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Effects of iron concentration on pigment composition in *Phaeocystis antarctica* grown at low irradiance

G. R. DiTullio · N. Garcia · S. F. Riseman · P. N. Sedwick

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Abstract Interpretation of photosynthetic pigment data using iterative programs such as CHEMTAX are widely used to examine algal community structure in the surface ocean. The accuracy of such programs relies on understanding the effects of environmental parameters on the pigment composition of taxonomically diverse algal groups. *Phaeocystis antarctica* is an important contributor to total autotrophic production and the biogeochemical cycling of carbon and sulfur in the Southern Ocean. Here we report the results of a laboratory culture experiment in which we examined the effects of ambient dissolved iron concentration on the pigment composition of colonial *P. antarctica*, using a new *P. antarctica* strain isolated from the southern Ross Sea in December 2003. Low-iron (<0.2 nM dissolved Fe) filtered Ross Sea seawater was used to prepare the growth media, thus allowing subnanomolar iron additions without the use of EDTA to control dissolved iron concentrations. The experiment was conducted at relatively low irradiance (~20 μE m^{-2} s^{-1}), with *P. antarctica* primarily present in the colonial form—conditions that are typical of the southern Ross Sea during austral spring. Relative to the iron-limited control treatments (0.22 nM dissolved Fe), iron addition mediated a decrease in the ratio of 19′-hexanoyloxyfucoxanthin to chlorophyll *a*, and an increase in the ratio of fucoxanthin to chlorophyll *a*. Our results also suggest that the ratio of 19′-hexanoyloxyfucoxanthin to chlorophyll c_3 (Hex:Chl c_3 ratio) may be a characteristic physiological indicator for the iron-nutritional status of colonial *P. antarctica*, with higher Hex:Chl c_3 ratios (>3) indicative of Fe stress. We also observed that the ratio of fucoxanthin to 19′-hexanoyloxyfucoxanthin (Fuco:Hex ratio) was highly correlated ($r^2 = 0.82$) with initial dissolved Fe concentration, with Fuco:Hex ratios <0.05 measured under iron-limited conditions (dissolved Fe <0.45 nM). Our results corroborate and extend the results of previous experimental studies, and, combined with pigment measurements from the southern Ross Sea, are consistent with the hypothesis that the interconversion of fucoxanthin and 19′-hexanoyloxyfucoxanthin by colonial *P. antarctica* is used as a photo-protective or light-harvesting mechanism, according to the availability of dissolved iron.

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

G. R. DiTullio (✉) · N. Garcia · S. F. Riseman
Hollings Marine Laboratory, College of Charleston, 331 Fort Johnson, Charleston, SC 29412, USA
e-mail: ditullioj@cofc.edu

P. N. Sedwick
Bermuda Biological Station for Research, Ferry Reach, St. George's, Bermuda GE01, Bermuda

Introduction

Phaeocystis antarctica is an important phytoplankton species in the Southern Ocean, although the relative contribution of *Phaeocystis* to total autotrophic biomass and primary production in this vast oceanic region is poorly constrained, for a number of reasons. First, *P. antarctica* has a complex heteromorphic life cycle, alternating between gelatinous colonies and free-living flagellate cells, which hampers identification using simple microscopy (Rousseau et al. 1994). Second, blooms of *P. antarctica* display pronounced temporal and spatial variability, especially near the Antarctic continental margins (El-Sayed et al. 1983; Palmisano et al. 1986). And finally, although numerous studies have focused on this species in near-surface waters, *P. antarctica* may also dominate algal biomass in the lower euphotic zone in the Southern Ocean (DiTullio et al. 2003a, b). In addition, the role of *P. antarctica* in the biogeochemical cycling of carbon, sulfur and nutrient elements in the Southern Ocean remains to be elucidated. Studies on the Antarctic continental shelf suggest that *P. antarctica* plays a significant role in the regional cycling of carbon (Schoemann et al. 2005; DiTullio et al. 2000) and sulfur (Gibson et al. 1990; DiTullio and Smith 1995), as well as impacting the stoichiometry of macronutrient cycling in Southern Ocean waters (Arrigo et al. 1999; Arrigo 2005). Thus there are compelling reasons to improve our understanding of the abundance, distribution and growth requirements of *P. antarctica*.

One of the primary methods for determining algal community structure in polar waters is based on the analysis of photosynthetic pigments by high-performance liquid chromatography (HPLC). Chemotaxonomic interpretations of HPLC data using programs such as CHEMTAX (Mackey et al. 1996) rely on the input of environmentally representative initial pigment ratios for taxonomically diverse phytoplankton groups. To apply such chemotaxonomic methods to *P. antarctica* in the Southern Ocean thus requires the use of laboratory culture studies in order to establish representative pigment ratios for this species under various growth conditions. Previous studies (e.g., Buma et al. 1991) have demonstrated that *P. antarctica* contains the two accessory pigments 19′-hexanoyloxyfucoxanthin (Hex) and fucoxanthin (Fuco), although diatoms, also common in the Southern Ocean, are also known to contain Fuco. The presence of Hex in significant concentrations relative to other photosynthetic pigments has been used to identify *P. antarctica* blooms in Antarctic waters (e.g., DiTullio and Smith 1995; Crocker et al. 1995), and a strong correlation has been observed between Hex concentrations and *P. antarctica* cell number estimated by microscopy in the southern Ross Sea (DiTullio et al. 2003b), which is dominated by *P. antarctica* blooms during the austral spring (November–December; El-Sayed et al. 1983; Arrigo et al. 1998; Smith et al. 2003).

