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Temperature effects on growth, colony development and carbon partitioning in three *Phaeocystis* species

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ABSTRACT: *Phaeocystis* is an ecologically important marine phytoplankton genus that is globally distributed. We examined the effects of temperature on the 3 most common species: *P. globosa*, *P. antarctica*, and *P. pouchetii*, which grew at 16–32, 0–6, and 4–8°C, respectively. *P. pouchetii* did not form colonies; *P. globosa* formed colonies at 16, 20, and 24°C, and *P. antarctica* colonies were observed at all temperatures. More cells were partitioned into the colonial form at lower temperatures than at higher temperatures for *P. globosa* and *P. antarctica*. *P. globosa* colony size decreased with temperature, whereas *P. antarctica* colony size showed no distinct response to temperature. Numbers of cells per unit of colony surface area of *P. globosa* and *P. antarctica* were lowest at temperatures where highest growth rates and colonial abundances were observed; more organic carbon was partitioned into solitary cell biomass at higher temperatures, whereas the carbon concentration of colonies was not affected by temperature. Maximum quantum yield of *P. antarctica* and *P. globosa* exhibited subtle responses to temperature, whereas that of *P. pouchetii* was relatively invariant within the growth temperature range. Future changes in sea surface temperature may dramatically alter the ecology and biogeochemical cycles of systems dominated by *Phaeocystis* spp. and result in further degradation, via oxygen depletion and altered food web structure.

KEY WORDS: *Phaeocystis* spp. · Temperature · Colony formation · Carbon partitioning

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INTRODUCTION

The genus *Phaeocystis* (Prymnesiophyceae) is one of the most widespread marine phytoplankton taxa, and plays a significant role in global carbon and sulfur cycles, food web structure, and climate regulation (Lancelot et al. 1998, Schoemann et al. 2005). It has the ability to form massive blooms that have substantial impacts on coastal systems, and it has a complex polymorphic life cycle that involves both colonial and solitary forms. The solitary cells, either flagellated or non-flagellated, are typically 3 to 9 µm in diameter. The colonial stage, with cells embedded in a polysaccharide gel matrix, may reach up to 3 cm in diameter (Rousseau et al. 1994, Chen et al. 2002). The organic matrix may provide an energy or nutrient source during periods of darkness or heterotrophic growth by the cells (Lancelot & Mathot 1985, Schoemann et al. 2001), but also represents a considerable carbon and energy demand for the individual cells. The success of *Phaeocystis* is often attributed to its ability to form colonies, which potentially provide protection for the colonial cells against grazers, viruses, and bacteria (Hamm et al. 1999, Jakobsen & Tang 2002, Brussaard et al. 2005). Of the 6 described *Phaeocystis* species, 3 are widespread and regularly develop blooms in colonial form: *P. globosa*, *P. pouchetii*, and *P. antarctica* (Medlin & Zingone 2007).

Phaeocystis is also a harmful algal genus in coastal waters (Lancelot et al. 1998). It forms dense blooms in the eutrophic North Sea (Lancelot et al. 1987), although observations and continuous plankton records show that it has occurred in the North Atlantic since the early 19th century (Cadée & Hege-

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man 2002, Gieskes et al. 2007). The blooms reach a high biomass and generate both hypoxic zones at depth (Peperzak & Poelman 2008) and dense organic films on beaches (Lancelot et al. 1987). They have both an ecological impact on the local food web and a negative economic influence on tourism. Dense blooms have also been observed off the coasts of southeast China (Qi et al. 2004), Vietnam (Tang et al. 2004), and Norway (Larsen et al. 2004). The clones isolated from Chinese and Norwegian waters have hemolytic properties as well (Shen et al. 2004, van Rijssel et al. 2007).

Different *Phaeocystis* species occur over different temperature ranges in the ocean (Schoemann et al. 2005). For example, *P. pouchetii* grows at temperatures below 5°C, and the reported optimal temperature range for North Sea *P. globosa* is 15 to 20°C (Schoemann et al. 2005). The temperature range of *P. pouchetii* overlaps with that of *P. globosa* (Baumann et al. 1994), such that *P. pouchetii* is also found in warm waters (Philippart et al. 2000). *P. antarctica* is confined to low temperatures and ceases to grow above 7°C (Buma et al. 1991). In general, *P. globosa* blooms in temperate and tropical regions, *P. pouchetii* in subarctic waters, and *P. antarctica* in the Southern Ocean (Schoemann et al. 2005). Temperature affects physiological responses of *Phaeocystis* in addition to altering growth. For example, *P. antarctica* releases more dimethyl sulfide at lower temperatures (Baumann et al. 1994). Furthermore, the cellular carbon content of *P. pouchetii* is greater at low than at high temperatures (Jahnke 1989), and solitary cells of *P. pouchetii* have higher photosynthetic and excretion rates than colonial cells at low temperatures (Verity et al. 1991). The carbon to chlorophyll *a* (chl *a*) ratio and cell size of *P. pouchetii* solitary cells vary inversely with temperature, whereas colonial photosynthesis is positively related to temperature (Verity et al. 1991). When subjected to a rapid temperature reduction, colonial cells of *P. pouchetii* develop flagella, become motile, and emigrate from the colonies (Verity et al. 1988).

While these individual studies collectively suggest that temperature plays a key role in *Phaeocystis* biology and ecology, the different strains and experimental designs used by the investigators make it difficult to compare and predict the response of different *Phaeocystis* species to temperature. In addition, because of the complex life history of the genus, it remains uncertain how temperature might affect the relative abundances of solitary and colonial cells, as well as its influence on photosynthetic capacity and carbon partitioning. These questions are particularly relevant to understanding how *Phaeocystis* may affect the marine food web and biogeochemistry in future climate scenarios.

