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Carbon acquisition and growth of Antarctic sea ice diatoms in closed bottle incubations

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ABSTRACT: Mixed cultures of 4 polar diatoms regularly found in Antarctic pack ice were grown over 20 d in closed bottles at high light (200 to 250 µmol photons m^{-2} s^{-1}) and at 0°C in order to investigate growth physiology and biomass production under conditions simulating the sea ice habitat during summer. Species tested were: *Chaetoceros* cf. *neogracile*, *Fragilariopsis cylindrus*, *Thalassiosira antarctica* and *Porosira pseudodenticulata*. Initially, all species grew exponentially, but exponential growth ceased for *P. pseudodenticulata* and *T. antarctica* after 6 d, for *F. cylindrus* after 8 d, and for *C.* cf. *neogracile* after 10 d. Slight increases in cell number were observed for all species 2 d later. Peak biomass amounted to 140 µg chl *a* (850 µmol particulate organic carbon, POC) l^{-1}. At the same time, concentrations of dissolved inorganic carbon (DIC) were reduced by 1000 µM, oxygen concentrations increased to 1400 µM, and pH increased to 10.5. At this stage, a substantial decline in plasma-containing cells was recorded for *F. cylindrus*. *C.* cf. *neogracile* accounted for 80%, and *C.* cf. *neogracile* and *F. cylindrus* accounted for >95% of total carbon biomass. The carbon isotope composition of POC (expressed as $\delta^{13}C$) increased from −24 to −9‰ during the experiment. Model calculations showed that diffusive uptake of dissolved CO_2 satisfied cellular carbon demand for all species except *P. pseudodenticulata* at CO_2(aq) concentrations >0.5 µM, whereas direct HCO_3^- utilization was observed for *C.* cf. *neogracile* below this concentration. Our data confirm that intense photosynthetic carbon assimilation may lead to profound chemical changes in isolated interstitial brine solutions, with significant consequences for sea ice biota. We propose that the capacity to efficiently utilize ambient DIC, possibly mediated by virtue of favorable surface to volume ratios as well as active pathways of inorganic carbon acquisition, favors growth of small diatoms, and may be an important factor driving ice algal species succession during summer blooms. Since only 2 species continued to grow in fresh medium following experimental incubation (*C.* cf. *neogracile* and *P. pseudodenticulata*), differential tolerance to chemical variations may influence the seeding potential of ice algae following release into the open water

KEY WORDS: Antarctica · Sea ice microalgae · Carbon uptake · Growth · Dissolved inorganic carbon · pH · Oxygen

INTRODUCTION

Studies on the ecophysiology of Antarctic sea ice diatoms have often concerned responses to environmental conditions pertinent to the autumn/winter period, i.e. reduced temperature and light at increased salinities (Palmisano & Sullivan 1982, Aletsee & Jahnke 1992, Gleitz & Thomas 1992). However, productivity of sea ice algae during winter is greatly hindered by adverse environmental conditions, and daily production rates are usually 1 to 2 orders of magnitude lower than rates determined during spring and summer (Garrison & Buck 1991, Garrison & Close 1993, Gleitz & Thomas 1993).

Microbial assemblages in Antarctic pack ice are generally distributed throughout floes as surface (Garrison & Buck 1991) or internal assemblages (Ackley et al. 1979). Recent investigations have shown that during summer, exchange of dissolved components between the water column and the interstitial brine of intact pack ice may be severely restricted, creating a temporarily isolated system (Gleitz et al. 1995). Influx of components taken up by intense primary productivity may not balance algal consumption, causing shifts in the chemical composition of the brine, which will again backlash on algal growth physiology.

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Several investigators have speculated that biomass and productivity of sea ice assemblages may be controlled by the availability of major nutrients (nitrate, phosphate and silicate), which may fall to very low levels during summer in the Antarctic pack (Clarke & Ackley 1984, Garrison et al. 1990, Dieckmann et al. 1991). However, depletion of major nutrients will proceed in parallel to other chemical changes, in particular depletion of dissolved inorganic carbon (DIC), accumulation of dissolved oxygen and a substantial increase of pH. This is especially notable since dominant sea ice diatoms have recently been shown to photosynthesize and assimilate carbon even at exhausted nutrient concentrations, depleting DIC concentrations to levels much lower than would be expected from ambient nutrient concentrations (Gleitz et al. 1995, Gleitz et al. 1996). Hence, in addition to restraints placed on algal growth by declining nutrient concentrations, chemical variations related to the carbonate system, oxygen concentrations and/or pH may control growth and species composition of ice algal assemblages during summer blooms. This hypothesis, however, has so far never been studied systematically.

