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COLONY SIZE OF *PHAEOCYSTIS ANTARCTICA* (PRYMNESIOPHYCEAE) AS INFLUENCED BY ZOOPLANKTON GRAZERS[1]

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The haptophyte *Phaeocystis antarctica* G. Karst. is a dominant phytoplankton species in the Ross Sea, Antarctica, and exists as solitary cells and mucilaginous colonies that differ by several orders of magnitude in size. Recent studies with *Phaeocystis globosa* suggest that colony formation and enlargement are defense mechanisms against small grazers. To test if a similar grazer-induced morphological response exists in *P. antarctica*, we conducted incubation experiments during the austral summer using natural *P. antarctica* and zooplankton assemblages. Dialysis bags that allowed exchange of dissolved chemicals were used to separate *P. antarctica* and zooplankton during incubations. Geometric mean colony size decreased by 35% in the control, but increased by 30% in the presence of grazers (even without physical contact) over the 15 d incubation. The estimated colonial-to-solitary cell carbon ratio was significantly higher in the grazing treatment. These results suggest that *P. antarctica* colonies would grow larger in the presence of indigenous zooplankton and skew the carbon partitioning significantly toward the colonial phase. While these observations show that the colony size of *P. antarctica* was affected by a chemical signal related to grazers, the detailed nature and ecological significance of this signal remain unknown.

Key index words: **chemical signal; colony; grazing; *Phaeocystis*; Southern Ocean**

Abbreviations: **ANOVA, analysis of variance; JGOFS, Joint Global Ocean Flux Study**

Despite the vast diversity of phytoplankton morphology occurring in the world's oceans, most fall within a relatively narrow size range of a few to tens of micrometers, mainly due to constraints of diffusive nutrient uptake and sinking (Malone 1980, Kiørboe 1993). These constraints may explain why small, motile phytoplankters are generally prevalent in oligotrophic waters, whereas large, nonmotile diatoms tend to dominate more nutrient-rich waters (Legendre and Le Fèvre 1989). Many diatoms are large and form colonies such that their effective cell size (volume-to-surface ratio) increases considerably. Besides diatoms, another prominent member of colony-forming phytoplankton belongs to the genus *Phaeocystis*. *Phaeocystis* occurs throughout the world's oceans (Lancelot et al. 1998), from the extreme north (e.g., Greenland Sea, Bering Sea) to temperate seas (e.g., North Sea, North Atlantic Ocean), tropical systems (e.g., Arabian Sea, coastal Vietnam), and waters surrounding Antarctica (e.g., Ross Sea, Weddell Sea). Six species have been identified (Medlin and Zingone 2007), three of which are the most widespread and intensively studied: *P. globosa* and *Phaeocystis pouchetii* in the Northern Hemisphere, and *P. antarctica* in the Southern Ocean. The three species have similar life cycles that involve motile or nonmotile solitary cells and colonies composed of small cells held together by a mucilaginous sheath (Rousseau et al. 1994).

A healthy *Phaeocystis* colony is spherical and three dimensional, compared to the generally two-dimensional diatom chain. *Phaeocystis* solitary cells are only a few (~4) μm in size, whereas colonies can reach a few mm in diameter (Schoemann et al. 2005). Much effort has been devoted to understanding how physicochemical factors, particularly light and nutrients, affect colonial abundance and size (Lancelot et al. 1998, Schoemann et al. 2005). Grazing mortality of *Phaeocystis* is highly dependent on its size, such that solitary cells and small colonies tend to be grazed heavily, whereas large colonies are relatively free from grazing due to size mismatch (i.e., the colonies are bigger than the handling capacity of most grazers) (Nejstgaard et al. 2007). An oft-discussed hypothesis is that *Phaeocystis* colony formation and size increase is a defense mechanism against small grazers (Weisse et al. 1994, Schoemann et al. 2005), although empirical evidence was not available until recently. Jakobsen and Tang (2002) demonstrated that *P. globosa* colonies grew to significantly larger size in the presence of grazers. A subsequent study by Tang (2003) further showed that the response was triggered by a chemical signal released from ambient grazing activities, and that the signal was not necessarily specific to grazing mortality of *Phaeocystis* cells. Long et al. (2007) also determined that grazing on solitary cells by ciliates enhanced colony formation in *P. globosa*. Relative to *P. globosa* and *P. pouchetii*, the trophic

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interactions between *P. antarctica* and zooplankton grazers are poorly known, despite the importance of the species to the Southern Ocean ecosystem.

