

Hypotheses to explain high-nutrient conditions in the open sea

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Abstract

Oceanic high-nutrient, low-chlorophyll waters are characterized principally by the persistence of major nutrients at the sea surface. This condition indicates control of autotrophic production by something other than NO_3 or PO_4 , but the nature of this control is at present unresolved. The range of hypotheses to explain the high-nutrient condition is illustrated by the grazing hypothesis (specific growth rates of phytoplankton are maximal and environmental stability allows development of a balanced food web that maintains low standing crops of phytoplankton) and the iron hypothesis (standing crop of plankton is constrained by availability of Fe: if more Fe were available, the standing crop of phytoplankton would increase and NO_3 would be depleted, despite grazing).

The iron hypothesis has been examined experimentally in the subarctic and equatorial Pacific and in Antarctic waters. In each environment, Fe enrichment enhanced the final yield of phytoplankton biomass after incubations of many days. Interpretation of these experiments is contentious because containment in bottles is unnatural. Nonetheless, recent studies in the laboratory and in the field indicate that Fe and possibly other trace elements exert selective pressures on oceanic phytoplankton and that enrichment of high-nutrient waters with Fe would change the species composition of phytoplankton and food-web interactions, thereby enhancing utilization of NO_3 . The magnitude of this enhancement cannot be predicted with confidence.

Results from the central equatorial Pacific indicate that the specific growth rates of phytoplankton are adequate to overcome physical forcing and to deplete ambient NO_3 in the euphotic zone. It is suggested that grazing controls the populations of the dominant, small cells. However, the supply of Fe might ultimately regulate nutrient utilization by limiting the specific growth rates of larger cells that might otherwise escape grazing control and bloom. Observations from the subarctic Pacific are consistent with this view, but the regulation of phytoplankton growth and nutrient utilization might not be the same in cold, physically perturbed Antarctic waters.

The depletion of major nutrients (NO_3 and PO_4) near the sea surface is the natural consequence of the growth of phytoplankton. It follows that these nutrients persist in the upper layer of the ocean only when autotrophic development is somehow retarded (Minas et al. 1986). Thus, the presence of excess nutrients at the surface connotes reg-

ulation of autotrophic processes by something other than NO_3 or PO_4 .

The causes of excess nutrients are sometimes readily ascribed. During deep winter mixing, mean irradiance in the mixed layer is low. Entrained nutrients are not exploited because photosynthetic production does not exceed community respiration. Under these conditions light limits primary productivity and the utilization of nutrients. However, other factors, such as temperature and the trophic composition of the plankton, influence the quantitative relationship between net production and irradiance and thus the timing of the spring bloom (Sverdrup 1953; Smetacek and Passow 1990).

Coastal upwelling can establish a succession from freshly upwelled, high-nutrient, low-chlorophyll (HNLC) water nearshore to high-chlorophyll, low-nutrient (HCLN) water offshore (Minas et al. 1986). Upwelling is the principal cause of excess nutrients near the surface, but the spatial pattern of nutrients, phytoplankton, and primary productivity depends on a complex interaction of

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physiological adaptation, growth, grazing, mixing, and horizontal advection (Jones et al. 1988; Dugdale and Wilkerson 1989). These interactions are sometimes inscrutable: in the coastal upwelling off Peru, Strickland et al. (1969) found an isolated patch of HCLN "brown" water surrounded by HNLC "blue" water, with boundaries sharp enough to be easily visible. They recognized that the blue waters with unexploited nutrients were anomalous and they examined several hypotheses to explain why phytoplankton populations did not reach bloom proportions, concluding that control by grazing was likely. During the following discussion it will be useful to remember that Strickland and colleagues found the HNLC condition in coastal waters where Fe is thought not to limit biological processes (Martin 1990 as pointed out by Minas and Minas in prep.).

Here we are concerned with the open ocean, particularly three large regions where major nutrients persist in the surface layer throughout the year: the subarctic Pacific, the equatorial Pacific, and the Southern Ocean. We seek explanations for why autotrophic processes fail to exploit NO_3 and PO_4 . These explanations are needed to describe the regulation of primary productivity in large parts of the ocean and thus they are essential to models of marine biogeochemical cycling in the context of global change. For example, if it is found that eolian flux of Fe to the ocean regulates nutrient utilization (and therefore influences the oceanic sink for CO_2 ; cf. Martin 1990), then models of past and future climate change must be fundamentally altered.

What is the high-nutrient condition?

Something seems wrong with open-ocean HNLC waters: phytoplankton and excess nutrients coexist indefinitely in well-lit surface waters. The condition is of great interest to those studying biogeochemical cycling in the ocean because we do not know why "primary productivity and chlorophyll levels appear to be lower than expected for the high ambient nutrient levels" (U.S. JGOFS Steering Comm. 1990, p. A-I-18). Implicit in this statement is the assumption that chlorophyll and primary productivity

should be positively correlated with nutrient concentration. Actually, although large-scale patterns of marine primary productivity clearly reflect the input of nutrients (Yentsch 1974, 1980; Reid et al. 1978; Berger 1989), the expected relationship between chlorophyll and nutrient concentration changes with time so that it is impossible to predict chlorophyll or productivity on the basis of nutrient concentration alone (Fig. 1).

It is more accurate to state that in HNLC waters, chlorophyll levels and primary productivity are lower than expected if all inorganic N had been assimilated (Thomas 1979). The implicit expectation here is that nutrients should be depleted at the surface and something prevents that from happening. Both McAllister et al. (1960) and Strickland et al. (1969) recognized that nutrients persisted because autotrophic processes were somehow kept in check. Thus, it is the persistence rather than the presence of unused major nutrients that defines the HNLC condition as an oceanographic problem.

Walsh (1976) inferred the persistence of major nutrients by comparing horizontal gradients of nutrient concentration: they were sharp in coastal waters, whereas in oceanic divergences, the gradients were very weak. Thomas (1979) suggested persistence by pointing out that water at 8° – 12°S was advected thousands of kilometers from the Peru Current yet nutrients were not depleted by phytoplankton. Minas et al. (1986) determined the temporal component quantitatively by calculating from temperature and water mass characteristics the age of upwelled water at the surface (cf. Broenkow 1965). At the Costa Rica Dome, nutrients were still high in surface waters even though the calculated age was 83 d. Clearly, utilization of major nutrients was restricted. One objective of this symposium is to describe the nature of this control.

What controls phytoplankton production in nutrient-rich areas of the open sea?

The utilization of NO_3 is a consequence of the growth of phytoplankton, and the growth of phytoplankton is primary production, so it is natural to equate the failure of phytoplankton to exploit major nutrients with the limitation of phytoplankton growth

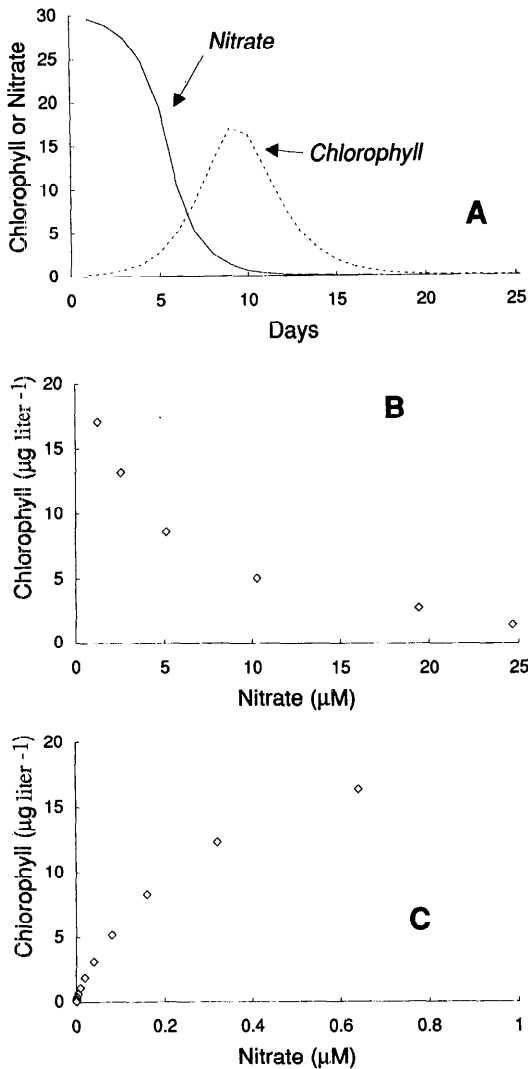


Fig. 1. The relationship between ambient NO_3 and phytoplankton biomass (chlorophyll) from a generic model of a phytoplankton bloom terminated by NO_3 depletion and grazing. A. NO_3 ($\mu\text{g-atoms liter}^{-1}$) and chlorophyll ($\mu\text{g liter}^{-1}$) during the course of the bloom. It is assumed that the assimilation of $1 \mu\text{g-atom liter}^{-1}$ NO_3 yields $1 \mu\text{g liter}^{-1}$ chlorophyll and that 50% of grazed phytoplankton nitrogen is regenerated. These assumptions are not critical to the principal observation, which is that NO_3 and chlorophyll are inversely related during the initial phase of the bloom (B) and positively related during the decline of the bloom (C). It is therefore quite difficult to make a prediction of chlorophyll concentration solely on the basis of ambient NO_3 concentration.

or primary productivity (e.g. Martin 1990). One should nonetheless be cautious when using terms such as limitation, growth, or primary production in hypotheses to explain the HNLC condition (Table 1): these terms are chameleonic and their use can generate controversies (e.g. Banse 1990; Martin et al. 1990) that hinge as much on terminology as on interpretation of results. It follows that the title of this symposium, "What controls phytoplankton production in nutrient-rich areas of the open sea?" could be an invitation to ambiguity. When constructing or discussing hypotheses, precision is essential. For example, a factor that regulates the rate of nutrient regeneration in the euphotic zone might have a strong influence on net primary productivity, independent of changes in the rate of new production.