The objective of this study was to investigate the growth and biological responses of the 3 major *Phaeocystis* species (*P. globosa*, *P. pouchetii*, and *P. antarctica*) to temperature under defined experimental conditions. Colonial and solitary cell abundances, colony abundance and size, quantum yield, and particulate organic carbon contents of the 3 species grown under a range of temperatures were determined. We hypothesized that temperature would significantly influence the growth of cells, colony formation, photosynthesis, and the partitioning of organic carbon among solitary cells, colonial cells, and the colonial matrix.

MATERIALS AND METHODS

Stock maintenance. *Phaeocystis globosa* (CCMP 1528; originally isolated from the South Pacific) and *P. antarctica* (CCMP 1871; originally isolated from the Bellingshausen Sea) were obtained from the Culture Collection at Bigelow Laboratory (Maine, USA). A culture of *P. pouchetii* (AJ 01; originally isolated from the Norwegian coast) was provided by J. Nejstgaard (University of Bergen, Norway). These strains were used in previous studies of *Phaeocystis* biology and ecology (Brussaard et al. 2001, Tang 2003, Elliott et al. 2008, Wang et al. 2010). *P. globosa* CCMP 1528 and *P. antarctica* CCMP 1871 regularly form colonies, while *P. pouchetii* only grows as solitary cells in the laboratory. The 3 species were maintained at 20, 0, and 8°C, respectively, in *f*/2 medium (Guillard & Ryther 1962) with a salinity of 32 under an irradiance of 100 µmol photons m^{-2} s^{-1} on a 12:12 h light:dark cycle. The stocks were maintained in exponential growth by regular dilutions with fresh medium.

Experimental cultures. We first grew the 3 *Phaeocystis* species under defined conditions to establish growth curves and determine when biomass became maximal. Solitary *Phaeocystis* cells were collected by passing the stocks twice through a 10 µm nylon sieve under gravity, and were placed in triplicate 1 l flasks at an initial cell density of 10^4 cells ml^{-1} (500 ml $flask^{-1}$). All cultures were grown in growth chambers using *f*/2 medium with a salinity of 32 under an irradiance of 350 µmol photons m^{-2} s^{-1}, but at different temperatures (±1°C): *P. globosa* at 20°C, *P. antarctica* at 0°C, and *P. pouchetii* at 8°C (Table 1). During incubations, the flasks were shaken gently by hand daily to avoid excessive cell settlement. *In vivo* chl *a* was measured daily in triplicate flasks at each temperature to establish growth curves from lag through senescent phases, and the maximum instantaneous growth rates were determined from the inflection point of the growth curves. Next, 3 sets of new flasks were initiated under the same conditions using the previous cultures as

Table 1. *Phaeocystis* spp. Temperatures and maximum growth rates (mean ± SD; n = 3). Starting temperatures for each species are shown in bold. Temperature was altered by 2 to 4°C between experiments. NG: no growth

	Temperature (°C)	Maximum growth rate (d^{-1})
P. globosa	32	0.54 ± 0.00
	28	0.59 ± 0.04
	24	0.50 ± 0.08
	20	1.10 ± 0.38
	16	0.19 ± 0.11
	12	NG
	8	NG
P. antarctica	8	NG
	6	0.12 ± 0.03
	4	0.35 ± 0.02
	2	0.17 ± 0.04
	0	0.16 ± 0.00
	–2	NG
P. pouchetii	16	NG
	12	NG
	8	0.50 ± 0.06
	6	0.19 ± 0.01
	4	0.23 ± 0.01
	2	NG
	0	NG
	–2	NG

inocula, and the cultures were grown to maximum biomass based on the previously determined growth curves. They were then harvested for the determination of biomass and photosynthetic parameters. Once an experiment for a specific temperature was completed, remaining aliquots were inoculated with fresh medium and transferred to a different temperature. The temperature was altered by 2 to 4°C between experiments for a number of generations, allowing the phytoplankton to gradually acclimate to the new temperature (Table 1). Once the cultures resumed active growth at the new temperature, aliquots were taken to initiate new growth curves and experiments.

Microscopy and chlorophyll measurements. Samples for microscopic enumeration of cell and colony abundances were preserved in Lugol's solution. Solitary cell concentrations were measured using Sedgwick-Rafter chambers (Tang 2003). Colony concentration, colony size, and cells per colony were enumerated in 24-well multi-plates using a Nikon inverted microscope with a calibrated micro-ruler (Tang 2003). Aliquots of known volume were filtered through Whatman GF/F filters to obtain total chl *a*, and through 20 µm polycarbonate membranes (Poretics) to obtain the >20 µm fraction, which was taken as representative of colonial chlorophyll (Tang et al. 2008). All chl *a* samples were extracted in darkness at 0°C for 24 h in 7 ml of 90% acetone; fluorescence of extracted chl *a* was measured on a TD-700 fluorometer (Turner Designs) before and after acidification (Parsons et al. 1984). The fluorometer was calibrated using commercially prepared chl *a* (Turner Designs, purity 98.0%).

Maximum quantum yield of PSII (F_v/F_m). To assess the response of potential photosynthesis to temperature, we measured the potential quantum yield using fluorescence techniques. *Phaeocystis* cultures were dark-adapted for 30 min, after which the minimal (F_0) and maximal (F_m) fluorescence were measured before and after addition of DCMU solution (final concentration of 10^{-5} M) on a TD-700 fluorometer. Maximum quantum yield of PSII was calculated as: $F_v/F_m = (F_m–F_0)/F_m$, where F_v is variable fluorescence (Vincent 1980).

