

Influence of zinc and iron enrichments on phytoplankton growth in the northeastern subarctic Pacific

*D. W. Crawford*¹

School of Ocean and Earth Science, University of Southampton, Southampton Oceanography Centre, Southampton SO14 3ZH, England

M. S. Lipsen

Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

D. A. Purdie, M. C. Lohan, and P. J. Statham

School of Ocean and Earth Science, University of Southampton, Southampton Oceanography Centre, Southampton SO14 3ZH, England

F. A. Whitney

Section for Ocean Climate Chemistry, Institute of Ocean Sciences, Sidney, British Columbia V8L 4B2, Canada

J. N. Putland

Department of Oceanography, Florida State University, Tallahassee, Florida 32306-4320

W. K. Johnson and N. Sutherland

Section for Ocean Climate Chemistry, Institute of Ocean Sciences, Sidney, British Columbia V8L 4B2, Canada

*T. D. Peterson and P. J. Harrison*²

Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

C. S. Wong

Section for Ocean Climate Chemistry, Institute of Ocean Sciences, Sidney, British Columbia V8L 4B2, Canada

Abstract

Near-surface seawater from the northeastern subarctic Pacific was incubated on deck for 8 d, supplemented with (1) control, no additions (2) +Zn (3) +Fe (4) +Zn+Fe. Concentrations of total Zn and Fe at time zero (t_0) and in the control remained at ~ 0.1 – 0.2 nmol L⁻¹. In the control, chlorophyll (< 0.3 mg m⁻³), ¹⁴C uptake into POC and PIC, and inorganic nutrients all remained relatively constant. Addition of Zn slightly but significantly increased chlorophyll ($p < 0.05$), decreased phosphate ($p < 0.01$) and nitrate ($p < 0.05$), and in P versus E experiments, increased $P_m > 10$ -fold and P_m^{chl} 2–3-fold. The abundance of small diatoms and coccolithophores was higher in the +Zn treatment compared to the control. The +Fe and +Zn+Fe treatments, compared to the control, both showed > 10 -fold increases in chlorophyll and ¹⁴C uptake into POC and PIC and complete removal of nitrate (≤ 0.2 mmol m⁻³). However, differences were observed in size-fractionated data; the +Zn+Fe treatment had significantly lower percent chlorophyll in the > 20 - μ m fraction ($p < 0.01$) and a higher percentage in the 0.2–5- μ m fraction ($p < 0.01$) than the +Fe treatment. In P versus E experiments, both +Fe treatments increased P_m and α around 100-fold and P_m^{chl} and α^{chl} by 5–10-fold compared to the control. The +Fe treatment showed a slightly higher α^{chl} and slightly lower P_m^{chl} than the +Zn+Fe treatment. Abundance of large diatoms, small diatoms, small flagellates, and coccolithophores all increased substantially (~ 7 – $1,000$ -fold) in response to Fe addition, whereas dinoflagellate abundance only doubled. The +Zn+Fe treatment had higher abundances of small diatoms and small flagellates than the +Fe treatment. We conclude that Zn additions had limited influence on conventional indices of phytoplankton growth compared to Fe, but that there might be subtle influences of Zn that require further attention.

Limitation of biomass and primary production of phytoplankton by dissolved iron (Fe) in high-nutrient, low-chlo-

rophyll (HNLC) areas of the world's oceans is now well established (e.g., Coale et al. 1996; Boyd et al. 2000). There

¹ Present address: 60, Newlands Avenue, Shirley, Southampton SO15 5ES, England.

² Present Address: Atmospheric, Marine, and Coastal Environment Program, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Acknowledgments

We thank the Captain and crew of the R/V *John P. Tully*. This research was funded by NERC grant GR3/11971. The comments of Philippe Tortell and an anonymous reviewer greatly improved an earlier version of the manuscript.

has also been considerable speculation regarding potential limitation by other trace metals such as zinc (Zn) (e.g., Morel et al. 1994), which is an essential element for marine phytoplankton.

The total concentration of dissolved zinc (Zn_T) in the surface waters of the open Pacific and Atlantic oceans is often around the same order ($\sim 0.1 \text{ nmol L}^{-1}$ or $10^{-10} \text{ mol L}^{-1}$) as that of Fe (e.g., Bruland et al. 1978; Bruland 1989; Martin et al. 1989; Ellwood and Van den Berg 2000; Kremling and Streu 2001; Lohan et al. 2002). However, the presence of organic ligands reduces the activity of free Zn ions [Zn^{2+}] to a small fraction ($\sim 10^{-11}$ – $10^{-12} \text{ mol L}^{-1}$) of the total Zn_T (Bruland 1989; Ellwood and Van den Berg 2000). It is the availability of this free [Zn^{2+}] that appears to limit phytoplankton growth, and it is clear from laboratory studies that the growth rate of many neritic species is limited below [Zn^{2+}] of $\sim 10^{-11}$ – $10^{-11.5} \text{ mol L}^{-1}$ (Brand et al. 1983). Despite this, several open ocean species have been observed to grow almost maximally at the lowest [Zn^{2+}] tested ($\sim 10^{-12}$ – $10^{-13} \text{ mol L}^{-1}$), suggesting acclimation to low Zn conditions (Brand et al. 1983; Sunda and Huntsman 1992). These studies are often cited to support the argument that Zn limitation of phytoplankton in the ocean is unlikely (e.g., Ellwood and Van den Berg 2000). However, Zn can be replaced functionally by cobalt (Co) (Price and Morel 1990; Sunda and Huntsman 1995), and the studies of Brand et al. (1983) and Sunda and Huntsman (1992) were carried out under saturating concentrations of Co. Zinc might therefore limit oceanic phytoplankton more than previously implied by these culture studies, because the low concentrations of Co in oceanic waters (e.g., Donat and Bruland 1995) could preclude effective replacement. Indeed, Ellwood and van den Berg (2001) have reported total Co concentrations in the open ocean of $\sim 25 \text{ pmol L}^{-1}$ and free [Co^{2+}] of $< 5 \text{ fmol L}^{-1}$ —levels likely to severely limit the biological availability of Co. Experiments by Sunda and Huntsman (1995) have confirmed that, when grown without Co in the culture medium, the oceanic species *Thalassiosira oceanica* and *Emiliania huxleyi* were more limited by low [Zn^{2+}] than previously implied by Sunda and Huntsman (1992). Cadmium (Cd) can also restore the growth of Zn-stressed phytoplankton (Price and Morel 1990; Lee and Morel 1995). Cd:C ratios in phytoplankton increase with decreasing [Zn^{2+}] (Sunda and Huntsman 2000), possibly explaining the apparent preferential uptake of Cd with respect to phosphorus over large areas of predominantly Zn-depleted surface oceans (Elderfield and Rickaby 2000; Kremling and Streu 2001).

The principal use of Zn by phytoplankton remains unclear, although one important role is as a cofactor in carbonic anhydrase (CA), an enzyme that enhances the rate of dehydration of bicarbonate ions (HCO_3^-) to free CO_2 . Indeed, there appears to be an increased demand for Zn, Co, or both, and Cd under low- pCO_2 conditions (Morel et al. 1994; Cullen et al. 1999). The rate of diffusion of free CO_2 into the cell can be a limitation for some species, even in air-equilibrated seawater (Riebesell et al. 1993), a point of particular potential relevance to larger cells (with longer diffusion paths) that might alternatively be directly using HCO_3^- (Goldman 1999). Indeed, recent field evidence for HCO_3^- use by phytoplankton (Tortell et al. 1997) does support the case for Zn-depend-

ent HCO_3^- use. Another potential role for Zn is in silicification by diatoms (e.g., Rueter and Morel 1981; De La Rocha et al. 2000), although Ellwood and Hunter (2000) have recently shown that only 1–3% of Zn in diatoms was found in the opal frustule. Despite the fact that Fe limitation increases silicification of individual diatom cells (e.g., Takeda 1998), the biomass stimulation by Fe will increase total Si uptake. Therefore, if Zn indeed is involved in silicification, subtle Fe and Zn coeffects are possible during Fe fertilization.

To date, the few field studies in which incubations of surface HNLC ocean water have been supplemented with Zn have suggested minimal effects on phytoplankton growth. In the equatorial Pacific, Southern Ocean, and subarctic Pacific, the addition of Fe always has the most pronounced effect, with addition of Zn alone having little or no effect relative to controls (Buma et al. 1991; Coale 1991; Coale et al. 1996; Scharek et al. 1997; Franck et al. 2000; Olson et al. 2000; Gall et al. 2001). When Zn and Fe were added together, no notable differences were observed compared to Fe alone (Coale et al. 1996; Scharek et al. 1997; Franck et al. 2000; Olson et al. 2000). It should be noted that Zn_T in the Southern Ocean is relatively high (e.g., $> 2 \text{ nmol L}^{-1}$; Frew et al. 2001), so limitation by Zn is less likely than in the other HNLC regions.

Although field evidence to date provides little evidence for Zn limitation of phytoplankton growth, the theoretical and laboratory evidence discussed above suggests that more subtle effects, possibly size or species specific, could merit attention. Ocean Station Papa (OSP) is situated in the northeastern subarctic Pacific, a HNLC area where phytoplankton growth is limited by the availability of Fe (e.g., Martin and Fitzwater 1988; Boyd et al. 1996). Levels of Zn_T in near-surface water are $< 0.1 \text{ nmol L}^{-1}$ at OSP (Martin et al. 1989; Lohan et al. 2002), so with $\sim 98\%$ of Zn_T potentially bound to ligands (Bruland 1989), [Zn^{2+}] certainly could be in the growth-limiting range. Coale (1991) has carried out incubations at OSP in which natural populations were supplemented with Zn and with Fe, although Zn was added alone and not in combination with Fe. The present study examines the influence of supplementation with Zn and Fe, both alone and together, on surface-water phytoplankton at OSP in September 1999.

Methods

Location and initial sampling—This study was conducted on cruise 9921 of the research vessel *C.C.G.S. John P. Tully* during the regular program along line P to OSP (Station P26) undertaken by the Institute of Ocean Sciences (IOS), Sidney, British Columbia (see Lohan et al. 2002 for map of area). The cruise began 23 August 1999 from IOS, arriving at OSP during the early morning of 1 September 1999. During the night of 1–2 September, seawater from 15 m was pumped into an on-deck PVC laminar clean-air flow hood using an air-driven Teflon bellows pump (PFD-1 Asti) fitted with reinforced Teflon tubing coated in PVC. The tubing was attached with plastic tape to a Kevlar line weighted with resin-coated lead weights and deployed off the side of the vessel.

Inside the flow hood, the water was pumped into 24, 4-liter low-density polyethylene (LDPE) flexible cubic containers (cubitanors). For filtered samples at time zero (t_0), the water was pumped through a Durapore 0.45- μm pore size filter in a Teflon holder. Samples were also taken from the pumped supply at t_0 for chlorophyll, nutrients, ^{14}C uptake, and total dissolved Zn and Fe.

Trace metal cleaning protocol—The rigorous cleaning protocol for sampling and incubation equipment is outlined in Table 1. All critical handling steps on the ship were conducted in the laminar flow hood and onshore in a Class 100 laminar flow hood or in the clean room. All laboratory apparatus was also subjected to stringent cleaning procedures. Polycarbonate bottles used for ^{14}C incubations were soaked in 10% HCl for about 24 h prior to use, followed by several rinses of MilliQ and surface ocean water. Ultraclean techniques were unnecessary because in 24-h incubations, the presence of Fe has a negligible effect on productivity estimates compared to those conducted using clean techniques (Martin et al. 1989). However, reasonable precautions were taken to avoid any inhibitory effects caused by trace metal contamination.

Time zero (t_0) analyses—Triplicate t_0 samples were taken for chlorophyll, nutrients, ^{14}C uptake into particulate organic (POC) and inorganic carbon (PIC), and total dissolved Fe (Table 2). Total dissolved Zn samples were taken from a routine profile at OSP (see Lohan et al. 2002). Additional single samples (i.e., not triplicated; see Table 2) were taken at t_0 for an experiment on photosynthesis and calcification versus irradiance (P vs. E and C vs. E curves) and for taxonomic analysis of the phytoplankton assemblage.

Experimental design—The 24 cubitanors consisted of two identical sets of four triplicated (A, B, C) treatments (4×3); the first set was run for 4 d and the second set for 8 d (Table 2). Pumping and filling of cubitanors took most of the night, so it is conceivable that the community species composition could have changed slightly during that time. Therefore, the filled cubitanors were randomly labeled and then supplemented with trace metals; this was in order to avoid, for example, three of the first filled cubitanors being used as control, and three of the later ones being used as one of the treatments. The triplicated treatments were as follows.

1. control—no additions
2. +1.8 nmol L⁻¹ Fe (FeCl₂:EDTA 1:1 molar ratio)
3. +10 nmol L⁻¹ Zn (ZnCl₂)
4. +1.8 nmol L⁻¹ Fe and +10 nmol L⁻¹ Zn

It should be noted that the concentrations given are target concentrations because the cubitanors were flexible with a nominal volume of ~4 liters. Zn (10 nmol L⁻¹) was added because Coale (1991) added 0.75 nmol L⁻¹ but speculated that the lack of observed response could have resulted from the low level of added Zn in combination with the presence of a strong Zn binding ligand (Bruland 1989). Phytoplankton growth appears to be saturated by Fe within the 1–

Table 1. Protocols for trace metal cleaning of sampling, incubation, and filtration equipment.

