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Metal enrichment experiments in the Weddell-Scotia Seas: Effects of iron and manganese on various plankton communities

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Abstract

During the European *Polarstern* Study (EPOS 1988/1989) in the Weddell and Scotia Seas, five series of metal enrichment experiments were carried out with natural plankton communities under ultraclean conditions. Despite a clear stimulation of growth by the addition of Fe, control bottles (no additions) also showed rapid buildup of Chl *a* and complete utilization of a major nutrient within 2 weeks, indicating nonlimiting ambient Fe levels. Effects of Mn additions were less pronounced or absent, whereas extra additions of Zn and Cu in one experiment showed little or no effect. The species composition of the plankton community, monitored by HPLC pigment analysis and microscopic observations, changed in favor of diatoms when Fe was added. The addition of Fe also caused an increase in microzooplankton densities and concentrations of pigment breakdown products. However, metal-mediated shifts in the plankton community were minor compared to major changes resulting from incubation. Changes were most pronounced in experiments where microzooplankton was strongly developed, presumably as a result of excluding mesozooplankton from the bottles. Fe had an impact on plankton growth and species composition, but other factors seem to be responsible for keeping phytoplankton productivity far from its potential in these Antarctic waters.

Over the past few years a lively discussion has come about on the matter of the presumed limiting role of Fe in various oceanic, high nutrient level ecosystems (e.g. Martin and Gordon 1988; Banse 1990; Broecker 1990; Martin 1990; Martin et al. 1990*a*; Davies 1990; Joos et al. 1991, Peng and Broecker 1991). Although the hypothesis of Fe limitation in Antarctic waters dates back half a century (Hart 1934), until now only two studies have been carried out to test this hypothesis. These studies, carried out in different regions of the Antarctic, yielded comparable results, but different conclusions (de Baar et al. 1989, 1990; Martin et al. 1990*b*).

It is well known that suboptimal Fe levels derange biochemical and life cycle processes of various phytoplankton species (Verstraete et al. 1980; Marschner 1986; Rueter and Ades 1987; Doucette et al. 1989; Doucette and Harrison 1990; Rueter et al. 1990). In the field changes in natural Fe levels may trigger red tide blooms (Wells et al. 1991). Metal enrichment studies carried out in the Gulf of Alaska revealed that Fe affects the species composition of the phytoplankton by selectively promoting either diatom growth or the growth of coccolithophorids depending on the water mass under investigation (Coale 1988; Martin et al. 1989). Laboratory studies have shown that suboptimal Fe levels probably restrict the development of large phytoplankton cells (i.e. mainly diatoms) more than small cells (i.e. nanoplankton and picoplankton) (Hudson and Morel 1990), indicating that low Fe levels favor pico- and nanoplankton growth in open oceans, whereas for inshore "Fe(rtilized)" waters shifts toward diatom-dominated communities can be expected. On the other hand, oceanic diatom species may have a lower demand for Fe than neritic species (Brand et al. 1983). When our study started data on the role of Fe or other

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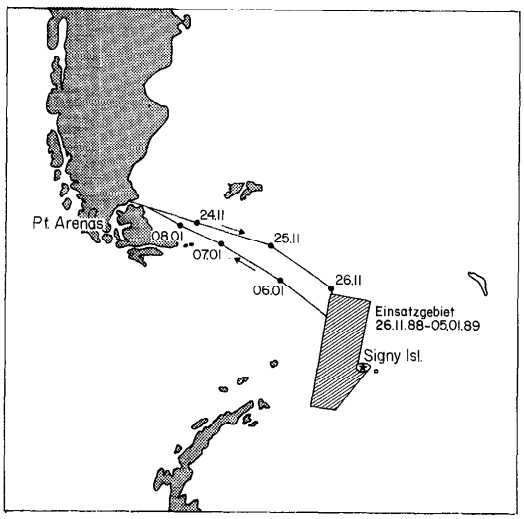

Fig. 1. Hatched area: region where initial samples were collected for metal enrichment experiments during EPOS leg 2 (1988–1989).

metals in determining species composition and succession in Antarctic plankton communities were lacking.

The EPOS program (European *Polarstern* Study) was designed to increase our understanding of the functioning of the various ecosystems in the Weddell and Scotia Seas. During the second leg of EPOS (November–January 1988–1989) integrated physical, chemical, and biological studies focused on factors determining pelagic productivity in the area (Hempel et al. 1989). Within this context enrichment experiments were conducted to study the effect of metal additions on both phytoplankton growth and community structure. Five experimental series were done covering different water masses and plankton communities. Part of this work has been published (de Baar et al. 1989). deBaar et al. (1990) concluded that despite the stimulating effect of Fe on nutrient uptake and buildup of Chl *a* and POC, Fe deprivation cannot explain the low productivity of the area during EPOS. Untreated controls showed rapid accumulation of Chl *a* to concentrations >10 μg liter^{-1} and virtual depletion of nutrients. Ambient Fe levels appeared adequate to support more than moderate phytoplankton growth in the experimental bottles, which is confirmed by data on ambient dissolved Fe concentrations in the research area (Nolting et al. 1991).

In this paper we assess the possible effects of Fe and Mn on growth and species composition in the various plankton communities of the region, relying on a combination of microscopic observations and specific pigment analysis.

Material and methods

The area of survey where water was taken for metal enrichment studies is shown in Fig. 1; general information on the sampling stations and metal additions in the experiments is given in Table 1. The hydrology and ice conditions were described by Cederløf et al. (1989) and Veth (1991). Sampling, which was carried out in daytime, relied on 12–17 modified Teflon-coated Go-Flo bottles mounted on an all-Teflon-coated stainless steel CTD/rosette frame. This package was used successfully to study nanomolar Fe and Mn distributions (Saager et al. 1989; de Baar 1991). The package was sent down to 500 m to flush out any possible remaining contamination after which the samplers were tripped during the upcast at depths of 20–50-m, which was done because fluorescence was maximal at these depths and also to avoid metal contamination from the ship.

After recovery, the bottles were mounted outside a Class-100 clean-air laboratory van, then the water was led through all-Teflon (PTFE, FEP) lines into 20-liter polycarbonate vessels in the clean van. Dissolved Fe, Mn, Cu, and Zn with or without EDTA were added from analytical stock solutions (*see Table 1*). After addition of metal solutions the bottles were capped, sealed with plastic bags, and incubated in a second refrigerated van. During incubation the vessels were slowly revolved (1–2 rpm) under artificial light at a saturating (Lancelot and Mathot 1989) intensity of 100 μEinst m^{-2} s^{-1} at a fixed temperature of 3.5°C. Vessels were brought into the Class-100 van daily or on alternate days to sample for nutrients, Chl *a*, and cell counts. Directly after collecting the initial water sample ($t = 0$) and at the end of the experiments additional samples were taken for particulate organic C (POC)

Table 1. General information on the five culture series. Number of bottles with and without (controls) additions. Concentrations (nM) of trace metals and EDTA in the culture solutions in parentheses. Additions usually at $t = 0$ except for EPOS 145 (addition of 1 nM at $t = 0$ and a second addition of 10 and 20 nM at $t = 7$ d) and EPOS 158 (additions at $t = 2$ d); 6 nM additions (EPOS 158) were done by subsequent daily additions of 1 nM over 6 d.