Previous laboratory culture experiments have shown that relative concentrations of Fuco and Hex in *P. antarctica* vary as a function of ambient dissolved Fe concentration in the growth media (van Leeuwe and Stefels 1998). However, these experiments were performed under relatively high dissolved Fe concentrations (nM–μM), compared to open Antarctic surface ocean waters, and, as in most other culture studies, the synthetic organic chelating compound EDTA was used to buffer free dissolved Fe concentrations. Gerringa et al. (2000) have presented a strong argument for the need to conduct such culture experiments using realistically low dissolved iron concentrations without the use of synthetic chelating compounds such as EDTA. Following these suggestions, we have attempted to examine the effect of iron availability on the growth and pigment composition of *P. antarctica*, using culture experiments conducted at low (i.e., sub-nanonolar) dissolved iron concentrations and without using EDTA (or other chelators) to control dissolved iron levels in the growth media. These experiments were performed using a new strain of *P. antarctica*, which was isolated from native phytoplankton collected in the southern Ross Sea during December 2003. Here we report the results of an experiment that examines the effect of sub-nanomolar iron additions on the pigment composition of colonial *P. antarctica* under relatively low irradiance—conditions that are typical of the southern Ross Sea during early austral spring—and discuss the ecological

implications of our results within the context of field observations from this region.

Methods and materials

Isolation of *Phaeocystis antarctica*

Algal cultures of *P. antarctica* were isolated from native phytoplankton assemblages collected in the Ross Sea, Antarctica, during cruise NBP03-05A of the RV/IB *Nathaniel B. Palmer* in December 2003. For our experiments, we used a strain of *P. antarctica* isolated from phytoplankton collected in the polynya region of the southern Ross Sea at ca. 76°05′S, 170°08′E. The mixed field assemblage was maintained at 0°C in filtered Ross Sea seawater amended with L1 growth medium (Guillard and Hargraves 1993), and then transported to the Hollings Marine Laboratory, where a uni-algal culture of *P. antarctica* was subsequently isolated. This *P. antarctica* strain was then maintained in semicontinuous culture at 0°C, in primarily colonial form, using L1-amended filtered Ross Sea seawater as a growth medium. In addition, a large volume of 0.2 μm-filtered, low-iron (<0.2 nM dissolved Fe) seawater was collected from the same area of the southern Ross Sea in December 2003, using a trace-metal clean protocol (Sedwick et al. this issue), and then transported to the Hollings Marine Laboratory for use in our culture experiments.

Laboratory iron-addition dose-response experiment

As described by Sedwick et al. (this issue), a semicontinuous culture of the Ross Sea *P. antarctica* strain was successively diluted with low-iron (<0.2 nM dissolved Fe) filtered Ross Sea seawater, over a period of several weeks, using a rigorous trace-metal-clean protocol. Using this process, we obtained an 'inoculum culture' of colonial *P. antarctica* in L1 medium diluted 9,140-fold with filtered, low-iron (0.17 nM dissolved Fe) Ross Sea seawater, for which we calculate dissolved Fe and EDTA concentrations of 1.42 nM and 1.25 nM, respectively. Prior to commencing the experiment described here, this inoculum culture was acclimated to a constant irradiance of ~20 μE m^{-2} s^{-1} for 11 days at 0°C. The experiment was initiated by diluting 770 ml of this inoculum culture with 37.8 l of filtered, low-iron (0.17 nM dissolved Fe) Ross Sea seawater that had been chilled overnight to 0°C, and this diluted inoculum culture ('starting seawater') was then dispensed into multiple acid-cleaned 1.2 l polycarbonate bottles, using a rigorous trace-metal clean protocol. As detailed by Sedwick et al. (this issue), bottles were then amended with iron in four different treatments: control (no added Fe), +0.2 nM Fe, + 0.6 nM Fe, and + 1.8 nM Fe. These treatments correspond to initial dissolved iron concentrations of 0.22 nM (measured in the starting seawater), 0.42 nM, 0.82 nM and 2.02 nM, respectively. All bottles were then incubated at a temperature of 0°C and a constant irradiance of ~20 μE m^{-2} s^{-1}, using a refrigerated incubator fitted with cool white fluorescent lights.

