

Stenn

The High Nutrient Low Chlorophyll (HNLC) Phenomenon
and the Iron Hypothesis

by

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A CRITICAL ESSAY

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ABSTRACT

With rising CO₂ levels in the atmosphere it becomes increasingly more important to understand the nature of the oceans as a sink for CO₂ as well as the mechanisms that transport carbon from the atmosphere to the oceans. Regions of ocean in the subarctic Pacific, eastern equatorial Pacific and the southern Ocean have been recognized as being abnormally low in total biomass and yet they maintain high levels of available macronutrients. Due to the characteristic high nutrient low chlorophyll content of these regions, they have been dubbed HNLC. The 'biological pump' concept is a proposed mechanism serving as a carbon sink and was assumed to be limited by nitrogen. Early shipboard container experiments demonstrated that iron might be the limiting nutrient and not nitrogen. The data from the early experiments proved to be inconclusive due to imprecise methodology. In the late 1980's there was renewed interest in HNLC areas championed by J.H. Martin. He maintained that HNLC regions exhibit Liebig limitation by iron, where, standing crops of phytoplankton are constrained by availability of iron: if iron were available, the standing crops of phytoplankton would increase and nitrate would be depleted despite grazing. Others argue that HNLC regions are a manifestation of active grazing in a steady state ecosystem. An intermediate camp claims that HNLC regions are a result of combined physical and biological processes that prevent the utilization of the surface macronutrients. The debate surrounding this issue prompted Martin and colleagues to perform the IronEx experiment: the *in situ* use of iron to enhance an HNLC patch in the Galapagos region of the Pacific. The ecosystem demonstrated an unequivocal response to iron; however, macronutrients were still relatively abundant after the experiment. Subsequent studies have revealed that iron impacts all cell size groups of phytoplankton and constrains new production in HNLC areas. What remains unclear is the effect of grazing within these ecosystems.

INTRODUCTION

The 'greenhouse effect', a product of increased accumulations of CO₂ in the atmosphere, has prompted interest in identifying the possible global sinks for CO₂. The 'biological pump' concept was proposed as a possible global sink for CO₂. The oceans are a major reservoir of CO₂ and one mechanism for increasing the storage of CO₂ in the ocean is the 'biological pump', proposed by Longhurst (1991). According to this hypothesis, primary productivity in the surface waters is stored as organic matter in the ocean's interior. CO₂ depletion by primary production leads to further uptake of atmospheric CO₂ at the ocean-atmosphere interface. Ultimately, the amount of carbon that can be stored in this way depends on the concentration of limiting nutrients (Longhurst 1991). Traditionally, nitrogen has been viewed as the main element limiting oceanic primary production (Berger et al. 1991; Longhurst 1991). However, the frequent occurrences of regions where surface waters have high levels of nitrate and low levels of chlorophyll has led to the idea that something else limits primary production or the accumulation of organic matter (Cullen 1992)

Open ocean environments characterized by high concentrations of major macronutrients at the sea surface but with very low total biomass were noticed as early as 1934 (Hart 1934). They were designated High Nutrient Low Chlorophyll (HNLC) regions by Minas et al.(1986). The subarctic Pacific, eastern equatorial Pacific and the southern Ocean are considered HNLC regions.

Over the years researchers have proposed various hypotheses attempting to explain the dynamics of HNLC regions. Martin (1988) proposed that HNLC regions exhibit "Leibig limitation" by iron. In other words both standing stocks of phytoplankton and primary productivity are constrained by the availability of iron. If more iron were available, the phytoplankton biomass would increase and nitrate would be depleted (Martin 1988; Martin et al. 1989; Martin 1990; Martin et al. 1994). A counter argument suggests that standing crops of phytoplankton are grazer controlled: rates of primary

production are maximal and biomass is maintained at low levels by a balanced food web (Walsh 1976; Banse 1990).

Other explanations for HNLC conditions are more complex. These consider and attempt to integrate low biomass production of phytoplankton, specific growth rates of phytoplankton; losses due to sinking, grazing, and/or the proportion of grazed material that is exported. Presently, the scientific community seeks explanations for why autotrophic processes fail to exploit nitrate and phosphate in HNLC regions. 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 (Cullen 1991). This paper will review the current status of the iron hypothesis and discuss alternative hypotheses which attempt to explain the occurrence of HNLC regions. First, I will briefly review two important processes: (i) carbon cycling and (ii) iron biogeochemistry in marine ecosystems.

Global CO₂ Cycling

On a global scale, the carbon cycle is important because it is responsible for consuming excess CO₂ in the atmosphere. CO₂ levels in the atmosphere have been increased by 25% since the preindustrial period (1750-1800) (Takahashi et al. 1992). This rapid development of the "greenhouse effect" has made it increasingly important to understand carbon sinks, their saturation points and the possibilities for reducing atmospheric buildup of CO₂.

There are three major reservoirs of carbon: (i) atmospheric, (ii) oceanic and (iii) terrestrial. Of the three, the oceanic contains the largest amount of carbon (Post et al. 1990). There are an estimated 37,000 gigatons of dissolved inorganic carbon, 1,000 gigatons of dissolved organic carbon and 30 gigatons of particulate organic carbon in the oceans (Post et al. 1990). Physical, biological, and chemical processes help to regulate the carbon flux in the oceans. Because of the effectiveness of convective wind flow over the ocean, CO₂ in the atmosphere is exchanged rapidly across the sea-air interface. This results in an approximate equilibrium between the partial pressures of CO₂ in the atmosphere and in the surface ocean water. Below surface waters CO₂ exists as a weakly

dissociated acid and as the dissolved ions of carbonate and bicarbonate. In shallower warmer waters, CO_2 is less soluble and will precipitate out as calcium carbonate. Whereas, deeper waters, 4500-6000 meters and below, are richly dissolved with CO_2 and are able to dissolve calcium carbonate much more readily (Murray 1994; Longhurst 1991).

The concept of the “biological pump” has attempted to explain the downward flux of carbon which is maintained by gravitational settling of carbonates and organic carbon produced in the surface layer by phytoplankton. It has been assumed that the flux is constrained by the supply of nutrients, specifically inorganic nitrogen as NO_3^- (Longhurst 1991). Recently, however, Martin (1988) suggested that, over geologic time, oceanic primary production is limited by iron and not nitrogen. Martin suggests that temperatures rose during times of high atmospheric CO_2 which led to greater geographical expanses of arid regions, and changes in frequency and intensity of prevailing wind patterns. Iron delivered to the Southern Oceans by massive iron-rich eolian dust deposits boosted phytoplankton productivity, which in turn created greater CO_2 undersaturation in the oceans and led to CO_2 drawdown (Martin 1988, 1990). While this hypothesis is consistent with the paleoclimate record of Fe at CO_2 concentration obtained from the Vostok Ice cores (Fig. 1), sediment cores from the Southern Ocean do not show evidence of enhanced deposition of organic matter at the appropriate times (Berger et al. 1991). Overall, the “Iron Hypothesis” as Martin’s theory is called, has created much discussion and controversy since its publication in 1990. At one point, the addition of iron into the world’s oceans was considered as a potential solution for controlling the “greenhouse effect” (Martin et al. 1990). Since then, enthusiasm supporting this action has diminished due to the discovery that only about 10% of CO_2 drawdown can be attributed to primary productivity (Watson et al. 1994).

Iron Biogeochemistry

In terms of cellular requirements, iron is intermediate between a macronutrient and micronutrient. It is a cofactor for many enzymes and coenzymes: superoxide dismutase, nitrate/nitrite reductase (Shkolnik 1984), pigments, ferredoxins and cytochrome c. It also helps to regulate the abundance of the D1 protein of photosystem II

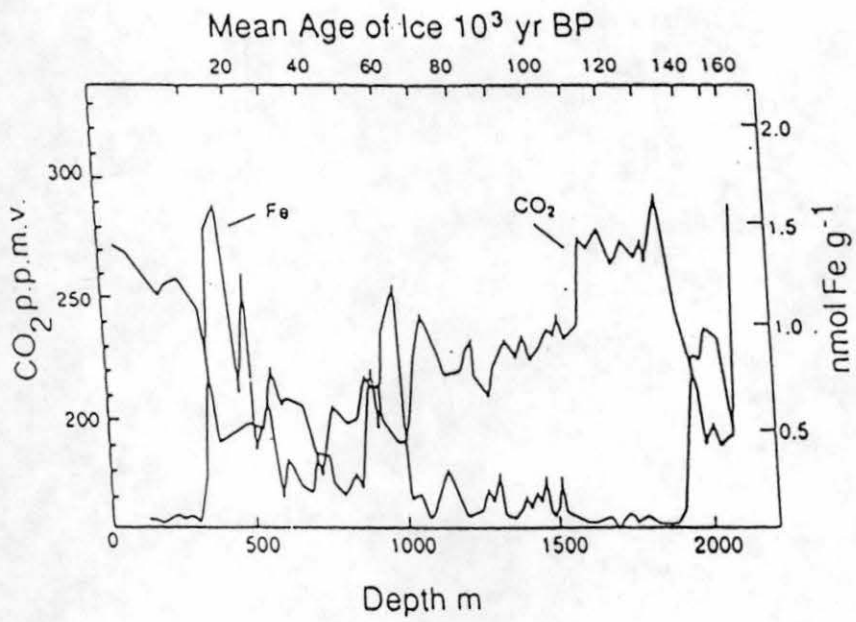


Figure 1. Iron concentration as a function of real depth in Antarctic Vostok ice core, together with mean age of air. (Martin, J.H. (1990)).

(Greene et al. 1991). Iron has a variety of valence states and is soluble as Fe(II) and insoluble as Fe(III). However, Fe(III) is biologically available when chelated. Total Fe concentration varies from 25 pM (Wells et al. 1994) to 7 nM concentration (de Baar et al. 1995) in the world's oceans.