	EPOS Exp.				
	145	158	159	169	182
Region or sea	Confluence	Confluence	Scotia Sea	Weddell Sea	Scotia Sea
Position	58°S, 49°W	59°S, 49°W	57°S, 49°W	62°S, 47°W	57°S, 49°W
Date (1988)	27 Nov	8 Dec	12 Dec	17 Dec	27 Dec
Ice cover (%)	0	0	0	>90	0
Sampling depth (m)	35	20	50	40	40
Water temp. (°C)	−0.6	−1.3	0.1	−1.8	1.6
Duration of exp. (d)	10	8	12	9	8
No. 20-liter cultures	4	7	2	6	7
No. 2.5-liter cultures	0	0	0	0	4
20-liter additions					
Controls (no additions)	2	2	1	1	1
Fe (nM)	2 (10, 20)	3 (1, 6, 10)	1 (10)	1 (10)	1 (10)
Fe + EDTA (nM + nM)	0	0	0	1 (10+15)	1 (10+30)
EDTA control (nM)	0	0	0	1 (15)	1 (30)
Mn (nM)	0	2 (6, 10)	0	1 (10)	1 (1)
2.5-liter additions					
Controls (no additions)	0	0	0	0	1
Fe + EDTA (nM + nM)	0	0	0	0	1 (1 + 30)
Fe + EDTA + Cu (nM + nM + nM)	0	0	0	0	1 (1 + 30 + 1)
Fe + EDTA + Zn (nM + nM + nM)	0	0	0	0	1 (1 + 30 + 1)

measurements and HPLC analysis of pigments (not in 2.5-liter bottles, i.e. Cu and Zn additions with Fe + EDTA). Shipboard analysis of nutrients and Chl *a*, as well as POC measurements, were performed with standard methods (*see* de Baar et al. 1990; POC following Cadée 1982); <3 μM SiO_4 was determined with a manual heteropoly-blue method (Strickland and Parsons 1972).

Because light microscopy may underestimate the nanophytoplankton abundance, HPLC pigment measurements were used to estimate nanophytoplankton groups by quantifying their specific pigments, whereas specific Chl *a* breakdown products (pheophorbides) provided information on zooplankton activity. HPLC analysis of pigments followed the methods of Gieskes and Kraay (1986). Five liters of seawater were filtered on 47-mm GFF Whatman glass-fiber filters and stored frozen for later analysis in the laboratory. Detection of peaks was established at 436 and 658 nm. Peak areas were calculated with a peak integrator except for pheophorbide peaks (usually 2–5) which were integrated by hand. Identification and calibration of pigments was performed as described by Gieskes and Kraay (1983). HPLC Chl *a* measurements were lower (mean of 30%) than the spectrofluorometric shipboard Chl *a* measurements, reported by de Baar et al. (1990), which is expected because the latter method also includes Chl *a* isomers (Gieskes and Kraay 1984).

Samples for cell abundance were fixed with a mixture of glutaraldehyde and acid Lugol's solution and stored at low temperature in the dark until counted in the laboratory (light microscopy). Counting was done with sedimentation chambers with volumes of 5–100 ml. Ciliates were counted twice in 100-ml chambers, diatoms in 10 or 20 ml, and small phototrophic cells in 5- or 10-ml volumes. Small micrograzers like choanoflagellates were usually very abundant and increasing during the experiments, but were not quantified due to identification problems. Net diatom division rates (growth minus grazing) were determined in EPOS 145 and 159 from changes in cell numbers during growth with data from five subsequent samples taken during the experiments (not done in the other experiments). Calculation was done by linear regression analysis. Cell counts were converted to biovolume with stereometric shapes and formulas given by Edler (1979). Mean cell volumes were calculated for each species by measuring cell size in each experiment for each species. When necessary two size classes were distinguished. A complete data table of HPLC pigment measurements, cell countings, and biovolume measurements is available on request.

Results

General trends for Chl a *and nutrients*—As reported previously, all five experimental series showed an increase of Chl *a* synthesis and nutrient assimilation after the addition of different amounts of Fe (with or without EDTA) as compared to the controls. At the same time, in four of five experiments final POC levels were significantly higher in bottles enriched with Fe (*t*-test, $P < 0.05$). Effects of Mn addition were modest in all respects (de Baar et al. 1989, 1990). In spite of the obvious favorable effect of Fe enrichment on phytoplankton development in the experimental bottles, good phytoplankton growth was also found in all control bottles, as evidenced by rapid decreases in nutrient concentrations as well as increases in Chl *a* levels. Concentrations of Chl *a* and nutrients at the end of the experiments in the control bottles were much higher and lower respectively than "normal" in the area of research as concluded from in situ measurements carried out during EPOS (Neth. Inst. Sea Res. 1989; de Baar et al. 1990).

In the final series (EPOS 182, Scotia Sea) additional experiments in 2.5-liter bottles were carried out to study the effect of Cu and Zn (1 nM) on the growth stimulation by Fe and EDTA (Fig. 2). The effect of Fe was not influenced by adding 1 nM Cu. The expected toxic effect of Cu on ciliate activity (Pacific Ocean, Coale 1988) or diatom growth (Florence and Stauber 1986) was not observed after adding 1 nM Cu. Upon later analysis of ambient seawater, Cu levels of 2–5 nM were found (Nolting et al. 1991), i.e. in retrospect it is possible that growth was not influenced by adding 1 nM Cu.

Adding Zn led to a modest increase in Chl *a* buildup and nutrient utilization (superimposed on the Fe effect), as shown in Fig. 2. Ambient Zn levels are quite high (Nolting et al. 1991) but may not be directly available due to strong complexation (Bruland 1989; Donat and Bruland 1990).

Community structure in experiments from the Scotia Sea (EPOS 159 and 182)—The first Scotia Sea experiment (EPOS 159) showed the most pronounced effect of an Fe addition compared to the other experimental series. Despite this clear response to adding 10 nM Fe, the growth of phytoplankton in the control bottle was also high leading to $[NO_3^-]$ of 16.4 μM and $[PO_4^{3-}]$ of 0.79 μM at t = 12 d (de Baar et al. 1990). The initial $[SiO_4^{4-}]$ of 27 μM had dropped to <0.3 μM at t = 14 d, suggesting SiO_4^{4-} limitation at the end of the experiment. Calculation of division rates based on cell counts showed Fe-mediated increases in division rate of most diatom species, which for three species were significant (Wilcoxon-test, $P < 0.05$, Table 2). However, the high division rates calculated for the control again confirmed the notion that, even at this station, with the lowest Fe levels recorded in surface waters during the cruise (Nolting et al. 1991), in situ Fe levels must have been sufficient for rapid phytoplankton growth. Calculated division rates in the control were well within ranges given by others for cold-adapted plankton (Jacques 1983; Rivkin and Putt 1987; Mortain-Bertrand 1989; Sommer 1989), whereas in a few cases they agreed with maximal specific growth rates as predicted by Eppley (1972) for high-latitude algae, especially when assuming that division rates, notably of small phytoplankton species, were underestimated due to microzooplankton grazing activity (small *Nitzschia* spp., Table 2).