Particulate organic carbon. All glassware, vials, and GF/F filters used to measure particulate organic carbon (POC) were pre-combusted (4 h, 450°C). An aliquot of 20 to 40 ml from each experimental flask was filtered through GF/F filters to collect the total POC (POC_T; µg ml^{-1}). To obtain the solitary cell POC fraction (POC_{SC}; µg ml^{-1}), solitary cells were collected by filtering 20 ml aliquot through a 10 µm sieve under gravity and then onto a GF/F filter. Based on known solitary cell concentration (see above), we estimated the carbon content of individual solitary cells. An additional 50 ml aliquot of a mixture of colonies and solitary cells was vortexed for 5 to 10 min; microscopic examination confirmed that this procedure released some (but not all) of the colonial cells while keeping the organic gel matrix relatively intact. The vortexed sample was passed through a 10 µm sieve to collect all single forms (that is, solitary + released colonial cells) in the filtrate. The abundance of released colonial cells was then obtained by subtracting the corresponding number of solitary cells based on the independently determined solitary cell abundance in the same aliquot volume before vortexing. The filtrate (solitary + released colonial cells) was filtered onto GF/F filter to obtain POC, from which the corresponding amount of solitary cell carbon was subtracted to calculate POC per colonial cell. This value was then scaled up to the total colonial cell abundance in the experimental flask (see above) to obtain the colonial cell POC fraction (POC_{CC}; µg ml^{-1}). The POC fraction from matrix material was then calculated as POC_M (µg ml^{-1}) = POC_T – POC_{SC} – POC_{CC}.

All samples for POC were rinsed with ca. 5 ml of 0.01N HCl in seawater to remove any inorganic carbon adsorbed to the filter (Gardner et al. 2000), and then dried in glass vials at 60°C. Dried filters were analyzed for carbon by flash combustion using a Carlo-Erba Model EA 1108 elemental analyzer. Similar amounts of culturing medium were filtered and processed in the same manner as blanks to correct the sample carbon values.

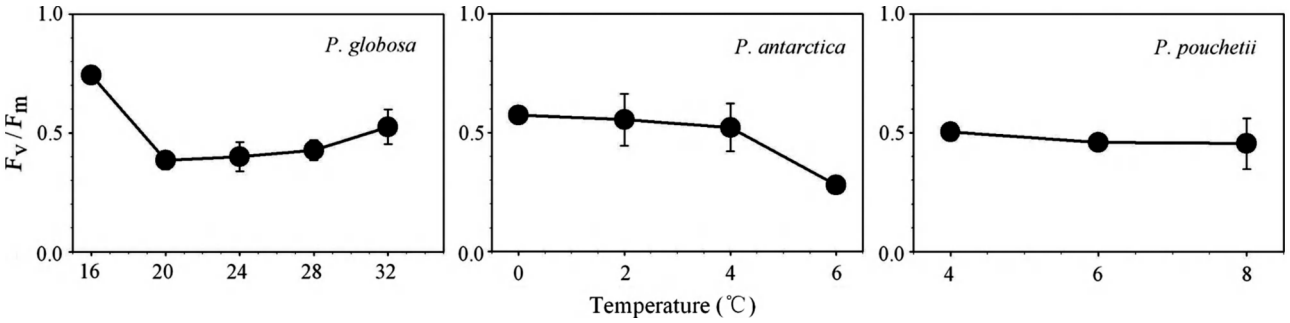

Fig. 1. *Phaeocystis* spp. Quantum yields (F_v/F_m; mean ± SD; n = 3; see 'Materials and methods' for definitions of terms) at different temperatures

Statistical analysis. SigmaStat (v. 3.50 SPSS) and Minitab (v. 15) were used for statistical analyses. Statistical comparisons of the effects of temperature on growth, F_v/F_m, cell abundance, partitioning of cells, chlorophyll, and POC were made by 1-way ANOVA. Prior to ANOVA, percentage data were normalized by an arcsine transformation (Zar 1984). In cases where data did not satisfy the criteria for parametric tests, 1-way ANOVA on ranks was used. *Post hoc* comparison following ANOVA was done by Dunn's method, the Holm-Sidak method, or a Tukey test. Linear regressions were fit to log cells colony^{-1} versus log colony diameter, and comparison of the slopes of regressions between temperatures was done by 1-way analysis of covariance (ANCOVA). Correlation between different parameters was tested by a Pearson correlation test. The significance level for all statistical tests was *a priori* set at a critical p value of 0.05.

RESULTS

Growth and maximum quantum yield

The temperature ranges over which *Phaeocystis globosa*, *P. antarctica*, and *P. pouchetii* grew were 16 to 32, 0 to 6, and 4 to 8°C, respectively (Table 1). Within these temperatures, the maximum growth rates based on *in vivo* chlorophyll measurements were observed at 20, 4, and 8°C, respectively, for the 3 species (Table 1). The growth of *P. globosa* decreased significantly from 20 to 16°C ($p < 0.05$); however, there was no significant difference in growth among 24, 28, and 32°C, although all 3 were less than the maximum observed at 20°C. The growth rate of *P. antarctica* increased significantly from 0 to 4°C ($p < 0.05$), and then decreased at 6°C. Similarly, the growth of *P. pouchetii* was also affected by temperature ($p < 0.01$), and reached a maximum at 8°C.

The differences in F_v/F_m among temperatures were not significant for *Phaeocystis pouchetii* ($p > 0.05$) and ranged from 0.46 to 0.51 (Fig. 1). F_v/F_m of *P. globosa* was markedly higher at 16°C, but decreased at 20°C before increasing slightly at 32°C ($p < 0.001$). F_v/F_m of *P. antarctica* was significantly lower at 6°C than at other temperatures ($p < 0.05$; Fig. 1).