For each different iron treatment, bottles were terminally sampled in duplicate or triplicate after incubation periods of 16, 25 and 31 days. Visual observations indicated that all treatments were dominated by colonial (rather than solitary) *P. antarctica* cells during the entire course of the experiment. Measurements of dissolved nitrate + nitrite, particulate chlorophyll *a* (Chl *a*) and photosynthetic efficiency in subsamples from the incubation bottles (Sedwick et al. this issue; Garcia et al. manuscript in preparation) suggest that exponential growth phase commenced at around day 16, whereas days 25 and 31 correspond to the early and late stages of the exponential growth phase. For measurements of photosynthetic pigments in the incubation cultures, duplicate 40 ml subsamples were passed through GF/F filters, which were then stored at –80°C until analysis by HPLC (see below). The low biomass observed after 16 days incubation (chlorophyll *a* concentration ~0.2 μg l^{-1}; Sedwick et al. this issue) precluded analysis of accessory pigments in the day–16 incubation bottles. Owing to low biomass in the starting seawater, initial concentrations of photosynthetic pigments were calculated from the analysis of material filtered from 40 ml subsamples of the inoculum culture

immediately prior to dilution with the low-Fe Ross Sea seawater.

Ross Sea pigment samples

Water-column samples for the analysis of phytoplankton pigments were collected during three cruises in the Ross Sea as part of the the Research on Ocean-Atmosphere Variability and Ecosystem Response in the Ross Sea (ROAVERRS) program. In this paper, we focus on samples collected aboard the RV/IB *Nathaniel B. Palmer* during the ROAVERRS III expedition in mid spring-early summer (November 11–December 11, 1998), when colonial *P. antarctica* blooms typically dominate the southern Ross Sea polynya (Arrigo et al. 1998, 1999; Smith et al. 2000, 2003). Water samples were collected using 10-l Bullister bottles attached to a conductivity-temperature-depth (CTD) rosette. From these water samples, 0.2–2.2 l aliquots were filtered under low-light conditions onto GF/F filters, quick-frozen in liquid nitrogen, and then stored at –80°C for on-shore processing and subsequent HPLC analysis (see below). The pigment measurements discussed in this paper are mean values for water-column samples collected within the 30–50 m depth range (within or below the base of the surface mixed layer), where mean in-situ irradiance is expected to be comparable to that (20 μE m^{-2} s^{-1}) used in our laboratotry experiment (Smith and Van Hilst 2003).

HPLC pigment analyses

Prior to analysis, filters were homogenized with 1.5 mL 100% acetone and extracted for 2–4 h at –20°C. Extracts were then centrifuged at 0°C, filtered, and then injected into a Hewlett Packard Series 1050 liquid chromatograph using an autosampler. The system was equipped with HP 1050 photodiode array and HP 1046A fluorescence detectors. The pigment samples from the laboratory iron-addition experiment were analyzed using a modification of the Zapata et al. (2000) method, as described in DiTullio and Geesey (2002), whereas the Ross Sea pigment samples were measured using a modification of the ammonium acetate pairing method (Wright et al. 1991) as described by DiTullio et al. (1993). HPLC pigment separation employed a Phenomenex ODS II Spherisorb C18 column for the ammonium acetate gradient method and a Waters C8 Symmetry column for the Zapata et al. (2000) gradient elution method. Purified pigment standards were isolated from uni-algal cultures. The coefficient of variation on replicate standard injections was typically <3%. Pigments were identified from relative peak retention times. In addition, visible-light absorption spectra from each eluted peak were compared to stored library spectra of purified pigment standards, to verify the identity and relative purity of eluted pigments. Pigment ratios were calculated by weight. CHEMTAX analyses were performed on the ROAVERRS III data using initial pigment ratios optimized for the Ross Sea. Those initial pigment ratios for the Ross Sea are described elsewhere (DiTullio et al. 2003b). During this austral spring cruise the phytoplankton Chl *a* biomass was dominated (>95%) by *P. antarctica* (DiTullio et al. 2003b).

Results and discussion

Hex:Chl *a* ratios

Our experimental results show that sub-nanomolar additions of dissolved Fe had a significant effect on the pigment ratios of our Ross Sea *P. antarctica* culture. The ratio of Hex to chlorophyll *a* (Hex:Chl *a* ratio) decreased from approximately 1.10 ± 0.20 under low-iron (0.22 nM dissolved Fe) conditions to 0.56 ± 0.02 under high-iron (2.02 nM dissolved Fe) conditions (Fig. 1a). The range of Hex:Chl *a* ratios observed in this experiement are consistent with the overall mean Hex:Chl *a* ratio measured in water samples collected in the Ross Sea during all three ROAVVERS expeditions (DiTullio et al. 2003b). In addition, the trend of decreasing Hex:Chl *a* ratios with increasing dissolved Fe concentration observed in our laboratory experiment is qualitatively consistent with the data trend for the ROAVERRS field samples: during the three ROAVERRS cruises, the highest Hex:Chl *a* ratio (1.08) was observed during the

