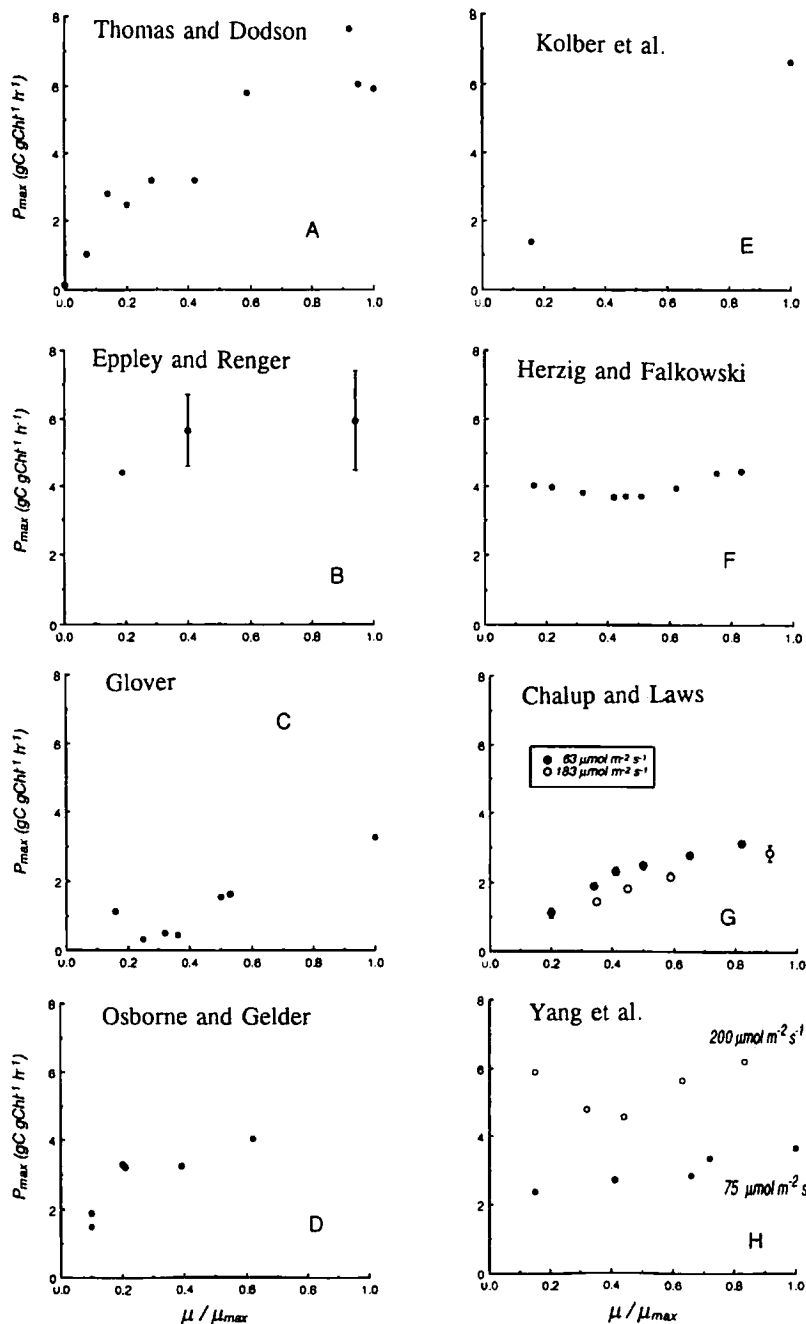


Fig. 3. Relationships between P_{max} and nitrogen-limited growth rate (converted to relative growth rate by normalizing to the nutrient saturated rate at that temperature and irradiance, Goldman, 1980): nitrogen-limited continuous cultures. Oxygen-based measurements of gross photosynthesis were converted using photosynthetic quotients in the original publications (but 0.8 mol C mol O_2^{-1} was assumed for Kolber et al., 1988). References and experimental details are presented in Table 1.

Table 1. Description of experiments described in Fig. 3. Irradiance measurements have been converted for comparison.

Reference	Species	Nutrient	Illumination	Other	Steady-state	Method	Result
Thomas and Dodson (1972)	<i>Chaetoceros gracilis</i> (oceanic)	10 μM NH_4 modified F	Continuous cool-white 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated and stirred 25°C	Successive cell counts	3 h ^{14}C uptake maximum about 320 $\mu\text{mol m}^{-2} \text{s}^{-1}$	P_{max} was a strong function of nitrate-limited growth rate
Eppley and Renger (1974)	<i>Thalassiosira oceanica</i> (13-1) (oceanic)	25 μM NH_4 + 25 μM NO_3 IMR/2	12h:12h quartz-iodide 225 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated 20°C	Run for many days prior to measurements, some variation noted	1 h ^{14}C -uptake	P_{max} was not clearly a function of nitrate-limited growth rate
Glover (1980)	<i>Phaeodactylum tricornutum</i> (Cambridge)	20 μM NO_3 F-1	Continuous warm-white ~240 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Stirred 20°C	48 h constant cell density	^{14}C uptake over 3 min after resuspension in NO_3 free medium	P_{max} was a strong function of nitrate-limited growth rate, with one higher point at the lowest growth rate
Osborne and Geider (1986)	<i>Phaeodactylum tricornutum</i> (SMBA strain 14)	100 μM NO_3 modified Aquil	Continuous cool-white 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated 23-25°C	Cell count \pm 10% for 3 days	O_2 electrode	P_{max} was a strong function of nitrate-limited growth rate, especially at low growth rates
Kolber <i>et al.</i> (1988)	<i>Thalassiosira pseudonana</i> (3H) (neritic)	75 μM NH_4 F/2	Continuous cool white 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated 18°C	One week	O_2 electrode	P_{max} was much lower in the nitrate-limited culture
Herrig and Falkowski (1989)	<i>Isochrysis galbana</i> (SERI ISOCH1)	150 μM NO_3 F/2	Continuous cool-white 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated 18°C	At least a week, measurements made on three successive days	O_2 electrode	P_{max} remained high at low nitrate-limited growth rates
Chalup and Laws (1990)	<i>Pavlova lutheri</i> (SIO MONO L)	25 μM NO_3 IMR medium	Continuous cool-white, 63 and 189 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated and stirred 22°C	Cell count \pm 5% 2 or more days, measurements repeated 3 days	30 min ^{14}C -uptake	P_{max} was a strong function of nitrate-limited growth rate
Yang, MacIntyre and Cullen (unpubl.)	<i>Thalassiosira pseudonana</i> (3H) (neritic)	50 μM NO_3 modified F/2	12h:12h via-light, 75 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Aerated and stirred 20°C	Several parameters steady for several days - measurements repeated twice	20 min ^{14}C -uptake, 5 times during photocycle	P_{max} remained high at low nitrate-limited growth rates. Possibly some nutrient effect for lower-irradiance series

Falkowski (1989), assimilation number was largely independent of nutrient-limited growth rate in continuous light. The largest discrepancies between experiments are at low dilution rates, when it is difficult to establish steady-state conditions.