Colony and cell abundances

Unlike in nature, colony development did not occur in our *Phaeocystis pouchetii* culture. *P. globosa* formed colonies at 16, 20, and 24°C, whereas *P. antarctica* colonies were observed in all 4 temperature treatments (Fig. 2). Colony abundance was highest at 20°C for *P. globosa*, and at 4°C for *P. antarctica*. Solitary cell abundance of *P. globosa* differed significantly between temperatures ($p < 0.01$), and was highest at 28°C (Fig. 3). The solitary cell abundances of *P. antarctica* and *P. pouchetii* were also affected by temperature ($p < 0.05$).

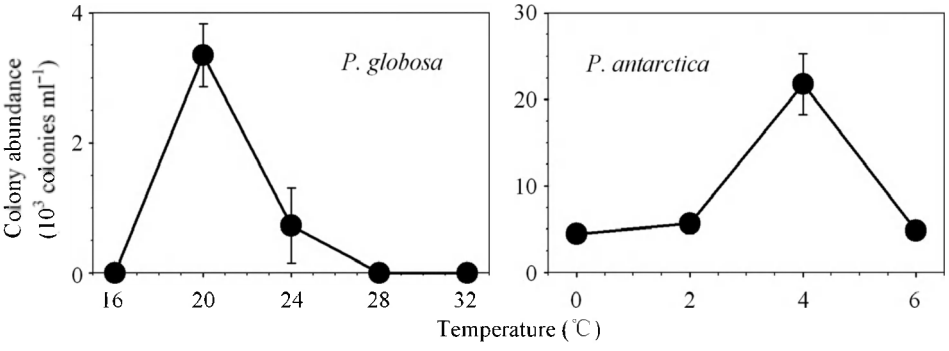

Fig. 2. *Phaeocystis* spp. Colony abundances (mean ± SD; n = 3) at different temperatures

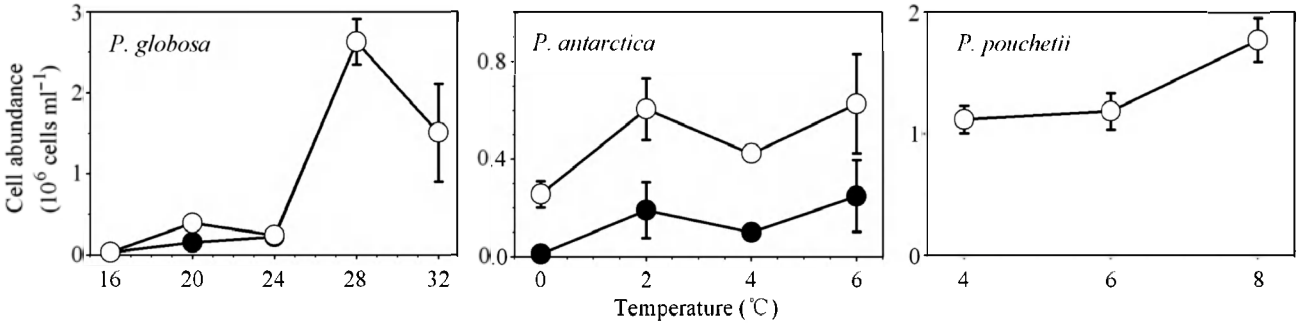

Fig. 3. *Phaeocystis* spp. Solitary cell (solid circles) and total cell (open circles) abundance at different temperatures (mean ± SD; n = 3)

Highest solitary cell concentration was at 6°C for *P. antarctica* and at 8°C for *P. pouchetii*. The total cell abundances (solitary + colonial) of *P. globosa* at 16, 20, and 24°C were far lower than the total cell abundances at higher temperatures ($p < 0.01$; Fig. 3). Unlike *P. globosa*, the total cell abundance of *P. antarctica* was not affected by temperature ($p > 0.05$).

Partitioning of cells and chlorophyll

Temperature strongly influenced the partitioning of cells between solitary and colonial forms (Fig. 4). *Phaeocystis globosa* existed exclusively as solitary cells at 28 and 32°C, and <10% of the total cells were in colonial form at 16 and 24°C. However, colonial cells contributed >60% to the total *P. globosa* cells at 20°C ($p < 0.001$; Fig. 4A). For *P. antarctica*, the colonial form was most dominant at 0°C, accounting for >95% of the total cells, but this percentage decreased at the higher temperatures, so that slightly over 60% of the cells were in colonial form at 6°C ($p < 0.05$; Fig. 4B).

Size-fractioned chlorophyll was also significantly different among temperatures. The proportion of the >20 µm chlorophyll fraction in *Phaeocystis globosa* was highest at 20°C ($p < 0.01$), contributing >55% to the total chlorophyll (Fig. 4C). This size fraction accounted for 51% of the total chlorophyll in *P. antarctica* at 0°C, and the percentage decreased to a minimum of 8% at 4°C (Fig. 4D). Because some small colonies could have passed through the 20 µm membrane filters, it was expected that the >20 µm chlorophyll size fraction