P. antarctica is distributed widely in the Southern Ocean and forms extremely dense accumulations in the Ross Sea and in Prydz Bay (East Antarctica) (Arrigo et al. 1999, Smith et al. 2000). In the Ross Sea, chl concentrations within blooms of *P. antarctica* can exceed 15 $\mu g \cdot L^{-1}$ (Smith et al. 1996, Smith and Asper 2001), and 65%–85% of the spring production and 36%–45% of the annual production can be attributed to this species (Smith et al. 2006). Although both colonial and solitary forms co-occur, in many cases, the assemblages are dominated by colonies with colonial cell concentration exceeding solitary cell concentration by one to two orders of magnitude (Mathot et al. 2000). Colonies of up to 10^7 μm^3 in volume containing hundreds of cells have been observed (Mathot et al. 2000). It remains unclear if *P. antarctica* is actively ingested by herbivores of the Ross Sea, since few studies have quantified in situ grazing rates. Caron et al. (2000) found no significant microzooplankton grazing at 82% of the stations they sampled, which were mostly dominated by *P. antarctica*. Smith et al. (2003) reanalyzed the data from stations that showed positive grazing and suggested that microzooplankton ingested only solitary cells and not colonies; thus, grazing primarily affected the *P. antarctica* population size structure more so than the absolute biomass. Unlike the more frequently studied Northern Hemisphere species, the factors that regulate colony development of *P. antarctica* are unknown. In this paper, we report on how the presence of zooplankton grazers affects colony size development in *P. antarctica*. On the basis of previous studies with *P. globosa* (Jakobsen and Tang 2002, Tang 2003), we hypothesized that the presence of zooplankton grazers in the water would stimulate *P. antarctica* colonies to grow bigger. However, unlike the previous work, we used indigenous *P. antarctica* and zooplankton grazers in this study such that the results would provide us with direct insights into the ecological processes in the region.

MATERIALS AND METHODS

Grazing treatment and control. About 100 L of surface mixed layer water was collected in early December 2006 from the southern Ross Sea (77°19.5′ S, 171°07.8′ E) from the RVIB N. Palmer during a dense phytoplankton bloom dominated by *P. antarctica*. The water was maintained at ~90 μmol photons $\cdot m^{-2} \cdot s^{-1}$ and constant light (temperature = −1°C) in an environmental room after collection and transported to McMurdo Station within 5 d for use in the experiment. Upon arrival in McMurdo, the water was maintained at −1°C under continuous light (~95 μmol photons $\cdot m^{-2} \cdot s^{-1}$). Microscopic (Nikon TS 100, Tokyo, Japan) observations confirmed that the assemblage was almost entirely composed of *P. antarctica* solitary cells and colonies. Prior to experiments, the Ross Sea water was gravity filtered through a 63 μm nylon screen (SeaGear, Melbourne, FL, USA) to remove large colonies, then diluted with 0.2 μm filtered (Pall-Gelman in-line filter cartridge; Pall Corp., Port Washington, NY, USA) McMurdo Sound surface water (collected from the flowing seawater system) to provide an initial chl concentration of ~2 $\mu g \cdot L^{-1}$, and amended with f/10 nutrients (final concentration). This water was used to fill six clear polycarbonate carboys of 10 L each. Aliquots were taken in triplicate for measuring initial size-fractioned chl, particulate carbon, *P. antarctica* solitary cell concentration, and *P. antarctica* colonial concentration and size.

