JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 104, NO. C6, PAGES 13,395–13,408, JUNE 15, 1999

Role of iron, light, and silicate in controlling algal biomass in subantarctic waters SE of New Zealand

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Abstract. Phytoplankton processes in subantarctic (SA) waters southeast of New Zealand were studied during austral autumn and spring 1997. Chlorophyll *a* (0.2–0.3 μg L^{-1}) and primary production (350–650 mg C m^{-2} d^{-1}) were dominated by cells <2 μm (cyanobacteria) in both seasons. The photochemical efficiency of photosystem II (F_v/F_m) of cells was low (0.3), indicating physiological stress. Dissolved Fe (DFe) levels in surface waters were subnanomolar, and the molecular marker flavodoxin indicated that cells were iron stressed. In contrast, Subtropical Convergence (STC) and subtropical waters had higher algal biomass/production levels, particularly in spring. In these waters, DFe levels were >1 nmol kg^{-1}, there was little evidence of Fe-stressed algal populations, and F_v/F_m approached 0.60 at the STC. In addition to these trends, waters of SA origin were occasionally observed within the STC and north of the STC, and thus survey data were interpreted with caution. In vitro Fe enrichment incubations in SA waters resulted in a switch from flavodoxin expression to that of ferredoxin, indicating the alleviation of Fe stress. In another 6-day experiment, iron-mediated increases in chlorophyll *a* (in particular, increases in large diatoms) were of similar magnitude to those observed in a concurrent Si/Fe enrichment; ambient silicate levels were 4 μM. A concurrent in vitro Fe enrichment, at irradiance levels comparable to the calculated mean levels experienced by cells in situ, resulted in relatively small increases (approximately twofold) in chlorophyll *a*. Thus, in spring, irradiance and Fe may both control diatom growth. In contrast, during summer, as mean irradiance increases and silicate levels decrease, Fe limitation, Fe/Si colimitation, or silicate limitation may determine diatom growth.

1. Introduction

Subantarctic (SA) waters form a ring which occupies 10°–20° of latitude between the Subtropical Convergence (STC) and the Polar Front (PF) [*Longhurst*, 1995; *Banse*, 1996]. This circumpolar band is comparable to the areal extent of the open Southern Ocean south of the PF. Whereas the majority of studies have focused on the waters south of the PF, comparatively little is known about SA waters [*Longhurst*, 1995; *Banse*, 1996]. Data are available on phytoplankton biomass (primarily inferred from coastal zone color scanner (CZCS) remote sensing and data archives [*Longhurst*, 1995; *Banse*, 1996; *Banse and English*, 1997], production [*Laubscher et al.*, 1993; *Bradford-Grieve et al.*, 1997; *Clementson et al.*, 1998], and taxonomic composition [*Chang and Gall*, 1998] in SA waters. However, little is known about the role of environmental factor(s) in controlling phytoplankton growth and/or determining the size structure of the algal assemblage in SA waters.

SA waters have been described as high nitrate, low chlorophyll (HNLC) by *Banse and English* [1997]. Furthermore, as SA waters have excess nitrate relative to silicate, this region is further defined as high nitrate, low silicate, low chlorophyll (HNLSLC [*Dugdale et al.*, 1995]); the relatively low silicate (Si) levels are thought to be due in part to the circumpolar character of the water mass [*Zentara and Kamykowski*, 1981] and to an elevated Si uptake rate relative to that of nitrate [*Minas and Minas*, 1992]. Despite this HNLSLC classification, evidence from paleoceanographic proxies suggest that during the Last Glacial Maximum (LGM), the Atlantic sector of the SA region was characterized by high rates of both primary and export production [*Kumar et al.*, 1995] relative to the present. Confirmation of the nature of the mechanisms required for such elevated rates during the LGM may be provided by contemporary studies.

Previous research in the Pacific sector of SA waters in the vicinity of New Zealand indicate that chlorophyll *a* (chl *a*) levels decline south of the STC and that the algal assemblage is dominated by cyanobacteria [*Bradford-Grieve et al.*, 1997, 1999; *Chang and Gall*, 1998] which are under heavy grazing pressure [*Hall et al.*, 1999]. Although Fe limitation of phytoplankton growth has been demonstrated in other HNLC regions [*Coale et al.*, 1996a, b; *LaRoche et al.*, 1996], at present there is only circumstantial evidence for such a limitation mechanism in SA waters in the Pacific sector. Studies south of New Zealand provide evidence for low phytoplankton quantum yields, indicative of physiological stress, in SA waters characterized by relatively high macronutrient levels [*Hawes et al.*, 1997]. A survey of the SA waters south of Australia reported subnanomolar Fe levels which may potentially limit rates of

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Paper number 1999JC900009.
0148-0227/99/1999JC900009$09.00

primary production [*Sedwick et al.*, 1997]. Moreover, the region is also characterized by low Si levels in summer [*Vincent et al.*, 1991] and relatively deep mixed layers in spring and autumn (M. Hadfield, personal communication, 1998). *Heath and Bradford-Grieve* [1980] suggested that irradiance levels may limit algal growth over the Campbell Plateau (south of New Zealand in SA waters), whereas *Banse* [1996], on the basis of an analysis of chlorophyll *a* levels versus mixed layer depth (MLD), reports that underwater irradiance did not influence seasonal mean chlorophyll *a* levels. Thus the environmental factors controlling phytoplankton growth in SA waters over the annual cycle are as yet unresolved and may be complex [see *Dugdale et al.*, 1995; *Sunda and Huntsman*, 1997; *Maldonado et al.*, 1999].

The aims of the present study were to contrast the magnitude of phytoplankton biomass and production in SA, STC, and subtropical (ST) waters, to elucidate the environmental factor(s) (grazing was not considered) that controls algal biomass in SA waters and relates them to physical and chemical data for this region, and to assess how the control mechanism(s) impacts the seasonal cycle of algal production, biomass, and size structure.

2. Methods and Materials

Two survey (May 21 to June 2 and September 24 to October 13, 1997) and two process voyages (April 28 to May 8 and October 16 to November 6, 1997) transected the 178°30′E meridian from 42°20′S to 47°S (Figure 1). Six short-term process stations (1–2 days) and two longer-term process stations (5–6 days) were occupied during the April/May (austral autumn) and October/November (austral spring) voyages, respectively (Figure 1). Both discrete (conductivity-temperature-depth (CTD) rosette) and underway (nontoxic pumped seawater supply) samples were collected on all voyages. Temperature and salinity vertical profiles were obtained using Seabird 9/11 plus CTD sensors calibrated periodically with discrete samples. Underway thermosalinograph data for the voyages were not available (see section 4.4). Chlorophyll *a* and active fluorescence measurements were performed on discrete samples taken underway using a calibrated Turner Designs Fluorometer and a Chelsea Instruments Fastracka fast repetition rate fluorometer (FRRF [*Kolber and Falkowski* [1993]), respectively. Samples for macronutrients (Si, nitrate, and phosphate) were taken underway and analyzed by Flow Injection Analysis (Quikchem 8000 instrument) following the procedures of *Lachat Instruments* [1996].

On the process voyages, additional water samples for the determination of chlorophyll *a* and primary production were obtained concurrently from acid-cleaned 10 L Niskin bottles with nylon-covered stainless steel springs. Incident and underwater photosynthetically active radiation (PAR) were measured using calibrated LiCor collectors (Models Li190 (cosine) and Li194 (spherical), respectively). Size-fractionated chlorophyll *a* and production were measured using fluorimetry [*Parsons et al.*, 1984] and the ^{14}C method outlined by *Joint et al.* [1993], respectively. Samples were filtered in series (20, 5, 2, and 0.2 μm, 47 mm diameter polycarbonate filters) through a fractionation cascade [*Joint and Pomroy*, 1983]. For ^{14}C uptake rates, samples were incubated for 24 hours, commencing before dawn, in a simulated in situ deck incubator (1–100% I_0 (percentage of incident irradiance), where water temperature was maintained by flowing seawater. After 24 hours, samples were filtered through the cascade and prepared for liquid scintillation counting by the channels ratio method.

Water samples for dissolved iron (DFe) and for in vitro perturbation experiments were obtained using 2.5 L and 30 L Teflon-lined Go-flo bottles, respectively, suspended on Kevlar line and tripped with plastic messengers. Water samples for DFe were sampled concurrently with those for algal Fe stress but up to 24 hours after those for algal biomass/production (see section 4.4). Within the shipboard clean laboratory, samples for DFe were drawn through acid-cleaned polycarbonate filters (0.45 μm) with a peristaltic pump connected via Teflon tubing to the Go-flo samplers (which were racked outside). One liter of filtrate was discarded prior to collection of a 1 L sample in a precleaned, fluorinated sample bottle without acidification. Fe was analyzed within 30 days of the end of the voyage by graphite furnace atomic absorption spectrophotometry (GFAAS) after solvent extraction of dithiocarbamate-chelated metals into $CHCl_3$ in the manner described in detail by *Frew and Hunter* [1995].