Pooled phytoplankton biovolume measurements as well as pigment signatures measured in both bottles (control and Fe addition) at the end of experiment 159 revealed that nanoflagellates were suppressed during incubation, notably Prymnesiophyceae (19′hexanoyloxyfucoxanthin, Fig. 3) and (or) Chrysophyceae (19′butanoyloxyfucoxanthin, not shown). High relative amounts of Chl *c* and fucoxanthin (in general, diatom pigments) indicated that diatoms prevailed at the end of both incubations, which was consistent with biovolume measurements (Fig. 3). The observed shifts

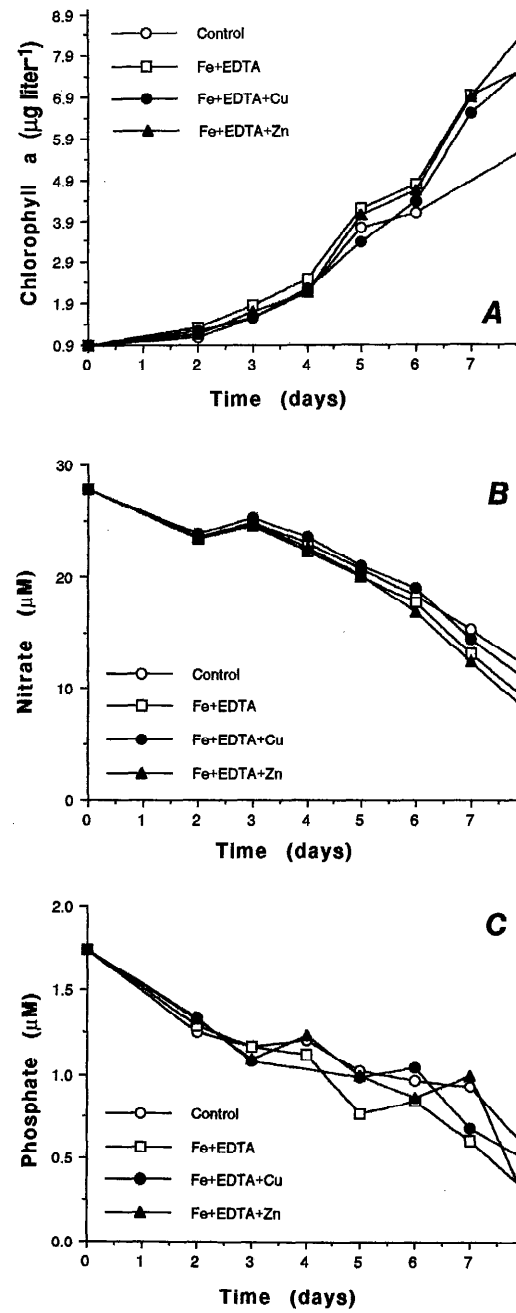

Fig. 2. Addition of Cu and Zn with Fe and EDTA to a natural phytoplankton community at EPOS station 182 (Scotia Sea, 27 December 1988). Incubations in 2.5-liter bottles (*see Table 1*).

Table 2. Division rates of the most abundant diatoms during metal enrichment experiments EPOS 145 and EPOS 159 (div. d^{-1}). Coefficient of correlation in parentheses. All regression analyses significant except for *Rhizosolenia* spp. (control bottle) of EPOS 159. (s)—Significant differences between control and Fe addition following Wilcoxon test ($P < 0.05$).

Phytoplankton sp.	Control (d^{-1})	Fe addition (d^{-1})
EPOS 145, WSC		
Corethron sp.	0.67 (0.98)	0.72 (0.98)
Chaetoceros neglectus	0.95 (0.99)	1.00 (0.99)
Nitzschia fragilaria large (s)	0.57 (0.93)	0.69 (0.93)
Thalassiothrix sp.	0.36 (0.93)	0.35 (0.93)
EPOS 159, Scotia Sea		
Corethron sp. (s)	0.68(0.97)	0.73(0.90)
Thalassiothrix sp.	0.50(0.92)	0.65(0.95)
Chaetoceros spp. large	0.76(0.92)	0.63(0.96)
C. neglectus (s)	0.71(0.94)	0.75(0.96)
Rhizosolenia spp.	0.52(0.84)	0.59(0.92)
cf. *Thalassiosira* spp. (s)	0.36(0.95)	0.46(0.89)
N. fragilaria large	0.66(0.93)	0.72(0.92)
N. fragilaria small	0.44(0.99)	0.38(0.88)
Nitzschia seriata small	0.54(0.96)	0.61(0.97)

in favor of diatoms in both phytoplankton communities seemed to be due to strong microzooplankton grazing on small-sized phytoplankton species.

Ciliates were abundant throughout the experiment, increasing from 1,775 ind. liter^{-1} at $t = 0$ to >8,000 liter^{-1} at the end of the incubation ($t = 14$ d). In these and all other experiments abundant and increasing numbers of choanoflagellates were observed but could not be properly quantified. At the same time, the concentration of pheophorbides (a specific product of zooplankton grazing) was high (Fig. 3), as was the C:Chl *a* ratio, being 320 ($t = 0$), 231 (control, end value), and 216 (Fe addition, end value). The latter values suggest the presence of high nonphototrophic biomass. The increase in microzooplankton abundance could be explained by the exclusion of large grazers (mesozooplankters like copepods and krill) from the bottles at the time of initial sampling. For the same reason (i.e. the absence of mesozooplankton), large diatoms could accumulate in the bottles. Finally the enclosure of diatoms in the experimental bottles would retain the cells in the light despite their tendency toward set-

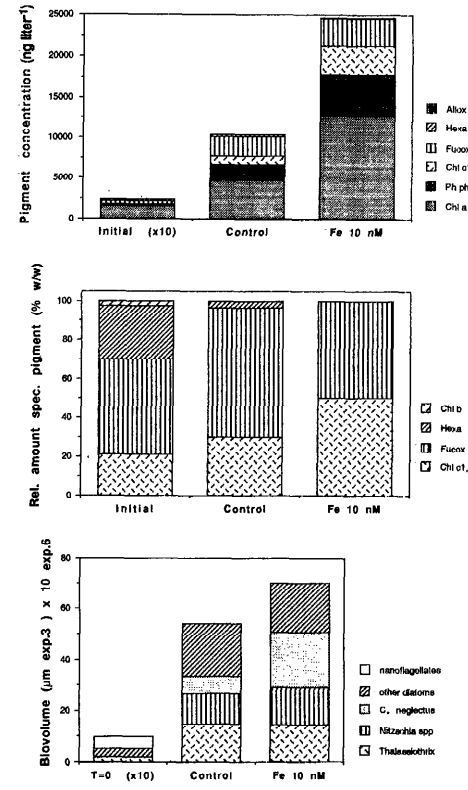

Fig. 3. The effect of Fe additions (Table 1) on phytoplankton species composition at EPOS station 159 (Scotia Sea, started 12 December 1988). Absolute (upper) and relative (middle) amount of (specific) pigments [abbreviations (all in ng liter^{-1}): Allox—alloxanthin; Hexa—19′hexanoyloxyfucoxanthin; Fucox—fucoxanthin; Chl *c*1,2—chlorophyll *c*1,2; Ph phorb—pheophorbides]. Phytoplankton biovolumes (lower) calculated from cell counts at $t = 0$ and $t =$ end (12 d).

tling, which might have caused floristic differences compared to the field. Under the stable weather conditions during EPOS (Veth 1991), these large diatoms likely would have disappeared from the euphotic zone through sinking.

