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Size-fractionated photosynthesis/irradiance relationships during *Phaeocystis antarctica*-dominated blooms in the Ross Sea, Antarctica

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In the Ross Sea, there are two major phytoplankton functional groups: diatoms and prymnesiophytes (dominated by Phaeocystis antarctica). Phaeocystis antarctica often occurs in colonial form, but also as solitary cells, and the two forms have distinct ecological roles. A comparison of the growth characteristics of solitary and colonial forms of Phaeocystis sp. is essential to understanding the differential impact each has on biogeochemical and ecological processes, and to allow parameterization of each form in numerical models. We measured the biomass and photosynthetic responses of two size fractions (>20 and <20 μm), representing colonies and solitary cells, at locations dominated by P. antarctica to assess the relative photosynthetic potential of the two forms. While the relative contribution of each form to total P. antarctica biomass differed among years, there were no significant differences between maximum photosynthetic rates of colonial and solitary forms within years. Substantial interannual variations in biomass and maximum photosynthetic rates normalized to chlorophyll a (P_m^B) and initial light-limited rates of photosynthesis (α) were observed among years for the colonial fractions; however, interannual variations in maximum rates of photosynthesis or α of solitary cells were not observed. A laboratory experiment with P. antarctica, together with field data, showed that growth stage of colonies strongly affected the maximum photosynthetic rates. Under nutrient-replete conditions and exponential growth, colonial cells had higher maximum photosynthetic rates than solitary cells, but as growth rate declined and senescence began, the solitary cells' rates became greater. This may be a reason for the high abundance of colonies that is often found in the Ross Sea during austral spring. Our results suggest that photosynthetic rates may influence the composition of the morphotypes of Phaeocystis, but do not appear to be the sole factor in regulating this critical biological variable.

INTRODUCTION

Waters of the Southern Ocean have a disproportionate effect on the global carbon and sulfur cycles (Sarmiento *et al.*, 2000), and because the composition of phytoplankton regulates energy flow within food webs and material flow in biogeochemical cycles (Boyd and Newton, 1999), knowledge of the impact of assemblage structure is especially critical there. The Ross Sea has a relatively predictable phytoplankton bloom due to the physical properties of annual sea ice retreat, polynya formation and water column stratification (Arrigo *et al.*, 1999; Smith *et al.*, 2006). The waters have a narrow range of environmental variables and are known to

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have a relatively restricted diversity of functional groups (Arrigo *et al.*, 1999). Specifically, there are two major functional phytoplankton groups: diatoms and prymnesiophytes, with the latter being dominated by *Phaeocystis antarctica* (Arrigo *et al.*, 1999; Smith and Asper, 2001). *Phaeocystis antarctica* has two morphotypes (Rousseau *et al.*, 1994): a colonial form and a solitary, single-celled form. Colonies are distinguished by being large (up to 2 mm), relatively unavailable to grazers (Caron *et al.*, 2000), and are major components of blooms in the Ross Sea (Arrigo *et al.*, 1999); conversely, solitary cells are small (ca. 5 μm), and a component of the microbial food web (Smith *et al.*, 2003), and can represent more than 50% of the total *P. antarctica* numbers during summer (Mathot *et al.*, 2000). Understanding the differences between the two forms is essential to understanding their spatial and temporal distributions in the Southern Ocean.

Three species of *Phaeocystis*, including *P. antarctica*, exist as single, flagellated cells or as non-flagellated cells in colonies (Rousseau *et al.*, 1994; Schoemann *et al.*, 2005). There are several life stages in which a motile cell with a flagellum can develop into a hollow, spherical colony with a diameter >2 mm and with active division of the cells within the matrix (Mathot *et al.*, 2000). In all assemblages of *Phaeocystis* in nature, both solitary and colonial forms co-exist, and in some cases one dominates over the other. For example, Mathot *et al.* (Mathot *et al.* 2000) found that spatial and temporal trends in relative abundance occurred between solitary and colonial cells in the southern Ross Sea. Most *P. antarctica* cells were associated with colonies during the austral spring through the time of maximum biomass (mid-December), and thereafter the number of solitary cells increased. Wassmann *et al.* (Wassmann *et al.*, 2005) found that single cells dominated at most times in Norwegian fjords, as well as in the White and Barents Seas. Indeed, they found numerous blooms in which colonies were nearly absent. Smith *et al.* (Smith *et al.*, 2003) investigated the percentage of cells associated with colonies in 1996–97, and found that <10% were colonial in late October, but more than 98% of the cells were in colonies at the time of the maximum chlorophyll concentration (mid-December), although this percentage subsequently declined to ~50% in colonies by February. Integrated over the entire growing season, solitary cells contributed 33% of the total *P. antarctica* abundance. Smith *et al.* (Smith *et al.*, 2003) suggested that the abundance of solitary cells might be controlled by grazing microzooplankton and heterotrophic flagellates, whereas the growth and abundance of colonial cells may be controlled by iron. Although evidence is contradictory about the environmental factors that control colony formation and subsequent release of solitary cells from colonies of *P. antarctica*, inorganic nutrient concentrations, micronutrients, grazing and irradiance all have been suggested to influence the form of *Phaeocystis* sp. (Verity *et al.*, 1988; Smith *et al.*, 2003; Tang *et al.*, 2008).