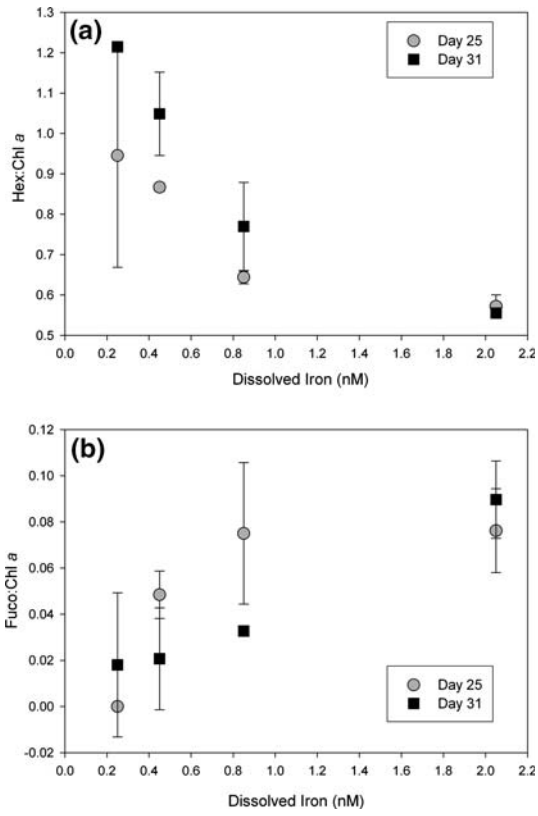

Fig. 1 (**a**) Hex:Chl *a* and (**b**) Fuco:Chl *a* ratios of Ross Sea colonial *P. antarctica* culture as a function of initial dissolved iron concentration, after incubation for 25 days (duplicate bottles sampled) and 31 days (triplicate bottles sampled) at an irradiance of 20 μE m^{-2} s^{-1}. The data points show mean values ± one standard deviation

austral summer period (DiTullio et al. 2003b), when dissolved Fe concentrations in the upper water column of the southern Ross Sea polynya are typically low (<0.2 nM; Fitzwater et al. 2000; Sedwick et al. 2000; Coale et al. 2005); whereas the lowest Hex:Chl *a* ratio (0.62) was observed in the southern Ross Sea during the early spring (DiTullio et al. 2003b), when dissolved Fe concentrations in the upper water column are typically higher than the summer values (Fitzwater et al. 2000; Sedwick et al. 2000; Coale et al. 2005). Although these field samples were collected in regions dominated by *P. antarctica* (as verified by microscopy; DiTullio et al. 2003b), the likely co-occurrence of diatoms, which will contribute to Chl *a* but not Hex to the field pigment samples, means that these field ratios represent lower limits for the Hex:Chl *a* ratio in native *P. antarctica*.

Fuco:Chl *a* ratios

The concentrations of Fuco in our Ross Sea *P. antarctica* isolate remained relatively low for all iron treatments used in our laboratory iron-addition experiment. However, the ratio of Fuco to chlorophyll *a* (Fuco:Chl *a* ratio) increased eightfold in response to iron addition, from approximately 0.01 ± 0.02 under iron-limited conditions (0.22 nM dissolved Fe) to 0.08 ± 0.02 under high-iron (2.02 nM dissolved Fe) conditions (Fig. 1b). In comparison, the mean Fuco:Chl *a* ratio in the *P. antarctica*-dominated region of the Ross Sea during the ROAVERRS expeditions was somewhat higher than our experimental values, at 0.12 ± 0.05 (DiTullio et al. 2003b). In bulk field samples, however, this pigment ratio is sensitive to the presence of diatoms, since these organisms contain Fuco but not Hex. Whereas Hex in the Ross Sea is diagnostic for the occurrence of *P. antarctica*, the presence of Fuco is not necessarily highly correlated with the presence of diatoms, since *P. antarctica* are known to produce Fuco.

Chl c_3:Chl *a* ratios

In our laboratory experiment, iron addition had mediated a decrease in the ratio of chlorophyll c_3 to chlorophyll *a* (Chl c_3:Chl *a* ratio) at day 25 (from ~0.27 at low iron to ~0.13 at high iron). This pigment ratio, however, had reached a relatively constant value of ~0.25 in all iron treatments during the late exponential growth phase (Fig. 2a). In comparison, analyses of the Chl c_3:Chl *a* ratio in *P. antarctica*-dominated regions in the Ross Sea during the three ROAVERRS cruises showed an average Chl c_3:Chl *a* ratio of 0.24 ± 0.11 (DiTullio et al. 2003b). The data trend for our laboratory experiment at day 25 are qualitatively consistent with the seasonal trend in the ROAVERRS field data: the highest mean Chl c_3:Chl *a* ratio (0.34) was observed during the summer, and the lowest mean Chl c_3:Chl *a* ratio (0.12) was observed during the spring (DiTullio et al. 2003b), when dissolved Fe concentrations