Zooplankton and McMurdo Sound water were collected from below the sea ice through a dive hole just in front of the McMurdo Station (77°52.8′ S, 166°43.8′ E). Zooplankton were collected by vertical haul of a 200 μm net and kept in buckets under low irradiance until use. McMurdo Sound water was collected by a submersible pump below the surface meltwater layer and stored in acid-washed polycarbonate carboys. Upon return to the laboratory, the seawater was divided into two portions. To the first, we added an aliquot of the zooplankton sample. To further enhance grazing activity, we enriched the water by adding *Dunaliella tertiolecta* (Chlorophyceae) and *Rhodomonas salina* (Cryptophyceae) each to a final concentration of ~10 μg chl $\cdot L^{-1}$. This portion of the water was used for the grazing treatment. The other portion was passed through an in-line 0.2 μm filter cartridge to remove all particles and then enriched with the same amounts of *D. tertiolecta* and *R. salina*; this subset was the control. Triplicate aliquots were taken from the experimental container for measurements of initial size-fractioned chl, particulate carbon, and zooplankton concentration.

Two liters of water for the grazing treatment and the control was added to dialysis bags (Spectra/Por® 2 dialysis membrane, Spectrum Laboratories, Rancho Dominguez, CA, USA; molecular weight cutoff 12–14 kDa). The bags were sealed and submerged into the carboys with Ross Sea water. A total of three carboys of grazing treatment and three carboys of control were set up. The dialysis bags prevented physical contact between *P. antarctica* in the carboy and the organisms inside the bags, but chemical molecules smaller than 12–14 kDa were freely exchanged between the two compartments. Meso- and microzooplankton grazers inside the dialysis bags were expected to generate a chemical-based signal that would diffuse out of the bags and affect colony formation in *P. antarctica*. All carboys were incubated in a running seawater aquarium at −1°C under a nominal light intensity of 95 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (measured at the surface of the carboys). The water flow in the aquarium provided gentle mixing to the carboy contents. The carboy content was also gently mixed prior to sampling. On days 5, 10, and 15, measurements were repeated for the outside of the dialysis bags (Ross Sea water). On the last day of the experiment (day 15), aliquots were also drawn from the inside of the dialysis bags for measuring the final chl and particulate carbon.

Size-fractioned chl. Aliquots of known volume were filtered through Whatman GF/F filters (Whatman, Kent, UK) to obtain the >0.7 μm size fraction, and through 20 μm polycarbonate filters (Osmonics, Livermore, CA, USA) to obtain the >20 μm size fraction. We assumed that only colonies were collected on the 20 μm filters, and that the GF/F filters collected both colonies and solitary cells. We recognized the separation by size is not perfect, since some small colonies may pass through a 20 μm filter, but determined that such a separation provided a rapid means of differentiating the two forms. The <20 μm size fraction, presumably dominated by solitary cells, was calculated as the difference between the chl on the GF/F filter and that on the 20 μm filter. Chl *a* was extracted by placing the filters in 90% acetone at −20°C for 24 h, and the fluorescence measured with Turner Designs TD-700 fluorometer (Turner Designs, Sunnyvale, CA, USA) before and after acidification. The

fluorometer was calibrated with a standard chl *a* solution (from Turner Designs) initially and with a secondary (solid) standard routinely during the experiments to ensure consistency.

P. antarctica *solitary cell and colonial concentrations.* Samples to assess the concentrations of *P. antarctica* solitary cells and colonies were preserved and processed immediately according to Jakobsen and Tang (2002). Water samples were preserved in ~4% Lugol's solution. Depending on the cell and colonial concentrations, 0.3–3 mL aliquots of the preserved samples were drawn with a wide-mouth pipette and added to a 24-well multiplate. The wells were topped off with filtered seawater, and the materials were allowed to settle for at least 10 min, after which each well was closed with a coverslip to exclude air bubbles. The solitary cells and colonies were allowed to settle for another 1 h before counting with an inverted microscope (Nikon TS 100). Twenty to thirty colonies were randomly chosen from each sample and sized with a calibrated ocular meter under × 100 magnification. Colony size was measured as the diameter across the mucous envelope of the colony. This simple method was adequate for our purpose since a majority of the colonies were spherical (cf. Rousseau et al. 1990).