Mathot *et al.* (Mathot *et al.*, 2000) also found that cell size and carbon content differed between the solitary and colonial *P. antarctica* cells, with solitary cells being smaller and having only 25% of the cellular carbon of colonial cells. Veldhuis *et al.* (Veldhuis *et al.*, 2005) found that colonial cells had much higher growth rates than did solitary cells of *P. globosa* and *P. pouchetii* (up to 3.8-times greater), but Perperzak *et al.* (Peperzak *et al.*, 2000) found only a 10% difference. No data exist on the relative growth rates of *P. antarctica* colonial and solitary cells; similarly, no data are available on the morphotypes' relative rates of photosynthesis and nutrient uptake. Specific metabolic rates and phytoplankton cell size have a strong relationship (Banse, 1976), so these observations of larger colonies growing more rapidly are unexpected. Lancelot and Mathot (Lancelot and Mathot, 1985) found that the mucous envelope formed could act as a reserve for the cells and was reabsorbed during darkness by colonial cells of *P. pouchetii*, providing a potential means of enhanced growth and survival of colonies and colonial cells.

It has been suggested that this relatively restricted phytoplankton assemblage in the Ross Sea results from a differential photosynthetic response of the two groups, with *P. antarctica* being able to more effectively photosynthesize (and presumably grow) at lower irradiances than diatoms (Arrigo *et al.*, 1999). Arrigo *et al.* (Arrigo *et al.*, 1999) and Smith and Asper (Smith and Asper, 2001) found that *P. antarctica* abundance was greater in deeper mixed layers (and hence experienced lower irradiances). However, van Hilst and Smith (van Hilst and Smith, 2002) found no statistically significant differences in the measured photosynthetic parameters between the two groups, and concluded that other factors were also important in generating the spatial differentiation. They suggested that iron might play an important role, as it is reduced to extremely low concentrations by biological removal during the austral spring and summer. Iron has been shown to limit phytoplankton growth in summer within the Ross Sea (Sedwick and DiTullio, 1997; Sedwick *et al.*, 2000; Olson *et al.*, 2000), and it has been speculated that variations in input of iron might give rise to spatial variations in assemblage composition (Sedwick *et al.*, 2000). Colonial *P. antarctica* has been shown to have a greater iron requirement than diatoms (Sedwick *et al.*, 2007), and it has been suggested that small cells have an advantage over large cells and

colonies by virtue of their increased cell surface:volume ratios (Sunda and Huntsman, 1997; Raven, 1998).

The goals of this study were to assess the relative photosynthetic potential of solitary and colonial *P. antarctica* cells from both *in situ* phytoplankton assemblages as well as cultures. Although there have been studies on how *P. antarctica* and other phytoplankton groups compare with respect to photosynthesis, it is not known how single and colonial cells differ. It has been suggested that *P. globosa* and *P. pouchetii* colonial cells have much greater rates of growth than do solitary cells, but these results were confounded by variations of chlorophyll within cells (Veldhuis *et al.*, 2005). Chlorophyll-specific photosynthetic measurements of solitary and colonial forms should help characterize the physiological acclimation and shifts in size-specific phytoplankton carbon fixation rates. Since light-limited rates of photosynthesis are affected by physiological differences, a comparison between solitary and colonial forms will provide information on which form would perform better under lower irradiances. Similarly, light-saturated rates of photosynthesis are affected by temperature and nutrients, so differences among forms might clarify strategies used in stratified or high irradiance environments. Such a comparison will elucidate the potential physiological differences between forms that ultimately may be a factor in controlling the relative abundance of colonies and solitary cells in the environment.

METHOD

Study site and field measurements

Water samples were collected from the southern Ross Sea as part of the Interannual Variations in the Ross Sea (IVARS) program conducted from 2001 to 2006 (Fig. 1; Smith *et al.*, 2006). Photosynthesis/irradiance (P/E) measurements were completed in November–December during the period of maximum biomass. Additional data were collected during cruise NBP06-08 to the southern Ross Sea in November–December 2006. The euphotic zone was sampled using a SeaBird 911 + CTD/rosette system from which samples for nutrients, biomass and rate determinations were collected. The depth of the euphotic zone was determined from a BioSpherical Instruments PAR sensor mounted on the rosette, and at least seven depths were sampled within the euphotic zone. Chlorophyll *a* was determined by fluorescence after filtering the samples through Whatman GF/F filters and extracting in 90% acetone for 24 h at −20°C (Smith *et al.*, 2006). Chlorophyll extracts were read on a Turner Designs Model 10AU fluorometer that had been calibrated with a known concentration of commercially purified chlorophyll *a* (Sigma Chemical). Samples for HPLC pigments, particulate organic carbon/nitrogen concentrations, biogenic silica concentrations, and samples for phytoplankton abundance were also collected (Smith *et al.*, 2006). Phytoplankton samples were preserved in parformaldehyde solution (1–2% final concentration) and were stored in amber glass bottles at 4°C until cells could be stained with acridine orange (1% final concentration). A subset of the samples (1–20 mL) was filtered onto a black 0.8 μm polycarbonate filter which was then mounted onto a slide for epifluoresence microscopy. Thirty fields of view and 100–200 cells were counted per slide (J. Peloquin, Switzerland, personal communication).

Separate samples of natural phytoplankton assemblages were size-fractionated by filtering from 50–250 mL through 20 μm polycarbonate filters (Poretics). The material retained on the filter was assumed to represent colonial cells for *P. antarctica* dominated assemblages, as the size of colonies is substantially >20 μm (from 50 to 2000 μm; Mathot *et al.*, 2000). An independent study found that only 0.3% of the total *Phaeocystis* sp. colonial fraction passed through 11 μm mesh (Jakobsen and Tang, 2002). The fraction that passed through the 20 μm filters was assumed to represent solitary cells, although it would also contain a few small colonies. The 20 μm polycarbonate filters were extracted directly in acetone and kept at −20°C for 24 h before fluorometric analyses. Hereafter, the >20 μm fraction is referred to as colonial *Phaeocystis*, whereas the <20 μm fraction is considered to be solitary *P. antarctica*. *Phaeocystis antarctica*-dominated stations were identified based on cell abundances for solitary and colonial cells (Table I; J. Peloquin, Switzerland, personal communication).