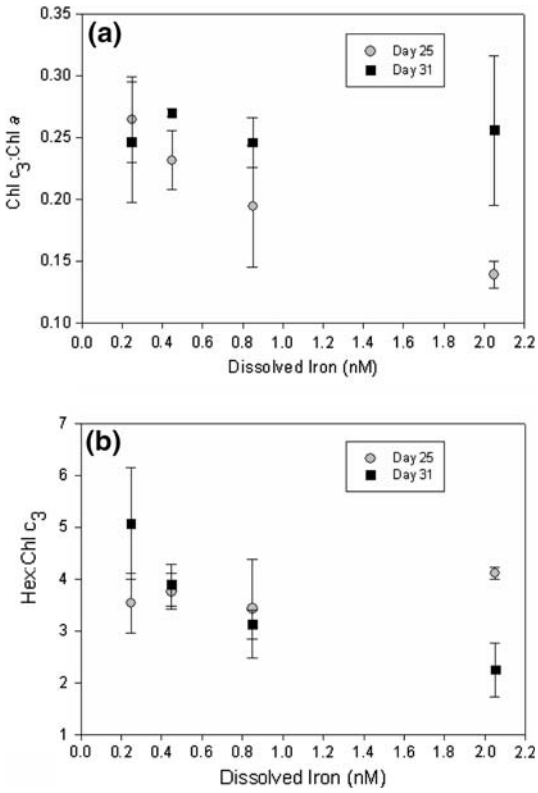

Fig. 2 (**a**) Chl c_3:Chl *a* and (**b**) Hex:Chl c_3 ratios of Ross Sea colonial *P. antarctica* culture as a function of initial dissolved iron concentration, after incubation for 25 days (duplicate bottles sampled) and 31 days (triplicate bottles sampled) at an irradiance of 20 μE m^{-2} s^{-1}. The data points show mean values ± one standard deviation

are typically low (<0.2 nM) and high (>0.2 nM), respectively (Fitzwater et al. 2000; Sedwick et al. 2000; Coale et al. 2005). Thus, in the southern Ross Sea, there may be a general seasonal increase in the Chl c_3:Chl *a* ratio of colonial *P. antarctica* that is associated with a corresponding seasonal decrease in dissolved iron availability, although these trends are not entirely consistent with our experimental results.

Hex:Chl c_3 ratios

Since diatoms and *P. antarctica* dominate the algal community in the southern Ross Sea polynya (DiTullio and Smith 1996; Arrigo et al. 1999), a potentially unique pigment signature for *P. antarctica* populations in this region is the ratio of Hex to chlorophyll c_3 (Hex:Chl c_3 ratio), because diatoms produce neither of these accessory pigments. In our laboratory iron-addition experiment, during the early exponential growth phase (i.e., day 25), the Hex:Chl c_3 ratio was relatively constant (~3.5) with respect to dissolved Fe concentration (Fig. 2b). However, by day 31, as Chl *a* biomass accumulated during the later exponential growth phase, lower Hex:Chl c_3 ratios were observed at higher dissolved Fe concentrations (Fig. 2b). Van Leeuwe and Stefels (1998) have reported a similar relationship between the Hex:Chl c_3 ratio and dissolved Fe concentration for a Weddell Sea *P. antarctica* culture grown at both high (110 μE m^{-2} s^{-1}) and low (25 μE m^{-2} s^{-1}) irradiance. Although the iron concentrations used in that study were significantly higher than those employed in our experiment, the results of van Leeuwe and Stefels (1998) are consistent with our experimental data, and there are no clear differences that might be attributed to the different *P. antarctica* strains (Weddell Sea versus Ross Sea) used in the two experimental studies.

The results of both studies indicate that Hex:Chl c_3 ratios are greater than ~3 for ambient dissolved Fe concentrations below ~1 nM, an iron concentration at which the growth rate of colonial *P. antarctica* appears to be significantly reduced at an irradiance of 20 μE m^{-2} s^{-1} (Sedwick et al. this issue). The fact that Hex:Chl c_3 ratios in the lower euphotic zone of the southern Ross Sea were ~3–5 during the ROAVERRS III spring-early summer cruise (Fig. 4d) is suggestive of the importance of iron availability in limiting the growth and biomass of colonial *P. antarctica*, as discussed by Sedwick et al. (this issue). However, we note that the experiments of van Leeuwe and Stefels (1998) also demonstrate a significant effect of irradiance on the Hex:Chl c_3 ratio of colonial *P. antarctica*, with growth under iron-replete conditions, producing Hex:Chl c_3 ratios of 0.6 at high irradiance (110 μE m^{-2} s^{-1}) and 0.06 at low irradiance (25 μE m^{-2} s^{-1}). These values, however, were approximately an order of magnitude lower compared to the high Hex:Chl c_3 ratios (3–5) observed under iron-limited conditions. Thus, it is clear that iron limitation results in significantly higher Hex:Chl c_3 ratios in *P. antarctica* compared to iron-sufficient conditions. Because these two

pigments are diagnostic pigments for *P. antarctica* in the Ross Sea, the Hex:Chl c_3 ratio may serve as a physiological indicator of iron stress for *P. antarctica* in this region. Further work comparing this ratio with dissolved iron concentrations in the region should provide a good test for this hypothesis.