Stage	Teflon pump and tubing	LDPE sample bottles	LDPE cubitanors*	Teflon filter holder and tubing	Durapore filters
Detergent soak	5% Extran 24 h, MQ† rinse	2% Micro 7 d, MQ rinse	—	5% Extran at 40–50°C, 24 h, MQ rinse	—
Acid soak	1 N HNO ₃ 24 h, MQ rinse	50% HCl 7 d, MQ rinse	10% HCl, 24 h, MQ rinse	3 mol L ⁻¹ HCl, at 20°C, 24 h, MQ rinse	1 N HCl at 35°C overnight, DMQ‡ rinse
Acid soak	0.1% HCl, 24 h, MQ rinse	50% HNO ₃ , 7 d, MQ rinse, quartz subboiled distilled water rinse	1% Seastar HCl 24 h, MQ rinse	0.3 mol L ⁻¹ HNO ₃ at 40–50°C, 24 h, MQ rinse	0.3 mol L ⁻¹ HCl/HNO ₃ /HF cocktail at 35–50°C overnight, DMQ rinse
Final storage and transport	Transported to sea wet with acid. Flushed with ocean water and left to soak 2 d before arriving at OSP	Air dried on clean bench, double bagged in clean resealable polyethylene bags	0.1% Seastar acetic acid, 7 d, emptied but taken to sea wet with acid. Rinsed several times in open ocean water, then left to soak 2 d before arriving at OSP. Rinsed with OSP water three times prior to filling	0.1 mol L ⁻¹ HCl at 20°C, 24 h, MQ rinse. Double bagged in clean resealable polyethylene bags	Stored in 0.05% HCl prior to use

* For cleaning, the flexible LDPE cubitanors were maintained in the flattened shape in which they were supplied; this minimized internal volume and reduced volume of soaking solutions required.
 † MilliQ water (18 M Ω cm⁻¹). ‡ Double MilliQ water.

Table 2. Sampling regime and experimental design for supplementation of Zn and Fe to 4-liter cubitanors incubated for 4 and 8 d. Cubitanors were incubated on deck at 30% ambient surface irradiance using multiple mesh screens.†

	Sampling																				
	4 d						8 d														
	t ₀		Control		+Zn		+Fe		+Zn+Fe		Control		+Zn		+Fe		+Zn+Fe				
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Chl	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Nuts	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PPOC	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PPIC	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
P versus E	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C versus E	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Phyto	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Fe	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Zn	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

† Chl, total and size-fractionated chlorophyll concentration (mg m⁻³); Nuts, concentration of nitrate, phosphate, and silicate (mmol m⁻³); PPOC, total and size-fractionated ¹⁴C uptake into particulate organic carbon (mg C m⁻³ d⁻¹); PPIC, total and size-fractionated ¹⁴C uptake into particulate inorganic carbon (mg C m⁻³ d⁻¹); P versus E, photosynthesis versus irradiance curves (mg C m⁻³ h⁻¹); C versus E, calcification versus irradiance curves (mg C m⁻³ h⁻¹); Phyto, counts of major taxonomic groupings of phytoplankton (cells L⁻¹); Fe, total concentration of dissolved Fe (nmol L⁻¹); Zn, total concentration of dissolved Zn (nmol L⁻¹).

2 nmol L⁻¹ range added here (Martin et al. 1989; Blain et al. 2002).

Before dawn, the cubitanors were placed inside water-cooled incubators held on deck. These were maintained at ambient temperature by means of a continuously pumped supply of surface seawater and at ~30% of surface irradiance using neutral-density screens wrapped around the incubators. As required, cubitanors were removed from incubators in the early morning; subsamples for measurements were decanted from the cubitanors. For the day 8 incubations, in order to minimize contamination, the first subsamples out of the cubitanors were for total dissolved Zn and Fe, and these were decanted into LDPE sampling bottles inside the laminar flow hood. It was not logistically feasible to make all measurements after 4 d nor to triplicate all measurements after 8 d (summarized in Table 2). For dissolved measurements, one sample from each cubitanor was taken. For size-fractionated chlorophyll and POC and PIC production, one sample was taken from each cubitanor, giving triplicated measurements for each fraction and for the total.

Chlorophyll—Samples were decanted into 300-ml bottles and stored in the dark in the cold room for 1–2 h prior to filtration. Samples were then poured into a filtration stack consisting of a sequence (20, 5, and 0.2 μm) of 47-mm-diameter polycarbonate membrane filters (Poretics). Water passed through the upper two filters by means of gravity and was drawn gently (<100 mm Hg vacuum) through the lower filter using a vacuum pump. Filter holders were rinsed twice with filtered seawater, and filters were stored for up to several days in glass vials in the freezer prior to extraction. Chlorophyll was extracted from the filters by adding 10 ml of 90% acetone to the vials, capping them, and leaving them for 24 h in the freezer. The samples were read (before and after acidification) against an acetone blank on a Turner Designs model 10 fluorometer calibrated with chlorophyll *a* (Chl *a*, Sigma) standardized on a spectrophotometer, and the concentration of Chl *a* was calculated using standard formulae (e.g., Strickland and Parsons 1972).

Nutrients—Unfiltered samples (filtered vs. unfiltered samples gave no significant difference in nutrient concentrations) were placed in polycarbonate tubes in the refrigerator for up to 24 h prior to analysis on a Technicon autoanalyzer using a modification of standard Technicon procedures as outlined in Barwell-Clarke and Whitney (1996).

Primary production—Samples were decanted from cubitanors into 250-ml polycarbonate bottles (previously acid soaked and rinsed). A single dark polycarbonate bottle was also filled. All polycarbonate bottles were supplemented with 10 μCi of NaH¹⁴CO₃ (100 μl of alkaline stock solution) and then capped and mixed. Subsamples of 100 μl were then removed from three randomly selected bottles from the 4 × 3 treatments, and then placed in 100-μl phenylethylamine in glass scintillation vials for counting of final specific activity of added ¹⁴C. The 250-ml bottles were then returned to the on-deck incubators at 30% surface irradiance and incubated for 24 h. The size-fractionated filtration protocol was the same as for chlorophyll, using a sequence of polycarbonate

membrane filters of 20, 5, and 0.2 μm . Because ^{14}C uptake into PIC was to be measured in addition to uptake into POC, a careful rinsing protocol had to be followed. After excess water had passed through each filter, either under gravity or gentle vacuum, the filter was rinsed with two washes of ~ 20 ml filtered seawater. The filter tower was then removed, and the rim of the filter was rinsed twice with a few milliliters of filtered seawater to remove residual dissolved $^{14}\text{CO}_2$. All filters were finally sucked dry for several seconds using gentle vacuum (<100 mm Hg vacuum). This protocol ensured that residual dissolved $^{14}\text{CO}_2$ was low and relatively constant. This allowed dissolved inorganic counts on dark bottle filters to be subtracted from those from the light bottle filters to give ^{14}C -labeled PIC by difference. Filters were placed in glass scintillation vials, capped, and frozen to await further processing.

Filters were processed according to an acidification/trapping protocol similar in principle to that of Paasche and Brubak (1994). Vials and filters were thawed, and a small glass tube containing 1 ml of 10% KOH was placed inside each large scintillation vial. The filter in the outer vial was then acidified with 1 ml 0.1 N HCl, and the vial was capped and left for 24 h. This converted any ^{14}C -labeled PIC and residual dissolved inorganic ^{14}C on the filter to free CO_2 , which then diffused out and was caught in the alkaline trap. POC labeled with ^{14}C was retained in the outer vial on the filter, thus giving two separate fractions of fixed particulate carbon for each filter. After 24 h, the inner tube was removed, the outside rinsed back into the outer vial with 1 ml distilled water, and the contents emptied into a standard scintillation vial, followed by a distilled water (1 ml) wash of the inside of the inner tube. Either Wallac Hi-safe or Fisher Scinti-safe scintillation cocktails (10 ml) were then placed in all vials, including those for specific activity. These two cocktails were tested and found to have very low chemiluminescence properties. Chemiluminescence was further minimized by allowing samples to stand for at least a day after adding the cocktail. All vials were counted on a Packard scintillation counter with quench correction using internal standards. Counts for the alkaline trap samples represented uptake of ^{14}C into PIC, whereas the counts for the filter vial represented ^{14}C uptake into POC. All rates for POC and PIC fixation were corrected to uptake in the light by subtraction of dark bottle counts. In the case of PIC formation, this meant that the low and relatively constant counts (disintegrations min^{-1} [dpm]) on both light and dark filters (arising from residual dissolved $^{14}\text{CO}_2$) was removed by subtraction to give PIC fixation in the light. POC and PIC fixation rates were calculated according to the standard formula (e.g., Peterson 1980) using a value for total CO_2 of 2.1 mmol L^{-1} (varies by $<5\%$ at OSP throughout the year, Wong and Johnson pers. obs.), the specific activity of the ^{14}C added, the dpm counts into fixed POC or PIC, and an isotopic discrimination factor of 1.05.

Production and calcification versus irradiance experiments—P versus E experiments were carried out on 2-liter subsamples decanted from the B cubitanors at the end of the 8-d incubations. The filters produced from these experiments were processed and acidified as described for productivity

samples above; this gave data for both P versus E and C versus E. Previously acid-soaked and rinsed polycarbonate bottles (18×70 ml plus 1×70 ml dark bottle) were rinsed and filled from this subsample. The bottles were then spiked with 10 $\mu\text{Ci NaH}^{14}\text{CO}_3$, capped, and mixed, and 100- μl subsamples were removed from three bottles for estimation of specific activity (described above). The 18 bottles were then placed in a light gradient in a horizontal darkened box with a dark lid, with the dark bottle placed at the low-light end. Seawater from the ships' supply was allowed to flow through the box to cool the samples to ambient temperature. Light was supplied from one end of the box from a halogen projector lamp, and the light attenuated through the series of incubation bottles. Irradiance was measured between each bottle using a Biospherical irradiance meter and 4π probe. Irradiance for each bottle was calculated as the mean of the values in front of and behind the bottle. Samples were incubated for 3–4 h before the light was turned off and the exact incubation time recorded. All samples were filtered (not size-fractionated) through 0.2- μm , 47-mm-diameter polycarbonate membrane filters (Poretics) using gentle vacuum (<100 mm Hg). Filtration towers and filter rims were carefully rinsed as described above, then filters were placed in glass scintillation vials and frozen. All vials were processed using the acidification/trapping method (*see above*) and the POC and PIC production rates calculated as above. An iterative routine (Sigmaplot) was used to fit standard P versus E equations of Webb et al. (1974) and Platt et al. (1980) to the P versus E data.

Dissolved Zn and Fe—Samples at t_0 for total dissolved Zn and Fe were taken directly from the Teflon pump and fed through a 0.45- μm , 90-mm-diameter membrane (Durapore, Millipore) filter in a Teflon filter holder. For the 8-d incubations, the logistics of subsampling from cubitanors for so many parameters dictated that samples for trace metals could not be cleanly filtered. Analyses of these samples therefore represent total dissolved plus "acid-labile particulate" concentrations of trace metals. For the control and +Zn treatments, this was likely to be of little consequence because little growth occurred and most trace metals would remain in the dissolved phase. For the two +Fe treatments, there was significant biological growth, so total dissolved Fe or Zn might have been overestimated somewhat by metals from particulate material removed by the acid into solution. However, this approach was justified because the trace metal measurements were primarily a check on contamination in the cubitanors.

Total dissolved Zn was determined by cathodic stripping voltammetry (Van den Berg 1985; Donat and Bruland 1990) as described in Lohan et al. (2002). Fe measurements were based on the chemiluminescent technique of Obata et al. (1993, 1997). Seawater samples were buffered to pH 3.2, and Fe was preconcentrated on an 8-hydroxyquinoline resin column. The metal was eluted and mixed with luminol, ammonia solution, and hydrogen peroxide. Emitted light was measured by photomultiplier tube and related to Fe concentration in the sample. The detection limit of Zn and Fe was 0.02–0.03 nmol L^{-1} , and the precision of triplicate measure-

ments from a single sample was <5% relative standard deviation.

Enumeration of phytoplankton—Samples for phytoplankton counts were preserved and analyzed according to Booth et al. (1993). Samples (250 ml) were preserved with hexamine-buffered formalin (final concentration 0.4%), and 50-ml (for control and +Zn) or 10-ml (for +Fe and +Zn+Fe) subsamples were sedimented in settling chambers. Broad taxonomic groupings of phytoplankton were counted on an inverted microscope at $\times 400$ magnification. The t_0 count for small flagellates and ciliates was made by epifluorescence microscopy (Booth et al. 1993). The sample was preserved with 1% (final concentration) glutaraldehyde and filtered onto a 0.2- μm pore size black polycarbonate membrane filter prior to mounting and examination.

It should be emphasized that phytoplankton counts were based on samples from single cubitanors; therefore, statistical comparisons could not be made between treatments.