Particulate carbon concentrations. Aliquots for particulate carbon measurements were filtered through precombusted (450°C for 2 h) Whatman GF/F filters. Following the JGOFS protocols (Joint Global Ocean Flux Study [JGOFS] 1996), the filters were dried and stored at 60°C until pyrolysis on a Fisons 254 elemental analyzer (Carlo Erba, Milan, Italy). Blanks were filters placed under another filter that were processed in an identical fashion as above.

Zooplankton concentration. Aliquots (100 mL) of the water used for the grazing treatment and control were taken at the beginning of the experiment and preserved in 4% formaldehyde (final conc.). Upon return to Virginia, the samples were examined under dissecting microscope (Nikon SMZ 1000, Tokyo, Japan), and zooplankton species and concentration determined.

RESULTS

The zooplankton assemblage was dominated by small copepods (e.g., *Oithona similis, Microcalanus pygmaues,* and *Oncaea curvata*; Table 1). One of the control bags contained some small copepods that had been accidentally introduced into the dialysis bag (80 individuals in the 2 L bag; Table 1). The initial mean concentration of chl *a* among the triplicate dialysis bags did not differ significantly between the grazing treatment and the control (23.5 vs. 22.9 $\mu g \cdot L^{-1}$; *t*-test; $P = 0.916$); however, the initial particulate carbon concentration inside the dialysis bag was significantly higher in the grazing treatment (mean ± SD; 0.29 ± 0.08 $mmol \cdot L^{-1}$) than in the control (0.04 ± 0.01 $mmol \cdot L^{-1}$; *t*-test; $P = 0.006$), indicating a substantial presence of nonphytoplankton particles (Fig. 1). The specific growth rate calculated based on changes in particulate carbon concentration over the 15 d incubation was 0.07 ± 0.00 d^{-1} for the grazing treatment, which was significantly lower than that for the control (0.16 ± 0.12 d^{-1}; *t*-test; $P = 0.002$), likely a result of zooplankton feeding activities.

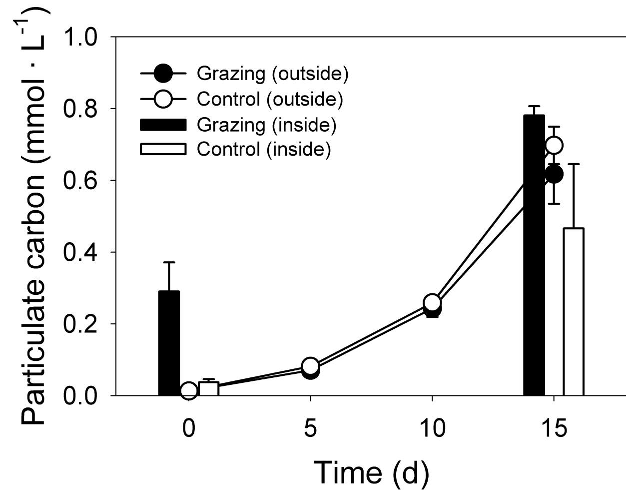


Fig. 1. Particulate carbon concentration inside (mean + SD; $n = 3$) and outside (mean ± SD; $n = 3$) the dialysis bags in the grazing treatment and the control.

Outside the dialysis bags the >20 μm size fraction, presumably dominated by colonies (see below), accounted for 70% of the total chl at the beginning of the experiment (Fig. 2). Growth rate was calculated based on chl changes in the >20 μm and the <20 μm fractions. Because two different aliquots were used for the 0.7 μm filter and the 20 μm filter, variation between the aliquots led to cases where the >20 μm chl was higher than the >0.7 μm chl in two of the replicates in the control on day 15. These two replicates were excluded in the calculation of growth rates. Average growth rate was 0.28–0.30 d^{-1} for the >20 μm fraction and 0.25–0.29 d^{-1} for the <20 μm fraction and was not significantly different between the grazing treatment and the control (*t*-test; $P = 0.07$).

Table 1. Initial zooplankton composition and concentration (m^{-3}) inside dialysis bags (2 L volume) in grazing treatment and control.