All sample manipulations to the point of GFAAS were carried out in a Class-100 clean laboratory. Water samples were acidified (2 mL of 10 *M* ultrapure HCl per liter of sample) 24 hours before analysis to desorb metal ions from the bottle surfaces. The sample aliquot to be extracted was generally 150 g, and duplicate aliquots were analyzed for each sample. Extraction efficiencies were 95 ± 4%, blanks were <0.02 n*M*, and the detection limit was 0.05 n*M* (2 σ); analytical precision as estimated by the standard deviation of duplicate extractions of the same sample was <5% in each case. Reported results have been corrected for recovery/blanks.

In situ algal Fe stress was measured on 50 L samples from both underway sampling along 178°30′E, and using subsamples from in vitro Fe enrichment experiments carried out in SA waters (May and October). Samples were filtered, and total protein was extracted, as previously described by *LaRoche et al.* [1995]. Protein concentration in the extract was measured using the bicinchoninic acid method [*Smith et al.*, 1985]. Equal amounts of proteins (20 μg) were loaded on 15% polyacralamide gels, separated by electrophoresis, and transferred to nitrocellulose by electroblotting. The blots were challenged sequentially with rabbit polyclonal antisera raised against flavodoxin [*LaRoche et al.*, 1995] and ferredoxin [*McKay et al.*, 1999] from diatoms, and immunoreactive proteins were detected by chemiluminescence (Amersham), as described previously by *McKay et al.* [1997]. In the in vitro perturbation experiments, raw seawater was added to clean 25 L polycarbonate carboys, handled following procedures outlined by *LaRoche et al.* [1996], and subsampled generally every 48 hours for flavodoxin and ferredoxin. Two additional in vitro experiments were conducted in October in SA waters to assess the effects of DFe and/or Si enrichment and those of DFe enrichment under differing PAR levels. In the former the supply of DFe (added to a final concentration of 2 nmol kg^{-1} after *Boyd et al.* [1996] and/or Si (4 μmol kg^{-1} added, contaminants removed by Chelex treatment) was amended. Samples were incubated under 50% I_0 in a seawater-cooled incubator (Si-only treatment was lost). In the latter the supply of DFe (final addition of 2 nmol kg^{-1}) and the light climate (neutral density screens to provide 30% I_0 and 10% I_0) were varied. No DFe measurements were made on the water from these experiments.

Algal Si uptake over the growth season (assumed to be 180 days, October to March) was estimated (see section 4.6). For this calculation it was assumed that diatoms take up the ma-

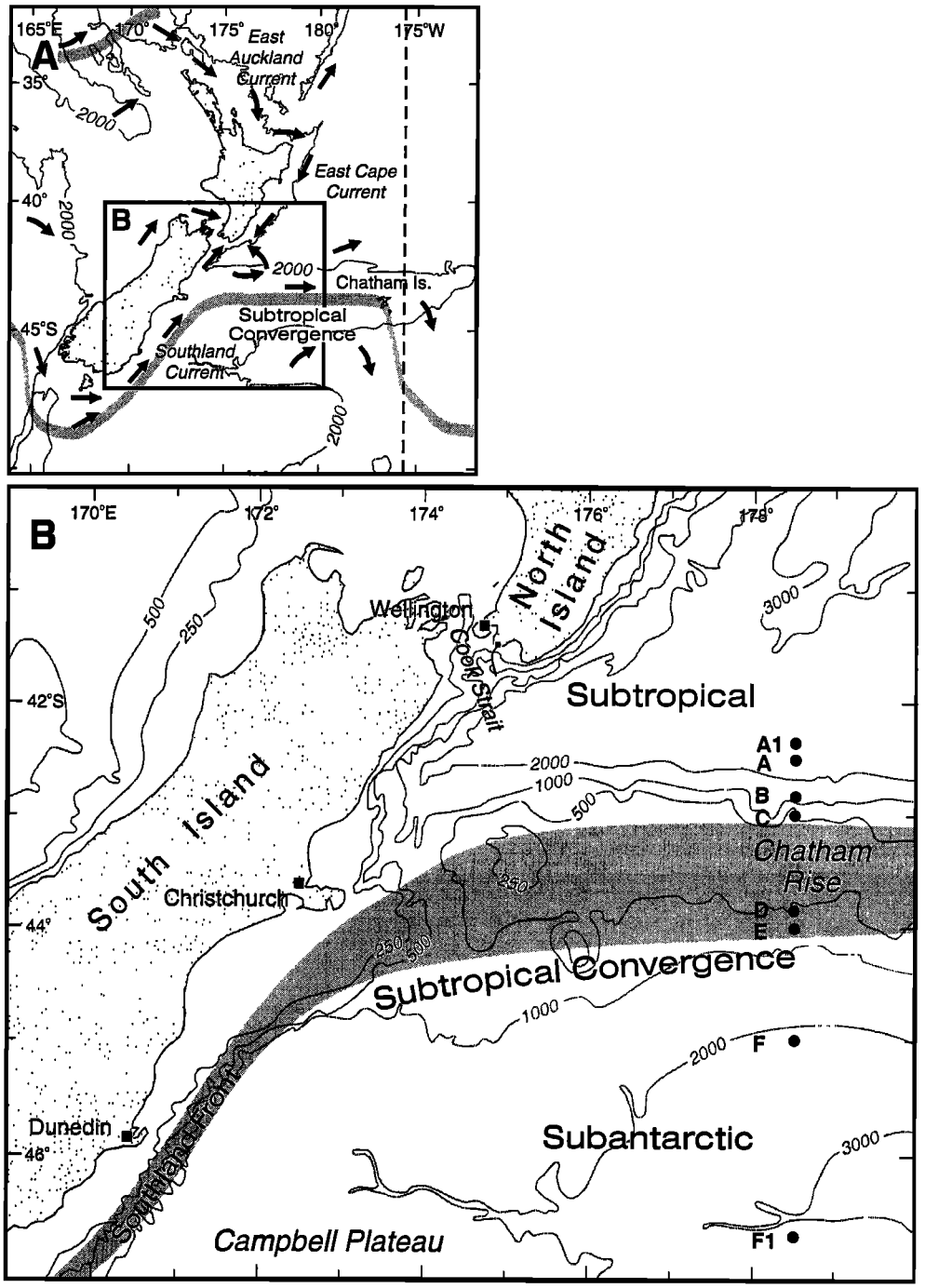

Figure 1. (a) The study area in relation to New Zealand and the U. S. Joint Global Ocean Flux Study (JGOFS) Southern Ocean Process Study (AESOPS) transect line (176°W meridian, denoted by dashed line). (b) Map of the process study sites showing the April/May (stations A–F) and October/November (A1 and F1) 1997 voyages. Underway data were collected on N-S or S-N transects along 178°30′E in early May, late May, September, and October 1997.

jority of Si in SA waters. Carbon fixation by large and small diatoms was derived from size-fractionated rates of production for the >20 μm (100%) and 5–20 μm (20%) classes, respectively; microscopical analysis indicated that most cells >20 μm were diatoms, whereas 5–20 μm diatoms composed ~20% of the 5–20 μm biomass (data not shown); *Tremblay and Legendre* [1994] have shown that in most oceanographic provinces the partitioning of both phytoplankton biomass and production into size fractions is comparable. It was also assumed that owing to the low seasonal amplitude in rates of primary production in HNLC waters [*Boyd and Harrison*, 1999], production rates from austral spring (October 1997) represent a mean production rate applicable for the growth season. Note that this approach is unable to take into account daily, seasonal, or species-specific variability in the algal uptake of C and Si. Algal Si requirements were calculated using Si:C ratios, an approach