Fuco:Hex ratios

The results of our laboratory experiment show that sub-nanomolar iron additions to our low-iron (0.22 nM dissolved Fe) growth medium mediated an increase in the Fuco:Chl *a* ratio and a decrease in the Hex:Chl *a* ratio of colonial *P. antarctica* at an irradiance of 20 μE m^{-2} s^{-1}. Not surprisingly, our data reveal a strong correlation (r^2 = 0.82) between the ratio of Fuco to Hex (Fuco:Hex ratio) after 25 and 31 days incubation and the initial dissolved Fe concentration of our experimental treatments (Fig. 3a). This observed increase in the Fuco:Hex ratio with dissolved iron availability appears to extend well above the range of dissolved iron concentrations used in our laboratory experiment. For instance, when grown under iron-replete conditions (~500 nM dissolved Fe, in a 20-fold seawater dilution of L1 medium) at an irradiance of 20 μE m^{-2} s^{-1}, the same strain of colonial *P. antarctica* exhibited a Fuco:Hex ratio of ~0.45 (Fig. 3b), which was around 40 times higher than the Fuco:Hex ratio (ca. 0.01) in our experimental control treatments (0.22 nM dissolved Fe). In addition, two older *P. antarctica* isolates that had been maintained under iron-replete conditions for more than 15 years displayed Fuco:Hex ratios > 1 (Fig. 3b), which is consistent with other reported data for *P. antarctica* laboratory cultures (Buma et al. 1991).

Our experimental results also corroborate the findings of van Leeuwe and Stefels (1998), who have reported an increase in the Fuco:Hex ratio of a Weddell Sea *P. antarctica* isolate with increasing iron availability, based on experiments using growth media with EDTA-buffered iron concentrations on the order of 1 nM ('iron-deplete' conditions) and 1 μM ('iron-replete' conditions). However, in contrast to this earlier work, our experimental results may be directly compared with field measurements of pigments in Antarctic surface waters. Specifically, we have used relatively low dissolved iron concentrations (~0.2–2 nM) that are typical of Antarctic surface waters during spring and summer, without using EDTA to control dissolved iron concentrations, thereby allowing iron speciation to be controlled by the natural iron-binding ligands present in Antarctic surface seawater (see discussion by

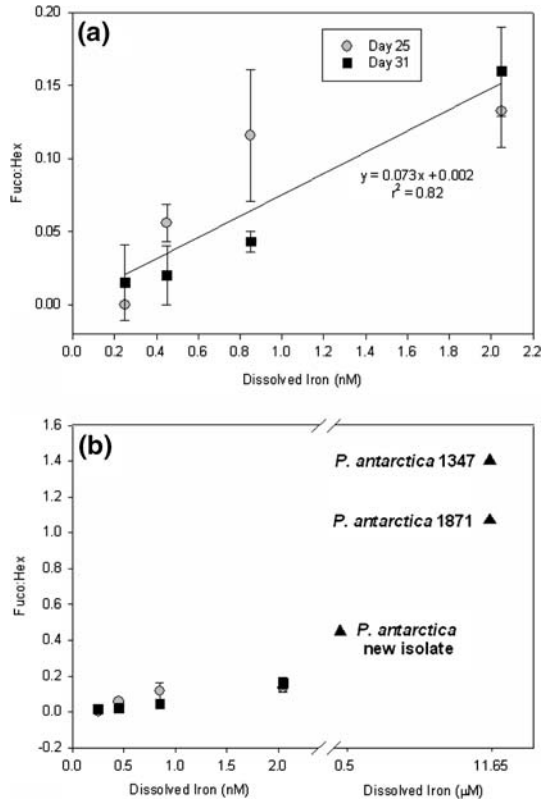

Fig. 3 (**a**) Fuco:Hex ratios of Ross Sea colonial *P. antarctica* culture as a function of initial dissolved iron concentration, after incubation for 25 days (duplicate bottles sampled) and 31 days (triplicate bottles sampled) at an irradiance of 20 μE m^{-2} s^{-1}. The data points show mean values ± one standard deviation. A linear regression using all data points yields the following relationship: $y = 0.073x + 0.002$ ($r^2 = 0.82$), where y is the Fuco:Hex ratio and x is the initial dissolved Fe concentration in nM. (**b**) Same data as figure (**a**), as well as Fuco:Hex ratios measured for two strains (1347 and 1871) of *P. antarctica* grown under iron-replete conditions (obtained from the Bigelow culture collection) and our Ross Sea *P. antarctica* culture (new strain) grown at a dissolved Fe concentration of 0.5 μM and an irradiance of 20 μE m^{-2} s^{-1}. Note the scale break on the x-axis

Gerringa et al. 2000). In addition, our experiment was conducted using a recently isolated strain of *P. antarctica*, in an effort to avoid any genetic and/or physiological biases that might arise when algal clones are maintained over a period of years under unnatural conditions (e.g., relatively high dissolved iron concentrations).