	Oithona similis	*Microcalanus pygmaues*	*Oncaea curvata*	*Metridia gerlachi*	*Ctenocalanus vanus*	Copepod nauplii	*Limacina antarctica*	Cladocerans
Grazing treatment								
Mean	2.3×10^5	1.1×10^5	1.4×10^5	3.3×10^3	3.3×10^3	1.0×10^4	3.3×10^3	2.0×10^4
SD	4.4×10^4	4×10^4	5.1×10^4	5.8×10^3	5.8×10^3	BDL	5.8×10^3	2.6×10^4
Control								
Mean	6.7×10^3	3.3×10^3	3.3×10^3	BDL	BDL	BDL	BDL	BDL
SD	1.2×10^4	5.8×10^3	5.8×10^3	BDL	BDL	BDL	BDL	BDL

SD, standard deviation of triplicate; BDL, below detection limit.

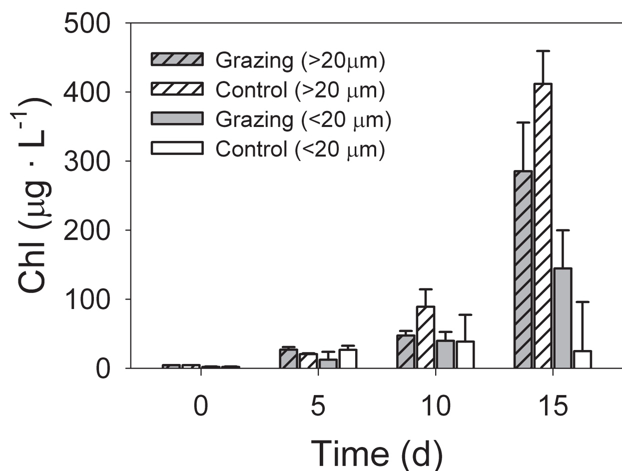
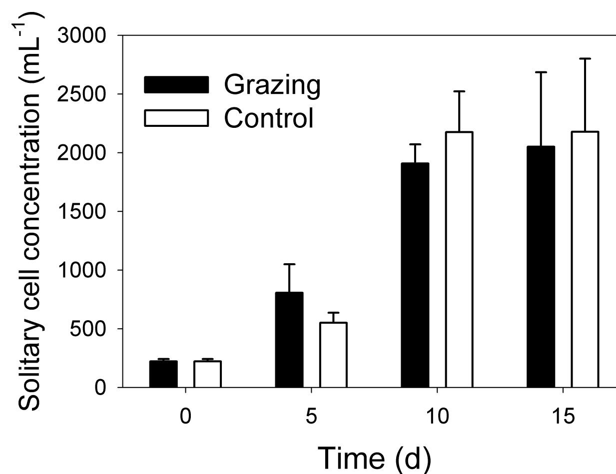


Fig. 2. Size-fractioned chl *a* concentration (mean + SD; $n = 3$) outside the dialysis bags in the grazing treatment and the control.

Microscopic counts confirmed that the phytoplankton outside the dialysis bags was overwhelmingly *P. antarctica*, and only a few diatoms were observed in some of the samples toward the end of the experiment. Solitary cell concentration increased from 0.2×10^3 to 2×10^3 cells · mL^{-1} over the 15 d incubation (Fig. 3). The solitary cell growth rate was not different between the control and the grazing treatment (0.15 ± 0.02 d^{-1}; *t*-test; $P = 0.72$). Colonial concentrations increased from 32 colonies · mL^{-1} to >150 colonies · mL^{-1} by day 10 and then decreased slightly toward the end of the experiment (Fig. 2). While there was an overall significant change in colonial concentration over time, there was no significant difference between the grazing treatment and the control (two-way analysis of variance [ANOVA]; $P = 0.17$ for treatment; $P = 0.75$ for interaction). Similarly, particulate carbon concentration outside the dialysis bag increased exponentially in both the grazing treatment and the control (Fig. 1), and the calculated growth rates were not significantly different between the two (0.26 ± 0.01 d^{-1} vs. 0.27 ± 0.01 d^{-1}; *t*-test; $P = 0.24$).