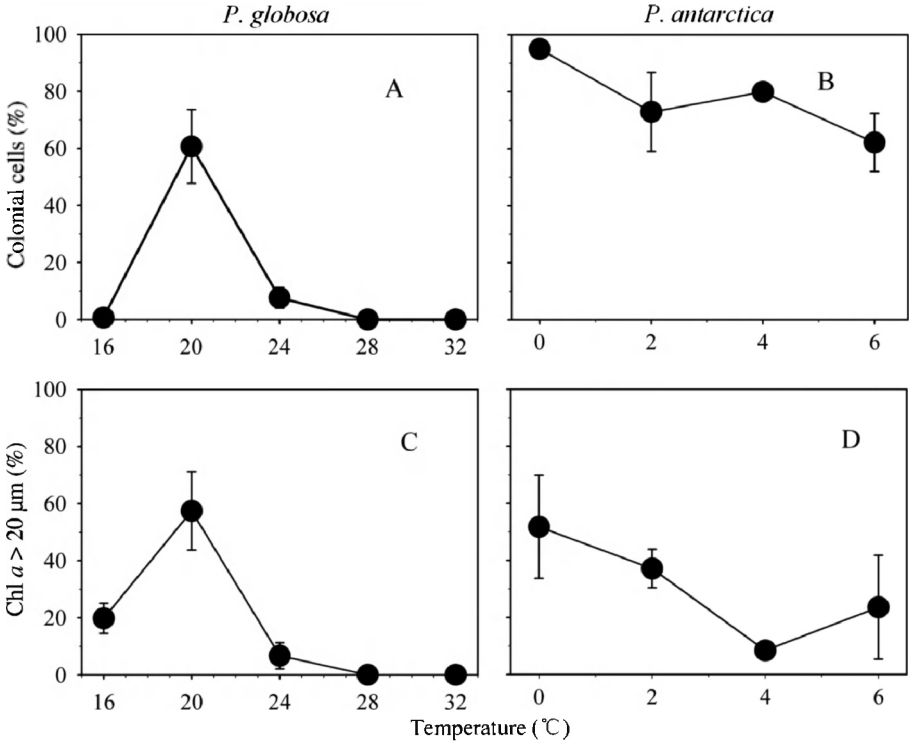

Fig. 4. *Phaeocystis* spp. Percentages of colonial cells to total cells for (A) *P. globosa* and (B) *P. antarctica*, and percentages of >20 µm chlorophyll to total chlorophyll for (C) *P. globosa* and (D) *P. antarctica* (mean ± SD; n = 3)

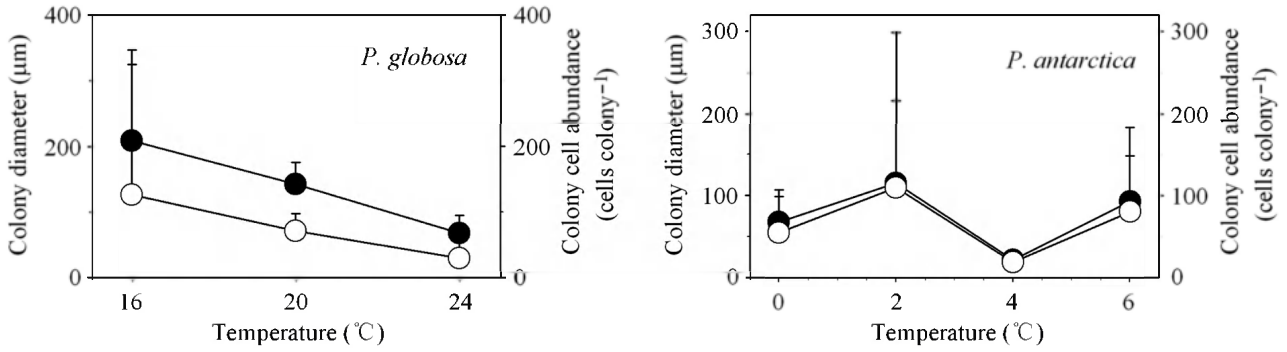

Fig. 5. *Phaeocystis* spp. Colony diameters (solid circles) and colonial cell abundances (open circles) of *P. globosa* and *P. antarctica* at different temperatures (mean + SD; n = 3)

underestimated colony abundances. Nevertheless, the trend of chlorophyll size distribution was significantly correlated to the distribution of cell types ($p < 0.001$), with the highest percentages of >20 µm chlorophyll coinciding with the highest colonial cell percentages.

Colony morphology

Colony diameters of *Phaeocystis globosa* were inversely correlated to temperature ($p < 0.01$), and decreased from an average of 208 µm at 16°C to 67 µm at 24°C (Fig. 5A). The response of *P. antarctica* colony size to temperature showed a different trend (Fig. 5B): colony diameter was smallest at 4°C, and largest at 2 and 6°C ($p < 0.001$). In all cases where colonies developed, there was a significant linear log-log relationship between the number of cells colony^{-1} and colony diameter ($p < 0.001$) with a slope <2, indicating that the number of cells per unit of colony surface area decreased as the colonies increased in size (Fig. 6). For *P. globosa*, the slope of the regression was significantly smaller at 20°C (1.28) than at 16 (1.65) and 24°C (1.64; $p < 0.01$). Thus, colonies at 16 and 24°C had more cells per unit of colony surface area than at 20°C. Temperature also affected cell distribution in *P. antarctica* colonies, and the number of cells per unit of colony surface area decreased at 4°C relative to other temperatures ($p < 0.01$).

Partitioning of POC

The concentrations of both total carbon and solitary cell carbon were higher at higher temperatures for all 3 species. For example, the total carbon and single cell carbon levels in *Phaeocystis globosa* were lower at 16, 20, and 24°C than at 28 and 32°C ($p < 0.05$). For *P. antarctica*, total carbon and solitary cell carbon concentration at 0°C were lower than at 2, 4, and 6°C. The carbon concentration of *P. pouchetii* also increased with temperature ($p < 0.001$). In all cases, the differences in colonial cell and matrix carbon concentrations between temperatures were not significant ($p > 0.05$), suggesting that temperature did not affect the colonies' carbon concentration.

Temperature affected the partitioning of POC in *Phaeocystis antarctica* and *P. globosa* ($p < 0.05$; Table 2). Except for *P. globosa* at 16°C, the percentages of solitary cell carbon were higher at higher temperatures for both species. For example, the percentage of solitary cell carbon of *P. antarctica* increased from 1.9% at 0°C to 68% at 6°C ($p < 0.01$). The percentages of colonial cell carbon and matrix carbon in *P. antarctica* and *P. globosa* were also affected by temperature ($p < 0.01$; Table 2).

