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von Liebig's Law of the Minimum and Plankton Ecology (1899-1991)

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Abstract – The Law of the Minimum was originally formulated by Justus von Liebig, as one of the 50 interlinked laws concerned with agriculture. The original writings of J. von Liebig often were misinterpreted by his successors. BRANDT (1899) took this one law out of its context and proposed that limitation by nitrogen is a dominant factor in plankton ecology, far beyond its original application to agriculture. This was opposed by NATHANSOHN (1908) who suggested instead a dynamic balance of growth and loss terms. Towards validating, or eventually falsifying Brandt's hypothesis, Atkins, Harvey, Cooper and others developed the chemical methods necessary for re-defining ocean nutrient cycling and growth limitation. The major exception to these modern perspectives was the Antarctic Paradox of high nutrients and low chlorophyll which inspired Gran, Atkins, Harvey and Cooper to pioneer the concept of iron limitation. An exhaustive overview is given of efforts to define Fe in seawater and its controlling effect on *in situ* plankton growth, for the 1920-1984 period. Somewhat parallel work in the laboratory on single species of algae in chelation-controlled media has provided much insight, but is sketched only briefly. Martin and contemporaries developed the chemical methods necessary for defining the ocean chemistry of Fe and its role for *in situ* growth. These developments are sketched for the 1982-1991 period. Once again the Law of the Minimum and associated bold hypotheses served, albeit briefly, to bring a nutrient element in the forefront of research. This, and the recent awareness of CO_2 as rate limiting factor, underline the conclusion that advances in sciences often hinge on advances in technology, confirming KUHN (1962). In this case the new analytical techniques developed by Atkins, Harvey, Cooper, Martin and their associates have proven revolutionary for plankton ecology. Some observations in plankton ecology may be reminiscent of the agricultural Law of the Minimum, but this would not warrant its direct application, beyond its original context and agriculture, to plankton ecology. Rather the net rate of increase of phytoplankton is the dynamic balance of multiple growth and loss terms, together also determining the biomass at given time and space.

CONTENTS

1. INTRODUCTION

July 27, 1676, was a warm sunny day with a gentle onshore breeze. A curious gentleman had visited the seaside at Schevelinge (now Scheveningen) in the Netherlands, where he noticed various very tiny animals in the sea-water. He had bought a new glass bottle which he gave to a man who was going into the sea to wash himself, asking him to rinse it two or three times and then fill it with seawater. He sealed the bottle and returned home to nearby Delft. There he viewed it through his microscope and saw a very small animal. This was the first ever observation of a marine plankton species (VAN LEEUWENHOECK, 1676) which, from the description, is now tentatively identified as *Mesodinium rubrum* Lohmann (TAYLOR, BLACKBOURN and BLACKBOURN, 1971; TAYLOR, 1981). During the following days very little was found in the bottle, but on 31 July he clearly saw some 100, but now much smaller (i.e. phytoplankton) and also of different shape and very clear. These small specimens were seen again on 2 and 4 August, but by 8 August only a very few and minute ones were left, and so he abandoned his observations. This constituted the first, albeit unintentional, bottle incubation, where a population, first unnoticed, had apparently multiplied in exponential fashion (VAN LEEUWENHOECK, 1676; incomplete translation published in 1677; see also DOBELL, 1932).

Some 200 years later, Victor Hensen in Kiel became convinced that such microscopic algae were the base of the marine foodchain. In contrast some of his contemporaries, like Haeckel, were still adhering to the belief that all (organic) foodstuff was brought into the sea by rivers. Hensen with fine precision had shown the abundance of plankton collected in his standardized nets. Also at Kiel junior colleague Lohmann demonstrated that the sea contained many, many more very small organisms dubbed nanoplankton (erratically Lohmann used the word nannoplankton). This inevitably highlighted the inadequacy of the net-sampling protocols of Hensen which previously had seemed rigorous. Nevertheless, between them, Hensen and Lohmann, and several others, observed that plankton varies widely in abundance and by region and season. So what factors could

be responsible for these variations in phytoplankton biomass? Ever since then this has been one of the central issues in marine ecology:

"What governs the growth and biomass of phytoplankton?"

Throughout the past century this problem has led repeatedly to intense scientific debate between those advocating one limiting factor and others contemplating the complex interaction of many environmental controls. The early history (1870-1960) of biological oceanography has been described most extensively and admirably by MILLS (1989; for comment on Lohmann, see BANSE, 1990b). Here only the issue of the control of phytoplankton will be briefly sketched in chronological order, focusing on the intense controversies centred around the chemical elements nitrogen and phosphorus. The complete historical development is given for the emergence of iron as a prime limiting factor of the oceanic plankton community, leading to the major breakthroughs in the decade 1982-1992. The Leitmotiv (red thread) is the remarkable parallel between the hypotheses of BRANDT (1899) and MARTIN and FITZWATER (1988). Finally I will summarize some common trends in the evolution of our knowledge which can now be discerned.

With hindsight one may easily see the 'truth' where in their time earlier researchers remained perplexed. Inevitably the reader and the author will tend to take sides when witnessing this historic debate. Yet this account is meant as a tribute to all the actors, to Brandt, Gebbing and Nathansohn, to Atkins, Cooper and Harvey, and last but not least to John Martin, a man of remarkable intellect and drive who stirred up great excitement and controversy. He has prematurely vacated the center stage, leaving his work incomplete but it is still a stimulating legacy which will continue to generate considerable admiration and debate amongst his contemporaries and successors.

1.1 Concepts of phytoplankton growth and biomass

When discussing limitation of algae it is crucial to define whether it is the growth rate or the biomass that is of concern. For a given area or volume of sea-water the mass balance of algal biomass is the difference between growth rate of phytoplankton and various loss terms:

$$\text{NET INCREASE} = \text{PRIMARY PRODUCTION} - \text{LOSS TERMS} \tag{1}$$

or

$$\text{NET INCREASE} = (\text{PHOTOSYNTHESIS-RESPIRATION})_{\text{Algae}} - (\text{GRAZING} + \text{SEDIMENTATION} + \text{LYSIS}) \tag{2}$$

The importance of losses resulting from grazing cannot be underestimated (FROST, 1987; BANSE, 1992; FROST and FRANZEN, 1992), especially where size selection can exert control on the community structure (RIEGMAN, KUIPERS, NOORDELOOS and WITTE, 1993). Similarly loss by sedimentation is commonly observed during bloom conditions and lysis, even in the absence of grazers, is conceivable, in which viruses may play a role (BRATBAK, EGGE and HELDAL, 1993). For the sake of the argument changes resulting from physical transport (advection, mixing) are here neglected. In general, however, it is the variations in the rates of production and loss that determine the standing stock (RILEY, 1946). Nevertheless when in a situation where the algae are growing faster than they are being eaten, upon several days an essential nutrient, e.g. nitrogen, will run out so that growth ceases; the classical spring bloom scenario. Just about at this time the phytoplankton biomass has then reached its maximum yield, a concept often, but incorrectly (as discussed below) associated with the name of J. von Liebig (e.g. PARSONS, TAKAHASHI and HARGRAVE, 1984; CULLEN, YANG and MACINTYRE, 1992).

This mass balance (Eqs 1 and 2) is a simplified description, which ignores multiple and complex interactions between phytoplankton, zooplankton, bacteria as well as the organic and inorganic

substrates of the real ecosystem (Fig.1). The intricate and ever changing structure of the pelagic foodweb deserves considerable attention (e.g. FASHAM, 1984; HEWES, HOLM-HANSEN and SAKSHAUG, 1985; SHERR and SHERR, 1988; BJO/RNSEN and KUPARINEN, 1991a,b; LANCELOT, BILLEN, VETH, BECQUEVORT and MATHOT, 1991) and is currently the subject of intense investigation, in projects ranging from detailed study by an individual scientist to large international programmes like the Joint Global Ocean Flux Study (DE BAAR, FRANSZ, GANSSEN, GIESKES, MOOK and STEL, 1989; DUCKLOW and HARRIS, 1993).

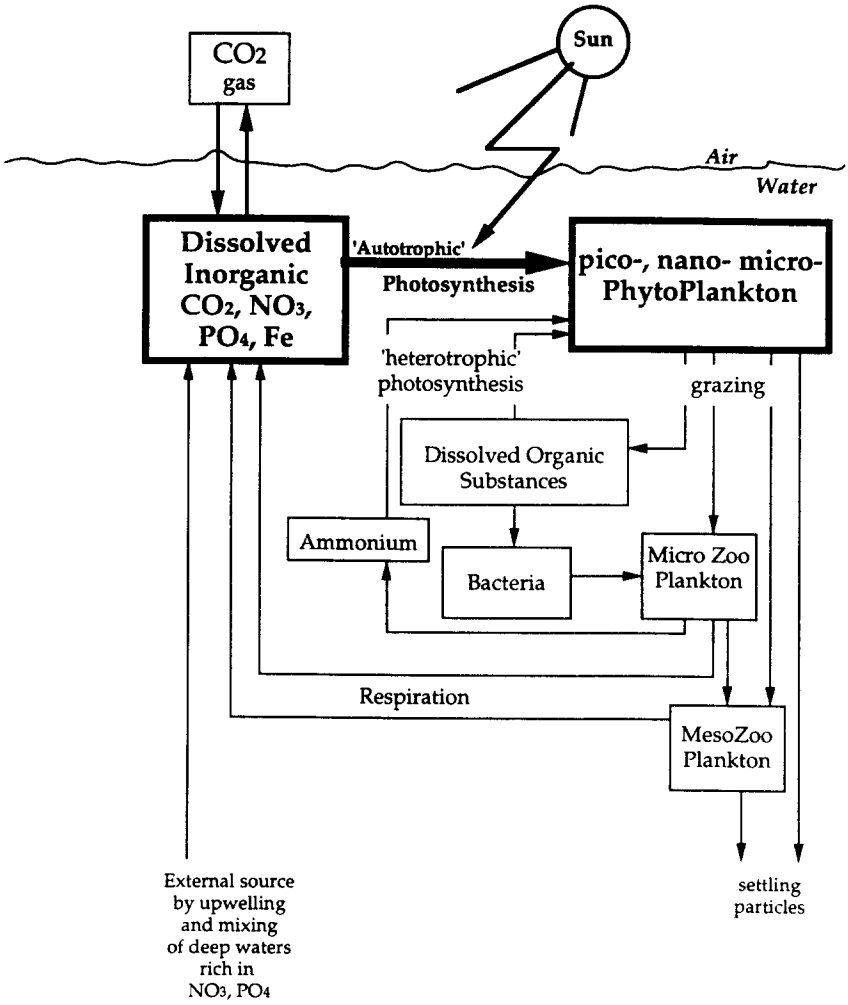


Fig.1. Simplified scheme of the plankton foodweb in the pelagic ecosystem. In reality there is a multiplicity of cycles and interactions, where several organisms are also capable of operating various trophic functions at once. Yet when, irreverently, viewing the system as a black box, then it is obvious that the ultimate constraints are the abiotic factors (light, temperature, inorganic nutrients) for autotrophic primary production. Latter process, as indicated in the fat printed boxes, is the focus of this paper. (NB: The silica-cycle, albeit important in the real ocean, is not considered here; yet much of what is written about N and P for algae in general also applies to Si as a limitation for those algae producing opaline skeletal parts, i.e. diatoms).

For example, from the chemical perspective, the cycling of nitrogen alone involves many coexisting chemical species, including nitrate, nitrite, N_2, ammonia, urea, various amines and dissolved amino acids. Each of these may serve as a source of nutrient for photosynthesizing plankton organisms. In the dynamic ecosystem most of these chemical forms are often short-lived intermediates in the cycles (Fig.1). For example ammonia is very important in regulating phytoplankton growth but its supply to seawater is by production within the food web itself. In essence for nitrogen, only nitrate and N_2 are considered the ultimate substrates for truly 'chemolithotrophic' or 'autotrophic' primary production; all other N-forms are considered 'recycled' substrates for 'heterotrophic' production.

While acknowledging the complexity of the ecosystem, it is obvious that the primary production term is very important (the highlighted box in Fig.1). By focusing on just this term, and ignoring heterotrophic production, the current paper is merely intended to review the historic efforts to assess the ultimate chemical (and physical) limits set to the ecosystem. In other words, the latter abiotic factors are seen as the 'external' constraints within which plankton communities are allowed, and are known, to develop: different communities of plankton species and (organic) chemical intermediates, adapted to cope with different abiotic (inorganic) conditions. By restricting ourselves to this, admittedly narrow, perspective, we can single out the handful of factors controlling the rate of autotrophic productivity: light regime, ambient temperature and inorganic nutrients.