Results

Replication and errors—The coefficient of variation for Chl *a* or POC fixation from triplicate cubitanors was 15–20% (standard error as a percentage of the mean). This probably reflects true variation between these large-volume treatments as a result of, for example, variation in irradiance in the incubators and variable initial community composition. Experimental errors could also have been enhanced by the size fractionation procedures, which compound the error in the totals. For the PIC fixation rates, the coefficient of variation was around 40%, probably because absolute rates were low. The coefficient of variation for nutrient concentrations was much less at <1% for t_0 and control samples, whereas variation between triplicates where significant growth had occurred increased to 1–10%. A typical coefficient of variation between cubitanors for Zn and Fe analyses was 1–15%, ignoring values suspected of contamination (see Table 5).

Bulk changes in chlorophyll, primary production, and nutrients—Total Chl *a* at t_0 and in the control remained between 0.2 and 0.3 mg m^{-3} throughout and was significantly higher at 0.5–0.6 mg m^{-3} in the +Zn treatment ($p < 0.05$; *t*-test) after 4 d (Fig. 1A). Chl *a* in the +Fe and +Zn+Fe treatments was clearly significantly higher than the control after both 4 d ($p < 0.05$) and 8 d ($p < 0.01$), with mean final concentrations reaching around 5 mg m^{-3} . However, there was no significant difference between the two Fe treatments. In the +Zn treatment, POC fixation increased to a maximum of 33 $\text{mg C m}^{-3} \text{d}^{-1}$ after 4 d (Fig. 1B) and was considerably higher than the rate in the control. However, because of variability between cubitanors, this difference was not statistically significant. In the +Fe and +Zn+Fe treatments, rates of POC fixation increased to >100 $\text{mg C m}^{-3} \text{d}^{-1}$, clearly significantly higher than the control after 4 d ($p < 0.05$) and 8 d ($p < 0.001$). However, there was no significant difference between the two Fe treatments.

PIC fixation showed a similar pattern to that for POC (Fig. 1C), although the absolute rates of carbon fixation were an

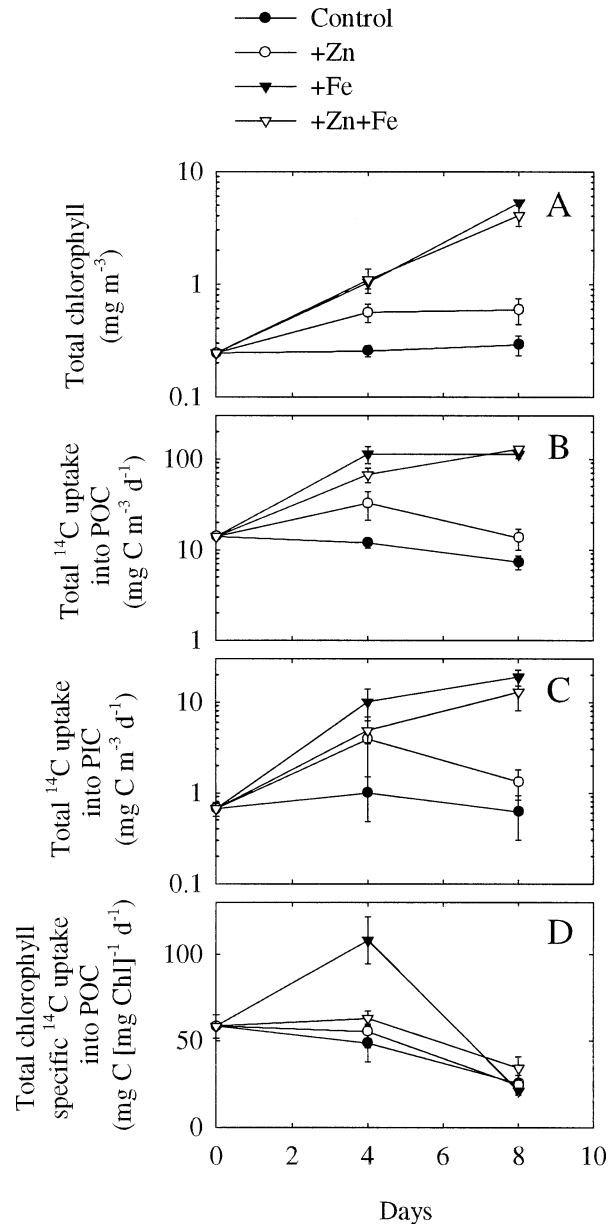


Fig. 1. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. Response of (A) total chlorophyll concentration, (B) total ^{14}C uptake into POC, (C) total ^{14}C uptake into PIC, and (D) total chlorophyll-normalized ^{14}C uptake into POC. Error bars represent standard errors from triplicate incubations.

order of magnitude lower. Mean PIC fixation in the control remained at 0.5–0.6 $\text{mg C m}^{-3} \text{d}^{-1}$ throughout. There was a notable peak, though not statistically significant, in the +Zn treatment after 4 d to around 4 $\text{mg C m}^{-3} \text{d}^{-1}$. Significant increases over the control occurred in the +Fe and the +Zn+Fe treatments ($p < 0.05$), which reached >13 $\text{mg C m}^{-3} \text{d}^{-1}$. Again, rates in the two Fe treatments were not significantly different from each other. For chlorophyll-specific POC fixation, the t_0 value of 58 $\text{mg C (mg Chl)}^{-1} \text{d}^{-1}$ fell in all treatments to ~ 20 –30 $\text{mg C (mg Chl)}^{-1} \text{d}^{-1}$ (Fig.

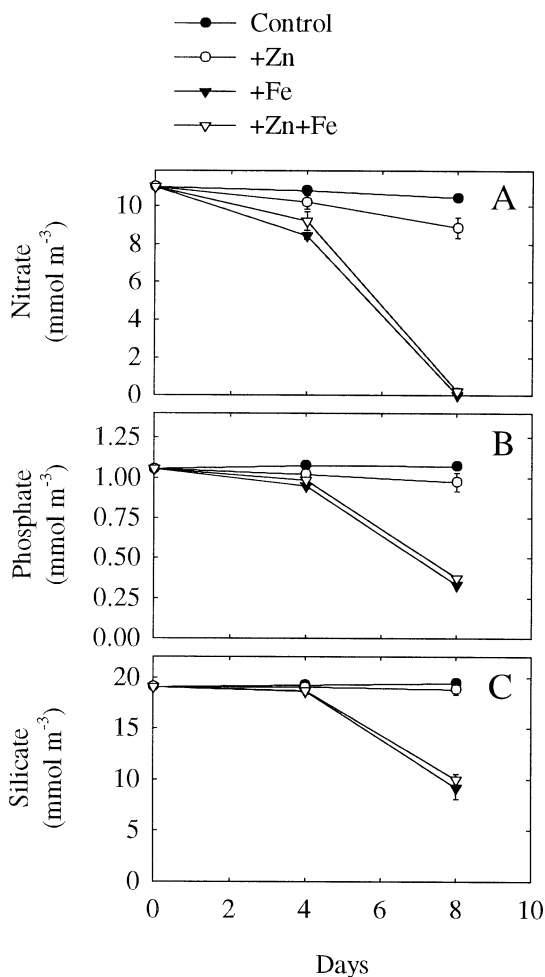


Fig. 2. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. Response of (A) nitrate, (B) phosphate, and (C) silicate. Error bars represent standard errors from triplicate incubations.

1D). The exception was the +Fe treatment, which peaked after 4 d at over $100 \text{ mg C (mg Chl)}^{-1} \text{ d}^{-1}$.

The nutrient data (Fig. 2) reflected the above changes in Chl *a* and POC fixation, with all nutrients significantly lower in the two +Fe treatments than the control after both 4 and 8 d ($p < 0.05$ or lower). There was no significant difference in nutrient concentrations between the +Fe and the +Zn+Fe treatment. Nitrate decreased only slightly in the control and was significantly lower ($p < 0.05$) in the +Zn treatment after 8 d (Fig. 2A). Nitrate was depleted to around detection limit ($\leq 0.2 \text{ mmol m}^{-3}$) in the +Fe and +Zn+Fe treatments by day 8. Maximum phosphate removal occurred in the +Fe and +Zn+Fe treatments and was $< 70\%$ of the t_0 concentration (Fig. 2B). After 4 d, phosphate was significantly lower in the +Zn treatment than the control ($p < 0.01$). Silicate was removed to $\sim 50\%$ of the t_0 concentration in the +Fe and +Zn+Fe treatments (Fig. 2C). Silicate showed a slight increase in the control, and there was no significant difference between this and the +Zn treatment. Expressing the nutrient removal as uptake ratios averaged over the 8 d, the

+Zn treatment showed a Si:N ratio of 0.14, whereas the +Fe and +Zn+Fe treatments gave Si:N ratios of 0.99 and 0.89, respectively. The +Zn treatment showed a N:P ratio of 27.0, whereas the N:P ratios for the +Fe and +Zn+Fe treatments were close to Redfield at 14.7 and 15.5, respectively.

Size-fractionated chlorophyll and primary production—All size fractions for Chl *a* and POC fixation showed similar patterns to those of totals, with minimal growth in the control, slight stimulation (particularly after 4 d) in the +Zn treatment, and near exponential growth in the +Fe and +Zn+Fe treatments (Fig. 3A–C). The two Fe treatments were significantly higher than the control in Chl *a* ($p < 0.05$) and POC fixation ($p < 0.01$) for the 0.2–5- μm and 5–20- μm fractions after both 4 and 8 d. However, for the $>20\text{-}\mu\text{m}$ fraction, there was no significant difference between either +Fe treatment and the control until the end of the incubation, both for chlorophyll ($p < 0.01$) and POC fixation ($p < 0.005$). The slight stimulation in the +Zn treatments was not statistically significant, either for Chl *a* or POC fixation in any of the fractions, possibly because of the additional experimental error introduced by the fractionation procedure. After 8 d, Chl *a* was significantly higher ($p < 0.01$) in +Fe treatment than the +Zn+Fe treatment for the large fraction, but not significantly different in the other fractions.

Size-fractionated PIC fixation (Fig. 3G–I) showed a similar pattern to that of POC fixation, although it is notable that PIC fixation in the 0.2–5- μm fraction in the +Fe treatments continued to increase when POC fixation was already declining (Fig. 3G). For the 5–20- μm fraction, PIC production was significantly greater than the control for both the +Fe ($p < 0.05$) and the +Zn+Fe treatments ($p < 0.05$). The apparent increase in PIC fixation in the +Zn treatment was not significantly greater than in the control, and there was no significant difference between the +Fe and the +Zn+Fe treatments for any of the fractions.

The high initial increase in total chlorophyll-specific POC fixation already noted for the +Fe treatment (Fig. 1D) after 4 d was mainly a result of high values, particularly in the 0.2–5- μm fraction and, to a lesser extent, the $>20\text{-}\mu\text{m}$ fraction (Fig. 3J–L). Some stimulation could also be seen in the +Fe and +Zn+Fe treatments after 4 d for the two larger fractions. It is noteworthy that in the +Zn+Fe treatment by the end of the incubation, the chlorophyll-specific POC fixation remained very high at almost $100 \text{ mg C (mg Chl)}^{-1} \text{ d}^{-1}$ for the $>20\text{-}\mu\text{m}$ fraction.