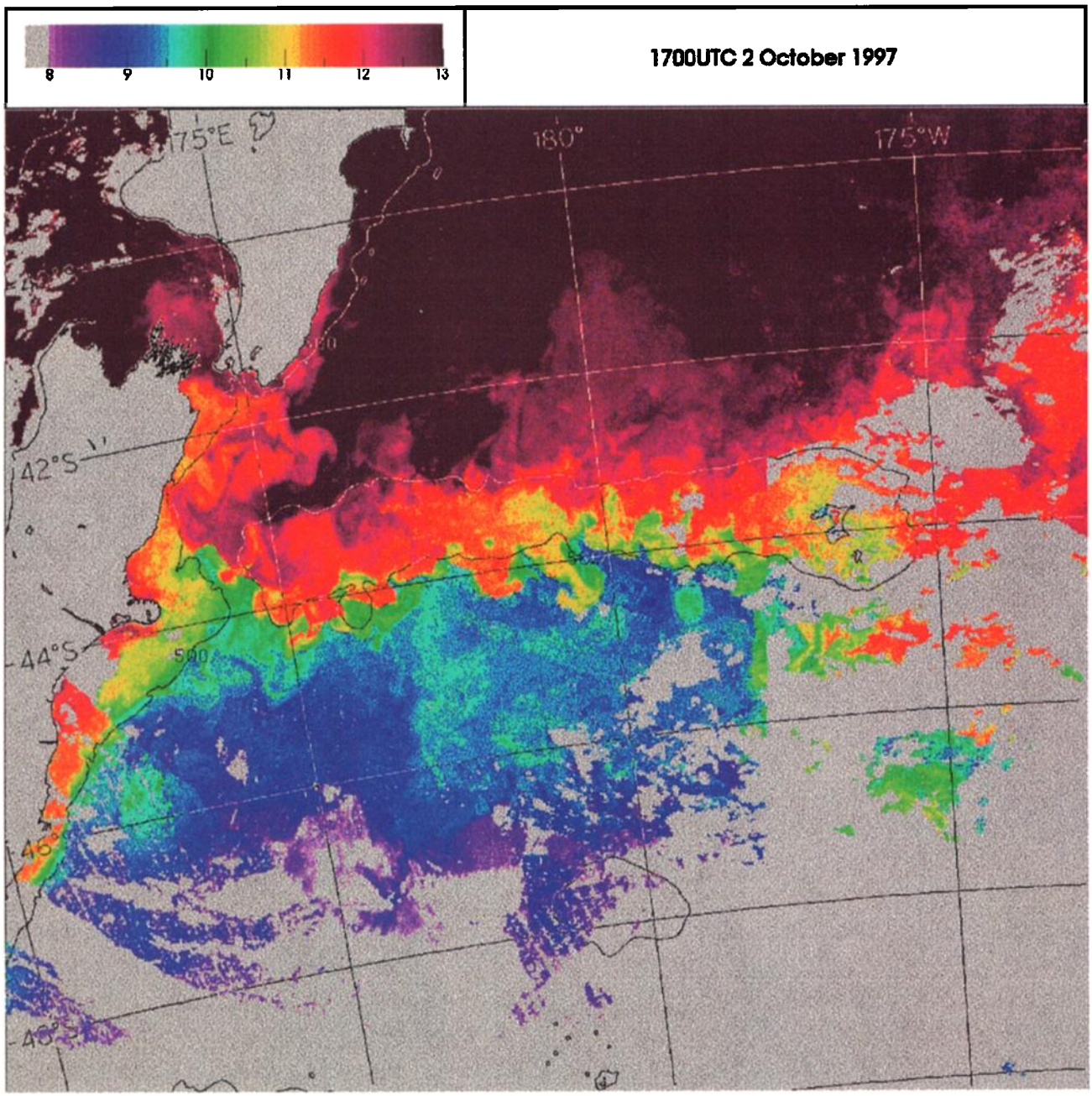

Plate 1. Advanced very high resolution radiometer (AVHRR) sea surface temperature (SST) image from 1700 UT October 2, 1997, showing the approximate position of the Subtropical Convergence (STC) SE of New Zealand (in the vicinity of 44°S) and the characteristic meanders and filaments on the southern boundary that were likely responsible for anomalous water mass characteristics observed in May (stations D and E) and September 1997. Image courtesy of M. Uddstrom (National Institute of Water and Atmospheric Research). Temperature scale is in °C.

advocated by *Brzezinski* [1985]. As the ratios reported by *Brzezinski* [1985] probably apply to cells under iron-replete conditions, the effect of Fe limitation on the Si:C ratio [*Hutchins and Bruland*, 1998] was also considered.

3. Results

3.1. Water Mass Properties

The north to south transects along 178°30′E crossed two main water masses separated by a frontal boundary (see Plate 1), which are described by the temperature/salinity (T/S) properties of warm, salty ST waters; intermediate salinity (coldest) STC waters; and cold, less saline SA waters (Figure 2). The atypically cold surface waters observed at the STC were due to an intrusion of a tongue of cooler surface water (P. Sutton, unpublished data, 1998.) Macronutrient levels were generally higher in SA waters than in STC or ST waters (Table 1); however, this south to north trend was followed to a lesser degree by Si. In general, macronutrient values in austral spring were higher than those in autumn at each site. DFe levels were available for May only and ranged from 3 nmol kg^{-1} in ST waters to subnanomolar levels at station F (Table 1). Although

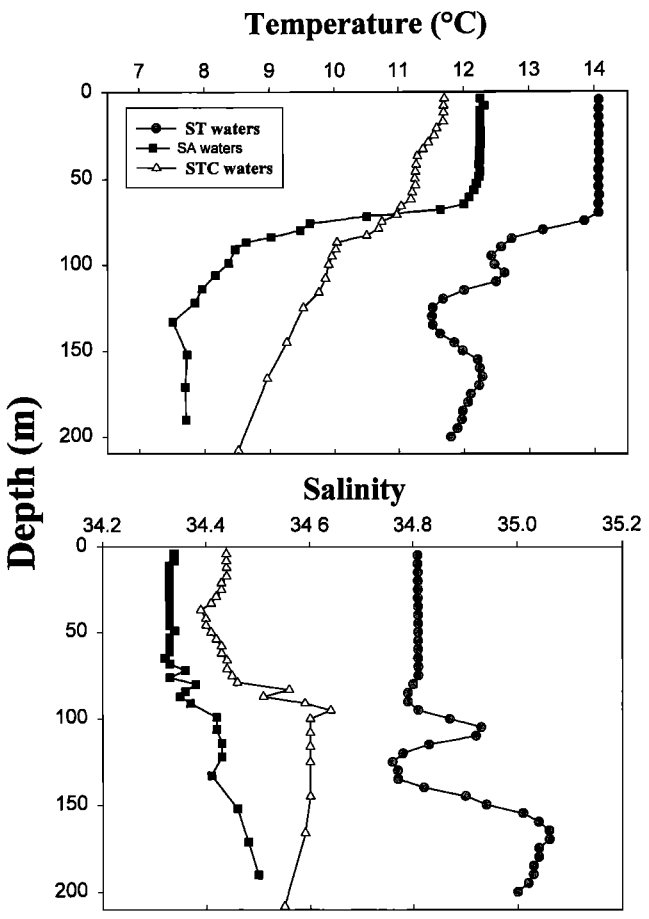

Figure 2. Upper ocean vertical profiles of temperature and salinity for Subtropical (ST) (station A), STC (station C), and Subantarctic (SA) waters (station E) in early May 1997.

DFe levels generally decreased from north to south, they were variable (0.2–1.2 nmol kg^{-1}) within the STC region (see section 4.4).

3.2. Bio-optical Properties

The MLD, arbitrarily defined as the depth (nearest the surface) where temperature first decreases by 0.1°C over a 1 m or greater depth interval, was greater in spring than in autumn for SA waters; the opposite trend was observed for ST waters (Table 2). In May, MLDs of ~60–70 m were characteristic of SA and ST waters, whereas the STC region had shallower MLDs. With the exceptions of station A1 in spring and station B in autumn, the depth of the euphotic zone (arbitrarily defined as 1% I_0) was greater than or equal to the MLD at each site. Incident PAR ranged from 7–20 mol quanta m^{-2} d^{-1} in autumn to 24–36 mol quanta m^{-2} d^{-1} during spring. Chlorophyll *a* levels in October were relatively high in ST and STC waters compared with the low and constant stocks in SA waters (Table 2). In all waters, chlorophyll *a* levels showed no evidence of subsurface features/maxima (Figures 3a–3c and Figures 4a and 4b). The photochemical efficiency of photosystem II (F_v/F_m) of resident algae was ~0.5 during spring in ST waters, higher (0.55) in the vicinity of the STC, and low (~0.3) in SA waters (Table 2).

3.3. Algal Size Structure and Production

The algal assemblage in ST waters in May was dominated by cells <2 μm (mainly cyanobacteria (J. Hall, personal communication, 1998)), which made up >50% of community biomass, whereas cells >20 μm composed ~10% (Figure 3a). This trend was also observed for the partitioning of production in ST waters (Figure 3d). Such a pattern was also observed in "ST" waters (see section 4.2) during the first 3 days at this site in October (data not shown), whereas from days 3 to 6 after a water mass change, large diatoms were present in ST waters at this site (Figure 4a). In STC waters in May, large cells (revealed by microscopy to be diatoms such as *Chaetoceros* spp.) dominated the assemblage (Figures 3b and 3e), whereas in SA waters, cells <1 μm (mainly cyanobacteria (J. Hall, personal communication, 1998)), composed 50% of algal biomass in both seasons (Figures 3c and 4b). Size-fractionated chlorophyll *a* levels varied seasonally in ST and STC waters but remained low and relatively constant in SA waters. These trends for each water mass were also apparent for the partitioning of production by size (Figures 3 and 4). In October, column-integrated production rates ranged from 350 to 650 mg C m^{-2} d^{-1} in SA waters (data not shown), whereas at the ST site, rates were >2 g C m^{-2} d^{-1} (data not shown) when diatoms dominated the assemblage.

3.4. Algal Fe Stress and Perturbation Experiments

Expression of the protein flavodoxin was used as a proxy to assess algal Fe stress (Figure 5). In May, flavodoxin was undetectable in total protein extract from ST waters suggesting that the assemblage was not Fe stressed (Figure 5a). However, because of the specificity of the flavodoxin antibody for dia-

Table 1. Surface Mixed Layer Micronutrient and Macronutrient Levels

	ST		STC		SA	
Parameter	Station A (A1)	Station B	Station C	Station D	Station E	Station F (F1)
Nitrate, μM	0.65	1.34	3.41	9.12	9.74	17.81
	(4.55)	(4.48)	(7.19)	(5.57)	(11.7)	(11.7)
Silicate, μM	1.34	1.62	1.29	1.21	1.34	3.94
	(2.02)	(1.79)	(3.98)	(2.84)	(1.63)	(3.90)
Phosphate, μM	na	0.11	0.18	0.58	0.57	1.41
	(0.38)	(0.36)	(0.75)	(0.64)	(1.31)	(1.26)
DFe, nmol kg^{-1}	3.0	1.5	1.2	0.2	1.2	0.6

Macronutrient (underway sampling, mean of four replicates, μM) and micronutrient (on-station sampling (20 m depth), no replicates, nmol kg^{-1}) levels are for May (stations A–F) and October 1997 (rows with values in parentheses; stations A1 and F1 and underway sampling between these stations). No DFe data were available for October 1997. ST, STC, and SA denote water mass; na denotes no data available.