Our experimental estimates of growth rate as a function of iron concentration yielded a half-saturation constant for growth of 0.45 nM dissolved Fe for colonial *P. antarctica* at an irradiance of ~20 μE m^{-2} s^{-1} (Sedwick et al., this issue). These results imply that at this relatively low irradiance, colonial *P. antarctica* was iron-limited (sensu Morel et al. 1991) at ambient dissolved Fe concentrations < 0.45 nM (Fuco:Hex < 0.035), and was still significantly iron-stressed (sensu Morel et al. 1991) at dissolved iron concentrations of 1–2 nM (Fuco:Hex ~0.075–0.15). Thus, the mean Fuco:Hex ratio of 0.076 measured in subsurface, *P. antarctica*-dominated waters of the Ross Sea during mid spring and early summer 1998 (DiTullio et al. 2003b; Figs. 4a, b) would suggest that ambient dissolved Fe concentrations were limiting growth. Although no iron measurements were made during the ROAVERRS III cruise, the results of other studies in the southern Ross Sea suggest that dissolved Fe concentrations in the upper water column are typically subnanomolar during mid spring-early summer (Fitzwater et al. 2000; Sedwick et al. 2000; Coale et al. 2005).

When comparing our experiental data with field observations, an important caveat that must be considered is the potential effect of irradiance on the pigment composition of *P. antarctica* in relation to iron availability. The experiments of van Leeuwe and Stefels (1998) revealed relatively low Fuco:Hex ratios (ca. 0.3 and 0.7) for *P. antarctica* grown under low iron conditions at high irradiance (110 μE m^{-2} s^{-1}) and low irradiance (25 μE m^{-2} s^{-1}), respectively. Under

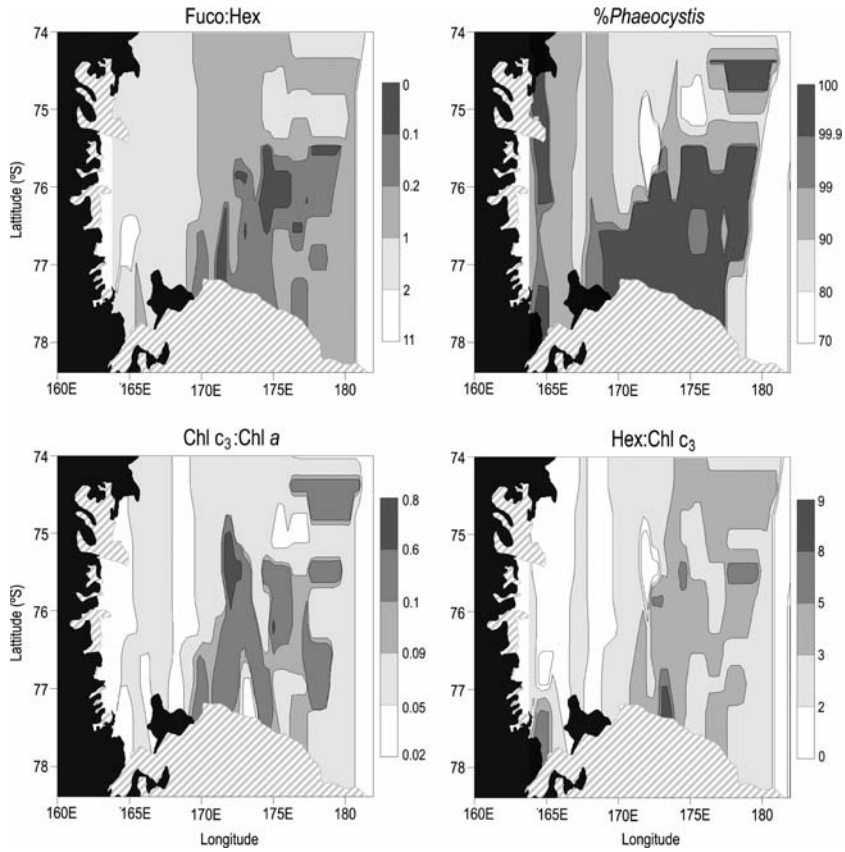

Fig. 4 Pigment ratios and *P. antarctica* abundance for 30–50 m depth in the Ross Sea during the ROAVERRS III expedition in November–December 1998: (**a**) Fuco:Hex ratios; (**b**) % *P. antarctica* as estimated by CHEMTAX analyses and corroborated by microscopy (see DiTullio et al. 2003b for details); (**c**) Chl c_3:Chl *a* ratios; and (**d**) Hex:Chl c_3 ratios