Size fractionated chlorophyll data were expressed as a percentage of the total occurring in each fraction (Fig. 4A–C). At t_0 , 51% of the chlorophyll was in the 0.2–5- μm fraction, with 18% in the $>20\text{-}\mu\text{m}$ fraction and 31% in the 5–20- μm fraction. The proportion of chlorophyll in the 0.2–5- μm fraction (Fig. 4A) remained relatively unchanged at around 50% in the +Zn+Fe treatment throughout, but this fell sharply in the +Fe treatment to around 21%; the difference was significant at 4 d ($p < 0.01$) and at 8 d ($p < 0.01$). By the end of the incubation, the proportion of chlorophyll in the large $>20\text{-}\mu\text{m}$ fraction (Fig. 4C) only increased to 28% for the +Zn+Fe treatment, but increased to 62% for the +Fe treatment; again, the difference was significant at 4 d ($p <$

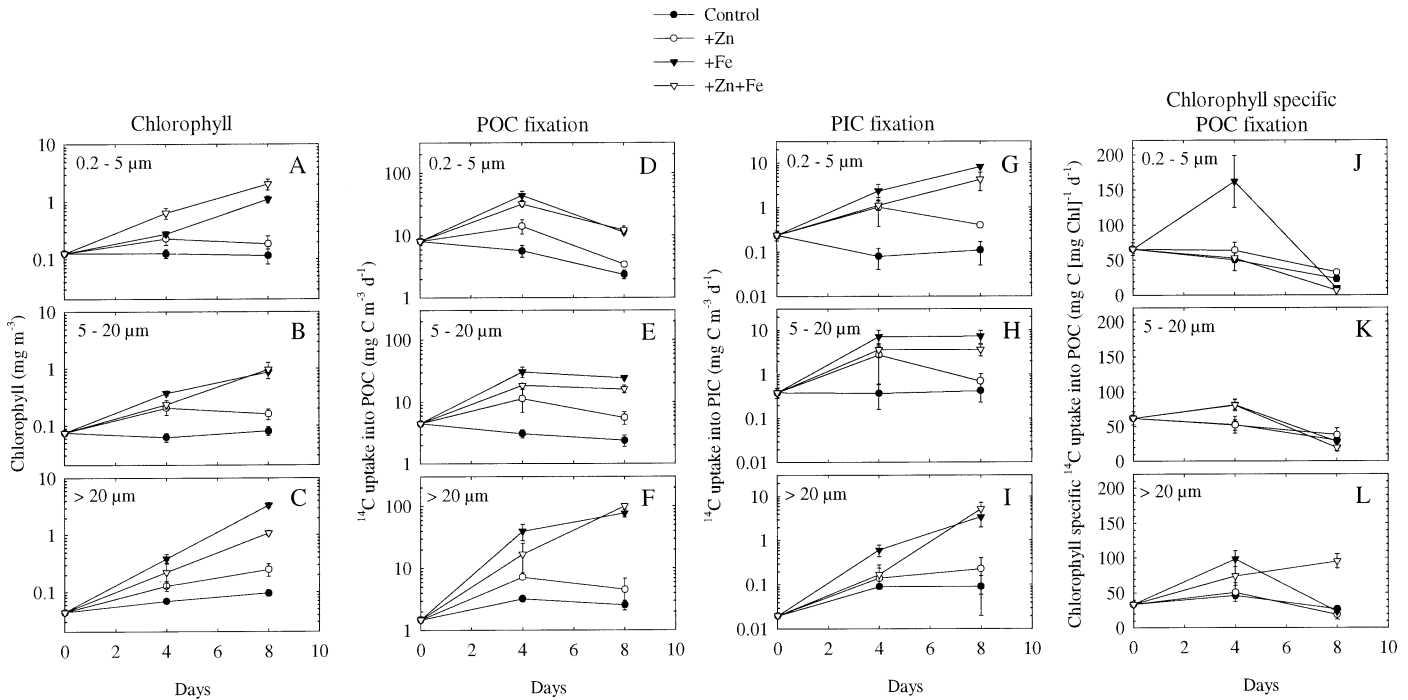


Fig. 3. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. As in Fig. 1, but with data size-fractionated into 0.2–5 (A, D, G, J), 5–20 (B, E, H, K), and >20 μm (C, F, I, L) fractions. Error bars represent standard errors from triplicate incubations.

0.05) and at 8 d ($p < 0.01$). In the 5–20- μm fraction (Fig. 3B), the differences between the +Fe and the +Zn+Fe treatments were less marked; there was a significant difference after 4 d ($p < 0.01$), but not after 8 d. Differences between the control and the +Zn treatment were never significant.

The POC fixation was similarly expressed as a percentage of the total for each fraction (Fig. 5A–C). Although this showed a shift to enhanced productivity in the larger fraction on addition of Fe, there was no significant difference between the +Fe and +Zn+Fe treatments, except for the 5–20- μm fraction after 8 d, where the percentage of production was about twofold higher ($p < 0.05$) in the +Fe than in the +Zn+Fe treatment.

Ratio of calcification to photosynthesis—The POC and PIC fixation data from Figs. 1B,C were used to give total PIC:POC fixation ratios (Fig. 6D). The ratio of total PIC:POC fixation was of the order 0.05–0.2 for all treatments. There appeared to be an increase in the Fe treatment, but because of the variability between replicates, this was not statistically significantly different from the control. The size-fractionated POC (Fig. 3D–F) and PIC data (3G–I) were also used to give PIC:POC ratios for the three size fractions (Fig. 6A–C). Again, despite an apparent gradual increase, particularly in the smaller fractions, there was no significant difference between any treatment and the control. However, it should be emphasized that the high point for +Fe at 8 d in the small fraction (Fig. 6A) was based on a single data point (two replicates were spoiled), so a statistical test could not be carried out. This point could therefore represent a signif-

icant increase in PIC:POC ratio in the +Fe 0.2–5- μm fraction.

Photosynthesis and calcification versus irradiance curves—The P versus E curves for each treatment are shown in Fig. 7, and the derived parameters of maximum rate of photosynthesis (P_m) and initial slope (α) are given in Table 3. Compared to t_0 and control values, P_m increased 10-fold in the +Zn treatment, although α only increased twofold. The P versus E curve was conducted on the B replicate of the +Zn treatment, which appeared to have a high contaminating Fe level (Table 5), so it is conceivable that this caused the increase in P_m . However, because α did not change (Table 3) and because chlorophyll and POC productivity for the B replicate were not the highest of the three +Zn replicates despite the high Fe (Table 5), it is likely that contamination was at the subsampling stage for Fe (see footnotes to Table 5). The increase in P_m was probably therefore a real effect of adding Zn. In the +Fe treatment, P_m and α both increased dramatically ~ 100 -fold (Fig. 7; note the change of scale for the two +Fe treatments). In the +Zn+Fe treatment, P_m increase was only slightly lower than that for +Fe, but the increase in α was only ~ 50 -fold.

These patterns for POC fixation in the P versus E curves were mirrored in the C (calcification) versus E curves, although the rates of PIC fixation (as in Figs. 1, 3, 6) were about an order of magnitude lower than for POC fixation. Maximum PIC fixation rate might have been slightly higher in the +Zn treatments compared to t_0 and control values. However, the poor precision of the method at these low ab-

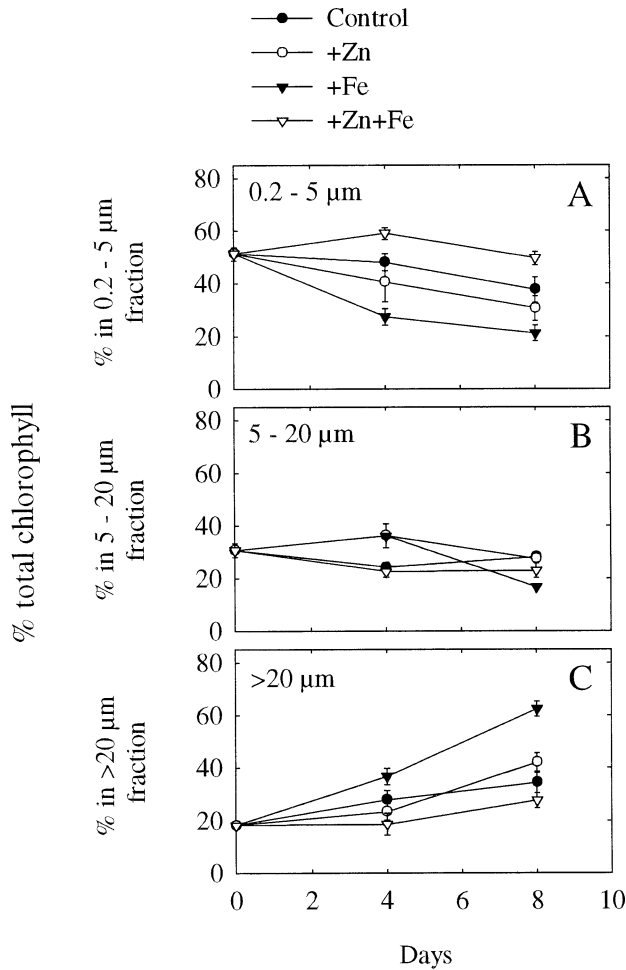


Fig. 4. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. Chlorophyll data from Figs. 1 and 3 expressed as percent total chlorophyll occurring in each of the three size fractions: (A) 0.2–5, (B) 5–20, and (C) >20 μm . Error bars represent standard errors from triplicate incubations.

solute rates of calcification introduced considerable noise in the data, and meaningful parameters were not generated. Maximum PIC fixation rate in the +Fe treatment was >20-fold higher at t_0 than in the control, with some inhibition at higher irradiance. In the +Zn+Fe treatment, the maximum rate was around half that of the +Fe treatment, and the C versus E curve had an initial slope lower than that for the +Fe treatment.

To some extent, the increases in P_m shown in Fig. 7 and Table 3 were the result of increases in biomass of phytoplankton. P versus E rates were recalculated (Fig. 8) as chlorophyll-normalized P_m (P_m^{chl} , or assimilation number) and α (α^{chl}), and these chlorophyll-normalized parameters are shown in Table 4 for two separate curve-fitting procedures. P_m^{chl} clearly increased two- to fourfold in the +Zn treatment over t_0 and control values, although α^{chl} was largely unaffected. P_m^{chl} increased only slightly more (two- to fivefold over control and t_0) in the +Fe treatment, but increased three- to eightfold in the +Zn+Fe treatment. α^{chl} increased

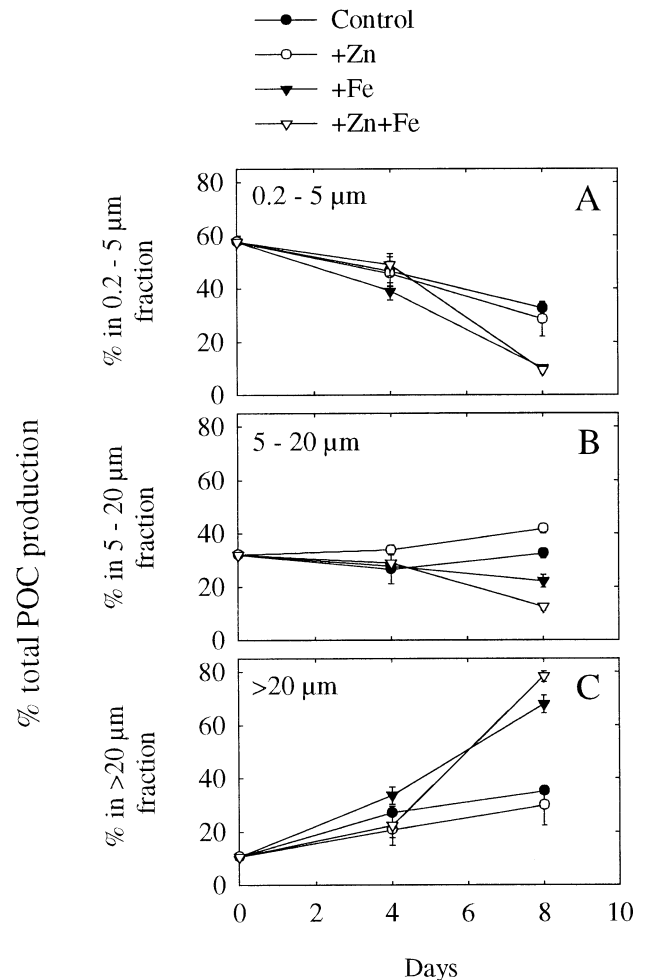


Fig. 5. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. POC production data from Figs. 1 and 3 expressed as percent total POC production occurring in each of the three size fractions: (A) 0.2–5, (B) 5–20, and (C) >20 μm . Error bars represent standard errors from triplicate incubations.

around eightfold over control and t_0 values in the +Fe treatment and around fivefold in the +Zn+Fe treatment.

Trace metal concentrations—The mean concentration of Zn at t_0 was 0.11 nmol L⁻¹ (Table 5), and after 8 d, the control concentration was 0.14 nmol L⁻¹. In the +Fe treatment, the mean Zn concentration was 0.20 nmol L⁻¹, skewed perhaps by a high and suspect value of 0.36 nmol L⁻¹ in the A replicate (see footnotes to Table 5). The concentration of Zn in the +Zn and +Zn+Fe treatments was much higher at 1.34 and 1.18 nmol L⁻¹, respectively.

A couple of Fe data points were suspected of contamination during subsampling (see footnotes to Table 5). The t_0 sample had a similar single high Fe value, and this supports the argument for contamination during subsampling because the t_0 samples were not taken from cubitanors. The mean concentration of Fe at t_0 was 0.13 nmol L⁻¹ and was 0.10 nmol L⁻¹ in the control. In the +Zn treatment, the Fe concentration was 0.21 nmol L⁻¹. In the +Fe and +Zn+Fe

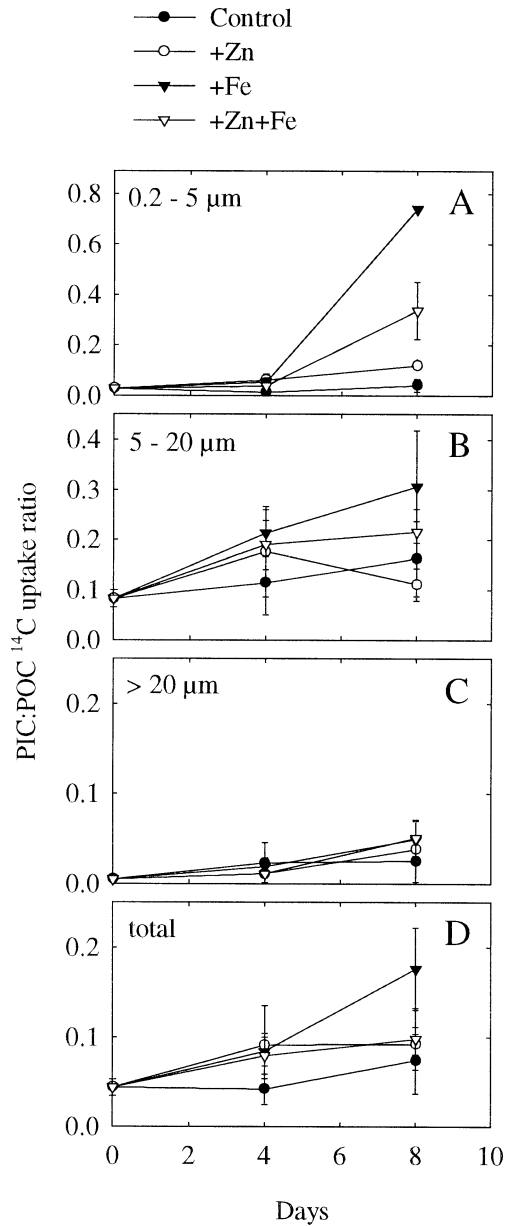


Fig. 6. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. Production data from Figs. 1 and 3 expressed as PIC:POC ratio in each of the three size fractions—(A) 0.2–5, (B) 5–20, (C) >20 μm—and in (D) the total PIC:POC ratio. Error bars represent standard errors from triplicate incubations. Note the change of scale from A to B and to C and D.

treatments, the Fe concentration was 1.2 and 1.08 nmol L⁻¹, respectively.