DISCUSSION

Global distribution

The objective of this study was to understand how changes in temperature might influence the growth of *Phaeocystis*, especially in view of the current and anticipated warming of the world's oceans. The 3 species of *Phaeocystis* exhibited very different growth responses to temperature. In our study, *P. globosa* grew at 32°C, which is higher than the previously reported maximum growth temperature (30°C; Schoemann et al. 2005). Blooms of *P. globosa* occur in coastal waters of the South China Sea, where colonies up to 3 cm in size were reported in waters with temperatures of 30°C (Shen et al. 2004). Our clone was originally isolated from the tropical South Pacific, so that its ability to grow at high temperatures may reflect its origin. Peperzak (2003) reported that *P. globosa* derived from the North Sea ceased to grow when temperature increased from 22 to 26°C. Its temperature range was similar to that of the isolate we used, but the absolute

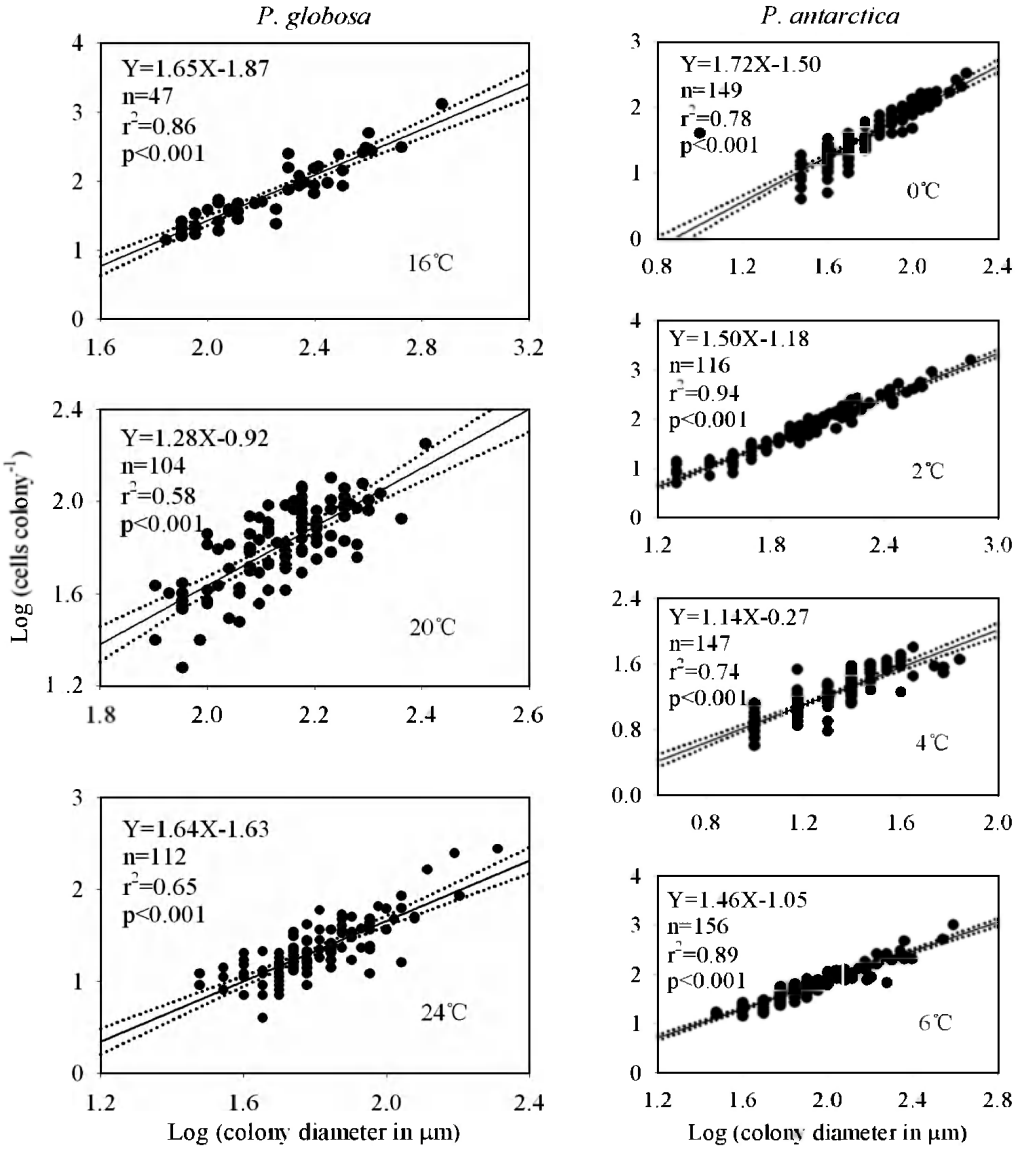

Fig. 6. *Phaeocystis* spp. Relationship between cell abundance per colony and colony diameter of *P. globosa* (left) and *P. antarctica* (right) at different temperatures. Solid lines are linear regressions, and dotted lines are the 95% confidence intervals. n: number of colonies measured

Table 2. *Phaeocystis* spp. Concentrations (mean ± SD; n = 3) and percentages (mean of triplicates) of total particulate organic carbon (POC; in μg C ml^{-1}) for solitary cells, colonial cells, and the colonial matrix of *Phaeocystis* at maximum biomass as a function of temperature (*T*); NC: no colonies