Table 2. Summary of Bio-optical Parameters From the Water Masses Sampled Along the North-South Transect

	ST		STC		SA	
Parameter	Station A (A1)	Station B	Station C	Station D	Station E	Station F (F1)
Mixed layer depth, m	70 (60)	78	40	25	62	60 (85)
Euphotic zone depth, m	85 (42)	42	33	53	65	105 (90)
Attenuance, m^{-1}	0.05 (0.09–0.12)	0.11	0.14	0.08	0.07	0.04 (0.05–0.07)
Chlorophyll *a*, $\mu g\ L^{-1}$	0.26 (0.85)	0.57 (0.41)	0.29 (0.8)	0.57 (0.9)	0.34 (0.31)	0.20 (0.21)
F_v/F_m	(0.51 ± 0.05)	(0.45 ± 0.03)	(0.57 ± 0.13)	(0.55 ± 0.16)	(0.35 ± 0.07)	(0.28 ± 0.02)

Water masses were sampled along the N-S transect in May (stations A–F) and October 1997 (values in parentheses; A1 and F1 and underway sampling between these stations). Mixed layer depth, euphotic zone depth, and attenuance were obtained from one vertical profile at each station. For underway sampling of chlorophyll *a* and F_v/F_m, values represent the mean of >20 (1 min intervals) and 4 (10 min interval readings), respectively. The variability in chlorophyll *a* levels was <10% of the mean value presented. Error bars for fast repetition rate fluorometer (FRRF) data represent ±1 standard deviation of the mean. ST, STC, and SA denote water mass. No FRRF data (F_v/F_m) were available in May 1997.

toms, undetectable levels of flavodoxin can also be due to an underrepresentation of diatoms in the phytoplankton assemblage (see section 4.3). In the vicinity of the STC, immunological evidence supported algal Fe stress, whereas evidence for Fe stress was variable in SA waters (see section 4.3). In SA waters close to the STC (station E), there was no immunological evidence of algal Fe stress (Figure 5a). In contrast, at 46°S (station F), there was a marked flavodoxin signal and, in addition, no expression for ferredoxin (Figure 6, time $t = 0$), indicating an Fe-stressed population. In late May, when more comprehensive spatial coverage was available, antibody staining provided no evidence for algal Fe stress in ST waters, whereas evidence in support of Fe stress was obtained for both STC and SA waters (Figure 5b). Algal Fe stress was also observed in SA waters (but not in the STC region) in September and in October (Figure 5c); however, at these times, there was evidence of an Fe-stressed assemblage north of 42°S (see section 4.4) and one instance of an atypically low expression of algal Fe stress in SA waters. In early May, despite observed geographical variations in algal Fe stress, DFe levels were inversely related to the magnitude of algal Fe stress (Figure 5d).

The addition of Fe to carboys of seawater at the SA site appeared to alleviate algal Fe stress within 48 hours during May, when a switch from the initial expression of flavodoxin to ferredoxin was observed (Figure 6a). Although no switch from flavodoxin to ferredoxin was observed in the controls, neither was there sustained expression of flavodoxin. Algal species composition data, obtained from subsampling the carboys, indicated a floristic shift toward picophytoplankton in the control, whereas diatoms such as *Nitzschia* and *Chaetoceros* spp. dominated the Fe-amended carboys (data not shown). In October, Fe enrichment had a transient effect on algal Fe stress, reducing accumulation of flavodoxin by day 2 and increasing flavodoxin accumulation thereafter (Figures 6b and 6c). The reasons for variations, between experiments and the extent of the return to Fe limitation, are not presently understood. It is not possible to comment on variations in the degree of Fe stress at $t = 0$, as samples from different voyages were run on different gels.

3.5. Fe and Fe/Si Enrichments

Chlorophyll *a* levels increased in both the DFe and the DFe/Si treatments from ~0.25 $\mu g\ L^{-1}$ to >1.6 $\mu g\ L^{-1}$ (Figure 7). In the controls, chlorophyll *a* levels increased threefold (likely because of inadvertent Fe contamination). The comparable increase in chlorophyll *a* levels in both the DFe and DFe/Si treatments suggests that the presence of additional Si

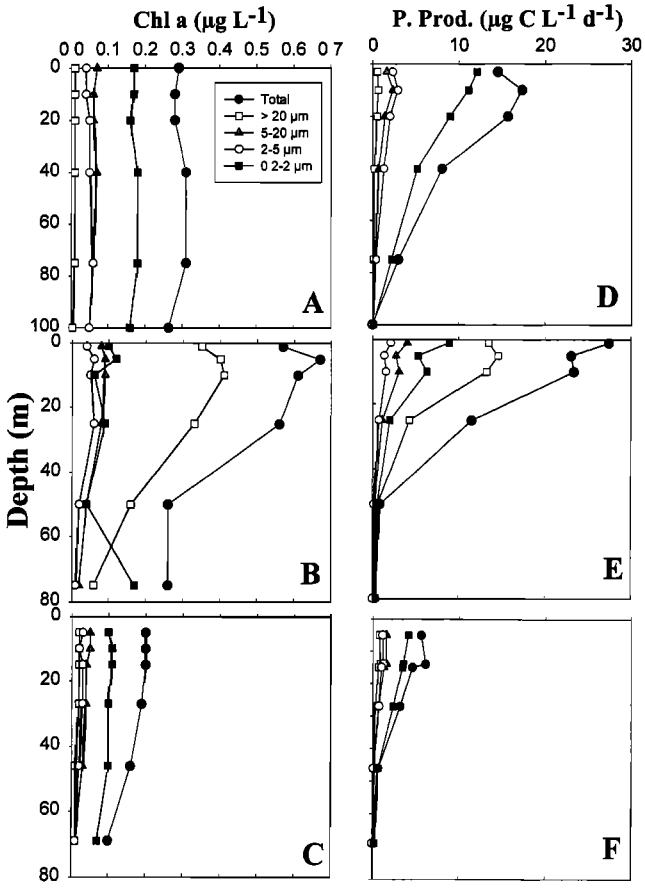

Figure 3. Upper ocean vertical profiles of the partitioning of chlorophyll *a* (four size fractions) for (a) ST (station A in Figure 1b), (b) STC (station D), and (c) SA (station F) waters in early May 1997. The partitioning of algal production is presented for these stations in (d) ST, (e) STC, and (f) SA waters. Variations in chlorophyll *a* (mean of two replicates) and primary production (mean of three replicates) were <15% and are not shown.

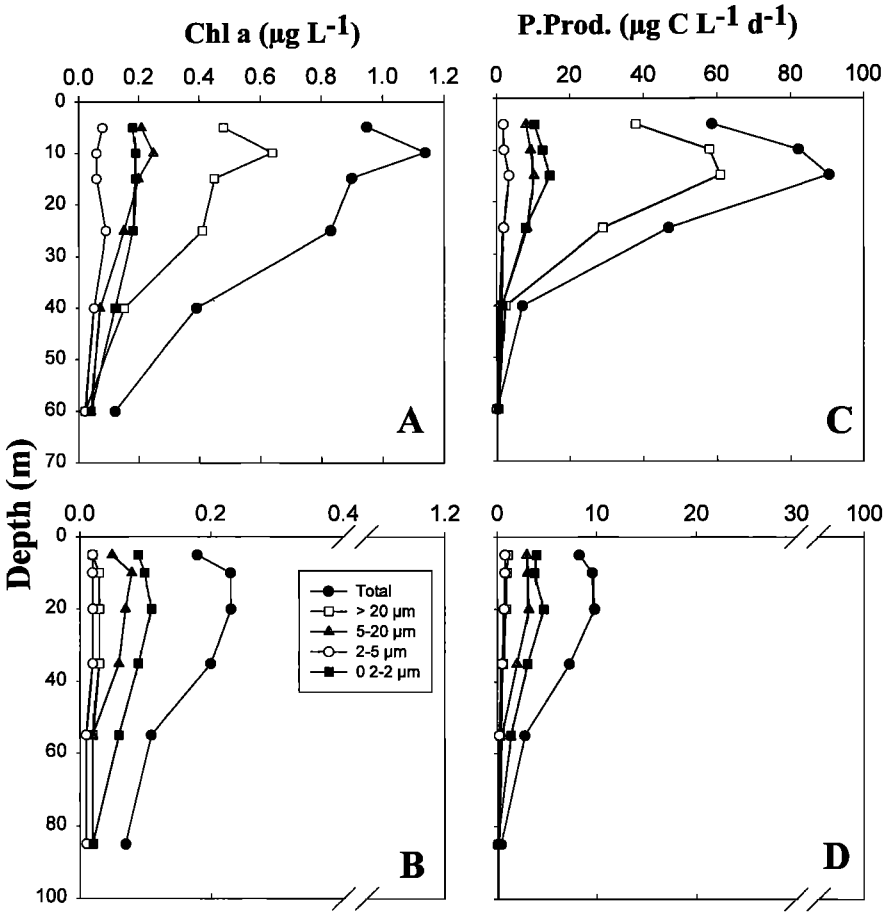

Figure 4. Upper ocean vertical profiles of the partitioning of algal biomass (four size fractions) for (a) ST (station A1 in Figure 1b) and (b) SA (station F1) waters in October 1997. The partitioning of algal production is presented for these stations in (c) ST and (d) SA waters. Variations in chlorophyll *a* (mean of two replicates) and primary production (mean of three replicates) were <10% and are not shown.