Even if the central question of this symposium were carefully constrained with specific definitions, it would have no simple answer. Quotes from two important contributors to the debate illustrate why. Hart (1934) described the distributions of phytoplankton in different regions of the Southern Ocean and discussed extensively the factors that might influence primary production. He presented an impressive list of factors, including stability of the water column, light, nutrients, the interaction of ice formation and water circulation with the life cycles of diatoms, and even the effects of UV radiation on photosynthesis. Fe was identified as one possible factor, and that insight has been recently recognized (Martin et al. 1989; de Baar et al. 1990). In his summary, however, Hart stated

It cannot be too strongly emphasized that in all probability phytoplankton production is always governed by a complex of inter-dependent factors, rather than by one or two which are clearly definable. [P. 193]

De Baar et al. echoed this sentiment.

Walsh (1976) also discussed many of the factors that might regulate the utilization of nutrients in the sea. His caveat merits repeating in the context of this discussion:

simple consideration of single control factors of marine primary production such as light, nutri-

Table 1. Different meanings associated with general terms. Because these general terms can be interpreted in many different ways, it is essential to be explicit and precise when discussing the factors that might influence primary production or the growth of phytoplankton.

General term	Specific terms	Comments (references*)
Primary production	Gross primary production	Important for understanding light limitation (1)
	Net primary production	Net rate of synthesis of the organic constituents of plant material in water (2)
	New production	Net accumulation plus export (3)
	Net small particle production	Measured in bottle incubations (4, 5)
	Net community production	Equivalent to new production (4, 6)
Growth	Standing crop of phytoplankton	Net result of phytoplankton growth; definitions of biomass differ (7)
	Potential standing crop	Terminal yield of bioassays (8)
	Specific growth rate of phytoplankton	Omits mortality and dispersal (9)
	Net growth rate of phytoplankton	Includes mortality and dispersal (10)
	Standing crop of plankton or net growth rate of plankton	Includes bacteria and grazers (4)
Control of primary production or control of phytoplankton standing crop†	Direct limitation of phytoplankton specific growth rate	Blackman concept (11, 12)
	Limitation of primary standing crop	Liebig-type (3) or a complex response (12)
	Colimitation of rate process	e.g. N ₂ and N (13)
	Proximate control	Direct regulation (14)
	Ultimate control	Indirect action through links in the ecosystem (12, 14)

* 1—Smetacek and Passow 1990; 2—Strickland 1965; 3—Dugdale and Goering 1967; 4—Platt et al. 1984; 5—Siegel et al. 1989; 6—Minas et al. 1986; 7—Cullen 1982; 8—Martin and Fitzwater 1988; de Baar et al. 1990; Banse 1990; 9—Lande et al. 1989; 10—Hecky and Kilham 1988; Banse 1991b; 11—Blackman 1905; 12—Thingstad and Sakshaug 1990; 13—Morel et al. 1991; 14—Cullen et al. 1992.

† Thingstad and Sakshaug 1990.

ents, mixing, chelation, or grazing can lead to rather naive and perhaps inaccurate conceptualizations of the dynamics of nonlinear aquatic ecosystems. [P. 1]

It is important to bear these comments in mind when comparing alternate hypotheses. Although one factor might have a profound influence on primary productivity and nutrient utilization, the interaction of many factors determines how the system works. The words of T. C. Chamberlin (1965, p. 756) provide useful guidance.

We are so prone to attribute a phenomenon to a single cause, that, when we find an agency present, we are liable to rest satisfied therewith, and fail to recognize that it is but one factor, and perchance a minor factor, in the accomplishment of the total result.

Chamberlin proposed a method of multiple working hypotheses that seems especially appropriate for studying the control of primary productivity in the open ocean.

Hypotheses to explain the high-nutrient condition

New production (Dugdale and Goering 1967; Eppley and Peterson 1979; *see also* Legendre and Gosselin 1989) has two components: accumulation of organic matter in the euphotic zone and export of organic matter from the euphotic zone. As defined here, new production is the same as net community production (NCP: Minas et al. 1986; Minas and Minas in prep.). New production consumes nutrients in the surface layer. Thus, the rate of new production or NCP is much more relevant to the high-nutrient condition than net primary production (Table 1). We want to know what keeps new production low. The reasons may differ in different regions.

Several factors influence new production: the biomass of phytoplankton, their specific growth rates, losses to dilution and sinking, grazing, and the proportion of grazed material that is exported. The influence of these

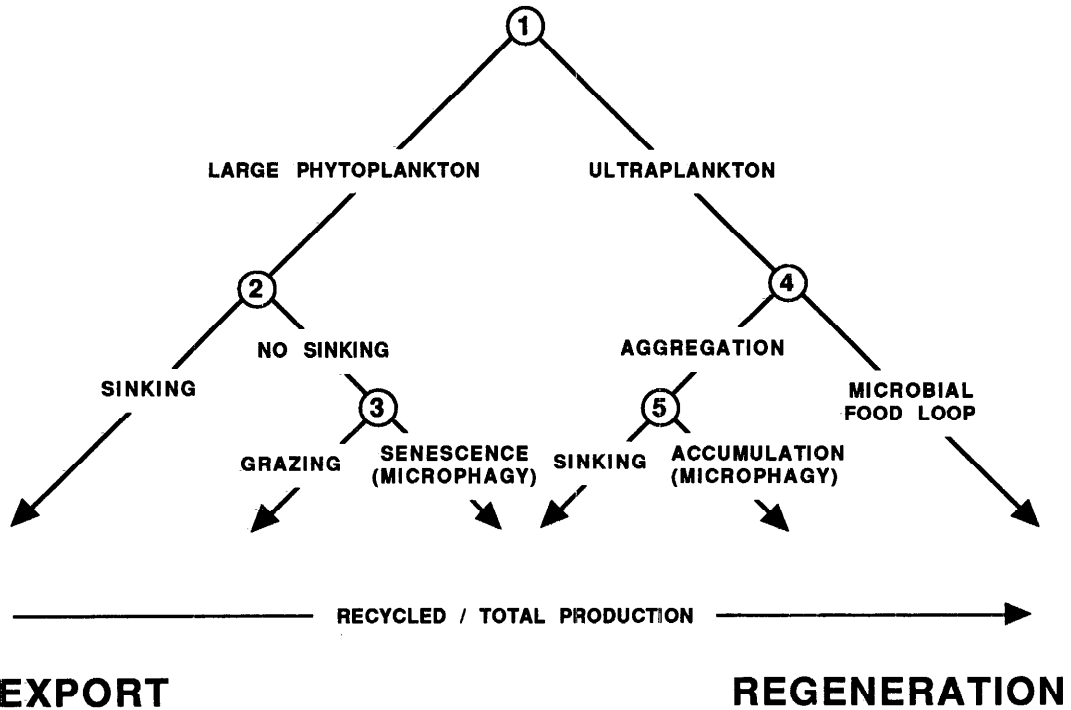


Fig. 2. Relationships between food-web structure and regenerated vs. new production. This diagram is modified from Legendre and Le Fèvre (1989) at branches 3 and 5. They call the branch points hydrodynamic singularities. These branches illustrate the consequences of different paths in the flow of nitrogen or organic material. Branches to the left indicate a higher probability of export. The characteristics of HNLC environments are consistent with branches to the right (regenerated production). (See also Michaels and Silver 1988.)

factors has been conceptually integrated by Legendre and Le Fèvre (1989; *see also* Michaels and Silver 1988). In the three HNLC environments discussed here, most of these factors tend to minimize new production (Fig. 2). The biomass of phytoplankton is low but dilution does not seem to be the principal cause; persistent stratification and small horizontal gradients minimize losses to mixing in the subarctic Pacific (Miller et al. 1988; Gargett 1991) and the equatorial Pacific (Carr et al. 1991; Cullen et al. 1992), although in the Southern Ocean, vertical mixing can influence the concentration and growth rates of phytoplankton (Mitchell et al. 1991). Phytoplankton are generally small (subarctic Pacific: McAllister et al. 1960; Miller et al. 1988; equatorial Pacific: Chavez 1989; Peña et al. 1990; Southern Ocean: Weber and El-Sayed 1987), so losses to sinking could not significantly reduce the net growth rates of the dominant phytoplankton (Bienfang 1985). Grazing has a

strong effect on phytoplankton standing crop, as exemplified in the relative constancy of chlorophyll despite active autotrophy, at least in the subarctic Pacific (McAllister et al. 1960; Miller et al. 1988; Frost 1991) and the equatorial Pacific (Walsh 1976; Cullen et al. 1992). When the small phytoplankton in oceanic HNLC waters are consumed, they enter the microbial loop and little of the grazed material is transported to the deep sea (Michaels and Silver 1988; Miller et al. 1988; Goldman 1988; Legendre and Le Fèvre 1989; Fig. 2). Regenerated production (NH_4 assimilation as opposed to NO_3 assimilation; Wheeler and Kokkinakis 1990) dominates in such systems.