We feel that no set of results can be considered "right" or "wrong"; rather, they differ for reasons that have not been identified or explicitly studied. It is curious and unsettling to discover that the laboratory experiments central to our understanding of nitrogen limitation and photosynthetic physiology can have such disparate results, for no obvious reason. A systematic examination of this problem is warranted.

Because P^B can remain high even when growth rates are severely limited by nitrogen supply, we conclude that assimilation number is an unreliable diagnostic of nitrogen limitation (Laws and Bannister, 1980; Herzig and Falkowski, 1989). That is, geographical uniformity of assimilation number cannot be assumed to reflect uniform nutritional status. However, when temperature and light are held constant in laboratory

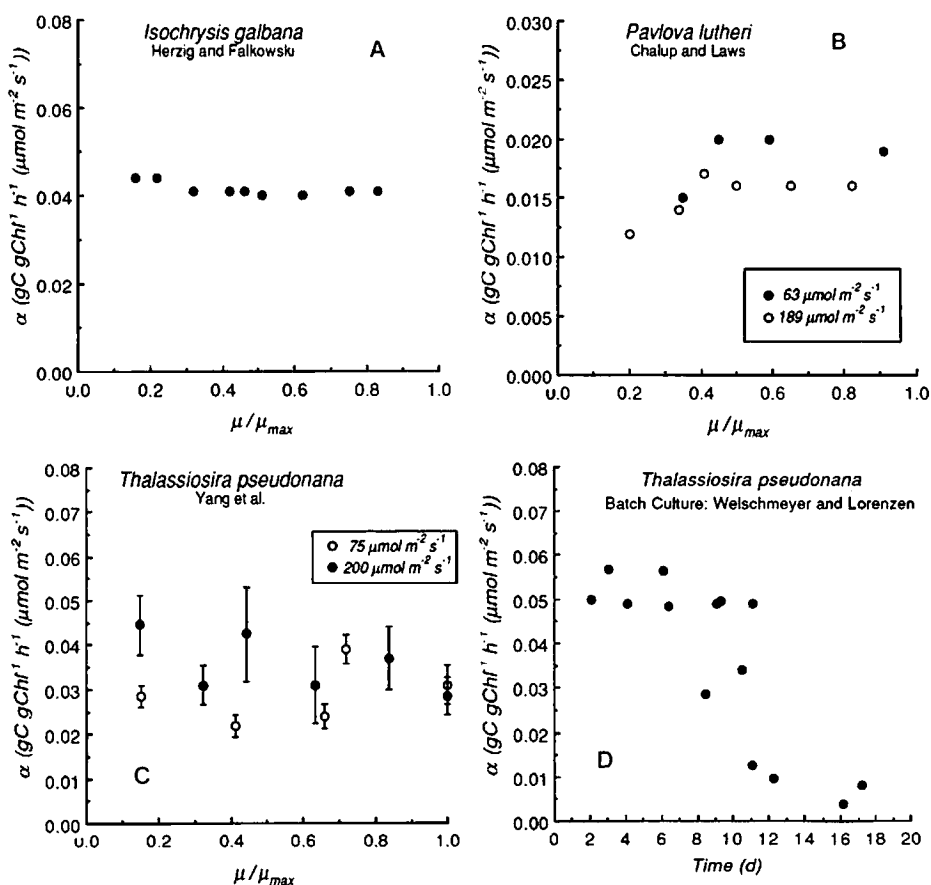


Fig. 4. Effects of nutrition on the initial slope of the P versus I curve, α . Results from continuous cultures described in Table 1: (A) Herzig and Falkowski (1989); (B) Chalup and Laws (1990); and (C) Yang et al. (unpubl.). The error bars represent diel variation of α : they are averages of the standard deviations of replicate series of 5 measurements during the light period. (D) An example from a batch culture (Welschmeyer and Lorenzen, 1981), showing the decline of α upon depletion of nitrate.

studies of cultures, low assimilation numbers implicate nutritional deficiency. Thus, regional differences of assimilation number at similar temperatures and irradiance (Fig. 1) are consistent with nutritional differences, but rigorous demonstration of causality would require more information.

Initial slope, α . The initial slope of the P versus I curve, [α : commonly used units, $\text{g C (g Chl)}^{-1} \text{h}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) $^{-1}$], is the product of the specific absorption coefficient for chlorophyll *a*, a_p ($\text{m}^2 \text{mg Chl}^{-1}$), and the quantum yield for photosynthesis ϕ_{max} [$\text{mol C (mol photons)}^{-1}$] (Falkowski, 1980; Dubinsky, this volume). It is a measure of photosynthetic efficiency, but it is not equivalent to quantum yield. The initial slope is more difficult to measure accurately than is maximum photosynthesis, and its magnitude is sensitive to the spectral quality of the light source and the absorption characteristics of the phytoplankton. Nonetheless, variability in α has been studied systematically in the field and in the laboratory.