Iron is introduced into ocean systems from a number of different sources: riverine systems, coastal upwelling, and through eolian depositions. Greater than 50% of the usable iron is introduced through these eolian depositions (Fig. 2; Kumar et al. 1995; Duce et al. 1991; Martin 1990). Recently the northern hemisphere has received the bulk of the eolian iron deposits, originating from Asia (Duce et al. 1991) and Africa (Prospero et al. 1977). The southern hemisphere receives iron from the Patagonian deserts (Kumar et al. 1995). Between 10-15% of atmospheric iron entering the world's oceans appear to dissolve when the mineral matter enters the ocean (Brand 1991; Duce et al. 1991). Evidence of past iron deposition and atmospheric content comes from the Vostok Ice cores which show a significant period of high atmospheric iron prior to the glacial period 125 mya (Martin 1990). Much of this atmospheric iron is present as Fe(II) due to photochemical reduction reactions that take place during transport (Duce et al. 1991). Iron is insoluble in oxygenated seawater, since it is rapidly oxidized from the soluble Fe(II) to the highly insoluble Fe(III) state (Sung et al. 1980). Both Anderson & Morel (1982) and Coale (1991) point out that Fe(II) also becomes photooxidized to Fe(III) with a half-life of about 2 minutes in the surface ocean waters (Johnson et al. 1994). In the water column, iron increases from the surface waters to depths of about 100m (Fig. 3; Martin et al. 1989), where it exists as colloidal particles or attached to detrital matter. Iron availability is thought to be influenced by competition for binding sites both on the phytoplankton and organic ligands. It was suggested that copper competes with manganese and iron for binding sites (Murphy et al. 1984). When copper (Cu^{++}) displaces Fe(II) from organic ligands this could lead to an increase in available iron. Wood (1983) hypothesized that copper in natural systems is regulated by the presence of complexing ligands (inorganic, organic and particulate complexing agents) which remove copper from

the free ion pool; it is possible that similar interactions occur with regard to iron. However, Coale and Bruland (1988) and Bruland (1989) demonstrated that particular ligands show a higher affinity for copper, zinc and cadmium but not for iron, so the degree to which complexation affects iron availability will be influenced by the precise mixture of complexing ligands.

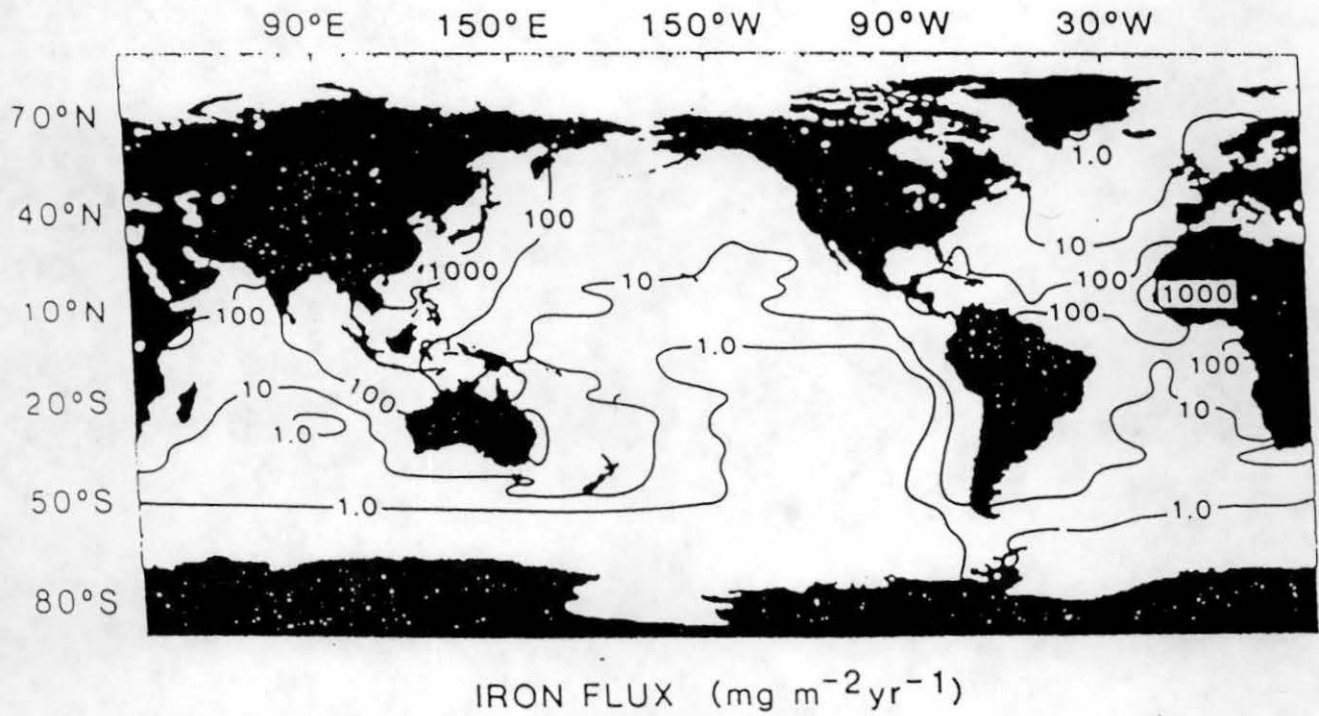


Figure 2. The calculated flux of total (particulate plus dissolved) Fe from the atmosphere to the ocean (adapted from Donaghay et al. 1991). (Duce et al. (1991)).

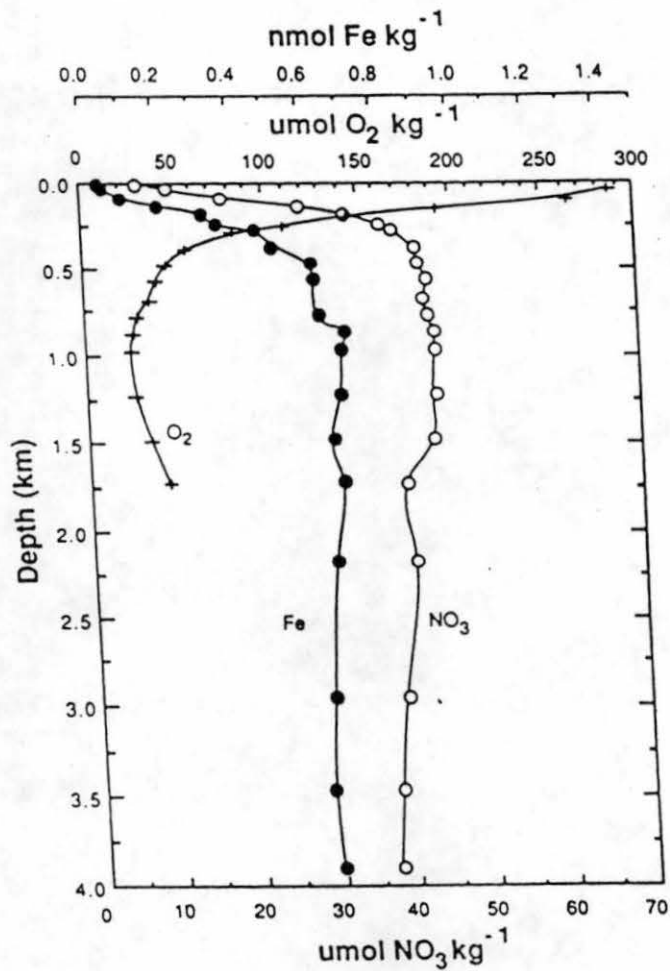


Figure 3. Vertical distribution of total dissolved iron (extracellular and colloidal), oxygen, and nitrate at the Gulf of Alaska Ocean Station PAPA (50°N; 145°W). (Martin et al. (1989)).

EVOLUTION OF THE IRON HYPOTHESIS

Iron limitation was first proposed as an explanation for lower productivity in the Southern Ocean early in this century (Gran, 1925, as summarized in Martin 1990). Hart (1934) commented that neritic influences were stronger around South Georgia than elsewhere in the Southern Ocean and that phytoplankton concentrations were ten times higher there than in other Antarctic regions. His interpretation suggested that nutrient limitation might be a constraining factor in waters which did not have neritic influence, and that iron might be one of the limiting nutrients (Hart, 1934, as summarized in Martin, 1990).

In the late fifties and early sixties a series of incubation experiments were conducted to identify the limiting nutrients in oceanic waters (Ryther & Guillard 1959; Ryther & Yentsch 1959; Menzel & Ryther 1961; Menzel et al. 1963; Thomas 1969). In these studies, samples of seawater were inoculated with varying amounts of nutrients and incubated in containers. Productivity over time was measured as an indication of the effect of experimental manipulation. In the first of these studies (Ryther & Guillard 1959), surface seawater and samples comprised of half surface seawater and half deep water were spiked with a complete enrichment medium that included all major macronutrients, a vitamin mix, and an iron-trace metal mix. In order to determine which of the added nutrients might cause an effect, some samples were spiked with an incomplete enrichment (i.e. one component of the enriched mixture left out). Samples were incubated for 24 hours under constant illumination and, in all experiments, only the samples spiked with the iron-trace metal mixture showed an enhancement of ^{14}C incorporation relative to the controls.

In a follow-up experiment, components of the iron-trace metal mixture were added individually to seawater samples (Menzel and Ryther 1961). Data from the Sargasso Sea and the Caribbean showed that iron alone significantly enhanced ^{14}C incorporation for up to 24 hours after metal additions but that this growth rapidly depleted other available macronutrients. Enhancement of ^{14}C incorporation by iron addition only persisted as long

as 72 hours if nitrogen, phosphates and iron were added at the beginning of the experiment.

Further experiments showed that addition of nitrogen or phosphate could enhance phytoplankton growth (when compared to unenriched controls), but only after an extended period of time (>7 days, Menzel et al. 1963). Apparently, addition of these macronutrients selected for a small phenotype of the diatom *Skeletonema costatum*. This phenotype which had never been observed previously, dominated the nitrogen- and phosphate-enriched carboys after nine days. An important result of this study (Menzel et al. 1963), is the realization that the phytoplankton of the Sargasso Sea probably included many rare taxa that were physiologically suited to a wide range of nutrient levels.

In the Pacific Ocean, the results of early iron addition experiments do not generally agree with the studies conducted in the Atlantic [compare Thomas (1969) and references cited therein with Ryther and Guillard (1959), Menzel and Ryther (1961), and Menzel et al. 1963)]. In the eastern equatorial Pacific, addition of nitrate and phosphate alone enhanced ^{14}C incorporation by 700% relative to unenriched controls (Thomas 1969). There was no difference between the ^{14}C incorporation rates of samples spiked with nitrate and phosphate (25 μM at 2.5 μM , respectively) and samples spiked with the same nitrogen and phosphate mixture plus added iron (0.2 μM as NaFeEDTA).