The aim of the study presented here was to determine the upper limit for growth with respect to DIC, dissolved CO_2 [CO_2(aq)], and O_2 concentrations and pH for 4 Antarctic sea ice diatoms in a completely isolated system. Secondly, we wanted to test if there were species-specific differences concerning the capacity to assimilate DIC under such isolated conditions. Additionally, we monitored changes in the $^{12}C/^{13}C$ composition of the biomass produced, and asked if growth in a closed system could adversely affect cell integrity to such an extent as to prevent recolonization of the water column following ice melt.

MATERIALS AND METHODS

Culture material and experimental set up. The diatoms *Fragilariopsis cylindrus* [surface equivalent cell radius (SER) 1.7 µm], *Thalassiosira antarctica* (SER 16.9 µm), *Porosira pseudodenticulata* (SER 35.5 µm) and *Chaetoceros* cf. *neogracile* (SER 3.2 µm) were isolated from Weddell Sea surface water and were subsequently grown in unialgal cultures in double-filtered (0.2 µm), nutrient-enriched Antarctic seawater at 0°C in the home laboratory. Prior to the experiments, stock cultures (not axenic) were grown at 0°C, 200 µmol photons m^{-2} s^{-1} (continuous light) in 3 l of double-filtered Antarctic seawater enriched with nutrients according to Von Stosch & Drebes (1964), resulting in nitrate, silicate and phosphate concentrations of roughly 500, 200 and 30 µM respectively. These concentrations ensured that nutrient limitation would not influence growth performance of the algae. Aliquots equivalent to 5 µg chl *a* (800 to 1800 ml) were withdrawn from each stock culture during early logarithmic growth, and diluted in fresh, nutrient-enriched medium to a final volume of 20 l, resulting in an initial chl *a* concentration of 1 µg l^{-1} (0.25 µg chl *a* l^{-1} of each species). The suspension was mixed and a subsample was taken for t_0 (time zero) analyses. The culture was then filled into 20 borosilicate glass bottles (ca 600 ml), stoppered without air space above the liquid, and incubated for 2 to 20 d in a temperature-controlled incubator at conditions specified above for stock cultures. A continuous irradiance of 200 µmol photons m^{-2} s^{-1} ensured that photosynthetic rates would be light-saturated all day long, which we consider a realistic approximation of summer conditions in the high Antarctic (e.g. Gleitz et al. 1996). In order to test if self-shading influenced growth rates, light intensity was increased to 250 µmol photons m^{-2} s^{-1} at t_{14}. Every 2 d, 2 bottles were randomly withdrawn for chemical analyses, $\delta^{13}C$, particulate organic carbon (POC) and chl *a* determinations and cell counts. Bottles were shaken prior to sampling and once per day. Aliquots (10 ml) were taken from t_{20} bottles, resuspended in fresh media (3 l), and incubated for another 10 d under experimental light and temperature conditions in open bottles in order to test the capacity of the algae to grow following experimental exposure.

Determinations of cell number, chl *a*, $\delta^{13}C$ and POC. Subsamples (25 ml) were fixed with acidified Lugol solution, and cell numbers were determined with a Zeiss IM 35 inverted microscope using the Utermöhl method (Utermöhl 1958). About 100 to 300 cells of *Porosira pseudodenticulata* and *Thalassiosira antarctica* respectively were enumerated at a magnification of 63×, and the entire chamber was counted once. The other 2 species were counted at a magnification of 400×, and 4 strips were counted, each with 200 to 500 cells of each species (cell numbers were initially lower in the resuspension cultures). Growth rates were calculated by optimizing linear regressions in semi-logarithmic plots of cell number vs time.

Subsamples of 50 to 1000 ml were filtered on pre-combusted (5 h at 450°C) glass fiber filters for chl *a* and POC determinations, and frozen at −27°C. Chl *a* was determined fluorometrically after homogenization and 4 h extraction in 90% acetone at 4°C in the dark (Evans et al. 1987). Prior to POC determinations, filters were exposed to fuming hydrochloric acid for 15 min and dried overnight at 60°C. They were then transferred into tin vials, and analyzed using a combination gas chromatograph/mass spectrometer (ANCA-SL 20-20, Europa Scientific). Isotopic composition of POC is reported in δ-notation relative to PeeDee belemnite (PDB):

$$\delta^{13}C = \left(\frac{[^{13}C]/[^{12}C]_{sample}}{[^{13}C]/[^{12}C]_{PDB}} - 1 \right) \times 1000 \quad (1)$$

