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ORIGINAL PAPER

Evidence for high iron requirements of colonial *Phaeocystis antarctica* at low irradiance

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Abstract We have carried out field and laboratory experiments to examine the iron requirements of colonial *Phaeocystis antarctica* in the Ross Sea. In December 2003, we performed an iron/light-manipulation bioassay experiment in the Ross Sea polynya, using an algal assemblage dominated by colonial *Phaeocystis antarctica*, collected from surface waters with an ambient dissolved Fe concentration of ~0.4 nM. Results from this experiment suggest that *P. antarctica* growth rates were enhanced at high irradiance (~50% of incident surface irradiance) but were unaffected by iron addition, and that elevated irradiance mediated a significant decrease in cellular chlorophyll *a* content. We also conducted a laboratory iron dose–response bioassay experiment using a unialgal, non-axenic strain of colonial *P. antarctica* and low-iron (<0.2 nM) filtered seawater, both collected from the Ross Sea polynya in December 2003. By using rigorous trace-metal clean techniques, we performed this dose–response iron-addition experiment at ~0°C without using organic chelating reagents to control dissolved iron levels. At the relatively low irradiance of this experiment (~20 μE m^{-2} s^{-1}), estimated nitrate-specific growth rate as a function of dissolved iron concentration can be described by a Monod relationship, yielding a half-saturation constant with respect to growth of 0.45 nM dissolved iron. This value is relatively high compared to reported estimates for other Antarctic phytoplankton. Our results suggest that seasonal changes in the availability of both iron and light play critical roles in limiting the growth and biomass of colonial *Phaeocystis antarctica* in the Ross Sea polynya.

Keywords Iron · Light · *Phaeocystis antarctica* · Ross Sea

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Introduction

The colonial prymnesiophyte *Phaeocystis antarctica* is a keystone species on the Antarctic continental shelf, where it plays an important role in the biogeochemical cycling of carbon, sulfur and nutrient elements (Gibson et al. 1990; Smith et al. 1991; DiTullio and Smith 1995; Arrigo et al. 1999; DiTullio et al. 2000; Sweeney et al. 2000; Schoemann et al. 2005). In the polynya region of the southern Ross Sea, extensive blooms of colonial *P. antarctica* typically form during spring and early summer, then wane during the mid to late summer (Smith and

Gordon 1997; Arrigo et al. 1998; Smith et al. 2000; Mathot et al. 2001; Arrigo and van Dijken 2004). Field observations have documented a general decrease in the depth of the surface mixed layer during the growing season, from more than 100 m in early spring to less than 25 m in summer (Arrigo et al. 1998; Gordon et al. 2000; Sweeney 2003; Worthen and Arrigo 2003; Smith and Van Hilst 2003). The results of several studies suggest that this seasonal change in stratification is accompanied by a decrease in the dissolved iron concentration of surface waters, from values of around 0.5 nM or more in spring to less than 0.1 nM in late summer (Sedwick and DiTullio 1997; Fitzwater et al. 2000; Sedwick et al. 2000; Coale et al. 2005). These dissolved iron levels are low enough to limit algal community growth rates in the Ross Sea during the summer (Martin et al. 1990; Sedwick and DiTullio 1997; Sedwick et al. 2000; Coale et al. 2003). Hence it has been argued that seasonal changes in irradiance and/or iron availability—bottom-up controls—regulate the timing and location of colonial *Phaeocystis antarctica* blooms in the Ross Sea (Arrigo et al. 1998; Boyd 2002; Smith et al. 2003; Worthen and Arrigo 2003; Arrigo and Tagliabue 2005; Tagliabue and Arrigo 2005). As yet, there are few published data to support this assertion.

With regard to irradiance, the results of field and laboratory studies indicate that *P. antarctica* can readily adapt to a wide range of ambient light conditions (Palmisano et al. 1986; Matrai et al. 1995; Hong et al. 1997; Moisan and Mitchell 1999; Robinson et al. 2003; Schoemann et al. 2005). However, the field and laboratory measurements discussed by Van Hilst and Smith (2002) and Smith and Van Hilst (2003) suggest that there is little difference between the photosynthesis-irradiance characteristics of colonial *Phaeocystis antarctica* and diatoms in the Ross Sea, implying that other factors, such as iron availability and grazing, must control the spatial and temporal distribution of the two main phytoplankton taxa in this highly productive shelf region.

So far there has been only limited research addressing the iron requirements of colonial *P. antarctica*. Sedwick et al. (2000) performed a shipboard iron-addition experiment with a colonial *Phaeocystis*-dominated algal assemblage collected from the southern Ross Sea in spring 1994, with their results suggesting that the ambient dissolved iron concentrations of ~0.8 nM were sufficient to meet the growth requirements of the native *P. antarctica* colonies. Evidence for iron limitation of *Phaeocystis antarctica* was reported by Olson et al. (2000), who measured the photosynthetic competency of individual *P. antarctica* cells in the shipboard iron-addition experiments that Coale et al. (2003) performed in the Ross Sea during late summer 1997. In these experiments, Coale et al. (2003) observed an increase in the abundance of prymnesiophytes, mainly *Phaeocystis*, in response to iron addition. The dose–response experiments performed by Coale et al. (2003) in the Ross Sea allowed them to estimate half-saturation constants (with respect to growth) of 0.005–0.043 nM dissolved Fe for prymnesiophytes, suggesting that the iron requirements of *Phaeocystis* in the Ross Sea may be quite low, although their experimental results suggest similarly low values for diatoms. However, the results described by Olson et al. (2000) and Coale et al. (2003) pertain to bottle incubations performed at full surface irradiance (i.e., light levels that were probably much higher than mean surface mixed-layer irradiance), and presumably apply to solitary *Phaeocystis antarctica* cells; therefore, their results may not be representative of the colonial *Phaeocystis antarctica* that bloom in the southern Ross Sea during spring and early summer.