For individual phytoplankton species, each factor can be studied separately in controlled incubations (continuous cultures) in the laboratory. The typical curve of growth as function of a single limiting factor is shown in Fig.2. For over a century many of such studies have been carried out and they continue to be done. In addition the synergistic/antagonistic effect of two or more abiotic factors have been investigated with such parameterization, albeit rarely (ICHIMURA, 1967; ARUGA, 1965; as cited by PARSONS, TAKAHASHI and HARGRAVE, 1984). Concepts have been developed by BLACKMAN (1905) and DROOP (1983), also investigating simultaneous effects of multiple factors (BLACKMAN, 1905). Concepts taken from microbiology (MONOD, 1942; MICHAELIS and MENTEN, 1913) have proved useful and were first applied by CAPERON (1967) and DUGDALE (1967) for describing nutrient kinetics of phytoplankton. For comparison of various concepts see also O'BRIEN (1972); DROOP (1983); PARSONS *et al* (1984); RIEGMAN and MUR (1984); MOREL (1987); KILHAM and HECKY (1988); HECKY and KILHAM (1988).

1.2 The scientific method

Testing of hypotheses is the crux of science, non-verifiable hypotheses are non-science. CHAMBERLIN (1890) was well aware of this truism and devised the industrious method of assessing multiple working hypotheses, by testing each one independently until the right idea is proven. Later on POPPER (1972) realised that while it is possible to falsify a hypothesis, it is almost impossible to provide firm proof. Hence methodology emerges of falsifying a series of multiple hypotheses until just one remains unfalsifiable. Such an approach appears ideally suited to our problem of multiple limiting factors, if it were not for the fact that these growth-limiting factors are interrelated, hence cannot be tested independently.

1.3 Physical factors

The light regime in the surface ocean shifts its spectrum as function of depth, suspended material and self-shading; while its intensity varies in time over several orders of magnitude depending on insolation, cloud cover, wind mixed layer depth, seasonal ice cover. Unquestionably variation of

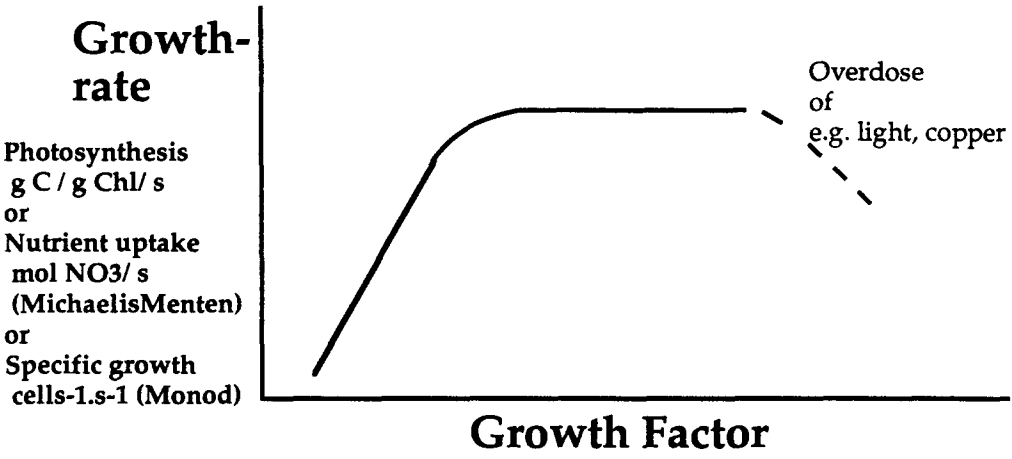


FIG.2. Schematic representation of the growth curve of a single plankton species as function limiting factor on horizontal axis. The factor can be the concentration of a limiting nutrient in the bulk medium (Monod and Michaelis-Menten) or intracellular (Droop), or the intensity of light with well-defined spectrum (P vs. I curve). The response (vertical axis) can be the specific increase in number of cells (day^{-1}; Monod, Droop) or an indication thereof (e.g. Chl *a*), the rate of uptake of a nutrient (mol $m^{-3}s^{-1}$; Michaelis-Menten), and in the case of light, an overall or specific photosynthetic rate (e.g. mol C $m^{-3}d^{-1}$ or mol C mol Chl $a^{-1}d^{-1}$).

light is a dominant factor controlling the growth rate of algae.

Each algal species has its own optimum temperature for growth. Combining all these optima (doubling rate [day^{-1}] versus optimum T [°C]) EPPLEY (1972) found a modest temperature dependence of about 1.88 per 10°C. GOLDMAN and CARPENTER (1974) found a similar dependence of about 2.08 per 10°C. Given the oceanic range from about -1.5°C to +30°C the optimum growth rate would vary within about one order of magnitude, significant but not enough to be considered a dominant control. However, these compilations rely on a variety of published studies which are neither necessarily compatible with one another nor with respect to modern experimental technique. For example, BANSE (1991c) in a recent overview showed some deviations of the temperature relation of EPPLEY (1972), in which the temperature dependence appears to be less pronounced than in the EPPLEY (1972) relationship, although the difference may be partly ascribed to different treatments of the data.

Summarizing the physical factors, light is too important to be debatable, temperature on the other hand appears relatively trivial. One may now consider the inorganic chemical factors: N, P, Fe and C, in that historical chronology.

2. NITROGEN: BRANDT VERSUS NATHANSOHN, GEBBING

Karl Brandt started working in Kiel in 1887 and soon after focused on the solution of our problem. He is credited with being the first to focus on chemical factors, reaching beyond traditional biology to encompass ideas from agricultural chemistry where J. von Liebig had made great advances. A problem which had been encountered all over Europe was that after many years of harvesting productive farmland eventually went infertile. J. VON LIEBIG (1855) had demonstrated that year after year essential nutrient elements had been removed along with the crop. Under the title 'Principles of Agricultural Chemistry' he promulgated 50 numbered statements, from which the thirty-third later was singled out as the famous Law of the Minimum:

When a given piece of land contains a certain amount of all the mineral constituents in equal quantity and in an available form, it becomes barren for any one kind of plant when, by a series of crops, one only of these constituents - as for example soluble silica - has been so far removed, that the remaining quantity is no longer sufficient for a crop.

BRANDT (1899) reckoned that the Law of the Minimum would also govern growth of oceanic algae and, from the list of various essential elements mentioned by von Liebig, he selected nitrogen (N) as the common limiting factor. Moreover he suggested that the supply of N (as either ammonia or nitrate) from land via rivers into the sea was crucial (Fig.3). Both hypotheses were based largely on deductions, i.e. the presumed analogy to the land. There were only very few nutrient values available, BRANDT (1899) mentions in a footnote the very scant analyses done on his behest, albeit in lakes. Published values for seawater were known to Brandt, but were not very reliable as the values were near the detection limit of the method (NATTERER, see MILLS, 1989).

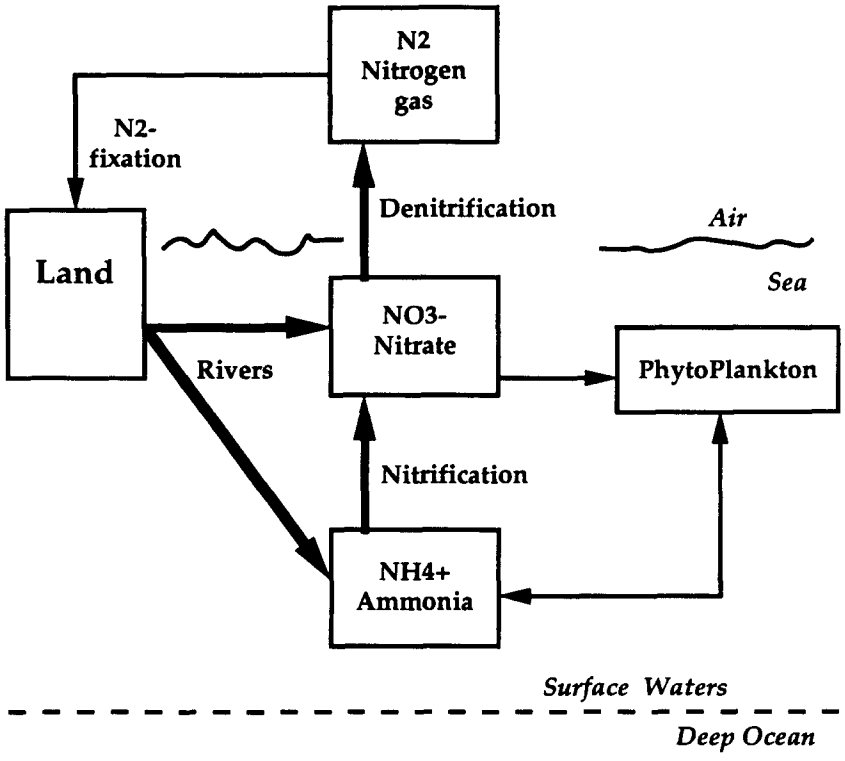


FIG.3. Simple scheme of the marine N-cycle as proposed by BRANDT (1899).

The first hypothesis of limitation by a single factor was soon to be challenged, notably by NATHANSOHN (1908) and GEBBING (1910). Under the provocative header 'Gilt für die Produktion im Meere das Gesetz vom Minimum?' ALEXANDER NATHANSOHN (1908) directly pinpointed the working hypothesis of BRANDT (1899), a remarkably direct approach at the turn of the century. NATHANSOHN (1908) simply argued that Liebig's Law of the Minimum was intended for, and was only applicable to, arable agriculture, and had no relevance for other issues such as cattle farming, ocean plankton or fisheries. In the ocean, crops are not harvested, but many grazers and 'composting' bacteria are fertilizing the sea again by recycling of nutrient elements. NATHANSOHN (1908, §2 on p60) highlights the differences in approach:

"When we now turn to the study of external, especially chemical, conditions for the plankton and its quantitative distribution, we will clearly recognise the great variety of problems that ensue once we consider the continual growing and dying of the plankton, and once we keep in mind that its biomass results from the balance of these two processes. It makes a great difference whether we study a nutrient solution in respect to its maximal potential of production (today we would write: its carrying capacity) *as Brandt strived too, or in respect to the rate with which the production in it proceeds, as we will have to do when we want to clarify the conditions of plankton development. The first variable is, in accordance with the repeatedly mentioned Liebig's Law, always only dependent on the one nutrient which happens to be in the minimum, while the latter* (the rate of production) *can be affected by all possible components."*

Simultaneous control of the rates of growth and loss terms is what NATHANSOHN (1908) wanted us to investigate. Further on in the paragraph curiously enough the effect of 'heavy metals' is contemplated.

As an example, it is virtually a general rule that toxins at very low concentrations are beneficial for plant growth. Such influence is for example exerted by the heavy metals, and it is therefore well conceivable that the concentration of such elements is not at all without significance. Most metals exist in minimal traces in seawater, and we cannot assess how big the impact of these substances and their uneven distribution is on the dominance of one (algal) *species over the others in a given watermass."*

Some 80 years later we know a bit more, but still can hardly assess this issue (BRULAND, DONAT and HUTCHINS, 1991).

GEBBING (1910, p.65) very much liked the multifactorial focus on the (dynamic) rates of production. In his final summary an independent judgement on the issue is given, in some, but not all, respects agreeing pointedly (MILLS, 1989) with Nathansohn:

"It appears to me to go far too far to negate the applicability to the sea of the Law of the Minimum altogether, as A. Nathansohn has done. Nevertheless I believe that the Law of the Minimum does by no means have the Effect on Production in the Sea the way Brandt envisions. It is a Factor though, which works along with many others, in one Place may be more, in another may be less pronounced. One singular Cause does not exist in Nature. In the Failure to appreciate this Fact is also the major Mistake of K. Brandt to be sought. Recently (i.e. in 1910!) *the Law of the Minimum is interpreted much too narrow anyway. In this narrow Meaning, as also used by Brandt, it does not control the Productivity of Farmland either. Liebig (Chemische Briefe, S. 35-50) himself had taken the Law of Production in a much wider sense for Farmland, yet in the course of Time this had been deteriorated into the, on its own rather fruitless, Law of the Minimum. The original Law of Production was in Essence something as: "The Yield (E=Ertrag) of a Soil depends on the Nutrient (N) which is in Minimum supply and the Resistance (W=Widerstand) that its Uptake by Plants encounters":*

E=N-W (50. Brief)

Here are all determining Factors combined. In this we do not only see the Potential for Productivity but also, or rather instead, the Rate of Production. Further scrutinising of this interesting Issue, a more accurate Definition of Minimum than hitherto commonly used, can be found in my full Paper (i.e. GEBBING, 1909)"

The earlier concepts of von Liebig (Chemical Letter No.50; first edition in 1844a,b,c, verified in later editions; VON LIEBIG, 1861, 1865) may be true, yet later on VON LIEBIG (1840, 1842, 1843, 1855) defined his Law of the Minimum as cited above. However the Law of the Minimum was published, i.e. meant to be understood, within the context of overall 50 laws, so the later publication of VON LIEBIG (1840, 1842, 1843, 1855) is consistent with his earlier 'Law of Production' in the Chemische Briefe No.50, and also consistent with these pronounced conclusions of GEBBING (1910). After all VON LIEBIG (1855, p.34) himself concluded that all his 50 propositions are contained in one proposition, and his final sentence tends to reiterate the above formulation (E=N-W). Indeed the Letter No.50 was intended to summarize all the previous letters (VON LIEBIG, 1844a,b,c, 1861, 1865). It should be noted though that VON LIEBIG (1844a,b,c, 1861, 1865) defines W as a resistance for uptake of a nutrient element from the soil (e.g. a solid phase resistant to the necessary dissolution for uptake) and not the various loss terms (e.g. grazing as in above Eqs 1 and 2) which GEBBING (1910) tended to include also in the W-term. Otherwise the candid disagreement with Brandt is remarkable. Nevertheless, phytoplankton production is envisaged as being controlled by several nutrients rather than one, and maintained at a constant level by the equilibrium between phytoplankton growth and grazing (see also MILLS, 1989, p.107).