Problems with Early Methodology

A variety of methodological problems in these early trace/metal iron enhancement experiments have been recognized in recent years. Among these, the most important are: trace-metal contamination; problems with the methods used to estimate growth, and the exclusion of various trophic levels from the containers.

Contamination of the containers used in the experiments poses a concern because of the inadequate methodology for removing trace metal from the containers; therefore, trace metal quantity was not rigorously controlled (Fitzwater et al. 1982). Coale (1991) points out that levels of dissolved metals in bottles could be expected to change at natural pH due to adsorption, leaching of the container walls or photochemical transformations of metal species during the course of the incubation. The lack of instrumentation and

methodology prevented the detection of nano- as well as picomolar quantities of trace-metal. Banse (1991) contends that the detection of the biologically available iron, in itself, might not be accurate. These problems remained until the mid-80s.

The second main criticism involves the use of chlorophyll as a means of determining growth rates and as an indicator of nutrient limitation (Banse 1991; Cullen 1991). While none of the studies relied exclusively on changes in chlorophyll concentration to show the effects of iron addition, Thomas (1969) used ^{14}C incorporation to measure the effect of added nutrient in only one of ten experiments. In all other cases, changes in biomass, measured as chlorophyll concentration, were used to assess the effects of macronutrient as well as trace metal additions. There are two problems with this approach. The first, which involves problems associated with measuring chlorophyll in the presence of detrital matter, can be circumvented by fluorometric as well as specific spectrophotometric methods (Ryther and Yentsch 1957; Yentsch and Menzel 1963). The second set of problems results from the physiological parameters unrelated to cell or population growth. Additionally, Greene et al. (1991) demonstrated that there is potentially more chlorophyll per cell in an iron limiting environment because of lower photosystem II efficiency. Iron deficiency was found to have increased the *in vivo* absorption of chlorophyll a but decreased the maximum quantum yield of photosynthesis. Immunoassay of proteins separated electrophoretically indicated a reduction in the relative abundance of D1, the photosystem 2 reaction center protein. Changes in the abundance of D1 proteins relative to the light harvesting chlorophyll proteins (which were unaffected by the iron deficiency) suggest an increase in the fraction of nonfunctional reaction centers under iron-limited conditions. This is in contrast to the general thought that higher chlorophyll levels are indicative of healthy cells. Chlorophyll also fluctuates diurnally in nature and delivers varying results due to the changing contents within an organism within the mixed zone. Problems also arise because of the variability that exists between photosynthesis and the amount of chlorophyll produced between major groups and perhaps between species of phytoplankton.

Third, many of the early experiments used biomass as a means of determining nutrient limitation. Biomass measurements do not provide an accurate measurement of nutrient limitation and do not allow accurate conversion into growth rates (Banse 1991). Banse (1990) contends that the earlier results would be more conclusive if cell specific division rates had been examined. The problem with biomass measurements as indicators of nutrient stress (in container experiments) is that containers can create unbalanced growth conditions where photosynthesis proceeds producing carbohydrates but the synthesis of critical cellular constituents such as proteins are restricted by the limiting nutrient(s). Biochemical and physiological capabilities change, as does the rate of cell division.

Finally, the use of containers presumably excludes some trophic level(s) or perhaps disrupts a tightly coupled interaction between the phytoplankton and grazers which may impact phytoplankton standing crops (Cullen 1991). In addition to excluding grazers the use of containers removes other disturbances which constrain phytoplankton growth (de Baar 1990). When Martin and Fitzwater (1988) examined iron deficiency in the northeast subarctic Pacific, a 2-day lag period followed the iron addition; this was assumed to be the result of physiological shock. It is possible that this was caused by over-exposure to supersaturating intensities of solar irradiance (Cullen 1992), which thus illustrates the artificial aspects of container experiments.

RECENT FIELD EXPERIMENTS

New container experiments have demonstrated that iron has a particularly positive effect on phytoplankton biomass (Martin et al. 1988, 1989, 1990, 1994). What distinguishes these experiments from the earlier ones is that trace-metal clean methods were used (Fitzwater et al. 1982).

Subarctic Pacific

The first of these "clean" trace metal addition experiments was performed at station PAPA, in the northeast subarctic Pacific. Overall, chlorophyll in the containers increased in proportion to the amount of iron added (Martin et al. 1988). Enrichments with nanomolar amounts of dissolved iron resulted in a nearly complete utilization of excess NO_3^- ; whereas phytoplankton in the control consumed only 25% of the available NO_3^- . Martin and Fitzwater (1988) used NO_3^- uptake rates to estimate production rates of the different size fractions (Coale 1991; Cullen 1991; Banse 1990), and community division rates were estimated under the assumption that the decrease of nitrate was linearly related to algal growth (Banse 1991; Martin 1989). Banse (1991) cautions that nitrate uptake is a poor substitute for direct growth measurements because calculations are heavily affected by the precision of the initial nitrate determinations. The activity is apt to be constant during exponential growth if phytoplankton in balanced growth, and uptake of all nutrients occurs in constant proportions relative to the increase in cell number. Depletion of nitrate is a more reliable indicator of autotrophic biomass production than increases in chlorophyll concentration, since it is less sensitive to the effects of grazing. Grazers can deplete chlorophyll concentration rapidly, to an underestimate of the available biomass production. If, however, a constant relationship between biomass production and nitrate uptake, can be assumed, then the rate of primary production can be estimated from measurements of nitrate depletion, regardless of the ultimate fate of the new phytoplankton biomass. The doubling rate of cumulative nitrate uptake should be faster than that of chlorophyll increase which has been found to be the case (Banse 1991).

Martin conducted iron enrichment container experiments in conjunction with VERTEX (Vertical Transport and Exchange) in the Gulf of Alaska and showed that atmospheric Fe input rates were sufficient to support moderately high growth rates of primary producers. However, not enough iron was available to support the high growth rates that would lead to major nutrient depletion (Martin et al. 1989). In this study, hydrographic patterns were linked with abundance of iron and macronutrients. Enhanced iron input occurred along the Alaska continental margin, where normal NO_3^- surface depletion was observed (Fig. 4) but HNLC conditions prevailed in most of the Gulf of Alaska .

Martin et al. (1989) conducted iron-enrichment container experiments in conjunction with VERTEX. They observed change in phytoplankton community structure once the treatments were enriched with iron. At one station the addition of iron stimulated the growth of a *Nitzschia* species instead of the coccolithophores which had grown up in response to added iron at a more offshore station. In another case, the natural community, (consisting of *Nitzschia* species, choanoflagellates, and *Chaetoceros atlanticus*) changed to one dominated by *C. atlanticus* upon addition of iron. Nitrate utilization patterns were similar to those observed at station PAPA (Martin et al. 1989); the controls used only 25% of available NO_3^- , but, upon an addition of iron, 98% of the available NO_3^- was exhausted in 5 days. Once again, they noticed that the amount of chlorophyll in the phytoplankton increased in proportion to the iron added and they concluded that iron may be more important than available nitrogen in determining global rates of new production by phytoplankton (Martin et al. 1989).

Southern Ocean

Martin's most recent container experiments were performed in 1990 in the Antarctic (Martin, Gordon and Fitzwater 1990): these tested the hypothesis that iron deficiency prevents phytoplankton from blooming and using up the 'luxuriant' supplies of the major nutrients found in vast areas of the Southern Ocean. High productivity ($3 \text{ g C m}^{-2} \text{ d}^{-1}$) was observed in the neritic waters of the Gerlache Strait ($\text{Fe } 7.4 \text{ nmol kg}^{-1}$) and low productivity ($0.1 \text{ g C m}^{-2} \text{ d}^{-1}$) was observed in the Drake Passage where dissolved iron was

very low ($0.16 \text{ nmol kg}^{-1}$). Since, Mn has also been demonstrated to enhance photosynthesis (de Baar et al. 1990; Coale 1991), it should be noted that Mn levels in the Drake Passage were also abnormally low ($0.08 \text{ nmol kg}^{-1}$). Martin et al. (1990) concluded

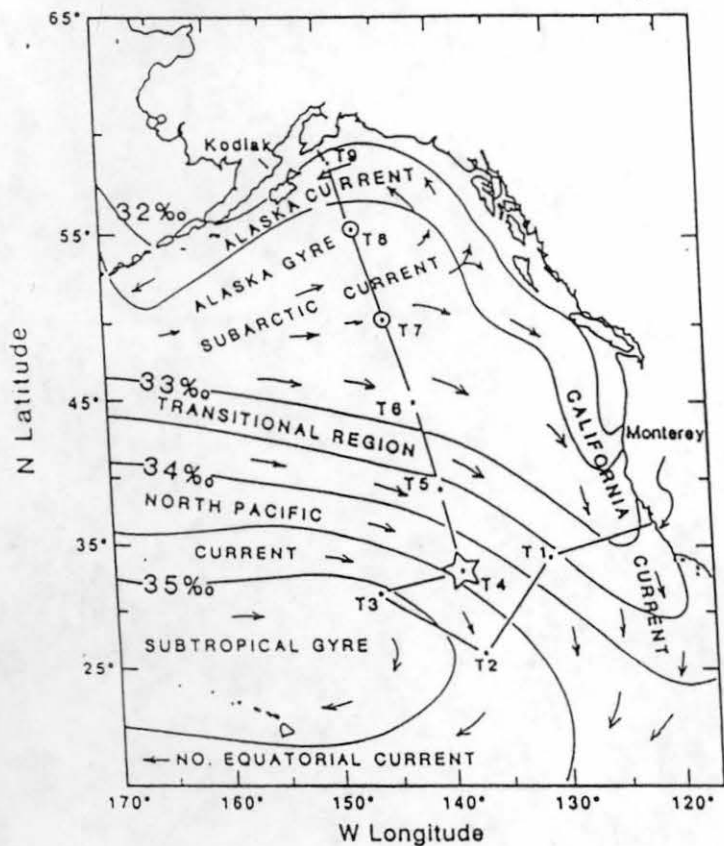


Figure 4a.