did not further enhance biomass levels. In both the DFe and the DFe/Si treatments, cells >20 µm were responsible for most of the increase in biomass (Figures 7b and 7c), whereas in the controls, cells <2 µm accounted for most of the observed increases in chlorophyll *a* (Figure 7a). Instantaneous changes in chlorophyll *a* were used as a proxy for growth rate (assuming balanced growth) and indicated that net growth of the assemblage in the Fe and Fe/Si treatments was ~0.5 d^{-1}; this compares with an algal gross growth rate, from microzooplankton dilution experiments, of 0.4 d^{-1} under ambient conditions in SA waters in spring 1993 [*James and Hall*, 1998]. The large cells (mainly diatoms, data not shown) had estimated growth rates of ~0.8 d^{-1} in both the DFe and DFe/Si treatment.

3.6. Fe/Irradiance Perturbation Experiment

This experiment was designed by first estimating the mean light levels received daily by cells and was based on the approach of *Maldonado et al.* [1999]. Their approach uses a published model [*Denman and Gargett*, 1983] to estimate the mean passage time for cells through a water column constrained with a basal density gradient in conjunction with data on incident PAR, MLD, wind speed, and water column attenuation coefficients. The estimated mean PAR level experienced by cells in SA waters (85 m MLD) in October was ~10% I_0 and was used to set two mean PAR levels (10% and 30% I_0) in the pertubation experiment. Fe-mediated increases in chlorophyll *a* over 5 days were greatest (more than fourfold) in the 30% I_0 treatment and were twofold in the 10% I_0 treatment (Figure 8). The increases in chlorophyll *a* in the 30% I_0 treatment after 5 days were 20% less than those recorded in the DFe or DFe/Si treatments (50% I_0) at this time. Increases in chlorophyll *a* in the Fe/PAR treatments were not uniform over time, and thus growth rates were not estimated. In the 30% I_0 treatment, chlorophyll *a* levels (which were not used as a proxy for algal biomass because of the possible confounding effects of photoacclimation on chlorophyll *a*) in the <2 µm fraction increased threefold over 5 days but changed little in the 10% I_0 treatment. In contrast to the DFe and DFe/Si experiments, increases in the chlorophyll *a* levels of the cells >20 µm were not observed until after day 4.

4. Discussion

4.1. Water Mass Characteristics

The T/S signature (with the exception of the surface intrusion in the STC), macronutrient, and chlorophyll *a* levels observed in each water mass were within the ranges reported previously for waters south of New Zealand [*Butler et al.*, 1992; *Bradford-Grieve et al.*, 1997, 1999]. The MLDs and attenuation coefficients for each water mass in the present study were

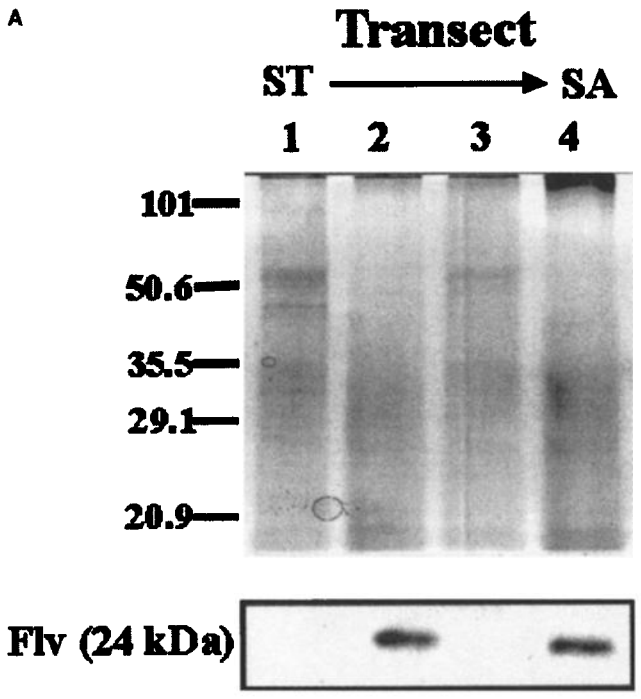

Figure 5. Expression of the algal Fe stress marker flavodoxin for natural populations of diatoms. (a) Example of flavodoxin detection in total proteins extracted from particulate samples collected during the May 1997 process cruise along a north-south transect (178°30′E) from ST to SA. Lanes 1 to 4 correspond to station A (ST(N)), station D (STC), station E (SA(N)) and station F (SA(S)), respectively. (top) The silver-stained gel, and (bottom) the corresponding western blots challenged with antiflavodoxin antisera. (b) Relative abundance of flavodoxin per unit of total protein from underway samples along 178°30′E during the late May survey. (c) Relative abundance from underway samples along the transect during the September survey (solid circles), and from the process stations A1 and F1 in late October. (Dashed lines denote the approximate position of the STC.) (d) A plot of flavodoxin relative abundance (normalized from the maximum flavodoxin abundance for each transect; flavodoxin relative abundance between gels cannot be directly compared) versus dissolved iron (DFe) levels for SA waters (from early May 1997, data from stations A, D, E, and F). Data from the NE subarctic Pacific [*LaRoche et al.*, 1996] are presented for comparison.

consistent with those recorded in 1992–1993 for each parameter [*Howard-Williams et al.*, 1995; *Hawes et al.*, 1997].

No previous data on DFe levels are available for open ocean waters SE of New Zealand; however, the suprananomolar levels in ST waters in the present study are higher than those reported by *Nakayama et al.* [1995] in ST waters in the Tasman Sea (north and NW of New Zealand) and measured by *Sedwick et al.* [1997] in ST waters south of Australia. The elevated DFe levels in STC and ST waters SE of New Zealand relative to those south of Australia may be related to the proximity of landmasses to New Zealand waters; although data are limited, DFe levels of ~6 nmol kg^{-1} have been reported in shelf waters east of the South Island of New Zealand and are thought to be supplied primarily from the resuspension of shelf sediments [*Croot*, 1994]. However, evidence of decreasing DFe/PO_4 ratios with distance offshore in these New Zealand waters may mean that offshore transport of high DFe shelf waters may not be as significant as first thought [*Croot and Hunter*, 1998]. Furthermore, the role of atmospheric deposition in the supply of Fe to SA waters SE of New Zealand is at present uncertain; *Halstead* [1996] reports no clear relationships between air mass trajectories and trace metal concentrations in this region.

Sedwick et al. [1997] measured surface layer DFe levels of ~0.4 nmol kg^{-1} in the vicinity of the STC (45°S, 140°E) and 0.2 nmol kg^{-1} in SA waters (50°S, 140°E). These values are of the same order as those observed in waters defined as SA (see section 4.4) in the present study. Despite the proximity of land to SA waters south of New Zealand, there does not appear to be a marked influence, such as an island effect, on these waters; the STC off the South Island of New Zealand, known as the Southland Current (see Figure 1), likely acts as a barrier to neritic waters.

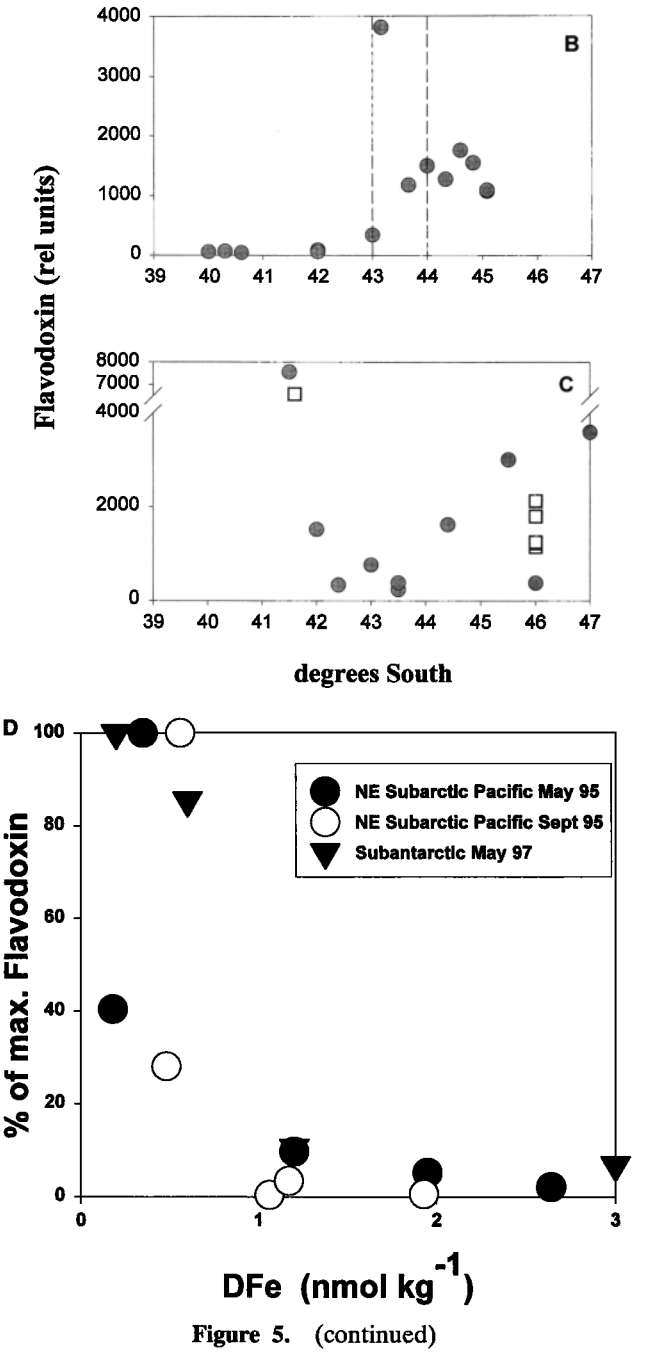

Figure 5. (continued)