Despite these contemporary criticisms, BRANDT's (1899) paradigm, based on taking one of von Liebig's laws out of context and misapplying it in another scientific discipline, has somehow survived for most of the twentieth century. Presumably the rather theoretical contributions of NATHANSOHN (1908) and GEBBING (1910) left less of an impression than the results of industrious experiments and measurements at sea from the Kiel Institute. The reason was perhaps as much that neither Nathansohn nor Gebbing were Chairholders (Ordinarius in German), of whom there were 24 in Zoology in pre-World-War-I Germany, one per university, and whose influence is nowadays difficult to imagine (BANSE, personal communication). BRANDT (1902, 1920, 1925) produced a stream of reports and publications which must all have appeared consistent with his authoritative treatises and in which he firmly rebuts his critics and restates his long-held belief in the single limiting factor.

However, his second hypothesis (BRANDT, 1899), namely that supply from land was limiting N-availability, did not survive. After three decades of research based on the first sets of more or less reliable measurements of nitrate in seawater (Natterer of Vienna, Raben with Brandt at Kiel, Harvey at Plymouth, Wattenberg's observations in the Meteor expedition) BRANDT (1929) conceded that the deep ocean is in fact the major store of dissolved nitrate, and that its impact on plankton growth is dependent on the rate of supply to euphotic surface waters provided by upward mixing (Fig.4).

The controversy concerning the route of supply of nitrogen is vividly re-enacted in MILLS (1989), noting the influence of Nathansohn who, already in 1906, had highlighted the importance of (upward) vertical transport of water as a means to bring essential nutrients into surface waters. One stumbling block for Brandt had been the conflicting evidence for the bacterial nitrification necessary in deep waters. Only after Brandt died in 1931 did COOPER (1935a) report decomposition experiments. In addition to studies mentioned specifically by MILLS (1989), the experiments of BRAND, RAKESTRAW and associates (1937-1942) demonstrated formation of nitrite and nitrate which was tentatively ascribed to *Nitrosomonas* and *Nitrobacter* species respectively. As late as

1946 ZOBELL (1946) stated that there was still a lack of firm evidence to support the tacit assumption that nitrification takes place at large scale in the deep sea. Furthermore, it was not until 1981 and 1982 that it was shown that photo-inhibition may have led to the spurious results obtained in earlier studies of marine nitrification (OLSON, 1981a,b; WARD, OLSON and PERRY, 1982). This remains highly plausible in the light of laboratory studies (ALLEMAN, KERASINDA and PANTEA-KISER, 1987; HOOPER and TERRY, 1974; BOCK, 1965), although the *a priori* photo-inhibition can never completely be proven for all marine nitrification (POPPER, 1972).

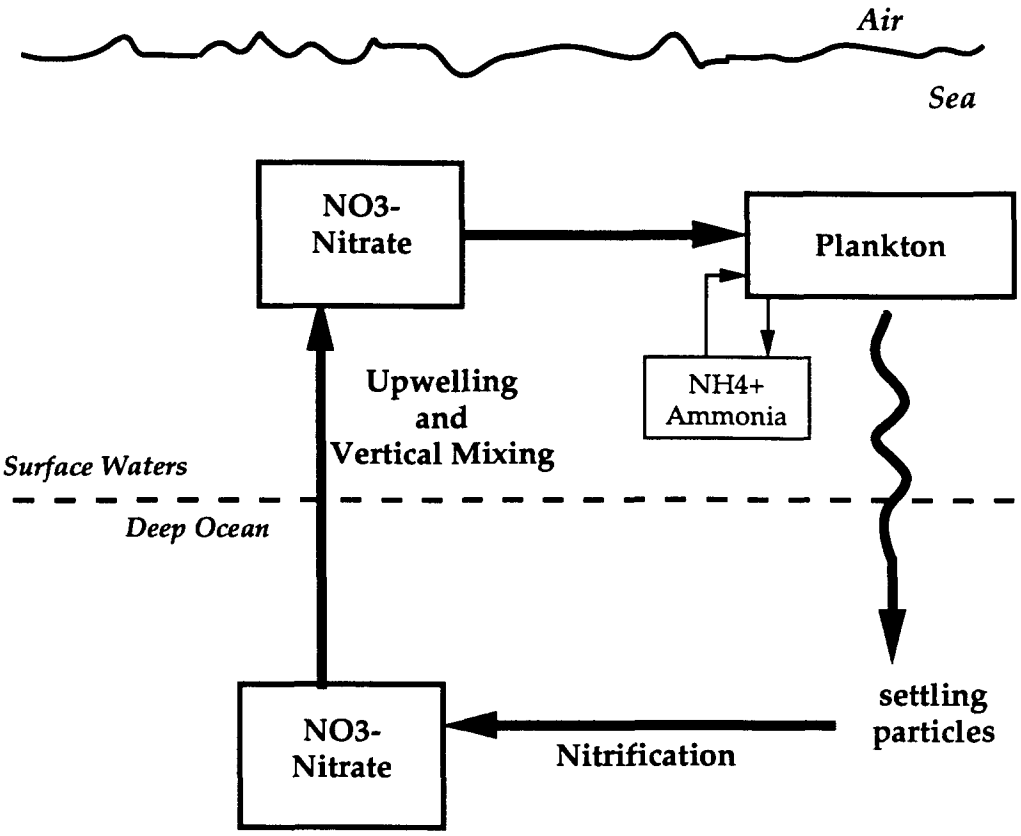


FIG.4. Simple scheme of the marine N-cycle as seen from 1929 onwards.

3. ATKINS, COOPER AND HARVEY: PHOSPHATE AND HYDROGRAPHY

Somewhat in parallel, and strongly influencing the developments described above, were the studies at the Marine Biological Association (M.B.A.) at Plymouth, and the emphasis being placed in Norway on the role of hydrography and vertical mixing. MATTHEWS (1916) had come across a sensitive colorimetric technique for phosphate (POUGET and CHOUCHAK, 1909) and reported phosphate levels in the English Channel which were fourfold lower than those measured by Raben in the Baltic Sea. These findings went unnoticed until in 1921 W.R.G. Atkins, a chemist, was appointed at the M.B.A. With his strong chemical background (see MILLS, 1989) Atkins realized the importance of Matthews' results, and using further improved methods first confirmed Matthews' results, and then some years later demonstrated that Raben's values were indeed too high (ATKINS, 1923, 1925a). In retrospect, it appears that Raben had inadvertently included arsenate in his analysis. Conceivably the high values of phosphate reported by Raben could have mislead Brandt into believing that phosphate could not be limiting phytoplankton growth. In Plymouth, all effort then went into studying phosphate as the limiting factor. In a suite of field studies in the Channel it was shown that, just at the end of the spring bloom, not only had phosphate become depleted, but also that once the surface waters had warmed, the resulting vertical stratification prevented upward mixing of underlying phosphate-rich waters (ATKINS, 1923, 1924, 1925a). The concept of nutrient limitation by vertical stratification was consistent with earlier observation of the upper mixed layer in lakes, known as the epilimnion (BIRGE and JUDAY, 1911, 1922). As early as 1900-1901 H.H. Gran had investigated the Norwegian Sea and concluded that hydrographic mixing and upwelling were important for productivity (GRAN, 1902a,b). Even earlier the relationship between regions of higher productivity and upwelling had already been recognised by PUFF (1890) in his dissertation. This thesis and the work of Gran had greatly influenced NATHANSOHN (1906) when advocating the role of vertical mixing, and in his later papers (NATHANSOHN, 1909, see further MILLS, 1989).

Additional analyses of water samples collected in the temperate and tropical Atlantic yielded phosphate values which were very low in surface waters, but high in deep waters. Now Atkins combined this information with knowledge about vertical mixing to produce a suite of classical papers (ATKINS, 1924, 1925a,b, 1926) in which he contrasted the permanent stratification seen in tropical waters with the seasonal stratification in subpolar waters (see also MILLS, 1989, pp.157-160). Sheina Marshall and A.P. Orr quickly seized upon the technique, and they undertook weekly measurements of phosphate and phytoplankton in the Clyde throughout 1926, thereby demonstrating that phosphate was limiting for diatoms (MARSHALL and ORR, 1927). Phosphate then appeared to be the major limiting nutrient.

Nitrate analyses were still based on the cumbersome distillation method of Raben. In 1920 a large fisheries biology project was started at the M.B.A. and the director E.J. Allen appointed a chemist, H.W. Harvey to study this topic. Soon a novel strychnine-sulphate method was developed for nitrate, based on a technique devised by DENIGÈS (1911) and used during the same cruises that Atkins was measuring phosphate (HARVEY, 1926). Soon nitrate concentrations in surface waters were also shown to be much lower than those reported by Raben. Together these measurements by Atkins and Harvey provided the foundation for the concept of co-limitation by phosphate and nitrate resulting from restricted vertical mixing. Independently WATTENBERG (1929; see also RAKESTRAW, 1958) had also developed the phosphate and nitrate methods and used these during the famous Meteor expedition from 1925 to 1927. His observations, when compiled into a large database for phosphate in tropical waters (HENTSCHEL and WATTENBERG, 1930), confirmed the findings of Atkins. In addition, a small number of nitrate values were measured with the method of HARVEY (1926). At a special ICES meeting held on June 4th 1928 in Copenhagen on "The

Estimation of Phosphates and Nitrogenous Compounds in Sea Water", HARVEY (1929), WATTENBERG (1929), BUCH (1929), GIRAL (1929), SCHREIBER (1929) and SUND (1929) all reported their results, and KARL BRANDT (1929) conceded. In October 1928, ICES also sponsored a workshop on the methods, which was held at J. Hjort's laboratory in Oslo (HJORT, 1929). Quite remarkable were the bioassays developed by SCHREIBER (1927, 1929; see also HJORT, 1929).

The work in Plymouth continued, and in 1930 the group was reinforced with the appointment of a third chemist, L.H.N. Cooper. COOPER (1933) developed reliable techniques for measuring ammonia and nitrite and at sea demonstrated that not only can algae use these forms, but also often actually preferred ammonia to nitrate, thus confirming laboratory observations of HARVEY (1933). Through the 1930s, Cooper established a time series of nutrient measurements in the English Channel, which verified the suggestion of HARVEY (1926) that algae take up nitrate and phosphate in constant proportion. Initially Cooper reported the N:P ratio ranged between 20:1 to 16:1, but later, after applying some corrections for salt effects, finally suggested a range of 16:1 to 15:1. This concept had been developed more extensively by A.C. REDFIELD (1934) and has now become one of the cornerstones of ocean science (HARVEY, 1940).

Atkins, Harvey and Cooper are now seen as pioneers of marine ecology. All three of them were chemists who, by improving the analytical techniques, were able to sort out the correct theory from a number of conflicting, long-term hypotheses.

4. THE ANTARCTIC PARADOX (RUUD AND GRAN)

All issues appeared to have been resolved, oceanic productivity being controlled by upward vertical mixing of nutrients phosphate and nitrate which are cycling in constant proportion of 1:16. Until today this is the major truism in virtually all textbooks, or is it?