Measurements on continuous cultures indicate that α is not very sensitive to nitrogen limitation (Fig. 4A, B, C). This result contrasts with observations on batch cultures subjected to nitrogen starvation (Welschmeyer and Lorenzen, 1981; Fig. 4D), illustrating the fundamental difference between nitrogen limitation in continuous cultures and nitrogen starvation in batch cultures. The relative constancy of α over a broad range of nitrogen-limited growth rates does not mean that photosynthetic efficiency stays high during nutrient limitation. In fact, it has been observed that the quantum yield for photosynthesis is depressed at low nitrogen-limited growth rates; however, this effect on α is more-or-less compensated by a concomitant increase in the specific absorption coefficient as a consequence of reduced chlorophyll per cell (Herzig and Falkowski, 1989; Chalup and Laws, 1990; Dubinsky, this volume). Thus, α is a poor diagnostic of nitrogen limitation, but published results indicate that quantum yield (hence, photosynthetic energy conversion efficiency; see Kolber et al., 1988) is low when nitrogen limits growth rate, whether in balanced or unbalanced growth. The efficiencies of photosynthetic energy

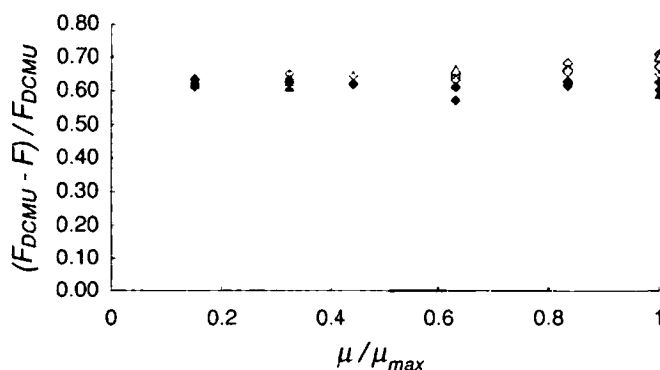


Fig. 5. *Thalassiosira pseudonana* (3H): variation of DCMU-enhanced fluorescence, $(F_{DCMU} - F) / F_{DCMU}$, with nitrate-limited relative growth rate at $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Yang et al., Table 1). Symbols represent the 5 different sampling periods during the light period with filled symbols indicating lights on and lights off.

conversion of natural phytoplankton, measured with a pump-and-probe fluorometer, were strongly related to the supplies of dissolved inorganic nitrogen in the Gulf of Maine (Kolber et al., 1990), leading to the suggestions that rates of photosynthesis were nitrogen-limited and that non-invasive measurements could be used to assess relative growth rates of phytoplankton *in situ*.

An apparently anomalous set of observations complicates our understanding of the relationship between nitrogen limitation and photosynthetic energy conversion. Dark-adapted fluorescence enhancement with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) is a crude measure of the photochemical conversion efficiency of photosystem II (Vincent et al., 1984). Relative enhancement, expressed as $(F_{\text{DCMU}} - F)/F_{\text{DCMU}}$, reliably reflects the degradation of photosynthetic quantum yield during nitrogen starvation (Welschmeyer and Lorenzen, 1981; Cleveland and Perry, 1987). In contrast, the magnitude of this parameter was unaffected by nitrogen limitation in continuous cultures of *Thalassiosira pseudonana* (Fig. 5). It would be very interesting to make the simple measurements of DCMU-enhanced fluorescence concurrent with pump-and-probe fluorometry to find out if culture conditions, species, or the type of measurement are responsible for the discrepant results.

Interpreting Spatial Patterns of Normalized Photosynthesis

The data reviewed here show that the photosynthetic characteristics of phytoplankton, normalized to chlorophyll, can be insensitive to large variations in nutrient-limited specific growth rates of phytoplankton. Yet, in nature, assimilation number can vary substantially in patterns that seem strongly related to nutrient supply (e.g., Curl and Small, 1965; Barber and Chavez, 1991; Fig. 1). How should these patterns be interpreted?

We recommend a cautious approach. It is instructive to compare patterns in assimilation number to inferred patterns in nutrient supply. For example, if there is a strong correlation between inferred nutrient supply and assimilation number, nutrient limitation of primary productivity is suggested, even though nutrient-limited specific growth rates cannot be specified. However, normalized photosynthesis can be insensitive to nutrition, and, as we show in the following section, little or no pattern might be observed over a strong gradient of nutrient supply, so that measurements of photosynthesis do little to resolve questions about the specific growth rates of phytoplankton.

Our review has a practical message: there is no experimental justification for converting measured values of P^b directly into estimated growth rates by assuming a constant C:Chl over an environmental gradient. Simply, P^b is not a reliable proxy for relative growth rate.

PHOTOSYNTHESIS - LIGHT MODELS

Having established that in some circumstances P^b can be nearly independent of nutrient-limited growth rate, we ask if nutrients need be incorporated into models of photosynthesis as a function of chlorophyll and light. The application considered here is remote sensing to estimate primary productivity. Bio-optical models and growth models will not be discussed. The approach is empirical; we will examine P versus I relationships in natural phytoplankton of the northwest Atlantic Ocean to see if inferred differences in nutrient supply influence P^b . If the effects of nutrient supply on P^b are small, the utility of geographical representations of P versus I (Platt et al., 1988) is enhanced.

Photosynthesis versus Irradiance near the Gulf Stream

We measured P versus I on samples from the vicinity of the Gulf Stream, including "green water" (surface chlorophyll $\geq 0.3 \text{ mg m}^{-3}$) and "blue water" (surface chlorophyll $< 0.3 \text{ mg m}^{-3}$). The arbitrary distinction reflects differences in nutrient supply to the euphotic zone associated with vertical mixing and sloping isopycnals across the Gulf Stream (Yentsch, 1974). If nutrient supply strongly influenced P versus I (as it does photosynthetic energy conversion efficiency; Kolber et al., 1990), then P versus I on samples of "green water" would differ from that in samples of "blue water".

Temperature can have a strong influence on seasonal (Harrison and Platt, 1980) and latitudinal (Harrison and Platt, 1986) comparisons of P versus I. However, during this cruise, most of the samples came from water in a narrow temperature range (22°C - 26°C), so temperature was not an important factor.

A composite presentation (Fig. 6A) may look like a jumble, but much of the variability in P versus I is easily explained by invoking light regime as the principal influence. The curves with low maximum rates and pronounced susceptibility to photoinhibition are from deep samples; shallow samples have higher P_{max} and less susceptibility to photoinhibition (cf. Harrison and Platt, 1986). The pattern at a station with near-surface stratification (Fig. 6B) is consistent with photoacclimation, essentially the same as previously described (e.g., Falkowski, 1980; Richardson et al., 1983; Harding