Here we report the results of two experiments that provide further information on the iron requirements of colonial *Phaeocystis antarctica* in the Ross Sea. The first is a shipboard iron- and light-manipulation experiment, which was performed using a native algal assemblage dominated by colonial *P. antarctica*, collected in the northern Ross Sea polynya during December 2003. The second is a laboratory dose–response iron-addition experiment in which we used a pure culture of colonial *P. antarctica* and low-iron (<0.2 nM) filtered seawater, both collected from the southern Ross Sea polynya in December 2003. To our knowledge, this experiment represents the first time a dose–response iron-addition experiment has been carried out with colonial *Phaeocystis antarctica* using realistic, sub-nanomolar

iron concentrations, and without using iron-complexing ligands such as Ethylene Diamine Tetra Acetic Acid (EDTA) to control dissolved iron concentrations in the growth medium.

Methods

Field collections

In late December 2003, we collected seawater and resident plankton from several sites in the Ross Sea polynya (Fig. 1) aboard the research vessel *Nathaniel B. Palmer*. At station A (ca. 73°24′ S, 173°14 E), in ice-free waters at the northern edge of the polynya, we used a Zodiac inflatable boat to collect surface seawater and resident plankton in an acid-cleaned 50-l polyethylene carboy. The seawater and plankton were collected by submerging an acid-cleaned 10-l polyethylene carboy from the bow of the work boat while slowly underway, then transferring the seawater from the 10-l carboy into the 50-l carboy on the work boat, using a protocol designed to avoid contamination of the seawater during the transfer process. The 50-l carboy of seawater and resident plankton were then stored for ~2 h in a shipboard cold room maintained at 2°C, prior to subsampling and processing for the shipboard iron-light manipulation experiment described below.

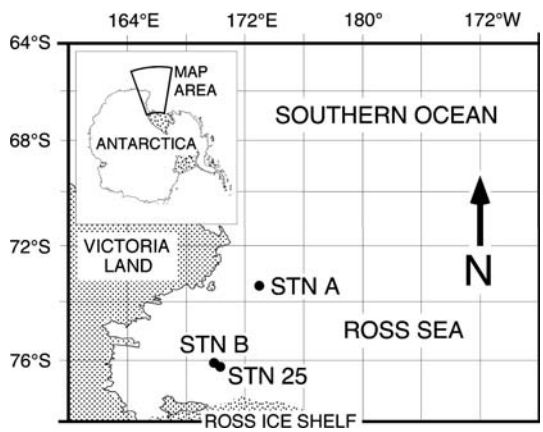

Fig. 1 Location of sampling sites in the Ross Sea, Antarctica, where seawater and plankton were collected for the experiments described in this paper. Seawater and resident plankton used in the shipboard iron-light experiment were collected at station A; uncontaminated seawater used in the laboratory iron experiment was collected at station B; the *P. antarctica* culture used in the laboratory iron experiment was isolated from water collected at station 25

Station 25 (ca. 76°05′ S, 170°08′ E), in the central polynya, was one of several stations where we collected near-surface seawater and resident plankton in 10-l Niskin bottles lowered on a rosette. Whole seawater samples from these stations were maintained in polystyrene culture flasks inside a shipboard incubator at ~0°C, prior to return to the Hollings Marine Laboratory (South Carolina, USA) for isolation of *Phaeocystis* cultures. In the nearby, ice-free waters at station B (ca. 76°02′ S, 169°53′ E), we collected ~4,000 l of seawater from the upper water column through acid-rinsed polyethylene tubing using an electrically operated Jabsco polypropylene-Santoprene double-diaphragm pump. The tubing inlet was lowered to ~10 m water depth from the side of the ship using a polyaramid line and epoxy-coated end weight, then seawater was pumped aboard while slowly underway at 1–2 knots. The pumped seawater was filtered through in-line, acid-rinsed 1-μm/0.2-μm high-capacity pleated-polypropylene cartridge filters (Cole-Parmer, Inc.), and then collected in acid-cleaned 125-l blue polyethylene barrels with airtight polyethylene screw closures. Subsequent measurements of iron in subsamples of this filtered seawater showed dissolved Fe concentrations of 0.07 ± 0.02 nM ($n = 4$). The barrels of low-iron filtered seawater (hereafter 'Station B seawater') were returned to the Hollings Marine Laboratory for use in the laboratory dose–response iron-addition experiment described below.

Shipboard iron-light manipulation experiment

Shipboard microscopy suggested that phytoplankton in seawater collected from Station A was dominated by healthy colonies of *P. antarctica*; this was later verified by high-performance liquid chromatography (HPLC) pigment analysis (see section ''Laboratory dose–response iron-addition experiment''). Using the 50-l of cleanly collected seawater and resident plankton, we carried out a bioassay experiment that was designed to evaluate the effects of iron and light availability on the growth rate of colonial *Phaeocystis antarctica*. The

seawater was gently mixed in the 50-l carboy, then transferred into acid-cleaned 1.2-l polycarbonate bottles, leaving ~200 ml headspace in each bottle, under class-100 filtered air using the rigorous trace-metal clean techniques described by Sedwick et al. (2000, 2002). Three iron treatments were used for these experimental incubation bottles: (1) 'high-Fe', to which 3 nM Fe was added as a 17.9 μM solution of ferric nitrate (the added nitrate was negligible relative to the ambient seawater nitrate concentration of ~20 μM); (2) 'control', to which there was no Fe added (initial ambient dissolved Fe concentration = 0.38 ± 0.03 nM); and (3) 'low-Fe', to which 3 nM of the iron-chelating ligand deferoxamine (DFOB or desferal) was added as a 38.4 μM solution of deferoxamine mesylate salt (Sigma Chemical).