Early on a contradictory situation had been identified in polar and subpolar waters. The Norwegians were eager to learn (HJORT, 1929) and immediately applied the new analytical methods. Thus in 1930 Johan T. Ruud wrote:

> *From September 7th 1929 to April 7th 1930 I accompanied Professor Hjort as his assistant on board the floating factory S.S. "Vikingen", on a voyage to the whaling grounds along the edge of the ice in the Weddell Sea.* (RUUD, 1930)

In contrast with elsewhere, the concentrations of phosphate and nitrate in the surface waters proved to be very high (Fig.5). Yet throughout the austral summer the phytoplankton was hardly blooming. This combination of high nutrients with low phytoplankton biomass was dubbed the "Antarctic Paradox", i.e. it was seen as an exception to the rule. Later similar observations were made in Pacific subArctic and Arctic waters, and in the Equatorial upwelling zone of the Pacific Ocean. In fact ~40% of the Pacific Basin was found to have higher nutrient levels in the surface waters, than in the 'normal' ocean where nutrients become depleted in surface waters. GRAN (1931) reflecting on this expedition wondered about an explanation for these observations:

> *Another investigation just finished seems to indicate that the growth of the plankton diatoms is determined by other factors than the concentration of phosphates and nitrates besides light and temperature. Mr Johan Ruud ...*

In the preceding pages Gran had described his experiments at Oslo in the week of 16-22 March 1930 (Ruud still at work aboard *S.S. Vikingen*!) in which algal cultures had been enriched with soil extracts (i.e. containing iron) as well as iron additions. That summer Gran had been working at the biological station at Friday Harbour of the University of Washington. Culture experiments conducted in the week of 14-19 July 1930 with various iron additions appeared to give a positive result

> ... *but many more experiments will have to be made before the question can be solved.*

Overall the findings were not conclusive, in retrospect this is understandable in that the iron concentrations (~10mg per cbm = ~20nM) as determined by collaborator Klem (BRAARUD and KLEM, 1931) were about two orders of magnitude higher than ambient levels now known to occur. Nevertheless, GRAN (1931) was the first to postulate and attempt to test the concept of Fe limitation, thus marking the start of a long series of efforts spanning six decades.

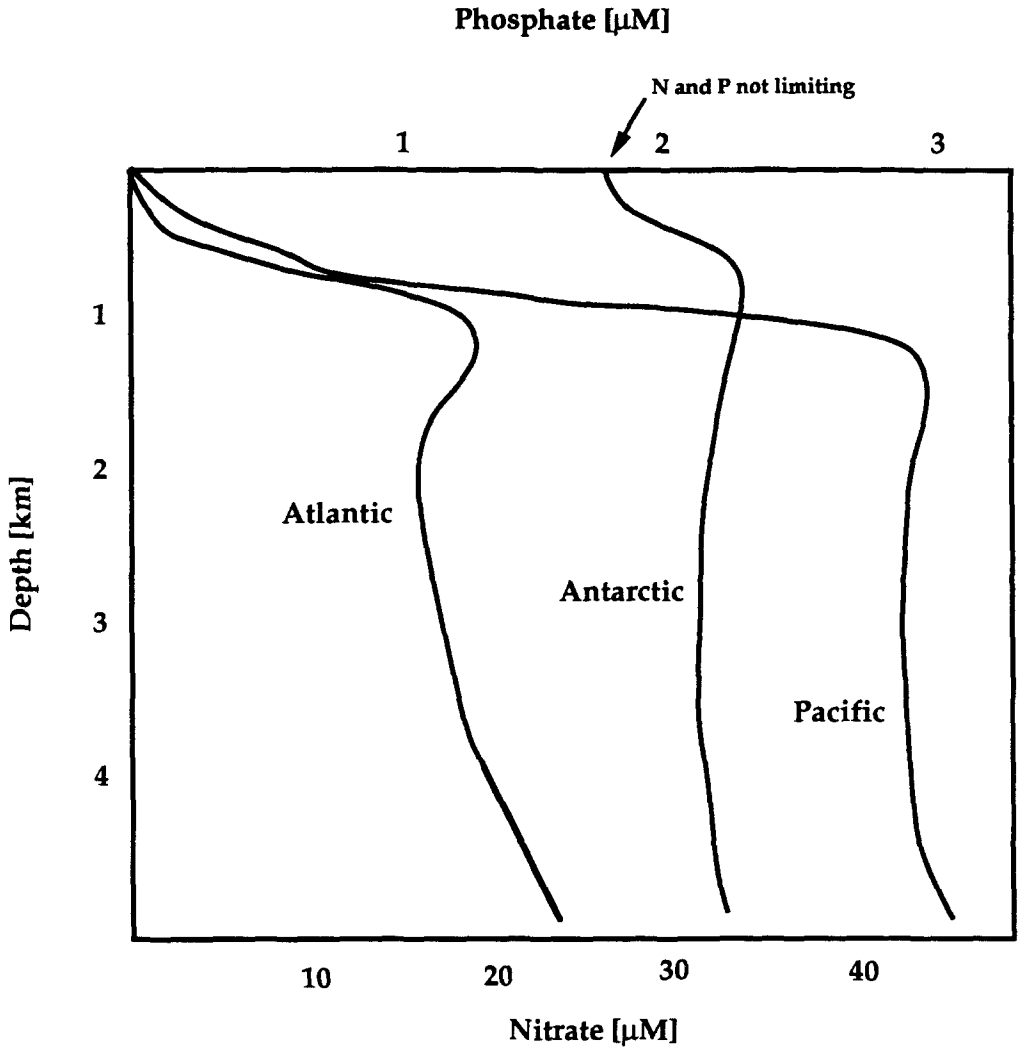


FIG.5. Vertical distribution of phosphate or nitrate in the central basins of the oceans. The ratio is virtually PO_4:NO_3 = 1:16 (REDFIELD, 1934) in the Atlantic, Indian and Pacific and slightly lower around 1:14 in the Antarctic Ocean.

5. HARVEY, COOPER AND IRON LIMITATION

Meanwhile in Plymouth, H.W. Harvey appears to have been the more reflective character (COOPER, 1972). Apparently for a while he had already sensed that there had to be much more to the control of plankton, than just vertical mixing of nitrate and phosphate. Already in 1924, Harvey was clearly aware of iron in seawater, when searching for the natural catalyst for oxidation of hydrogen peroxide he reported concentrations of 0.003 to 0.006mg Fe per litre seawater (i.e. 60 to 120nM; HARVEY, 1925). When developing the nitrate method of DENIGÈS (1911) for seawater he was aware that iron occurred in sufficiently low concentration in seawater not to interfere with the analysis (HARVEY, 1926).

The suggestion of GRAN (1931) that iron might play a role in plankton growth was rapidly picked up, and from 1932 onwards the M.B.A. group worked continuously on the iron-issue as part of their overall investigations. HARVEY (1933) in the classical paper "On the Rate of Diatom Growth" describes, among other experiments on the effects of light, phosphate, silicate, nitrate and ammonia, the effect of iron (as ferric ammonium citrate) on the number of algal cells. Remarkably upon addition of 1, 3 and 5mg m^{-3} Fe (~20, ~60, ~100nM), the number of cells of *Nitzschia closterium* present after 15 days' incubation had increased dramatically, the rate of growth doubling for each ~20nM Fe added. Inspired by GRAN (1931), incubations with soil extracts were also conducted, the growth stimulating effect being ascribed not only to the Fe and Si contents, but also to organic moieties. In 1935, Harvey addressed control by grazing, thus completing the suite of various control mechanisms (HARVEY, 1933; HARVEY, COOPER, LEBOUR and RUSSELL, 1935) which have been debated ever since.

Meanwhile GRAN (1933) was working in the Gulf of Maine on board *Atlantis*, the elegant vessel of the Woods Hole Oceanographic Institution. Upon advice of Waksman, the effect of synthetic ferri-ligno-protein (as proxy of natural humates) was studied, also with additional $MnCl_2$ added. The oceanic species *Rhizoselenia alata* hardly responded to such treatment, whereas the typical neritic species *Leptocylindricus danicus* increased its cell division rate by 30-40% upon the addition of either the ferri-ligno-protein or the soil extract. Some 58 years later SUNDA, SWIFT and HUNTSMAN (1991) reported a similar contrast between an oceanic and a coastal diatom species. However, the iron levels in this latter study were likely to have been much lower than those in Gran's experiments (the moles of Fe added can be calculated, see also WAKSMAN and IYER, 1932; but knowing the 1932 state of the art the possibility of further contamination cannot be excluded).

COOPER (1935b) determined iron in various fractions of seawater as well as in marine plankton. The total iron (sum of all fractions) in seawater was found to be highly variable ranging from 4 to 25mg m^{-3}, i.e. about 80 to 500nM, and was compared with previous studies (see below, on 1920-1984 period). The iron in plankton, expressed per m^3 of sea water, is 0.24-2.3mg m^{-3}, i.e. about 5-50nM. The ratio Fe:P = ~4:1 in plankton appeared only slightly higher than Fe:P = 1:1.41 previously reported by BRANDT and RABEN (1920). From then available thermodynamic solubility studies COOPER (1937) calculated that the equilibrium concentration in seawater is 4×10^{-7}mg m^{-3} at pH 8 and 3×10^{-8}mg m^{-3} at pH 8.5, or ~8nM and ~0.6nM respectively. [Modern concepts predict an equilibrium concentration of Fe(III) = ~0.1nM and negligible Fe(II) = ~10^{-10}nM (BYRNE, KUMP and CANTRELL, 1988), but kinetic rates of e.g. photoreduction may well yield very different true concentrations in the real ocean.]

HARVEY (1937a) realized that, at such low equilibrium concentrations, the supply of Fe to diatoms, apparently containing 4 times as much Fe as P, could not be maintained. To the best of my knowledge, HARVEY (1937a) was the first to do a formal calculation of the rate of diffusion into the cell, an approach later on applied for macronutrients (MUNK and RILEY, 1952; PASCIAK and GAVIS, 1974), Fe (SCHENCK, MOREL and HUDSON, 1988; HUDSON and MOREL, 1990) and

CO_2 (RIEBESSELL, WOLF-GLADROW and SMETACEK, 1993). HARVEY (1937a) estimated that the rate of diffusion of Fe is about four orders of magnitude below the apparent rate of uptake by diatoms:

The big discrepancies found suggest that diatoms obtain iron by some other mechanism than diffusion of ions from the surrounding water.

With hindsight we know the "mechanism" was analytical contamination, currently the true Fe:P ratio in algal cells is now deemed to be less than 1:1000. Hence nowadays diffusion limitation of supply of Fe to (larger) cells (HUDSON and MOREL, 1990) is a feasible proposition again. Otherwise HARVEY (1937a) very cleverly suggested adsorption of ferric hydroxide particles on the diatom, hypothesizing that carboxyl groups of the lipid membrane maintain a microenvironment with a lower pH, which locally increases the solubility of Fe so that it passes more rapidly through the cell membrane in its dissolved ionic state. Alas, the fact that photosynthesis tends to increase the pH, would not help here. In an accompanying note, HARVEY (1937b) provided evidence that colloids in sea water may also help to maintain higher overall Fe levels (as required by the 'Fe-loaded' diatoms) than the ~8nM dissolved Fe at equilibrium. Although Harvey may have been misled by inaccurate data, remarkably though the various concepts which concerned him (diffusion limitation, equilibrium distribution, adsorption of metals on algae, colloids) are still very much in vogue nowadays. Most remarkably the recent observations by electrochemistry that >99% of dissolved Fe is strongly organically complexed (GLEDHILL and VAN DEN BERG, RUE and BRULAND, personal communications; TIMMERMANS, GLEDHILL, VAN DEN BERG, NOLTING and DE BAAR, 1994) have revived the notion that the remaining <1% dissolved free Fe (i.e. at pM concentrations) may be below diffusion limitation for at least some algal species, where direct uptake of organic moieties (e.g. siderophore complexes) as well as algae-colloid interactions (HARVEY, 1937a) are being invoked once again.

In 1925 the British had launched the first series of the *Discovery* expeditions to the Southern Ocean. The extensive and scholarly monographs of HART (1934, 1942) describing the plankton communities were concluded with a small chapter on the control of plankton growth. Hart, being aware of the work of Gran, Cooper and Harvey, in just one or two lines (HART, 1934, p.186; 1942, p.344) hypothesized that the more abundant diatoms in neritic (nearshore) waters may be favoured by coastal sources, i.e. Fe, Mn as well as organics. During the war the M.B.A. at Plymouth was extensively damaged by bombing and research virtually ceased. Nevertheless, HARVEY (1945) managed to summarize pre-war findings on iron concentrations (pp.34-36), discussing its supply (pp.136-139) to phytoplankton in the context of an important chapter on the fertility of ocean waters. It was not until 1946-1947 that Cooper made another attempt to determine Fe in seawater. The results, as in the earlier findings, were very scattered, and only by statistical treatment were some vague trends discernible (COOPER, 1948a,b). Cooper and Harvey then shifted their attention away from Fe, probably realizing that the problem was beyond the reach of the techniques then available.