Photosynthesis/irradiance measurements

P/E relationships were determined at 12 stations where *P. antarctica* overwhelmingly dominated. P/E relationships of solitary and colonial forms were determined by using a large-volume irradiance gradient incubator (Platt and Jassby, 1976). Water for P/E experiments was collected from the 50% light depth, after which 23 samples (265 mL each) were placed in Qorpak bottles, to which ca. 150 μCi $NaH^{14}CO_3$ were added to each. The samples were added to the P/E incubator (fitted with a high intensity xenon-arc light; Fig. 2) and incubations lasted ~2 h (Fig. 2). Surface seawater surrounded the samples and circulated through the

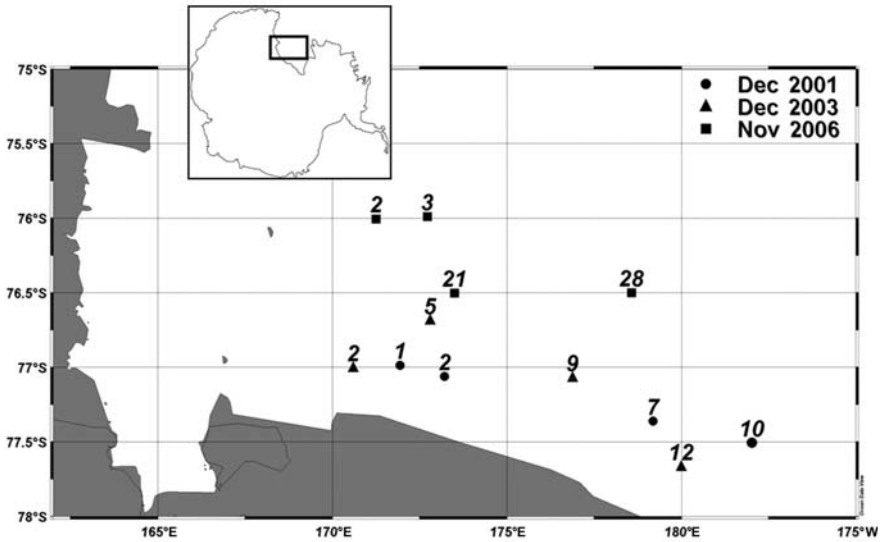

Fig. 1. *Phaeocystis antarctica* photosynthesis/irradiance experiments station locations.

Table I: The concentration of chlorophyll, its distribution among sizes and the cell counts (abundance) at the stations where photosynthesis/irradiance measurements were conducted (cell counts; unpublished data)

	<20 μm Chl *a* (μg L^{-1})	>20 μm Chl *a* (μg L^{-1})	>20 μm Chl *a* (%)	Solitary cells (%)	Colonial cells (%)	Total *P. antarctica* (%)
2001 Stations						
1	0[a]	2.22	100	69.6	29.4	99.0
2	1.68	3.07	64.6	79.3	16.7	96.0
7	6.99	1.53	18.0	78.4	14.4	92.8
10	3.36	2.20	39.6	89.2	8.0	97.2
Average ± SD	3.01 ± 2.99	2.26 ± 0.63	55.6 ± 35.2	79.1 ± 8.0	17.1 ± 8.0	96.3 ± 2.6
2003 Stations						
2[b]	4.38	5.97	57.7	nd	nd	nd
5	1.03	3.08	74.9	35.4	59.1	94.5
9	0.28	2.87	91.1	23.1	62.8	85.9
12	0.37	1.62	81.4	11.7	69.2	80.9
Average ± SD	1.52 ± 1.94	3.39 ± 1.84	76.3 ± 14.1	23.4 ± 11.9	63.7 ± 5.1	87.1 ± 6.9
2006 Stations						
2	3.43	0.81	19.2	68.8	25.7	94.5
3	1.55	0.50	24.3	51.8	32.4	84.2
20	2.48	0.52	17.4	74.7	21.9	96.6
28	2.90	0.90	23.6	64.8	17.4	82.2
Average ± SD	2.59 ± 0.79	0.68 ± 0.20	21.13 ± 3.4	65.0 ± 9.7	24.4 ± 6.3	89.4 ± 7.2

Chlorophyll *a* for the size fraction (<20 μm) was calculated by subtracting the 20 μm size fraction from the bulk GF/F (>0.7 μm) chlorophyll *a* concentration. Stations were selected based on microscopy observations and were considered to be dominated by *Phaeocystis antarctica* when abundance was >80%, or where biogenic silica concentrations were exceptionally low but chlorophyll levels were elevated (Station 2, 2003). nd, no data.

[a]>20 μm chlorophyll *a* concentration larger than GF/F (~0.7 μm) chlorophyll *a* concentration.

[b]Mean euphotic zone Chl/BSi ratio (wt.mol) was 7.89.

incubator to maintain samples at ambient temperatures (flow ca. 10 L min^{-1}). The light also passed through a heat sink, which consisted of two plates of tempered glass that held flowing surface seawater (5 cm thick) between the incubator and light source, to insure that no heating of the samples occurred. Because the light was mounted on one end of the incubator, an irradiance gradient naturally occurred as distance from the light increased. A bottle wrapped in aluminum foil was used as a dark control. All external surfaces were blackened to minimize external light from entering. Irradiance was measured within each bottle with a BioSpherical Sensor quantum meter. After incubation, each sample was size-fractionated by filtering an aliquot of known volume through a 20 μm Poretics filter, and a separate volume through a 25 mm GF/F filter

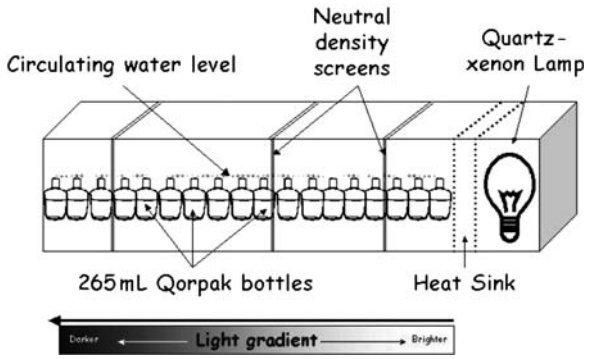

Fig. 2. Photosynthetron with Quartz-Xenon lamp located on the right side of the holding tank for the sample bottles. This provided a gradient of irradiance (brighter to darker). The holding tank had surface seawater circulated continuously to maintain a constant temperature. Drawing not to scale.