4.2. Phytoplankton Biomass, Size Structure, and Production

Clear trends in the magnitude of algal biomass, the size of the dominant cells, and rates of production were observed along 171°30′E. Generally, ST waters were most variable with respect to the size structure of the assemblage. In April, when nitrate levels were probably limiting algal growth [*Eppley et al.*, 1969], the community was dominated by small cells which were probably using regenerated nutrients. In spring the observed high algal biomass and production, relatively unimpaired photochemistry (F_v/F_m) = 0.5), and a diatom-dominated community probably resulted from the availability of both high DFe and macronutrient levels.

The STC in spring was characterized by relatively high biomass with a near maximal F_v/F_m (~0.6) and a community dominated by large diatoms. In the STC region of the Atlantic sector of the Southern Ocean, *Laubscher et al.* [1993] have similarly observed a community dominated by large algal cells associated with high levels of chlorophyll *a*. SA waters had low levels of algal biomass and production, comparable to those reported by *Clementson et al.* [1998] for SA waters south of Tasmania. Cells showed evidence of nutrient stress (F_v/F_m = 0.3) and were dominated by cyanobacteria which accounted for the majority of biomass and production. These findings concur with reported biomass levels and the dominant flora in

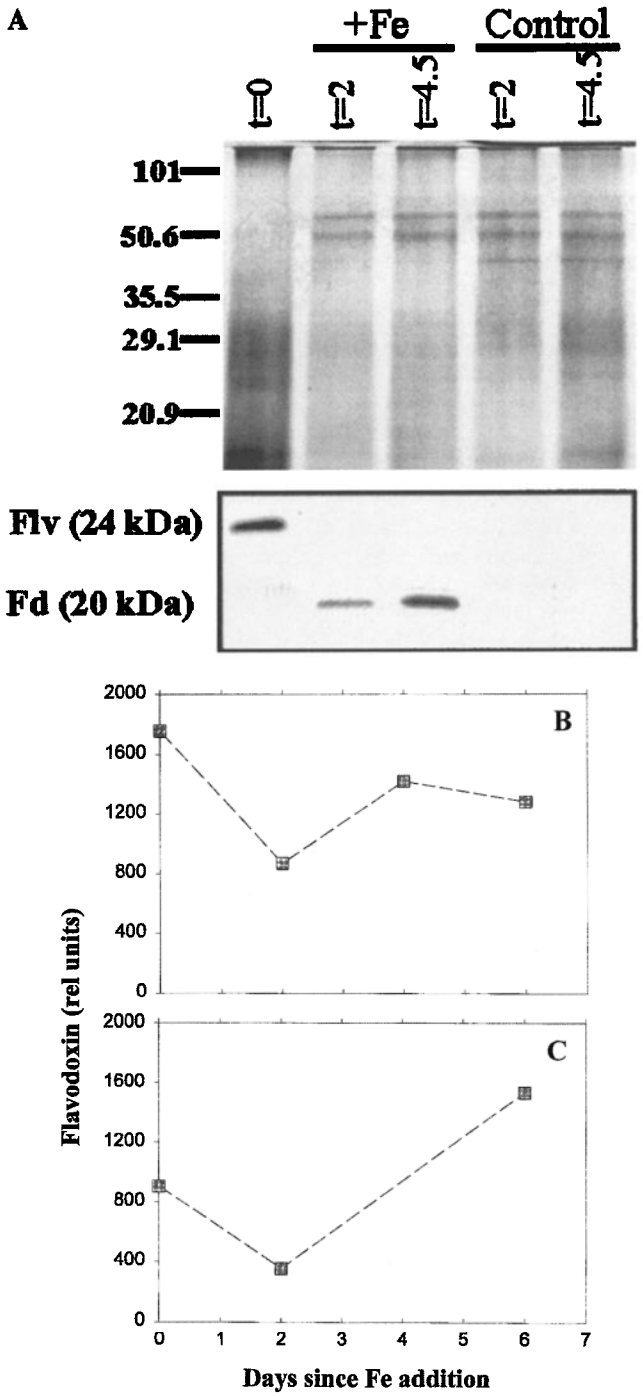

Figure 6. Time series for the relative abundance of the algal Fe stress markers flavodoxin and ferredoxin (May only) in total protein extracts during in vitro Fe enrichments. (a) Example of flavodoxin and ferredoxin detection at station F (SA waters) in early May 1997. (top) Silver-stained gel and (bottom) western blots treated with antiflavodoxin and antiferredoxin. (b) Station F1 in October 1997. (c) Station F1 during a second experiment several days later in October.

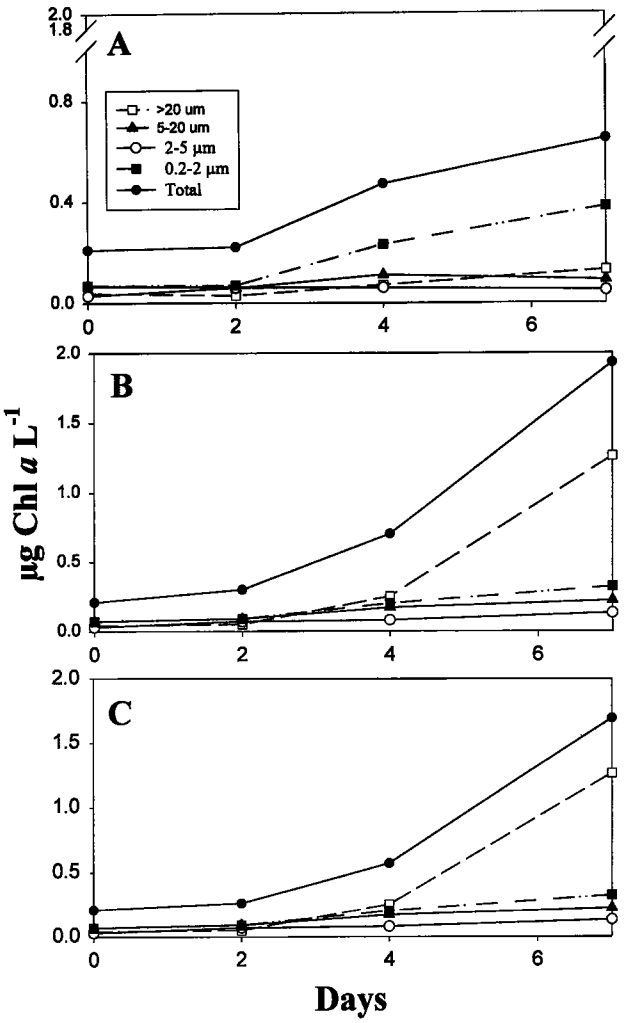

Figure 7. Time series of changes in the magnitude of chlorophyll *a* (mean of two replicates, variation was <10%) and the partitioning of chlorophyll *a* between size fractions during in vitro Fe enrichments in October at station F1 (SA waters) for the following treatments: (a) control, (b) Fe only, and (c) Fe/Si enrichment.

