Rapid and early export of *Phaeocystis antarctica* blooms in the Ross Sea, Antarctica

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The Southern Ocean is very important for the potential sequestration of carbon dioxide in the oceans[1] and is expected to be vulnerable to changes in carbon export forced by anthropogenic climate warming[2]. Annual phytoplankton blooms in seasonal ice zones are highly productive and are thought to contribute significantly to pCO_2 drawdown in the Southern Ocean. Diatoms are assumed to be the most important phytoplankton class with respect to export production in the Southern Ocean; however, the colonial prymnesiophyte *Phaeocystis antarctica* regularly forms huge blooms in seasonal ice zones and coastal Antarctic waters[3]. There is little evidence regarding the fate of carbon produced by *P. antarctica* in the Southern Ocean, although remineralization in the upper water column has been proposed to be the main pathway in polar waters[4,5]. Here we present evidence for early and rapid carbon export from *P. antarctica* blooms to deep water and sediments in the Ross Sea. Carbon sequestration from *P. antarctica* blooms may influence the carbon cycle in the Southern Ocean, especially if projected climatic changes lead to an alteration in the structure of the phytoplankton community[6,7].

As part of the Research on Ocean–Atmosphere Variability and Ecosystem Response in the Ross Sea (ROAVERRS) programme, we found extensive blooms of *Phaeocystis antarctica*, a colonial prymnesiophyte. *P. antarctica* allocates ~50% of its carbon to the extracellular mucus that makes up its colonial matrix[8]. Nutrients drawn down in regions dominated by *P. antarctica* have ratios of carbon to phosphorus that are significantly greater than the Redfield (C/P = 106) ratio, indicating the greater potential of *P. antarctica* to export carbon relative to diatoms[6,7]. It has been assumed that senescing *Phaeocystis* blooms are remineralized within the upper water column (depth <100 m) and hence do not influence carbon sequestration[5]. Here we suggest, however, that in the Ross Sea *P. antarctica* blooms are exported rapidly during the early spring bloom.

Before ROAVERRS NBP-96-6 sampling (18 December 1996 to 8 January 1997), satellite images of ocean colour[7] showed a large phytoplankton bloom along the Ross Ice Shelf (RIS) as early as 8 November 1996. However, images collected only five days later showed that levels of chlorophyll associated with this bloom had already declined. We measured (24–26 December 1996) the integral