Phytoplankton counts—The numbers of cells in broad taxonomic groupings of phytoplankton are shown in Table 6. Compared to t_0 and control values, the two Fe treatments showed a stimulation of ~7–1,000-fold in the abundance of large diatoms, small diatoms, small flagellates, and coccolithophores. Compared to the control, the abundance of dinoflagellates only doubled and ciliates increased two-

threefold in the two Fe treatments. Comparing the +Zn treatment with the control, the abundance of small diatoms approximately doubled and coccolithophores tripled. Comparing the +Zn+Fe with the +Fe treatment, the presence of Zn resulted in increases in the abundance of small diatoms and small flagellates. In both treatments, the increase in small diatoms category was dominated by one small (as yet unidentified) diatom species. One surprising result was a higher abundance of several groups in the control than at t_0 despite the relative lack of increase in chl *a* in the controls. This was particularly notable for large diatoms where the abundance in the control was 10 times higher than at t_0 . The +Zn+Fe treatment decreased abundance of coccolithophores compared to +Fe, although the +Zn treatment alone had increased abundance compared to the control. Both +Zn treatments decreased the abundance of ciliates relative to the control and the +Fe treatment, respectively.

Discussion

Trace metal concentrations—The concentrations of Zn and Fe at t_0 (Table 5) were similar to levels previously reported for this region (e.g., Martin et al. 1989). The control showed remarkably little change with both Zn and Fe remaining at around 0.1 nmol L⁻¹ (Table 5), suggesting little if any contamination from handling or containment. There was also no significant evidence of contamination from stock solutions in the supplemented treatments; levels of the unsupplemented metal (e.g., Zn in +Fe; Fe in +Zn) remained close to control and t_0 values.

Control incubations—Chlorophyll, POC fixation, and nutrient drawdown (Figs. 1, 2) all showed remarkably little change in the control treatments from t_0 throughout the 8 d. The low concentrations of trace metals in the control (Table 5) confirmed that this likely was due to trace metal limitation. This contrasts with some earlier studies at OSP (e.g., Martin and Fitzwater 1988; Martin et al. 1989; Boyd et al. 1996), where some growth occurred in the controls and might have been the result of a small amount of Fe contamination. Given that the interpretation of some of the earlier experiments at OSP has been complicated by this growth and nutrient removal in the controls, the present data are encouraging. However, the difference in abundance of large diatoms between t_0 and control was surprising. It is possible that the average size of “large diatoms” was much smaller in the control (Table 6), because total chlorophyll (Fig. 1A) and >20-μm fraction chlorophyll (Fig. 3C) did not increase sufficiently to account for such an increase in large diatoms.

Trace metal: carbon uptake ratios—Some interesting points emerged from the trace metal data. In the two Fe treatments, the drawdown in total dissolved Fe was ~0.7–0.8 nmol L⁻¹, whereas in the two +Zn treatments, the drawdown in total dissolved Zn was almost 9 nmol L⁻¹. Although this could potentially result to some extent from adsorption to cubitanor walls, the possibility of such a high biological demand for Zn cannot be ruled out. For example, in the +Zn+Fe treatment, assuming a decrease in Zn of around 8.9 nmol L⁻¹, a chlorophyll increase of 3.85 μg L⁻¹ against

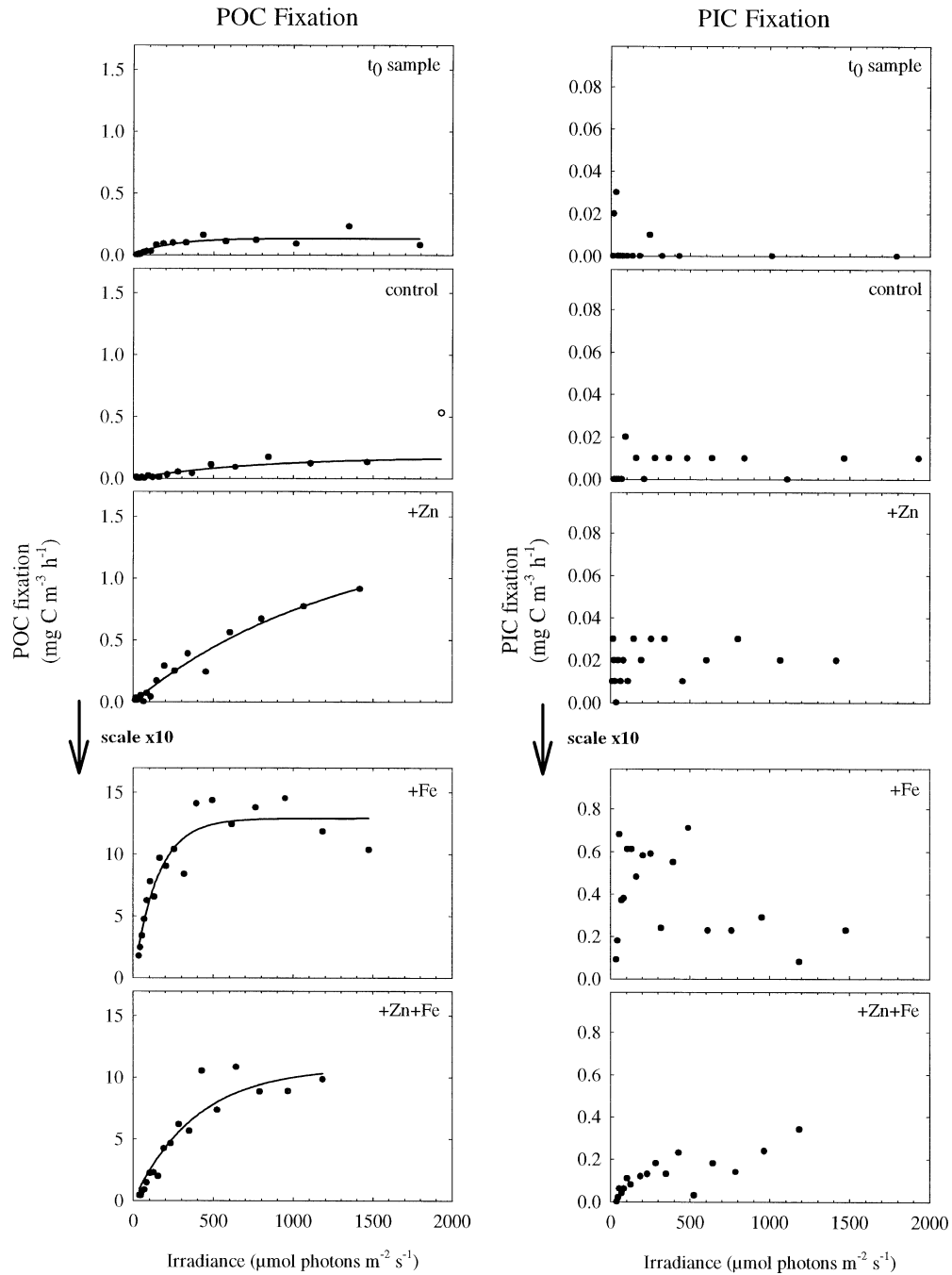


Fig. 7. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. Photosynthesis versus irradiance (P versus E) and calcification versus irradiance (C versus E) curves conducted at t_0 and from incubations of 8 d for the control, +Zn, +Fe, and +Zn+Fe treatments. Note the change of scale for the two +Fe treatments. Solid lines represent best curve fits to POC fixation data according to Webb et al. (1974); parameters of curve fits given in Table 3 for each treatment. The single unfilled data point in the control treatment was omitted from the analysis because curve fitting could not be satisfied with it present. No curve fits were undertaken for PIC fixation data because the data were noisy and the C versus E curve has not been well parameterized in the literature (*but see* Balch et al. 1992).

Table 3. Physiological parameters derived from the P versus E curves shown in Fig. 7. P_m is the maximum rate of ^{14}C uptake into POC (photosynthesis), α is the initial slope of the P versus E curve. The parameters were derived using the approach of Webb et al. (1974). Standard error of derived parameter given in parentheses.

Treatment	P_m ($\text{mg C m}^{-3} \text{ h}^{-1}$)	α ($\text{mg C m}^{-3} \text{ h}^{-1}$ [$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$] $^{-1}$)
t_0	0.14(0.02)	0.0006(0.0002)
Control	0.18(0.04)	0.0002(0.0000)
+Zn	1.38(0.30)	0.0011(0.0010)
+Fe	12.90(0.62)	0.0852(0.0104)
+Zn+Fe	10.85(1.15)	0.0277(0.0039)

t_0 , and a C:Chl ratio of around 100 in summer (including both autotrophic and heterotrophic C; Booth et al. 1993), this gives an estimated Zn:C ratio in “new” organic material of around 250 $\mu\text{mol}:\text{mol}$. This compares favorably with Zn:C ratios in cultures maintained under high $[\text{Zn}^{2+}]$ conditions (Sunda and Huntsman 1995; e.g., see their fig. 2B). In the +Zn treatment, the high removal of Zn is more puzzling because of the lower growth of phytoplankton. However, such enhanced uptake by Zn-starved cells is still not inconceivable in the light of the recent observation that winter Zn_T levels at OSP are maintained as low as in summer (Lohan et al. 2002) despite vertical mixing of high Zn_T water from below. The equivalent Fe drawdown in the two Fe treatments gives Fe:C ratios of 14–22 $\mu\text{mol}:\text{mol}$, well within the range of 2–47 $\mu\text{mol}:\text{mol}$ reported by Takeda (1995) for equatorial Pacific phytoplankton in response to additions of Fe. For an oceanic diatom in culture, Sunda et al. (1991) reported Fe:C of only 2 $\mu\text{mol}:\text{mol}$, but the values reported here, like those of Takeda (1995), are probably higher because of enhanced uptake by Fe-replete cells compared to Fe-limited cells.

Confirmation of Fe limitation of growth—The experiments here clearly confirm and extend earlier studies (e.g., Martin and Fitzwater 1988; Coale 1991; Boyd et al. 1996) showing that Fe limits phytoplankton growth and production at OSP. Chlorophyll concentration increased exponentially to around 5 mg m^{-3} (Fig. 1; Table 5) when supplemented with Fe, and POC production also increased sharply to around 100 $\text{mg C m}^{-3} \text{ d}^{-1}$. This is lower than the value of >200 $\text{mg C m}^{-3} \text{ d}^{-1}$ given by Boyd et al. (1996) but similar to that reported by Coale (1991). The chlorophyll-specific POC production for the +Fe treatment peaked at around 100 $\text{mg C (mg Chl)}^{-1} \text{ d}^{-1}$ and is in good agreement with that reported in a similar experiment after 6 d (Boyd et al. 1996). As observed by Martin and Fitzwater (1988), Fe stimulated complete removal of nitrate by day 8. Nitrate removal was very low in the controls (Fig. 2A) and averaged $\sim 75 \text{ nmol L}^{-1} \text{ d}^{-1}$ (0.58 $\mu\text{mol L}^{-1}$ over 8 d), agreeing well with typical HNLC nitrate transport rates at OSP of 84 $\text{nmol L}^{-1} \text{ d}^{-1}$ (Dugdale and Wilkerson 1991). Average transport rates in the +Fe treatments were around 1,400 $\text{nmol L}^{-1} \text{ d}^{-1}$ (11 $\mu\text{mol L}^{-1}$ over 8 d), which corresponds with rates in productive upwelling areas (Dugdale and Wilkerson 1991) and supports the contention that Fe plays a major role in the