The geometric mean colony size significantly differed between the grazing treatment and the control over time (two-way repeated measures ANOVA; $P = 0.003$ for time, 0.003 for treatment, 0.006 for interaction; $n = 3$) (Fig. 4). The range of colony size increased through time in the control, but the geometric mean of the colonial diameter actually decreased from 106 to 69 µm, and the median diameter decreased from 102 to 49 µm, indicating that the colony size distribution shifted toward smaller sizes as the experiment progressed. An opposite trend was observed in the grazing treatment: the geometric mean colonial diameter increased from 106 to 137 µm, and the median colony diameter increased from 102 to 156 µm.



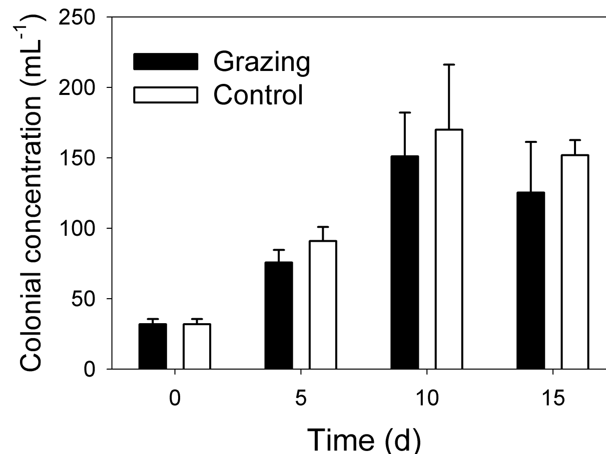


Fig. 3. *Phaeocystis antarctica* solitary cell (upper panel) and colony (lower panel) concentrations (mean + SD; $n = 3$) outside the dialysis bags in the grazing treatment and the control.

Using the colonial volume to cell abundance relationship described by Mathot et al. (2000) for *P. antarctica*, we estimated the colonial cell abundance from the geometric mean colonial diameter. We also calculated the total solitary and colonial cell carbons based on the data of Mathot et al. (2000). The calculated mean colonial volume was on the order of 10^5–10^6 µm^3, well within the range observed by Mathot et al. (2000). The estimated cell abundance per colony increased by 59% in the grazing treatment, but decreased by 54% in the control (Table 2). Colonial cell concentration increased by 5-fold in the grazing treatment, but only doubled in the control. When combined with the solitary cell concentration data, we estimated that from day 5 onward, cellular partitioning skewed toward the colonial phase significantly more in the grazing treatment than in the control (Table 2; paired *t*-test, $P = 0.022$). The ratio of colonial:solitary cell carbon was much greater than 1, and significantly higher in the grazing treatment than in the control from day 5 onward, indicating that significantly more cell