Thus, new production is low in oceanic HNLC environments because phytoplankton biomass is low, the net growth rate of phytoplankton (cell division minus grazing, cf. Banse 1991a,b) approaches zero, and production is more likely regenerated than exported. The reason for the low net growth

rate is unresolved, and it forms the basis of the high-nutrient controversy: is phytoplankton standing crop relatively constant due principally to low specific growth rates of phytoplankton or due to effective control by herbivores?

Hypotheses invoking low specific growth rates of phytoplankton or impaired nitrogen assimilation

Minor-nutrient limitation—Various hypotheses have been presented to explain low specific growth rates or low specific rates of NO_3 assimilation in HNLC phytoplankton. Several of them involve minor nutrients. Vitamins have been mentioned (Carlucci and Cuhel 1977; Thomas 1979), but trace elements have been more prominent. As mentioned above, Hart (1934) identified Fe as one of many possible limiting factors in Antarctic waters. Barber and Ryther (1969) were more emphatic: they found a local minimum in productivity index [$\text{g C}(\text{g Chl})^{-1} \text{h}^{-1}$] in the eastern equatorial Pacific and suggested that specific growth rates of phytoplankton were lower there because of physiological impairment associated with upwelled waters that were low in natural chelators. These chelators facilitate trace element nutrition and protect the phytoplankton from trace element toxicity (Huntsman and Sunda 1980). Thomas (1969, 1972, 1979) identified minor nutrients as possible limiting factors in the eastern tropical Pacific.

Subsequently it has been recognized that early tests of these hypotheses (discussed below) were compromised by contamination and trace-metal interactions resulting from unrealistically high additions (Huntsman and Sunda 1980). The ideas persisted, however, and recent studies with cleaner techniques and more realistic enrichments (de Baar et al. 1990; Bruland et al. 1991; Buma et al. 1991; Coale 1991; Martin et al. 1991; Price et al. 1991) have given new life to established, but unresolved hypotheses. Martin has taken the role as the champion of the "iron hypothesis," and the work of his group has stimulated the interest that generated this symposium.

Shift-up of NO_3 assimilation—Dugdale and Wilkerson (1989, 1991) postulated that

phytoplankton in HNLC waters were physiologically impaired, but the cause was not related to minor nutrients. They suggested that phytoplankton in upwelling systems such as the equatorial Pacific fail to exploit supplies of NO_3 because initial concentrations of NO_3 are too low to support a rapid shift-up of assimilatory pathways. The resulting low rates of new production are therefore the result of physical forcing (i.e. rate of upwelling coupled with NO_3 concentration in the source water). Changes in these conditions would alter the development of phytoplankton populations by changing their specific growth rates.

Recently, Garside (1991) has challenged the shift-up concept, attributing it to a weakness of the ^{15}N -tracer method of measuring the specific rate of nitrogen assimilation: nitrogen assimilation is normalized to particulate N rather than to phytoplankton N. Because the contribution of phytoplankton to particulate N increases during bloom development (Eppeley et al. 1977), an apparent acceleration of normalized uptake rate can be observed even if the kinetics of cellular uptake remain unchanged. This artifact could be termed "algebraic shift-up."

Effects of low temperature—In the Southern Ocean, inefficient utilization of major nutrients, low photosynthetic rates, and slow growth of phytoplankton may be the consequence of low temperatures (Tilzer et al. 1986; Dugdale and Wilkerson 1989). For example, Jacques (1983) and Sommer (1986) have reported extremely high half-saturation constants for the uptake of SiO_4 and NO_3 at 0°C , indicating that the fairly high concentrations of macronutrients in Antarctic waters might nonetheless be low enough to regulate the growth rates of some phytoplankton. Later, Sommer (1991) found evidence in the distributions, growth responses, and cell quotas of Antarctic diatoms that the ambient concentrations of major nutrients influenced competitive interactions, even though those concentrations would be saturating in temperate waters. There are at least two implications of low-temperature effects: phytoplankton in the high-nutrient Southern Ocean might be growing so slowly that they cannot consume ambient nutrients, regardless of grazing; and uptake capacity may not be saturated, so

that the concentrations of some major nutrients might actually limit the specific growth rates of some phytoplankton.

Taxonomic considerations—A different explanation for the apparent impairment of NO_3 assimilation in oceanic high-nutrient waters is that the neritic bloom-forming diatoms which characterize coastal upwelling systems (Smetacek 1985) are absent from the offshore regions, possibly because of inadequate seed stocks (equatorial Pacific: Chavez 1989) or because they are poor competitors for Fe (cf. Martin et al. 1989; Banse 1991a,b; Chavez et al. 1991; Coale 1991; Cullen et al. 1992). Unlike those diatoms, the small, competitive oceanic phytoplankters (cf. Brand et al. 1983; Sunda et al. 1991) that dominate in HNLC waters may be inherently incapable of rapid assimilation of NO_3 , which would not be surprising considering that some species have low quotas for Fe (Sunda et al. 1991), an element that is required for NO_3 assimilation (Morel et al. 1991). In this context, relatively high Fe quotas for picoplanktonic cyanobacteria (Brand 1991) are difficult to reconcile with their abundance in high-nutrient, low-Fe oceanic environments (Booth 1988; Chavez et al. 1990).

The grazing hypothesis: Insensitive to the growth rates of phytoplankton

Walsh (1976) disputed the suggestion that the growth rates of phytoplankton were depressed in oceanic upwelling systems and countered with his hypothesis that herbivory regulated the standing crops of phytoplankton in oceanic HNLC environments. He felt that the critical factor distinguishing coastal systems from oceanic ones was physical disruption of the planktonic system on the 5–10-d time scale—a temporal scale critical to the development of phytoplankton: herbivore (e.g. diatom: copepod) interactions. In coastal waters, events such as storms disrupted herbivore control whereas in oceanic waters herbivore control could develop and persist because water motions were less variable on the critical scale. Grazing control has been identified by others, before and after Walsh (McAllister et al. 1960; Strickland et al. 1969; Minas et al. 1986; Miller et al. 1988; Wheeler and

Kokkinakis 1990; Banse 1991a,b; Frost 1991; Miller et al. 1991).

The protozoan consumers of small phytoplankton are capable of extremely fast specific growth rates, exceeding those of their prey and allowing rapid development of tight coupling between herbivores and small phytoplankton (Banse 1982; Goldman et al. 1985). Thus, Walsh's physical-disruption hypothesis can apply only to larger phytoplankton and slower-to-respond metazoan herbivores. The hypothesis is still relevant to the high-nutrient condition, because the growth of large phytoplankton is very important to new production (Goldman 1988; Michaels and Silver 1988; Legendre and Le Fèvre 1989; Fig. 2) and the factors that keep larger phytoplankton in check may be the principal control of nutrient utilization (cf. Barber and Chavez 1991; Chavez et al. 1991). Consistent with this view, Strickland et al. (1969) found that the presence of large diatoms distinguished a nutrient-depleted brown patch from the surrounding nutrient-rich blue water.

Identifying the high-nutrient controversy

Although the high-nutrient condition is determined by ecological interactions of great complexity, one fundamental question is simple: Do nutrients persist because Fe is in short supply? The opposite poles of this unresolved question are exemplified by the iron hypothesis (e.g. Martin and Fitzwater 1988; Martin et al. 1989; Martin 1990) and the grazing hypothesis (Walsh 1976). Basically, they correspond to bottom-up vs. top-down control of nutrient utilization (cf. Lehman 1991).

The iron hypothesis maintains that the standing crop of phytoplankton is low and the net growth rate is near zero because there is no Fe available to support an increase in planktonic biomass: if grazing pressure were relaxed, the standing crop of phytoplankton would change little. Conversely, if more available Fe was supplied, the standing crop of phytoplankton would increase and NO_3 would be depleted, despite grazing.

The grazing hypothesis, in the extreme, holds that the specific growth rates of phytoplankton are maximal. Herbivores effectively maintain the standing crop of phy-

toplankton at the lowest possible level because environmental stability allows development of a balanced phytoplankton-herbivore system. Changes in the specific growth rates of phytoplankton, due to seasonality in irradiance for example, would not strongly influence the standing crop of phytoplankton.

Factors such as light and temperature in the Southern Ocean (Mitchell et al. 1991) and trace-element speciation and interactions (Morel et al. 1991; Bruland et al. 1991) may prove to be important. Still, much of the current controversy about oceanic HNLC waters could be resolved by testing the following hypothesis.

An increase in the rate of supply of iron to the surface layer of the ocean will reduce to depletion the unused macronutrients, nitrate and phosphate.

This hypothesis was formulated by participants at a workshop convened by the Board on Biology of the U.S. National Research Council in 1990 (summary: Zaborsky unpubl.). It is consistent with hypotheses presented by Martin and colleagues and here it will be considered *the Fe hypothesis*. It could be tested by performing an unbounded experimental fertilization with either Fe or a mix of trace elements (Watson et al. 1991) or possibly by studying sites of natural enrichment with metals, such as the Galapagos upwelling (Minas et al. 1990; Barber and Chavez 1991; Martin et al. 1991).