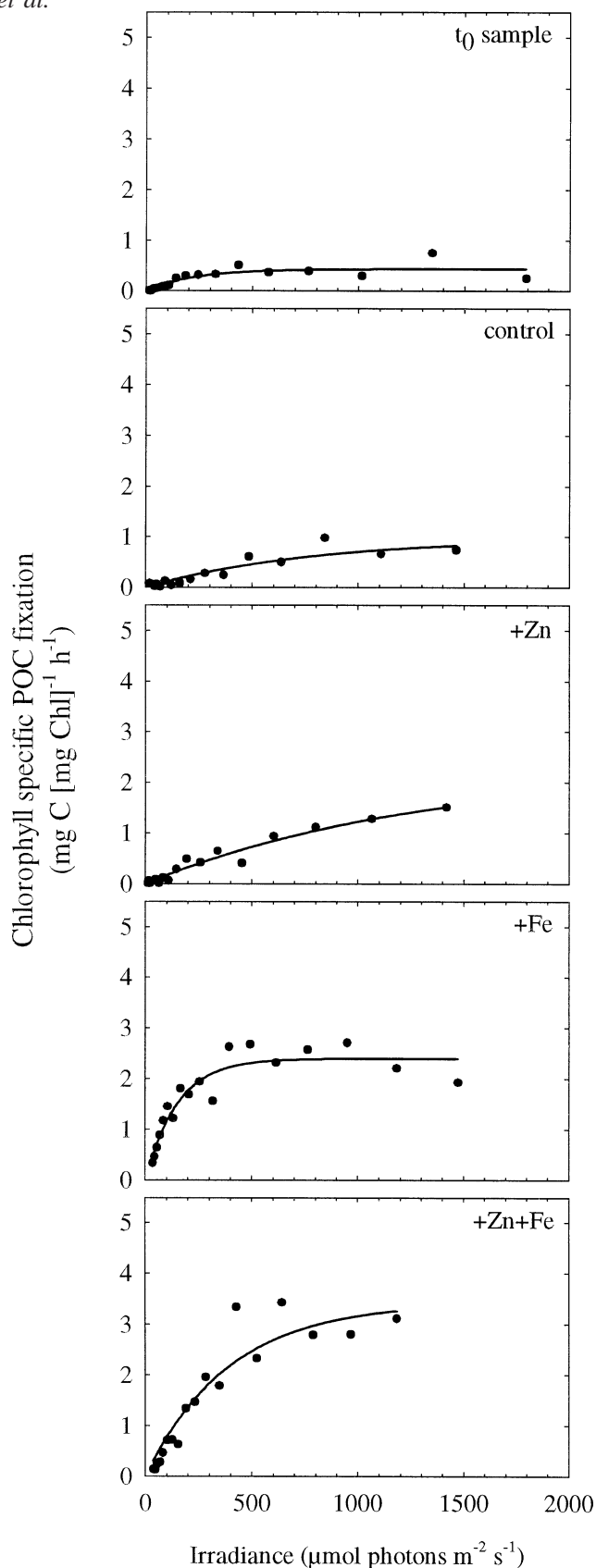


Fig. 8. Additions of +Zn, +Fe, and +Zn+Fe to on-deck incubations of surface water from Ocean Station Papa in the NE subarctic Pacific in September 1999. P versus E curves as for Fig. 7, but normalized to chlorophyll. Curve fits as for Fig. 7 and parameters generated are given in Table 4 for each treatment.

Table 4. Physiological parameters derived from the chlorophyll-normalized P versus E curves shown in Fig. 8. P_m^{chl} is the maximum photosynthesis normalized to chlorophyll, α^{chl} is the initial slope of the P versus E curve, β^{chl} is the photoinhibition parameter, and E_K is the light saturation parameter. The parameters were derived twice, first using Webb et al. (1974), which assumes no photoinhibition and therefore does not allow derivation of β^{chl} . Webb et al. (1974) was used however because this gives a reasonable error estimate on P_m^{chl} and because photoinhibition was minimal. The second approach was that of Platt et al. (1980), which derives P_s^{chl} and from this P_m^{chl} ; however, P_s^{chl} can have a large error term which is then transferred to P_m^{chl} . Note that the derived parameters for the two approaches are very similar. The error terms for P_s^{chl} and P_m^{chl} have not been shown for the derivations from Platt et al. (1980). The parameters derived from the equations of Platt et al. (1980) were simply shown to verify those derived using the equations of Webb et al. (1974).

Treatment	Webb et al. (1974)			Platt et al. (1980)				
	P_m^{chl}	α^{chl}	E_K	P_s^{chl}	P_m^{chl}	α^{chl}	E_K	β^{chl}
t_0	0.43(0.06)	0.0019(0.0006)	228	0.73	0.44	0.0016(0.0005)	273	0.0003(0.0008)
Control	0.98(0.23)	0.0013(0.0003)	752	34.44	0.84	0.0013(0.0004)	648	0.0189(25.48)
+Zn	2.27(0.49)	0.0018(0.0002)	1,260	2.72	2.19	0.0018(0.0004)	1,215	0.0001(0.0061)
+Fe	2.40(0.12)	0.0159(0.0019)	151	3.48	2.52	0.0136(0.0015)	185	0.0013(0.001)
+Zn+Fe	3.43(0.36)	0.0088(0.0012)	390	196.80	3.13	0.0084(0.0016)	373	0.0190(275.5)

* Units are P_m^{chl} (mg C [mg Chl a]⁻¹ h⁻¹), α^{chl} (mg C [mg Chl a]⁻¹ [μ mol photons m⁻² s⁻¹]⁻¹ h⁻¹), β^{chl} (mg C [mg Chl a]⁻¹ [μ mol photons m⁻² s⁻¹]⁻¹ h⁻¹), E_K (μ mol photons m⁻² s⁻¹).

Table 5. Concentrations of Zn and Fe (nmol L⁻¹) before (t_0) and after an incubation of 8 d; concentrations are given for time zero (t_0) and for each of four treatments involving supplementation with various combinations of Zn and Fe. Final t_0 concentrations in the supplemented treatments were 10 nmol L⁻¹ Zn and 1.8 nmol L⁻¹ Fe. Values represent means of triplicate incubations with the standard errors of the means in parentheses. The chlorophyll and primary production of each corresponding replicate is also shown for comparison (from Fig. 1).

Treatment	Zn (nmol L ⁻¹)		Fe (nmol L ⁻¹)		Chlorophyll (mg Chl m ⁻³)		Production (mg C m ⁻³ d ⁻¹)	
	Conc.	Mean (SE)	Conc.	Mean (SE)	Conc.	Mean (SE)	Rate	Mean (SE)
t_0								
A			0.11					
B		0.11*	0.15	0.27(0.14)		0.24(0.02)†		14.00(0.80)†
C			0.55‡	[0.13]§				
Control								
A	0.16		0.10		0.37		9.22	
B	0.17	0.14(0.02)	0.10	0.10(0.00)	0.18	0.29(0.06)	4.98	7.30(1.24)
C	0.10		0.11		0.33		7.69	
+Zn								
A	1.31		0.11		0.33		11.27	
B	1.37	1.34(0.02)	0.86‡	0.43(0.39)	0.61	0.59(0.15)	8.90	13.54(3.52)
C	1.33		0.31	[0.21]§	0.85		20.44	
+Fe								
A	0.36¶		1.14		5.77		136.05	
B	0.14	0.20(0.08)	1.04	1.20(0.10)	5.37	5.31(0.28)	103.23	112.74(11.72)
C	0.09		1.41		4.79		98.93	
+Zn+Fe								
A	1.10		1.45		5.75		121.25	
B	1.24	1.18(0.04)	0.87	1.08(0.51)	3.16	4.09(0.83)	126.72	129.42(5.66)
C	1.20		0.90		3.36		140.28	

* Estimate of concentration at 15 m from the values of 0.04 nmol L⁻¹ at 10m and 0.42 nmol L⁻¹ at 40m, given by extrapolation assuming linear increase with depth (Lohan et al. 2002). The profile of dissolved Zn at OSP given in Lohan et al. (2002) was taken 18 h earlier on the same cruise as reported here and using the same sampling equipment. † For the t_0 samples, the A, B, and C triplicates for Fe did not correspond with samples for chlorophyll and POC production, which were taken from regular profiles at OSP; therefore, the mean chlorophyll and POC production values have simply been listed with the corresponding standard error in parentheses. ‡ Samples suspected of contamination during subsampling for trace metals. § Means calculated without contribution of samples suspected of contamination. || Note that the high Fe value for the B sample was not associated with the highest chlorophyll and POC production of the triplicates for the +Zn treatment. This suggests that the high Fe value was probably contaminated (like the high C sample for t_0) during subsampling for trace metals, rather than during the incubation (i.e., from the Zn stock solution or from the container walls). If contaminated during the incubation, a much larger growth of chlorophyll might have been expected. ¶ Possible contamination from cubitanor walls or during subsampling, but not from Fe stock solution because B and C replicates had Zn values similar to the control.

Table 6. Abundance of broad taxonomic groupings of microplankton at OSP at time zero (t_0), and subsequently after 8 d on-deck incubation in the trace metal-supplemented treatments.

Treatment	Abundance (10^4 cells L^{-1})					
	Large diatoms	Small diatoms*	Dinoflagellates	Small flagellates	Coccolithophores†	Ciliates
t_0	1.6	0.5	17.2	(35.9)‡	18.9	(0.24)‡
Control	18.2	1.1	14.4	74.9	26.5	3.03
+Zn	13.1	2.1	12.6	71.3	75.5	1.57
+Fe	905.2	170.3	29.2	306.6	554.8	9.73
+Zn+Fe	1,022.0	425.8	28.4	488.3	335.0	5.68

* Small diatoms were differentiated from large diatoms below a diameter of $\sim 10 \mu\text{m}$. † Coccolithophores were dominated by *Emiliania huxleyi* (>95% numerically) in t_0 and in all treatments. ‡ Count for t_0 was made using epifluorescence microscopy (see Methods). This count was adjusted to include only flagellates $>2.5 \mu\text{m}$ in diameter (autotrophic) in order to allow for comparison with settling chamber counts. The small flagellate counts for the four treatments were taken from inverted microscopy (see Methods) and so could include autotrophic and heterotrophic forms. Therefore, direct comparison between the t_0 count and the treatments was not possible, and the difference between t_0 and control or +Zn count could simply result from difference in methodology. Similarly, for the ciliates, the epifluorescence count at t_0 represented only mixotrophic ciliates, whereas the counts for the other treatments represented total ciliates. Therefore, a direct comparison between ciliate abundance at t_0 and in the other treatments cannot be made.

contrasting production rates between OSP and productive upwelling zones. Phosphate and silicate also showed strong drawdowns in response to Fe but were not completely removed. Silicate removal seemed to occur relatively late in the incubation with almost no difference between the control and +Fe treatments at day 4. In terms of nutrient removal ratios, the +Fe treatment gave a Si:N ratio of 0.99 and a N:P ratio of 14.7, agreeing well with ratios given by Takeda (1998). However, our control data did not allow calculation of these ratios, either because the changes were too small or because the nutrients actually increased.

When Fe was supplied, increases in chlorophyll (Fig. 3A–C) and POC fixation (Fig. 3D–F) were highest in the largest size fraction. Marked increases also occurred in percent chlorophyll and percent POC production in the large fraction in the +Fe treatment (Figs. 4C, 5C), together with corresponding decreases in percent chlorophyll and POC fixation in the small fraction (Figs. 4A, 5A). These data, together with the strong silicate removal occurring relatively late in the +Fe incubation, clearly support the contention (Martin et al. 1989; Boyd et al. 1996) that at OSP, and in other HNLC areas (e.g., Takeda 1995), large diatoms dominate when Fe is supplied. This is confirmed by the numerical dominance of large diatoms at the end of the +Fe incubation (Table 6).

Compared to t_0 and control values, addition of Fe alone increased α and P_m approximately 100-fold (Fig. 7; Table 3). Normalized for chlorophyll (Fig. 8), P_m^{chl} and α^{chl} increased 5–10-fold (Table 4), suggesting either a real increase in the photosynthetic efficiency in response to Fe, a shift in species composition, or both. In Fe-deficient cultures of the diatoms *Phaeodactylum tricornutum* and *Thalassiosira weissflogii*, Greene et al. (1991) and McKay et al. (1997) showed that addition of Fe only approximately doubled P_m^{chl} for either species, with all values falling within the range $1.9\text{--}6.4 \text{ mg C (mg Chl)}^{-1} \text{ h}^{-1}$ (Green's data converted assuming Chl molecular mass of ~ 900 and photosynthetic quotient of 1.25). These two studies also showed that α^{chl} was not greatly influenced by availability of Fe for the two species, with all values within the range $0.015\text{--}0.039 \text{ mg C (mg Chl)}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1} \text{ h}^{-1}$, close to α^{chl} values reported for the +Fe treatment here ($0.014\text{--}0.016 \text{ mg C [mg}$

$\text{Chl}]^{-1} [\mu\text{mol photons m}^{-2} \text{ s}^{-1}]^{-1} \text{ h}^{-1}$, Table 4). Because both P_m^{chl} and α^{chl} were relatively invariant in culture in response to Fe, the “apparent” physiological changes observed here could well result from shifts in species composition from populations dominated by small cells at t_0 and control (Table 6), to populations dominated by large diatoms (Table 6).