(~0.7 μm). Colonial carbon fixation was assumed to be retained on the 20 μm filter, and the difference between GFF and 20 μm filters was the carbon fixation of solitary forms. Only particulate organic carbon production was measured, as DOC release in 2 h is generally much less than 10% (unpublished data). Each filter was placed in a 7 mL scintillation vial, and 0.25 mL of 10% HCl solution was added to degas any inorganic carbon on the filter. After ventilation for at least 24 h, 5 mL Ecolume® (ICN) was added, and after another 24 h in the dark, all samples counted on a liquid scintillation counter. Total inorganic ^{14}C-bicarbonate available during incubation was assessed by counting a 0.1 mL aliquot (to which 0.05 mL β-phenethylamine, a CO_2 trap, was added) directly in Ecolume.

Laboratory culture experiments

Phaeocystis antarctica (CCMP 1374) cultures were grown in environmental rooms at Crary Laboratory, McMurdo Station, Antarctica for 16 d in 2005–06. An acid-cleaned, 50-L polycarbonate carboy with filtered (<0.2 μm) McMurdo Sound seawater was inoculated with *P. antarctica* culture (initial chlorophyll ~2 μg L^{-1}). The culture was grown in acid-cleaned polycarbonate carboys at ca. −2°C under 50 μmol photons m^{-2} s^{-1} irradiance (constant irradiance) to simulate natural conditions. Every fourth day, P/E experiments were performed in the same manner as the field samples (total volume removed 6 L each sampling period), with the incubation being completed in a −2°C environmental room to keep samples at a constant temperature. Additional volumes for nutrients, numerical abundance and chlorophyll determinations were collected. The photosynthetron used in the laboratory experiments utilized high output fluorescent lights rather than a xenon-arc light to minimize heat output, and although the two types of lights were not directly compared, their irradiance outputs were similar in both quality and quantity. All samples processed as described above, and the data were fitted to a photosynthesis–irradiance model.

Data analysis

Since photoinhibition was not significantly different from zero in our study, photosynthetic rates were fitted to the Webb *et al.* (Webb *et al.*, 1974) empirical model:

$$P^B = P_m^B\left[1 - e^{-\alpha E/P_m^B}\right] \quad (1)$$

where P^B is the rate of photosynthesis normalized to chlorophyll *a* [mg C (mg chl *a*)$^{-1}$ h^{-1}], P_m^B the maximum rate of photosynthesis in the absence of photoinhibition, α is the initial, light-limited, photosynthetic rate [mg C (mg chl *a*)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})] and E the irradiance (μmol photons m^{-2} s^{-1}). The data were fitted to this equation using SigmaPlot (Version 10). After the data were fitted to the Webb *et al.* (Webb *et al.*, 1974) model, plots of the photosynthetic responses for each experiment were generated, and the regression estimates for P_m^B and α computed; those values are reported only when $P < 0.05$. A parameter from these variables, E_k (light saturation index), is derived by:

$$E_k = \frac{P_m^B}{\alpha} \quad (2)$$

The parameter values resulting from the non-linear regressions were compared using the non-parametric Kruskal–Wallis analysis of variance (ANOVA) and a posteriori Mann–Whitney tests, as the data did not meet the normality and variance assumptions of the ANOVA. A critical *P*-value of 0.05 was selected a priori to evaluate the effects of temporal variations in the parameters and significant differences between size fractions. Systat (Version 12) was used to perform the statistical analyses. For the laboratory data, a comparison of the 95% confidence interval was used to compare parameter values between size fractions.

RESULTS

Species composition during field studies

Microscopic abundance data show that *P. antarctica* was most abundant during austral spring of 2001, 2003 and 2006 (Table I). However, the size structure of the

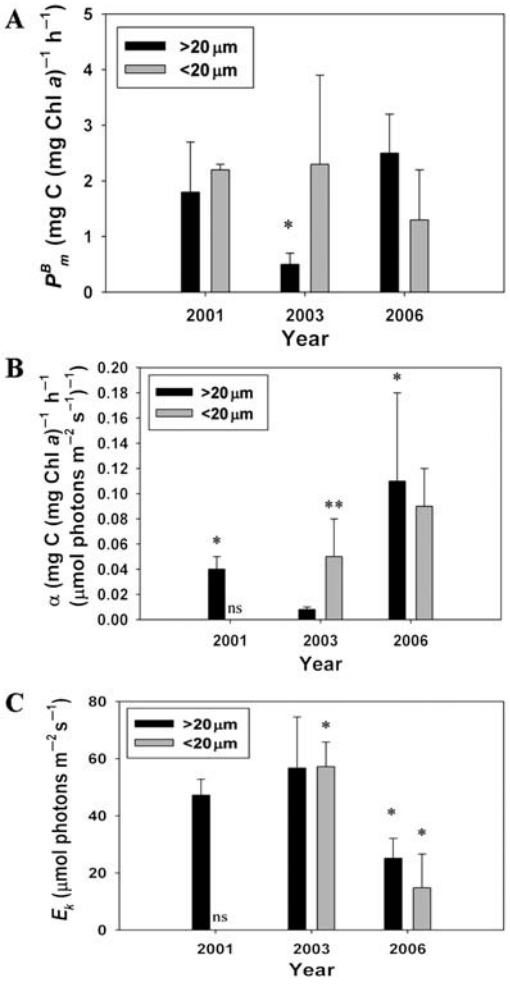

Fig. 3. (**A**) P^B_m (maximum photosynthetic rates), (**B**) α (light limited rate) and (**C**) E_k (light saturation index of colonial (>20 μm) and solitary (<20 μm) *Phaeocystis antarctica* assemblages in 2001, 2003, and 2006. * denotes a significant difference using Kruskal–Wallis and *a posteriori* Mann–Whitney tests.