Testing the iron hypothesis with bioassay experiments

Martin (1990; Martin et al. 1991) has assembled observational (chemical and paleoceanographic) and experimental evidence to support the iron hypothesis. The paleoceanographic evidence is discussed elsewhere (Berger and Wefer 1991). The experimental work, which has generated considerable discussion (Banse 1990, 1991a,b; de Baar et al. 1990; Buma et al. 1991; Coale 1991; Cullen et al. 1992) is examined here.

Bioassays of Liebig limitation—Nutrients can control the growth of phytoplankton in several ways. For example, nutrient availability can regulate rate processes such as photosynthesis (Blackman 1905; Thomas

and Dodson 1972) or the final yield of a plant crop (Liebig limitation). The important difference between the two types of limitation was once clearly recognized (Browne 1942). However, limitation of final yield and regulation of rate processes are not mutually exclusive in ecological systems, and the fundamental distinction between Liebig limitation of yield and Blackman's rate-limiting factor has been blurred in ecological studies (Odum 1971). As a result, the term "limitation of phytoplankton growth" has assumed many meanings, including limitation of the specific growth rates of phytoplankton or limitation of standing crop (Table 1). Limiting-nutrient bioassays will be examined here first in the context of Liebig limitation of standing crop.

Trace-element and minor-nutrient bioassays have been performed many times in oceanic waters (e.g. Menzel and Ryther 1960; Tranter and Newell 1963; Menzel et al. 1963; Thomas 1969; Barber and Ryther 1969; *see also* Goldman 1965). The principal objective was to determine which nutrients influenced the final yield of phytoplankton enclosed in bottles. The early experimental field studies on minor-nutrient limitation in the ocean are mentioned here not because results were conclusive (technical obstacles prevented that; *see* Huntsman and Sunda 1980), but because of their conceptual bases and experimental designs.

Curiously, much of the early experimental work on minor-nutrient limitation was done in oligotrophic waters where the ambient concentrations of N and P are very low so that relief of purported trace-element limitation would change the standing crop of phytoplankton only slightly before N or P became limiting. Nonetheless, it was acknowledged that if Fe limitation were real and it was relieved, N or P would become limiting "at a higher rate of photosynthesis" (Ryther and Guillard 1959; *see also* Young et al. 1991). This establishes the ecological relevance of Liebig limitation in terms of primary productivity. The same observation holds for high-nutrient waters, but the increase of net primary productivity upon relief of Fe limitation would be large and major nutrients would be depleted.

An important feature of the early experiments (cf. Ryther and Guillard 1959; Menzel and Ryther 1960; Menzel et al. 1963; Thomas 1969) was that the influence of nutrients was examined not only by adding several different nutrients individually, but also by omitting them individually from otherwise complete nutrient enrichments. This procedure is standard for documenting Liebig-type limitation of final yield (see Cullen in press), but unfortunately, the concentrations used in the minor-nutrient enrichments were orders of magnitude too high to be realistic. Significant contamination and unanticipated trace-element interactions were likely. The older results are thus uninterpretable in a realistic ecological context. For example, Menzel et al. (1963) found that enrichments with Al (an element that is not a plant nutrient) stimulated ^{14}C uptake and the increase of cell numbers just as Fe did.

Through meticulous attention to detail, the formidable obstacles of contamination were overcome (Bruland et al. 1979; Fitzwater et al. 1982; Martin et al. 1991) and some consistent results have been obtained. Most notably, Fe has been identified as a nutrient that strongly influences the utilization of other nutrients and the final yield of chlorophyll during experimental incubations, with final yields of chlorophyll proportional to added Fe (Martin et al. 1991). These results are consistent with Liebig-type limitation by Fe, but other results, such as enhancement of phytoplankton yield by Cu and the apparent stimulation of diatom growth by Mn (Coale 1991) are not. Experiments have not been reported in which uncontaminated, unenriched controls are compared to those enriched with complete nutrients minus Fe. Such a design might be logistically impossible.

It is important to recognize that even if Fe is the first nutrient to run out during an experiment, grazing might nonetheless control the standing crops of plankton, i.e. Fe would limit the standing crop of plankton only if grazing did not (cf. Banse 1991a; Buma et al. 1991; Cullen et al. 1992). This hypothesis would be tested by relaxing grazing pressure on natural unenriched phytoplankton and observing subsequent changes in standing crop. If the standing crop re-

mained constant after grazing pressure was reduced, the grazing hypothesis could be rejected.

It seems that the appropriate tests with reduced grazing pressure have already been performed: when samples are prepared for enrichment experiments, relatively rare larger herbivores are excluded by sampling. Also, microzooplankton are notoriously fragile and subject to mortality during handling, so their grazing may be disrupted. This artifactual relaxation of grazing pressure is apparently responsible for substantial increases in chlorophyll observed over the first few days in unenriched control samples (de Baar et al. 1990; Buma et al. 1991; Price et al. 1991). If Fe were indeed responsible for Liebig limitation of the standing crop of phytoplankton in situ, biomass would not increase in these uncontaminated controls, and it certainly would not exceed the maximum concentrations observed in the local environment (de Baar et al. 1990). Simply, if standing crop increases without added Fe, the strict hypothesis of Liebig limitation by Fe in situ is rejected. The question of inadvertent contamination of controls is addressed below.

Martin et al. (1991) frequently observed an initial decline of chlorophyll, both in their controls and in enriched samples. This decline is quite likely a response to light-shock from being incubated in full sunlight attenuated only by three clear plastic bags. On the same cruise in the equatorial Pacific, Price et al. (1991) eliminated this problem by attenuating light with neutral density screen; they found substantial initial growth in controls, with no difference between controls and enrichments over the first 2 d.

We are left to conclude that Liebig-type limitation of standing crop by Fe has not been rigorously demonstrated. However, there is a strong case for the following hypothesis.

There is not enough available iron in high-nutrient waters to support the net community production necessary for depletion of the major nutrients, N and P.

Fe and specific growth rates of phytoplankton—Another way to examine the reasons for low biomass in high-nutrient waters is to look at the growth rates of phytoplank-

ton rather than terminal yields. Perhaps grazing keeps pace with phytoplankton cell division only because specific growth rates of phytoplankton are retarded because of Fe limitation. Ideally, one would test this hypothesis by measuring the specific growth rates of phytoplankton and determining the influence of Fe enrichment on these rates (Banse 1991b). A direct influence of Fe availability on net community production would be indicated if specific growth rates of phytoplankton were low in unenriched controls and increased proportionately to Fe enrichment. By reporting results as doubling rates and showing higher rates in Fe-enriched samples, Martin and colleagues (summarized by Martin et al. 1991) suggest that this is indeed the case. The measurement and interpretation of growth rates is contentious, however.

There are several problems with estimating growth rates during incubations, such as unbalanced growth and lag periods (Eppley 1968; Fig. 3). A more fundamental uncertainty arises from the fact that increases in phytoplankton biomass reflect net growth (cell division minus grazing) and one cannot determine on the basis of changes in biomass the degree to which the rate of cell division or the rate of grazing has responded to experimental treatment (Banse 1990, 1991b; Dugdale and Wilkerson 1990; Buma et al. 1991; Coale 1991). For example, net rates of biomass accumulation may be greater in Fe-enriched samples because Fe stimulates the growth of species that are un ingestible to the small grazers in the bottles (Dugdale and Wilkerson 1990; Fig. 3B,D). Also, manipulations of trace elements influence the microzooplankton in complicated ways that affect the net growth rates and species composition of the phytoplankton (Banse 1990; Dugdale and Wilkerson 1990; de Baar et al. 1990; Buma et al. 1991; Coale 1991). Finally, exclusion of larger grazers changes food-web structure in the bottles, thoroughly complicating the interpretation of growth minus grazing.

To summarize, the response of larger herbivores is not assessed in bioassay experiments, so regardless of uncharacterized effects on microzooplankton, results are inconclusive. Even though Fe has been shown to influence the accumulation of in-

cubated phytoplankton from high-nutrient waters, the iron hypothesis (as defined here) can be neither accepted nor rejected on the basis of experimental measurements presented to date. As pointed out by Banse (1991b), methods exist to estimate species-specific rates of cell division in situ and in response to nutrient enrichments (Carpenter and Chang 1988). These estimates might resolve critical uncertainties.

Fe and species composition—The results of enrichment experiments may not be conclusive, but they are nonetheless extremely informative. They reinforce the predictions from laboratory studies (reviewed by Huntsman and Sunda 1980; Morel et al. 1991) and measurements in the field (Bruland et al. 1991) that trace elements should exert profound selective pressures on oceanic phytoplankton. It is noteworthy that enrichments with Fe (and other trace metals: Coale 1991) strongly influenced the species composition of phytoplankton (Martin et al. 1989; Buma et al. 1991; Chavez et al. 1991; Coale 1991), consistent with a fundamental change in food-web relationships toward a system with larger phytoplankton cells and higher rates of new production (cf. Michaels and Silver 1988). For example, in the equatorial Pacific, the natural food web is largely based on regeneration and is dominated by small cells (Chavez 1989; Peña et al. 1990; Chavez et al. 1990) with feeble NO_3 assimilation (Dugdale et al. 1992; Price et al. 1991). Enrichment with Fe stimulated the growth of a distinctly different assemblage of phytoplankton (Chavez et al. 1991), capable of assimilating NO_3 (Price et al. 1991) and increasing in biomass as long as nutrients were available.