For the calculation of $\delta^{13}C_{DIC}$ at different measuring intervals (t_i = 2 to 20), we used a $\delta^{13}C_{DIC}$ at t_0 of 1.5‰, a value determined for surface seawater at 60°S (Rau et al. 1982), and assumed the same $\delta^{13}C$ value for DOC (not measured) as for POC:

$$\delta^{13}C_{DIC_{t_i}} = \frac{\delta^{13}C_{DIC_{t_0}} - \delta^{13}C_{POC_{t_i}} \times f''}{f'} \quad (2a)$$

where

$$f' = \frac{[DIC_{t_i}]}{[DIC_{t_0}]} \quad (2b)$$

$$f'' = \frac{\Delta[DIC]}{[DIC_{t_0}]} \quad (2c)$$

$$\Delta[DIC] = [DIC]_{t_0} - [DIC]_{t_i} \quad (2d)$$

Chemical analyses. Initially, 4 volume-calibrated glass bottles (60 ml) were filled, and 2 of these were used for determination of oxygen concentrations according to the Winkler method (Grasshoff 1983). The other 2 bottles were stored and allowed to warm to 15°C for pH determinations. The pH meter was calibrated daily prior to the measurements with NBS buffers 6.865 and 9.180. After calibration, a combination electrode was immersed in an aliquot of sample water for 2 h. Thereafter, the electrode was immersed in the (previously stoppered) sample, and a stable pH reading (drift <0.1 mV $30 s^{-1}$) was recorded.

Concentrations of DIC were calculated from measurements of pH and total alkalinity (TA). TA was determined using a potentiometric titration method (Almgren et al. 1983) at t_0, t_{14} and t_{20}. For these analyses, the pH meter was calibrated with NBS buffers 4.001 and 6.865. A subsample (50 ml) was titrated with hydrochloric acid (0.035 N), the normality of which had been determined beforehand by iodometric titration. No change of TA was observed between t_{14} and t_{20}, and this value was also used for t_{16} and t_{18} determinations. For sampling dates t_2 to t_{12}, TA was raised in accord with PON concentrations (assumed to equal nitrate removal) in the media. The second equivalence point of carbonic acid was calculated by linear regression analyses of Gran plots constructed from 18 to 26 pH measurements in the range of pH 4 to 3, determined by adding 0.1 ml increments of acid and recording pH. The carbonate system was calculated using the carbonic acid dissociation constants of Mehrbach et al. (1973). *In situ* pH was calculated from DIC and TA at a temperature of 0°C.

Testing for the source of inorganic carbon. In order to test for the source of inorganic carbon utilized during photosynthesis, photosynthetic carbon uptake (U_{DIC}) for each species, determined from carbon quota and instantaneous growth rate, was compared with the potential maximum flux of CO_2(aq) to the cell surface (Q_a) for consecutive measuring intervals, the latter calculated according to (Riebesell et al. 1993):

$$Q_a = 4\pi r D \left(1 + r\sqrt{\frac{k'}{D}}\right)(c_\infty - c_r) \quad (3)$$

where r is the (surface equivalent) cell radius, D the diffusivity of CO_2(aq), k' the rate constant for conversion of HCO_3^- to CO_2(aq), assuming that HCO_3^- conversion is purely chemical, c_∞ the CO_2(aq) concentration in the bulk medium, and c_r the CO_2(aq) concentration at the cell surface (here assumed to equal zero to allow for maximum flux). If $U_{DIC}/Q_a \leq 1$, cellular carbon demand is less than diffusive CO_2(aq) transport can supply, and photosynthetic carbon uptake can rely on CO_2(aq) as the only source of inorganic carbon. If $U_{DIC}/Q_a > 1$, carbon demand exceeds the potential maximum flux of CO_2(aq), which we consider as evidence for direct use of HCO_3^-.

RESULTS

Cell counts and biomass production

All 4 species grew exponentially during the early phase of the experiment with growth rates of 0.64, 0.60, 0.49 and 0.32 d^{-1} for *Chaetoceros* cf. *neogracile*, *Fragilariopsis cylindrus*, *Porosira pseudodenticulata* and *Thalassiosira antarctica* respectively (Fig. 1). Exponential growth was maintained for 10 d by *C.* cf. *neogracile*, for 8 d by *F. cylindrus*, and for 6 d by the other 2 species. Slight increases in cell numbers were recorded for all species 2 d after exponential growth had ceased. Thereafter, cell numbers remained essentially constant, but decreased substantially in *F. cylindrus* cultures after t_{16}.