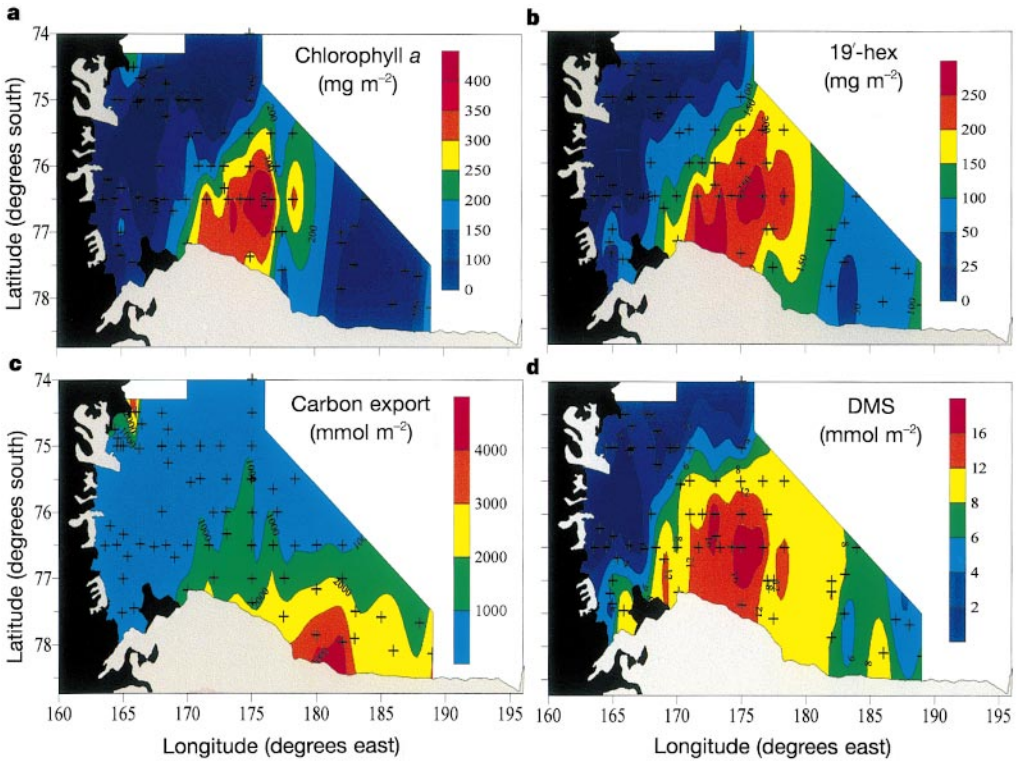

Figure 1 Integral values (depth 0–150 m) of chlorophyll *a* (**a**), 19′-hexanoyloxyfucoxanthin (19′-hex) (**b**), carbon export (**c**) and dimethylsulphide (DMS) (**d**) measured during the ROAVERRS-96-6 cruise (18 December 1996 to 8 January 1997). Carbon export was estimated from the carbon deficit (ΔTDIC − POC) in the water column assuming a winter[11] TDIC value of 2,287 mmol m^{-3}.

chlorophyll values to be 25% of those measured in the central bloom area (>400 mg chlorophyll per m^2; Fig. 1). Fluorescence profiles from this region showed deep (400–550 m) phytoplankton peaks at several stations along the RIS. These fluorescence peaks were associated with dimethylsulphide (DMS) concentrations up to 300-fold higher (4–15 nM; Fig. 2) than those previously measured in the deep waters (depth >400 m) of the Ross Sea[9]. DMS is an effective chemical indicator of the recent presence of *P. antarctica* in the Ross Sea[9]. High *P. antarctica* cell concentrations (about 5×10^6 cells per litre) were observed in this deep water (Fig. 2), demonstrating that it was not simply the colonial matrix that sedimented out of the upper water column in the Ross Sea, as was observed for *Phaeocystis pouchetii* blooms in the Balsfjord[10].

The photochemical quantum efficiency of photosystem II (variable/maximal fluorescence (F_v/F_m)) for *P. antarctica* cells obtained from 450–500 m depth ranged from 0.20 to 0.25 (Fig. 2). Although these values indicate some physiological stress, the similarity to surface values (about 0.3–0.4) indicates that the photosynthetic apparatus of deep *P. antarctica* cells was not significantly degraded relative to cells at the surface. The active physiological state of this deep *P. antarctica* population implies that they were rapidly exported from nearby surface waters. High sinking speeds (much greater than 200 m day^{-1}) have been measured for *P. antarctica* aggregates during bloom conditions in the Ross Sea[4], meaning that cells could theoretically reach deep water in only 1 to 2 days. This rapid export probably accounts for the physiological viability of the deep water cells. Relatively low current velocities (<10 cm s^{-1}) measured at nearby instrument moorings and a sharp pycnocline imply that water mass advection and convection were relatively unimportant with respect to vertical flux, and that sinking was the main cause of the export.

In December 1996, surface NO_3^- concentrations measured near the RIS were reduced from their winter[11] values of ~30 μM to 11 μM. It is likely that seasonal iron limitation terminated this bloom[12]. The silicate concentrations in these waters were unchanged from their winter[11] values (about 76 μM), indicating that most of the NO_3^- drawdown was due to *P. antarctica* rather than to diatoms. Only ~20% of the CO_2 drawdown, as estimated from the change in total dissolved inorganic carbon (ΔTDIC) in the upper 150 m of the water column, could be accounted for by suspended particulate organic carbon (POC) (Fig. 1). On the basis of previous measurements, it is unlikely that either dissolved organic carbon (DOC) production[13] or zooplankton grazing[14,15] contributed significantly to the POC deficit in the water column along the RIS. Instead, ROAVERRS data indicate that this deficit may have been due to the rapid vertical export of carbon between 8 November and 24 December 1996.