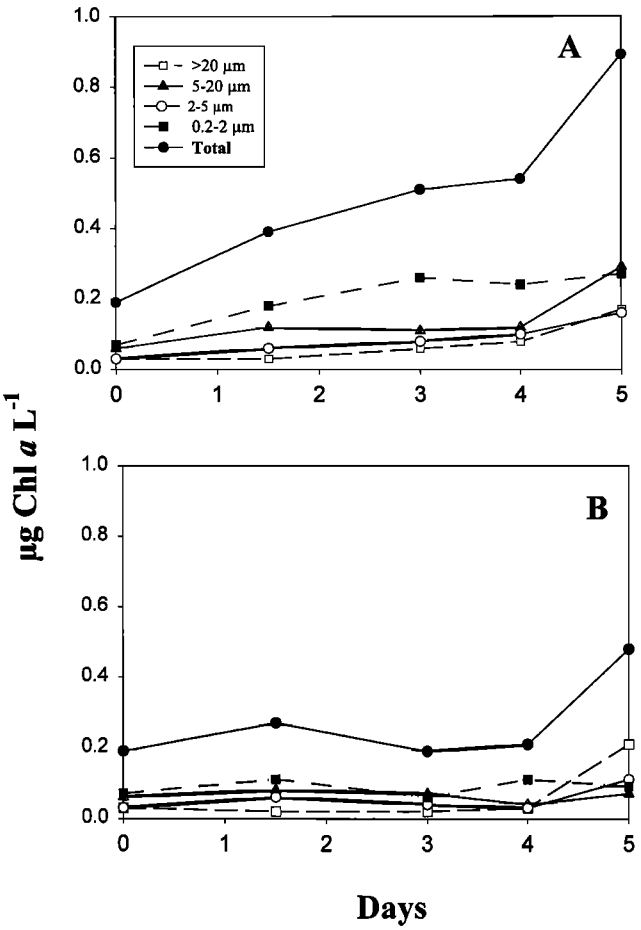

Figure 8. Time series of changes in the magnitude of chlorophyll *a* (mean of two replicates, variation was <10%) and the partitioning of chlorophyll *a* between size fractions during in vitro Fe enrichments at station F1 (SA waters) in October during the following treatments: (a) Fe enrichment at 30% I_0 and (b) Fe enrichment at 10% I_0.

SA waters [*Bradford-Grieve et al.*, 1997, 1999]. In addition, the magnitudes of chlorophyll *a* levels in the present study for SA waters agree with those derived, in SA waters, from CZCS (box 1 of *Banse and English* [1997]). Indeed, it may be possible to use the findings of the present study to further interpret observations on other SA Pacific regions (CZCS boxes 4 and 6) described by *Banse and English* [1997].

The magnitude of algal biomass in ST waters appears to be controlled by macronutrient availability, which becomes limiting after the development of the spring bloom. Conversely, in STC waters, algal biomass levels of generally >0.5 µg Chl *a* L^{-1} in both seasons and measured F_v/F_m which approach the theoretical maximum are suggestive of a micronutrient- and macronutrient-replete system over most of the year. In SA waters, low DFe levels and resultant algal Fe stress are one of the probable causes (grazing was not considered in this study) of the low seasonality in chlorophyll *a* levels relative to ST and STC waters. Other HNLC regions are characterized by low seasonality in chlorophyll *a* levels [*Landry et al.*, 1997; *Boyd and Harrison*, 1999].

4.3. Phytoplankton Fe Stress

A diatom-specific immunological probe has been previously used to detect the presence of flavodoxin in in situ samples from the NE subarctic Pacific [*LaRoche et al.*, 1996], equatorial Pacific [*LaRoche et al.*, 1995], and Polar Front [*Timmermans et al.*, 1998]. It was used here in conjunction with other hydrographic and diagnostic measurements (e.g., F_v/F_m) to gain additional insight on the iron nutritional status of the resident phytoplankton in SA waters where iron limitation has been suspected previously [*Banse*, 1996; *Sedwick et al.*, 1997]. Diatoms in ST (~10% of algal biomass) and STC (>40% of algal biomass) waters were present in greater or equal proportions to those in SA waters (~10% of algal biomass). As such, the generally observed trend of no expression of flavodoxin in ST or STC waters indicated that the assemblage was not Fe stressed, as opposed to being underrepresented by diatom biomass. Relatively high DFe concentrations and higher F_v/F_m levels for the algal community in ST and STC waters, relative to SA waters, lend support to this interpretation.

The gradients in algal Fe stress from (generally) low expression in ST waters (high DFe levels) to high expression in SA waters (low DFe levels) along 178°30′E were comparable with those noted along the line P transect in the NE subarctic Pacific (which extends east to west from high to low DFe levels [*LaRoche et al.*, 1996]). However, as might be expected, transects across a dynamic frontal boundary in the present study resulted in gradients in algal Fe stress which were less uniform that noted along line P (see section 4.4). *Banse and English* [1997] suggest that much of the Fe supply to SA waters may be derived from atmospheric sources in Australia. Clearly, more work is required on the seasonality of dust supply to SA waters and the resultant temporal-spatial impact on Fe stress of the resident phytoplankton.

The relationship between algal Fe stress and DFe levels indicated that ~1 nmol Fe kg^{-1} was the threshold, above which algal Fe stress, as indicated by flavodoxin accumulation, became markedly reduced (see Figure 5d). This was also the case in the NE subarctic Pacific [*LaRoche et al.*, 1996] and has been reported, albeit using different methods, for the equatorial Pacific [*Coale et al.*, 1996b]. The antiflavodoxin polyclonal antisera used as a probe in this study has been shown to be specific to diatoms in laboratory experiments [*LaRoche et al.*, 1995]. The flavodoxin antibody has so far shown cross-reactivity with all species of diatoms tested in the laboratory [*LaRoche et al.*, 1995, J. LaRoche and R. M. L. McKay, personal communication, 1998]. Additionally, single-cell immunofluorescence assays have demonstrated the cross-reactivity and specificity of the flavodoxin antibody to natural populations of diatoms [*LaRoche et al.*, 1996]. In contrast, the ferredoxin antibody, although specific to diatoms also, is much more variable in its response to different diatom species [*McKay et al.*, 1999]. Given the relative abundance of flavodoxin per microgram of protein extracted from total phytoplankton biomass, it is clear that the immunological results presented here must be interpreted with caution in regions where species composition shifts from diatom- to picoplankton-dominated assemblages.

4.4. Anomalous Water Mass Characteristics in ST and STC Regions?

In general, well-defined north to south gradients in algal Fe stress, micronutrient, and macronutrient levels were observed across the STC. The absence of a pronounced N-S gradient in Si is probably due to its deficit, relative to nitrate, that typifies SA waters [*Zentara and Kamykowski*, 1981]. However, some of the micronutrient and algal Fe stress data along 178°30′E appear to be anomalous, such as in May when the southern

boundary of the STC (station D) is characterized by low DFe levels and high algal Fe stress, a condition more characteristic of SA waters. Furthermore, the northern boundary of SA waters (station E) appears to be more characteristic of STC waters. The nature of the relationship between algal Fe stress and DFe levels (Figure 5d) suggests that station D is likely representative of SA waters and station E is representative of STC waters. Although underway thermosalinograph data were not available for the present study, an SST image (Plate 1) provides evidence of the heterogenity of the STC SE of New Zealand, such that the southern boundary of the STC is not well defined, with northward meanders of SA waters and southward extending filaments of STC waters. Furthermore, the difficulties in sampling in the vicinity of this dynamic region are illustrated by rapid changes in the surface water properties at station D within a 24 hour period; on May 4, waters were characterized by high chlorophyll *a* (mostly diatoms) indicative of Fe-replete conditions, whereas 24 hours later, low DFe levels and a diatom assemblage with high algal Fe stress were observed. Moreover, the algal Fe stress of cells within the STC region shows considerable spatial variability, which probably reflects the heterogenous nature of this region.

Occasionally, waters exhibiting algal Fe stress (or high picophytoplankton biomass) were observed north of the STC. Although there is no direct evidence, a likely explanation is that of the movement north of cold-core eddies of SA origin [*Chiswell and Sutton*, 1997]. Indeed, such features are persistently observed in SST images SE of New Zealand (M. J. Uddstrom and N. A. Oien, On the use of high-resolution satellite data to describe the spatial and temporal variability of sea surface temperatures, submitted to the *Journal of Geophysical Research*, 1999). *Froneman and Perissinotto* [1996] report the movement south of warm-core eddies across the STC region of the South Atlantic.

4.5. In Vitro Perturbation Experiments in SA Waters

Fe supply alleviated Fe stress in the resident cells in the October experiments, with a gradual return to Fe stress after 48 hours; this trend has also been observed in similar experiments in the NE subarctic Pacific [*LaRoche et al.*, 1996]. Fe supply also altered the magnitude of algal biomass and mediated shifts in the size structure of the community toward <2 μm and >20 μm cells in the control and Fe-amended carboys, respectively. Elevated chlorophyll *a* levels of <2 μm cells in the control (some minor Fe contamination is suspected) but not in the Fe-enriched treatments may reflect the ability of small cells to sequester Fe more efficiently [*Raven*, 1990; *Sunda and Huntsman*, 1997]. However, as <2 μm cells are subject to strong grazer pressure in SA waters [*Hall et al.*, 1999], how are they able to escape grazer control? *Banse and English* [1997] suggest that such conditions may occur in situ in SA waters if mesozooplankton abundances increase. This would result in elevated grazing pressure on microzooplankton, leading to reduced microzooplankton herbivory and, in turn, to elevated picophytoplankton biomass. The in vitro abundance of mesozooplankton in the carboys was not assessed in the present study, and thus the reason for the observed increases in picophytoplankton biomass is not presently known.