6. IRON, SEAWATER AND PHYTOPLANKTON (1920-1990)

6.1 Iron in seawater: 1920-1950

In parallel to the Plymouth school, various investigators were attempting to determine the iron content of seawater, but very few studied iron in plankton. BRANDT and RABEN (1920) reported on Fe in seawater and their high ratio Fe: of 1:1.41 was mentioned above. For two seawater samples ORTON (1923) gave values of ~0.1 and ~0.2mg l^{-1}. VERNADSKY (1924) proposed iron concentrations of ~1.4mg l^{-1} citing Schmidt, presumably the same fellow-citizen of St Petersburg who had

published in 1874 and 1877 (according to LEWIS and GOLDBERG, 1954). WATTENBERG (1927, p.308) in preparing a method for expected Fe levels in the ~1.5mg l^{-1} range, found only 0.06mg l^{-1}, near the lower detection limit of his method, and he suggested that the true concentration was likely to be even lower. The findings of BRAARUD and KLEM (1931) have been mentioned above. THOMPSON, BREMNER and JAMIESON (1932) measured ~0.03 to ~0.08mg l^{-1} in waters of Puget Sound, with higher concentrations up to 0.28mg l^{-1} near the bottom. Further improvements of the method were reported by THOMPSON and BREMNER (1935a) accompanied by a paper (1935b) reporting seasonal variation of Fe off Friday Harbour, as well as vertical profiles in the northeast Pacific Ocean. The latter study yielded concentrations of 40-200nM soluble Fe and 300-960nM total Fe. SEIWELL (1935) collected water from the Gulf of Maine with Nansen bottles deployed from Atlantis and reported concentrations of 0.005 to 0.040mg l^{-1}, i.e. ~100 to ~800nM. RAKESTRAW, MAHNCKE and BEACH, (1936) developed an iron-sulphide precipitation and reported 1-20mg m^{-3}, i.e. ~20-400nM, for filtered seawater off the east coast and at Woods Hole itself. HARVEY (1945, pp.34-37) summarized most of these findings and the underlying methods.

6.2 Iron in the marine ecosystem: 1952-1984

GOLDBERG (1952), at the beginning of a career studying virtually every chemical element in seawater (e.g. GOLDBERG, KOIDE, SCHMITT and SMITH, 1963; which covered 13 elements at once) investigated the assimilation of iron radiotracers ^{55}Fe and ^{59}Fe by marine diatoms. Adsorption of iron onto the walls of the culture vessels was prevented by 'Desicote' coating, similar to 'silanizing' nowadays when use of glass cannot be avoided. Ferric citrate added to seawater was observed to decompose under light and produce soluble ferrous Fe(II) ions. This light effect is similar to that reported by PELTZ and LYNN (1938) and more recently has been observed to occur in natural seawater (WAITE and MOREL, 1984a,b; HONG and KESTER, 1986; KUMA, NAKABAYASHI, SUZUKI, KUDO and MATSUNAGA, 1992). The experiments yielded Fe uptake by *Asterionella japonica* in the ratio Fe:P = 3.6:1 in keeping with results of COOPER (1935b), i.e. too high from modern perspective. It was concluded that Fe is taken up from particulate and/or colloidal form, again consistent with HARVEY (1937a,b). The promised follow-up study of distribution of particles in the Pacific Ocean utilized novel membrane filters (LEWIS and GOLDBERG, 1954). They reported soluble Fe at ~3.4μg l^{-1} (i.e. ~60nM) and particulate Fe at ~4.5μg l^{-1} (i.e. ~80nM) in the deep north Pacific Ocean (cf. modern values of about 0.2-1.8nM for both fractions, MARTIN, GORDON, FITZWATER and BROENKOW, 1989). LEWIS and GOLDBERG (1954) also provided a bibliography of all previous iron determinations in marine waters, among which are some 19th century efforts (USIGLIO, 1849; MARCHAND, 1855; THORPE and MORTON, 1871; SCHMIDT, 1874, 1877), eighteen papers over the 1935-1951 period by anonymous authors from Japan, and some more, then recent, studies (TANITA, KATO and OKUDA, 1951a,b; KAWAMATO, 1952).

Simons, Monogham and TAGGART (1953) used the colorimetric indicator o-phenantroline for direct determination of Fe in seawater and reported values of 2-7μg l^{-1} for surface waters collected from the tanker *Esso Cumberland* with a canvas bucket loaded with cement. ARMSTRONG (1957) reported total Fe concentrations ranging from 4-424μg l^{-1} (80-8000nM) consistent with some earlier values for particulate Fe in the 42-210μg l^{-1} range (ARMSTRONG and ATKINS, 1950). LAEVASTU and THOMPSON (1958) realized that the membrane filters as used by LEWIS and GOLDBERG (1954) were superior to the Whatman filters used previously by THOMPSON and BREMNER (1935b), and attempted a revision of the measurements of Fe in waters off the coast of Washington State. MOKIYEVSKAYA (1959) provided a fresh overview of studies on Fe in seawater.

In his later textbook HARVEY (1966, pp.142-146) does not mention these postwar findings. His

treatise on factors influencing the growth of plants mentions only briefly the supply of Fe and Mn (HARVEY, 1966, PP. 98-99), apparently ignoring his own earlier experiments. In contrast, in the oceanographic community along the east coast of the USA there was a revival of the interest in Fe limitation. RYTHER and GUILLARD (1959) incubated Sargasso Sea water with a series of enrichment media from which in each instance just one essential component had been omitted. The medium without (Fe+trace metals) showed the lowest ^{14}C uptake, similar to that of the untreated control and the medium without silicate.

> *Rather than nitrogen and phosphorus, the nutrients limiting photosynthesis in these waters appear to be silicate or one or more of the components of the iron - trace metal mixture. This does not imply, of course, that if these substances were present in excess, nitrogen or phosphorus would eventually, perhaps very quickly, become the limiting factors. It does imply, however, that they would become limiting at a higher rate of photosynthesis, which is another way of saying that the rate of primary production in the Sargasso Sea, at the time of our observations, was limited by nutrients other than nitrogen or phosphorus* (RYTHER and GUILLARD, 1959).

Note that actual rates were deemed crucial, with the possibility of multiple control by a sequence of elements. The obvious next step was to distinguish the critical element in the trace metal mixture. MENZEL and RYTHER (1961) reported this to be iron, which alone enhanced ^{14}C uptake in the first 24 hours, but required the addition of nitrogen and phosphorus to maintain the effect over a three-day period. Meanwhile RYTHER and KRAMER (1961) investigated 5 coastal and 4 oceanic species of plankton algae and found that:

> *The oceanic species attained their maximum populations at levels of iron capable of supporting either no growth or a small fraction of the potential growth of the coastal species .*

Apparently, they were confirming the same working hypothesis of GRAN (1933) and later on BRAND, SUNDA and GUILLARD (1983), SUNDA *et al* (1991) as well as BRAND (1991). In the same time MENZEL and SPAETH(1962a) reported soluble and particulate Fe concentrations at ~1$\mu g\ L^{-1}$ in the Sargasso Sea, in another paper contemplated vitamin B-12 (i.e. Co, see also DROOP, 1955) as another limiting factor (MENZEL and SPAETH, 1962b), an issue later taken up later again by CARLUCCI and CUHEL (1977) as a possible answer to the Antarctic Paradox. Next MENZEL, HULBURT and RYTHER (1963) suggested the effect of Fe to be catalytic, in view of the equally strong growth enhancement by Al (aluminium), an element which has no biochemical function, i.e. is not essential. In retrospect, they realized that all experiments of this era had inadvertently suffered from contamination (HULBURT, 1991, personal communication). With hindsight this may also be concluded for the independent, but very similar, experiments of TRANTER and NEWELL (1963) in the Indian Ocean. This notion of inadvertent contamination was apparently unknown to THOMAS (1969), when he conducted an extensive study of the effect of various enrichments on phytoplankton populations off Baja California and in the eastern Equatorial Pacific Ocean, in which iron enrichment never had the consistent effect shown by nitrogen enrichment. Meanwhile TOPPING (1969) had investigated a suite of metals in the northwest Indian Ocean and reported Fe concentrations typically at several $\mu g\ l^{-1}$. RILEY and TAYLOR (1968) had developed a pre-concentration technique with chelating resin followed by atomic absorption spectroscopy and soon reported values for Fe and other transition metals in the Atlantic Ocean (RILEY and TAYLOR, 1971). As part of the GEOSECS programme an effort was undertaken to analyze Fe and several other trace elements in seawater (SPENCER, ROBERTSON, TUREKIAN and FOLSOM, 1970; BREWER, SPENCER and ROBERTSON, 1972). Yet for GEOSECS, only the trace element Ba (barium) has eventually proven 'oceanographically consistent'; thus Ba may be considered to be the first trace

element for which reliable data were obtained. In his review BREWER (1975, his table 7.13) lists five, then recent, studies which with hindsight have subsequently become obsolete. SUGIMURA, SUZUKI and MIYAKE (1978) reported that dissolved Fe (~2-4μg l^{-1}) was 80 to 90% in organic form, an observation mostly of historical interest; as was the remarkable statistical co-variance of the later findings by the same investigators of Dissolved Organic Carbon (DOC) with Apparent Oxygen Utilization (SUGIMURA and SUZUKI, 1988; DE BAAR, BRUSSAARD, HEGEMAN, SCHIJF and STOLL, 1993; SUZUKI, 1993).

After THOMAS (1969) no further Fe enrichments were conducted for oceanic plankton communities until SUBBA RAO and YEATS (1984) in essence repeated the experiments of MENZEL and RYTHER (1961), but this time using modern ultraclean sampling equipment and aware of protocols of FITZWATER, KNAUER and MARTIN (1982) for clean incubations. Chelated iron was added in amounts from 0.5 to 100μg l^{-1}, i.e. ~10 to ~2000nM, with background concentrations in the mixed layer reportedly ranging from 0.37-0.91μg l^{-1}, i.e. 6-16nM as compared to about 0.5-2nM in the northwest Atlantic Ocean (SYMES and KESTER, 1985) and <0.3nM recently reported in the northeast Atlantic Ocean (MARTIN, FITZWATER, GORDON, HUNTER and TANNER, 1993). After 4 hours incubation no effect was seen, but after 72 hours photosynthesis was enhanced by 40% and 75%, independently of the amount of Fe added. Finally JACQUES, FIALA and ORIOL (1984) investigated the trace element limitation hypothesis as the answer to the 'Antarctic Paradox'. No effect of metal addition was observed, yet in retrospect this lack of response is again ascribed to inadvertent contamination (JACQUES, personal communication).

6.3 Growth response of individual phytoplankton species

This review is focusing on the concentration of Fe in seawater, and its effect on the *in situ* plankton community. Although the description of past developments is now complete, for proper understanding of its effect at the ecosystem level, it is also desirable to conduct complementary studies of the growth response and physiology of individual species of phytoplankton. Although this line of research is beyond the limited scope of the current review, some of these studies have already been mentioned (e.g. GOLDBERG, 1952) and below some more, but by no means all, relevant findings are summarized.

Briefly, the introduction of the use of chelating agents in incubations of phytoplankton allowed the experimental reduction of concentration of free metal ions down to the levels necessary for plant growth (PROVASOLI, MCLAUGHLIN and DROOP, 1957). Without clean techniques, the total concentrations of metals like Cu or Fe were high in the media. Without chelation control, this could have either toxic or stimulating effect on algal growth, and hence would, for example, bias a study of the growth response to ambient macronutrient (N, P, Si) or light regime.

Manipulations with chelators like EDTA have also allowed studying the growth effect of metals themselves, including iron. Many of the initial studies focused on the role of chelators in maintaining Fe in solution for plant growth (DROOP, 1961; PROVASOLI, 1963; JOHNSTON, 1964; DUURSMA and SEVENHUYSEN, 1966). JOHNSTON (1964) reckoned that algae required an Fe:P ratio of 1:10, below the ratio of 1:~0.25 of HARVEY (1937a) and GOLDBERG (1952) but well above the true ratios of 1:>1000 (DE BAAR, BUMA, NOLTING, CADÉE, JACQUES and TRÉGUER, 1990). Experimental investigations of individual algal species were done by DAVIES (1970), SAKSHAUG and HOLM-HANSEN (1977) and others.