Parsimonious assessment of enrichment assays—An interpretation of observations to date is that the availability and proportions of trace elements influence the speciation of phytoplankton in high-nutrient waters; enrichment of these waters with Fe would change food-web structure and stimulate new production. However, phytoplankton would bloom and rapidly deplete nutrients *if and only if* the phytoplankton that respond to Fe could escape grazing control. The uncertainty about grazing, and therefore the validity of the iron hypothesis, is at present unresolved.

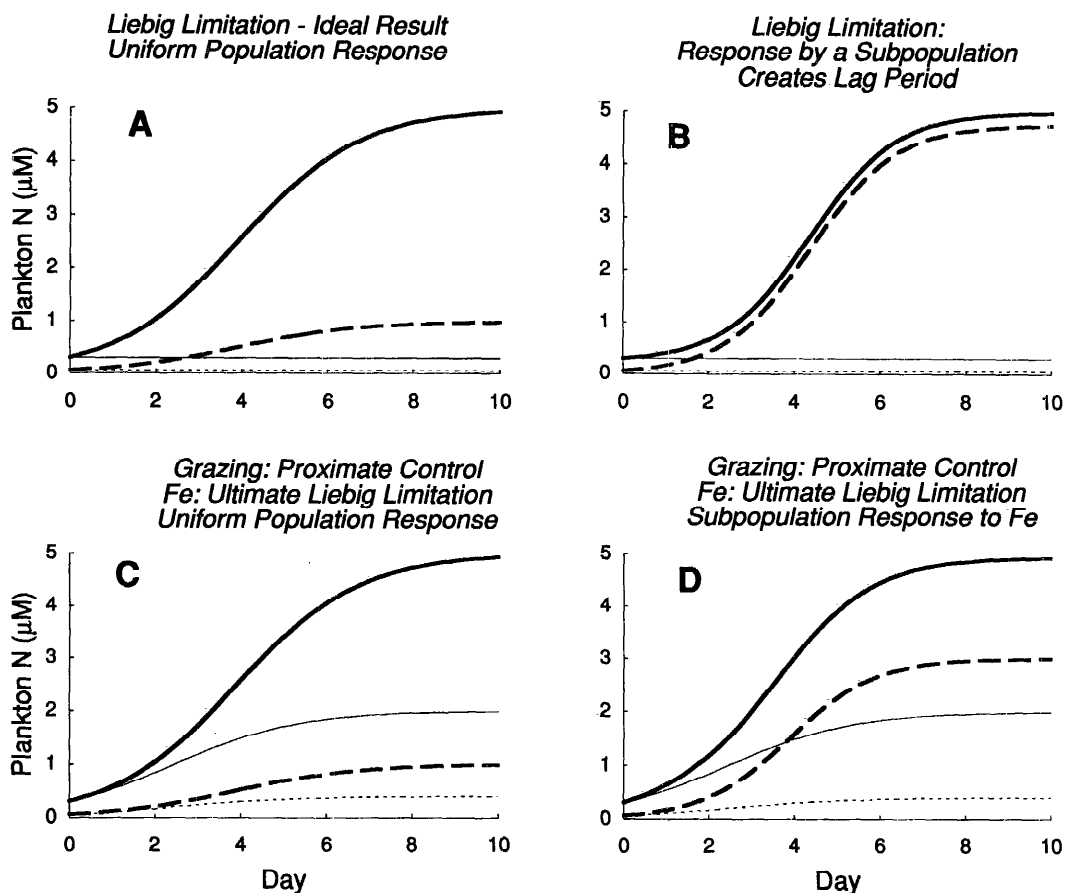


Fig. 3. Possible results of Fe enrichment experiments in HNLC waters ($5 \mu\text{g-atoms liter}^{-1} \text{NO}_3$): biomass (plankton N, $\mu\text{g-atoms liter}^{-1}$) vs. time. Solid lines indicate total biomass, dashed lines represent an idealized subpopulation (e.g. diatoms) that might be particularly responsive to Fe enrichment. The light lines are for controls and the heavy lines show samples enriched with Fe. A. The ideal expected response if available Fe limited the standing crop of phytoplankton in situ. Even though grazing is relaxed because of exclusion of some herbivores, there is no growth in the controls because no Fe is available. Biomass increases exponentially in the enriched sample, but it slows as NO_3 is depleted. B. Fe limits the standing crop of phytoplankton in situ, and response to enrichment is primarily due to growth of diatoms: rapid increase of total biomass is delayed 1–2 d as the responsive subpopulation accumulates. Here the subpopulation represents 20% of the biomass at time zero. If the initial biomass had been lower, the apparent lag would have been longer. C. The expected response if grazing is the proximate control of phytoplankton biomass but there is insufficient Fe to support depletion of NO_3 : biomass in the control increases because metazoan grazers are excluded and possibly because some microzooplankton are disrupted during sampling. After a few days, Fe is depleted before all NO_3 is utilized whereas in the enriched sample, net growth continues until NO_3 is depleted. It could also be argued that this result was the consequence of unavoidable contamination with minute amounts of Fe. D. Grazing controls the biomass of the dominant phytoplankton, but there is insufficient Fe to support depletion of NO_3 : enrichment with Fe differentially stimulates the subpopulation of diatoms, so species composition changes dramatically in response to enrichment. The exclusion of larger herbivores from the bottles compromises this result. One cannot reject the hypothesis that the diatom population is controlled by herbivores in nature, regardless of Fe-limited specific growth rate.

Because natural systems involve complex interactions and because small-scale experiments exclude important natural factors such as grazing and vertical mixing, no one observation or experimental result is likely

to prove or disprove the iron hypothesis. However, it may be possible to determine what controls nutrient utilization in high-nutrient environments by measuring or estimating maximum yield of plankton and

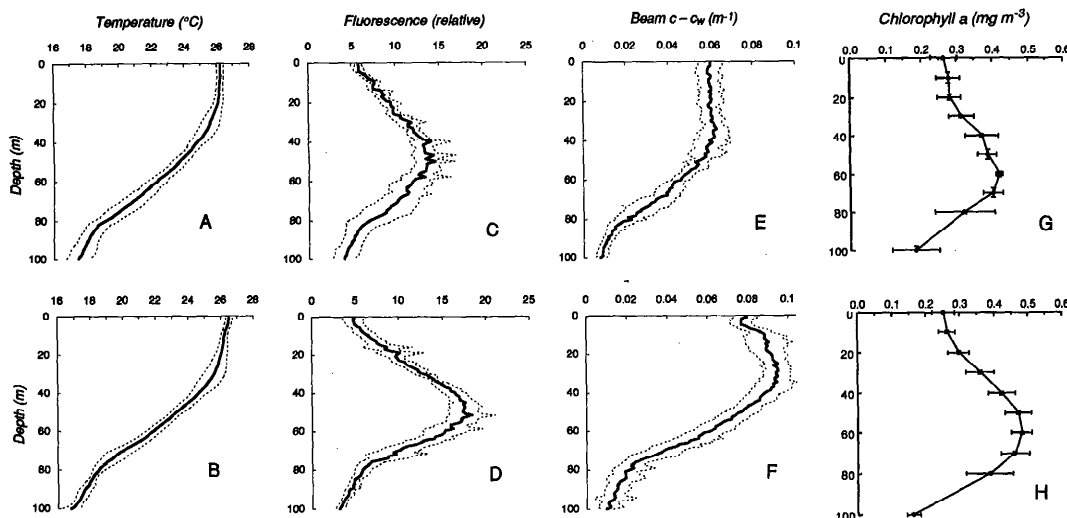


Fig. 4. Mean vertical profiles ($\pm$ SD) from an equatorial station at 150°W , 2–7 March 1988 (Cullen et al. 1992). A–F. Data from a bio-optical profiling system, with morning profiles in the top row, afternoon profiles in the bottom row: temperature at 0830 and at 1330 hours, in situ fluorescence, and beam attenuation, $c - c_w$, (i.e. corrected for pure water). G, H. Data from bottle casts: Chl *a* at 0600 and at 1200 hours.

total nutrient uptake during experimental incubations, the specific growth rates of phytoplankton in situ, the specific growth rates of phytoplankton in response to experimental manipulations, grazing rates in situ and in response to enriched phytoplankton, sinking rates, and residence times of water in the surface layer (i.e. rates of mixing and advection). This approach involves testing many specific hypotheses with the objective of rejecting or accepting the central hypothesis. It may not be possible to come up with a conclusive answer (prediction of grazing responses is a real problem), but much can be learned in the process.

Testing hypotheses in the equatorial Pacific

A recent study in the central equatorial Pacific (Cullen et al. 1992) represents an approach that might be useful in describing the high-nutrient condition: specific, falsifiable hypotheses were tested to examine processes that might regulate primary productivity. Neither the grazing hypothesis nor the Fe hypothesis was addressed directly, but much was learned about the regulation of autotrophic processes in those waters. It will be emphasized that the analysis per-

tains to the small cells that dominate in this environment and does not resolve why larger cells do not bloom (cf. Chisholm in press).