The addition of Fe caused both an increase in pheophorbide concentration (Fig. 3) and a slight increase in microzooplankton numbers, finally reaching 10,077 ciliates (mainly tintinnids) liter^{-1} in the Fe 10 nM addition vs. 8,027 liter^{-1} in the control. At the same time, a selective advantage for diatoms was found. Absolute and relative concentrations of diatom pigments (fucoxanthin, Chl *c* 1,2) were higher in the Fe-treated bottle, whereas nanoflagellate pig-

ment (19′hexanoyloxyfucoxanthin) was lower than in the control (Fig. 3). Fe addition also resulted in higher diatom biovolumes (Fig. 3) and growth rate (Table 2). Summarizing, Fe simultaneously stimulated diatom growth and microzooplankton density, the latter leading to decreased nanoflagellate abundance. This combination created a stronger shift toward diatom dominance compared to the control bottle.

The second experiment in the Scotia Sea (EPOS 182) began 15 d later (Table 1). The initial plankton community consisted of a mixture of diatoms and flagellates (prymnesiophytes), as monitored by their pigments (Fig. 4), accompanied by lower zooplankton abundance as compared to EPOS 159 (POC : Chl *a* ratio of 148; initial ciliate numbers, 356 liter^{-1}). During the incubation, the phytoplankton community shifted in favor of diatoms (Fig. 4), probably for the same reasons found for EPOS 159. However shifts were less pronounced compared to EPOS 159 presumably due to the lower initial SiO_4^{4-} level (19.3 μM) leading to limiting values already at t = 8 d (0.35–0.40 μM). Moreover, top-down control by microzooplankton was of minor importance in this experiment, as monitored by fairly low and constant pheophorbide concentration (Fig. 4) and modest ciliate abundances. Therefore heterotrophic activity did not control nanoplankton biomass to the extent found for EPOS 159.

The effect of Fe was less pronounced in EPOS 182 than in EPOS 159. In EPOS 182 the amount of nanoflagellate pigments (19′hexanoyloxyfucoxanthin) was not influenced by the addition of Fe. The amount of diatom pigments however (fucoxanthin and Chl *c*1,2) increased upon the addition of Fe, which resulted in a small shift toward diatom dominance in one Fe-enriched bottle compared to the others. The more pronounced effect of Fe in EPOS 159 compared to EPOS 182 is probably due on the one hand to differences in orthosilicic acid availability for diatom growth and on the other hand to differences in microzooplankton abundance, the latter being much higher in EPOS 159.

Community structure in the experiments from the Weddell Scotia Confluence (EPOS 145 and 158)—The first experiment in the Weddell Scotia Confluence (EPOS 145) started with an initial Chl *a* value of 0.63 μg liter^{-1} accompanied by high nutrient levels (27.9 μM NO_3^-; 1.86 μM PO_4^{3-}; 61 μM SiO_4^{4-}). The initial phytoplankton community was composed mainly of flagellates from the Prasinophyceae (containing Chl *b*), Cryptophyceae (containing alloxanthin), and Prymnesiophyceae (containing 19′hexanoyloxyfucoxanthin), accompanied by some diatoms (Fig. 5). Nonphototrophic biomass was high (POC : Chl *a* ratio, 238) but it cannot be accounted for by ciliates whose initial abundance was only 270 liter^{-1}. During the incubation SiO_4^{4-} values dropped to final values of 42 μM (control) and 35 μM (20 nM Fe addition) at t = 10 d; at that time NO_3^- had become undetectable in the Fe bottles (de Baar et al. 1990). At the same time, a shift was observed in all bottles toward a diatom (*Corethron criophilum*) dominated community (Fig. 5), probably again resulting from bottle effects like diatom containment and the absence of mesozooplankton, the latter also allowing microzoo-

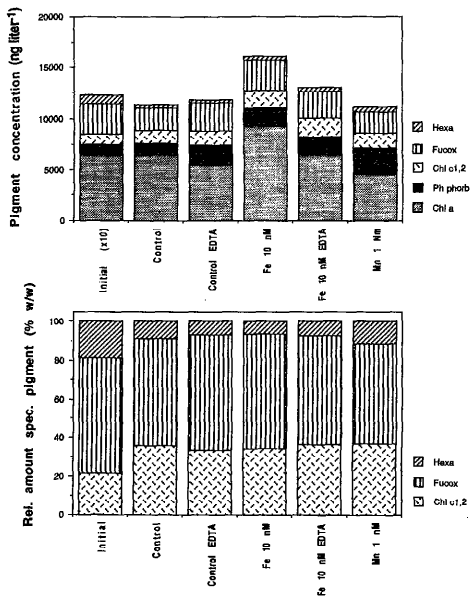

Fig. 4. The effect of Fe and Mn additions (Table 1) on phytoplankton species composition at EPOS station 182 (Scotia Sea, started 27 December 1988). Absolute (upper) and relative (lower) amounts of (specific) pigments (abbreviations same as Fig. 3). Phytoplankton biovolumes not measured.

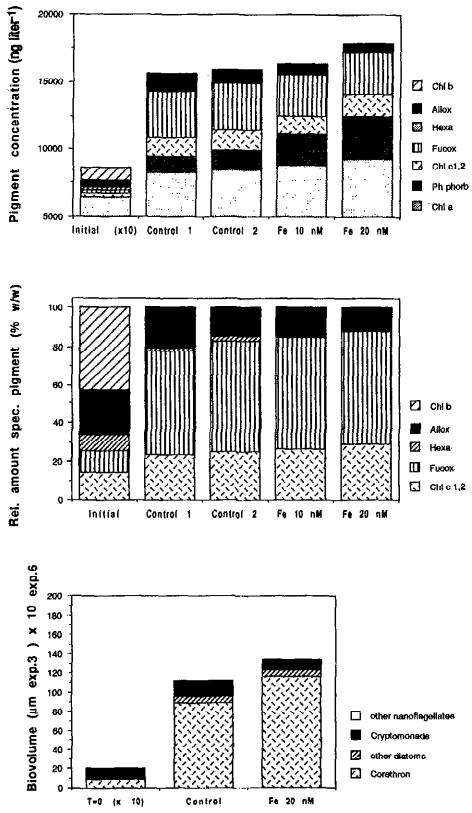

Fig. 5. The effect of Fe (Table 1) additions on phytoplankton species composition at EPOS station 145 (Weddell Scotia Confluence, started 27 November 1988). Absolute (upper) and relative (middle) amount of (specific) pigments (abbreviations same as Fig. 3). Phytoplankton biovolumes (lower) calculated from cell counts at $t = 0$ and $t =$ end (10 d).

plankton development and grazing (*see EPOS 159*). Microzooplankton numbers (here ciliates) were very high at $t = 10$ d (20,600–26,200 liter^{-1}).