The classical demonstration by SUNDA and GUILLARD (1976) that the activity of free ionic Cu (copper), rather than its total concentration, is the variable best representing biological availability of a metal, has led to the consensus that this is also the case for iron and other metals. Most dissolved

Fe(III) exists as the dominant ferric hydroxide species ($Fe(OH)_3^0$ and $Fe(OH)_2^+$) which in seawater at virtually constant pH ~8.2 occur in constant proportion to the much smaller activity of the truly free Fe^{3+} ion. Hence, for most biological experiments, albeit usually expressed in terms of free Fe^{3+} activity, one cannot in fact discriminate between the ferric hydroxide species and the free Fe^{3+} ion as the bioactive agents. The rate of diffusion to the cell wall would be very similar for all inorganic species. In terms of kinetics the hydroxide complexes would still have to dissociate for Fe^{3+} to bind to the transport moiety of the cell wall. More recently HUDSON and MOREL (1990, 1993) have argued that inorganic hydrolysis complexes of Fe may well be easily (rapid kinetics) dissociated and available, i.e. the total concentration of all inorganic Fe, rather than only the free ionic concentration (or activity), is equivalent to bioavailability. The dissolved organic complexes would have very slow kinetics and, therefore, would not be bioavailable, which is in keeping with the demonstrated control by concentration of chelator (e.g. EDTA). Thus far, firm conclusions are precluded because the experimental quantification of both equilibrium and kinetic parameters for the overall Fe-system in seawater is incomplete. Moreover the assumption of nearly constant pH of seawater is invalidated by the effects of photosynthesis, most notably in micro-environments of algal aggregates and colonies (RICHARDSON, AGUILAR and NEALSON, 1988; DAVIDSON and MARCHANT, 1987; LUBBERS, GIESKES, CASTILHO, SALOMONS and BRIL, 1990).

Diffusion limitation as a general principle was suggested by MOREL and HUDSON (1985), illustrated for Fe with a calculated diffusive flux matching the growth requirement of *Thalassiosira weissflogii*. For the green algae *Chlamydomonas variabilis*, in fairly acidic (pH 5) freshwater, SCHENCK, TEISSIER and CAMPBELL (1988) have calculated that diffusion to the cell wall is more than adequate when applied to all inorganic Fe(III) species, but not when applied solely to the free ionic Fe^{3+} ion. Similarity with the exercise carried out 50 years before (HARVEY, 1937a) was apparently overlooked. HUDSON and MOREL (1990) from experimental findings were able to argue the case for the likelihood of there being diffusion limitation of marine algae. Having revisited Redfield, (MOREL and HUDSON, 1985) had unwittingly revisited the concept of HARVEY (1937a). If diffusion limitation is indeed quite common, then an adaptive strategy for a cell would be to increase its specific surface area, either by staying very small or by modifying its morphology away from being spherical (PASCIAK and GAVIS, 1974; e.g. pennate diatoms as suggested by CHISHOLM, 1992). Alternatively, the Fe requirement per unit C (carbon) may be minimized (SUNDA, SWIFT and HUNTSMAN, 1991), especially if reliance is placed on ammonia as the nitrogen source (PRICE, ANDERSEN and MOREL, 1991) since nitrate reduction may account for about 60% of the cellular Fe requirement (RAVEN, 1990).

The earlier hypotheses that algae have to assimilate, directly or indirectly, particulate iron (HARVEY, 1937a; GOLDBERG, 1952) had been dictated by the erratic data of those days, and have now been abandoned. Nevertheless, colloids and fine particles may still play a role (WELLS, 1990), serving as a source (through, e.g., photoreduction) of readily supplied dissolved iron, also as Fe(II), towards maintaining the truly dissolved free ionic activity (also of Fe(II)) required for steady growth (WELLS, ZORKIN and LEWIS, 1983; WAITE and MOREL, 1984; FINDEN, TIPPING, JAWORSKI and REYNOLDS, 1984; WELLS and MAYER, 1991a,b; WELLS, MAYER and GUILLARD, 1991). Dissolution of the aeolian input of continental dust may also be supplementing the pool of dissolved iron in some regions (MOORE, MILLEY and CHATT, 1984; DUCE, 1986; DUCE and TINDALE, 1991).

Recent studies based on chelation manipulation, like those done by ANDERSEN and MOREL (1982), BRAND, SUNDA and GUILLARD (1983), HARRISON and MOREL (1983, 1986), RUETER and ADES (1987), have provided considerable insight into the physiological role of iron (see also the intriguing synthesis by MOREL and HUDSON, 1985). Nowadays it is possible to work at total Fe

concentrations of ~1nM in the media, so very low ionic activities of inorganic Fe can be attained using relatively small additions of chelator (PRICE, personal communication; BRAND, 1991; TIMMERMANS, STOLTE and DE BAAR, 1994). Natural seawater contains dissolved organic moieties, some of which likely serve as ligands for Fe, and help maintain Fe in solution. In particular the siderophores are very strong ligands, being excreted "intentionally" by microorganisms (MURPHY, LEAN and NALEWAJKO, 1976; TRICK, ANDERSEN, PRICE, GILLAM and HARRISON, 1983; KERRY, LAUDENBACH and TRICK, 1988; REID and BUTLER, 1991). These siderophores may conceivably have a significant effect on the *in situ* marine ecosystem.

7 IRON LIMITATION AND THE LAW OF THE MINIMUM (1982-1991)

Within the algal cell, Fe has various physiological functions, for example the Fe-S and heme proteins (cytochromes) present in the photosynthetic and respiratory chains are electron transport proteins. They are the most abundant metal redox proteins and account for most of the Fe in the cell (HEWITT, 1983; RAVEN, 1988). Note that at low light conditions the cell requires much more Fe for maintaining growth (RAVEN, 1990) i.e. the growth limiting factors Fe and light are interrelated. In addition, the synthesis of chlorophyll *a* requires catalytic Fe at two steps of the synthetic pathway.

Other Fe-containing proteins are nitrate reductase, nitrite reductase and sulphite reductases, catalase, peroxidase, superoxide dismutase, and nitrogenase (SUNDA, 1990; MOREL, HUDSON and PRICE, 1991). The initial steps in the conversion of nitrate towards amino acids also require iron, hence the role of Fe as limiting factor is also interrelated with nitrate as a growth-limiting factor. Algae utilizing ammonia as N-source not only have an energetic advantage but also require less Fe than those utilizing nitrate. For the conversion of elemental nitrogen (N_2) large amounts of Fe are needed for the enzyme nitrogenase, and Mo is also required (RAVEN, 1988). Hence N_2-fixation as a route to alleviate N deficiency, to the extent that P would become the ultimate limiting macronutrient in seawater is strongly dependent on Fe availability (e.g. BRAND, 1991, pp. 1766-1769).

It was not until 1981, that the first reliable vertical profiles of dissolved Fe were available (e.g. LANDING and BRULAND, 1981) and were eventually published (GORDON, MARTIN and KNAUER, 1982; LANDING, 1983; LANDING and BRULAND, 1987). The rigorously ultraclean methodology developed at the same laboratories (BRULAND, FRANKS, KNAUER and MARTIN, 1979) was successful for Fe as well. The review of BRULAND (1983) was in time to mention the first of these reliable findings, including concentrations of about 1.5nM for the central Arctic Ocean (MOORE, 1983). In the intense oxygen-minima of the East Equatorial Pacific and the NorthWest Indian Oceans very similar dissolved Fe maxima were observed (LANDING and BRULAND, 1987; SAAGER, DE BAAR and BURKILL, 1989). In surface waters, dissolved Fe was found to be very low at ~0.05 to 5nM in surface waters of the Atlantic, Indian and Pacific Oceans (see also SYMES and KESTER, 1985; HONG and KESTER, 1986). FITZWATER, KNAUER and MARTIN (1982) demonstrated that ^{14}C uptake determination of the rate of primary production has to be done with similar ultraclean techniques if artefacts caused by contamination with either toxic or stimulating metals, are to be avoided.

7.1 Subarctic North Pacific

On August 1 1987, the R/V *Wecoma* had arrived at station T-6 (45.00°N, 142.87°W) as part of the VERTEX VII program in the subarctic northeast Pacific Ocean. In this region surface waters

always contain appreciable concentrations of macronutrients (N, P, Si), so some other factors appear to be limiting phytoplankton growth. Here the team from Moss Landing (Fitzwater, Gordon, Tanner and Elrod) started a series of experiments using the natural plankton community cultured in ambient seawater enriched with 1, 5, 10nM Fe, as compared with untreated controls and an enrichment of 10nM Fe + 1 nM Mn + 0.1nM Co. For the first four days of deck incubations in the 2 litre bottles there was no enhancement of phytoplankton growth as a result of iron enrichment, but after 5.5 days the iron enriched cultures began to outgrow the controls, showing increases of chlorophyll *a* (Chl *a*) and more rapid uptake of nitrate, phosphate and silicate (MARTIN *et al*, 1989).

At the next station T-7 (Ocean Station Papa, 50°N 145°W) the experiment was repeated on 6th August 1987. Here Kenneth Coale started an independent experiment, where in various, larger 20 litre, bottles 0.89nM Fe, 1.8nM Mn, 3.9nM Cu and 0.75nM Zn were added. From day 2 onwards the Fe enrichments of the Moss Landing team showed significant enhancement over the controls; the controls actually showed an initial decrease in chlorophyll *a* at days 2 and 3. By the end (day 6) the Chl *a* in all Fe enrichments was much higher, and nutrients lower, the trend increased in proportion to the amount of Fe added (MARTIN and FITZWATER, 1988; MARTIN *et al*, 1989). The experiments of Coale showed a steady growth in both the control and the enriched bottles, at day 2 both the Fe and the Mn enrichments contained significantly higher Chl *a*, and by the end (day 6) the Fe treated bottle contained much higher phytoplankton biomass, whereas the biomass in the other enrichments was only modestly enhanced compared to the control (COALE, 1988, 1991).

Finally, on 10th August, at station T-8 (55.5°N, 147.5°W) the Moss Landing experiment was repeated for the third time. As before at T-7, by days 2 and 3 the enrichments had already outgrown the control, again the latter actually having decreased at day 3. By days 4 and 4.5 the control had recovered and was in excess of the initial field values of Chl *a*, but the enrichments had really taken off, again more or less in proportion with the amount of Fe added.

In early September 1987 the planning meeting for the JGOFS North Atlantic Pilot Study was being held in Paris. On the final day there was some time for presentations of individual research. John Martin presented his now famous plots on the overhead, and soon the audience became much excited. *Veni, vidi, vici.* On 28 January 1988 the paper describing the T-7 experiment of the Moss Landing team appeared in print (MARTIN and FITZWATER, 1988). Also shown were the results for chlorophyll *a* of the other two experiments at T-6 and T-8. Hypotheses with grandeur reminiscent of KARL BRANDT (1899) were postulated convincingly:

> *These data, provide consistent evidence that Fe limits phytoplankton growth in the northeast Pacific subarctic. With appropriate independent confirmation this area could become a classical marine example of Liebig's law of the minimum.*

This was, in itself, already impressive enough, but an even broader perspective was given. Iron availability in oceanic waters was suggested as being important in determining global atmospheric CO_2 levels; notably in the Antarctic Ocean where persistent high concentrations of macronutrients in surface waters (the Antarctic Paradox) was suggested to result from Fe-limitation in its offshore regions away from coastal iron sources. Conversely, during the last glacial there was more dust input into the Antarctic region, so that productivity may have been higher, and indeed atmospheric CO_2 was lower (RAYNAUD, JOUZEL, BARNOLA, CHAPELLAZ, DELMAS and LOVIUS, 1993).

The paper stimulated great excitement and was actively debated. Firstly, the authors tended to emphasize the effect of iron on biomass yield. The implicit assumption that the experiments provided evidence of limitation of growth rates was criticized by BANSE (1990c), who calculated the growth rates from the complete data tables published by MARTIN *et al*, (1989). However, his interpretation was firmly rejected by MARTIN, BROENKOW, FITZWATER and GORDON (1990) and

so BANSE (1991a) produced a more extensive re-analysis of the data, which once again was rejected (MARTIN, FITZWATER and GORDON, 1991). The closing comment by BANSE (1991b) showed that unfortunately no consensus had been reached, possibly as a result of the humorous floating of an untestable hypothesis (MARTIN *et al*, 1991) on iron and mermaids (BANSE 1990a) obfuscating the reasoning. Conceptually the debate resembled that between BRANDT (1899) and NATHANSOHN (1908), which initially suffered from a total absence of data. The Martin versus Banse controversy had data available which in itself was not in contention, but they never reached agreement on how properly to handle and interpret the actual data.