After filling, the bottles were tightly sealed with polyvinyl chloride (PVC) tape, then set in circulating surface seawater at ~0°C in deckboard incubator baths shaded to two light levels using neutral-density screening: (1) 'low-light', shaded to ~15% of incident deckboard irradiance, and (2) 'high-light', shaded to ~50% of incident deckboard irradiance. The low-light treatment was chosen so as to fall within the range of mean in situ irradiance experienced by phytoplankton in the surface mixed layer, whereas the high-light treatment was intended to approximate the expected maximum value for mean in-situ irradiance (see discussion in section ''Conclusions and directions for future research''). Our shipboard experiment thus incubated bottles containing seawater and resident plankton with six different treatments: (1) low-light, low-iron; (2) low-light, control-iron (this treatment was intended to approximate in situ irradiance and iron concentration); (3) low-light, high-iron; (4) high-light, low-iron; (5) high-light, control-iron; and (6) high-light, high-iron. Duplicate bottles for each treatment were harvested over an 80-h period, and subsampled for dissolved macronutrients, chlorophyll *a*, phytoplankton pigments, and other parameters.

Laboratory dose–response iron-addition experiment

At the Hollings Marine Laboratory, a pure culture of colonial *P. antarctica* was isolated from the native phytoplankton assemblage collected at Station 25, and subsequently maintained in nutrient-replete semicontinuous batch cultures, using Station B seawater amended with L1 medium (Guillard and Hargraves 1993). For the dose–response iron-addition experiment described here, we used a culture of this *P. antarctica* strain (predominantly colonial) that had been maintained for two months in L1 medium diluted 457-fold with station B seawater.

Eleven days before commencing the experiment, 50 ml of this culture was diluted to 1 l with 0.2 μm-filtered station B seawater in an acid-cleaned polycarbonate bottle, using a stringent trace-metal clean protocol. A peristaltic pump was used to transfer the Station B seawater from the 125-l polyethylene barrel (in which it had been stored since collection) through acid-cleaned silicone tubing and an acid-rinsed 0.2-μm CritiCap Supor capsule filter (Pall Corporation) into the 1-l polycarbonate bottle under class-100 filtered air. Subsamples of this 0.2-μm filtered station B seawater were collected for subsequent iron measurements, which confirmed that dissolved Fe concentrations were relatively low (0.17 ± 0.1 nM, $n = 4$). Based on the concentrations of $FeCl_3$ (11.65 μM) and EDTA (11.71 μM) in the L1 medium, the resultant 1-l inoculum of colonial *P. antarctica* had initial dissolved Fe and EDTA concentrations of approximately 1.42 and 1.25 nM, respectively.

After acclimating this *P. antarctica* inoculum to an irradiance of ~20 $\mu E\ m^{-2}\ s^{-1}$ for 11 days at ~0°C, approximately 230 ml was removed for measurements of 'initial' pigment concentrations and qualitative observations, which showed the inoculum to be dominated by colonies rather than solitary cells. The remaining 770 ml of the *P. antarctica* inoculum was added to 37.8 l of station B seawater (~0.17 nM dissolved Fe) that had been filtered (0.2 μM Supor capsule filter) then chilled to ~0°C in an acid-cleaned 50-l polyethylene carboy. The calculated dissolved Fe concentration in the resulting large-volume *P. antarctica* inoculum was ~0.20 nM, which was subsequently confirmed by measurement of 0.22 ± 0.00 nM ($n = 2$) dissolved Fe in filtered subsamples, whereas the final concentration of EDTA in this solution was negligibly low

(~0.02 nM). This large-volume inoculum was gently mixed, and then used to fill 32 acid-cleaned 1.2-l polycarbonate incubation bottles, leaving 200 ml of headspace in each bottle. In addition, subsamples were taken from the 50-l carboy for measurements of initial chlorophyll *a* and dissolved macronutrient concentrations. In order to avoid inadvertent contamination with iron, the preparation of the large-volume *P. antarctica* inoculum and filling of the 1.2-l bottles were carried out using stringent trace-metal clean techniques, inside a plastic enclosure under positive pressure of class-100 filtered air; in addition, all materials contacting the station B filtered seawater and *P. antarctica* inoculums were rigorously cleaned using methods similar to those described by Sedwick et al. (2000).

Of the 32 incubation bottles, eight received no iron additions ('control treatments'), eight received an addition of 0.2 nM Fe ('+0.2 nM treatments'), eight received an addition of 0.6 nM Fe ('+0.6 nM treatments'), and eight received an addition of 1.8 nM Fe ('+1.8 nM treatments'). Iron was added as an aqueous 17.9 μM solution of ferric nitrate in 0.1% hydrochloric acid; the added nitrate and hydrochloric acid had negligible effect on the nitrate concentration and pH of the inoculum. The four iron treatments correspond to initial dissolved Fe concentrations of 0.22 nM ('control treatments'), 0.42 nM ('+0.2 nM treatments'), 0.82 nM ('+0.6 nM treatments'), and 2.02 nM ('+1.8 nM treatments'). The 32 incubation bottles were then placed in an incubator maintained at an irradiance of ~20 μE m^{-2} s^{-1} and a temperature of ~0°C. For each different iron treatment, duplicate 1-l bottles were terminally sampled after incubation periods of 16, 25 and 31 days, with subsamples taken for measurements of dissolved macronutrients, chlorophyll *a*, and other chemical and biological parameters. These sampling times were chosen by following the concentration of particulate chlorophyll *a* in dedicated monitoring bottles (one of the eight bottles prepared for each iron treatment), which were repeatedly subsampled for chlorophyll measurements during the course of the experiment. Axenic conditions were not maintained in this experiment; however, in an effort to minimize bacterial contamination, the low-iron seawater used to prepare the experimental inoculums was filtered through the 0.2-μm CritiCap Supor capsule filter immediately prior to use, and the 50-l inoculum carboy and 1-l incubation bottles were thoroughly rinsed with this filtered seawater under class-100 air before filling.