4.6. HNLSLC Condition

Although SA waters are characterized as HNLSLC, the addition of 4 μM Si did not elevate algal biomass further during the Fe and Fe/Si experiments in the present study. However, these experiments were run in spring when Si levels were close to winter reserve levels and comparable to the highest K_s (half-saturation constant of nutrient uptake) for Si reported for laboratory-cultured phytoplankton [*Paasche*, 1973]. Thus conditions in October may be better described by HNLC rather than HNLSLC conditions. In summer, Si levels in SA waters of 1 μM have been recorded [*Hawes et al.*, 1997] and thus may be limiting, i.e., less than K_s for many cultured diatom species. However, Si levels for SA waters (Table 3a), albeit from a limited data set, indicate that summertime levels vary considerably (1–4 μM) during the growth season. This trend is supported by upper ocean Si levels (<1–4 μM), measured along a World Ocean Circulation Experiment (WOCE) transect in SA waters SE of Tasmania during midsummer and late summer (S. Rintoul, unpublished data, 1997.) Thus, even in midsummer both HNLC and HNLSLC conditions may be observed.

Table 3a. Published Si Levels in the Surface Mixed Layer in Open Subantarctic Waters

Date	Silicate, μM	Source
October 1993	2.0–2.5	*Bradford-Grieve et al.* [1997]
October 1997	3.9	this study
Mid-December 1973	0.9–4	unpublished data[a]
Late February 1995	1–4	*Hawes et al.* [1997]
May 1989	1.6–2.3	*Vincent et al.* [1991]
May 1989	1.8–2.6[b]	*Butler et al.* [1992]
May 1997	3.9	this study
June–July 1993	3.2	*Bradford-Grieve et al.* [1997]

[a]Data made available by F. J. Taylor.
[b]This site was on the Campbell Plateau (see Figure 1).

Estimates of the annual Si uptake in SA waters, estimated using diatom carbon uptake rates (present study) in conjunction with published diatom Si:C ratios, suggest that 151–817 mmol Si m^{-2} yr^{-1} (Table 3b) are taken up. Assuming that the winter reserve concentration is 400 mmol Si m^{-2} (4 mmol m^{-3} in a 100 m MLD) and that summer levels are 25 mmol m^{-2} (0.5 mmol m^{-3} in a 50 m MLD), then 375 mmol m^{-2} are potentially available, depending on the affinity for Si of the resident cells, over the growth season. The lower rate is calculated after *Brzezinski* [1985], who reports that the use of this ratio to estimate Si requirements is reliable within a threefold margin of error. Thus the estimated annual Si requirement appears to be balanced by available Si. However, the upper Si uptake rate, which takes into consideration how Fe limitation may elevate Si:C uptake ratios [*Boyle*, 1998], is more than twofold higher than the Si available (advection excluded) over the annual cycle. Although a number of assumptions are made in this calculation, the outcome suggests that a marked supply of Si to surface waters is required over the year. However, if Si was supplied vertically, it would also result in upwelled DFe, which would likely permit "Fe-limited" diatoms to take up more Si.

Banse and English [1997] report evidence, from the CZCS archive, of occasional algal blooms (>1 μg chl *a* L^{-1}) in open SA waters in late summer/autumn and invoke either aerosol Fe supply (resulting in elevated diatom biomass) or a relaxation of microzooplankton herbivory (resulting in elevated biomass of <2 μm cells) to explain their onset. The chlorophyll *a* levels attained in the perturbation experiments in the present study suggest that the former is more probable. However, if this is so, how can diatoms acquire the Si needed in order to bloom in

Table 3b. Calculated Si Requirements by Algal Cells Under Ambient

Day in October 1997	>20 μm Diatom Column-Integrated Production, mmol C m^{-2} d^{-1}	>20 μm Diatom Si Uptake, mmol m^{-2} d^{-1}	<10 μm Diatom Column-Integrated Production, mmol C m^{-2} d^{-1}	<10 μm Diatom Si Uptake, mmol m^{-2} d^{-1}	Total Si Uptake, mmol m^{-2} d^{-1}	Total Si Uptake, mmol m^{-2} yr^{-1}
16	6.27	0.94	5.70	0.51	1.45	261
		(2.07)		(1.88)	(3.95)	(711)
17	8.47	1.27	5.29	0.48	1.75	315
		(2.79)		(1.75)	(4.54)	(817)
18	3.01	0.45	4.30	0.39	0.84	151
		(0.99)		(1.42)	(2.41)	(434)
19	4.08	0.61	4.08	0.37	0.98	176
		(1.34)		(1.34)	(2.69)	(484)
20	4.06	0.61	3.13	0.28	0.89	160
		(1.34)		(1.03)	(2.37)	(427)
21	4.56	0.68	3.04	0.27	0.96	173
		(1.51)		(1.00)	(2.51)	(452)
Fe enrichment (t = 5 days)	133.4	20.0	0	0	20.0	
		(30.7)			(30.67)	

Molar ratios of Si:C composition used for large diatoms of 0.15 and for small diatoms of 0.09 [*Brzezinski*, 1985]. Values in parentheses represent Si requirements estimated using Si:C ratios (Si used is particulate organic carbon produced) for Fe-deplete cells (0.33, mean of 2 values from Californian coastal upwelling regime [*Hutchins and Bruland*, 1998] and perturbed conditions (i.e., Fe enrichment, using a molar ratio of Si:C of 0.225 [*Hutchins and Bruland*, 1998]). The column-integrated production resulting from an Fe enrichment was estimated by scaling column-integrated production (chlorophyll *a*-normalized) in SA waters to chlorophyll *a* levels at t = 5 days in Figures 7b and 7c.

late summer if ambient Si levels are 1 μ*M* or less? Calculations, using data from Fe enrichment experiments (present study), suggest that large diatoms will require 20–30 mmol Si m^{-2} d^{-1}; that is, during late summer conditions this would potentially (if they have a high affinity for Si) utilize all of the mixed layer Si in 24 hours. Thus both the calculated annual algal Si uptake and the possibility of the occurrence Fe-mediated blooms in summer require that the advection of Si into surface SA waters takes place. Although there is no direct evidence, the marked spatial variability in Si levels in SA waters south of New Zealand and Australia supports this hypothesis. More research is required on the physical supply of Si to surface waters, determination of the affinity for Si of cells in SA waters, and the relationship between these factors.

It is of interest to note that the change in mixed layer Si levels over a year (advection excluded) is ~3.5 μ*M* in SA waters. This compares with 6–11 μ*M* yr^{-1} for the open NE subarctic Pacific [*Whitney and Freeland*, 1999]. This large difference in annual Si uptake between these regions is puzzling since algal biomass levels, production rates, and the contribution of large diatoms to biomass/production are comparable [*Boyd and Harrison*, 1999; this study]. In addition, the ratio of Si:NO_3 uptake should be similar as both regions are HNLC [*Takeda*, 1998].

4.7. Fe and PAR Colimitation

Most in vitro Fe enrichments [e.g., *Coale*, 1991; *Boyd et al.*, 1996] have been performed at light levels which may not necessarily mimic those experienced by cells in situ. In the present study, when cells were incubated at 10% I_0 (comparable to the mean light climate in SA waters in spring), chlorophyll *a* levels increased by twofold over 5 days in response to Fe enrichment. This result is comparable to that reported by *Maldonado et al.* [1999] for a winter deep MLD in the NE subarctic Pacific. This relatively slow increase in chlorophyll *a* in the present study, in particular for large diatoms, may explain why episodic algal blooms are observed, from the CZCS archive, only in late summer/autumn [*Banse and English*, 1997] when the MLD is relatively shallow. Analysis of hydrographic data [*Levitus and Boyer*, 1994] suggests that the MLD in SA waters south of New Zealand ranges from 150 m (midwinter) to 40 m (late summer) over the year.

5. Overview: Seasonality in the Factors Controlling Algal Biomass in SA Waters?

Variations in MLD in conjunction with seasonal changes in Si availability may result in seasonality of the environmental factor(s) controlling algal biomass (in particular, of diatoms) in SA waters. In winter/spring, light limitation, or colimitation by Fe and light, may control algal growth. Although *Banse* [1996] reports that algal pigment concentrations in summer are independent of MLD, pigments may not accurately reflect algal biomass due to photoacclimation; *Bradford-Grieve et al.* [1999] report greater than twofold changes in the C:chl *a* ratio in SA waters. With the shoaling of the mixed layer, there may be a corresponding reduction in algal cellular Fe requirements [*Raven*, 1990] such that Fe supply may control algal growth rates in late spring/early summer. During summer, if Si levels become vanishingly low, or less than the K_s of the resident cells, then Fe/Si colimitation or, indeed, Si limitation of algal growth rates may occur.

Acknowledgments. We wish to thank the officers and crew of the NIWA research vessel *Tangaroa*. Comments on the manuscript were kindly provided by J. Bradford-Grieve, P. Sutton, S. Chiswell (NIWA, New Zealand), and K. Hunter (University of Otago, New Zealand). SST image is courtesy of M. Uddstrom and P. Sutton (NIWA, New Zealand). We are grateful to the insights and suggestions offered by three anonymous reviewers, which helped to improve the manuscript. We acknowledge the assistance of D. Mackie, J. Sherrard, M. Elwood, and P. Polak (University of Otago, New Zealand) at sea and in the laboratory. S. Rintoul (CSIRO, Hobart, Australia), J. Hall, M. Hadfield (NIWA, New Zealand), and F. J. Taylor (University of Auckland, New Zealand) kindly provided unpublished observations. This work was funded by FORST (New Zealand) through the Ocean Fronts programme CO1623.

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(Received September 6, 1998; revised December 22, 1998; accepted January 5, 1999.)