Perhaps the best evidence for the raid export of carbon by *P. antarctica* comes from the concentrations of particulate dimethylsulphoniopropionate ($DMSP_p$; an intracellular precursor of DMS) measured in surface sediments. Hydrolysis of DMSP produces DMS and acrylic acid in equimolar ratios[16] through grazing[17] and DMSP-lyase activity[18]. $DMSP_p$ concentrations in the sediments of the Ross Sea can be used as a diagnostic indicator of *P. antarctica* export from surface waters. $DMSP_p$ concentrations along the RIS were about 15 μg DMSP per gram dry sediment weight, equivalent to ~300 mg DMSP per m^2 or 105 mg carbon per m^2 as DMSP. These concentrations represent about 1.75 g carbon per m^2 of *P. antarctica* carbon (assuming that 6% of carbon in *P. antarctica* cells are present as DMSP (ref. 19)). Because some fraction of the *P. antarctica* export flux is remineralized during export[4,5] or at the sediment–water interface, the estimates of carbon-flux to the ocean floor on the basis of the $DMSP_p$ content of the sediment represent a lower boundary to the true carbon export value associated with *P. antarctica* blooms. However, sediment focusing in this region could complicate the interpretation of the areal $DMSP_p$ fluxes[20].

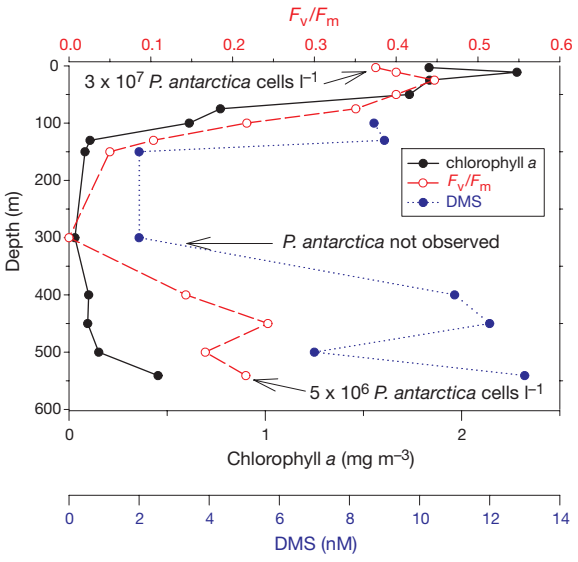

Figure 2 Vertical profiles of *P. antarctica* cell numbers, chlorophyll *a* and DMS concentrations, and F_v/F_m near the Ross Ice Shelf (76.1° S, 174° W) on 24 December 1996. DMS concentrations in the upper 100 m of the water column were not included as these values were more than an order of magnitude higher than those for samples taken below a depth of 100 m. Note that the DMS minimum layer corresponds to the F_v/F_m and chlorophyll *a* minimum layers.