Analytical methods

Dissolved inorganic nitrate + nitrite, phosphate and silicic acid were determined in samples filtered through 0.45-μm Acrodisc Supor syringe filters (Pall Corporation), using standard flow analysis methods by the Marine Science Institute Analytical Laboratory, University of California, Santa Barbara. Chlorophyll *a* was measured by fluorometry in a 90% acetone extract of particles collected under 1–2 psi vacuum on a Whatman GF/F filter, using a Turner Designs 10-AU fluorometer and standard Joint Global Ocean Flux Study (JGOFS) protocols (Knap et al. 1996). Water samples for the analysis of iron were collected and processed using the rigorous trace-metal clean protocols described by Sedwick et al. (1997, 2000, 2005), which are required to avoid inadvertent sample contamination. Dissolved iron was determined in 0.4 μm-filtered, acidified water samples by flow injection analysis, modified after the method of Measures et al. (1995), as described by Sedwick et al. (2005). Phytoplankton pigments were measured in particles collected on Whatman GF/F filters by high-performance liquid chromatography using the methods described by DiTullio and Geesey (2002).

Interpretation of experimental results

As in previous studies that have used bioassay growout experiments to examine resource limitation of phytoplankton growth (e.g. Martin et al. 1990; De Baar et al. 1990; DiTullio et al. 1993; Fitzwater et al. 1996; Hutchins et al. 2001; Sedwick et al. 2002), we infer relative net growth rates in our incubation bottles from the accumulation of algal biomass. Because changes in light intensity and iron availability are known to effect variations in the ratio of chlorophyll *a* to cell carbon for *Phaeocystis antarctica* (Van Leeuwe and Stefels 1998; Stefels and van Leeuwe 1998;

Schoemann et al. 2005), measurements of chlorophyll *a* may not provide a reliable estimate of relative changes in cell numbers (i.e., growth rate) in our incubation bottles. In addition, there are potential problems in using particulate organic carbon (POC) to estimate changes in the cellular biomass of colonial *Phaeocystis*, as a result of the POC that resides in the colonial mucilage rather than in the *Phaeocystis* cells (Schoemann et al. 2005). Thus, for the experiments reported here, we estimate relative net growth rates from net decreases (drawdown) in dissolved nitrate + nitrite, which are assumed to be proportional to net increases in cell carbon and cell number of colonial *Phaeocystis antarctica*, assuming balanced, exponential growth.

The experiments described here are principally diagnostic in nature: where cellular biomass was significantly enhanced in bottles after resource (iron or light) amendment, relative to control (or other) treatments, we infer that algal growth rates in the control (or other) treatments were limited by a deficiency in that resource. The statistical significance of differences between mean values of parameters measured in different treatments were assessed using a two-tailed *t*-test for comparisons between two treatments, or a one-way analysis of variance (ANOVA) for comparisons between three or more treatments, at a confidence level of 95% ($P = 0.05$).

Results and discussion

Shipboard iron-light manipulation experiment

The starting seawater collected for this experiment contained high concentrations of dissolved inorganic macronutrients that were typical of surface waters in the Ross Sea during early summer: ~20 μM nitrate + nitrite, ~1.5 μM phosphate, and ~55 μM silicic acid. Analysis of iron in 0.4-μM filtered subsamples of the starting seawater using methods described by Sedwick et al. (2005) revealed an initial dissolved Fe concentration of 0.38 ± 0.03 nM ($n = 2$). Thus, our additions of DFOB to the low-iron treatments were nearly an order of magnitude higher than the ambient dissolved iron concentration, on a molar basis. Initial phytoplankton biomass was relatively high, with ~4 μg l^{-1} chlorophyll *a*. Shipboard microscopy and subsequent HPLC measurements of the molar ratios of 19′-hexanoyloxyfucoxanthin to fucoxanthin (~3.8) and chlorophyll *c3* to chlorophyll *a* (~0.3) in the starting seawater indicate that algal biomass was overwhelmingly dominated by *Phaeocystis antarctica* (DiTullio and Smith 1996; Van Leeuwe and Stefels 1998; DiTullio et al., this issue).

The results from this shipboard incubation experiment are presented in Fig. 2, which shows the concentrations of dissolved nitrate + nitrite, particulate chlorophyll *a*, and particulate 19′-hexanoyloxyfucoxanthin:fucoxanthin in the incubation bottles during the course of the 80-h experiment. There was a significant decrease (drawdown) in dissolved nitrate + nitrite in all treatments during the experiment (Fig. 2a), relative to the starting seawater, suggesting net growth and accumulation of biomass in all treatments. There was no statistically significant difference between nitrate + nitrite drawdown among the different iron treatments at a given light level, suggesting that algal community growth rates were not increased by iron addition, nor decreased by DFOB addition. However, after 80 h incubation there was a significantly lower ($P < 0.05$) concentration of nitrate + nitrite (mean = 14.6 ± 0.6 μM) in the six high-light treatments than in the six low-light treatments (mean = 16.6 ± 0.2 μM), implying higher net growth rates in the bottles that were exposed to an irradiance higher than estimated in situ levels.

