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AR Shields
Virginia Institute of Marine Science

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

AMY R. SHIELDS†* AND WALKER O. SMITH

VIRGINIA INSTITUTE OF MARINE SCIENCE, COLLEGE OF WILLIAM AND MARY, GLOUCESTER PT, VA 23062, USA

†PRESENT ADDRESS: UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, REGION 7, WATER, WETLANDS AND PESTICIDE DIVISION, 901 N, 5TH STREET, KANSAS CITY, KANSAS 66101.

*CORRESPONDING AUTHOR: shields.amy@epa.gov

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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 (PmaxB) 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 (Boyed 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
have a relatively restricted diversity of functional groups (Arrigo et al., 1999). Specifically, there are two major functional phytoplankton groups: diatoms and pyrnesiophytes, 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., 2003) 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. (Perperzak 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 (IV ARS) 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 paraformaldehyde 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 epifluorescence 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 0.5% 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 μg NaH14CO3 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
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. 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)

<table>
<thead>
<tr>
<th></th>
<th>&lt;20 μm Chl a (μg L$^{-1}$)</th>
<th>&gt;20 μm Chl a (μg L$^{-1}$)</th>
<th>&gt;20 μm Chl a (%)</th>
<th>Solitary cells (%)</th>
<th>Colonial cells (%)</th>
<th>Total P. antarctica (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001 Stations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0*</td>
<td>2.22</td>
<td>100</td>
<td>69.6</td>
<td>29.4</td>
<td>99.0</td>
</tr>
<tr>
<td>2</td>
<td>1.68</td>
<td>3.07</td>
<td>64.6</td>
<td>79.3</td>
<td>16.7</td>
<td>96.0</td>
</tr>
<tr>
<td>7</td>
<td>6.99</td>
<td>1.53</td>
<td>18.0</td>
<td>78.4</td>
<td>14.4</td>
<td>92.8</td>
</tr>
<tr>
<td>10</td>
<td>3.36</td>
<td>2.20</td>
<td>39.6</td>
<td>89.2</td>
<td>8.0</td>
<td>97.2</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>3.01 ± 2.99</td>
<td>2.26 ± 0.63</td>
<td>55.6 ± 35.2</td>
<td>79.1 ± 8.0</td>
<td>17.1 ± 8.0</td>
<td>96.3 ± 2.6</td>
</tr>
<tr>
<td>2003 Stations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>4.38</td>
<td>5.97</td>
<td>57.7</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>5</td>
<td>1.03</td>
<td>3.08</td>
<td>74.9</td>
<td>35.4</td>
<td>59.1</td>
<td>94.5</td>
</tr>
<tr>
<td>9</td>
<td>0.28</td>
<td>2.87</td>
<td>91.1</td>
<td>23.1</td>
<td>62.8</td>
<td>85.9</td>
</tr>
<tr>
<td>12</td>
<td>0.57</td>
<td>1.62</td>
<td>81.4</td>
<td>11.7</td>
<td>69.2</td>
<td>80.9</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>1.52 ± 1.94</td>
<td>3.39 ± 1.84</td>
<td>76.3 ± 14.1</td>
<td>23.4 ± 11.9</td>
<td>63.7 ± 5.1</td>
<td>87.1 ± 6.9</td>
</tr>
<tr>
<td>2006 Stations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.43</td>
<td>0.81</td>
<td>19.2</td>
<td>68.8</td>
<td>25.7</td>
<td>94.5</td>
</tr>
<tr>
<td>3</td>
<td>1.55</td>
<td>0.50</td>
<td>24.3</td>
<td>51.8</td>
<td>32.4</td>
<td>84.2</td>
</tr>
<tr>
<td>20</td>
<td>2.48</td>
<td>0.52</td>
<td>17.4</td>
<td>74.7</td>
<td>21.9</td>
<td>96.6</td>
</tr>
<tr>
<td>28</td>
<td>2.90</td>
<td>0.90</td>
<td>23.6</td>
<td>64.8</td>
<td>17.4</td>
<td>82.2</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>2.59 ± 0.79</td>
<td>0.68 ± 0.20</td>
<td>21.3 ± 3.4</td>
<td>65.0 ± 9.7</td>
<td>24.4 ± 6.3</td>
<td>89.4 ± 7.2</td>
</tr>
</tbody>
</table>

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.

bMean euphotic zone Chl/BSi ratio (wt:mol) was 7.89.
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 Econulum® (ICN) was added, and after another 24 h in the dark, all samples counted on a liquid scintillation counter. Total inorganic 14C-bicarbonate available during incubation was assessed by counting a 0.1 mL aliquot (to which 0.05 mL β-phenethylamine, a CO2 trap, was added) directly in Econulum.