P. antarctica assemblages differed markedly between years. Mean chlorophyll concentrations in the colonial size fraction for the 3 years were 2.26, 3.39 and 0.68 μg L^{-1}, which represented 56, 76 and 21% of the total phytoplankton chlorophyll, respectively (Table I). These variations in the absolute amounts of chlorophyll were associated with the date of collection and the development of the spring *Phaeocystis* bloom. The rest of the assemblage was composed largely of diatoms, including *Chaetoceros* sp., *Fragilariopsis* sp. and *Pseudonitzschia* sp. (unpublished data).

Photosynthetic relationships of colonial and solitary *P. antarctica*

Colonial and solitary maximum photosynthetic rates also reflect the development of the spring bloom (Fig. 3A). During 2001, the P^B_m for colonies averaged

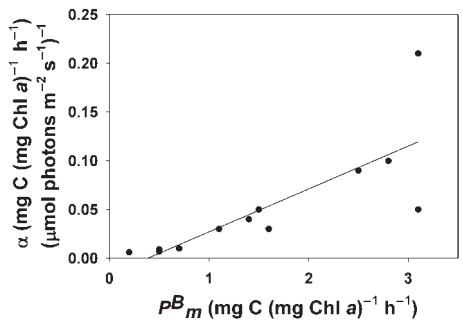

Fig. 4. P^B_m (maximum photosynthetic rates) relationship with α (light limited rate) in >20 μm *Phaeocystis antarctica* dominated assemblages in 2001, 2003 and 2006.

1.8 ± 0.7 mg C (mg chl *a*)$^{-1}$ h^{-1} and ranged from 1.1–2.8 mg C (mg chl *a*)$^{-1}$ h^{-1}, whereas that of solitary cells averaged 2.2 ± 0.1 mg C (mg chl *a*)$^{-1}$ h^{-1} and ranged from 2.1–2.2 mg C (mg chl *a*)$^{-1}$ h^{-1}. During 2003, colonial P^B_m averaged 0.5 ± 0.2 mg C (mg chl *a*)$^{-1}$ h^{-1}; solitary cells averaged 2.3 ± 1.6 mg C (mg chl *a*)$^{-1}$ h^{-1}. During November 2006, colonial and solitary cell P^B_m averaged 2.5 ± 0.7 and 1.3 ± 0.9 mg C (mg chl *a*)$^{-1}$ h^{-1}, respectively. P^B_m for 2001 and 2006 were significantly higher than 2003 for colonies (Fig. 3A; Kruskal–Wallis and *a posteriori* Mann–Whitney tests, $P = 0.019$), but no significant difference was found for solitary cells (Kruskal–Wallis, $P = 0.764$).

The initial light-limited rate of photosynthesis (α) for colonies was also significantly lower in 2003 than during the other 2 years (Fig. 3B, Kruskal–Wallis and *a posteriori* Mann–Whitney tests, $P = 0.008$). There was no significant difference in the solitary cell α between 2003 and 2006 (Kruskal–Wallis, $P = 0.079$). During 2003, solitary α was significantly higher than colonial α (Fig. 3B, Mann–Whitney, $P = 0.034$). E_k (the index of photoadaptation) for colonies was significantly lower in 2006 than in 2001 and 2003 (Fig. 3C, Kruskal–Wallis and *a posteriori* Mann–Whitney tests, $P = 0.023$). The E_k of the solitary cell-size fraction during November 2006 was significantly less than 2003 (Kruskal–Wallis and *a posteriori* Mann–Whitney tests, $P = 0.034$). Lastly, linear regressions were performed to assess the relationship between P^B_m and α. The colonial fraction exhibited a significant relationship between P^B_m versus α (Fig. 4; $r^2 = 0.63$; $P = 0.002$), while solitary cells showed no significant relationship between the two ($r^2 = 0.05$, $P = 0.63$).

When the percentage of colonial cells within an assemblage was compared with the colonial P^B_m with a least squares linear regression, a significant, negative relationship was found (Fig. 5A; $r^2 = 0.40$; $P = 0.03$). Similarly, when the colonial *P. antarctica* maximum photosynthetic rate was compared with the percentage of chlorophyll in the >20 μm fraction, a clear negative

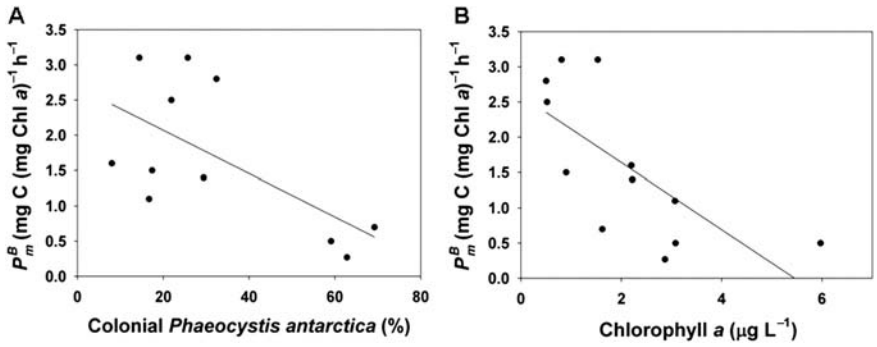

Fig. 5. Relationship between (**A**) percentage *Phaeocystis antarctica* colonial cells and (**B**) >20 chlorophyll *a* concentrations with P_m^B (maximum photosynthetic rates) in 2001, 2003 and 2006.

relationship was observed (Fig. 5B; $r^2 = 0.49$, $P = 0.011$). These data suggest that colonial photosynthetic capacity (e.g. P_m^B rates) decrease when chlorophyll and colonial contributions becomes maximal, and presumably reflect decreased growth rates at the onset of growth limitation by either irradiance or nutrients.