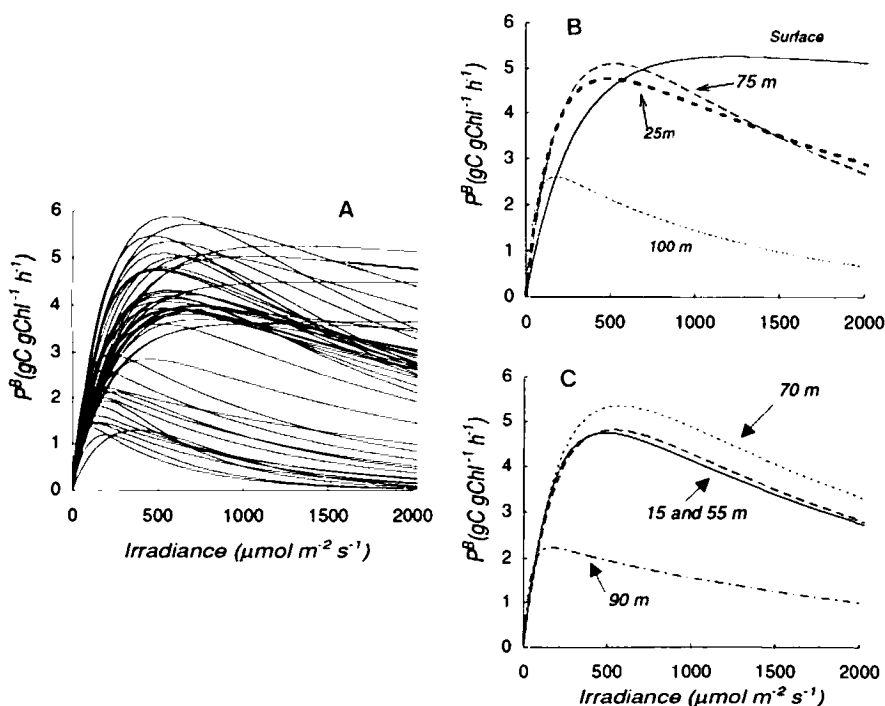


Fig. 6. Photosynthesis versus irradiance in and near the Gulf Stream. Curve-fits of 24-point P versus I curves (Lewis and Smith, 1983) to the model of Platt et al. (1980). A) A composite of all results from "blue water" during the Biosynop cruise, 12-21 October 1988. B) Station 68, where weak stratification permitted photoacclimation of the phytoplankton assemblage. C) Station 71, with an apparently active mixed layer to 75 m.

et al., 1987), whereas P versus I in an actively mixing surface layer (Fig. 6C) is uniform because the rate of vertical mixing exceeds the rate of photoacclimation (Steemann Nielsen and Hansen, 1959; Falkowski, 1983; Lewis et al., 1984; Cullen and Lewis, 1988).

For cultures of phytoplankton, if P^B is little influenced by nutrition, then a plot of P^B against growth irradiance will conform to a saturating function of irradiance (Bannister and Laws, 1980; see Fig. 2 in Cullen, 1990). For samples from the field, we do not know growth irradiance, but we can compare nutrient regimes by plotting P^B as a function of *in situ* irradiance. That is, for each curve, P^B is calculated at the irradiance corresponding to the depth of sampling, estimated from measured extinction coefficients assuming surface irradiance of $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$. The result is gratifying if not remarkable (Fig. 7): P^B is clearly a function of irradiance, with very little scatter around a saturation curve. In fact, the data are consistent with the model of Ryther and Yentsch (1957) as reformulated by Cullen (1990). Rates for "green water" are higher than those for "blue water", but the differences are not great, and the sample size for the more eutrophic stations is small.

Apparently, in this region, nutrition had little influence on P versus I. This finding does not mean that growth rates were the same along the gradient of nutrient supply. Perhaps the more important observation is that a single P versus I relationship could describe the data with good precision. Such good agreement between measurements and chlorophyll-light models are not always obtained, however (Campbell and O'Reilly, 1988; Balch et al., 1989). Besides the possible physiological and ecological explanations for

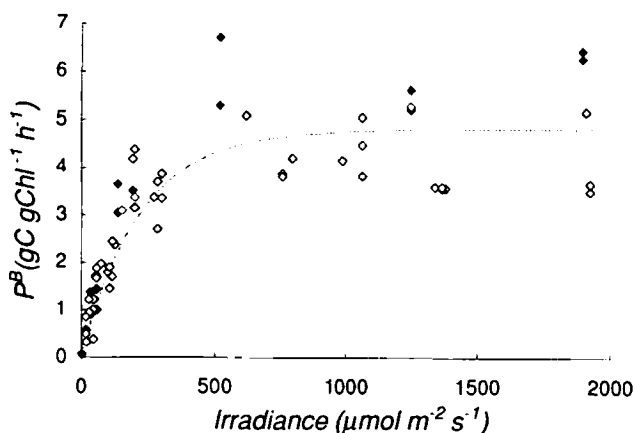


Fig. 7. Calculated P^B at *in situ* irradiance for samples from the Biosynop cruise in and near the Gulf Stream (see text). Closed symbols are from stations with surface chlorophyll $> 0.3 \text{ mg m}^{-3}$, open symbols from stations with surface chlorophyll $< 0.3 \text{ mg m}^{-3}$. The line is the chlorophyll-light model of Ryther and Yentsch (1957) as reformulated by Cullen (1990). Estimates from "green water" tend to be higher than the others, but the effect is small compared to the variability that might be expected from inspection of the individual P versus I curves (Fig. 6). This result suggests that the phytoplankton were adapted to their irradiance regimes and that nutrition had a small influence on P^B at growth irradiance (cf. Cullen 1990).

variable photosynthesis-light relationships for natural phytoplankton (Cullen, 1990), results from conventional *in situ* or simulated *in situ* incubations might be more variable than estimates from short-term P versus I estimates for methodological reasons, such as longer incubation times and fluctuating solar irradiance during conventional incubations.

EXPERIMENTAL ASSESSMENT OF NUTRIENT LIMITATION

Nutrients can control primary productivity in several ways. For example, nutrient availability can regulate rate processes, such as photosynthesis (Blackman, 1905), or the final yield of a plant crop (Liebig limitation). The two types of limitation are conceptually distinct (Browne, 1942), but they are not mutually exclusive in ecological systems. Perhaps that is why the fundamental distinction between the Liebig limitation of yield and Blackman's rate-limiting factor has been blurred in ecological studies (Odum, 1971). Consequently, the term "limitation of phytoplankton growth" has assumed many meanings, including limitation of the specific growth rates of phytoplankton or limitation of standing crop (Cullen, in press). To be effective, hypotheses should specify which type of limitation is acting. Our discussion, so far, has focussed on rate processes, and how certain short-term rate measurements on natural phytoplankton might be influenced by nutrient supply. Now, we discuss how nutrients might control marine photosynthesis by limiting the standing crop of phytoplankton.