Biomass increased from an initial value of 1 µg chl *a* (5 µmol POC) l^{-1} to a peak concentration of about 140 µg chl *a* (850 µmol POC) l^{-1} (Fig 2). Chl *a* peaked at t_{12}, whereas maximum POC concentrations were observed at t_{10}. Thereafter, chl *a* as well as POC declined to about 70 µg l^{-1} and 700 µmol l^{-1} respectively at t_{20}. The $\delta^{13}C$ of POC ($\delta^{13}C_{POC}$) increased from an initial −24‰ to −9‰ at t_{14}, and remained essentially constant thereafter. Over the same time interval, $\delta^{13}C_{DIC}$ increased to 9.5‰. Ratios of POC/particulate organic nitrogen (PON) (m/m) and POC to chl *a* (w/w) were 6.0 and 62 respectively at t_0 (data not shown). POC/PON decreased linearly to 5.0 until t_4, after which it remained constant. A marked increase of POC/chl *a* to a value of 120 was observed at t_{18}.

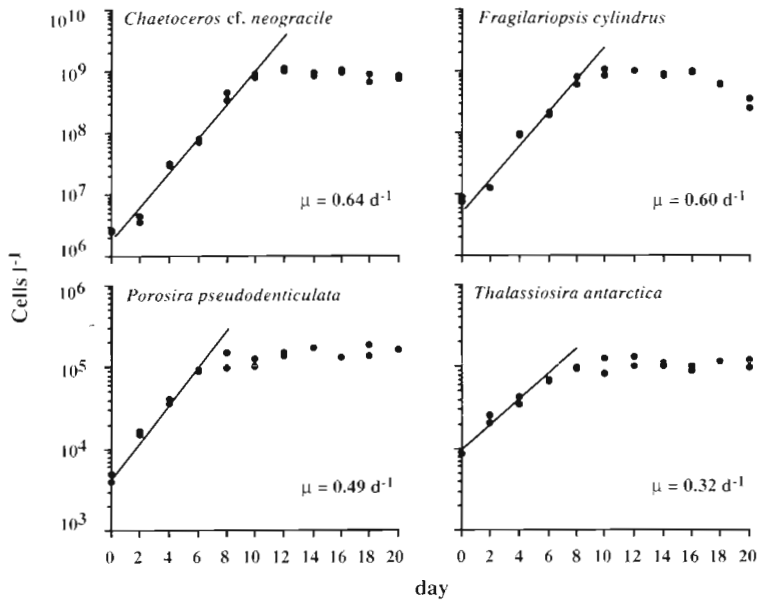

Fig. 1. Cell concentrations of 4 polar diatoms grown in mixed batch cultures at 200 (from t_{14} onwards: 250) μmol photons m^{-2} s^{-1} and 0°C in closed bottle incubations for 20 d. (●) Data points (2 replicates). Solid lines represent best fit of linear regressions during exponential growth. μ: growth rate

Following resuspension of aliquots into fresh media, an increase in cell numbers was only observed for *Chaetoceros* cf. *neogracile* and *Porosira pseudodenticulata* (Fig. 3). After an initial lag phase, growth rates were similar to those observed in closed bottles early in the experiment (0.65 and 0.41 d^{-1} for *C.* cf. *neogracile* and *P. pseudodenticulata* respectively). No increase in cell numbers was recorded for the other 2 species.

Chemical analyses

DIC concentrations decreased from 2200 μM to 1220 μM at t_{16} (Fig. 4). CO_2(aq) concentrations decreased from the initial 5 μM to <0.01 μM at t_{12} (Fig. 5). Oxygen concentrations increased from 310 μM to a peak value of 1400 μM at t_{12}, and declined thereafter to 1200 μM at t_{20}. Gas bubbles steadily increasing in size were visible in the bottles from t_8 onwards. The pH increased from 8.77 at t_0 to about 10.50 at t_{16}, and was slightly lower again at t_{20}.