Adding Fe (especially the 20 nM case) caused a further shift in the community in favor of diatoms. When considering relative specific pigment concentrations, these shifts were only due to decreased concentrations of nanoflagellate pigments (alloxanthin and 19′hexanoyloxyfucoxanthin) and not to a simultaneous increase in diatom pigment levels (Fig. 5). However, biovolume measurements (*Corethron,* Fig. 5) and calculated division rates of the most abundant species (Table 2) support the idea of a direct diatom growth stimulation by Fe. Adding Fe also enhanced ciliate abundance (20,600 liter^{-1} in control vs. 26,000 in the 20 nM Fe addition) and pheophorbide concentrations at $t = 10$ d (3,233 ng liter^{-1} for the 20 nM Fe addition). Nanoflagellate biovolume and pigment content was lower in the Fe (20 nM addition) bottle, presumably as a result of increased grazing activity.

The second experiment in the Weddell-Scotia Confluence area (EPOS 158) began with a relatively high Chl *a* concentration (2.24 µg liter^{-1}). Initial NO_3^- (26.0 µM) and PO_4^{3-} (1.55 µM) concentrations were slightly suppressed compared to EPOS 145 but SiO_4^{4-} was higher (67.6 µM). During incubation, SiO_4^{4-} dropped only slightly, reaching final concentrations of 63.2 µM in both controls and 61.3–62.9 µM in the Fe and Mn additions. Comparable with EPOS 145, the initial phytoplankton community contained mainly nanoflagellates (cryptophytes, Chl *b*-containing algae, and prymnesiophytes, Fig. 6), yet with low microzooplankton abundance (230 ciliates liter^{-1}) and a POC : Chl *a* ratio of 82.2.

Shifts in the community as a result of incubation were minor compared to EPOS 145 (Fig. 5). In the controls nanoflagellate biovolume as well as relative nanoflagellate pigment remained high throughout. Minor incubation effects might be due to lower microzooplankton grazing pressure (maximum ciliate number, 3,650 liter^{-1}) or to less dramatic containment effects (*see EPOS 159*), because the diatom community of EPOS 158 consisted mainly of small species like *Chaetoceros neglectus* and *Nitzschia* spp., whereas EPOS 145 was dominated by the large *Corethron* sp.

The 10 nM Fe addition caused shifts toward diatom dominance as monitored by biovolume and absolute and relative pigment concentrations, whereas the other Fe additions showed no effect (Fig. 6). Microzooplankton, mainly consisting of small aloricate ciliates, remained low during EPOS 158 compared to EPOS 145. However, as judged from higher values for ciliate abundance and pheophorbide content at the end of the experiment, Fe addition again must have had an impact on microzooplankton activity (control, 2,600 liter^{-1} vs. 10 nM Fe addition, 3,650 liter^{-1}). In summary, high

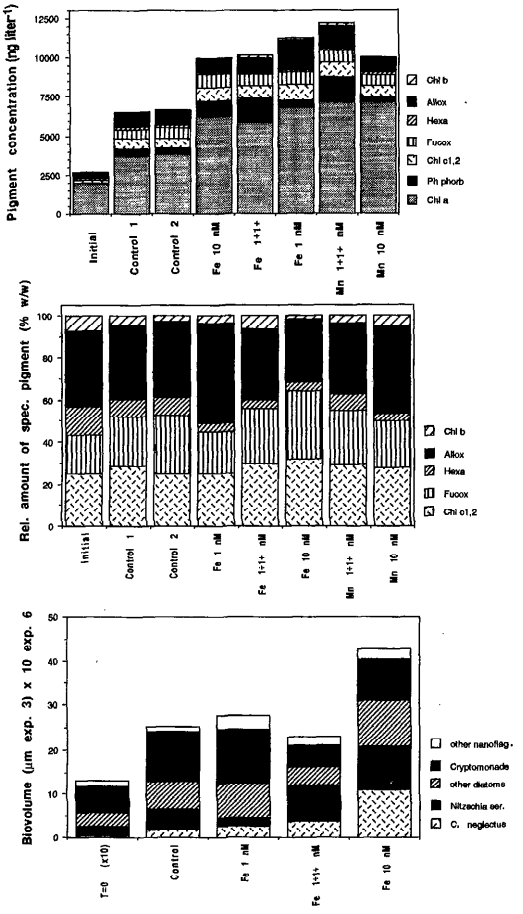

Fig. 6. The effect of Fe and Mn additions (Table 1) on phytoplankton species composition at EPOS station 158 (Weddell Scotia Confluence, started 8 December 1988). Absolute (upper) and relative (middle) amount of (specific) pigments (abbreviations same as Fig. 3). Phytoplankton biovolumes (lower) calculated from cell counts at $t = 0$ and $t =$ end (8 d).

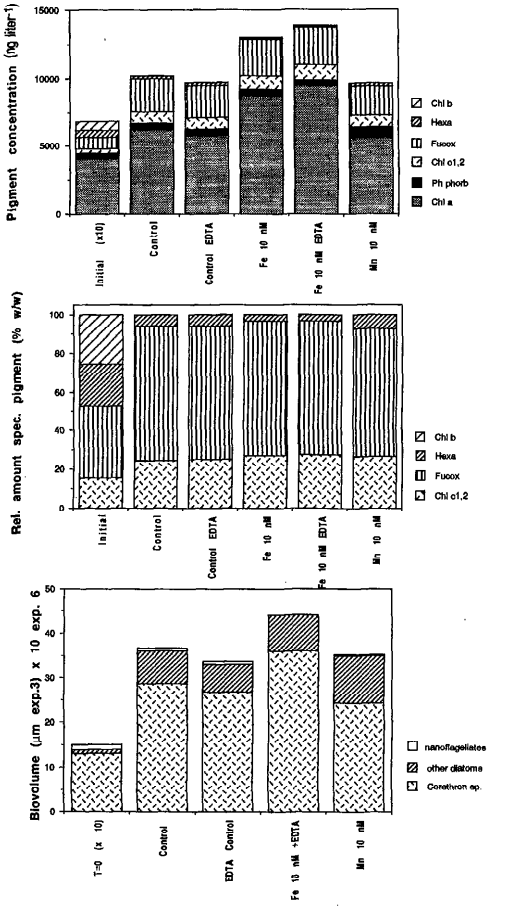

Fig. 7. The effect of Fe, Mn, and EDTA additions (Table 1) on phytoplankton species composition at EPOS station 169 (Weddell Sea, started 17 December 1988). Absolute (upper) and relative (middle) amount of (specific) pigments (Abbreviations same as Fig. 3). Phytoplankton biovolumes (lower) calculated from cell counts at $t = 0$ and $t =$ end (9 d).

(EPOS 145) microzooplankton abundances enhanced the effect of Fe additions on the phytoplankton community structure by accelerating the community shift toward diatom dominance compared to experiments where microzooplankton abundance was low (EPOS 158). This trend is consistent with experiments from the Scotia Sea (Figs. 3, 4).