P/E relationships from cultured *Phaeocystis*

The biomass and activity of colonial and solitary cells was monitored in a monoculture of *P. antarctica* to assess whether there was a difference in photosynthetic parameters and growth rates between the two morphotypes (Fig. 6). Both forms showed maximum growth between days 4 and 8 (Table II), but colonial growth rates were 2.8 times as great (0.38 versus 0.13 day^{-1}); after 8 days colonial growth ceased, while growth of solitary cells continued. Maximum photosynthetic rates for colonies were initially 1.47 mg C (mg chl *a*)$^{-1}$ h^{-1}, but declined by nearly an order of magnitude during the rest of the experiment. P_m^B of cultured *P. antarctica* was observed to be 1.02 ± 0.56 and 1.86 ± 0.76 in other studies (van Hilst and Smith, 2002). P_m^B of solitary cells were initially 0.60 mg C (mg chl *a*)$^{-1}$ h^{-1}, and the rates declined slightly with time, but not to the extent that they did for colonial forms. Although the maximum photosynthetic rates of solitary cells were lower than the colonial cells at the beginning of the experiment, they fall within the lower ranges of previous studies (van Hilst and Smith, 2002). During the first 8 days, the P_m^B was significantly higher for colonies, but on the 16th day solitary cells' P_m^B was greater than that of colonies (Fig. 6, 95% CI).

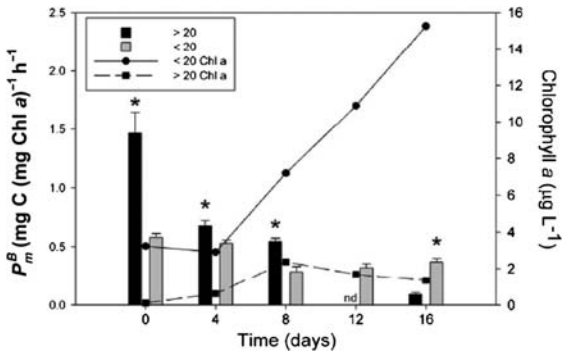

Fig. 6. *Phaeocystis antarctica* (CCMP 1374) colonial and solitary cells maximal photosynthetic rates over 16 days of growth. Chlorophyll *a* values are also presented. * denotes a significant difference between the 95% confidence interval of the slopes for the photosynthetic parameter. nd, no data.

Table II: Biomass and growth rate estimates of solitary and colonial cells of cultured Phaeocystis antarctica

Variable	Solitary cells	Colonial cells
Initial chlorophyll *a* (μg L^{-1})	3.2	0.11
Maximum chlorophyll (μg L^{-1})	15.3	2.4
Growth rate (day^{-1})	0.13[a]	0.38[b]

[a]Determined from 4 to 16 days.
[b]Determined from 0 to 8 days.

DISCUSSION

Distribution of *Phaeocystis antarctica*

Although the Ross Sea is one of the locations where large accumulations of *P. antarctica* have been repeatedly observed (Arrigo *et al.*, 1999; Smith and Asper, 2001), there are few data from the Southern Ocean on the temporal and spatial distributions of colonies (non-flagellated) and solitary forms (Mathot *et al.*, 2000), and none on variations in growth rates and photosynthetic parameters. A conceptual diagram that illustrates the seasonal relationships among environmental factors (irradiance, iron concentrations), biomass of colonial and solitary forms, and growth rates of the two morphotypes are presented (Fig. 7). The overall pattern is one characterized by initial, rapid growth of colonies

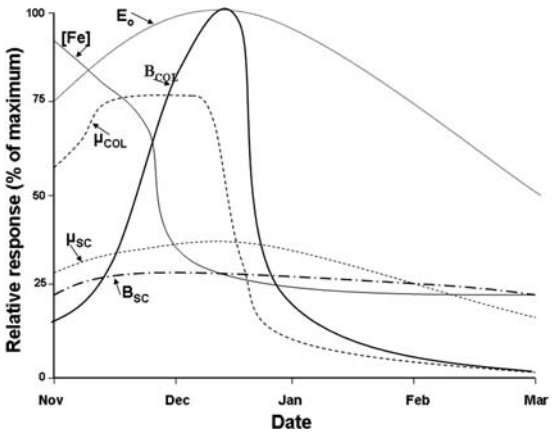

Fig. 7. Conceptual diagram of the temporal sequence of *Phaeocystis antarctica* growth rates, environmental variables (iron and irradiance) and biomass for both colonial and solitary forms. E_o, surface irradiance; [Fe], iron concentrations; B_{col}, biomass of colonies; μ_{col}, growth rate of colonies; B_{SC}, biomass of solitary cells; μ_{SC}, growth rate of solitary cells.