Does addition of Zn alone stimulate phytoplankton growth?—The addition of Zn alone stimulated a small but significant increase in total chlorophyll compared to that in the control, although the slight increase in POC production was not statistically significant (Fig. 1A,B). The growth stimulation in the +Zn treatment was apparent in all size fractions of chlorophyll (Fig. 3A–C) and POC fixation (Fig. 3D–F), but perhaps because of errors compounded by fractionation, these differences were not statistically significant. Growth stimulation by Zn was stronger in the early part of the incubation, suggesting that this influence might be unsustainable without an input of Fe. The significant stimulation by Zn on chlorophyll after 4 d was also confirmed by a phosphate concentration that was significantly lower than in the control. Nitrate concentration was also significantly lower in the +Zn treatment than the control, but only at the end of the incubation (Fig. 2A). These observations represent perhaps the first substantial evidence for Zn limitation in HNLC waters and suggest that the very low Zn_T at OSP (Martin et al. 1989; Lohan et al. 2002), probably in the presence of an organic ligand (e.g., Bruland 1989), reduces $[Zn^{2+}]$, at least for some species, into the growth-limiting range established in cultures (e.g., Sunda and Huntsman 1995). In terms of nutrient drawdown ratios, the Zn stimulation was accompanied by a Si:N ratio of 0.14 and a N:P ratio of 27—much lower and higher, respectively, than the ratios observed in the +Fe treatment. The coccolithophores were the group that showed the greatest absolute increase in abundance in the +Zn treatment (Table 6); given that *E. huxleyi* (the major coccolithophore) competes very well at low phosphate (e.g., Paasche and Brubak 1994), it might be speculated that the high N:P and low Si:N ratios were mainly associated with growth of this species. Small diatoms also increased in response to Zn addition (Table 6). This is

not entirely in agreement with the observations of Coale (1991), who also observed stimulation by Zn on the abundance of smaller phytoplankton, although this group excluded diatoms. Coale (1991) also found that Zn stimulated the abundance of small ciliates, whereas our counts suggest a slight decrease.

P_m increased approximately 10-fold in the +Zn treatment compared to that at t_0 and in the control, but without an accompanying increase in α (Fig. 7; Table 3). This is consistent with an increase in the rate of carbon fixation (e.g., Morel et al. 1994) but without a concomitant increase in efficiency of carbon fixation at low light, for which Fe is presumably required. This increase in P_m in the short-term P versus E experiment was relatively greater than that observed in POC fixation over 24 h (Fig. 1B). This suggests that respiratory or recycling processes over 24 h might also be relatively increased in the +Zn treatment. When P versus E curves were normalized to chlorophyll (Fig. 8; Table 4), a two- to threefold increase in P_m^{chl} (Table 4) was still evident on addition of Zn, again with no noticeable effect on α^{chl} . It is interesting to note that computed P_m^{chl} in the +Zn treatment was almost as high as that in the +Fe and +Zn+Fe treatments (Table 4).

The 10 nmol L⁻¹ added Zn could conceivably have had a toxic effect on the phytoplankton population. However, there seems little evidence for this; Table 6 suggests that abundance of most groups either remained constant or increased in response to Zn. The only exception to this was the ciliates, although these counts were based on a very low total number of cells counted. The concentration of 10 nmol L⁻¹ Zn_T added was chosen because Coale (1991) speculated that the 0.75 nmol L⁻¹ he added might not have been sufficient to stimulate growth because of the presence of a Zn-specific ligand. Even in the unlikely situation that the 10 nmol L⁻¹ added was all in the freely available form, the concentration of [Zn²⁺] would still only just reach the order of 10⁻⁸ mol L⁻¹ required for Zn toxicity to begin to be observed (e.g., Sunda and Huntsman 1992, 1995).

Does addition of Zn and Fe together influence growth compared to Fe alone?—In terms of total chlorophyll, total POC fixation (Fig. 1A,B), and nutrient concentrations (Fig. 2), there were no significant differences between the +Zn+Fe treatment and the +Fe treatment. Si:N and N:P ratios were also very similar in the +Zn+Fe treatment compared to the +Fe treatment. The chlorophyll-specific POC production did show a major difference with +Zn+Fe treatment after 4 d, being much lower than for the +Fe treatment (Fig. 1D). This resulted mainly from a very high value in the small fraction, although the reason for the difference between the +Fe and +Zn+Fe treatments in this small fraction is unknown. The highest chlorophyll-specific POC production at the end of the incubations was for the +Zn+Fe treatment in the large fraction, consistent perhaps with carbon limitation of large diatoms at relatively low pCO₂ at the end of the incubation; Zn could allow synthesis of CA and could therefore mitigate the potential CO₂ limitation to which larger cells with longer diffusion paths might be susceptible (e.g., Morel et al. 1994; Goldman 1999).

The most interesting difference between the +Fe and

+Zn+Fe treatments was in size-fractionated chlorophyll. The +Fe treatment had a significantly higher proportion (up to 60%) of total chlorophyll in the large fraction than the +Zn+Fe treatment (20–30%), and vice versa for the small fraction. These data provide convincing evidence for some difference in the size composition, species composition, or both between the +Fe and +Zn+Fe treatments. This is supported by cell count data (Table 6) where considerably more small diatoms and small flagellates were present in the +Zn+Fe treatment. Although the proportion of chlorophyll in the large fraction was much higher for the +Fe treatment, the actual abundance of large diatoms was quite similar (a little lower) to that in the +Zn+Fe treatment. In the context of this increase in chlorophyll in the small fraction in the +Zn+Fe treatment compared to +Fe alone, Olson et al. (2000) noted in the Southern Ocean that the +Zn+Fe (and +Zn) treatment showed the greatest stimulation of cell numbers, suggesting a subtle effect of Zn on small cells, as also noted by Coale (1991) at OSP. Although toxic effects of Zn (as discussed above) could have played a part in the shift in community composition, there is again little evidence for this. The only groups to show a decrease in abundance from +Fe to +Zn+Fe treatments were coccolithophores and ciliates (Table 6). However, coccolithophores actually increased in the +Zn treatment compared to the control, suggesting that toxicity was unlikely to be responsible. Similarly, both the +Fe and +Zn+Fe treatments increased the abundance of ciliates relative to the control (Table 6), suggesting that an acute toxic effect of Zn is unlikely to be responsible for the decrease in ciliates in the +Zn treatment compared to the control.

Theoretical analyses have suggested that Zn concentrations are low enough in the some parts of the ocean to cause diffusion limitation of cells >4 μm in diameter, but not smaller (Hudson and Morel 1993). In contrast, our data suggest that compared to Fe alone, the supply of Zn seems to stimulate growth of smaller cells and increases the proportion of chlorophyll within the small fraction.

The +Fe treatment showed higher values for P_m and α than the +Zn+Fe treatment (Fig. 7; Table 3), but when normalized to chlorophyll, the +Zn+Fe treatment had a higher assimilation number, P_m^{chl} , and lower α^{chl} than the +Fe treatment (Table 4; Fig. 8). Therefore in both the +Zn+Fe and +Zn treatments, there was evidence for enhanced biomass-normalized carbon fixation (compared to the corresponding treatment without Zn), again supporting the argument that carbon fixation rates might be limited by Zn availability (e.g., Morel et al. 1994). These enhanced rates could have been caused by a change in physiology of individual species, by a shift toward species that had previously been Zn limited, or by both. The increased α^{chl} in the +Fe treatment compared to the +Zn+Fe treatment (Fig. 8) might explain the increased chlorophyll-specific POC production rates after 4 d in the +Fe treatment (Figs. 1D, 3J–L), because the 24-h productivity incubations were carried out at relatively low irradiance (i.e., 30% surface irradiance).

How do Zn and Fe influence PIC production?—The addition of Zn alone appeared to slightly stimulate total PIC production compared to the control (Fig. 1C), particularly

early in the experiment, although this was not statistically significant. However, the increase in abundance of coccolithophores (Table 6) and the N:P ratio (discussed earlier) does suggest that there could have been a real effect. For the two +Fe treatments, PIC clearly increased significantly compared to the control (Fig. 1C). This occurred most significantly in the 5–20- μm fraction, suggesting that calcification by the dominant coccolithophore (Table 6; see also Okada and Honjo 1973), *E. huxleyi*, was mainly responsible. This was supported by an ~ 20 -fold increase in the abundance of coccolithophorids in the two Fe treatments.

When expressed as the ratio of PIC:POC fixation (Fig. 6), there appeared to be a steady but slight increase for the two +Fe treatments throughout the incubation in the 5–20- μm fraction and a sharp increase in the 0.2–5- μm fraction toward the end of the incubation, the latter consistent perhaps with shedding of recently formed coccoliths (e.g., see Balch et al. 1992). However, because of scatter in the data, the various treatments were not statistically different from the control. The total PIC:POC ratio at t_0 and in the control was on the order of 0.04–0.1, consistent with the lower end of values reported for shallow sediment traps at OSP (Wong et al. 1999; Wong and Crawford 2002). This ratio increased to almost 0.2 in the +Fe treatment in which PIC fixation was the highest. This ratio has been observed to reach the order of 1 at OSP in sediment traps (Wong and Crawford 2002) and in ^{14}C uptake experiments (Crawford unpubl. data) when coccolithophores were abundant.

These observations contradict some of the published observations that *E. huxleyi* and other coccolithophores might be relatively difficult to limit with Fe (Martin et al. 1989; Muggli and Harrison 1997). The data presented here even contradict those of Lam et al. (2001), a study carried out at OSP on the same cruise as the present study. However, there were differences in length of incubation between this study and that of Lam et al. (2001), and this complicates comparisons. The rates of PIC fixation and PIC:POC ratios were similar to those of Lam et al. (2001) in the controls. However, PIC fixation rates were higher here for the +Fe treatment in the 24-h incubations (Fig. 1C) than for the shorter term experiments of Lam et al. (2001).

The response of C versus E curves to metal enrichment (Fig. 7) was similar to that of the P versus E curves, but with more “scatter” resulting from the low PIC signal on filters. Because of this, it was difficult to resolve a shape to the curves for t_0 , control, and +Zn treatments. However, it is clear that maximum rates of PIC fixation in t_0 , control, and +Zn treatment were on the order of 0.01–0.03 $\text{mg C m}^{-3} \text{h}^{-1}$ or 0.1–0.4 $\text{mg C m}^{-3} \text{d}^{-1}$. These short-term rates were much lower than the daily rates given in Fig. 1, suggesting a discrepancy based on length of incubation, possibly as a result of dark processes. Although this discrepancy could contribute to the lack of agreement mentioned above between the shorter incubations of Lam et al. (2001) and the 24-h incubations in this study, it should be noted that maximum PIC fixation rates from C versus E experiments (Fig. 7) also showed a 10–20-fold increase in the +Fe treatments compared to the control.

Although the stimulation by Fe of 24 h and short-term PIC fixation rates does not support some recently published

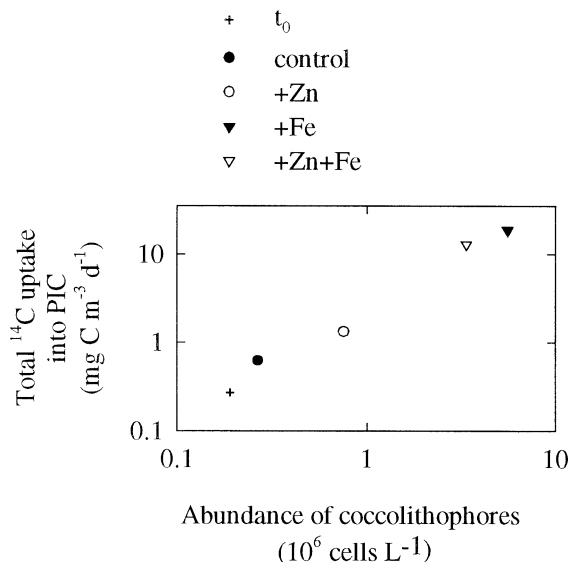


Fig. 9. Significant relationship ($p < 0.001$; $r^2 = 0.99$) between abundance of coccolithophores and measured PIC fixation rates at the end of the incubations. The slope of the relationship gives a mean PIC fixation rate of $3.6 \text{ pg C cell}^{-1} \text{ d}^{-1}$. Log scale used is simply to show the detail of the relationship at lower cell abundance.

information, our cell count data (Table 6) strongly supports the argument that coccolithophores were strongly stimulated by Fe. Moreover, the PIC fixation data from t_0 and from the four treatments also correlate remarkably well (Fig. 9) with abundance of coccolithophores (Table 6) at the end of each incubation. This suggests that the PIC fixation rates as measured are real and related to coccolithophore abundance. The PIC fixation rates of $3.6 \text{ pg C cell}^{-1} \text{ d}^{-1}$ from this relationship also agree well with published rates. There is also some anecdotal supporting evidence for our observations; for example, Martin et al. (1989) pointed out sharp increases in abundance of certain coccolithophores at some stations in response to Fe additions. We can offer little explanation for the lack of agreement between our study and that of Lam et al. (2001). The question of whether coccolithophores are limited by trace metals in the subarctic Pacific therefore seems to require further clarification.