Species	*T* (°C)	Solitary cells		Colonial cells		Colonial matrix		Total POC
		POC	%	POC	%	POC	%	
P. globosa	16	0.08 ± 0.00	~100	0	~0	0	~0	0.08 ± 0.00
	20	3.23 ± 0.53	56.7	2.00 ± 0.37	39.8	0.47 ± 0.52	8.2	5.70 ± 0.07
	24	1.80 ± 0.11	91.4	0.14 ± 0.01	7.1	0.02 ± 0.00	1.0	1.97 ± 0.13
	28	13.0 ± 0.88	100	NC	NC	NC	NC	13.0 ± 0.88
	32	8.48 ± 2.99	100	NC	NC	NC	NC	8.48 ± 2.99
P. antarctica	0	0.04 ± 0.03	1.9	1.74 ± 0.32	81.7	0.35 ± 0.20	16.4	2.13 ± 0.18
	2	1.63 ± 0.70	35.6	2.35 ± 0.69	51.3	0.60 ± 0.15	13.1	4.58 ± 1.54
	4	0.87 ± 0.09	24.3	1.75 ± 0.04	48.9	0.96 ± 0.04	26.8	3.58 ± 0.09
	6	2.57 ± 0.55	68.0	0.96 ± 0.51	25.4	0.25 ± 0.22	6.6	3.78 ± 0.17
P. pouchetii	4	4.54 ± 1.05	100	NC	NC	NC	NC	4.54 ± 1.05
	6	9.07 ± 2.10	100	NC	NC	NC	NC	9.07 ± 2.10
	8	13.50 ± 1.88	100	NC	NC	NC	NC	13.50 ± 1.88

growth temperature was lower. However, because in that experiment the phytoplankton was subjected to a sudden change in salinity (31 to 29 psu) and irradiance (150 to 600 µmol m^{-2} s^{-1}) at the same time, it is difficult to attribute the observations of Peperzak (2003) solely to a temperature effect.

Because we used only 1 *Phaeocystis globosa* clone in our experiments, extrapolating our results to other strains and environments should be done with caution. Nevertheless, our observations suggest that *P. globosa* (e.g. CCMP 1528) may grow at temperatures warmer than those where it has been observed, although whether it can survive at temperatures above 32°C remains to be tested. The *P. globosa* CCMP 1528 strain we used formed colonies regularly at 20°C, but not at temperatures above 24°C. This strain may lack the capability of forming colonies at high temperatures, unlike the clone found in the South China Sea. While the ability to form colonies may be influenced by temperature, the growth response appears to be minimally impacted (Fig. 3). On the other hand, lower growth temperature limits for *P. pouchetii* and *P. antarctica* observed in this study differed from previous studies. We found that these 2 coldwater species did not grow at temperatures below 4 and 0°C, respectively. However, it has been reported that *P. pouchetii* grew at 2°C and *P. antarctica* grew at –2°C (Jahnke 1989). In addition, *P. antarctica* is routinely found actively growing at an *in situ* temperature of –1.8°C (e.g. Smith et al. 2000). The observed differences in growth temperature ranges may also be due to the different strains studied, as well as long-term genetic drift in the cultures.

We found that growth of *Phaeocystis globosa* occurred over the greatest temperature range among the 3 species tested (over 16°C), which parallels the broad latitudinal distribution of this species (Schoemann et al. 2005). Maximum growth was observed at 20°C (Table 1). However, *P. globosa* also exhibited subtle responses to temperature; that is, its maximal biomass, morphology, and photosynthetic potential were all affected by temperature, and these responses were not necessarily coupled to the maximal growth temperature. Indeed, the temperature at which *P. globosa* attained maximal biomass was 28°C, significantly higher than the temperature for maximum growth rate. This response may partially reflect the carbon partitioning between cells and colonies, but cannot be directly determined from our data.

Phaeocystis species isolated from higher latitudes had narrower temperature tolerances than those at lower latitudes. *P. antarctica* had a narrower growth temperature range (6°C), which again is not surprising given the narrow *in situ* range found south of the Antarctic Circumpolar Current. In comparison, *P. pouchetii* grew within the narrowest temperature range in our study (only 4°C), but its physiological responses were relatively insensitive to temperature within this range. Our data suggest that the geographical ranges of *P. antarctica* and *P. pouchetii* are broadly restricted by temperature, whereas the ability of *P. globosa* to bloom in disparate environments may depend more on other factors, such as nutrients, grazing, and irradiance. The highest growth rate for *P. antarctica* was observed at 4°C in our experiments, which is higher than the *in situ* water temperature from –1.86 to 0°C at which *P. antarctica* blooms develop in the Ross Sea (Arrigo et al. 1999, van Hilst & Smith 2002, Shields & Smith 2008), suggesting that *P. antarctica* growth is more strongly regulated *in situ* by light and nutrients than by temperature. However, growth of *P. antarctica* during spring may also be a function of its ability to maximize its growth and photosynthesis during periods of low irradiance induced by high solar angles, relatively deep vertical mixing, and the presence of ice (Moisan & Mitchell 1999, Kropuenske et al. 2009). Its *in situ* distribution, when compared to its optimal growth temperature, suggests that temperature has a relatively minor role in explaining its appearance in coastal Antarctic systems, but would limit its growth outside the Southern Ocean.