The chlorophyll *a* data (Fig. 2b) also show no significant difference between the various iron treatments for a given light level after 80 h incubation, although significantly lower ($P < 0.05$) levels of chlorophyll *a* (mean = 6.1 ± 1.6 μg l^{-1}) were measured in the high-light treatments relative to concentrations in the low-light incubations (mean = 9.5 ± 0.5 μg l^{-1}). If chlorophyll *a* were to be used as a proxy for cell biomass, this result would run contrary to our conclusions based on nitrate drawdown. A likely explanation for this discrepancy is that chlorophyll *a* concentrations in the high-light treatments reflect light-driven changes in cellular chlorophyll *a* content, with cells producing less chlorophyll *a* in

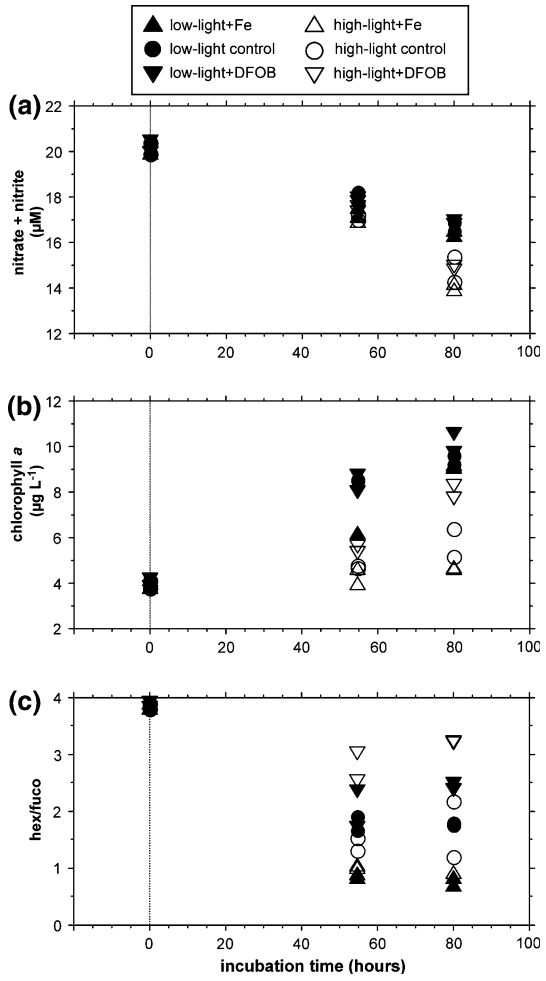

Fig. 2 Concentrations of (**a**) dissolved nitrate + nitrite, (**b**) particulate chlorophyll *a*, and (**c**) ratio particulate 19′-hexanoloxyfucoxanthin/fucoxanthin (hex/fuco) in the incubation bottles versus incubation time, from the shipboard iron-light manipulation experiment

the high-light treatments relative those incubated at low (approximately in-situ) irradiance, consistent with the experimental results of Van Leeuwe and Stefels (1998). This finding suggests that chlorophyll *a* is not a suitable proxy for cell biomass in such bioassay experiments with *P. antarctica*.

The ratio of 19′-hexanoloxyfucoxanthin (hex) to fucoxanthin (fuco) in the experimental bottles (Fig. 2c) showed a considerable range after 80 h incubation, from ~0.7 to ~3.2, with a general decrease relative to the ratio of ~3.8 in the starting seawater. In contrast to the data trends for nitrate + nitrite and chlorophyll *a*, there were no significant differences in the mean hex/fuco ratio of the low-light versus high-light treatments. However, there were statistically significant differences in the hex/fuco ratio between the high-iron, control-iron, and low-iron treatments after 80 h incubation (one-way ANOVA), with lower hex/fuco ratios observed in the Fe-amended bottles. These differences may reflect an increase in the relative abundance of diatoms following addition of iron (DiTullio and Smith 1996; Sedwick et al. 2000), and/or iron-driven changes in the hex/fuco ratio of *Phaeocystis* cells (Van Leeuwe and Stefels 1998; DiTullio et al., this issue). However, there was no significant drawdown of silicic acid in any of the treatments (data not shown), implying little growth by diatoms. This is consistent with our visual observations, and suggests that colonial *P. antarctica* dominated the algal biomass that accumulated in all incubation bottles. Thus the lower hex/fuco ratios in the Fe-amended bottles most likely reflect changes in the hex/fuco ratio of *Phaeocystis* cells, a conclusion corroborated by the results of pigment analyses from our laboratory dose–response iron-addition experiment (see next section and DiTullio et al., this issue).

Additions of nanomolar concentrations of the iron-chelating ligand DFOB to open-ocean algal assemblages have been shown to reduce the concentration of iron available to many types of phytoplankton (Wells et al. 1994; Wells 1999; Hutchins et al. 1999; Timmermans et al. 2001). However, there is evidence that some algal species can access DFOB-bound iron (Soria-Dengg and Horstman 1995; Hutchins et al. 1999; Maldonado and Price 1999; Eldridge et al. 2004; Maldonado et al. 2005). A potentially important observation from our shipboard experiment is that DFOB, when added to incubation bottles in significant excess (~10×) over dissolved iron, did not appear to limit the growth of colonial *Phaeocystis antarctica* over the course of the experiment. The majority of dissolved Fe in open-ocean surface waters is thought to be complexed by an excess of uncharacterized Fe(III)-binding organic ligands that have conditional stability constants $\left(K'_{\mathrm{FeL,Fe^{3+}}}\right)$ of around 10^{20}–10^{24} M^{-1} (Gledhill and van den Berg 1994; Rue and Bruland 1995,

1997; van den Berg, 1995; Wu and Luther 1995; Boye et al. 2005). A significant fraction of this organically complexed iron is thought to be available to phytoplankton (Rue and Bruland 1997; Hutchins et al. 1999; Maldonado and Price 1999, 2001; Boye et al. 2005; Maldonado et al. 2005). Assuming this to be the case for surface seawater at our Ross Sea station A, then the addition of 3 nM DFOB (which has a $K'_{FeL,Fe^{3+}}$ value similar to or higher than that of Fe-binding ligands in seawater; Rue and Bruland 1995; Maldonado et al. 2005) would have sequestered a significant fraction of the dissolved iron in the DFOB-Fe complex. Thus our experimental results imply that DFOB-bound iron, and potentially other organic-iron complexes, are, to some extent, biologically available to colonial *P. antarctica*. The mechanism by which *Phaeocystis* might access this DFOB-bound Fe remains unknown, but it may reflect the ability of haptophytes to produce specific Fe-binding ligands (C. Trick, personal communication, 2006).