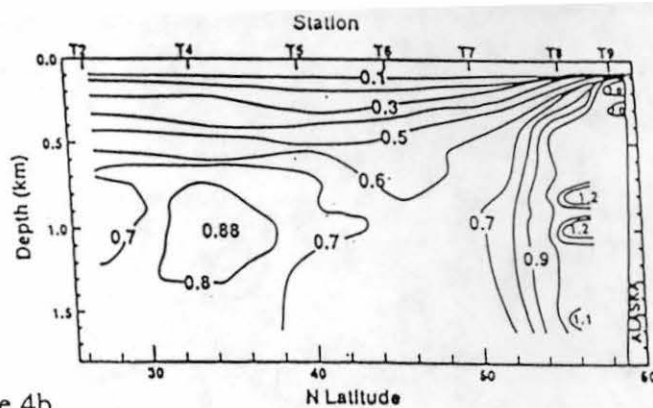


Figure 4b.

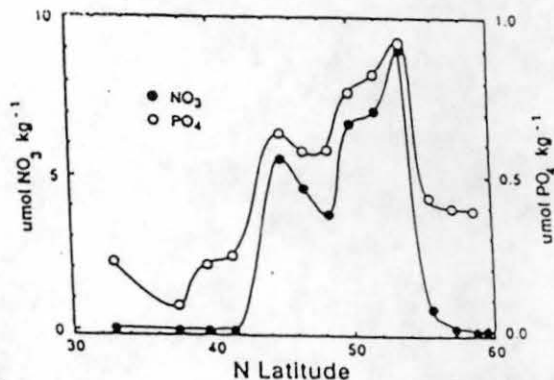


Figure 4c.

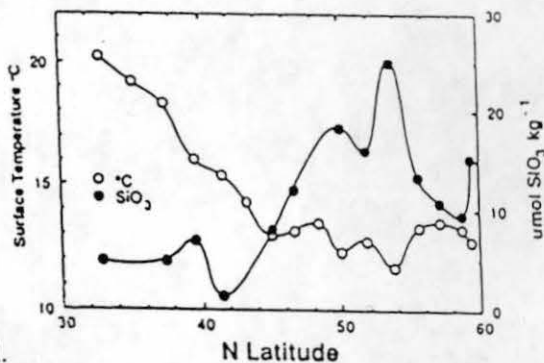


Figure 4d.

Figure 4. Hydrographic and nutrient data collected from the VERTEX study by Martin et al. 1989. Fig. 4a shows the Northeast Pacific surface current systems and surface haloclines. Fig. 4b shows the dissolved Fe section; station locations are shown in Fig. 4a. Fig. 4c & d shows the nitrate, phosphate and silicate concentrations and temperatures at 5 m depths vs. latitude on the Alaska transect. (Martin et al. (1989)).

that little iron was introduced by upwelling. Even though Martin et al (1990) comment on the fact that the world's lowest surface Mn concentrations occur in the Antarctic and suggest that Mn may limit primary production in the Southern Ocean, their focus in this and subsequent papers continue to be on iron limitation.

In the subarctic Pacific, nutrient assimilation rates suggest that phytoplankton are nutrient-stressed and therefore are not likely to be growing at their maximum rate (Falkowski 1980, as summarized in Coale 1991). Trace metals typically exist at concentrations of 0.1 nM Fe; 1.0 nM Mn; 1 nM Cu; 0.1 nM Zn (Coale 1991). A number of studies indicate that Zn, Mn, Fe and Cu are essential for growth of phytoplankton and suggest that any of these may act as ecological factors controlling phytoplankton distribution (Morel et al. 1991; Brand 1983; Gavis et al. 1981). In iron enhancement experiments in the Southern Oceans (Dugdale & Wilkerson 1991), showed that chlorophyll-specific uptake rates of nitrate were the same in controls and in the treatments. There was no difference between phytoplankton growth rates in containers where Mn, Cu or Zn were added in conjunction with Fe and containers where iron was added alone (Buma et al. 1991). However, in a different experiment, it was found that Mn and Fe had similar effects on growth as measured by the products of organic carbon (POC) to chlorophyll a ratio (de Baar 1990). Meanwhile, Coale (1991) observed that Mn additions enhanced chlorophyll by as much as 15%. In addition to complexation, certain trace metals can cause inhibitory effects: manganese can limit productivity, particularly for diatoms, possibly by interacting with other metals, and copper can have a deleterious effect on the growth of phytoplankton as well as that of grazers which would positively effect phytoplankton biomass (Coale 1991).

As noted earlier, de Baar et al. (1990) showed that added iron had a striking and positive influence on phytoplankton biomass from the Weddell Sea and surrounding waters. However, they also noticed that after seven days incubation, chlorophyll concentration in the unspiked containers had also increased relative to ambient values or *in situ* chlorophyll concentrations. The increase was not as great as that observed in

samples spiked with iron or manganese, but did suggest that experimental conditions in the controls were more favorable for phytoplankton growth than conditions in the field, obviously eliminating one (or more) growth suppressing factors which only exist in the field: such as turbulence regimes, grazing pressures by large herbivores (*Euphausia superba*) and a constant light regime. The usefulness of container experiments should not be discounted, however, as this design can help to examine species specific growth rates in response to iron additions. This type of experiment could be more informative if performed in parallel with natural enhancements to define or compare what actually accounts for the growth rates that deplete available macronutrients.

The Results Exhibit a Real Trend

Criticisms concerning the container methodology are valid in some respects. The evidence, however, of a physiological reaction when iron is added to a treatment is very pronounced. Trace-metal clean techniques have been developed which discount the theory that trace metal confounds any experimental results published after 1988 (Fitzwater et al. 1982; Martin et al. 1988; Cullen 1992). Secondly, the use of photosynthesis normalized to chlorophyll (P^B) relies on results derived from laboratory modeling. Two methods are most commonly employed: batch culture and continuous culture. Results from each model are valid but apply to different natural situations. Therefore one has to select carefully those data which determine the chlorophyll ratios from the appropriate laboratory model. So even though the use of chlorophyll increase as an indicator of specific growth rate can be a contentious issue, it has been used effectively in determining biomass growth rates (Dugdale & Wilkerson 1991). In relative terms, large increases in chlorophyll can still safely indicate biomass increase and indicate an effect of iron on community growth rates (Martin 1992).

IRONEX: THE FIRST OPEN OCEAN ENRICHMENT EXPERIMENT

The criticism of the experimental and methodological approach of the early container enrichment studies, discussed previously (Cullen 1991; Lloyd 1991; Sunda et al. 1991; de Baar et al. 1990; Banse 1990a, 1990b, 1991), prompted Martin et al. (1994) to perform an *in situ* enrichment experiment in the eastern Equatorial Pacific near the Galapagos Islands. Testing a hypothesis under natural conditions in the open ocean, had virtually never been attempted before (Cullen 1995). An HLNC region was delineated using GPS (global positioning system) attached to drogued buoys. Iron was added with an inert tracer, SF₆, which aided in tracking the patch of dissolved iron within the ecosystem. The experiment lasted 8 days during which the following parameters were extensively monitored within the iron-enriched patch: primary productivity; species composition; chlorophyll; FRR fluorometry; fluorescence; pigments; nutrients; particulate organic carbon; particulate organic nitrogen; dissolved organic carbon; dimethylsulphide; dimethylsulphoniopropionate; dissolved and particulate trace metals; halocarbons; and hydrographic and CO₂ system parameters. In addition to the enrichment study, a LNHC plume downstream from the islands was located using satellite imagery and hydrographic surveys. This was also monitored and served as a basis for comparison with the enrichment site (Martin et al. 1994).

The results demonstrated a direct and unequivocal biological response of the equatorial Pacific ecosystem to added iron. The biological response included a dramatic increase in fluorescence within 24 hours, an increase in all size fractions of phytoplankton, a three-fold increase of chlorophyll (0.24 µg l⁻¹ to 0.65 µg l⁻¹) as well as carbon (10-15 µg C l⁻¹ d⁻¹ to 48 µg C l⁻¹ d⁻¹), increases in DMSP (dimethylsulphoniopropionate), lower dissolved CO₂ in the patch than outside of it, and a decrease in ammonia from 0.45 µM to 0.12 µM within the patch.

The largest contributors to the plankton biomass were *Synechococcus*, red fluorescing picoplankton, and photoautotrophic dinoflagellates. Diatoms were a small

fraction of the total plankton biomass (17%) but demonstrated increases similar to the picoplankton (Martin et al. 1994). Heterotrophic biomass increased by 50% over the course of the patch experiment and was dominated by heterotrophic dinoflagellates and ciliates; however, levels of pheophorbide found in the samples did not indicate a lot of grazing overall (Martin et al. 1994). The increase in heterotrophic biomass was rapid and appeared to level off quickly (Martin et al. 1994; Banse 1995).

The chemical response of the enrichment was contrary to what one would expect; the nutrient measurements indicated little or no systematic difference in nitrate, phosphate and silica within the mixed layer inside and outside the iron-enrichment patch. As mentioned earlier, ammonia was consistently lower inside the patch than outside the patch ($0.45\mu\text{M}$ outside the patch; within the patch $0.12\mu\text{M}$).

The LNHC region was located west of the Galapagos Islands and in association with the nutrient-rich, westerly flowing south equatorial current. Because nutrients were depleted in this chlorophyll-rich water, an effort was made to determine if detectable anomalies of iron were present and, if so, how this related to increased levels of chlorophyll and primary production. High chlorophyll (from 0.7 to $13\mu\text{g l}^{-1}$) and high iron concentrations (1.3 - 3 nM) correlated with a nearly complete exhaustion of nitrate. The high iron found in the LNHC plume was associated with an upwelling water mass that contacted the Galapagos shelf and had a higher salinity and lower temperature than the surrounding water masses. The highest fluorescence signals always occurred at the interface between these upwelled water masses and the surrounding water (Martin et al. 1994), implying that iron limitation was relieved by iron input from the Galapagos shelf.

Other experimental techniques used to determine nutrient limitation and photosynthetic activity include Fast Repetition Rate (FRR) fluorometry and ^{14}C labeling. Greene et al. (1992) and Kolber et al. (1994) have demonstrated that iron does have an impact on photosynthetic efficiency and other physiological aspects in all sizes of phytoplankton. ^{14}C uptake, another technique used in the early container experiments, has proven to be fairly accurate (Minas et al. 1986). As yet, it is not unequivocally

demonstrated that grazing does or does not keep biomass at threshold levels. This is a very difficult interaction to illustrate using container experiments (Cullen 1995).