Characteristics of the environment—The equatorial Pacific is a highly dynamic environment, influenced by upwelling, strong shears, and vertical mixing (Carr et al. 1991; Gargett 1991). However, as suggested by Walsh (1976), the variability of these processes is such that planktonic dynamics in the equatorial system seem not to be disrupted on the time scale of days. The result is striking: despite a current shear of $\sim 100 \text{ km d}^{-1}$ between 0 and 60 m and a significant change in nutrient concentrations over a 6-d station at the equator (Carr et al. 1991), vertical profiles of important biological properties at 150°W showed very little variability day-to-day (Fig. 4). This apparent constancy suggests that zonal gradients on the equator are very weak (cf. Barber and Chavez 1991), that the net growth rate of plankton approaches zero, and that it is legitimate to pool measurements made over several days to increase statistical confidence.

Upwelling and adaptation of phytoplankton—One testable hypothesis concerning physical forcing and the physiological impairment involves the influence of upwell-

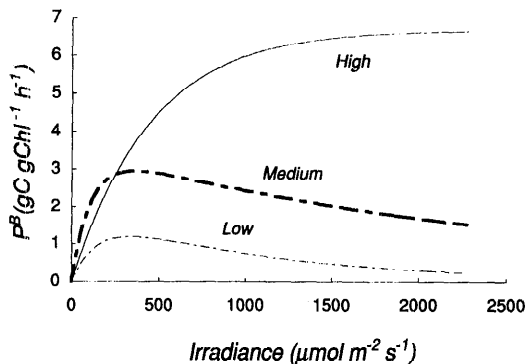


Fig. 5. The P vs. I relationship as a function of growth irradiance for the diatom *Thalassiosira pseudonana* (Clone 3H) from Cullen and Lewis (1988). Best-fits to the model of Platt et al. (1980). Low, medium, and high irradiance correspond to 20, 100, and 2,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Corresponding values of $I_k (= P_{\text{max}}/\text{initial slope})$ are 34, 102, and 423 $\mu\text{mol m}^{-2} \text{s}^{-1}$. If upwelled phytoplankton were not adapted to their photic environment, the curves would progress toward a higher light condition during a simulated in situ incubation.

ing on the photoadaptation of phytoplankton.

H_0 : Photosynthetic rates of the dominant phytoplankton in the central equatorial Pacific Ocean are reduced because the rate of upwelling exceeds the rate at which phytoplankton can adapt to the photic environment.

This hypothesis is comparable to the shift-up hypothesis (Dugdale and Wilkerson 1989), but it examines photosynthetic physiology rather than systems of NO_3 assimilation. It is tested by measuring the photosynthetic characteristics of phytoplankton to infer the degree to which they have adapted to their photic environment (see Cullen and Lewis 1988 and references therein).

Test: Measure photosynthesis vs. irradiance (P vs. I) on natural phytoplankton in vertical profile. Examine the results for evidence of photoadaptation. The hypothesis is rejected if complete photoadaptation is demonstrated.

The concept of complete photoadaptation is discussed below.

The results of measurements over 6 d show that the saturation parameter for photosynthesis (I_k ; Talling 1957; Platt et al. 1980), as well as other parameters of the P vs. I relationship (Cullen et al. 1992), showed a pattern with depth consistent with photoadaptation (Figs. 5, 6). This pattern does

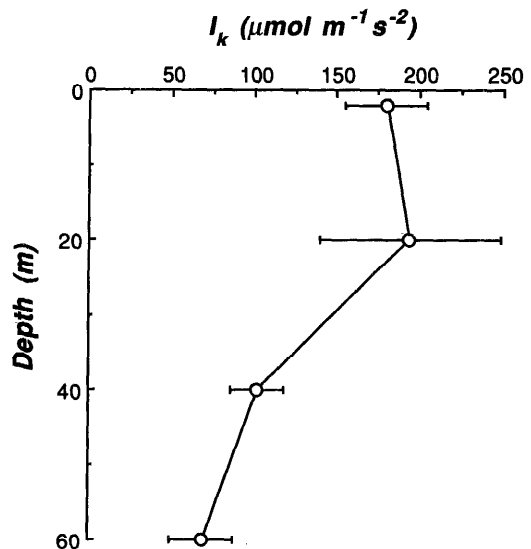


Fig. 6. Vertical distribution of the saturation parameter for photosynthesis, I_k ($\text{PAR} \pm \text{SE}$). This result is consistent with successful photoadaptation of the phytoplankton, with the influence of nocturnal mixing evident in the upper 20 m.

not resolve the degree of photoadaptation, however. To reject the hypothesis, it must be demonstrated that the phytoplankton assemblage was fully adapted to its photic environment.

A test was performed on a sample from the middle of the euphotic zone. Samples were incubated on deck at a similar irradiance to that encountered in situ. If upwelling had transported the assemblage upward through the euphotic zone faster than they could adapt, we would have observed further high-light adaptation of P vs. I during incubations. If the assemblage had already adapted to the ambient conditions in situ, no further adaptation would be observed. The result (Fig. 7) was very clear: the dominant phytoplankton at middepth in the euphotic zone were well adapted to the photic environment. The hypothesis is rejected. The assemblage is shifted-up with respect to C assimilation. One must infer the degree to which this result bears on the shift-up hypothesis of NO_3 assimilation.

Methodological considerations for P vs. I —It has been demonstrated that determinations of P vs. I can be very useful for testing hypotheses, but the tests are only as robust

as the measurements. One problem with the methodology used here (1-ml samples; Lewis and Smith 1983) is that the photosynthetic responses of large, rare cells or aggregates cannot be evaluated (they generate high outliers that are excluded from analysis). Accordingly, the hypotheses tested with P vs. I measurements pertain only to the small phytoplankton which dominate the biomass at the equator (Chavez 1989; Peña et al. 1990). This is unfortunate, because large particles might contribute disproportionately to new production (Goldman 1988), and larger phytoplankton dominate the response to experimental enrichment (Chavez et al. 1991).

Another problem with the P vs. I methodology is that samples were incubated for 1 h in soft glass scintillation vials, exposing the phytoplankton to substantial changes in trace element concentrations (cf. Fitzwater et al. 1982). Trace metal contamination would very likely influence measurements made over several hours, but the effects over 1 h are difficult to predict. We therefore addressed the contamination problem with the following hypothesis.

H_0 : Because of contamination, the activities of one or more trace elements artifactually influenced the rate of photosynthesis during the 1-h incubations.

Unless the hypothesis can be rejected, the results of the measurements should not be trusted. The test of the hypothesis follows procedures outlined by Sharp et al. (1980) and more thoroughly examined by Cullen et al. (1986).

Test: Compare control (purportedly contaminated) samples with parallel samples treated with EDTA. If there is no difference between the two, the hypothesis is rejected.

The premise of this test is that the chelator EDTA profoundly alters trace element speciation (Jackson and Morgan 1978). If, because of contamination, the activity of one or more divalent cations influenced the rate of photosynthesis during the measurement of P vs. I , then a radical alteration of this activity would alter photosynthetic rate, most likely by alleviating toxicity of Cu or Zn (Steemann Nielsen and Wium-Andersen 1970). This test is not particularly sophis-

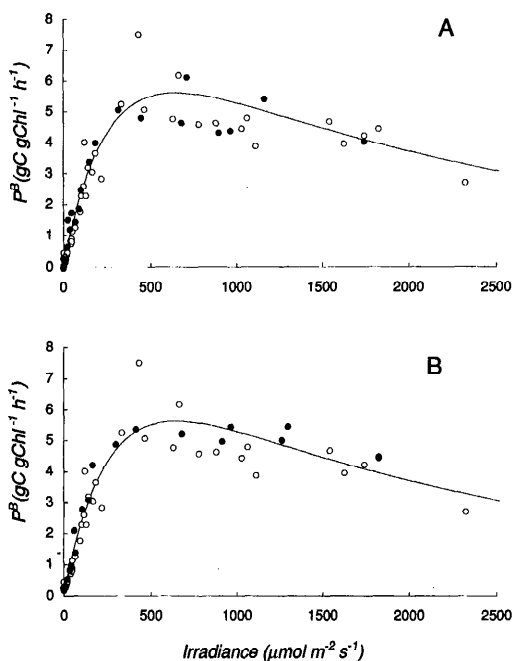


Fig. 7. Changes in P vs. I during simulated in situ incubations at 11% surface irradiance. Sample from the equatorial station (Cullen et al. 1992). In each plot, results from an experimental treatment (●) are plotted with the time-zero (○) measurements. The curve is the best-fit (Platt et al. 1980) for time-zero P vs. I . A. Control sample after 24 h. B. Control sample after 48 h. Possible influence of metal contamination is discussed in the text.

ticated, but it is direct: if P vs. I is unaffected by changes in the activities of divalent cations, then divalent cations did not regulate photosynthesis *during the measurement*.

The hypothesis was tested on a sample from the equatorial Pacific (Fig. 8), and the result is clear: 1 μ M EDTA had essentially no effect on photosynthesis, suggesting that trace metal contamination did not significantly influence the 1-h measurement of P vs. I .