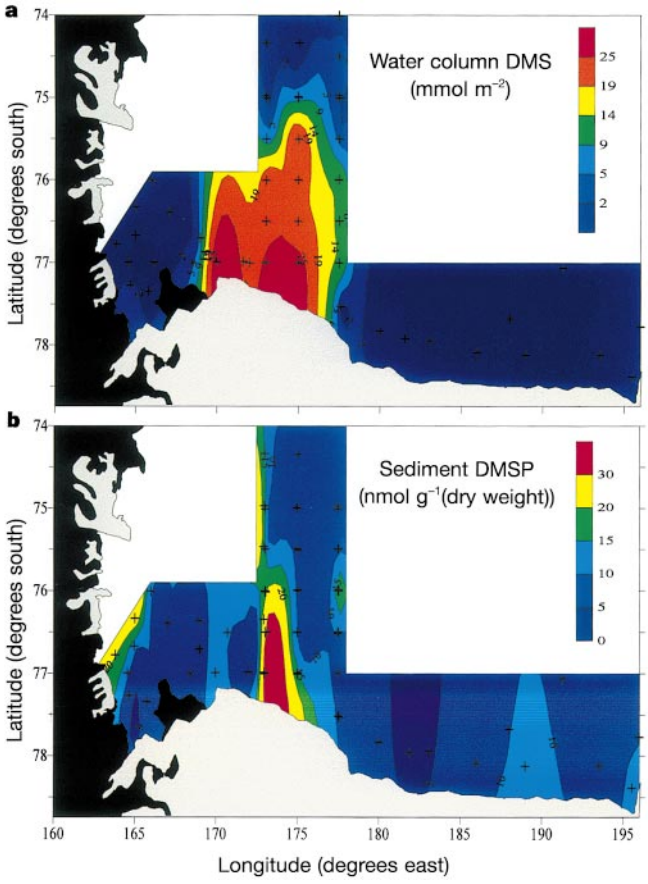

Figure 3 Spatial distribution of DMS concentrations in the integral water column (depth = 0–150 m) (**a**) and DMSP concentrations in the surface sediment (depth = 0–2 cm) (**b**) measured during the early spring bloom period (November 1998) in the Ross Sea (ROAVERRS-98-7).

In November 1998, we observed evidence for the export of *P. antarctica* from surface waters in early blooms. $DMSP_p$ concentrations in surface sediments were more than twice as high in November 1998 as in December 1996 (Fig. 3), implying a carbon export due to *P. antarctica* of ~4 g carbon per m^2. Fluorescence profiles and elevated DMS concentrations at various depths (300–650 m) from several stations in the central Ross Sea revealed the presence of deep *P. antarctica* populations. More importantly, a strong spatial correlation was observed between areal sediment DMSP and the developing *P. antarctica* bloom (Fig. 3). Although some lateral advection undoubtedly occurs, vertical export must be significantly faster than lateral processes to explain this strong spatial relationship (Fig. 3). Deep water fluorescence signals with elevated DMS concentrations were observed during four consecutive field seasons at various locations in the Ross Sea, indicating the common occurrence of *P. antarctica* flux events.

High carbon flux during early bloom conditions implies that senescence was not a prerequisite for *P. antarctica* export to deep water and sediments. Hence, carbon export from *P. antarctica* blooms in the Ross Sea was not dependent on the demise of the bloom itself. Moreover, because DOC concentrations and bacterial production in the spring are low in the Ross Sea[13], and because of the rapid sinking speeds of aggregates[4], a significant fraction of the carbon export associated with *P. antarctica* must escape remineralization in the upper water column (depth <100 m) and be exported to deep waters. On the basis of the carbon export that was recorded in November 1998, the large carbon deficit that was observed along the RIS during December 1996 probably did not result from a single episodic event due to bloom senescence. Rather, the numerous fluorescence peaks that were observed at various depths indicate that carbon export due to *P. antarctica* blooms results from multiple episodic export events. Also, rates of oxygen uptake by the sediment (which indicate carbon flux to the ocean floor) were at their highest in 1998 in the same region as the high-sediment DMSP. Interannual variability in the locations of regions where levels of oxygen uptake by the sediment are high is also consistent with multiple episodic export events.

Sediment traps were not installed adjacent to the central or eastern RIS from 1996 to 1998 as part of the ROAVERRS and US JGOFS (United States Joint Global Ocean Flux Study) field programmes. However, sediment trap fluxes were measured between January 1995 and January 1996 at 423 m at a site adjacent to the RIS in the central Ross Sea (78° S, 177° W) (ref. 21). The highest daily average for POC flux during the year occurred between 8 December and 23 December 1995, at a time when the trapped particles had relatively high organic carbon to silicon ratios. These findings are consistent with our own observations of *Phaeocystis* export.