IronEx: The Aftermath

The IronEx experiment demonstrated an unequivocal biological response to iron additions in the equatorial Pacific. The response observed in the fertilization experiment was similar in magnitude and character to the increased production and chlorophyll found in the LNHC Galapagos plume (Martin et al. 1994). Productivity on a unit carbon basis, as determined by estimates of cell biovolume, indicated a 50% increase in doubling rate (1.0 d^{-1} to 1.5 d^{-1}). Increases in DMSP, a metabolic product of some phytoplankton taxa, also indicated increased primary production, biomass, and/or predation levels within the patch. Microscopic examination of samples from inside and outside the patch showed that microheterotrophic biomass increased by 50% over the course of the patch experiment and accounted for the removal of most of the phytoplankton biomass (Martin et al. 1994).

Recent evidence supporting the iron hypothesis came from the use of Fast Repetition Rate fluorometry (FRR) and fungal siderophores to examine the effects of iron on the phytoplankton communities. FRR enabled Kolber et al. (1994) to examine the changes in photochemical energy conversion efficiency of the natural phytoplankton community before and after the addition of iron in a small area ($7.5 \times 7.5 \text{ km}$) of the equatorial Pacific Ocean. Their results showed that iron limits phytoplankton photosynthesis in all size classes in this region by impairing intrinsic photochemical energy conversion (Fig. 5). The conclusion is that the growth of phytoplankton in the equatorial Pacific is physiologically limited by iron. Iron-limited cultures of *Dunaliella tertilecta* and *Phaeodactylum tricornerutum*, had reduced cellular chlorophyll concentrations, but there was an increase in the *in vivo*, chlorophyll-specific, optical absorption cross-sections. Thus, although more light was absorbed per unit of chlorophyll, iron limitation reduced the transfer efficiency of excitation energy in photosystem II. The half-time for

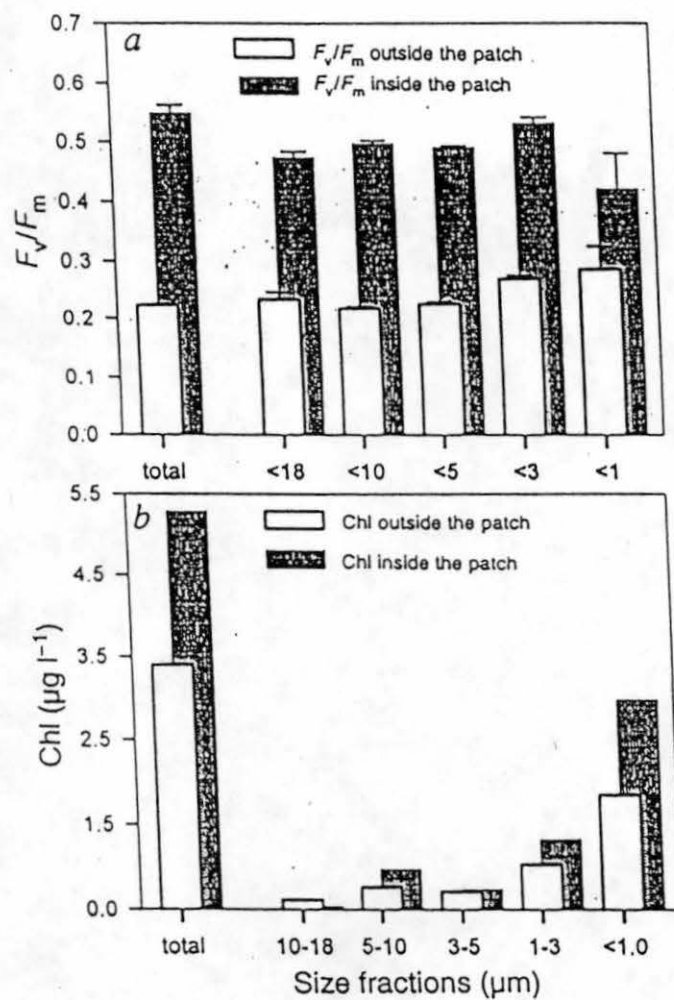


Figure 5. Changes in F_v/F_m (a) and chlorophyll concentrations (b) in 4 size fractions inside and outside the iron-enriched patch 2 days after the beginning of the iron enrichment. F_v/F_m , is a quantitative measure of the quantum efficiency of PSII for an ensemble of reaction centers. Note the relatively large and statistically significant increases in F_v/F_m in all size fractions in the iron-enriched waters, indicating ubiquitous physiological limitation of photosynthetic energy-conversion efficiency. (Kolber et al. (1994)).

the oxidation of the primary electron acceptor of photosystem II, calculated from the kinetics of decay of variable maximum fluorescence, increased two-fold under iron limitation (Greene et al. 1992). Using a fungal siderophore, Wells et al. (1994) showed that ambient levels of iron in equatorial Pacific HNLC surface waters are not strongly rate limiting for picoplankton communities. It is interesting to note that, even though iron appears to be a limiting nutrient for larger phytoplankton growth, there is evidence that suggests iron, like nitrogen and phosphorus, is recycled rapidly within the microbial loop allowing for maintenance of moderate growth rates of picoplankton, even in areas of low iron concentrations (Fig. 6; Hutchins et al. 1993).

Martin et al. (1994) proposes three hypotheses, each of which could potentially explain why nutrients were not fully depleted and chlorophyll levels did not increase more dramatically during the IronEx experiment: 1) the depletion of another micronutrient prevented further growth; 2) grazing increased in the patch as a result of increased production and the system rapidly reached a new steady state; 3) iron was lost from the system due to colloidal aggregation and/or sinking of larger particles containing iron.

Martin et al. (1994) discount the first hypothesis by recalling their earlier container experiments. These iron addition experiments (conducted in trace-metal clean containers) did not demonstrate similar trends as the IronEx patch experiment: nitrate was completely exhausted in these instances. Additional testing of waters both in and outside of the patch did not reveal significant differences in Zn, Cu, Ni or Cd (Martin et al. 1994). One point not mentioned in this argument is that Mn has been proven to affect photosynthesis and yet was not tested (or mentioned) as a possibly depleted micronutrient.

The second hypothesis, that increased grazing exerted some control on biomass accumulation, was also discounted by Martin et al. (1994). High primary productivity accompanied by large depletions of major nutrients has been observed at other stations over regions of the Galapagos platform (Chavez and Brusca 1991, as cited in Martin et al.

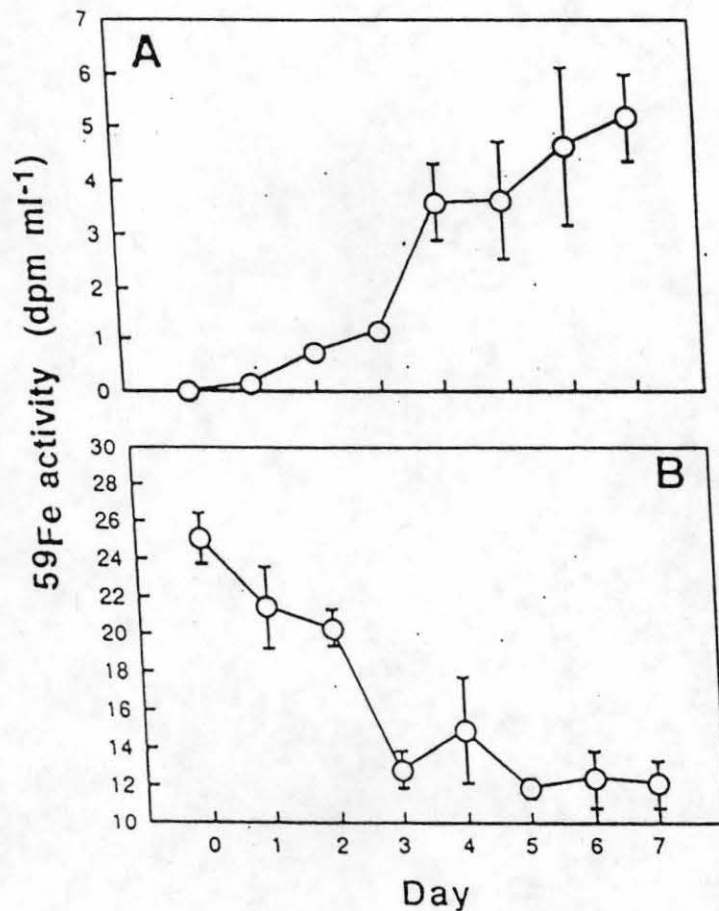


Figure 6. Transfer of ^{59}Fe activity from added *Synechococcus* WH7803 to the $> 5\mu\text{m}$ size fraction of equatorial Pacific seawater collected in August 1991. A. Activity in the $> 5\mu\text{m}$ size fraction. B. Activity in $0.2-5\mu\text{m}$ size fraction containing the added, labeled cyanobacteria. Error bars represent standard deviation of triplicate bottles. (Hutchins et al. (1993)).

1994). Although reduced grazing in bottle experiments is likely, there is no reason to expect a similar reduction in grazing pressures, over the shelf regions where high chlorophyll concentrations were observed. Interestingly enough, there was no mention of grazer studies performed within the LNHC plume. As this region demonstrated reduced levels of macronutrients (near exhaustion), valuable information concerning grazing could have been gathered.

Finally, the third hypothesis, that iron is lost from the system due to colloidal aggregations and/or sinking from large particles, was described as somewhat credible (Martin et al. 1994). Little is known about this loss term that occurs in open waters but does not occur in container experiments. Such loss term might result from the sinking of larger phytoplankton; this would mean that iron would be lost more rapidly from open ocean systems than from bottles or shallow waters. In shallow waters, the sinking iron should collect in a nepheloid layer near the bottom. Concentrations in the nepheloid layer along continental margins could then exceed 10 nM Fe and the upwelling of iron-rich water from a shallow nepheloid layer would continually resupply the euphotic zone with iron (Martin et al. 1994). This process may explain the presence of LNHC regions in shelf waters and could explain why macronutrients were not completely utilized in the IronEx patch experiment. Since the experiment was located in deep waters, sedimentation of added iron could have caused a return to iron-limiting conditions before macronutrients were consumed.