Solitary vs. colonial forms

Because the different morphotypes of *Phaeocystis* have very different trophic roles (e.g. Hamm et al. 2001, Smith et al. 2007), temperature-induced changes in cell and carbon partitioning between solitary and colonial forms will inevitably affect many of the ecological processes in systems dominated by *Phaeocystis*. We found that *P. globosa* reached its highest biomass at 28°C but exclusively in solitary form, whereas the colonial form dominated at 20°C with maximum growth rate (Table 1). It is noteworthy that for *P. globosa* and *P. antarctica* colonies, the lowest numbers of cells per unit of colony surface area coincided with the highest growth rates and highest colony abundances. This suggests that at high growth rate, *Phaeocystis* could build more colonies with fewer cells, which may further reduce grazing mortality (Nejstgaard et al. 2007). Because *Phaeocystis* solitary cells are more readily consumed by grazers relative to colonies (Nejstgaard et al. 2007), our results suggest that *P. globosa* might experience increased grazing pressure at higher temperature, and combined with the near-0 sinking rates of solitary cells, could result in a reduced *Phaeocystis* carbon flux to the sediments (Hamm & Rousseau 2003, Reigstad & Wassmann 2007) and increased POC retention within the pelagic food web (Hamm 2000). Reduced colony formation at higher temperature would also reduce the amount of mucilage

production and bacterial activities that are supported by it (Becquevort et al. 1998, Rousseau et al. 2000).

Growth and temperature-controlled physiological processes were not necessarily tightly coupled. *Phaeocystis pouchetii* had its maximal growth rate at 8°C, but did not exhibit significant variations in quantum yield among temperatures, suggesting that photosynthesis and growth were weakly coupled, if at all. In contrast, *P. antarctica* showed maximal growth at 4°C, but the maximum F_v/F_m was observed at 0°C. It also had its maximal colonial abundance at the temperature of maximum growth. F_v/F_m values had declined greatly at 6°C, suggesting that temperature stress was occurring for this species at the highest temperature. Both growth and F_v/F_m declined by approximately 40% despite their different temperature optima. Thus it appears that photosynthesis and growth are weakly coupled in *P. antarctica*. *P. globosa* showed a similar response; that is, its F_v/F_m temperature maximum (16°C) was slightly lower than the maximal growth temperature (20°C), suggesting weak coupling between photosynthesis and growth. Colony formation and growth were also tightly coupled in this species.

The colonial form of *Phaeocystis antarctica* was most important at the lowest tested temperature (0°C), suggesting that low temperatures favored colony formation relative to solitary cells. This may be explained by the higher metabolic demand and the likelihood of nutrient limitation of cellular processes at higher temperatures (Rhee & Gotham 1981), as colony formation is considered to decrease nutrient uptake by *Phaeocystis* cells due to the presence of diffusive boundary layers (Ploug et al. 1999). Also, the solitary form of *Phaeocystis* arguably has diverse nutritional strategies (Verity & Medlin 2003). This may at least in part explain the lower percentages of colonial cells at relatively high temperatures for both *P. globosa* and *P. antarctica*. Verity et al. (1991) suggested that low temperatures favor *P. pouchetii* colonies, whereas high temperatures favor solitary cells. They further suggested that solitary cells are more efficient in utilizing light for photosynthesis at high temperatures, whereas colonial cells excrete more dissolved organic carbon at increased temperatures, which potentially may reduce their growth. Shields & Smith (2009) showed that colonial cells had more rapid growth rates than solitary cells, and when coupled with the greater susceptibility to grazing losses (Smith et al. 2000), may explain why colonies form during spring (and at very low temperatures) in the Ross Sea. Mathot et al. (2000) also found that the contribution of colonial cells to total *P. antarctica* cell abundance was highest in late spring (at the biomass maximum), and that the percentage of solitary cells increased in summer when the sea surface temperature was increasing. While Mathot et al. (2000) suggested that iron limitation in summer resulted in colony senescence and solitary cell liberation, it is possible that elevated temperatures also enhanced the survival and growth of solitary cells as well.

Unlike *Phaeocystis globosa* CCMP 1528 and *P. antarctica* CCMP 1871 that we used, some strains of *Phaeocystis* cease forming colonies in culture (e.g. strains of *P. globosa* derived from the South China Sea and the Vietnam coast; Tang et al. 2004). *P. pouchetii* AJ01 also did not form colonies at all tested temperatures (confirming the results of Malin et al. 1998), suggesting that the role of temperature is minor in controlling the development of colonies. *P. pouchetii* forms colonies *in situ*, but the reasons it did not form colonies in culture (like other isolates) remains unclear and independent of temperature. Temperature apparently influenced the growth and abundance of solitary cells, suggesting temperature also would alter the carbon partitioning between solitary cells and colonies *in situ* in the anticipated warming scenario.

In a *Phaeocystis* population where solitary cells and colonies coexist, photosynthetically fixed particulate carbon is partitioned among the solitary cells, colonial cells, and the colonial matrix. When only colonies are considered, the ratios of matrix carbon (POC_M) to colony carbon ranged from 17.5 to 31.3% for *P. antarctica* and from 6.2 to 16.1% for *P. globosa*. The modest (but insignificant) difference in the ratio of matrix carbon to colony carbon among temperatures suggests that the partitioning of POC between colonial cells and the matrix was relatively insensitive to temperature. Our results are quantitatively consistent with the study by Mathot et al. (2000): during a *P. antarctica* bloom in the Ross Sea, matrix carbon represented 10 to 33% of colony carbon.

Global warming

Models have predicted that global annual temperature will be elevated by 1.5 to 5.8°C by 2070–2100 (Houghton et al. 2001), which would result in increased sea surface temperature (Bopp et al. 2001), and consequently influence phytoplankton dynamics. Ocean temperature increases have been documented in a number of locations (e.g. Levitus et al. 2000, Hansen et al. 2006, Montes-Hugo et al. 2009). Our results suggest that rising seawater temperature would directly influence *Phaeocystis* populations; that is, it would inhibit the growth of *P. antarctica* and *P. pouchetii*, whereas *P. globosa* colonies would become smaller or cease to form. However, the net biomass of *P. globosa* would increase, thus exacerbating some already eutrophic regimes. All of these potential changes would in turn alter the trophic interactions in that specific environment and the regional biogeochemical cycles.

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