Since then further enrichment experiments of similar design have been carried out by various investigators, and in different regions (see below). In all these studies, as in the experiment of COALE (1988, 1991), the control bottles always showed steady growth with healthy cell doubling rates, irrespective of how strong the growth responses in the Fe enrichments were. In retrospect the initial decrease of chlorophyll *a* in the T-7 and T-8 controls appears to have been most unusual (DE BAAR *et al*, 1990). CULLEN, YANG and MACINTYRE (1992) later suggested that the screening of deck incubators may have been inadequate so that the plankton community had suffered a light shock. Algae may take quite some time to recover from a sudden overexposure to light (BUMA, NOORDELOOS and LARSEN, 1993, and references therein). If so, then the Fe enrichments at T-7 and T-8 will have served mostly as a 'medicine' (Geritol, MARTIN, 1990) repairing the damage of the cellular photosystem resulting from the experimental artefact. Fe is crucial in synthetic reactions as well as in the operation of the photosynthetic apparatus of the cell, hence may also remedy such photo-damage. In retrospect the poor performance of the control experiments T-7 and T-8 might have misled the original authors to believe the real ecosystem is controlled by iron as the single factor, i.e. lead them to believe that they had confirmed Liebig's Law of the Minimum.

It was in 1988 that the accompanying superior quality seawater data for Fe and other metals were published (MARTIN and GORDON, 1988; MARTIN *et al*, 1989): the outstanding accomplishments lead to the grand extrapolations on the role of iron in the Southern Ocean in CO_2-cycling and climate. Judging from the similarity of vertical profiles of Fe and nitrate, together with the very low Fe concentrations in surface waters, iron is obviously intricately linked with the biogeochemical cycle.

7.2 The Southern Ocean

The first test of the hypothesis of iron limitation in the Southern Ocean was conducted by a European team during the austral spring 1988/1989 (DE BAAR, BUMA, JACQUES, NOLTING and TRÉGUER, 1989). In the Weddell and Scotia Seas a suite of five experiments was run from 27 November 1988 until 4 January 1989. In every experiment the Fe enrichments of 1nM, 10nM, 20nM, some also with EDTA, consistently showed enhanced growth relative to the controls. However, the controls also consistently outgrew the typical Chl *a* and POC levels found in ambient waters. This suggested that other factors, not limiting in the control experiment, were in fact limiting in the field. These waters may be neritic in the sense of their containing an ample supply of micronutrients including iron, but are not neritic in the sense of algal growth and biomass. Paraphrasing GRAN (1931) one might say that the growth of the plankton is determined by other factors than the concentration of iron, phosphates and nitrates. Optimal lighting of the experiments and exclusion of mesozooplankton grazers (krill, copepods) from the bottles seem to be the most likely explanations for the subsequent increases of the controls. Conversely, in the field suboptimal light and mesozooplankton grazing appear to play an important role in limiting phytoplankton from attaining appreciable standing stocks. This has been confirmed by several parallel studies during this European *Polarstern* Study (HEMPEL, 1993) and a model simulation validated by the overall

ecosystem dataset (LANCELOT, MATHOT, VETH and DE BAAR, 1993). Measurements onshore showed that ambient Fe concentrations in these 'neritic' waters were more than 1.5nM, i.e. ~2-4nM in the Scotia Sea and 4-6nM in the Weddell Sea. The findings were presented at the Ocean Sciences conference in January 1990 (BUMA, NOLTING, DE BAAR, CADÉE, JACQUES and TRÉGUER, 1990; NOLTING and DE BAAR, 1990) and published 2 August 1990 (DE BAAR *et al*, 1990), followed by publication of the suite of biological observations (BUMA, DE BAAR, NOLTING and VAN BENNEKOM, 1991) and the seawater metal concentrations (NOLTING, DE BAAR, VAN BENNEKOM and MASSON, 1991).

In the same austral spring and summer season, teams from Sweden and the USA independently collected seawater samples in the Weddell Sea and near the Antarctic Peninsula respectively. Dissolved iron levels in the Weddell Sea were in the 0.4-2.5nM range, with total Fe being higher (1-6nM) in the Weddell Sea, and on the shelves 1-25nM (WESTERLUND and OHMAN, 1991). In Gerlache Strait the dissolved iron concentrations of 4.7-7.4nM were consistent with the viewpoint that the extensive shelves around the Peninsula supply iron not only to these neritic waters, but also downstream to the Scotia/Weddell Confluence (NOLTING *et al*, 1991). On the other hand, iron in the upper water column of Drake Passage was as low as 0.1-0.4nM (while rejecting the data of 0.52-0.88nM, which were still subnanoMolar; MARTIN, GORDON and FITZWATER, 1990). The abstract of the latter paper (MARTIN, GORDON and FITZWATER, 1990) conveniently announced the preparation of another scenario which soon would create considerable turmoil:

> *It is also important because oceanic iron fertilization aimed at the enhancement of phytoplankton production may turn out to be the most feasible method of stimulating the active removal of greenhouse gas CO_2 from the atmosphere, if the need arises.*

The next austral summer season, January-February 1990, saw another suite of iron enrichments experiments, now in the Ross Sea, which, judging from the nearby continent and the high chlorophyll *a* concentrations seen from satellite, is another neritic region. In four experimental runs, Fe was again seen to stimulate growth, but once again the controls outgrew the field populations. The results were submitted at the end of August and appeared in print at the end of the year (MARTIN, FITZWATER and GORDON, 1990). The data were interpreted to imply that iron deficiency limits phytoplankton growth in Antarctic waters; the earlier observations to the contrary, as described above, were ignored. On the other hand, ways towards removal of anthropogenic CO_2 from the atmosphere were contemplated. The earlier announcement was confirmed by the suggestion that intentional

> *... iron fertilization of the southern ocean appears to be a feasible method at least in terms of the amounts required.*

Now two studies had been carried out in different regions of the Antarctic, yielding comparable results, but providing different conclusions (BUMA *et al*, 1991). The interpretations of MARTIN, FITZWATER and GORDON, (1990) were reanalyzed and rejected in the same issue (DUGDALE and WILKERSON, 1990). Grazing pressure, and changes therein in the incubations, appeared crucial, in keeping with the findings of BUMA *et al* (1991). At the special symposium in February 1991 organized by the American Society for Limnology and Oceanography a comparison of daily division rates in the then published Fe experiments versus literature values was presented by BANSE (1991c). Briefly the normal division rates in the control bottles underlined that there must be limitation by other processes *in situ* , and intense grazing was identified as a likely control.

7.3 Science Fiction

The intentional Fe fertilization scenario as to remove fossil fuel CO_2 from the atmosphere was the subject of a special workshop of the US National Research Council on 4-5 December 1989.

In April 1990 it was expanded upon in a Joint Global Ocean Flux Study newsletter (MARTIN, 1990), followed by the announcement in *Nature* (MARTIN, GORDON and FITZWATER, 1990) on 10th May, and an article in the Washington Post on 20 May (BOOTH, 1990). Subsequently calculations were published suggesting that if iron equivalent of half the capacity of a supertanker were to be spread over the Southern Ocean each year, it would halt the atmospheric increase of CO_2 (US JGOFS, 1990). The uncritical acclaim which followed in un-reviewed or fashionable periodicals, as well as the media, surely influenced the credulity of the oceanographic community (CHISHOLM and MOREL, 1991) and helped towards the realization of the special ASLO symposium on 22-24 February 1991. Meanwhile modellers were showing that, in the simulated case of 'an act of god' such as iron fertilization, all surface water phosphate and nitrate would soon be removed, so that the eventual impact on atmospheric CO_2 would still be modest (PENG and BROECKER, 1991a,b; JOOS, SARMIENTO and SIEGENTHALER, 1991a,b; SARMIENTO and ORR, 1991; KURZ and MAIER-REIMER, 1993). In a formal resolution by all participants at the ASLO meeting all governments were urged not to consider iron fertilization as a policy option (CHISHOLM and MOREL, 1991, p.viii). The proposal lingered on only briefly afterwards (KUNZIG, 1991). In scientific articles as presented at the meeting and elsewhere the fertilization policy option was hardly ever mentioned, but its ethical dilemma has been the subject of a thesis (CHEN, 1993) also providing an overview of the media-hype. Otherwise the hypothesis served another scientific purpose by paving the way for an *in situ* enrichment experiment (WATSON, LISS and DUCE, 1991; MARTIN and CHISHOLM, 1992), which was deemed to circumvent bottle effects suffered by all previous experiments.

7.4 Consensus

At the symposium in February 1991 it was obvious that the paradigm of the oligotrophic ocean being driven by the intense recycling small food web of pico- and nanoplankton also made sense with respect to Fe deficiency, since small cells with high specific surface area and low Fe requirement for ammonia utilization are likely to be at an advantage. The occasional supply of N and Fe from above (aeolian iron; DUCE, 1986) or below (e.g. EPPLEY, RENGER, VENRICK and MULLIN, 1988, for nitrate) would then trigger blooms of larger cells such as diatoms utilizing nitrate. These blooms are also the main source of export production. Supply of Fe alone would favour increased N_2-fixation, but its very low efficiency (RAVEN, 1988), also with respect to Fe requirement, renders N_2-fixation unlikely to have a major impact on the open oceans. Otherwise it was recognised that bottle incubations (as pioneered by Van Leeuwenhoeck) are subject to implicit artefacts and need to be complemented by other approaches, e.g. *in situ* enrichment experiments and development of biochemical assays for direct assessment of Fe starvation of the field population.

The special issue arising from the 1991 symposium marked the start of the current new era in plankton ecology, for which the developments can only be reviewed retrospectively in the future. Otherwise the link between iron and the Law of the Minimum (Liebig) was briefly expanded upon (MARTIN, 1991; KUNZIG, 1991) but received no mention in the more recent papers (MARTIN, 1992; MARTIN, FITZWATER, GORDON, HUNTER and TANNER, 1993).

7.5 Some recent developments

The first (DE BAAR, BUMA, JACQUES, NOLTING and TREGUER, 1989; DE BAAR *et al*, 1990; BUMA *et al*, 1991) and subsequent (MARTIN, FITZWATER and GORDON, 1990; HELBLING, VILLAFANE and HOLM-HANSEN, 1991) Fe/biota studies in the Antarctic Ocean were all in relatively nearshore 'neritic' waters. On one hand, these had shown (BANSE, 1991c; DUGDALE

and WILKERSON, 1990) that the vast and important ecosystems of the Weddell Sea and Ross Sea were not Fe-starved *per se*, i.e. the distinct limitation in these HNLC regions has largely been ascribed (BUMA *et al*, 1991) to other factors. However, the even larger, distinctly offshore, Antarctic Circumpolar Current (ACC) region had not yet been tested with regard to the Fe limitation hypothesis. Recently our group investigated the geochemistry and biological role of Fe in the ACC and its fronts. Briefly, Fe addition always stimulated the plankton community in incubation experiments (VAN LEEUWE, SCHAREK, DE BAAR, DE JONG and GOEYENS, 1994; SCHAREK, VAN LEEUWE, DE BAAR, DE JONG and GOEYENS, 1994), but the controls again demonstrated that primary productivity in the ACC is definitely not Fe-limited. This is confirmed by the ambient concentrations of dissolved Fe in surface and deep waters of the ACC. Higher ambient Fe in the Polar Frontal Zone (DE JONG, LÖSCHER and DE BAAR, 1994) is consistent with bloom conditions, the natural input with the Polar Frontal jet providing a continuous, rather than instantaneous (WELLS, 1994) impact of *in situ* Fe enrichment. Further confirmation will be sought in the Indian and Pacific sectors of the ACC proper, and in other seasons. From all evidence available thus far it can be concluded that iron is one of a suite of limiting factors, rather than being the sole one, controlling biological productivity in most, if not all, Antarctic ecosystems and seasons.