Community structure in EPOS 169 (Weddell Sea)—The initial phytoplankton community at EPOS 169 (Weddell Sea, Table 1) was composed of a mixture of diatoms (mainly *Corethron* sp. in biovolume, Fig. 7) accompanied by flagellates from various taxonomic classes: Chl *b*-containing organisms (*Pyramimonas* spp.) and prymnesiophytes (mainly *Phaeocystis* sp. flagellates). At the end of the experiment ($t = 9$ d), no nutrient limitation was observed; Chl *a* concentrations were still steadily increasing, and NO_3^- and PO_4^{3-} were 15.8–21.2 and 0.73–1.14 μM respectively. SiO_4^{4-} (initial concn, 81.3 μM) decreased to 75.4–75.8 μM at $t = 9$ d. Incubation for 9 d caused a pronounced relative reduction of nanoflagellate biovolume and pigments and a relative increase in diatom abundance in all

bottles (Fig. 7), which might have been caused by containment effects in combination with increased microzooplankton grazing, in accordance with EPOS 159 (Scotia Sea) and 145 (Weddell Scotia Confluence). Despite low initial zooplankton abundance (410 ciliates liter^{-1}, POC : Chl *a* ratio of 77.1), an increase of small aloricate ciliates was observed during incubation (maximum of 11,400 ind. liter^{-1} in 10 nM Fe bottle).

Adding Fe resulted in a further reduction of absolute and relative nanoflagellate pigment content, as well as nanoflagellate biovolume (Fig. 7), compared to the controls (one being an EDTA control) and the 10 nM Mn addition. At the same time, diatom abundance was enhanced (as observed by absolute and relative pigment content and biovolume). Adding Fe resulted in elevated microzooplankton abundances but pheophorbide concentrations were not enhanced (Fig. 7). Pheophorbide concentration as well as microzooplankton abundances were low compared to EPOS 145 and 159, but microzooplankton densities were higher than in EPOS 182 and 158.

Discussion

Questions remain on the matter of Fe speciation and bioavailability of Fe and Mn to plankton in these and other waters (e.g. Wells et al. 1983; Subba Rao and Yeats 1984; de Haan et al. 1985; Sunda and Huntsman 1988; Kester 1990). Nevertheless, ambient absolute and relative (cellular Fe requirement vs. PO_4; Anderson and Morel 1982) levels of dissolved (0.2-μm filtered) Fe and Mn are high enough to support rapid build-up of phytoplankton pigment and utilization of nutrients in the Weddell-Scotia Seas, as discussed before (de Baar et al. 1990). Surface levels of Fe were always >1.5 nM during the EPOS cruise (Nolting et al. 1991). Division rate calculations for various phytoplankton species in the EPOS 159 and 145 control bottles also indicate that phytoplankton are not significantly nutritionally limited in these waters (Table 2). Nevertheless addition of Fe seems to have a stimulating effect on both specific phytoplankton and microzooplankton growth. For another region, the equatorial Pacific, similar stimulation was found for mesozooplankton, i.e. copepods (Chavez et al. 1991).

The question of how Fe stimulates zooplankton growth cannot be answered conclusively. Besides a direct stimulating effect on zooplankton organisms, the possibility of an indirect effect remains open to speculation. Fe-enhanced microzooplankton growth could result from Fe-enhanced growth of small diatoms, such as small *Nitzschia* spp., which, judging from their low net division rates (Table 2), seem to be actively grazed down. Dugdale and Wilkerson (1990) advocated growth stimulation of diatoms through decreased grazing by Fe-inhibited micrograzers. This idea is not confirmed by our study. Diatoms were especially favored by added Fe, in agreement with earlier findings in the subarctic Pacific where diatoms were more strongly enhanced by Fe than were small organisms (Coale 1988). The findings of Coale (1988), as well as ours, are consistent with the suggestion by Hudson and Morel (1990) that large cells are more affected by low levels of Fe than are small cells. At the same time, the Fe-mediated stimulation of zooplankton (here ciliate) caused additional shifts in community structure toward dominance by large diatoms through selective grazing.

The apparent stimulatory effect of Fe on diatoms and microzooplankton abundance is obvious in our cultures. It is not clear whether this also applies to the field situation. We state here that experimental design (Coale 1988; Martin and Fitzwater 1988; this study) may significantly mislead notions of how Fe affects the in situ plankton community. The sampling procedure for this type of bottle experiment can lead to underrepresentation of mesozooplankton in the bottles. We never observed copepods or krill during sampling or on filters, whereas they were otherwise abundant in the field. The simultaneous development of large diatoms and ciliates in the bottles and the observed high numbers imply top-down control of these organisms in the field by the natural presence of mesozooplankton. Ciliates did develop in our bottles, likely as a result of the absence of larger grazers,

thereby more and more repressing the abundance of nanoflagellates or small diatoms in EPOS 159 and 145.

Our results support the three-compartment planktonic food-web model devised by Frost (1987) for the subarctic Pacific, where mesozooplankton grazing is in approximate balance with phytoplankton growth. There grazers control large diatoms as well as microzooplankton abundance (Frost 1987). In Antarctic waters, overall grazing is pronounced throughout. In the Scotia Sea and Drake Passage, low phytoplankton biomass was associated with high copepod biomass (Schnack et al. 1985; Tréguer et al. 1991). Copepod feeding could well be capable here of cropping phytoplankton biomass (Schnack et al. 1985). In the Weddell Sea and Weddell-Scotia Confluence, high numbers of tintinnids are common (Boltovskoy et al. 1989). The continuous presence of copepods and other grazers might keep large diatoms as well as microzooplankton abundance at low levels. Incidental grazing events of krill swarms would temporarily reduce diatom as well as ciliate abundances, thereby permitting development of phototrophic nanoflagellates (EPOS 158). Therefore the effect of Fe fertilization in the field cannot be predicted with regard to species composition, as long as information is lacking on how mesozooplankton controls its food resources.

Top-down control is further supported by the fact that we observed fast utilization of nutrients and rapid buildup of phytoplankton in our control experiments within 2 weeks, leading to Chl *a* and nutrient levels that are only rarely found in Antarctic waters (Burkholder and Sieburth 1961; El-Sayed 1971; Fukuchi et al. 1983; El-Sayed and Weber 1982). Similarly, in three of four recent experiments in the Ross Sea the controls did outgrow the field (Martin et al. 1990*b*). During EPOS leg 2 in situ Chl *a* levels seldom exceeded 2 μg liter^{-1} (Jacques and Panouse 1989), with a maximum of 4.5 μg liter^{-1}. These are realistic levels for this and other areas of the Antarctic Ocean (Allanson et al. 1983; Lutjeharms et al. 1983; El-Sayed 1988; Smith and Garrison 1990). During EPOS, ambient levels of major nutrients as well as Fe and other metals were quite high (Nolting et al. 1991). Thus nutrients were not exhausted despite high metal levels. The same was observed at station 3 in the study of Martin et al. (1990*c*) in inshore Bransfield Strait waters where an Fe concentration of 7 nM (surface water) was accompanied by [NO_3^-] and [PO_4^{3-}] of 23.8 and 2.19 μM respectively.

Among others factors, light limitation in well-mixed waters is generally considered to play a significant role in reducing phytoplankton production in Antarctic waters (El-Sayed 1988; Mitchell et al. 1991). During EPOS leg 2 total incident radiation was high and the mixed layer was shallow throughout the cruise (15–40 m), even in Scotia Sea waters (Veth 1991). Therefore, we assume that light conditions per se were not a crucial factor in determining phytoplankton production in the field nor an explanation for the biomass accumulation as observed in our experiments. Stratification is, however, a seasonal event restricted to the ice-edge zone. In general, light limitation is expected to occur in vast regions of the Scotia Sea and Drake Passage, regions where deep mixed layers prevail due to strong wind force accompanied by almost continuous cloud cover (Mitchell et al. 1991). So even in regions where Fe levels are low, like the Drake Passage (Martin et al. 1990*c*), Fe fertilization might not have the expected effect due to the overriding effects of, for instance, light limitation or grazing pressure, leaving aside the possibility of a prevailing abundance of oceanic phytoplankters with a low Fe demand in these areas (Brand et al. 1983). Factors limiting primary production may differ from region to region, because the Antarctic Ocean contains many ecosystems, each having its own physical, chemical, and biological characteristics (Hempel 1985; Fogg 1977).