Implications for OSP and other HNLC waters—This study supports previous work in demonstrating that availability of dissolved Fe strongly limits phytoplankton growth at OSP. The role of Zn is less clear, with a relatively minor though significant effect in stimulating growth and production, but with evidence also for an increase in short-term carbon fixation rates (i.e., supporting Morel et al. 1994). Compared to Fe alone, addition of Zn and Fe together resulted in significant shifts in physiology and size distribution of chlorophyll within the population. These changes were supported by increases in some of the smaller phytoplankton groups (Table 6). Although subtle, these effects are not necessarily inconsequential; Fe is unlikely to be supplied alone as a trace metal, either from atmospheric input or vertical mixing. Although the typical interannual variation in POC

export flux at OSP is thought to be driven principally by variations in irradiance (Wong et al. 1998), there are clearly some extremely high flux events (Wong et al. 1999) that could be driven by sporadic input of Fe. The question of how combinations of trace metals influence community composition and how this affects the export of POC therefore becomes a crucial question. With regard to PIC fixation, it is known that growth of coccolithophores decreases alkalinity and reduces the air-sea $p\text{CO}_2$ gradient generated by growth of noncalcifying phytoplankton (e.g., Crawford and Purdie 1997). The data presented here suggest that fixation of PIC is stimulated by Fe to a similar extent as fixation of POC, so that PIC:POC fixation ratios could be relatively unchanged by addition of Fe. Of more relevance perhaps is the question of the effect of trace metals on PIC:POC export ratios and thus the efficiency of the biological pump at OSP. Further study is clearly required in light of the interannual variation in PIC production and PIC:POC export ratios at OSP and a possible link with the El Niño cycle (Wong and Crawford 2002).

References

- BALCH, W. M., P. M. HOLLIGAN, AND K. A. KILPATRICK. 1992. Calcification, photosynthesis and growth of the bloom-forming coccolithophore, *Emiliania huxleyi*. *Cont. Shelf Res.* **12**: 1353–1374.
- BARWELL-CLARKE, J., AND F. WHITNEY. 1996. Institute of Ocean Sciences nutrient methods and analysis. *Can. Tech. Rep. Hydrogr. Ocean Sci.* **182**: 1–43.
- BLAIN, S., AND OTHERS. 2002. Quantification of algal iron requirements in the subantarctic Southern Ocean (Indian Sector). *Deep-Sea Res. II* **49**: 3255–3273.
- BOOTH, B. C., J. LEWIN, AND J. R. POSTEL. 1993. Temporal variation in the structure of autotrophic and heterotrophic communities in the subarctic Pacific. *Prog. Oceanogr.* **32**: 57–99.
- BOYD, P. W., AND OTHERS. 1996. In vitro iron enrichment experiments in the NE subarctic Pacific. *Mar. Ecol. Prog. Ser.* **136**: 179–193.
- , AND OTHERS. 2000. A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. *Nature* **407**: 695–702.
- 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.
- BRULAND, K. W. 1989. Complexation of zinc by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* **34**: 269–285.
- , G. A. KNAUER, AND J. H. MARTIN. 1978. Zinc in northeast Pacific water. *Nature* **271**: 741–743.
- BUMA, A. G. J., H. J. W. DE BAAR, R. F. NOLTING, AND A. J. VAN BENNEKOM. 1991. Metal enrichment experiments in the Weddell-Scotia Seas: Effects of iron and manganese on various plankton communities. *Limnol. Oceanogr.* **36**: 1865–1878.
- COALE, K. H. 1991. Effects of iron, manganese, copper, and zinc enrichments on productivity and biomass in the subarctic Pacific. *Limnol. Oceanogr.* **36**: 1851–1864.
- , AND OTHERS. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* **383**: 495–501.
- CRAWFORD, D. W., AND D. A. PURDIE. 1997. Increase of PCO_2 during blooms of *Emiliania huxleyi*: Theoretical considerations on the asymmetry between acquisition of HCO_3^- and respiration of free CO_2 . *Limnol. Oceanogr.* **42**: 365–372.
- CULLEN, J. T., T. W. LANE, F. M. M. MOREL, AND R. M. SHERRELL. 1999. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. *Nature* **402**: 165–167.
- DE LA ROCHA, C., D. A. HUTCHINS, M. A. BRZEZINSKI, AND Y. ZHANG. 2000. Effects of iron and zinc deficiency on elemental composition and silica production by diatoms. *Mar. Ecol. Prog. Ser.* **195**: 71–79.
- DONAT, J. R., AND K. W. BRULAND. 1990. A comparison of two voltammetric techniques for determining zinc speciation in northeast Pacific Ocean waters. *Mar. Chem.* **28**: 301–323.
- , AND ———. 1995. Trace elements in the Oceans, p. 247–281. *In* B. Salbu and E. Steinnes [eds.], *Trace Elements in Natural Waters*. CRC Press.
- DUGDALE, R. C., AND F. P. WILKERSON. 1991. Low specific nitrate uptake rate: A common feature of high-nutrient, low-chlorophyll marine ecosystems. *Limnol. Oceanogr.* **36**: 1678–1688.
- ELDERFIELD, H., AND R. E. M. RICKABY. 2000. Oceanic Cd/P ratio and nutrient utilization in the glacial Southern Ocean. *Nature* **405**: 305–310.
- ELLWOOD, M. J., AND K. A. HUNTER. 2000. The incorporation of zinc and iron into the frustule of the marine diatom *Thalassiosira pseudonana*. *Limnol. Oceanogr.* **45**: 1517–1524.
- , AND C. M. G. VAN DEN BERG. 2000. Zinc speciation in the northeastern Atlantic Ocean. *Mar. Chem.* **68**: 295–306.
- , AND ———. 2001. Determination of organic complexation of cobalt in seawater by cathodic stripping voltammetry. *Mar. Chem.* **75**: 33–47.
- FRANCK, V. M., M. A. BRZEZINSKI, K. H. COALE, AND D. M. NELSON. 2000. Iron and silicic acid concentrations regulate Si uptake north and south of the Polar Front Zone in the Pacific Sector of the Southern Ocean. *Deep-Sea Res. II* **47**: 3315–3338.
- FREW, R., A. BOWIE, P. CROOT, AND S. PICKMERE. 2001. Macro-nutrient and trace metal geochemistry of an in situ iron induced Southern Ocean bloom. *Deep-Sea Res. II* **48**: 2467–2481.
- GALL, M. P., R. STRZEPEK, M. MALDONADO, AND P. W. BOYD. 2001. Phytoplankton processes. Part 2: Rates of primary production and factors controlling algal growth during the Southern Ocean Iron Release Experiment (SOIREE). *Deep-Sea Res. II* **48**: 2571–2590.
- GOLDMAN, J. C. 1999. Inorganic carbon availability and the growth of large marine diatoms. *Mar. Ecol. Prog. Ser.* **180**: 81–91.
- GREENE, R. M., R. J. GEIDER, AND P. G. FALKOWSKI. 1991. Effect of iron limitation on photosynthesis in a marine diatom. *Limnol. Oceanogr.* **36**: 1772–1782.
- HUDSON, R. J. M., AND F. M. M. MOREL. 1993. Trace metal transport by marine microorganisms: Implications of metal coordination kinetics. *Deep-Sea Res. I* **40**: 129–150.
- KREMLING, K., AND P. STREU. 2001. The behaviour of dissolved Cd, Co, Zn, and Pb in North Atlantic near-surface waters (30 degrees N/60 degrees W–60 degrees N/2 degrees W). *Deep-Sea Res. I* **48**: 2541–2567.
- LAM, P. J., P. D. TORTELL, AND F. M. M. MOREL. 2001. Differential effects of iron additions on organic and inorganic carbon production by phytoplankton. *Limnol. Oceanogr.* **46**: 1199–1202.
- LEE, J. G., AND F. M. M. MOREL. 1995. Replacement of zinc by cadmium in marine phytoplankton. *Mar. Ecol. Prog. Ser.* **127**: 305–309.
- LOHAN, M. C., P. J. STATHAM, AND D. W. CRAWFORD. 2002. Total dissolved zinc in the upper water column of the subarctic North East Pacific. *Deep-Sea Res. II* **49**: 5793–5808.
- MARTIN, J. H., AND S. E. FITZWATER. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* **331**: 341–343.
- , AND OTHERS. 1989. VERTEX: Phytoplankton/iron studies in the Gulf of Alaska. *Deep-Sea Res.* **36**: 649–680.

- MCKAY, R. M. L., R. J. GEIDER, AND J. LAROCHE. 1997. Physiological and biochemical response of the photosynthetic apparatus of two marine diatoms to Fe stress. *Plant Physiol.* **114**: 615–622.
- MOREL, F. M. M., AND OTHERS. 1994. Zinc and carbon co-limitation of marine phytoplankton. *Nature* **369**: 740–742.
- MUGGLI, D. L., AND P. J. HARRISON. 1997. Effects of iron on two oceanic phytoplankters grown in natural NE subarctic Pacific seawater with no artificial chelators present. *J. Exp. Mar. Biol. Ecol.* **212**: 225–237.
- OBATA, H., H. KARATANI, AND E. NAKAYAMA. 1993. Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection. *Anal. Chem.* **65**: 1524–1528.
- , ———, M. MATSUI, AND E. NAKAYAMA. 1997. Fundamental studies for chemical speciation of iron in seawater with an improved analytical method. *Mar. Chem.* **56**: 97–106.
- OKADA, H., AND S. HONJO. 1973. The distribution of oceanic coccolithophorids in the Pacific. *Deep-Sea Res.* **20**: 355–374.
- OLSON, R. J., H. M. SOSIK, A. M. CHEKALYUK, AND A. SHALAPYONOK. 2000. Effects of iron enrichment on phytoplankton in the Southern Ocean during late summer: Active fluorescence and flow cytometric analyses. *Deep-Sea Res. II* **47**: 3181–3200.
- PAASCHE, E., AND S. BRUBAK. 1994. Enhanced calcification in the coccolithophorid *Emiliania huxleyi* (Haptophyceae) under phosphorus limitation. *Phycologia* **33**: 324–330.
- PETERSON, B. J. 1980. Aquatic primary productivity and the ¹⁴C–CO₂ method: A history of the productivity problem. *Ann. Rev. Ecol. Syst.* **11**: 359–385.
- PLATT, T., C. L. GALLEGOS, AND W. G. HARRISON. 1980. Photo-inhibition of photosynthesis in natural assemblages in marine phytoplankton. *J. Mar. Res.* **38**: 687–701.
- PRICE, N. M., AND F. M. M. MOREL. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. *Nature* **344**: 658–660.
- RIEBESSELL, U., D. A. WOLF-GLADROW, AND V. SMETACEK. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature* **361**: 249–251.
- RUETER, J. G., AND F. M. M. MOREL. 1981. The interaction between zinc deficiency and copper toxicity as it affects the silicic acid uptake mechanisms in *Thalassiosira pseudonana*. *Limnol. Oceanogr.* **26**: 67–73.
- SCHAREK, R., M. A. VAN LEEUWE, AND H. J. W. DE BAAR. 1997. Responses of Southern Ocean phytoplankton to the addition of trace metals. *Deep-Sea Res. II* **44**: 209–227.
- STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis, 2nd ed. Fisheries Research Board of Canada Bulletin 167.
- SUNDA, W. G., AND S. A. HUNTSMAN. 1992. Feedback interactions between zinc and phytoplankton in seawater. *Limnol. Oceanogr.* **37**: 25–40.
- , AND ———. 1995. Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications. *Limnol. Oceanogr.* **40**: 1404–1417.
- , AND ———. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. *Limnol. Oceanogr.* **45**: 1501–1516.
- , D. G. SWIFT, AND S. A. HUNTSMAN. 1991. Low iron requirement for growth in oceanic phytoplankton. *Nature* **351**: 55–57.
- TAKEDA, S. 1995. Response of equatorial Pacific phytoplankton to subnanomolar Fe enrichment. *Mar. Chem.* **50**: 219–227.
- . 1998. Influence of iron availability on nutrient consumption ratio of diatoms in oceanic waters. *Nature* **393**: 774–777.
- TORTELL, P. D., J. R. REINFELDER, AND F. M. M. MOREL. 1997. Active uptake of bicarbonate by diatoms. *Nature* **390**: 243–244.
- VAN DEN BERG, C. M. G. 1985. Determination of the zinc complexing capacity in seawater by cathodic stripping voltammetry of Zinc-APDC complex ions. *Mar. Chem.* **16**: 121–130.
- WEBB, W. L., M. NEWTON, AND D. STAR. 1974. Carbon dioxide exchange of *Almus rubra*. A mathematical model. *Oecologia* **17**: 281–291.
- WONG, C. S., AND D. W. CRAWFORD. 2002. Flux of particulate inorganic carbon to the deep subarctic Pacific correlates with El Niño. *Deep-Sea Res. II* **49**: 5705–5715.
- , R. J. MATEAR, F. A. WHITNEY, AND K. ISEKI. 1998. Enhancement of new production in the northeast subarctic Pacific Ocean during negative North Pacific index events. *Limnol. Oceanogr.* **43**: 1418–1426.
- , AND OTHERS. 1999. Seasonal and interannual variability in particle fluxes of carbon, nitrogen and silicon from time series of sediment traps at Ocean Station P, 1982–1993: Relationship to changes in subarctic primary productivity. *Deep-Sea Res. II* **46**: 2735–2760.

Received: 16 May 2002

Accepted: 17 December 2002

Amended: 24 February 2003