The "Iron Hypothesis" and Liebig Limitation of Standing Crop

Recently, it was suggested that iron limits the growth of phytoplankton in large parts of the ocean (Martin, 1990). Enrichment experiments have been used to assess the Liebig-limitation of phytoplankton standing crop by iron (Martin, in press). The results of these experiments, along with supporting measurements of dissolved iron concentrations, a variety of provocative paleoceanographic observations, and the suggestion that fertilization of the Southern Ocean with iron might mitigate the increase of atmospheric CO₂ (Martin, 1990), have generated intense interest and discussion (Chisholm and Morel, in press). The broad issues of the "iron hypothesis" (Martin, 1990, in press; Cullen, in press) are well beyond the scope of this presentation, but the interpretation of enrichment experiments is included as one more example of the ambiguity that can be encountered when trying to assess nutrient limitation in the ocean. Other aspects of nutrient-enrichment experiments are reviewed by Hecky and Kilham (1988).

The design of Martin's enrichment experiments is simple: samples of natural plankton are incubated in bottles and changes in chlorophyll, nitrate and other parameters are measured over several days. Samples enriched with small amounts of iron are compared to unenriched controls, and differences between experimental treatments and controls are attributed to iron. A particularly noteworthy achievement is in executing the experiments free from contamination. The results are consistent: in iron-poor waters, more phytoplankton accumulate in the iron-enriched bottles than in the unenriched controls. Ambient nitrate is depleted in the enriched samples, whereas some residual nitrate persists in the controls. Although the results are reported as growth rates (a contentious approach: cf. Banse, 1990; Martin et al., 1990), Martin interprets the experiments in the context of the Liebig limitation (Martin, 1990), with the fundamental observation being that there is not enough iron in the water to support the accumulation of phytoplankton and assimilation of residual nitrate.

More complete nutrient-enrichment experiments, with factorial design, were performed several times in the past (e.g., Ryther and Guillard, 1959; Menzel et al., 1963;

Thomas, 1969). It is now recognized that these experiments were compromised by contamination (Huntsman and Sunda, 1980), but their design bears examination. The standard of proof for such an experiment is stringent: to demonstrate Liebig-limitation, growth (relative to an unenriched control) must be stimulated by addition of the purported limiting nutrient and that nutrient alone, and growth should be unaffected when the limiting nutrient is omitted from a complete nutrient addition. The technical obstacles to performing such experiments without contamination are formidable, and the nature of oceanic trace-element nutrition (Morel et al., in press; Bruland et al., in press) is such that such a rigorous demonstration is unlikely.

There is another standard for evaluating the results of iron-enrichment experiments. Simply, the results should be consistent with the hypothesis that the availability of iron limits the standing crop of phytoplankton. Strictly interpreted, this means that there must be no increase of standing crop in the unenriched control. After all, if iron limited standing crop *in situ*, no increase in biomass would be observed during incubation of an ideal uncontaminated sample. Suppose that another factor, such as grazing or the balance between light-limited growth and grazing (cf. Banse, 1991), regulated the concentration of phytoplankton at some concentration below that which could be supported by iron. If grazing were diminished in bottles or if light-limited growth rates were increased during on-deck incubations, growth would be observed in controls until the nutrient in shortest supply was depleted. In fact, this is what regularly occurs: controls grow some, until iron is depleted, but iron-enriched samples grow much more (growth was indicated by disappearance of nitrate; Martin, in press). At face value, this result is a falsification of the null hypothesis that available iron limits the standing crop of phytoplankton *in situ*. Experimental artifacts, iron contamination, and second-order effects must be considered in a more rigorous interpretation.

Some control samples seemed not to grow (measurements of Chl: equatorial Pacific results of Martin, in press). It should be noted that the large initial decreases in chlorophyll were very likely the result of light-shock during on-deck incubations at supra-optimal irradiance: uncontaminated samples protected by neutral-density screens (Price et al., in press) showed increases of chlorophyll.

Interpretation of published results. Iron-enrichment experiments described to date show that in large regions of the ocean, iron is in short supply and that, isolated from allochthonous sources, iron would run out before nitrate was depleted. The experiments do not demonstrate that iron limits standing crop *in situ*, however. Also, these results do not exclude the possibility that iron limits the specific growth rates of larger phytoplankton (especially diatoms) and that an enhanced supply of iron would stimulate primary productivity and the utilization of nitrate (Cullen, in press). Simply, the bottle experiments are inconclusive.

Assessing Nitrogen Limitation of Standing Crop with a Factorial Experiment

Nitrogen is much easier to work with than iron, and Liebig-limitation can be examined with a factorial experiment. Results from a class exercise illustrate nitrogen limitation in Texas coastal waters. A sample from the Aransas Pass, Port Aransas, Texas was obtained near midday on an incoming tide. Sampling isolated the phytoplankton from the benthos, where much of the grazing pressure is thought to occur. A series of enrichments was prepared, and changes in chlorophyll *a*, particulate protein, particulate carbohydrate, nitrate, nitrite, ammonium, and phosphate were recorded during a 24-h incubation in 8-l polycarbonate bottles, water-cooled, and covered with neutral-density screen to simulate mid-depth irradiance. Changes of chlorophyll were consistent with the

other measurements (Fig. 8). There was no increase of chlorophyll in the control, which was not surprising because nitrogen is required for net chlorophyll synthesis and dissolved inorganic nitrogen was very low to begin with. In response to a complete nutrient addition (major and minor nutrients, nitrogen added as ammonium, $16 \mu\text{M}$), chlorophyll increased more than threefold over 24 h. Ammonium alone ($16 \mu\text{M}$) stimulated a substantial increase of chlorophyll, but phosphate was depleted within 12 h, and the +N treatment did not stimulate the same increase of biomass as the complete nutrient addition. The nutrient-omission treatment, complete minus N, was indistinguishable from the control, indicating clearly that N limited the standing crop of phytoplankton. It is not simple to relate these results of small-scale experiments to the functioning of the ecological system (Hecky and Kilham, 1988). It would be harder yet if the results were not so clear.

The limitation of standing crop by nitrogen was not necessarily accompanied by severe limitation of specific growth rates. In fact, the rapid increase in the enriched sample, with little lag period, suggests that specific growth rates were quite high. Thus, physiological diagnostics of nitrogen limitation might not reveal the control that nitrogen supply exerts on standing crop, hence primary productivity (Flynn, 1990). Clearly, a variety of approaches should be used when studying nutrient limitation of marine primary productivity.