DISCUSSION

Results obtained in this study demonstrate the physiological capacity of a mixed culture of 4 polar diatoms regularly found in pack ice (albeit with different abundances; Krebs et al. 1987, Garrison & Buck 1989) to remove about 1000 μmol DIC l^{-1} in closed bottle incubations before photosynthetic carbon assimilation subsided. In fair agreement with measured DIC depletion, total carbon biomass amounted to about 850 μmol POC l^{-1}; the missing 150 μmol must have been transferred to dissolved pools. However, growth kinetics (Fig. 1) and different species sizes suggest that POC production was not alike for each species during the experiment. Following the recommendations given in Edler (1979), we calculated carbon quotas of 2, 9, 500 and 2500 pg C $cell^{-1}$ for *Fragilariopsis cylindrus*, *Chaetoceros* cf. *neogracile*, *Thalassiosira antarctica* and *Porosira pseudodenticulata* respectively. Species-specific POC production calculated from cell counts and carbon quotas resulted in peak POC accumulations of about 800, 170, 30 and 5 μmol l^{-1} for *C.* cf.

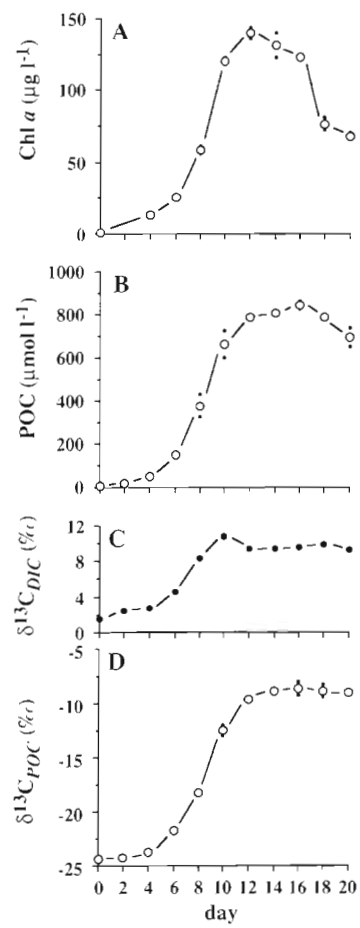

Fig. 2. (A) Chl *a* and (B) POC concentrations, and isotopic composition of (C) DIC and (D) POC (expressed in δ notation) in mixed batch cultures of 4 polar diatoms in closed bottle incubations vs experimental period (20 d). (○) Mean of 2 replicate incubations; (●) data points

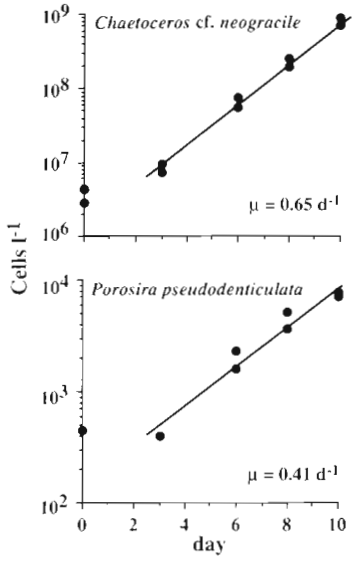

Fig. 3. Cell concentrations of the 2 polar diatoms that grew when resuspended in fresh media following a 20 d incubation in closed bottles. (●) Data points (2 replicates; only 1 replicate was counted at t_0 and t_3 for *Porosira pseudodenticulata*). Solid lines represent best fit of linear regressions during exponential growth. μ: growth rate

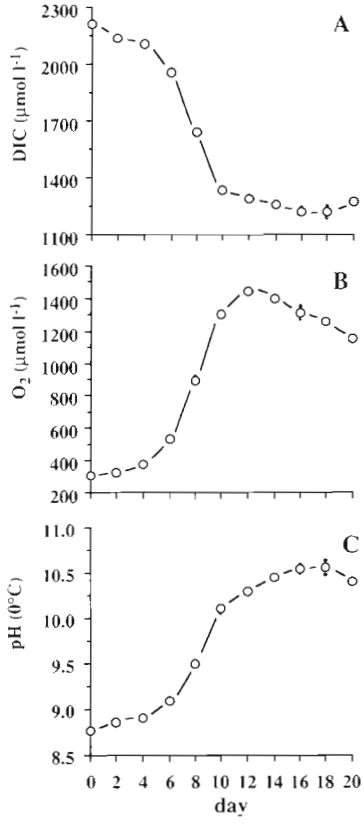

Fig. 4. (A) DIC and (B) O_2 concentrations, and (C) pH in mixed batch cultures of 4 polar diatoms grown over 20 d in closed bottles. Symbols as in Fig. 2