(and accumulation of biomass) under high iron concentrations, followed by a rapid decrease in growth rates of colonial cells, which results in the eventual relative dominance by solitary cells during high light, reduced iron concentration conditions. Smith *et al.* (Smith *et al.*, 2006) found that in both 2001 and 2003 prymnesiophytes represented ca. 80% of the phytoplankton chlorophyll (ca. 6 $\mu g\ L^{-1}$) in December, and that the contributions of *P. antarctica* to phytoplankton chlorophyll decreased markedly, to $<1\ \mu g\ L^{-1}$ by February. Microscopic abundance during November–December of 2001, 2003 and 2006 further suggest that *P. antarctica* dominated the Ross Sea polynya during those time periods (Table I). In some years, large diatom blooms occurred after the decline of the *P. antarctica* blooms, with a diatom bloom in equal magnitude to the spring bloom in February 2004 (Smith *et al.*, 2006; Peloquin and Smith, 2007). These large diatom blooms, possibly due to iron-enriched modified circumpolar deep water intrusions, appear to occur in summer approximately every 2–4 years (Peloquin and Smith, 2007). Even if diatoms do not reach high concentrations, during January and February they generally dominate the phytoplankton assemblage.

Variations between colonial and solitary cells

Our results are the first to suggest that colonial *P. antarctica* dominance (relative to solitary *P. antarctica*) in the Ross Sea may be due to higher growth and maximum photosynthetic rates of colonial cells during the onset of the bloom, rather than solely due to a grazing defense mechanism. Both the field and laboratory results support this contention (Fig. 3A–C, Fig. 6; Table II). The culture experiments further illustrate the capabilities of colonies, which exhibited significantly higher P_m^B values and a faster growth rate during the first 8 days of the study (Fig. 6). Other studies have suggested that *Phaeocystis* sp. colonies are capable of higher growth rates than solitary flagellates (e.g. Veldhuis *et al.*, 2005), but investigations of *P. antarctica* growth rates are limited. While this contrasts with theoretical models that suggest that solute exchange increases with decreased cell radius (Raven, 1998), other physiological mechanisms such as nutrient storage or increasing scalable components may give colonial *P. antarctica* cells an advantage. Large phytoplankton cells are often capable of higher growth rates or maximum carbon specific photosynthesis (under nutrient-replete conditions and high irradiance) than small-sized phytoplankton, but previous studies are not consistent (Furnas, 1991; Frenette *et al.*, 1996; Crosbie and Furnas, 2001; Cermeño *et al.*, 2005). Colonial cells of *P. globosa* have been found to divide at the same rate as motile cells (Veldhuis *et al.*, 2005); however, it has also been shown for *P. pouchetii* that colonies have lower specific growth rates. The variations in growth rates are important to consider in models of primary production in *P. antarctica*-dominated ecosystems, as well as their impacts on local ecology and biogeochemical cycles (Lancelot and Rousseau, 1994; Peperzak *et al.*, 2000).

It is plausible to ask why solitary cells do not more consistently dominate the assemblage, particularly under high-light, low iron conditions. Colonial biomass accumulation could be enhanced due to extensive grazing of solitary forms by heterotrophic microzooplankton (Caron *et al.*, 2000; Smith *et al.*, 2003). Such forms of phytoplankton grazers are common in the Ross Sea and display a clear seasonal increase (Dennett *et al.*, 2001). We suggest that the early growth of solitary cells can be near maximal, but that ultimately their abundance is limited by micro-heterotrophic ingestion. Interestingly, in the culture experiment, solitary cells did reach a higher biomass than did colonial cells under conditions where microzooplankton were substantially reduced in biomass. Colonial biomass did not reach concentrations found *in situ*, which may reflect a limitation by irradiance (either shading or the reduced photon flux density in the culturing environment). The environmental controls of the two forms appear to be different, with colony growth and abundance limited by bottom-up factors (iron limitation, enhanced aggregate formation and increased sinking), whereas solitary cell abundance is limited by loss rates (transformation into new colonies) and grazing. Laboratory studies, however,

suggest that some microzooplankton may be capable of grazing individual colonial cells, and further research on grazing and morphotype development is needed in the Ross Sea to fully understand *P. antarctica* dynamics (Shields and Smith, 2008).

Interannual variability of colonial *Phaeocystis antarctica* photosynthesis

Colonial *Phaeocystis antarctica* P_m^B rates were significantly lower in 2003 than during other years; however, there was no significant interannual variability among solitary *P.* antarctica P_m^B rates. While the lower colonial P_m^B in 2001 and 2003 could be due to differences in growth stage when compared with 2006, the lower colonial P_m^B during those time periods could also have resulted from iron limitation during the strongly stratified summer (Olson *et al.*, 2000; Smith and Asper, 2001). If such declines were indeed initiated by the onset of iron limitation, it is logical that colonies would reflect that limitation first, as single cells (by virtue of their greater surface area: volume ratio due to smaller size, as well as the absence of a mucoid sheath) (Mathot *et al.*, 2000) would likely be less stressed under low nutrient conditions than larger colonies (Smith *et al.*, 2003). Colonial P_m^B rates in 2003 tended to be lower (although not significantly lower; Kruskal–Wallis, $P = 0.083$) than those of solitary cells and may reflect iron stress, as has been shown in diatoms (Lindley *et al.*, 1995). While no measurements of iron concentrations were made, Peloquin and Smith (Peloquin and Smith, 2007) reported low maximum quantum yields (F_v/F_m) of 0.3–0.4 during 2003 and suggested the assemblages were under severe micronutrient stress. While we observed a decrease in P_m^B from austral spring to summer, van Hilst and Smith (van Hilst and Smith, 2002) observed an increase in P_m^B over time, and suggested that acclimation to *in situ* irradiance occurred from November to December, a period of rapidly increasing irradiance and declining ice concentrations. It is possible that iron limitation occurred earlier in our study, especially in 2003 (Peloquin and Smith, 2007), and this impacted photosynthetic parameters. The percentage of colonial *P. antarctica* in the phytoplankton assemblage also affects field measurements of P_m^B in the Ross Sea; that is, a lower P_m^B occurred when the percentage of colonial *P. antarctica* was highest (Table I; Fig. 5A and B). As colonial growth proceeds, it is likely that the degree of iron limitation also increases through time (Fig. 7). It is clear that models looking at phytoplankton dynamics in the Ross Sea must consider not only the morphotypes of *P. antarctica* separately, but the interannual differences in the environmental controls of photosynthetic parameters.