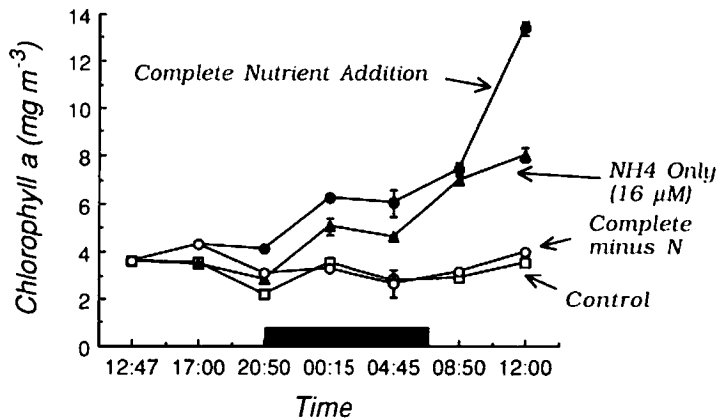


Fig. 8. A factorial nutrient-enrichment experiment, performed as a class exercise using water from the Aransas Pass, Texas (see text for details). Error bars are the range of duplicates. The solid bar indicates darkness.

FROM LABORATORY TO NATURE: PHYSIOLOGICAL VERSUS GENETIC VARIABILITY

We have discussed here studies performed in the laboratory and in nature. When the results of laboratory experiments on individual clones of phytoplankton are used to predict the effects of environmental variability on the photosynthesis, growth, or chemical

composition of phytoplankton in the sea, an implicit assumption is made: that for complex natural assemblages of phytoplankton responding to environmental variability, combined physiological responses (acclimation) and genotypic responses (including species succession and intraspecific changes in genotype frequency) will resemble the physiological responses of a phytoplankton clone in culture. So far, this assumption has served us fairly well. General trends have been described (e.g., Eppley and Renger, 1974; Goldman, 1980; Laws and Bannister, 1980; Kiefer and Mitchell, 1983; Geider, 1987) and different adaptational patterns have been identified (e.g., Richardson et al., 1983; Brand et al., 1983; Sakshaug et al., 1987). For some studies of marine processes, genetic responses need not be considered. In studies of diel variability or short-term physiological responses to vertical mixing, changes of species composition should be unimportant, so one need worry only about how well the physiological responses of cultured phytoplankton represent those in the natural setting. But, if geographical or longer-term temporal patterns are to be predicted or explained, we must recognize that in reaction to environmental change, species succession can dominate the responses of phytoplankton, and even if species persist, physiologically distinct genotypes might exchange dominance (Gallagher, 1982; Wood, 1988). For adaptation to temperature, a genetic component of phytoplankton response has been clearly recognized (Eppley, 1972). With respect to nutrient-limitation of photosynthesis, it seems warranted to examine in detail the possible genetic contributions to relationships between photosynthetic performance and nutrient-limited growth rate.

In a nutrient-limited system in which natural selection has had time to operate, the better competitors survive, and those that are most nutrient-limited become rare. Those rare species would probably exhibit the most pronounced physiological characteristics of nutrient limitation, and they would be likely to respond disproportionately to nutrient enrichment (cf. Martin, in press). Conversely, the dominant species would be least likely to show severe nutrient limitation. It would seem profitable, then, to use single cell methods, such as fluorescence microscopy or flow cytometry, to look for biochemical or physiological diagnostics of nutrient limitation of specific growth rates in the sea.

CONCLUSIONS

1. Models of nutrient limitation are based on laboratory experiments. There is a fundamental distinction between nutrient starvation (batch culture, unbalanced growth) and nutrient limitation (continuous culture, balanced growth over a photocycle), so the results of experiments should be interpreted and models should be generated with clear recognition of the stress imposed, and how it relates to oceanographic processes.

2. It is tempting to interpret geographical variability of P^b as a manifestation of nutrient limitation. However, in continuous culture, P^b can remain high despite nutrient limitation because phytoplankton adapt by regulating cellular chlorophyll concentration. Thus, although low values of P^b may, indeed, reflect nutrient stress, P^b is not a robust diagnostic of nutrient limitation and cannot be related directly to growth rate unless C:Chl is known.

3. Nutrient-limited algal cultures are used routinely to search for new diagnostics of nutritional status. With respect to assimilation number, the responses of phytoplankton cultures to nutrient limitation vary widely, and the source of this variation cannot be resolved. Because we do not know how the sources of variability would influence other potentially useful diagnostic parameters, it is essential to find the reason for the wide range of results.

4. Within some oceanic regions, P^B at ambient irradiance appears to be only weakly influenced by nutrient supply. Thus, models based on a regional P^B versus I relationship should have some utility.

5. The results of bioassays for iron-limitation, when interpreted strictly, reject the hypothesis of iron-limitation of standing crop and are inconclusive with respect to iron limitation of the specific growth rates of phytoplankton.

6. The responses of phytoplankton to environmental variability are often generalized on the basis of physiological responses to experimental conditions, i.e., how individual clones respond in the laboratory. In nature, though, genetic responses are undoubtedly important (natural selection). It may be instructive to question the conventional physiological approach. In the search for diagnostics of nutrient limitation in the sea, work should focus on the physiological characteristics and growth responses of rare species, which would flourish if purported nutrient limitation were alleviated.

ACKNOWLEDGEMENTS

This paper is dedicated to Dick Eppley, on the occasion of his retirement, with personal and professional gratitude. We thank Mary-Elena Carr for comments on the manuscript, A. Michelle Wood for assistance with the section on genetics, and Eric Mills for help with historical aspects of Liebig limitation. Supported by the Office of Naval Research (Oceanic Biology, Remote Sensing), NASA, and an NSERC Canada International Scientific Exchange Award. Bigelow Laboratory Contribution No. 91019.