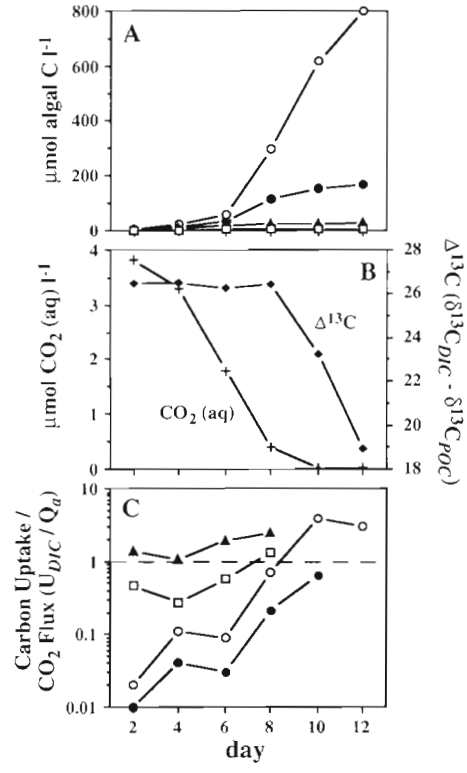

Fig. 5. (A) Algal carbon biomass, determined from cell density and cell quota (see text), (B) changes of $\Delta^{13}C$ and CO_2(aq) concentration, and (C) ratio of carbon uptake to CO_2(aq) flux between Day 2 and Day 12 of closed bottle incubations (for derivation see text). Symbols in (A) and (C): (○) *Chaetoceros* cf. *neogracile*; (●) *Fragilariopsis cylindrus*; (▲) *Porosira pseudodenticulata*; (□) *Thalassiosira antarctica*

neogracile, *F. cylindrus*, *P. pseudodenticulata* and *T. antarctica* respectively, demonstrating that about 80% of total DIC depletion was due to only 1, and >95% due to growth of only 2, species.

In brine solutions collected from intact pack ice in the Weddell Sea during summer, Gleitz et al. (1995) measured DIC depletions, oxygen concentrations and pH values of up to 1200 μmol DIC kg^{-1}, 933 μmol O_2 kg^{-1} and pH 9.89. These values are similar to those observed at the end of logarithmic growth in our study, verifying that the concept of the brine channel system being effectively isolated from the surrounding water column for time scales comparable to biological activity (days to weeks) is in fact legitimate. At present, we are not aware of chemical data from undiluted sea ice brine other than the reference cited above, thus it is difficult to evaluate the degree of isolation experienced by internal sea ice communities in nature. From the data presented by Gleitz et al. (1995), we conclude that during summer, brine chemical composition often deviates substantially from conditions found in surface seawater, but may not always attain peak variations as

observed in this study. This is related to the fact that complete isolation as applied here will not always be maintained under natural conditions for prolonged periods, and some replenishment of dissolved matter within brine channels from the underlying seawater will occur

Nutrient salts were added in excess in this study, and in nature, these will be depleted well before DIC concentrations drop to the levels observed here. Depletion of major nutrients will proceed in parallel to the chemical changes which were of principal concern in the present study (carbonate system, pH, oxygen), but it would be futile to differentiate between effects of the 2 processes on algal growth and survival in an experiment where both were allowed to change to potentially deleterious levels simultaneously. In order to ensure interpretability of the data, we considered it indispensable to avoid nutrient limitation in the experimental set-up, yet we realize that this may somewhat limit our ability to relate our results to the field situation. This, however, will not necessarily invalidate our argument, since sea ice algae have been shown to sustain photosynthesis even after macronutrients were fully depleted ('overflow production'). For instance, in isolated pools of meltwater trapped between brash ice floes in the Weddell Sea in January, Gleitz et al. (1996) measured photosynthetic rates of 3 to 30 µg C l^{-1} h^{-1} at nitrate concentrations ranging from undetectable levels to 0.8 µmol l^{-1}. The algal assemblages consisted almost entirely of *Fragilariopsis cylindrus* and *Thalassiosira antarctica*, and massive accumulation of carbon-rich storage material such as lipid was observed (Fahl & Kattner 1993). In these samples, pH ranged from 9.07 to 9.29, and ambient DIC concentrations were depleted by up to 700 µmol l^{-1}. Similarly, levels of DIC depletion observed in summer sea ice brine by Gleitz et al. (1995) were explained by sustained carbon assimilation of the internal algal assemblages at exhausted nutrient concentrations.