Although the addition of DFOB in excess of dissolved iron had no clear effect on the growth rate of colonial *Phaeocystis antarctica*, the data shown in Fig. 2c suggest that DFOB addition did result in a higher hex/fuco ratio in *P. antarctica* relative to cells grown in the control treatments, presumably in response to complexation of dissolved Fe by the DFOB. This observation is consistent with experimental results reported by Van Leeuwe and Stefels (1998), and with the pigment data from our laboratory dose–response iron-addition experiment (DiTullio et al., this volume), which indicate that the hex/fuco ratio of *P. antarctica* cells may vary significantly in response to changes in the concentration and/or speciation of dissolved iron, even though dissolved iron concentrations are not low enough to limit algal growth rate. Van Leeuwe and Stefels (1998) suggest that *P. antarctica* may convert fuco to hex as a photoprotective mechanism under conditions of decreased iron availability. Thus the higher hex/fuco ratio observed in our DFOB-amended incubation bottles, relative to control samples, may reflect photoprotective adaptation to a decrease in the concentration of readily available dissolved Fe.

In summary, the results of our shipboard iron-light manipulation experiment suggest that: (1) the proximate control on the growth of the colonial *P. antarctica* at station A was irradiance, with higher growth rates observed in colonies exposed to irradiance higher than the range of estimated in-situ values; and (2) the ambient dissolved Fe levels of ~0.38 nM were sufficient to meet the growth requirements of colonial *Phaeocystis* in surface waters at this site. However, one caveat that must be considered here is the relatively short duration (~3 days) of this incubation experiment, which was imposed by our cruise schedule. Results of other shipboard incubation experiments in the Ross Sea (e.g., Martin et al. 1990; Sedwick et al. 2000; Coale et al. 2003) have shown time lags of 2 or more days between iron amendment and biological response, in terms of increases in algal biomass and drawdown in macronutrients. Thus we cannot say definitively that the growth rate of colonial *P. antarctica* collected at station A did not change in response to additions of Fe or DFOB.

Laboratory dose–response iron-addition experiment

As well as the results of the shipboard iron-light experiment described in the preceding section, a preliminary laboratory experiment that we performed using *Phaeocystis* isolated from Ross Sea station 25 provided further evidence that colonial *P. antarctica* are able to access iron from seawater in which DFOB is present in ~10× excess of dissolved iron concentration (Garcia et al., manuscript in preparation). This finding, and evidence for the ability of phytoplankton to access Fe bound by other organic ligands, including EDTA, raise concerns regarding the use of chelating compounds, such as EDTA and DFOB, to maintain low concentrations of biologically available iron in algal culture experiments (e.g., Anderson and Morel 1982; Brand et al. 1983; Sunda et al. 1991; Sunda and Huntsman 1995, 1997). Indeed, Gerringa et al. (2000) have eloquently argued against the use of EDTA or other added ligands to control dissolved inorganic Fe(III) in such experiments.

With this issue in mind, we designed the laboratory iron-addition experiment that is described in the methods section ''Laboratory dose–response iron-addition experiment'', whereby our starting culture of colonial *Phaeocystis antarctica* was successively diluted with low-iron (~0.17 nM dissolved Fe) station B seawater using stringent trace-metal clean techniques. We thus produced a unialgal culture of predominantly colonial *P. antarctica* in low-iron (~0.2 nM) seawater containing a negligible concentration of EDTA (~0.02 nM), which was then used in a dose–response iron-addition experiment (see the methods section ''Laboratory dose–response iron-addition experiment''). To our knowledge, this represents the first successful application of an iron-clean culture experiment using colonial *Phaeocystis antarctica* in a land-based laboratory. A similar method has been applied to unialgal cultures of diatoms in shipboard experiments (Timmermans et al. 2001), and, more recently, in land-based laboratory experiments (Timmermans et al. 2004).

Visual observations indicated that *Phaeocystis* biomass was dominated by nearly spherical colonies in all incubation treatments during the course of our 31-day incubation experiment. Figure 3 shows the time course of dissolved nitrate + nitrite and particulate chlorophyll *a* concentrations in the incubation bottles. After 31 days incubation, the iron-amended bottles (except for the +1.8 nM Fe treatment) displayed a statistically significant decrease (drawdown) in dissolved nitrate + nitrite relative to the control treatments (Fig. 3a), with the +0.6 nM Fe treatments achieving the greatest mean drawdown in nitrate + nitrite (6.5 μM) relative to the starting seawater. Assuming that the drawdown in nitrate + nitrite reflects net accumulation of cellular biomass in the incubation bottles (see section ''Interpretation of experimental results''), we conclude that the growth rate of colonial *P. antarctica* in the starting seawater, which contained 0.22 nM dissolved Fe, was limited by iron deficiency. The chlorophyll *a* data (Fig. 3b) also indicate Fe-driven increases in chlorophyll biomass, with the highest mean chlorophyll *a* concentration (5.3 μg l^{-1}) achieved in the +0.6 nM Fe treatment after 31 days. These chlorophyll *a* data are wholly consistent with the nitrate + nitrite results, implying that iron addition did not mediate large changes in cellular chlorophyll *a*. This result supports our assertion that changes in cellular chlorophyll *a* during the shipboard iron-light incubation experiment (see previous section) were largely driven by changes in irradiance.