It is assumed that the effects of putative trace metal contamination on photosynthesis are slow enough to progress over the course of the experiment and that EDTA would react and alter these effects within the time scale of the experiment. These requirements were satisfied during an earlier study (Cullen et al. 1986, their figures 3, 4): after intentional poisoning of coastal phytoplankton with Cu, photosynthetic rate de-

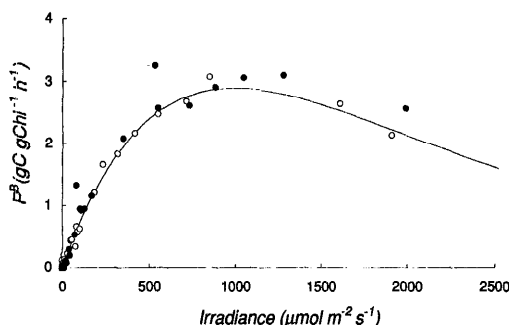


Fig. 8. Assessment of the effects of trace metal contamination on measurement of P vs. I . Sample from 18 m at 6°N, 150°W. Control sample (○): solid line is the best-fit as in Fig. 7. Parallel sample treated with 1 μ M Na_2EDTA (●). If toxicity from Cu or Zn had inhibited photosynthesis in the control, the EDTA treatment would show higher rates (Cullen et al. 1986).

clined fairly steadily over several hours in a process that was rapidly halted upon addition of 20 μ M EDTA. No such experiment was performed during our equatorial study. However, data indicate that both Cu (10-min reaction time) and Zn (30 min) would react with 1 μ M EDTA during the course of the experiment, whereas the activity of Ni (50-h reaction time with 1 μ M EDTA) would change much more slowly (Hudson et al. in prep.). Further experimentation, with uncontaminated controls, intentional additions of different trace metals, and treatment with different chelators could resolve uncertainties about this unconventional but inexpensive and potentially useful assessment of contamination artifacts.

Trace metals and specific growth rates of phytoplankton—Changes in the specific growth rates of phytoplankton are the manifestation of changes in physiological state, so we can test hypotheses concerning the growth rates by assessing physiological state. Measurements of P vs. I are sensitive indicators of the physiological state of phytoplankton (Thomas and Dodson 1972; Falkowski 1983; Prézelin and Matlick 1983; Cullen and Lewis 1988; Chalup and Laws 1990), so they can be used for testing hypotheses.

H_0 : In the central equatorial Pacific, the availability or activity of trace elements strongly limits the specific growth rates of the dominant phytoplankton in situ.

Test: Measure a very sensitive indicator of phytoplankton physiological state (P vs. I). Radically alter trace element activities and speciation, but do not change light and macronutrient regimes (Fig. 9). If P vs. I does not change over the time scale of cell division (hours to days), then the hypothesis is rejected.

Essentially, the time-zero sample is the control. The logic is that if trace elements with divalent cations regulated growth rates of the dominant phytoplankton at time zero, then changes in trace-element speciation would alter growth rates and influence P vs. I . The critical premise is that characteristics of the P vs. I relationship will change over a transient period of hours to days, when growth rate changes. Photosynthetic characteristics change on these time scales when irradiance is shifted (e.g. Cullen and Lewis 1988) and when major nutrients are depleted (Welschmeyer and Lorenzen 1981), but only a restricted set of conditions has been examined. In a study of Fe-limited phytoplankton, enrichment with Fe stimulated short-term photosynthesis (Glover 1977).

The results (Fig. 9) demonstrate quite clearly that the photosynthetic characteristics of the dominant phytoplankton were insensitive to trace element manipulations over 24–48 h. On the basis of these measurements and the explicit assumption about the relationship between P vs. I and growth, the hypothesis is rejected. If instead P vs. I had changed in response to these heavy-handed manipulations of trace elements, the results would be extremely difficult to interpret because trace element interactions could not be characterized (cf. Huntsman and Sunda 1980). It would be useful to repeat this approach with noncontaminating techniques and a suite of sophisticated diagnostics of phytoplankton physiology (Greene et al. 1991). It also bears repeating that this hypothesis refers only to the dominant phytoplankton, not any subpopulation that might be Fe limited.

Growth rates from changes in chlorophyll—The relative constancy of chlorophyll from day to day at the equator (Fig. 4) indicates that the net growth rates of phytoplankton were near zero, and the results from the P vs. I experiment suggest that the

specific growth rates of the dominant phytoplankton were not strongly regulated by trace element activities in situ, but neither observation reveals the specific growth rate of the dominant equatorial phytoplankton. Were specific growth rates low and is that why grazing was able to control standing crop?

One estimate of specific growth rate for the upper euphotic zone can be obtained from the daily productivity index [$60 \text{ g C (g Chl)}^{-1} \text{ d}^{-1}$, Cullen et al. 1992] and estimates of the C:Chl ratio of phytoplankton (58 g C:g Chl , Eppley et al. 1992): the result is 0.7 d^{-1} (see also Chavez et al. 1991; Barber and Chavez 1991). This growth rate, unfettered, would lead to a 35-fold increase in phytoplankton biomass over 5 d, enough to deplete NO_3 in the surface layer at the equator (calculation: initial Chl = $0.3 \mu\text{g liter}^{-1}$; initial $\text{NO}_3 = 6 \mu\text{M}$; $1 \mu\text{M NO}_3$ assumed to yield $1 \mu\text{g liter}^{-1}$ chlorophyll; residence time of water in the upper 30 m, $\sim 30 \text{ d}$, M.-E. Carr pers. comm. 1991). Instead, because loss processes largely balance cell division, chlorophyll and NO_3 concentration vary little.

An independent estimate of specific growth rate comes from changes in phytoplankton biomass during incubations: chlorophyll increased because grazing was reduced, and the observed rate of increase over the first day is a minimum estimate of the specific growth rate in situ. The estimate is low to the extent that grazing persists in the bottles and inaccurate to the extent that growth rates change in the bottles. If chlorophyll is used as a measure of biomass, it must be assumed that growth is balanced (i.e. the rate of increase of different cellular materials is the same over each sampling interval; Eppley 1968). This assumption is not likely to be satisfied when either irradiance (Cullen and Lewis 1988) or limiting nutrients (Sakshaug and Holm-Hansen 1977) are substantially altered. However, P vs. I measurements (Figs. 7, 9) are strongly consistent with balanced growth of the dominant phytoplankton over 2 d (see above). Thus, the net rate of increase of chlorophyll should be a reasonable minimum estimate of the specific growth rates of the dominant phytoplankton in situ.

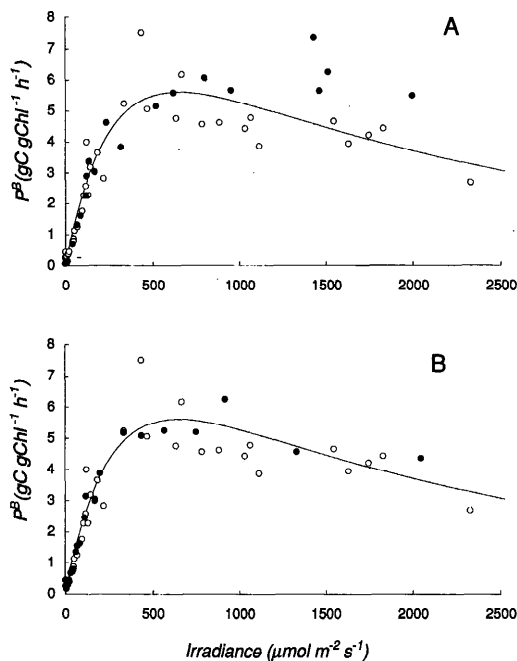


Fig. 9. Effects of trace metals on P vs. I during simulated in situ incubations at 11% surface irradiance as in Fig. 7. Results from an experimental treatment (●) are plotted with the time-zero (○) measurements. A. Cu-treated sample (5 nM) after 48 h. B. Fe-treated sample (10 nM) after 48 h. Possible influence of Fe contamination is discussed in the text.

The results of several experiments are consistent (Fig. 10): the initial net rate of increase of chlorophyll was 0.6 d^{-1} , and the rate was insensitive to perturbations of trace metals. Absent from these results is an uncontaminated control, so one might argue that all growth rates were overestimated due to inadvertent enrichment with Fe. This is not likely, because P vs. I did not change over 2 d (Fig. 7), and accelerated increase of chlorophyll was not observed until after day 2. Consistent with the conclusion that Fe contamination did not stimulate bulk photosynthesis or growth during the first 24–48 h of the incubations, Price et al. (1991) measured similar increases of chlorophyll in uncontaminated controls protected from light-shock and found no influence of intentional Fe enrichment during the first 1–2 d of the experiments. Also, Coale (1991), using clean techniques, found no significant effect of Fe enrichment on primary productivity during the first 24 h of incubation.