Conclusion

We conclude that it would be premature to consider Fe to be the main factor limiting phytoplankton production in Antarctic waters. Obviously it plays a role as one of several rate-limiting factors. Nevertheless, in

the Scotia and Weddell Seas during late spring and summer, grazing appears to be the dominant process in keeping nutrient concentrations high and phytoplankton standing stocks at a low level. More information is needed on how biological factors control primary productivity before we can answer the intriguing question: what keeps phytoplankton in these waters from reaching its full potential?

References

ALLANSON, B. R., B. BODEN, L. PARKER, AND C. D. RAY. 1983. A contribution to the oceanology of the Prince Edward Islands, p. 38–46. *In* W. R. Siegfried et al. [eds.], Antarctic nutrient cycles and food webs. Springer.

ANDERSON, M. A., AND F. M. M. MOREL. 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii* Limnol. Oceanogr. **27:** 789–813.

BANSE, K. 1990. Does iron really limit phytoplankton production in the offshore subarctic Pacific? Limnol. Oceanogr. **35:** 772–775.

BOLTOVSKOY, D., V. A. ALDER AND F. SPINELLI. 1989. Summer Weddell Sea microplankton: Assemblage structure, distribution and abundance, with special emphasis on the Tintinnina. Polar Biol. **9:** 447–456.

BRAND, L. E., W. G. SUNDA, AND R. R. L. GUILLARD. 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. Limnol. Oceanogr. **28:** 1182–1198.

BROECKER, W. S. 1990. Comment on "Iron deficiency limits phytoplankton growth in Antarctic waters" by John Martin et al. Global Biogeochem. Cycles **4:** 3–4.

BRULAND, K. W. 1989. Complexation of zinc by natural organic ligands in the central North Pacific. Limnol. Oceanogr. **34:** 269–285.

BURKHOLDER, P. R., AND J. M. SIEBURTH. 1961. Phytoplankton and chlorophyll in the Gerlache and Bransfield Straits of Antarctica. Limnol. Oceanogr. **6:** 45–52.

CADÉE, G. C. 1982. Tidal and seasonal variation in particulate and dissolved organic carbon in the western Dutch Wadden Sea and Marsdiep tidal inlet. Neth. J. Sea Res. **15:** 228–249.

CEDERLØF, U. E. B., S. OBER, R. SCHMIDT, A. SVANSSON, AND C. VETH. 1989. Scientific reports: Physics and chemistry. Ber. Polarforsch. **65:** 14–20.

CHAVEZ, F. P., AND OTHERS. 1991. Growth rates, grazing, sinking, and iron limitation of equatorial Pacific phytoplankton. Limnol. Oceanogr. **36:** 1816–1833.

COALE, K. H. 1988. Copper complexation in the North Pacific Ocean. Ph.D. thesis, Univ. Calif., Santa Cruz.

DAVIES, A. G. 1990. Taking a cool look at iron. Nature **345:** 114–115.

DE BAAR, H. J. W. [ed.]. 1991. RV *Tyro* JGOFS, 1989 leg 1. Data Rep. Neth. Inst. Sea Res.

———, A. G. J. BUMA, G. JACQUES, R. F. NOLTING, AND P. J. TRÉGUER. 1989. Trace-metals: Iron and manganese effects on growth. Ber. Polarforsch. **65:** 34–44.

———, AND OTHERS. 1990. On iron limitation of the Southern Ocean: Experimental observations in the Weddell and Scotia Seas. Mar. Ecol. Progr. Ser. **65:** 105–122.

DE HAAN, H., M. J. W. VELDHUIS, AND J. R. MOED. 1985. Availability of dissolved iron from Tjeukemeer, The Netherlands, for iron-limited growing *Scenedesmus quadricauda* Water. Res. **19:** 235–239.

DONAT, J. R., AND K. W. BRULAND. 1990. A comparison of two voltametric techniques for determining zinc speciation in northeast Pacific Ocean waters. Mar. Chem. **28:** 301–323.

DOUCETTE, G. J., A. D. CEMBELLA, AND G. L. BOYER. 1989. Cyst formation in the red tide dinoflagellate *Alexandrium tamarense* (Dinophyceae): Effects of iron stress. J. Phycol. **25:** 721–731.

———, AND P. J. HARRISON. 1990. Some effects of iron and nitrogen stress on the red tide dinoflagellate *Gymnodinium sanguineum*. Mar. Ecol. Progr. Ser. **62:** 293–306.

DUGDALE, R. C., AND F. P. WILKERSON. 1990. Iron addition experiments in the Antarctic: A reanalysis. Global Biogeochem. Cycles **4:** 13–19.

EDLER, L. 1979. Recommendations on methods for marine biological studies in the Baltic Sea. Baltic Mar. Biol. Publ. 5.

EL-SAYED, S. Z. 1971. Observations on phytoplankton bloom in the Weddell Sea, p. 301–312. *In* G. A. Llano and I. E. Wallen [eds.], Biology of the Antarctic seas. V. 4. AGU.

———. 1988. Seasonal and interannual variabilities in Antarctic phytoplankton with reference to krill distribution, p. 101–119. *In* D. Sahrhage [ed.], Antarctic Ocean and resources variability. Springer.

———, AND L. H. WEBER. 1982. Spatial and temporal variations in phytoplankton biomass and primary productivity in the Southwest Atlantic and the Scotia Sea. Polar Biol. **1:** 83–90.

EPPLEY, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. **70:** 1063–1085.

FLORENCE, T. M., AND J. L. STAUBER. 1986. Toxicity of copper complexes to the marine diatom *Nitzschia closterium* Aquat. Toxicol. **8:** 11–26.

FOGG, G. E. 1977. Aquatic primary production in the Antarctic. Phil. Trans. R. Soc. Lond. **279:** 27–38.

FROST, B. W. 1987. Grazing control of phytoplankton stock in the open subarctic Pacific Ocean: A model assessing the role of mesozooplankton, particularly the large calanoid copepods *Neocalanus* spp. Mar. Ecol. Progr. Ser. **39:** 49–68.

FUKUCHI, M., A. TANIMURA, AND H. OHTSUKA. 1983. Marine biological and oceanographical investigations in Lutzow-Holm-Bay, Antarctica, p. 52–60. *In* W. R. Siegfried et al. [eds.], Antarctic nutrient cycles and food webs. Springer.

GIESKES, W. W. C., AND G. W. KRAAY. 1983. Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected

by HPLC analysis of pigments. Mar. Biol. **75:** 179–185.

———, AND ———. 1984. Phytoplankton, its pigments, and primary production at a central North Sea station in May, July and September 1981. Neth. J. Sea Res. **18:** 51–70.