**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 photosyntheron 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:

\[ P^B_m = P^B_m \left[ 1 - e^{-C/E} \right] \]

where \( P^B_m \) is the rate of photosynthesis normalized to chlorophyll \( a \) [mg C (mg chl \( a \))⁻¹ h⁻¹], \( P_m \) the maximum rate of photosynthesis in the absence of photoinhibition, \( C \) 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 \( P^B_m \) and \( C \) computed; those values are reported only when \( P < 0.05 \). A parameter from these variables, \( E_k \) (light saturation index), is derived by:

\[ E_k = \frac{P^B_m}{C} \]

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
was composed largely of diatoms, including of the spring associated with the date of collection and the development of chlorophyll were plankton chlorophyll, respectively (Table I). These variations which represented 56, 76 and 21% of the total phytoplankton (Fig. 3A). During 2001, the $P_m$ for colonies averaged 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 $P_m$ 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 $P_m$ averaged 2.5 ± 0.7 and 1.3 ± 0.9 mg C (mg chl a)$^{-1}$·h$^{-1}$, respectively. $P_m$ 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 ($\alpha$) for colonies was also significantly lower in 2003 than during the other 2 years (Fig. 3B, Kruskal–Wallis and a posteriori Mann–Whitney, $P = 0.008$). There was no significant difference in the solitary cell $\alpha$ between 2003 and 2006 (Kruskal–Wallis, $P = 0.079$). During 2003, solitary $\alpha$ was significantly higher than colonial $\alpha$ (Fig. 3B, Mann–Whitney, $P = 0.034$). $E_2$ (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 $E_2$ 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 $P_m$ and $\alpha$. The colonial fraction exhibited a significant relationship between $P_m$ and $\alpha$ (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 $P_m$ 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. 3. A) $P_m$ (maximum photosynthetic rates), B: $\alpha$ (light limited rate) and C: $E_2$ (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.

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 $P_m$ for colonies averaged 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., Fragilaropsis sp. and Pseudonitzschia sp. (unpublished data).
relationship was observed (Fig. 5B; \( r^2 = 0.49, P = 0.011 \)). These data suggest that colonial photosynthetic capacity (e.g. \( P_m \) 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. \( P_m \) 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). \( P_m \) 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 \( P_m \) was significantly higher for colonies, but on the 16th day solitary cells’ \( P_m \) was greater than that of colonies (Fig. 6, 95% CI).

**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.
The onset of the bloom, rather than solely due to a maximum photosynthetic rates of colonial cells during the Ross Sea may be due to higher growth and biomass for both colonial and solitary forms. E, surface irradiance; [Fe], iron concentrations; B<sub>col</sub>, biomass of colonies; μ<sub>col</sub>, growth rate of colonies; B<sub>SC</sub>, biomass of solitary cells; μ<sub>SC</sub>, 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 μg L<sup>-1</sup>) in December, and that the contributions of P. antarctica to phytoplankton chlorophyll decreased markedly, to <1 μg L<sup>-1</sup> 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<sub>m</sub> 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; Cermeno 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 microplankton 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
decreases 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 con-
ditions than larger colonies (Smith *et al*., 2003). Colonial
\( P_m^B \) rates in 2003 tended to be lower (although not sig-
nificantly 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 (\( F_a/F_m \))
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 per-
centage 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 per-
centage 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 micronu-
trient 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
colonal 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 capa-
bilities. This shade acclimation and rapid growth at the
low irradiiances 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 \( \alpha \), has
been observed in a variety of other studies as well (e.g.
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 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\)) and elevated \( \alpha \) values (0.04 mg C
(mg chl a)\(^{-1}\) h\(^{-1}\)) (\( \mu \)mol photons m\(^{-2}\) s\(^{-1}\)) 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 (\( \alpha \)) 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 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\)) and high \( \alpha \) values (0.09 mg C
(mg chl a)\(^{-1}\) h\(^{-1}\)) (\( \mu \)mol photons m\(^{-2}\) s\(^{-1}\))>. Our \( \alpha \) 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 \( \alpha \) during 2001 than
previous observations. While it is clear that shade
acclimation does occur in spring, our results also
suggested 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, 2005). 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 \( \alpha \) 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 \( P_m^C \) and \( \alpha \) can exist temporally in assemblages dominated by one species. Harding et al. (Harding et al., 1987) described seasonal changes in \( P/E \) parameters for Procentrum mariaelebouriae and found a significant relationship between \( P_m^C \) and \( \alpha \), as we did between colonial \( P_m^C \) and \( \alpha \). Antarctic studies also demonstrate the covariation of \( P_m^C \) and \( \alpha \) (Claustre et al., 1997; Moline et al., 1998). The covariance between \( \alpha \) and \( P_m^C \) 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 \( P_m^C \) and \( \alpha \) 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 \( P_m^C \) and \( \alpha \) 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 \( P. antarctica \) \( \alpha \) 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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