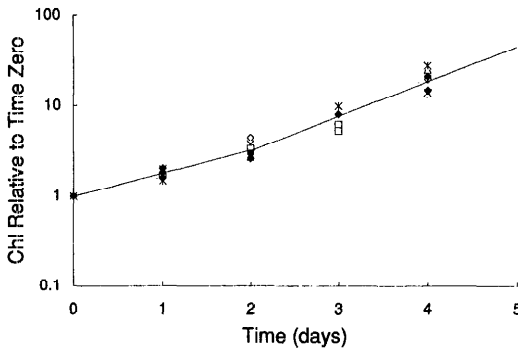


Fig. 10. Changes of Chl concentration during simulated *in situ* incubations, such as those presented in Figs. 7 and 9. Samples from between 2°S and 6°N, >10% I_0 ; treatments described by Cullen et al. (1992). Results normalized to Chl concentration at time zero. There were no consistent differences between treatments, so all were combined to calculate specific rates of increase. The lines are best-fits for days 1–2 (regression forced through 0, on log-transformed data: slope = 0.59 d^{-1} , $n = 21$, $r^2 = 0.82$) and days 2–4 (regression on log-transformed data, slope = 0.89 d^{-1} , $n = 21$, $r^2 = 0.92$). The initial rate of increase is interpreted as a minimum estimate of the specific growth rate of the dominant phytoplankton. The subsequent rate is a minimum estimate for a subpopulation that was stimulated during incubations, possibly by Fe contamination.

Note that the results showing strong growth with no lag (Fig. 10) are inconsistent with substantial trace metal toxicity at the time of sampling (Fitzwater et al. 1982).

These measurements of net growth reinforce the conclusion that the dominant phytoplankton in the central equatorial Pacific were growing vigorously and that they would deplete the ambient NO_3 in a matter of days, if it were not for a strong control on standing crop. It is possible that Fe would run out before NO_3 (Martin et al. 1991; Price et al. 1991), but because even trace-metal clean samples show net growth in unenriched controls (Price et al. 1991) and because the physiology of the dominant phytoplankton was insensitive to enrichment with Fe, Fe does not seem to be the proximate control. Rather, grazing keeps a tight rein on the dominant, small phytoplankton.

Evidence for grazing control—A third, independent methodology can be used to assess the specific growth rates of phytoplankton. The method is noninvasive and grazing rates are also estimated. Diel changes of

beam attenuation (beam c , m^{-1}) are assumed to reflect the photosynthetic production of ultraplanktonic biomass during the day, offset by losses through microzooplankton ingestion (Siegel et al. 1989). In the model, specific growth rates of phytoplankton are calculated, assuming exponential light-dependent growth reduced by constant grazing pressure. The method relies on several assumptions, including that beam attenuation is a precise indicator of particle concentration (see Siegel et al. 1989; see also Cullen et al. 1992).

At the equatorial station, the diel variation of beam c was quite large (Fig. 11A): at 28 m, the attenuation of light by particles increased 53% over 5 h, a net rate of increase of 0.085 h^{-1} . With this result and conservative assumptions, a specific growth rate for phytoplankton of 1.46 d^{-1} can be calculated (Fig. 11B,C). It is the minimum C-specific rate of increase necessary to support the diurnal accumulation of biomass indicated by changes in attenuation. Grazing is apparently responsible for consuming most of this growth, but phytoplankton respiration would be confused with grazing, leading to overestimates of specific growth rates (Geider *in press*). The optical methodology for studying particle dynamics is still under development, and many uncertainties must be resolved (e.g. Ackleson et al. 1990). Still, these noninvasive measurements suggest strongly that the dominant small phytoplankton in the central equatorial Pacific are dividing rapidly and that grazing controls the standing crop of small cells.

What controls new production in the equatorial Pacific?

The lines of evidence presented here suggest very strongly that in the central equatorial Pacific, the specific growth rates of the dominant, small phytoplankton are adequate to overcome physical forcing and to deplete ambient NO_3 in the euphotic zone. The observed growth in control bottles when grazing is artifactually diminished suggests that herbivores control the standing crop of these small cells, and that if grazing were reduced in nature, standing crop would increase before Fe ran out. However, it is not

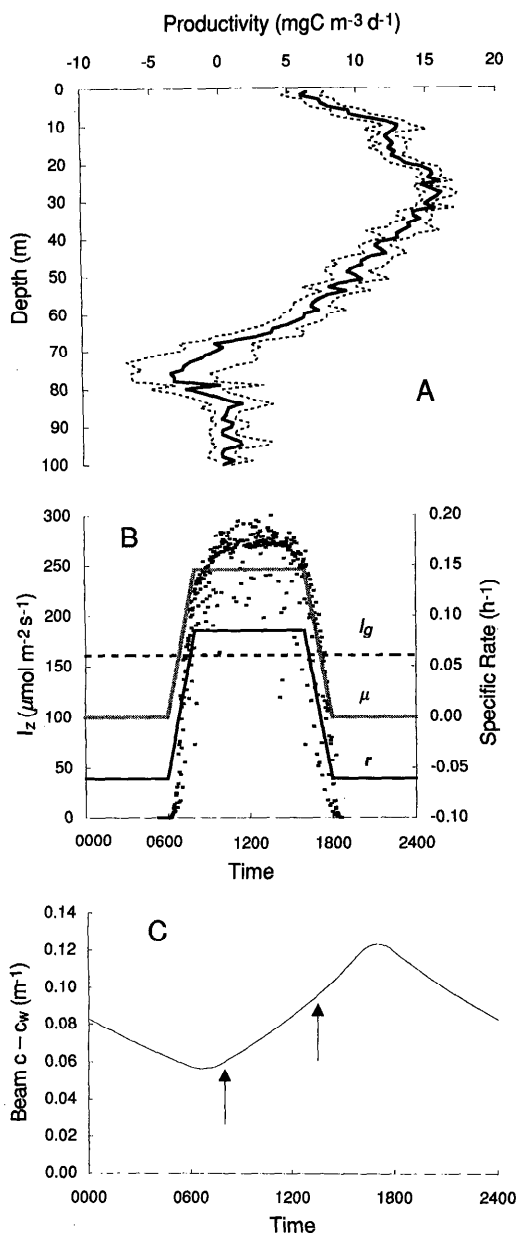


Fig. 11. Beam attenuation and particle dynamics at the equatorial station. A. Estimates of primary productivity (mean $\pm$ SD for 6 d) from changes in beam attenuation (method of Siegel et al. 1989, applied by Cullen et al. 1992). Note the small errors, indicating little day-to-day variability. B. Model of phytoplankton growth and grazing at the equatorial station, 28 m. The three lines represent the constant grazing rate (I_g), the light-dependent growth rate (μ), and the specific rate of particle production ($r = \mu - I_g$). The individual points show irradiance at 28 m, 10-min averages, observations over 6 d, calculated as 10% I_0 . C. Changes

at all clear what determines the balance between growth and grazing, and why a balance is reached with persistent NO_3 near the surface and nearly uniform chlorophyll concentrations over broad expanses of ocean (Chavez et al. 1991).

In the course of this analysis, it has become evident that although small cells dominate the planktonic system, the control of large cells is likely to determine the degree of nutrient utilization (see also Goldman 1988). We need to know why big cells are rare (Chisholm in press). It is reasonable to implicate Fe, which is demonstrably in short supply: larger cells are poorer competitors for nutrients, so they are much more likely than small cells to be limited by Fe or other trace elements (Morel et al. 1991). Thus, Fe might ultimately regulate productivity by influencing the specific growth rates of relatively large diatoms, thereby changing food-web structure. It remains to be seen if Fe enrichment would stimulate diatom blooms in the open ocean, in the presence of natural grazing pressure.

This view is broadly consistent with observations from the subarctic Pacific (summarized by Frost 1991; Miller et al. 1991) and interpretations of other investigators (e.g. Banse 1991a,b; Barber and Chavez 1991; Chavez et al. 1991; Coale 1991; Michaels unpubl.; Price et al. 1991). Antarctic waters might be different: careful analyses must be performed to determine if the specific growth rates of phytoplankton are adequate to overcome physical forcing and to deplete ambient NO_3 in the euphotic zone. Thus, a test of the iron hypothesis for one region should not be extrapolated to another. Also, experiments should be performed in the context of multiple working hypotheses (Chamberlin 1965) with the recognition that different components of the phytoplankton assemblage will be controlled

← of beam attenuation (corrected for pure water) from the model: the arrows indicate 0830 and 1330 hours, the times at which attenuation was measured. Averages of beam c at these times over 6 d constrained the model. This large diel amplitude is interpreted as describing a tightly coupled system of active growth controlled by grazing.

differentially by processes such as nutrient limitation, grazing, sinking, etc.

Conclusions

The iron hypothesis predicts that additions of Fe to high-nutrient waters would stimulate new production and lead to the depletion of major nutrients. The implications of this hypothesis are profound: new production in large parts of the ocean would be driven by eolian flux (cf. Duce and Tindale 1991), rather than by vertical exchange with deep water. If the iron hypothesis is true, models of biogeochemical cycling in the sea will have to be fundamentally altered. Predictions of global change in response to the buildup of greenhouse gases will certainly be influenced. And, if the iron hypothesis is validated for Antarctic waters, fertilization of the Southern Ocean might be considered as a means for reducing the atmospheric buildup of CO₂ (Joos et al. 1991; Sarmiento and Orr 1991; Peng and Broecker 1991). The results of such a manipulation are difficult to predict, but some scenarios are catastrophic (Fuhrman and Capone 1991). One thing is certain, however: fertilization with Fe can increase productivity only by changing fundamentally the food webs and vertical fluxes in high-nutrient waters, and these changes would have far-reaching consequences.

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