iron-replete conditions, the Fuco:Hex ratio in that study averaged 5 and 41 under high- and low-light conditions, respectively. Hence, similar to the trend in the Hex:Chl c_3 ratios, the effects of irradiance on the Fuco:Hex ratios were relatively small compared to the observed changes due to iron availability. Similarly, the results of a shipboard bioassay experiment in the Ross Sea using a *P. antarctica*-dominated assemblage suggest that the Fuco:Hex ratio of colonial *Phaeocystis* is more sensitive to changes in iron availability than to changes in irradiance (Sedwick et al., this issue). However, the relationship between iron availability and the pigment composition of *P. antarctica* may be complicated by the interrelated influence of iron and light on phytoplankton growth, whereby algal iron requirements are expected to decrease under increased irradiance (Raven 1990; Sunda and Huntsman 1997). Indeed, the results of recent culture experiments in our laboratory indicate that the half-saturation constant for the growth of colonial *P. antarctica* with respect to iron is significantly less than 0.45 nM at irradiances greater than 20 μE m^{-2} s^{-1} (Garcia et al. manuscript in preparation). This finding has potentially important implications for the pigment composition of *P. antarctica* in the southern Ross Sea, where there are seasonal decreases in dissolved iron concentration and increases in mean irradiance between the early spring and mid summer (see discussion by Sedwick et al., this issue).

Thus there is a need for further experimental work to assess the relationship between the pigment composition of *P. antarctica* and dissolved iron concentrations as a function of irradiance. To this end, we are currently undertaking the analysis of pigment samples from iron-addition culture experiments conducted at irradiances > 20 μE m^{-2} s^{-1}. In addition, there are other factors that may limit the utility of pigment ratios such as Fuco:Hex in assessing the physiological status and/or ambient growth conditions of *P. antarctica* in Antarctic waters. These factors include growth limitation/co-limitation by other resources, such as zinc (Coale et al. 2003) or vitamin B_{12} (Bertrand et al. in press); luxury uptake of iron; integrated effects of prior changes in availability of iron and/or light; physiological differences between genetically distinct *P. antarctica* ecotypes; and life-stage of *P. antarctica* (i.e., colonial versus solitary cells, each of which may have quite different growth requirements).

Physiological and ecological implications

The use of algal pigment measurements in field samples as a chemotaxonomic tool begs a mechanistic understanding of the environmental factors that impact the pigment composition of relevant phytoplankton species. With regard to understanding the distribution and ecology of *P. antarctica* in the Ross Sea and the wider Southern Ocean, it is of interest to consider the physiological basis behind the significant changes in the Hex:Chl *a*, Fuco:Chl *a*, Hex:Chl c_3 and Fuco:Hex ratios in response to sub-nanomolar iron additions that were observed in our culture experiment. These experimental trends are qualitatively consistent with the results of previous work, which has demonstrated decreases in both the cellular chlorophyll content and the Fuco:Hex ratio of colonial *P. antarctica* in response to decreased iron availability (van Leeuwe and Stefels 1998; Schoemann et al. 2005). This decrease in cellular chorophyll is readily explained by the involvement of iron in the synthesis of chlorophyll (Greene et al. 1992; Schoemann et al. 2005). With regard to the decrease in the Fuco:Hex ratio, van Leeuwe and Stefels (1998) have proposed that this reflects the conversion of Fuco to Hex, which acts as a photoprotective mechanism under conditions of low iron availability, when iron-deficient cells are more susceptible to photo-damage. Field measurements from the ROAVERRS cruises provide some support for this hypothesis, in that the Hex:Chl *a* ratio in *P. antarctica*-dominated waters was highest during the summer, when dissolved Fe levels are typically low and mean mixed-layer irradiance is relatively high (see discussion by Sedwick et al. this issue). Conversely, under conditions of high iron availability, Hex may be converted to the more-efficient light-harvesting pigment Fuco, in order to maximize the capture of light energy by the iron-replete cells (van Leeuwe and Stefels 1998). There is evidence for

this in the observed increase in the Fuco:Hex ratio of our *P. antarctica* cultures with increasing dissolved iron concentration, with the trend extending to dissolved Fe concentrations as high as 0.5 μM (Fig. 3b).

At present, it is not clear whether the Ross Sea *P. antarctica* strain used in our experiments is genetically distinct from other *P. antarctica* strains, particularly those isolated from open-ocean surface waters of the Antarctic circumpolar current (ACC), where dissolved iron levels are likely to be low (<0.5 nM) year-round (Martin et al. 1990; Coale et al. 2005), and mean summer mixed-layer depths are typically greater (hence mean irradiance lower) than those over the Antarctic continental shelf (Trull et al. 2001). It may be that our experimental data are readily applicable to *P. antarctica* in these ACC waters, given that our experiment was conducted using sub-nanomolar iron additions and relatively low irradiance. On the other hand, long-term adaptation of open-ocean *P. antarctica* strains to the iron and light regimes of the ACC, which differ significantly from those in the southern Ross Sea, may limit the application of our experimental results to *P. antarctica* ecotypes from the Ross Sea/Antarctic shelf region. Ongoing molecular studies of relevant *P. antarctica* isolates, as well as further experimental work using open-ocean *P. antarctica* strains, are expected to provide answers to these questions.

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