An alternative hypothesis implies that colonial *P. antarctica* may not exhibit iron stress before solitary cells. It has been shown that manganese, phosphate and possibly iron (Davidson and Marchant, 1987; Lubbers *et al.*, 1990; Veldhuis *et al.*, 1991) can be sequestered in the colonial matrix and subsequently reused during later growth, thereby giving colonies a competitive advantage over single cells during periods of micronutrient limitation. It may be possible that other negative effects associated with colonies (e.g. self-shading), rather than micronutrient limitation, were occurring in 2003. Robinson *et al.* (Robinson *et al.*, 2003) argued that colonial bloom development could cease due to excessive colonial carbon requirements restricting colony size. These reductions in both forms would be consistent with the onset of iron limitation earlier in 2003 than in 2001 (Smith *et al.*, 2006).

Adaptation to low irradiance by *Phaeocystis antarctica*

Tagliabue and Arrigo (Tagliabue and Arrigo, 2003) suggested that large colonial *P. antarctica* blooms during austral spring results from its shade acclimation capabilities. This shade acclimation and rapid growth at the low irradiances found in spring in the Ross Sea allows *P. antarctica* to become uncoupled with zooplankton grazing, resulting in blooms. Shade acclimation, denoted by low P_m^B and E_k values and a higher α, has been observed in a variety of other studies as well (e.g. Holm-Hansen and Mitchell, 1991; Boyd *et al.*, 1995). For example, Boyd *et al.* (Boyd *et al.*, 1995) measured P_m^B rates (0.70 mg C (mg chl *a*)$^{-1}$ h^{-1}), E_k (20 μmol photons m^{-2} s^{-1}) and elevated α values (0.04 mg C (mg ch1 *a*)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$) during an early spring bloom in the Bellingshausen Sea, Antarctica, and both *P. antarctica* and diatoms have been found to be well adapted to low-irradiance levels in the austral spring in the Ross Sea, indicated by higher photosynthetic efficiency (α) and a low E_k (van Hilst and Smith, 2002). During our study, the November 2006 *P. antarctica* solitary cells appear to have these shade acclimation characteristics, with low P_m^B rates (1.3 mg C (mg chl *a*)$^{-1}$ h^{-1}), low E_k (14.9 μmol photons m^{-2} s^{-1}) and high α values (0.09 mg C (mg ch1 *a*)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$). Our α values fall within the range of those observed by van Hilst and Smith (van Hilst and Smith, 2002), with the solitary *P. antarctica* cells having a higher α during 2001 than previous observations. While it is clear that shade acclimation does occur in spring, our results also

suggest that there is a significant temporal pattern in both the solitary and colonial fraction photosynthetic response (Fig. 7). While a lower E_k during the early spring could suggest low-irradiance acclimation, increased values during austral summer demonstrated that *P. antarctica* can also adapt to the higher irradiance levels encountered at that time. This is important to consider in primary production models that assume that *P. antarctica* is low-light adapted and less likely to compete with diatoms in high irradiance, stratified environments (Tagliabue and Arrigo, 2003). van Hilst and Smith (van Hilst and Smith, 2002) observed the lowest E_k values in cultured phytoplankton grown at the lowest irradiance, further suggesting their ability to adapt to the irradiance regime of high-latitude environments.

The variations in α values we observed could be due to the colonial cells changing concentrations of photosynthetically active accessory pigments, variable photosystem I: photosystem II ratios, or nonphotochemical quenching (Behrenfeld *et al.*, 2004). Behrenfeld *et al.* (Behrenfeld *et al.*, 2004) suggest that covariation in P_m^B and α can exist temporally in assemblages dominated by one species. Harding *et al.* (Harding *et al.*, 1987) described seasonal changes in P/E parameters for *Prorocentrum mariaelebouriae* and found a significant relationship between P_m^B and α, as we did between colonial P_m^B and α. Antarctic studies also demonstrate the covariation of P_m^B and α (Claustre *et al.*, 1997; Moline *et al.*, 1998). The covariance between α and P_m^B may suggest that nutrient limitation caused a shift in net carbon partitioning due to changes in the fraction of photosynthetically produced reductants. This observation is essential to a description of the temporal changes in colonial *P. antarctica* photosynthetic parameters and how these morphotypes may be affected more strongly by nutrient limitation than solitary cells of *P. antarctica*. Covariation of P_m^B and α may also represent the ability of phytoplankton to keep E_k relatively constant in an unchanging light environment. Assuming light is not changing substantially but nutrients are decreasing, phytoplankton will reduce both P_m^B and α to accommodate the lower nutrient supply (keeping E_k relatively unchanged), and thus the two will covary. Experiments will be needed to examine the biophysical mechanisms by which colonial *P. antarctica* α varies.

Conclusions

The forms of *P. antarctica* exist in a dynamic equilibrium in nature, and a distinct temporal trend occurs in these forms. Different controls of each morphotype likely exist, and hence the relative importance of these controls (bottom-up controls on colonies versus top-down controls on small flagellates), as well as the differential rates of photosynthesis and growth between them, will ultimately regulate their biomass within a bloom. Our data are relatively limited, but represent the first field data showing differences in the photosynthetic parameters and growth rates between the morphotypes of *P. antarctica*. Further study will clarify the importance of these differences, as well as the environmental and ecological regulation of the exchanges between the two forms. Understanding these differences will allow greater insights into the influence of phytoplankton composition on biogeochemical cycles in the Ross Sea.

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