REFERENCES

- Balch, W. M., Abbott, M. R., and Eppley, R. W., 1989, Remote sensing of primary production - I. A comparison of empirical and semi-analytical algorithms, *Deep-Sea Res.*, 36:281.
- Bannister, T. T., and Laws, E. A., 1980, Modeling phytoplankton carbon metabolism, in: "Primary Productivity in the Sea," P. G. Falkowski, ed., Plenum Press, New York.
- Banse, K., 1990, Does iron really limit phytoplankton production in the offshore subarctic Pacific?, *Limnol. Oceanogr.*, 35:772.
- Banse, K., 1991, Rates of phytoplankton growth, *Limnol. Oceanogr.*, Spec. Symp. Vol.: (in press).
- Barber, R. T., and Chavez, F. P., 1991, Regulation of primary productivity rate in the equatorial Pacific Ocean, *Limnol. Oceanogr.*, Spec. Symp. Vol.: (in press).
- Blackman, F. F., 1905, Optima and limiting factors, *Ann. Bot.*, 19:281.
- Brand, L. E., Sunda, W. G., and Guillard, R. R. L., 1983, Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron, *Limnol. Oceanogr.*, 28:1182.
- Browne, C.A., 1942, Liebig and the law of the minimum, in: "Liebig and after Liebig: A Century of Papers in Agricultural Chemistry," F.R. Moulton, ed., AAAS, Washington, D.C.
- Bruland, K., et. al., 1991, Interactive influences of bioactive trace metals on biological production in the ocean, *Limnol. Oceanogr.*, Spec. Symp. Vol.: (in press).
- Campbell, J. W., and O'Reilly, J. E., 1988, Role of satellites in estimating primary productivity on the northwest Atlantic continental shelf, *Cont. Shelf Res.*, 8:179.

- Chalup, M. S., and Laws, E. A., 1990, A test of the assumptions and predictions of recent microalgal growth models with the marine phytoplankter *Pavlova lutheri*, *Limnol. Oceanogr.*, 35:583.
- Chisholm, S. W., and Morel, F. M. M., 1991, What controls phytoplankton production in nutrient-rich areas of the open sea?, *Limnol. Oceanogr.*, Spec. Symp. Vol.: (in press).
- Cleveland, J. S., and Perry, M. J., 1987, Quantum yield, relative specific absorption and fluorescence in nitrogen-limited *Chaetoceros gracilis*, *Mar. Biol.*, 94:489.
- Cullen, J. J., and Lewis, M. R., 1988, The kinetics of algal photoadaptation in the context of vertical mixing, *J. Plankton Res.*, 10:1039.
- Cullen, J. J., 1990, On models of growth and photosynthesis in phytoplankton, *Deep-Sea Res.*, 37:667.
- Cullen, J. J., 1991, Hypotheses to explain high-nutrient conditions in the open sea, *Limnol. Oceanogr.*, Spec. Symp. Vol.: (in press).
- Cullen, J. J., Lewis, M. R., Davis, C. O., and Barber, R. T., 1991, Photosynthetic characteristics and estimated growth rates indicate grazing is the proximate control of primary production in the equatorial Pacific, *J. Geophys. Res.*, (in press).
- Curl, H., and Small, L. R., 1965, Variations in photosynthetic assimilation ratios in natural phytoplankton communities, *Limnol. Oceanogr.*, 10:R67.
- Dubinsky, Z., this volume.
- Eppley, R. W., 1972, Temperature and phytoplankton growth in the sea, *Fish. Bull.*, 70:1063.
- Eppley, R. W., 1980, Estimating phytoplankton growth rates in the central oligotrophic oceans, in: "Primary Productivity in the Sea," P. G. Falkowski, ed., Plenum Press, New York.
- Eppley, R. W., 1981, Relationship between nutrient assimilation and growth rate in phytoplankton with a brief view of estimates growth rate in the ocean., in: "Physiological Bases of Phytoplankton Ecology," T. Platt, ed., Ottawa.
- Eppley, R. W., and Renger, E. H., 1974, Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture, *J. Phycol.*, 10:15.
- Falkowski, P. G., 1980, Light-shade adaptation in marine phytoplankton, in: "Primary Productivity in the Sea," P. G. Falkowski, ed., Plenum Press, New York.
- Falkowski, P. G., 1981, Light-shade adaptation and assimilation numbers, *J. Plankton Res.*, 3:203.
- Falkowski, P. G., 1983, Light-shade adaptation and vertical mixing of marine phytoplankton: A comparative field study, *J. Mar. Res.*, 41:215.
- Falkowski, P. G., 1991, Molecular ecology of phytoplankton photosynthesis, in: "Primary Productivity and biogeochemical Cycles in the Sea," P.G. Falkowski and A. Woodhead, eds., Plenum, New York.
- Flynn, K. J., 1990, The determination of nitrogen status in microalgae, *Mar. Ecol. Prog. Ser.*, 61:297.
- Gallagher, J. C., 1982, Physiological variation and electrophoretic banding patterns of genetically different seasonal populations of *Skeletonema costatum* (Bacillariophyceae), *J. Phycol.*, 18:148.
- Geider, R. J., 1987, Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton, *New Phytol.*, 106:1.
- Glover, H. E., 1980, Assimilation numbers in cultures of marine phytoplankton, *J. Plankton Res.*, 2:69.
- Goldman, J. C., 1980, Physiological processes, nutrient availability, and concept of relative growth rate in marine phytoplankton ecology., in: "Primary Productivity in the Sea," P. G. Falkowski, ed., Plenum Press, New York.

- Harding, L. W. J., Fisher, T. R. J., and Tyler, M. A., 1987, Adaptive responses of photosynthesis in phytoplankton: specificity to time-scale of change in light, *Biol. Oceanogr.*, 4:403.
- Harrison, W. G., and Platt, T., 1980, Variations in assimilation number of coastal marine phytoplankton: Effects of environmental covariates, *J. Plankton Res.*, 2:249.
- Harrison, W. G., and Platt, T., 1986, Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations, *Polar Biol.*, 5:153.
- Hecky, R. E., and Kilham, P., 1988, Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment, *Limnol. Oceanogr.*, 33:796.
- Herzig, R., and Falkowski, P. G., 1989, Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). I. Photosynthetic energy conversion and growth efficiencies, *J. Phycol.*, 25:462.
- Horrigan, S. G., and McCarthy, J. J., 1981, Urea uptake by phytoplankton at various stages of nutrient depletion. *J. Plankton Res.*, 3:403.
- Huntsman, S. A., and Sunda, W. G., 1980, The role of trace metals in regulating phytoplankton growth, in: "The Physiological Ecology of Pphytoplankton," I. Morris, ed., University of California, Berkeley.
- Kiefer, D. A., and Mitchell, D. G., 1983, A simple, steady state description of phytoplankton growth based on absorption cross section and quantum efficiency, *Limnol. Oceanogr.*, 28:770.
- Kolber, Z., Zehr, J. R., and Falkowski, P. G., 1988, Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II, *Plant Physiol.*, 88:923.
- Kolber, Z., Wyman, K. D., and Falkowski, P. G., 1990, Natural variability in photosynthetic energy conversion efficiency: A field study in the Gulf of Maine, *Limnol. Oceanogr.*, 35:72.
- Laws, E. A., and Bannister, T. T., 1980, Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean, *Limnol. Oceanogr.*, 25:457.
- Lewis, M. R., and Smith, J. C., 1983, A small volume, short-incubation-time method for measurement of photosynthesis as a function of incident irradiance, *Mar. Ecol. Prog. Ser.*, 13:99.
- Lewis, M. R., Cullen, J. J., and Platt, T., 1984, Relationships between vertical mixing and photoadaptation of phytoplankton: Similarity criteria, *Mar. Ecol. Prog. Ser.*, 15:141.
- Malone, T. C., and Neale, P. J., 1981, Parameters of light-dependent photosynthesis for phytoplankton size fractions in temperate estuarine and coastal environments, *Mar. Biol.*, 61:289.
- Martin, J. H., 1990, Glacial-interglacial CO₂ change: The iron hypothesis, *Paleoceanography*, 5:1.
- Martin, J. H., this volume.
- Martin, J. H., Broenkow, W. W., Fitzwater, S. E., and Gordon, R. M., 1990, Yes it does: A reply to the comment by Banse, *Limnol. Oceanogr.*, 35:775.
- Martin, J. H., Gordon, R. M., Fitzwater, S., and Broenkow, W. W., 1989, VERTEX: phytoplankton/iron studies in the Gulf of Alaska, *Deep-Sea Res.*, 36:649.
- Menzel, D. W., Hulbert, E. M., and Ryther, J. H., 1963, The effects of enriching Sargasso Sea water on the production and species composition of the phytoplankton. *Deep-Sea Res.*, 10:209.
- Morel, F. M. M., Hudson, R. J. M., and Price, N. M., 1991, Trace metal limitation in the sea, *Limnol. Oceanogr.*, Spec. Symp. Vol. (in press).
- Odum, E. P., 1971, "Fundamentals of Ecology," W.B. Saunders Co., Philadelphia.