The IronEx study showed that added iron unequivocally increased primary production in the equatorial Pacific. However, a number of issues are left unresolved. For instance, what accounted for the slight decrease in major nutrients within the HNLC patch, but a near exhaustion of nutrient within the LNHC plume? Is it an issue of pulsed supply of iron versus a steady rate of supply? Is it an issue of micronutrient unavailability due to complexation or exhaustion? What accounts for the low representation of larger phytoplankton? Is the ecosystem nutrient or grazer controlled? What are the dynamics of a grazer-controlled system that exhibits nutrient depletion? If the HNLC ecosystems are grazer controlled, what accounts for the continual presence of high nutrients? Is removal

of iron due to sinking a real possibility in deeper waters, where the nepheloid layer tends not to be upwelled ?

ALTERNATIVE EXPLANATIONS FOR THE HNLC CONDITION

It has been argued that in all of the iron enhancement studies so far there has not been an unequivocal demonstration that HNLC regions exhibit Liebig limitation (Cullen 1995). There are however, a number of alternative hypotheses and explanations that may explain the HNLC condition. These include grazer control hypothesis and hypotheses based on the recycling rate, the presence and/or absence of other nutrients, curtailed division rates and/or the effects of physical processes on standing phytoplankton stocks and nutrient availability.

Ecosystem is Controlled by Grazers

This hypothesis states that standing crops of phytoplankton are maximal, and environmental stability allows for the development of a balanced food web that maintains low standing crops of phytoplankton (Walsh 1976; Banse 1990). Grazing has been suggested as a means of explaining the consistent low levels of chlorophyll despite active photoautotrophy and lack of net growth in the subarctic Pacific (Miller et al. 1991; Frost 1991) and equatorial Pacific (Walsh 1976; Cullen et al. 1992). As explained by Walsh (1976), biological processes are at a steady state when biomass remains at fixed levels. The picture illustrated by this argument centers around the structure of the phytoplankton community, the dominant types being the picoplankton which are grazed by microzooplankton. By discluding or disrupting one or more trophic levels from a container experiment, Price et al. (1991) found that grazing has a tight reign on the dominant small (picoplankton) phytoplankton. Estimated grazing rates in the subarctic Pacific account for approximately 70% removal of the picoplankton biomass (Miller et al. 1991; Frost 1991). Picoplankton are cropped quickly and kept at low-to-moderate levels (Frost 1987, 1991). Although no one has calculated a general figure for grazing in the eastern equatorial Pacific, Banse (1995) determined that 90% of the biomass generated during the IronEx patch experiment was removed by grazing. The response of the grazers was rapid, occurring within four days after the addition of the iron (Banse 1995; Martin et

al. 1994), and suggested that perhaps a new equilibrium had been created. Furthermore, the growth rate of unfettered phytoplankton would lead to an enormous increase in phytoplankton biomass within a short period of time which should easily deplete NO_3^- in the surface layer at the equator or anywhere else (Fig 7: Frost 1991). Instead, because loss processes largely balance cell division, chlorophyll and nitrate concentrations varied little (Walsh 1976; Price et al. 1991).

The Contribution of Other Nutrients to the HNLC Condition

Other nutrients may be more scarce than iron in the oceans. The annual cycle of nitrate shows a distinct seasonal cycle in the surface layer. Nitrate, for instance, was found to be the limiting factor off the coast of Baja California (Thomas 1969), and in the subarctic Pacific the build-up of nitrate has been shown to have a strong seasonal variation in properties of the physical environment which can have a significant impact on the specific growth rate of the phytoplankton (Miller et al. 1991). Nitrate is not fully depleted in the summer by the draw down because of the rapid recycling of nitrogen. This recycling could maintain the high surface nutrient levels seen in HNLC and the entire open subarctic Pacific (Anderson et al. 1969, as summarized in Frost 1991).

Even though ammonia is technically not a limiting nutrient in oceanic systems, it does exist at low concentrations (Fig. 8a). A preference for ammonia might account for the consistently high nitrate conditions observed within the HNLC regions (Frost 1991). Active regeneration of ammonia within the microbial food webs may maintain ammonium levels at near constant concentrations (Frost 1991; Martin et al. 1991; Miller 1991). Ammonia may be the preferred nitrogen source of the microphytoplankton (Price et al. 1991) and if high it suppresses nitrate uptake (Fig. 8b; Frost 1991). Ammonium is energetically less costly to utilize than nitrate and it also lowers cellular iron requirements because iron-containing nitrate and nitrite reductases are not required for nitrogen assimilation when ammonia is being utilized (Price et al. 1991).

Silicon, which is also required for diatom growth, can limit primary productivity by diatoms in the subarctic Pacific (Frost 1991) and may not be present at concentrations

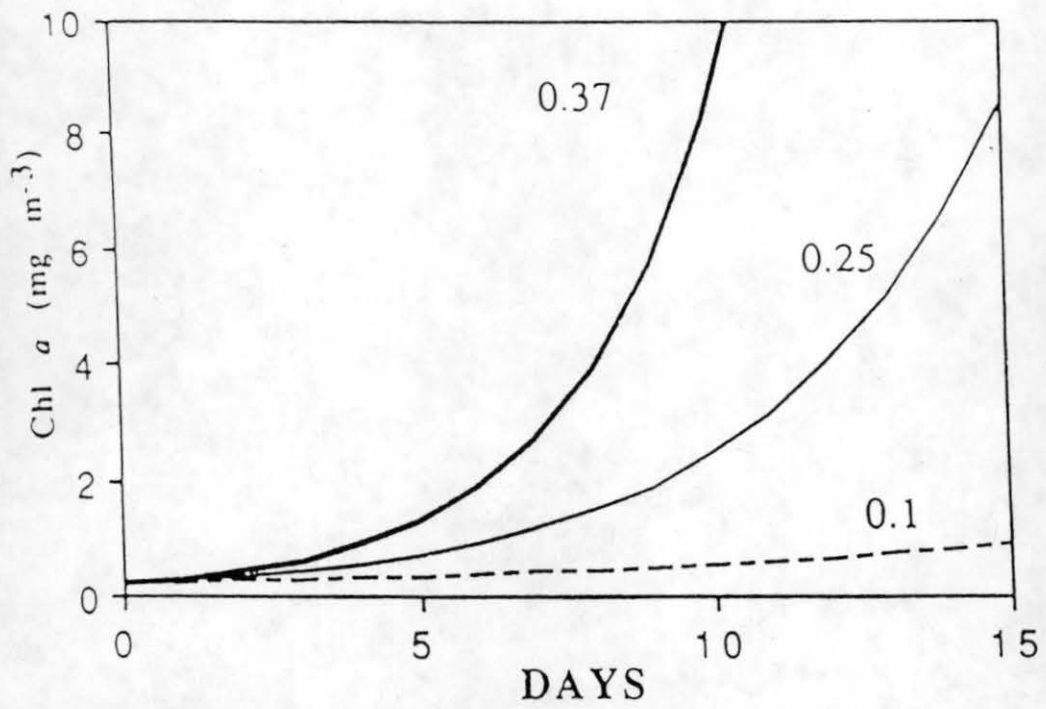


Figure 7. Increase in phytoplankton stock (initially at 0.2 mg Chl *a* m) projected for different net specific growth rates (d⁻¹) with no grazing. (Miller et al. (1991)).

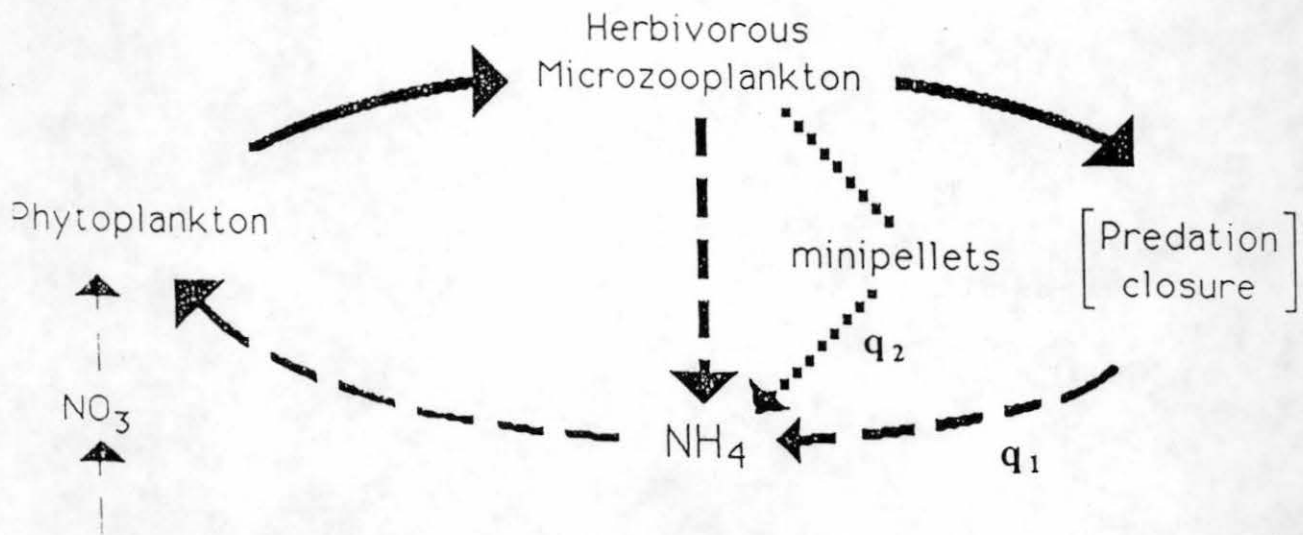


Figure 8a. Trophic interactions. Solid lines are phagotrophic links; broken lines are N recycling links [q_1 , fraction of herbivore mortality released as NH_4 ; q_2 , fraction of herbivore egestion ("mini-pellets") released as NH_4]; dashed line represents input of NO_3 by vertical turbulence and entrainment. (Miller et al. (1991)).