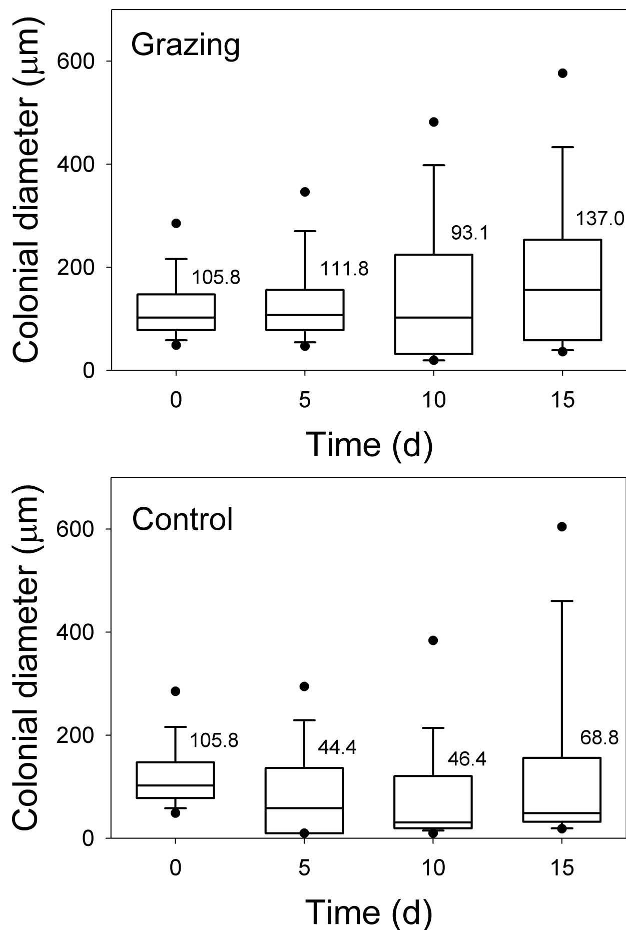


FIG. 4. *Phaeocystis antarctica* colony diameter outside the dialysis bags in the grazing treatment (upper panel) and the control (lower panel). For illustrative purposes, data are pooled from triplicates and presented in box-and-whisker plots showing 95, 90, 75, 50, 25, 10, and 5 percentiles; numbers next to each box indicate the geometric mean diameter of the pooled data. The geometric mean for individual replicate (n = 3) was used to test for differences between grazing treatment and control by two-way repeated measures ANOVA. See text for explanation.

carbon was allocated to the colonial phase in the grazing treatment (Table 2; paired t-test, $P = 0.015$).

DISCUSSION

Because most trophic processes among plankton are size dependent (Hansen et al. 1997), the ability of phytoplankton to increase and maintain large size, such as by forming colonies, could be an effective means of defense against small grazers. A well-studied example is the colony-forming freshwater chlorophyte *Scenedesmus* sp., which greatly increases in colony size in the presence of daphnid grazers (Lürling and van Donk 1997, Lürling 1999). Formation of mucilaginous colonies is one of the most remarkable features in the life cycles of *P. globosa*, *P. pouchetii*, and *P. antarctica* (Rousseau et al. 1994). Unlike the chain-forming colonies of *Scenedesmus* sp., *Phaeocystis* colonies are hollow spheres with individual cells embedded in a thin mucous skin. Colonial cell abundance and colony diameter exhibit a linear log-log relationship with a slope < 2 (Rousseau et al. 1990, Jakobsen and Tang 2002), indicating that cell spacing within the colonial matrix increases as the colony grows bigger. The ability of *Phaeocystis* to form colonies that are up to several orders of magnitude larger than solitary cells has led to the suggestion that colony formation is a defense strategy that creates a size-mismatch problem for small grazers (Weisse et al. 1994, Hamm et al. 1999). The presence of grazers has been shown to induce *P. globosa* to form significantly larger colonies (Jakobsen and Tang 2002). The present study also demonstrated, for the first time, a similar effect in *P. antarctica*—namely, that the presence of grazers resulted in significantly larger colonies. Because the dialysis bag physically separated the grazers from *P. antarctica* in the incubation, we conclude that the grazing effect was communicated via a chemical that diffused across the dialysis

TABLE 2. Estimated colonial volume, cell abundance per colony, colonial cell concentration, and carbon partitioning in the experiment.

	Day 0		Day 5		Day 10		Day 15	
	Grazing	Control	Grazing	Control	Grazing	Control	Grazing	Control
Colonial volume (μm^3)	6.20×10^5	6.20×10^5	7.33×10^5	4.58×10^4	4.22×10^5	5.23×10^4	1.35×10^6	1.71×10^5
Cell abundance per colony	79.34	79.34	87.71	16.68	63.06	18.05	126.34	36.67
Colonial cell concentration (mL^{-1})	2539	2539	6636	1518	9531	3069	15834	5574
Colonial cells (% of total)	92.0	92.0	89.2	73.3	83.3	58.5	88.5	71.9
Colonial-to-solitary cell carbon ratio	46.6	46.6	33.61	11.24	20.4	5.76	31.54	10.45

Colonial volume was calculated from geometric mean colony diameter using the formula for a sphere.

Cell abundance per colony was calculated from colonial volume following Mathot et al. (2000).

Colonial cell concentration was calculated as [Cell abundance per colony × Average colony concentration].

Percentage of cells in colonies was calculated as [Colonial cell concentration/(Colonial cell concentration + Solitary cell concentration)] × 100.