The sharp decrease in chl *a* after t_{12} was partly due to decrease of total cell density, but also to bleaching of intact (plasma-containing) cells. Also, empty cells of *Fragilariopsis cylindrus* were found in substantial numbers (30 to 60% of total cell counts) following t_{18}, showing that cell mortality had set in. For the other species, however, only few empty frustules were observed at this time (<5% of total cell counts). In our study, only *Chaetoceros* cf. *neogracile* and *Porosira pseudodenticulata* recovered from the experimental treatment and grew when suspended in fresh medium (Fig. 3). Thus, we suspect that deterioration of cell integrity at the end of the summer bloom may significantly influence recolonization of the water column following floe disintegration, provided that sea ice isolation is so effective as to allow environmental conditions to decline to extreme levels. This may have important consequences for species composition of ice-edge blooms, as it may prevent or delay growth of damaged species upon entering the open water.

From the data presented here it is difficult to specify factors that eventually caused termination of growth of the different species. It is feasible though to assume that high rates of photorespiration at oxygen oversaturation and strongly alkaline pH in conjunction with very low CO_2(aq) concentrations were major factors leading to stagnation of cell division. However, as noted above, growth subsided at different times for different species, suggesting a differential tolerance to abiotic variations.

We hypothesize that pathways of DIC acquisition may have been one critical factor determining growth capacity in our experiment. Monitoring carbon isotope fractionation during photosynthesis has helped to elucidate pathways of DIC acquisition in microalgal cultures (e.g Hinga et al. 1994, Laws et al. 1995). For instance, several lines of evidence suggest that the ^{13}C content of marine phytoplankton POC may vary as a function of external CO_2(aq) concentrations as the $\delta^{13}C_{POC}$ signal emanating from diffusive CO_2(aq) uptake is modified significantly when ^{13}C-enriched HCO_3^- ($\delta^{13}C_{HCO_3^-}$, ca 11.9‰ more positive than $\delta^{13}C_{CO_2}$ at 0°C; Mook et al. 1974) is taken up and/or β-carboxylations are dominant mechanisms of carbon acquisition (Descolas-Gros & Fontugne 1990, Raven et al. 1993, Goericke et al. 1994).

Antarctic phytoplankton is generally characterized by low $\delta^{13}C$ values of −24 to −35‰, possibly the result of high CO_2(aq) concentrations at low sea surface temperatures (Rau et al. 1989, Goericke et al. 1994). On the other hand, higher $\delta^{13}C$ values of up to −16.7‰ have been observed in the particulate organic matter obtained from sea ice or from waters closely associated with sea ice (Fischer 1991, Rau et al. 1991), which has been related to photosynthetic carbon assimilation taking place under the confined conditions thought to prevail within the brine channel system (Gleitz et al. 1995).

In this study, the isotopic composition of POC increased sharply from −24 to −9‰ during the experiment (Fig. 2). This change was most pronounced between t_6 and t_{12}, when >90% of the biomass production (i.e. DIC uptake) was due to growth of only 2 species, *Chaetoceros* cf. *neogracile* and *Fragilariopsis cylindrus* (Fig. 5A). During this time interval, CO_2(aq) decreased substantially to well below 0.01 µM (Fig 5B)

In order to ascertain concomitant changes in carbon isotope fractionation, we calculated $\Delta^{13}C$ by subtracting $\delta^{13}C_{POC}$ from $\delta^{13}C_{DIC}$ (Rau et al. 1996). This evaluation revealed that whereas the initial increase in

$\delta^{13}C_{POC}$ until t_8 was apparently related to ^{13}C enrichment of the source DIC (i.e. constant fractionation; Fig. 5B), a profound decrease in $\Delta^{13}C$ by about 8‰ was observed from t_8 until t_{12} when CO_2(aq) concentrations fell to <0.5 µM. In order to clarify if this substantial decrease in fractionation was related to changes in the form of DIC acquisition, we compared photosynthetic carbon uptake (U_{DIC}) for each individual species with the potential maximum flux of CO_2(aq) to the cell surface (Q_a) for consecutive measuring intervals.