Our data may have a direct bearing on the glacial iron hypothesis[22] because of the potential link between *Phaeocystis* and the biological pump. A recent study of the seasonal ice zones during the Last Glacial Maximum (LGM) concluded that biological production in the Southern Ocean contributed substantially to the lowering of atmospheric pCO_2 (ref. 23). In addition, radionuclide proxies have shown that POC export in the subantarctic zone was significantly higher during the LGM than during the Holocene epoch[24]. Because silicate utilization by diatoms was diminished during the LGM relative to the present interglacial[25], it is possible that a non-siliceous phytoplankton species may have been important at this time. Considering the high productivity[26] and carbon-export potential of *P. antarctica* blooms, this species may have been important during the LGM in sequestering atmospheric pCO_2 in the Southern Ocean. On the basis of the high levels of methanesulphonic acid (a product of the atmospheric breakdown of DMS) during the LGM[27] and its positive correlation with proxies of iron in ice cores[28], it seems that *P. antarctica* may represent an important link in the carbon, iron and sulphur cycles. Furthermore, our results indicate that the episodic sinking of colonial *P. antarctica* blooms may not require bloom senescence conditions, a fact that may facilitate the continuous drawdown of pCO_2 during the austral summer. Thus, early bloom conditions in the Ross Sea may be important with respect to carbon export from surface waters, particularly as *P. antarctic* blooms remove about twice as much CO_2 per mole of PO_4 relative to diatoms[7]. The next generation of ocean models must incorporate the possible effects of algal species composition on carbon export in the Southern Ocean, especially if rising levels of CO_2 lead to shifts in the composition of the phytoplankton community[6,7]. □

Methods

Water samples were collected using 10-litre Bullister Bottles attached to a conductivity temperature depth (CTD) rosette. Pigment concentrations were measured using high-performance liquid chromatography (HPLC) pigment analyses[9]. Concentrations of 19′-hexanoyloxyfucoxanthin are a proxy for the presence of *P. antarctica* in the Ross Sea[9]. The F_v/F_m measurements were made using a pulse-amplitude modulated fluorometer. Cells were adapted to the dark for 30 min before measurement. *P. antarctica* cell counts were determined by microscopy and the bright autofluorescence of cells at depth implied rapid vertical export as opposed to lateral advection. Surface sediments were collected with both box and Haps corers and sampled in duplicate at various sites on the core. Most sampling sites were reoccupied and the average sediment DMSP value was used. DMS concentrations were measured using cryogenic purge and trap gas chromatography[9]. Aliquots of sediment were subjected to base hydrolysis (6N NaOH) for 24 h in the dark to convert DMSP to DMS[16] and were analysed at sea. Sediments were dried and weighed in centrifuge tubes for normalization. For our estimate of the carbon deficit (Fig. 1), we assumed that DOC concentrations were constant during the early spring bloom period[13].

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Developmental cheating in the social bacterium *Myxococcus xanthus*

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Cheating is a potential problem in any social system that depends on cooperation and in which actions that benefit a group are costly to individuals that perform them[1–5]. Genetic mutants that fail to perform a group-beneficial function but that reap the benefits of belonging to the group should have a within-group selective advantage, provided that the mutants are not too common. Here we show that social cheating exists even among prokaryotes. The bacterium *Myxococcus xanthus* exhibits several social behaviours, including aggregation of cells into spore-producing fruiting bodies during starvation. We examined a number of *M. xanthus* genotypes that were defective for fruiting-body development, including several lines that evolved for 1,000 generations under asocial conditions[6] and others carrying defined mutations in developmental pathways[7–10], to determine whether they behaved as cheaters when mixed with their developmentally proficient progenitor. Clones from several evolved lines and two defined mutants exhibited cheating during development, being over-represented among resulting spores relative to their initial frequency in the mixture. The ease of finding anti-social behaviours suggests that cheaters may be common in natural populations of *M. xanthus*.

The myxobacteria are soil-dwelling prokaryotes that exhibit a social life cycle similar to that of the eukaryotic slime mould *Dictyostelium discoideum*[11,12]. Like the slime moulds, myxobacteria undergo multicellular development in response to starvation that yields fruiting bodies of remarkable morphological variety[13]. This process involves multiple intercellular signals specific to distinct stages of development, and it results in a minority of the original population becoming stress-resistant spores that germinate under favourable conditions[14]. Myxobacteria also exhibit social motility and social predation, swarming as a 'wolf pack' toward prey, which they kill and degrade by the secretion of extracellular compounds[15].