Cell carbon concentrations were calculated using the conversion factors 3.33 pg C per solitary cell and 13.60 pg C per colonial cell (Mathot et al. 2000).

membrane, similar to the observations by Tang (2003) and Long et al. (2007) for *P. globosa*.

A striking similarity between our results and those from the study by Tang (2003) is that the stimulatory effect was not necessarily related to grazing on *Phaeocystis*. In this study, non-*Phaeocystis* algae (indeed, nonhaptophyte algae) were used to enhance grazing activity inside the dialysis bags. The larger amount of non-chlorophyll-containing particulate carbon in the grazing treatment also suggests the significant presence of non-phytoplankton food source for the zooplankton. Indeed, *Oithona*, *Microcalanus,* and *Oncaea* spp. are generally considered to be omnivorous copepods. The present and earlier results suggest that the chemical signal in question is likely a general indicator of ambient grazing activity, rather than a signal specific to the mortality of *Phaeocystis* cells or colonies. We do not know the characteristics of this chemical, except that its molecular weight does not exceed 12–14 kDa (the molecular weight cutoff of the dialysis membrane). Also, while the chemical signal appeared to be related to zooplankton grazers, we could not rule out the possibility that the signal came from microbes associated with the grazers rather than the grazers themselves directly.

Another important observation in this study is that *P. antarctica* growth rates, in terms of chl, particulate carbon, or cell concentration, were not different between the grazing treatment and the control. Thus, our results show that grazing signal caused *P. antarctica* to shift its colony size without affecting the growth rate of the cells within the colony. Similar to the observations by Tang (2003) with *P. globosa*, *P. antarctica* maintained its colonial forms even in the absence of grazers. This finding indicates that grazing is not the only cause for colony formation. Also, the colony sizes observed in both studies were substantially smaller than the maximum colony sizes observed in nature, indicating that other environmental factors also play a role in regulating colony size in situ. There is, however, an important difference between the two studies. Tang (2003) reported that *P. globosa* colony size increased in both grazing treatment and control, although significantly less so in the control. In the present study, the mean size of *P. antarctica* colonies actually decreased in the control. If increasing colony size results from a defense mechanism by *P. antarctica* against grazers, one would indeed expect the colonies to reduce their size when ambient grazing activity is low (as we observed).

We cannot, however, directly extrapolate our results to the field, because many experimental conditions were substantially different from those encountered in the Ross Sea and other regions of the Antarctic. For example, the food and the grazer concentrations used to generate the chemical signal were both much higher than normally found in the Ross Sea. Iron concentrations were likely elevated over in situ conditions, which is important since iron has been suggested as a factor influencing colony formation (Sedwick et al. 2007). As we do not know the nature of the chemical, we cannot predict its half-life and turnover in situ. While the presence of grazers clearly influences the colony size of *P. antartica*, it does so along with other environmental factors, and the resultant size spectrum of the *P. antarctica* population is undoubtedly the result of a complex interaction among them all.

While these and other results (Jakobsen and Tang 2002, Tang 2003) are consistent with the argument that colony formation and enlargement is a defense strategy against small grazers, defense theory (Mole 1994, Agrawal 1998) suggests that there must be a cost associated with colony formation. Attempts to identify such a cost, however, have not been successful (Jakobsen and Tang 2002). We calculated the specific growth rates for solitary cells and colonial cells for the different time intervals in the grazing treatment and determined that there was no statistical difference between the two (paired *t*-test, $P = 0.694$, $n = 3$). Hence, there was no discernible cost in colony formation in terms of cell growth in the present study. It should, however, be emphasized that the colonial cell abundance was estimated based on a published algorithm (Mathot et al. 2000), and as such, the calculated specific growth rate for colonial cells may not be accurate enough to reveal small differences from solitary cells. Also, while there is no noticeable cost associated with cell growth rate over a short duration (15 d), there may be a cost over longer durations or with other cellular processes, such as nutrient uptake and photosynthesis. Regardless of the cost, enhanced colony size as influenced by ambient grazers would not only affect the planktonic trophic processes in regions dominated by *P. antarctica*, but also, by shifting the cell biomass partitioning, affect carbon biogeochemistry in the water column.

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