Our experimental data indicate that the cellular biomass of colonial *Phaeocystis* increased significantly in response to added Fe, with the notable exception of the +1.8 nM Fe treatments, which displayed a lesser biological response than the +0.2 nM and +0.6 nM Fe treatments after 31 days incubation. This unexpected result for the +1.8 nM Fe treatment may reflect an effective solubility limit for dissolved Fe in such experiments, whereby bottles with an initial dissolved Fe concentration greater than ~1–2 nM (in the absence of EDTA) experience a significant loss of biologically available Fe during the incubation period, via precipitation of iron hydroxides (Kuma et al. 1996, 1998; Nakabayashi 2002). Following this reasoning, we suggest that the final concentration of dissolved Fe in the +1.8 nM Fe treatments was perhaps much less than the initial concentration of 2.02 nM. In support of this explanation, we note that the concentration of dissolved Fe rarely exceeds ~1–2 nM in open-ocean surface waters, even in the presence of much higher concentrations of particulate iron (e.g. see Sedwick and DiTullio 1997; Brown et al. 2005; Sedwick et al. 2005). This effective solubility limit for dissolved Fe in ocean surface waters is thought to be determined by the concentration of naturally occurring Fe(III)-binding organic ligands, which allow for dissolved Fe(III) concentrations that far exceed the solubility of iron hydroxides in organic-free seawater (Kuma et al. 1996, 1998; Wu et al. 2001; Liu and Millero 2002; Nakabayashi 2002).

We have estimated nitrate-specific net growth rates (μ_N) of *Phaeocystis* for each experimental treatment between days 25 and 31, using the mean values of nitrate + nitrite drawdown, relative to the starting seawater, at these timepoints. This calculation assumes that: (1) growth was exponential in all bottles between days 25 and 31, and (2) decreases in nitrate + nitrite were directly

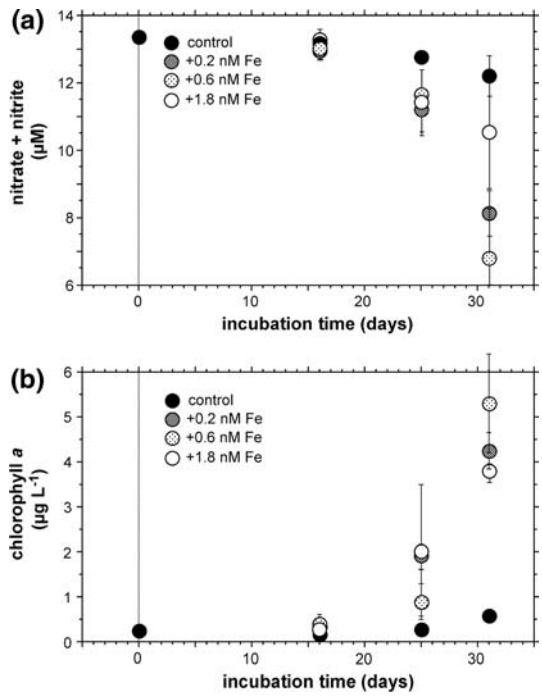

Fig. 3 Concentrations of (**a**) dissolved nitrate + nitrite, (**b**) particulate chlorophyll *a*, in the incubation bottles versus incubation time, from the laboratory dose–response iron-addition experiment. Data are plotted as the mean value of duplicate treatments, with error bars showing ± one standard deviation from the mean

proportional to net increases in the standing stock of colonial *Phaeocystis* cells (see the section ''Interpretation of experimental results''). In Fig. 4, we plot these μ_N values against the initial concentration of dissolved Fe in the incubation bottles. In other studies that have used bioassay experiments to examine the relationship between algal growth rate and dissolved Fe concentration (e.g. Coale et al. 1996, 2003; Blain et al. 2002; Timmermans et al. 2001, 2004), the experimental data have been described by a Monod (Michaelis–Menten) saturation function of the form $\mu = \mu_{max}[dFe/(K_\mu + dFe)]$, where μ is the net growth rate, dFe is the dissolved Fe concentration in the experimental treatments, μ_{max} is maximum Fe-replete growth rate, and K_μ is the half-saturation constant for growth with respect to dissolved Fe. An important assumption of these studies is that dissolved Fe concentrations do not change significantly during the course of growout experiments; as discussed above, we suggest that this assumption is not valid for our +1.8 nM Fe treatments. If we exclude results from the +1.8 nM Fe treatments on this basis, then a Monod function can be fitted to the experimental data plotted in Fig. 4.

To do this we use the Eadie–Hofstee linear transformation of the Monod hyperbolic function, which is a relatively robust approach with regard to error-prone data such as our μ_N values, because it gives equal weight to all data points (Zivin and Waud 1982). The Eadie–Hofstee plot of μ_N versus μ_N/dFe yields a least-squares best fit with a slope (=$-K_\mu$) of –0.45 nM, a *y*-intercept (=μ_{max}) of 0.33 d^{-1}, and an r^2 value of 0.85. This exercise yields a half-saturation constant for growth (K_μ) of 0.45 nM dissolved Fe for colonial *Phaeocystis antarctica* grown at an irradiance of ~20 μE m^{-2} s^{-1}. This K_μ estimate is within the range of experimental K_μ estimates that Timmermans et al. (2004) report for four species of Southern Ocean diatoms grown at an irradiance of 60 μE m^{-2} s^{-1} (K_μ = 0.19–1.14 nM Fe), but it is more than an order of magnitude higher than the K_μ values estimated for Ross Sea prymnesiophytes (presumably solitary *P. antarctica*) by Coale et al. (2003). It should be noted that the curve shown in Fig. 4 is constrained by only three data points, and that there are large uncertainties in the μ_N values calculated from the nitrate + nitrite data shown in Fig. 3a. As a result, there are large uncertainties associated with our estimates of K_μ and μ_{max}. Nonetheless, our experimental results clearly demonstrate that colonial *P. antarctica* require relatively high dissolved Fe concentrations—certainly well above 0.2 nM—to achieve maximum growth rates at the relatively low irradiance of ~20 μE m^{-2} s^{-1}.