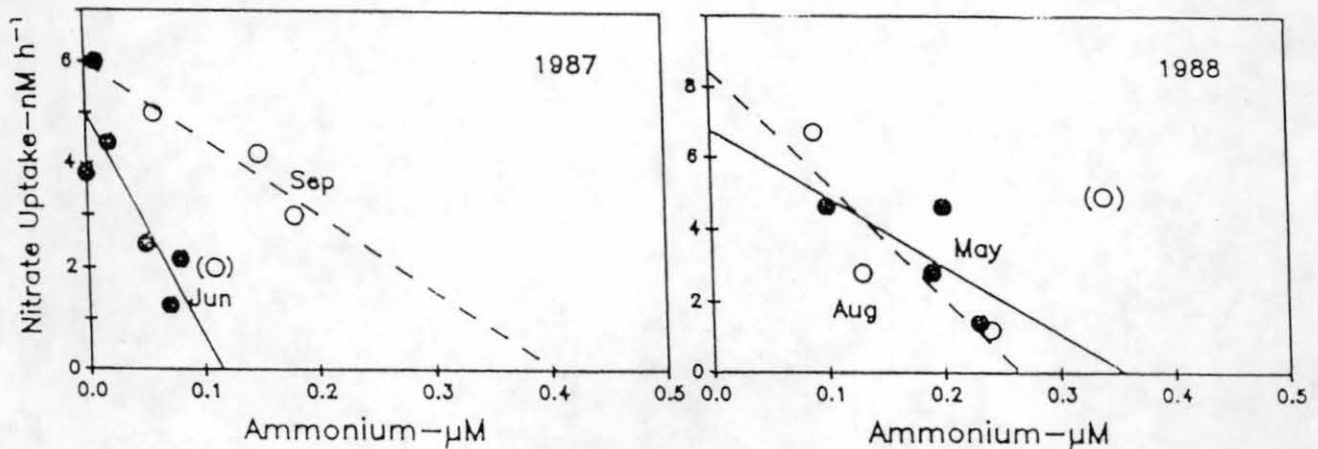


Figure 8b. Relationship between mid-incubation NH_4^+ concentration and NO_3^- uptake by phytoplankton during short-term incubations of natural phytoplankton populations in shipboard containers. Points in parentheses not included in regressions. NO_3^- uptake is suppressed by NH_4^+ concentrations frequently seen in the field. (Miller et al. (1991)).

high enough to induce silica uptake in other regions of the world ocean (Dugdale & Wilkerson 1991).

Temperature Limitation of Phytoplankton Division Rates

Division rates in the subarctic Pacific and Antarctic waters are similar to those in coastal, Fe replete, waters once the temperature is accounted for, according to Eppley's regression (1972: in Banse, 1991). It is possible that productivity is limited due to submaximal photosynthetic rates at low temperature, and this could be a reason why macronutrients are not being depleted. There are two implications of low temperature effects: (i) phytoplankton in the high-nutrient Southern Oceans might be growing so slowly that they cannot consume ambient nutrients, regardless of grazing pressures; and (ii) uptake capacity may not be saturated, so that the concentrations of some major nutrients might actually limit the specific growth rates of some phytoplankton. In the Antarctic, the half saturation rates were extremely high at 0°C, which indicates that the fairly high concentrations of macronutrients in the Antarctic waters might nonetheless be low enough to inhibit the growth rates of some phytoplankton (Cullen 1991).

The enhancement experiments conducted in the Southern Oceans exhibited less of an impact of iron on phytoplankton (division rates compared with the controls) (de Baar et al. 1990; Banse 1991; Frost 1991; Price et al. 1991; Cullen 1992). However, division rates went up by 50% (from 1.0 d⁻¹ to 1.5 d⁻¹) in the open ocean enrichment experiment, IronEx, when iron was added (Martin 1994). Directing his comments at the IronEx experiment, Banse (1994) argued that divisions of 1.0 d⁻¹ are near maximal and an increase to 1.5 d⁻¹ does not prove that iron markedly constrains phytoplankton growth (Banse 1991, 1995).

Physical Processes

Physical processes can also have a negative effect on biomass production. Deep winter mixing lowers the mean irradiance and entrained nutrients are not exploited because photosynthetic production does not exceed community respiration (Cullen 1991). Vertical mixing, as pointed out for the Southern Ocean by Mitchell et al. (1991), can also influence the concentration and growth rate of phytoplankton. However, in the subarctic

Pacific (Miller et al. 1991) and the equatorial Pacific (Cullen 1992), the effects of mixing on phytoplankton populations are minimal. Walsh (1976) suggested that persistent stratification and small horizontal gradients minimize losses due to mixing and these losses, due to dilution and sinking, are minimal. Thomas (1979) mentions that persistence due to advection takes place over thousands of kilometers off of Peru, yet nutrients are not depleted by phytoplankton. In comparing coastal to oceanic systems, Walsh (1976) mentions that a critical factor distinguishing coastal systems from oceanic ones was the greater physical disruption of the former on planktonic systems on the 5-10 day time scale (a temporal scale critical to the development of phytoplankton:herbivore interaction). It should be noted that the above mentioned disruptions to the predator:prey interaction primarily concerns the larger phytoplankton and slower-to-respond metazoan herbivores (i.e. diatoms vs. copepods) .

In comparing coastal to oceanic systems, one notices that neritic and oceanic diatoms, while differing in species assemblages, have adapted to the different levels of iron in the surrounding waters (Walsh 1976; Sunda 1991) with oceanic diatoms requiring a much lower external cell iron concentration for growth than do comparable coastal species (Sunda et al. 1991). Therefore, in open ocean systems the slower growing diatoms, with low iron requirements, dominate. When iron is added, the ubiquitous, faster growing coastal types, will dominate the blooms (Walsh 1976).

RECENT FIELD DATA SUPPORTING THE IRON HYPOTHESIS

Recently, de Baar et al., examined iron limitation in the Southern Ocean by comparing natural levels of productivity at sites in the Antarctic circumpolar current (ACC, well away from any shelf source influence) with those at sites in the Polar Front (PF, thought to be in contact with shelf sources). Total dissolved iron was measured in 120 seawater samples collected at 6 standard depths together with measurements of chlorophyll a, the partial pressure of CO₂ (P_{CO₂}) in seawater and air “along with observations by colleagues of many other variables”. These two areas differ markedly in overall iron availability and primary productivity. In the southerly branch, they suggest that upwelling of deep waters supplies sufficient iron to the surface to sustain moderate primary production but not enough to permit blooms to develop. Within the fast flowing, iron rich jet of the polar front (PF), phytoplankton biomass during the spring bloom was an order of magnitude greater than that in the southern ACC waters. As a result, the waters of the PF were marked by CO₂ undersaturation. The surface waters of the ACC had iron concentrations, measured as total dissolved iron, sometimes as low as 0.17 μM, whereas those of the PF were 2-4 μM. There was also a seasonal decrease in iron concentration at the PF which coincided with decreases in surface water CO₂ (10-15 μM), nitrate (1-2 μM), phosphate (0.1 μM), and silicate (10 μM) within the water column. Meanwhile chlorophyll levels increased from 0.8-1.6 to 1.2-2.4 μg l⁻¹ while the spring bloom evolved and rates of primary productivity were observed to be 1.2-3.0 g C m⁻²d⁻¹. Nevertheless, macronutrients were still available in high concentration (nitrate 24μM; phosphate 1.8μM; silicate 21 mM). Hence, their complete utilization was hampered by other factors such as light limitation due to intense storm mixing as well as grazing pressure. In comparison with the equatorial Pacific experiments, pulsed addition of iron caused only a small and transient CO₂ response, whereas the natural and continuous supply of iron in the PF produced a strong CO₂ response (de Baar et al. 1995).

INTEGRATING THE IDEAS

Critics of the IronEx study find fault with the accuracy of the growth rate determinations, which were calculated based on photosynthesis normalized to chlorophyll, and with the interpretation of the results, which discounted predation as a mechanism controlling the removal of macronutrients from the surface layer (Cullen 1991, Banse 1995). These criticisms have merit, but when used in a comparative context, results from the IronEx and subsequent field studies were meaningful. In particular, the findings from the Plume Study (Martin et al. 1994) and de Baar et al. (1995) data were powerful in that they reinforced the interpretation that the system was limited by iron which, if supplied at a steady rate, would create enough biomass to deplete the macronutrients and CO₂ in the surface waters. On the other hand there was evidence that something must be controlling biomass accumulation. Martin et al. (1994) call it a loss term while others attribute the high nutrient condition to grazing (Banse 1990) and others (Frost 1991; Miller et al. 1991) to permutations of nutrient limitation and grazing.

If one were to interpret the iron hypothesis strictly, then the evidence provided by the open ocean enrichment experiments nullifies an important prediction: all the nutrients should be used to depletion. This does not occur in either IronEx or the de Baar experiments (1995). However, there was some loss term in IronEx which cannot be accounted for (Martin 1994) and which may have caused phytoplankton to become iron limited before macronutrients were used up. Martin et al. (1994) discounted micronutrient complexation or colimitation and grazing as explanations for the fact that macronutrients were not exhausted in IronEx. If one examines the ambient growth rates (controls), they appeared to be moderately high and most certainly could deplete the available macronutrients if grazers were not there to crop them (Frost 1991). However, it was often argued by proponents of the Iron Hypothesis that, since new production was accounted for by the larger phytoplankton (specifically diatoms) nutrient limitation may be the main factor that keeps large phytoplankton in check even if grazing regulates standing

stocks of smaller phytoplankton (picoplankton and nanoplankton) (Chavez 1991; Barber and Chavez 1991). Growth rates of the larger phytoplankton were more rapid than those of their predators. The larger phytoplankton are not as tightly coupled to their grazers as were the picoplankton to their grazers. This would allow for rapid and near complete exhaustion of nutrients before the larger zooplankton were able to respond and constrain the bloom. If one assumes that the system was in a dynamic equilibrium, there might not be enough grazers around to respond rapidly to a bloom of large phytoplankton. Even so, not all large zooplankton subsist entirely on diatoms (Miller et al. 1991). However, it was not obvious that the larger phytoplankton were being controlled by grazing (Martin 1994).

Growth rates were maximal even in ambient conditions (within the controls), which means that there was enough iron to stimulate growth of adapted species (Buma et al. 1991; Sunda et al. 1991; Wells et al. 1994), but if iron were available continuously, then the more rapidly growing species would utilize the available macronutrients (Martin 1990).