———, AND ———. 1986. Analysis of phytoplankton pigments by HPLC before, during and after mass occurrence of the microflagellate *Corymbellus aureus* during the spring bloom in the open North Sea in 1983. Mar. Biol. **92:** 45–52.

HART, T. J. 1934. On the phytoplankton of the southwest Atlantic and Bellingshausen Sea, 1929–31. Discovery Rep. **8:** 1–268.

HEMPEL, G. 1985. On the biology of polar seas, particularly the Southern Ocean, p. 3–33. *In* Marine biology of polar regions and effects of stress on marine organisms. Proc. 18th Eur. Mar. Biol. Symp. Wiley.

HEMPEL, I., P. H. SCHALK, AND V. SMETACEK. 1989. The expedition Antarctic VII/3 (EPOS LEG 2) of RV "Polarstern" in 1988/1989. Ber. Polarforsch. **65:** 1–199.

HUDSON, R. J. M., AND F. M. M. MOREL. 1990. Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions. Limnol. Oceanogr. **35:** 1002–1020.

JACQUES, G. 1983. Some ecophysiological aspects of the Antarctic phytoplankton. Polar Biol. **2:** 27–33.

JOOS, F., J. L. SARMIENTO, AND U. SIEGENTHALER. 1991. Estimates of the effect of Southern Ocean iron fertilization on atmospheric CO_2 concentrations. Nature **349:** 772–774.

KESTER, D. R. 1990. The role of photochemical processes and hydrogen peroxide in iron redox marine chemistry, p. 108–109. *In* N. V. Blough and R. G. Zepp [eds.], Effects of solar ultraviolet radiation on biogeochemical dynamics in aquatic environments. Woods Hole Oceanogr. Inst. Tech. Rep. 90-09.

LANCELOT, C., AND S. MATHOT. 1989. Phytoplankton: Photosynthesis, growth and respiration. Ber. Polarforsch. **65:** 78–86.

LUTJEHARMS, J. R. E., N. M. WALTERS, AND B. R. ALLANSON. 1983. Oceanic frontal systems and biological enhancement, p. 11–22. *In* W. R. Siegfried et al. [eds.], Antarctic nutrient cycles and food webs. Springer.

MARSCHNER, H. 1986. Mineral nutrition of higher plants. Academic.

MARTIN, J. H. 1990. A new iron age, or a ferric fantasy. U.S. JGOFS Newsletter **1**(4): 5, 11.

———, W. W. BROENKOW, S. E. FITZWATER, AND R. M. GORDON. 1990*a*. Yes, it does: A reply to the comment by Banse. Limnol. Oceanogr. **35:** 775–777.

———, AND S. E. FITZWATER. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific Subarctic. Nature **331:** 341–343.

———, ———, AND R. M. GORDON. 1990*b*. Iron deficiency limits phytoplankton growth in Antarctic waters. Global Biogeochem. Cycles **4:** 5–12.

———, AND R. M. GORDON. 1988. Northeast Pacific iron distributions in relation to phytoplankton productivity. Deep-Sea Res. **35:** 177–196.

———, ———, AND S. E. FITZWATER. 1990*c*. Iron in Antarctic waters. Nature **345:** 156–158.

———, ———, ———, AND W. W. BROENKOW. 1989. VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Res. **36:** 649–680.

MITCHELL, B. G., E. A. BRODY, O. HOLM-HANSEN, C. MCCLAIN, AND J. BISHOP. 1991. Light limitation of phytoplankton biomass and macronutrient utilization in the Southern Ocean. Limnol. Oceanogr. **36:** 1662–1677.

MORTAIN-BERTRAND, A. 1989. Effects of light fluctuations on the growth and productivity of Antarctic diatoms in culture. Polar Biol. **9:** 245–252.

NETH. INST. SEA RES. 1989. Data Rep. EPOS-leg 2.

NOLTING, R. F., H. J. W. DE BAAR, A. J. VAN BENNEKOM, AND A. MASSON. 1991. Cadmium, copper and iron in the Scotia Sea, Weddell Sea and Weddell/Scotia Confluence (Antarctica). Mar. Chem. **35:** 219–243.

PENG T. H., AND W. S. BROECKER. 1991. Dynamic limitations on the Antarctic fertilization strategy. Nature **349:** 227–229.

RIVKIN, R. B., AND M. PUTT. 1987. Photosynthesis and cell division by Antarctic microalgae: Comparison of benthic, planktonic and ice algae. J. Phycol. **23:** 223–229.

RUETER, J. G., AND D. R. ADES. 1987. The role of iron nutrition in photosynthesis and nitrogen assimilation in *Scenedesmus quadricauda* (Chlorophyceae). J. Phycol. **32:** 452–457.

———, K. OHKI, AND Y. FUJITA. 1990. The effect of iron nutrition and photosynthesis and nitrogen fixation in cultures of *Trichodesmium* (Cyanophyceae). J. Phycol. **26:** 30–35.

SAAGER, P. M., H. J. W. DE BAAR, AND P. H. BURKILL. 1989. Manganese and iron in Indian Ocean waters. Geochim. Cosmochim. Acta **53:** 2259–2267.

SCHNACK, S. B., V. SMETACEK, AND B. VON BODUNGEN. 1985. Utilization of phytoplankton by copepods in Antarctic waters during spring, p. 65–81. *In* Marine biology of polar regions and effects of stress on marine organisms. Proc. 18th Eur. Mar. Biol. Symp. Wiley.

SMITH, W. O., JR., AND D. L. GARRISON. 1990. Marine ecosystem research at the Weddell Sea ice edge: The AMERIEZ Program. Oceanography **3**(2): 22–29.

SOMMER, U. 1989. Maximal growth rates of Antarctic phytoplankton: Only weak dependence on cell size. Limnol. Oceanogr. **34:** 1109–1112.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis, 2nd ed. Bull. Fish. Res. Bd. Can. 167.

SUBBA RAO, D. V., AND P. A. YEATS. 1984. Effect of iron on phytoplankton production in the Sargasso Sea. J. Exp. Mar. Biol. Ecol. **81:** 281–289.

SUNDA, W. G., AND S. A. HUNTSMAN. 1988. Effect of sunlight on redox cycles of manganese in the southwestern Sargasso Sea. Deep-Sea Res. **35:** 1297–1317.

TRÉGUER, P., AND OTHERS. 1991. Production of biogenic silica in the Weddell-Scotia Seas measured with ^{32}Si. Limnol. Oceanogr. **36:** 1217–1227.

VERSTRAETE, D., R. STORCH, AND V. L. DUNHAM. 1980. A comparison of the influence of iron and

nitrate metabolism of *Anabaena* and *Scenedesmus*. Physiol. Plant. **50**: 47–51.

VETH, C. 1991. The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia-Weddell Sea. J. Mar. Syst. **2**: 451–464.

WELLS, M. L., L. M. MAYER, AND R. R. L. GUILLARD. 1991. Evaluation of iron as a triggering factor for red tide blooms. Mar. Ecol. Progr. Ser. **69**: 93–102.

———, N. G. ZORKIN, AND A. G. LEWIS. 1983. The role of colloid chemistry in providing a source of iron to phytoplankton. J. Mar. Res. **41**: 731–746.