Conclusions and directions for future research

Our experimental results must be considered in the context of seasonal changes in mean irradiance and dissolved iron concentrations for ocean surface waters in our study region. Smith and van Hilst (2003) estimate mean mixed-layer irradiances of 96 ± 58 μE m^{-2} s^{-1} and 180 ± 110 μE m^{-2} s^{-1} in the southern Ross Sea for the periods November

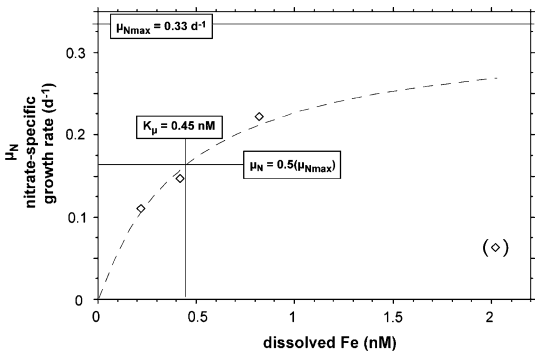

Fig. 4 Nitrate-specific growth rates (μ_N) of conlonial *Phaeocystis* in the incubation bottles between days 25 and 31, versus initial dissolved iron concentrations. The Monod hyperbola (dashed line) was fitted using an Eadie–Hofstee linear transformation ($r^2 = 0.85$), and excludes the +1.8 nM Fe datum (in parentheses). The half-saturation constant for growth (K_μ) and maximum nitrate-specific growth rate (μ_{Nmax}) are indicated

10–December 8, 1994 (mid spring–early summer) and December 20–January 15, 1995 (mid summer), respectively. Over these same periods, dissolved iron concentrations in the upper water column averaged 1.0 and 0.23 nM, respectively (Sedwick et al. 2000). It is also important to acknowledge the antagonistic relationship between the iron and light requirements of phytoplankton, whereby cellular iron requirements are expected to decrease as mean irradiance increases (Raven 1990; Sunda and Huntsman 1997). Thus, in the Ross Sea polynya, we would expect the cellular iron requirements of colonial *Phaeocystis* to decrease during the growing season, as the surface mixed-layer shoals and mean irradiance increases, and as dissolved iron concentrations decrease.

The results of our two experiments are consistent with these seasonal changes in the availability of light and iron, given the interrelated influences of irradiance and iron availability on phytoplankton growth rate. The data from our laboratory dose–response iron-addition experiment indicate a relatively high iron requirement for colonial *Phaeocystis* at an irradiance of ~20 μE m^{-2} s^{-1}, a value that is representative of the mean irradiance in the mixed layer of the southern Ross Sea during early spring (Smith et al. 2000; Smith and van Hilst 2003; Hiscock 2004). At that time, dissolved Fe concentrations are likely to exceed our estimated half-saturation constant of 0.45 nM dissolved Fe (Sedwick et al. 2000; Coale et al. 2005), thus the growth of colonial *P. antarctica* should not be strongly limited by iron availability. However, we would expect colonial *Phaeocystis* to have significantly lower iron requirements at the higher irradiance levels used in our shipboard iron-light manipulation experiment, which was conducted in late December. In the southern Ross Sea during mid summer, integrated clear-sky irradiance at the sea surface estimated from the model of Gregg and Carder (1990) is around 640 μE m^{-2} s^{-1} (Hiscock 2004), thus we estimate irradiance on the order of 100 μE m^{-2} s^{-1} and 300 μE m^{-2} s^{-1} for our low-light and high-light treatments, respectively. The expectation of lower iron requirements of *Phaeocystis* under such irradiance levels (compared with the much lower irradiance of 20 μE m^{-2} s^{-1} used in our laboratory dose–response experiment) are borne out by the results of our shipboard experiment, since we observed no evidence for growth limitation or iron stress at the ambient dissolved Fe concentration of 0.38 nM.

Boyd (2002) speculated that the growth of *Phaeocystis* in the Ross Sea may be limited by iron availability from spring through late summer, and by low irradiance from autumn through early spring, with the potential for colimitation by iron and light during spring and early fall. We propose a slightly different scenario, whereby the impact of decreasing Fe availability during the spring is mitigated by the increase in irradiance, which significantly lowers the cellular iron requirements of *Phaeocystis*, thus allowing blooms to develop through the spring and into the summer. Eventually, however, dissolved Fe concentrations decrease to such low levels as to limit the growth rate of colonial *Phaeocystis*, even under relatively high mean irradiance, and at this point the blooms are terminated. To test this conceptual model requires an understanding of the effect of irradiance on the iron requirements of colonial *P. antarctica*. Towards this goal, we are currently undertaking further dose–response iron-addition experiments with our *P. antarctica* culture at higher levels of irradiance.

With regard to quantitative simulations of the Ross Sea ecosystem, we note that recent

numerical models (Arrigo et al. 2003; Tagliabue and Arrigo 2005) have parameterized the growth of colonial *Phaeocystis* using a half-saturation constant of 0.01 nM for iron, which is more than an order of magnitude less than the K_μ value estimated from our laboratory experiment. Even allowing for a significant decrease in K_μ at the higher irradiance levels during late spring and into summer, it seems likely that the growth requirements of colonial *Phaeocystis* are closer to 0.1 nM dissolved Fe, similar to values estimated for Southern Ocean diatoms by Timmermans et al. (2001, 2004). The cellular C/Fe uptake ratio is another critical parameter for such numerical models, with a value of 450,000 (estimated from model diagnostics) used in the recent modelling study of Tagliabue and Arrigo (2005). The measurement of C/Fe uptake ratios in colonial *Phaeocystis* represents a formidable methodological challenge, because dissolved Fe is likely to be partitioned into both the *Phaeocystis* cells and surrounding mucus, and the extent to which mucus-bound Fe is available to the cells is unknown (Schoemann et al. 2005). Techniques that have been used to remove extracellular Fe for cellular Fe uptake measurements (e.g., Hudson and Morel 1989; Tovar-Sanchez et al. 2003; Tang and Morel 2006) might not be applicable to colonial *Phaeocystis*, and sophisticated analytical tools such as the synchrotron X-ray fluorescence microprobe (Twining et al. 2003) may be required to provide accurate estimates of cellular C/Fe uptake ratios.

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