For 3 out of the 4 species tested, our calculations suggest that diffusional CO_2(aq) flux was in fact sufficient to satisfy cellular carbon demand (Fig. 5C). The strong discrimination against ^{13}C at CO_2(aq) concentrations >0.5 µM is well in accord with photosynthesis relying primarily on diffusive CO_2(aq) uptake (Goericke et al. 1994, Rau et al. 1996). Only for the largest species employed (*Porosira pseudodenticulata*) does direct HCO_3^- utilization need to be invoked to account for the observed rate of photosynthetic carbon uptake (Fig. 5C). Its contribution to total DIC uptake, however, did not exceed about 20% until t_6, when exponential growth of this species subsided. On the other hand, the steep decrease in carbon isotope fractionation between t_8 and t_{12} coincided with direct HCO_3^- utilization in *Chaetoceros* cf. *neogracile*, exemplified by ratios of $U_{DIC}/Q_a > 1$ (Fig. 5C), which absorbed 70 to 90% of total DIC after t_8. Active uptake of HCO_3^- as opposed to CO_2(aq) diffusion results in a pronounced decrease of discrimination against ^{13}C, and hence less negative $\delta^{13}C$, in microalgae (Sharkey & Berry 1985). Thus, 2 independent lines of evidence (reduced fractionation against ^{13}C together with photosynthetic carbon demand exceeding diffusional CO_2 supply) substantiate the proposed HCO_3^- utilization in *C.* cf. *neogracile* at CO_2(aq) concentrations <0.5 µM and pH >9.5. This interpretation is consistent with the fact that this species has been shown to be a facultative HCO_3^- user in other experiments using the pH-drift technique (Lützenkirchen pers. comm.).

From these considerations, we infer that the physiological ability to actively assimilate HCO_3^- at very low CO_2(aq) concentrations may have been a decisive factor explaining the success of *Chaetoceros* cf. *neogracile* in our experiments. Induction of DIC concentrating mechanisms at low CO_2(aq) concentrations has been shown to result in less negative $\delta^{13}C_{POC}$ values in culture experiments with *Chlamydomonas reinhardtii* (Chlorophyta; Sharkey & Berry 1985). The ecological significance of active HCO_3^- uptake is evident from the fact that about 40% of the total DIC depletion observed in this study occurred after t_8 when CO_2(aq) concentrations decreased to <0.5 µM. Yet, it is clear that this physiological feature alone cannot entirely explain the differential growth patterns observed here, as *Porosira pseudodenticulata* apparently utilized HCO_3^- over its entire growth period. Hence, additional factors such as differential tolerance to other environmental constraints (e.g. increasing pH and strong oxygen oversaturation) may have also controlled growth performance. For instance, Hinga et al. (1994) noted that at high pH, it may be difficult for a cell to maintain a given internal pH when actively transporting charged HCO_3^- across the cell membrane.

In addition to species-specific mechanisms of carbon acquisition, our data along with the observation that sea ice algal blooms are frequently dominated by small diatoms (Garrison & Buck 1989) also suggest that small species may gain their competitive edge simply by virtue of favorable surface to volume ratios and outgrow larger species due to their capacity to sustain growth at much lower nutrient (including DIC) concentrations. It is clear though that biotic factors will additionally influence community structure in nature (e.g. composition of seed population, differential grazing pressure etc.). These, however, were beyond the scope of the present investigation.

CONCLUSIONS

Observations of substantial DIC and nutrient depletion, oxygen oversaturation, strongly alkaline pH together with ^{13}C-enriched POC in samples collected from Antarctic pack ice has prompted several investigators to suggest that the ice habitat may at times resemble a closed system. Our results confirm that polar diatoms have the capacity to bring about levels of DIC depletion, oxygen oversaturation and alkaline pH as were measured in the field. Moreover, >95% of DIC depletion was related to only 2 species with cell volumes ≤100 µm^3, indicating that large species may be diffusion limited at low nutrient and DIC concentrations and do therefore not attain high biomass levels during the spring ice algal bloom. DIC depletion was paralleled by substantial ^{13}C enrichment of POC, suggesting that at CO_2(aq) concentrations <0.5 µM, direct utilization of HCO_3^- was a dominant mechanism of carbon acquisition. Since DIC uptake at such low CO_2(aq) concentrations was almost exclusively due to *Chaetoceros* cf. *neogracile*, physiological differences related to pathways of inorganic carbon uptake may be a decisive factor controlling species diversity in sea ice assemblages during bloom maturation. Prolonged confinement of diatoms within sea ice under post-bloom conditions may impair physiological integrity to such an extent as to prevent growth following release into the open water. We conclude that perturbation of chemical equilibria in sea ice brine in the course of intense biological activity will significantly affect over-

all productivity of an ice algal assemblage, its community structure, and its biochemical composition as well as its potential to recolonize the water column following ice melt. Thus, biological processes related to the concept of the internal ice habitat being effectively isolated from the surrounding water should be considered as a major variable in future sea ice ecophysiological studies.

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