A developmentally defective mutant of *M. xanthus*, when mixed with a developmentally proficient wild type, has five possible fates during development. First, the defective strain may sporulate with the same efficiency as it does in pure culture. This outcome corresponds to null hypothesis H_1 here. Second, a partially defective genotype's sporulation in the presence of wild type may be inhibited even below its efficiency in pure culture. The third and fourth potential fates are partial and complete rescue (relative to wild type), respectively, of the defective genotype by extracellular complementation in the presence of wild type. Complete rescue to the wild-type sporulation efficiency corresponds to null hypothesis H_2 here. Fifth, a developmentally defective genotype may produce more spores in the presence of wild type than would a neutrally marked wild type introduced at the same initial frequency. This last outcome constitutes evolutionary cheating, because the defective mutant obtains disproportionate reproductive success.

We first compared the developmental performance, in pure culture, of six experimentally evolved clones with that of their wild-type ancestor DK1622 (ref. 16). In pure culture, all six clones, to varying degrees, showed defects in spore production relative to DK1622 (Fig. 1). Thus, these evolved clones were all defective for this group function. We then measured spore production of these evolved defective clones when they were each mixed with their ancestor at an initial frequency of 0.01. The performance of each minority genotype was then contrasted with the two distinct hypothetical outcomes, H_1 and H_2 (Table 1). For five out of the six

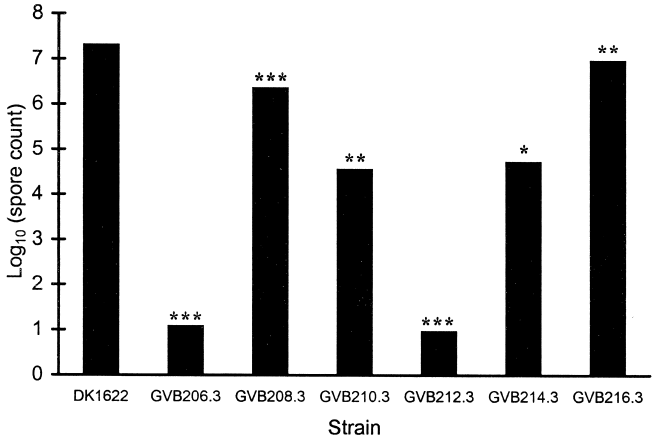

Figure 1 Spore production for six independently evolved clones[6] of *M. xanthus* and their common ancestor (DK1622). Asterisks indicate significant defects in an evolved clone's spore production relative to that of DK1622, calculated using Welch's approximate *t*-test which does not assume homogeneity of variance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, all one-tailed.

Table 1 Observed spore production of evolved clones when mixed at 1% with the wild-type ancestor compared with production expected under two hypotheses

Evolved clone	Observed	H_1 expected	Observed − H_1 expected	Observed − H_2 expected
GVB206.3	–0.31	–7.30	+6.99***	+1.69***
GVB208.3	–0.98	–2.96	+1.98***	+1.02***
GVB210.3	–3.32	–4.75	+1.43**	–1.32**
GVB212.3	–3.27	–7.30	+4.03**	–1.27*
GVB214.3	–0.93	–4.59	+3.66***	+1.07**
GVB216.3	–3.49	–2.35	–1.14***	–1.49***

All values are $\log_{10}$-transformed; an observed value of –3 thus indicates that an evolved clone produced 0.1% of the pure-culture wild-type spore count. According to H_1, a clone produces spores with the same efficiency when mixed with the ancestor as it does when alone. For example, a pure culture of GVB208.3 produces ~10% as many spores as does a pure culture of the wild type (Fig. 1). If GVB208.3 makes spores with the same 10% relative efficiency when mixed with its ancestor at a frequency of 1%, then it will produce 0.1% of the total spores. Expected values under H_1 vary among the evolved clones; a $\log_{10}$-transformed value of –7.30 represents the limit of detection. A positive difference between the observed spore production of an evolved clone and its expectation under H_1 indicates at least partial complementation of the evolved clone by the wild-type. According to H_2, an evolved clone behaves as would a neutrally marked variant of the wild-type; that is, it produces 1% of the total spores when mixed at 1% with the wild-type ancestor. The expected value under H_2 is thus –2 ($\log_{10}$ 0.01) for all clones. A positive difference between an evolved clone's observed spore production and the uniform expectation under H_2 demonstrates developmental cheating by the evolved clone. Asterisks denote that contrasts are significantly different from zero based on *t*-tests: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, all two-tailed.