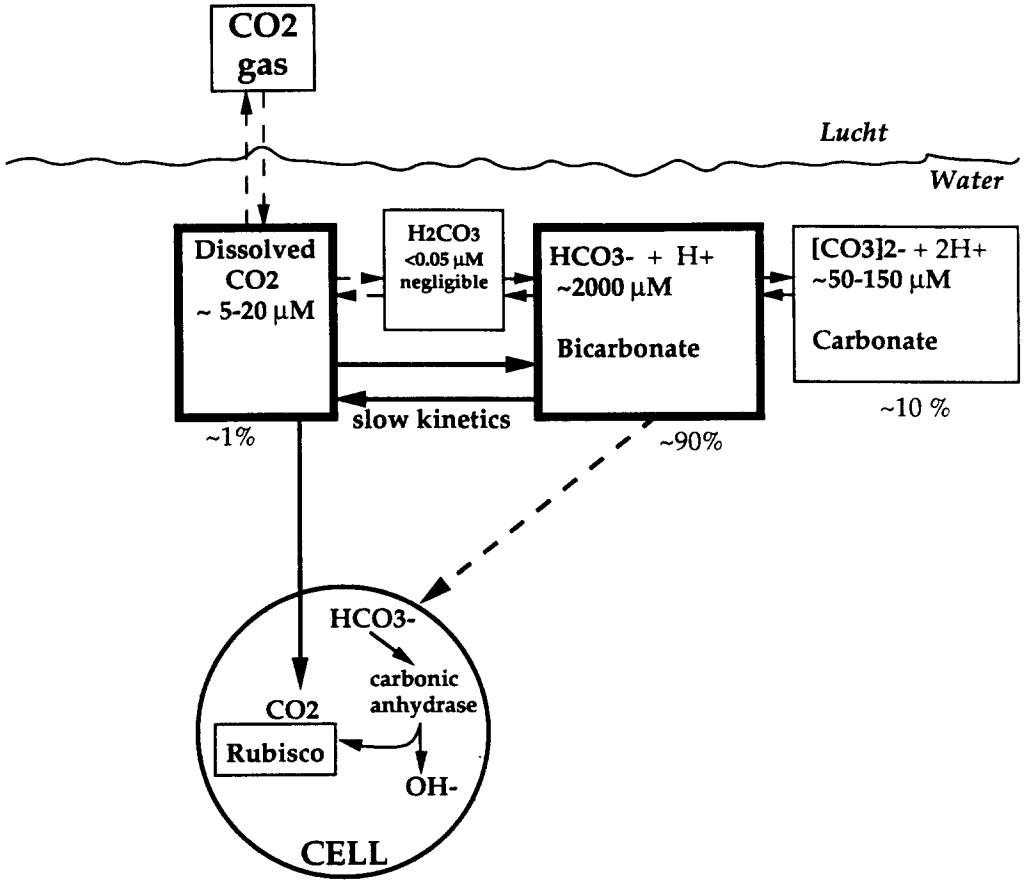
The *in situ* enrichments experiment as envisaged and organised by John Martin was eventually performed in October 1993 near the Galapagos Islands (IRONEX GROUP, 1994). Unfortunately Martin did not live to see it happen. Very convincing evidence was found, but the haphazard nature of a field experiment was felt, leading to re-appraisal of the virtues of bottle experiments (WELLS, 1994).

8. CARBON DIOXIDE LIMITATION

Throughout the literature it has always been assumed that the various dissolved forms of CO_2 in seawater are so abundant that its limitation of growth rate or biomass is unlikely. Early on NATHANSOHN (1909, pp.62-63) thought that the end of late season blooms in the Mediterranean might become CO_2 limited, but this *faux pas* was never heard of again. Algae require the undissociated $[CO_2]_{aqeous}$ which exists in seawater at ~5-20μM, which can be replenished from the large pool of bicarbonate (Fig.6). In terms of final biomass yield, or 'carrying capacity' if you wish, carbon dioxide seems unlikely to be a problem.

However, when reading von Liebig thoroughly, as well as NATHANSOHN (1908), GEBBING (1910), HECKY and KILHAM (1988), the important issue is the rate of growth. If that is true then CO_2 might become limiting for some species of algae (RAVEN and JOHNSTON, 1991). All reactions in the seawater CO_2 system (Fig.6) are very rapid, except the dissociation of HCO_3^- to $[CO_2]_{aqeous}$ (SKIRROW, 1975), which happens to be the one reaction the algae would have 'preferred' to be fast. Also, as a bloom progresses, because of the existing equilibria at virtually constant Alkalinity, the steady decrease of TCO_2 in the sea water will yield a decrease in the equilibrium concentration of $[CO_2]_{aqeous}$ as well. RIEBESELL, WOLF-GLADROW and SMETACEK (1993) recently have shown by model calculations that at realistic low $[CO_2]_{aqeous}$ the rate of CO_2 uptake may be more strongly limited by molecular diffusion than is the rate of nitrate and phosphate uptake. The approach resembles that pioneered by HARVEY (1937a) and appears consistent with observed $^{13}C/^{12}C$ fractionations (DEUSER, 1970; RAU, TAKAHASHI and DES MARAIS, 1989; RAVEN, JOHNSON and TURPIN, 1993). RIEBESELL *et al* (1993) also calculated the simultaneous diffusive supply of bicarbonate (HCO_3^-) to the cell, this being dissociated underway at the given slow kinetic rate. As such this supply route would only be a few percent of the direct supply of $[CO_2]_{aqeous}$ to the cell.

(Note that the parameterization would also apply for diffusive supply of various Fe(III) species to the cell, if only their kinetics of dissociation to free Fe^{3+} ion were known.) Experimental evidence was provided in support of this CO_2 limitation hypothesis. The authors are at pains to point out that CO_2 limitation concerns the rate rather than extent, i.e. biomass yield or export production (RAVEN, 1993). Obviously higher growth rates may help towards higher biomass or export production, but not necessarily. By avoiding the trap of equating limitation of rate with limitation of biomass, which, as described above, has lured so many predecessors when working



Cellular conversion:

$$HCO_3^- + H^+ \xrightarrow[\text{anhydrase}]{\text{carbonic -}} CO_2 + H_2O$$

Alternative is calcification (E. huxleyi) :

$$Ca^{2+} + 2\,HCO_3^- \longrightarrow CaCO_3 + CO_2 + H_2O$$

FIG.6. The carbon dioxide system in seawater and routes for uptake by algae.

on other limiting nutrient elements N, P and Fe, RIEBESELL *et al* (1993) nicely follow the lines of thought pioneered by Nathansohn, Gebbing and Harvey.

9. DISCUSSION

The original writings (Briefe) of von Liebig reflected an awareness that the rate of plant growth is crucial to the agriculture science of crops on farm lands. This was expanded into 50 propositions on agriculture, which were explicitly meant to be taken as variations on one overall theme of the rate of plant growth depending on nutrient availability versus the resistance against their utilization. Proposition 33 was most appealing for ecologists, who applied it as the Law of the Minimum, well beyond its original context in agriculture. The risk associated with such extrapolation is well recognised nowadays (POMEROY, 1974).

Ever since BRANDT (1899), the Law of the Minimum has been repeatedly invoked in plankton ecology, when studying N, P and Fe as limiting factors. Similarly NATHANSOHN (1908) was the first in a long series contesting its application in this context, arguing that the difference between rates of phytoplankton growth and rates of loss were the proper terms in a mass balance (RILEY, 1946; HECKY and KILHAM, 1988, P.798) of population growth rate. Fortunately in the case of the recent revival of CO_2-limitation, the Law of the Minimum has been avoided.

Surely, a given plankton bloom (read *'one kind of plant'*) at a given point in time and space may succumb for one overriding reason, e.g. shortage of one nutrient element, or light (e.g. self-shading, or deep mixing storm event), or grazing (e.g. krill swarm event, LANCELOT, MATHOT, VETH and DE BAAR, 1993). Surely such overriding factors are, more or less parallels to the Law of the Minimum. The well known succession of algal species which occurs during the North Atlantic spring bloom is a classical example of limitation of a single plant taxon (actually group of species, i.e. diatoms) by one element (Si), and this is then followed by N, P depletion limiting other plants. However, for the various pelagic ecosystems of the complete Antarctic Ocean, and other world oceans, when integrated over time and space, limitation of growth by a single factor appears unlikely.

With discussing the growth rate or standing stock of phytoplankton in the world ocean it is irrelevant to discuss whether one or the other of nutrient elements N, P, Fe and C, is the ultimate limiting element. Surely in the central gyres of the temperate zones both N and P appear to be severely limiting, but the loss terms (grazing, sedimentation) are also severe and the roles of Fe and C are poorly understood. In the so-called High Nutrient Low Chlorophyll regions (HNLC, e.g. ~40% of Pacific, REID, 1962) grazing most likely is to be important at all latitudes (FROST, 1991; BANSE, 1992; FROST and FRANZEN, 1992), and light limitation at high latitudes (TRANTER, 1982), while deficiency of Fe and C may also have an effect (RIEBESELL *et al*, 1993; DE BAAR *et al*, 1990). With respect to export production, or new production as its equivalent under steady state, it might be more sensible to contemplate whether it is P, or N or Fe as the ultimate global limitation (BRAND, 1991), or is this another irrelevant question triggered by the general application of the Law of the Minimum in ecology?

Another consequence of the extrapolation of the Law of the Minimum to ecology has been the debate on the ultimate limiting nutrient in freshwater versus marine environments (e.g. SMITH, 1984; HECKY and KILHAM, 1988). Here there appears to be a tendency to suggest that freshwater systems often are P-limited, whereas marine systems would be more prone to N-limitation, the latter in concordance with the original hypothesis of BRANDT (1899), albeit fortuitously. Whether or not freshwater systems generally are P-limited is beyond the scope of this study. For marine N-limitation, HOWARTH and COALE (1985) have sought to provide a mechanism by suggesting that

marine algae have difficulty taking up Mo (molybdenum). Both Mo and Fe are essential for nitrate reduction, and even more for N_2-fixation (RAVEN, 1988). The anion molybdate occurs in high concentration in sea water. Yet at even higher concentrations of major anion sulphate in sea water, the marine algae were assumed to have problems in distinguishing between the similar anions, i.e. the required preferential uptake of molybdate would be impaired. However elegant, the experimental evidence failed to provide firm validation of this marine Mo deficiency hypothesis. Nowadays it is clear that the concentration of Fe in freshwater systems is much higher than in the central ocean basins. Hence the Fe required for nitrate reduction or N_2-fixation is in short supply in the oceans, i.e. one argument in favour of N-limitation of marine algae, albeit indirectly as the underlying mechanism is actually Fe limitation. However here it has been argued that no one single limiting factor exists in nature, i.e. the past debate on P- versus N-limitation of freshwater versus marine ecosystems is deemed a non-issue.

The various concepts of control of plankton growth, or iron supply to the cell, have existed for many decades. The distinction between opposing concepts (hypotheses) can only be made by observational evidence. Some major breakthroughs in marine plankton ecology can be ascribed to the development and application of novel chemical techniques and analyses, thus allowing the observations to be made with the precision necessary. Atkins, Harvey, Cooper, Wattenberg, and Martin were all chemists, each one seeking to develop advanced techniques to solve the problems they considered to be important. Further study of the limiting role of CO_2 will also require sophisticated, accurate measurements of the CO_2 system both in seawater and within the cell. These developments are in keeping with the findings of KUHN (1962) in other sciences, who concluded that competing concepts persist for long periods, until new technology opens the way for their resolution, i.e. enabling the major breakthroughs in understanding to occur.

10. ACKNOWLEDGEMENTS

By consultation of previously published work an attempt has been made to sketch some of the lines of development of plankton ecology of the sea. Except for intrinsically personal interpretation, this is by no means seen as original research. Yet it is hoped this synthesis will be of some use. Virtually all books and periodicals were available in the fabulous historical collection of the library at the Netherlands Institute for Sea Research. Help and advice from the librarians, also at the Plymouth Marine Laboratory and the Free University in Amsterdam, is highly appreciated. The 1942 issued volume of the *Discovery* Reports (HART, 1942) was consulted at the P.M.L. as it was missing from the library at the Netherlands Institute for Sea Research, presumably it was either not mailed out or not received in occupied The Netherlands during those war days. In principle all cited work has been consulted, with occasional exceptions. At the time of type setting, the library search for some publications had regretfully not been successful. For this reason, some citations lack full titles (most notably some older works cited after LEWIS and GOLDBERG, 1954) in which case the source of reference is mentioned. The work of J. von Liebig has been published in so very many editions in several languages that it was not always possible to retract exactly the same edition as cited by other colleagues. Here it is assumed most editions of the same work were identical in their main message. Most grateful the historical monographs of RILEY (1965), VON ARX and MARTIN (1977), TAYLOR (1981), PARSONS (1981) and SNELDERS (1980) were consulted. Most of all the fascinating history written by ERIC MILLS (1989) with very wide ranging perspective, served as a valuable source for tracing just the narrow Liebig-line of development for elements N and P in the early period. As much as possible the original papers were cited in the text, but the influence of these historical monographs cannot be underestimated. If it were not for the fascinating findings and provocative hypotheses of John Martin and his collaborators, this, our earlier, and ongoing Fe-studies would not have been undertaken. Anita G.J. Buma, Gerhard C. Cadée, Maria A. van Leeuwe, Roel Riegman, Klaas R. Timmermans and Marcel J.W. Veldhuis kindly provided comments on an earlier draft of the manuscript. Parts were presented at a public lecture at the University of Groningen (11 May 1993) and published privately (DE BAAR, 1993) in the Dutch language. At the symposium commemorating the scientific quest of John Holland Martin a summary was presented (DE BAAR, 1994). Multi-cultural

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