- Osborne, B. A. and Geider, R. J., 1986, Effect of nitrate-nitrogen limitation on photosynthesis of the diatom *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae), *Plant Cell Environ.*, 9:617.
- Platt, T., Gallegos, C. L., and Harrison, W. G., 1980, Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton, *J. Mar. Res.*, 38:687.
- Platt, T., and Jassby, A. D., 1976, The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton, *J. Phycol.*, 12:421.
- Platt, T., Sathyendrenath, S., Caverhill, C. M., and Lewis, M. R., 1988, Oceanic primary production and available light: Further algorithms for remote sensing, *Deep-Sea Res.*, 35:855.
- Price, N. M., Andersen, L. F., and Morel, F. M. M., 1991, Iron and nitrogen nutrition of equatorial Pacific plankton, *Deep-Sea Res.*, (in press).
- Redalje, D. G., and Laws, E. A., 1981, A new method for estimating phytoplankton growth rates and carbon biomass, *Mar. Biol.*, 62:73.
- Richardson, K., Beardall, J., and Raven, J. A., 1983, Adaptation of unicellular algae to irradiance: an analysis of strategies, *New Phytol.*, 93:157.
- Ryther, J. H., and Guillard, R. R. L., 1959, Enrichment experiments as a means of studying nutrients limiting to phytoplankton populations, *Deep-Sea Res.*, 6:65.
- Ryther, J. H., and Yentsch, C. S., 1957, The estimation of phytoplankton production in the ocean from chlorophyll and light data, *Limnol. Oceanogr.*, 2:281.
- Sakshaug, E., Demers, S., and Yentsch, C. M., 1987, *Thalassiosira oceanica* and *T. pseudonana*: Two different photoadaptational responses, *Mar. Ecol. Prog. Ser.*, 41:275.
- Sakshaug, E., and Holm-Hansen, O., 1977, Chemical composition of *Skeletonema costatum* (Grev.) Cleve and *Pavlova (Monochrysis) lutheri* (Droop) Green as a function of nitrate-, phosphate-, and iron-limited growth., *J. Exp. Mar. Biol. Ecol.*, 29:1.
- Sakshaug, E., Kiefer, D. A., and Andresen, K., 1989, A steady state description of growth and light absorption in the marine planktonic diatom *Skeletonema costatum*, *Limnol. Oceanogr.*, 34:198.
- Shuter, B., 1979, A model of physiological adaptation in unicellular algae, *J. Theor. Biol.*, 78:519.
- Stemann Nielsen, E., and Hansen, V. K., 1959, Light adaptation in marine phytoplankton populations and its interrelation with temperature, *Physiol. Plant.*, 12:353.
- Strickland, J. D. H., Holm-Hansen, O., Eppley, R. W., and Linn, R. J., 1969, The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels., *Limnol. Oceanogr.*, 14:23.
- Thomas, W. H., 1969, Phytoplankton nutrient enrichment experiments off Baja California and in the eastern equatorial Pacific Ocean, *J. Fish. Res. Board Can.*, 26:1133.
- Thomas, W. H., and Dodson, A. N., 1972, On nitrogen deficiency in tropical Pacific oceanic phytoplankton. II. Photosynthetic and cellular characteristics of a chemostat-grown diatom, *Limnol. Oceanogr.*, 17:515.
- Vincent, W. F., Neale, P. J., and Richerson, P. J., 1984, Photoinhibition : algal responses to bright light during diel stratification and mixing in a tropical alpine lake, *J. Phycol.*, 20:201.
- Welschmeyer, N. A., and Lorenzen, C. J., 1981, Chlorophyll-specific photosynthesis and quantum efficiency at subsaturating light intensities, *J. Phycol.*, 17:283.
- Wood, A. M., 1988, Molecular Biology, single cell analysis, and quantitative genetics: new evolutionary genetic approaches in phytoplankton ecology, in: "Immunochemical approaches to coastal, estuarine, and oceanographic questions," C.M. Yentsch, F.C. Mague, and P.K. Horan, eds., Springer-Verlag.

- Yentsch, C. M., Yentsch, C. S., and Strube, L. R., 1977, Variations in ammonium enhancement, and indication of nitrogen deficiency in New England coastal phytoplankton populations, *J. Mar. Res.*, 35:537.
- Yentsch, C. S., 1974, The influence of geostrophy on primary production, *Tethys*, 6:111.
- Yentsch, C. S., Yentsch, C. M., Strube, L. R., and Morris, I., 1974, The influence of temperature on the efficiency of photosynthesis in natural populations of marine phytoplankton, *in*: "Thermal Ecology," J. W. Gibbons and R. R. Sharitz, eds., AEC, Oak Ridge, Tenn.