The addition of iron typically influenced species composition. Diatom species certainly exist within the HNLC at ambient iron concentrations and grow well as populations, however, these diatoms have adapted to low iron concentrations and availability (Sunda 1991). The addition of iron causes species compositional changes and enhances the growth of those species that are not really adapted to the HNLC ecosystem (Walsh 1976; Martin 1988, 1989; Cullen 1991, 1992; Miller 1991). The ambient iron concentrations meet the picoplankton's low requirements (Hutchins et al. 1993; Wells et al. 1994), and it has been demonstrated that picoplankton recycle iron and make excreted iron available for uptake by larger phytoplankton (Hutchins et al. 1993).

Size structure

Within the phytoplankton there are two fairly separate community dynamics occurring simultaneously: a microbial loop and a linear path of new production involving the larger phytoplankton. Within the microbial loop there is rapid recycling because of tight coupling between predator and prey. Typically the protozoan grazers have a rapid division rate and can respond quickly to increases in biomass (Banse 1991a,b). 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 (Miller et al. 1988). Primary production utilizing NH_4^+ rather than NO_3^- dominates in such systems (Wheeler and Kokkinakis 1990). New production, on the other hand, is associated with the larger phytoplankton, which are not tightly coupled in a predator:prey relationship. New production is low in oceanic HNLC environments because large phytoplankton biomass is low, the net growth rate of phytoplankton approaches zero and production is overall more likely regenerated than exported (Cullen 1991).

It has already been noted that phytoplankton species composition changes with the addition of iron to a treatment (Martin et al. 1989; Menzel 1963). Is this because an already existing low inoculum of neritic type diatoms will dominate the community composition when iron is available (Banse 1991; Cullen 1992) ? Is it that large diatoms are always present but have adapted to the low iron concentrations ? or utilize ammonium as a nitrogen source therefore requiring reduced amounts of iron (Banse 1994; Frost 1991; Cullen 1991) ? Walsh (1976) contends that there is a low inoculum of large phytoplankton that are neritic in nature and can utilize iron rapidly but subsist under low iron conditions. In an earlier paper, Banse (1991) had results suggesting that species are well suited to the environmental controls and that simultaneous, size dependent grazing pressure tends to dominate natural communities.

Is The HNLC a Grazer Controlled Nutrient Limited Ecosystem ?

In Hart's initial observations (1934) he states that "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." Recently there have been a number of experiments that have helped to piece a realistic picture of the dynamics within the HNLC regions (Miller et al. 1991; Price et al. 1994; Wells et al. 1994; de Baar et al. 1995; Kumar et al. 1995). These syntheses emphasize one or the other argument in this puzzle.

Miller et al. (1991) compared an HNLC in the Pacific and a LNHC system in the Atlantic, and made comparisons between them. He found that productivity in each is

different due to the nature of available nutrients. The subarctic Atlantic LNHC system is characterized by high mixing, low nutrients, and repeated physical disturbances. The subarctic Pacific HNLC system is characterized by shallow mixing, high nutrients, and exhibits relative steady-state equilibrium conditions. In each region the grazing strategy was different. In the Atlantic (LNHC), he found that the principal macrozooplankton were copepods (*Calanus finmarchicus*) that produced eggs which hatched according to the time of year and abundance of food. In the eastern subarctic Pacific (HNLC), the grazers, also copepods (*Neocalanus*), produce eggs that had less yolk, hatch year-round and produce nauplii that are able to begin active foraging. This suggests that the HNLC region had reached some sort of steady state. He also found that the growth rates of phytoplankton were similar, but that the conditions for year-round growth rates were very different, and he attributed this to the deep water mixing (LNHC) and intensity of storms which uncoupled the predator:prey interactions.

As has been mentioned previously the dominant primary producers in HNLC regions are the picophytoplankton, which are tightly coupled to their protozoan grazers. Iron requirements by this group of phytoplankton are minimal (Wells 1994) and tend to be met by regeneration (Hutchins et al. 1993) while the excesses of iron get absorbed by larger phytoplankton. The nitrogen source is also conserved within this microbial loop as ammonia (Frost 1991; Martin 1991; Miller et al. 1991), ammonia is the preferred nitrogen source and is energetically less expensive to utilize. It is also known that the picoplankton, being tightly coupled to their protozoan predators, are therefore constrained and prevented from utilizing all the available nutrients. New production in these ecosystems is accounted for by the larger phytoplankton and results in the consumption of nitrate. Iron is necessary to generate the enzymes to utilize nitrate/nitrite as a source of nitrogen. Since the larger phytoplankton are generally found in lower numbers and are not as tightly coupled to the grazers that feed upon them (Miller et al. 1991), it is then possible that the factors controlling the presence of the larger phytoplankton might be responsible for limiting new production in these regions.

This scenario suggests a “grazer-controlled phytoplankton population in an iron limited ecosystem” explanation for the occurrence of HNLC’s (Price et al. 1994). A study conducted in the equatorial Pacific Ocean was designed to assess the role of iron and grazing in regulating the use of nitrate by phytoplankton communities (Price et al. 1994). Ammonia concentrations acted as an inhibitory agent limiting nitrate uptake. When grazer activity was artificially suppressed, phytoplankton growth rates and biomass increased, and nitrate was consumed by rapidly growing photoautotrophs perhaps due to the decrease in ammonia levels caused by the lack of grazing. The observed rapid rates of iron uptake demonstrated that these rates were under physiological regulation and were elevated in response to the low iron concentrations. In this experiment the larger phytoplankton exhibited a physiological response whereas the biomass of the small phytoplankton were “unaffected” by the iron enrichments. They concluded that HNLC conditions favor growth of small phytoplankton that are grazed efficiently and use ammonia preferentially, while the larger phytoplankton are constrained due to limitation of iron. An alternative explanation is that there was an all size fraction response of phytoplankton to the iron additions, however, grazing amongst the picophytoplankton was so efficient that picophytoplankton numbers exhibited “no response”.

While this hypothesis provides an excellent context for experimentation and discussion, it is not amenable to simple comprehensive tests and does not explain all the results from the enrichment experiments (Cullen 1995). The explanation proposed by Price et al. (1994) fits a modified iron hypothesis that Cullen (1995) calls the “ecumenical iron hypothesis” where no single factor regulates primary production, but the interplay of factors is key. Based on imprecise criterion concerning the modeled values for growth rates in nutrient limited environments, (μ/μ_{max}), the distinction between physiological limitation by iron and grazing controls of small cells are fuzzy at best (Cullen 1995). A central tenet of the ecumenical iron hypothesis is that large cells will dominate the response to iron enrichments. IronEx, on the other hand illustrated a strong response of the smaller phytoplankton to the addition of iron. Kolber et al. (1994) also demonstrated an apparently strong physiological response to iron by the smaller phytoplankton.

CONCLUSION

The debate concerning the Iron Hypothesis can be described by the following quote: “we are 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, in the accomplishment of the total result.” (Chamberlain 1965).

The case for iron limitation in HNLC regions seems convincing since iron addition consistently stimulates biomass production. The major HNLC regions throughout the world's oceans have all been examined at some level, and similar results have been observed in each. The most dramatic of these are the findings by de Baar et al. (1995) who compared the Antarctic Circumpolar Current to the Polar Front and demonstrated high productivity in iron rich waters to the point of CO₂ undersaturation without depletion of the macronutrients of the surface waters. Additionally, IronEx demonstrated that iron enhancement in nature does stimulate biomass production, yet neither CO₂ nor macronutrients were depleted from the enriched patch. In contrast to de Baar's (1995) findings, the LNHC plume study illustrated that (i) nutrient levels, as indicated by NO₃⁻ measurement, were close to being completely exhausted and (ii) there was a significant reduction in CO₂.

The argument that grazing helps to create HNLC conditions is also compelling but this hypothesis is more difficult to unequivocally demonstrate experimentally (Cullen 1995). Without some control, phytoplankton populations in HNLC regions would multiply exponentially within a short period. Unchecked, exponential growth would result in high biomass accumulation, and would utilize all the available macronutrients (Frost 1991). This occurs in LNHC regions, where the system has lost equilibrium and nutrients had become depleted (Miller et al. 1991).

De Baar (1990) warns that each HNLC is different and that different mechanisms may operate in different HNLC regions. Cullen (1995) suggests that the macronutrient inputs to the equatorial Pacific Ocean might be episodic and patchy, and, consequently,

the nutritional needs of natural phytoplankton assemblages may vary in space and time. It should be recognized that the results of the bottle experiments, IronEx or subsequent enrichment experiments might not apply generally to all HNLC regions. However, these experiments aided in the understanding of an HNLC of the eastern equatorial Pacific. One *in situ* iron enhancement experiment such as the IronEx experiment could be considered too ephemeral and is certainly not applicable to the understanding of all HNLC regions. Therefore, multiple other experiments of similar nature should be undertaken in other HNLC regions throughout the world.

Based on the criticism of the previous container enrichment experiments, future experiments should utilize cell counts to estimate species-specific growth rates. Care should be taken to select model data from the appropriately executed laboratory experiment (or as close to the natural condition as possible; Cullen 1992). Inasmuch as it is possible to explore the physiology of nutrient limitation in the laboratory, of the two models most commonly used, batch and continuous culture, selection should be based on the site-specific hydrographic observations, such that the results mimic as much as possible the natural conditions. From the laboratory physiology experiments, one could develop new criterion in order to test nutrient limitations. Geider & La Roche (1994) suggested an alternative approach for diagnosing iron-limitation which uses molecules whose abundance increase in response to iron-limitation. These include cell surface iron transport proteins, and the electron transfer protein flavodoxin, which is known to replace the Fe-S protein ferredoxin in many iron-deficient algae and cyanobacteria.

Recommendations for Future Research

Future experiments should focus on the long term effects of iron enrichments. For instance, Cullen (1995) suggests creating a station where predetermined amounts of iron are regularly added directly into the surface layer or via an aerosol. Aerosol inputs might be favored in such an experiment since this is the most common form of natural input (Duce et al. 1991). The station should be available to all interested scientists, which would encourage interdisciplinary interactions, and hopefully, generate new viewpoints as well as active discussion. Enrichments could be subdivided into quarterly periods to

coincide with the seasonal fluctuations in biomass, physical processes, etc. Since one criticism of IronEx was that it was not truly 'oceanic', more effort would have to go into selecting a site meeting the criterion of 'open ocean'. It might be worthwhile to examine varying degrees of HNLC. For instance, one could examine how community dominance as well as trophic interactions vary throughout a transect of an HNLC region.

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