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High plasticity in inorganic carbon uptake by Southern Ocean phytoplankton in response to ambient CO₂

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ABSTRACT

The fixation of dissolved inorganic carbon (DIC) by marine phytoplankton provides an important feedback mechanism on concentrations of CO₂ in the atmosphere. As a consequence it is important to determine whether oceanic primary productivity is susceptible to changing atmospheric CO₂ levels. Among numerous other factors, the acquisition of DIC by microalgae particularly in the polar seas is projected to have a significant effect on future phytoplanktonic production and hence atmospheric CO₂ concentrations. Using the isotopic disequilibrium technique the contribution of different carbon species (CO₂ and bicarbonate) to the overall DIC uptake and the extent to which external Carbonic Anhydrase (eCA) plays a role in facilitating DIC uptake was estimated. Simultaneous uptake of CO₂ and HCO₃⁻ was observed in all cases, but the proportions in which different DIC species contributed to carbon assimilation varied considerably between stations. Bicarbonate as well as CO₂ could be the major DIC source for local phytoplankton assemblages. There was a positive correlation between the contribution of CO₂ to total DIC uptake and ambient concentration of CO₂ in seawater suggesting that Southern Ocean microalgae could increase the proportion of CO₂ uptake under future high atmospheric CO₂ levels. Results will be discussed in view of metabolic costs related to DIC acquisition of Southern Ocean phytoplankton.

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1. Introduction

The concentration of dissolved inorganic carbon (DIC) in seawater is generally several orders of magnitude higher than that of the other plant nutrients. As a result, DIC has for a long time not been regarded a limiting factor for oceanic primary productivity. This notion was challenged by Riebesell et al. (1993), who showed in a study combining model and laboratory data that due to the special chemistry of seawater, phytoplankton can become carbon limited when its DIC acquisition is based on the passive uptake of CO₂. A sufficient supply of DIC is crucial to phytoplanktonic cells, because of the characteristics of the main carbon-fixing enzyme in photosynthesis: ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco). Rubisco accepts CO₂ as well as O₂ as substrates and both compete for the same active site at the enzyme. However, only CO₂ is a suitable substrate in photosynthesis. Fixation of O₂ instead leads to photorespiration, a process wasting metabolic energy (Falkowski and Raven, 1997). Half-saturation carboxylation constants for Rubisco (i.e., the CO₂ concentration at which Rubisco

catalyses chemical reactions at 50% of its maximum rate) in microalgae vary between algal species (Badger et al., 1998), but are typically higher (20–70 μmol kg⁻¹) than the concentration of CO₂ in seawater (10–25 μmol kg⁻¹). At the typical pH range of seawater (7.9–8.3) dissolved CO₂ represents only a small fraction of the oceanic DIC pool (< 1%). The remaining bulk consists for about 90% of HCO₃⁻ and about 10% of CO₃²⁻ (Zeebe and Wolf-Gladrow, 2001), both unsuitable substrates for Rubisco, which need to be converted to CO₂ to become available for photosynthesis.

Research on carbon utilization has been dominated by studies on freshwater macrophytes and cyanobacteria, which experience a much wider range of CO₂ concentrations (Badger et al., 1978; Prins and Elzenga, 1989). However, since the late 1970s numerous laboratory studies have shown that also marine microalgae developed different ways for intracellular DIC accumulation (Beardall et al., 1976; Espie and Colman, 1986; Colman et al., 2002; Reinfelder et al., 2004). These strategies to enhance the concentration of CO₂ in the vicinity of Rubisco, and thus out-compete O₂ as a substrate, are commonly referred to as Carbon Concentrating Mechanisms (CCMs) (Beardall et al., 1998; Raven and Beardall, 2003; Giordano et al., 2005).

CO₂ molecules, which are small and do not carry an electric charge, can pass the cell membrane either via diffusion or active

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transport (Miller et al., 1988), whereas HCO_3^- ions carry a negative charge and solely can enter the cell via membrane bound symporters or antiporters (Colman et al., 2002; Giordano et al., 2005). Active transport of molecules against an electrochemical (or in case of CO_2 : chemical concentration) gradient requires energy; either in terms of light energy and/or nutrients (Raven and Lucas, 1985). These overall costs consist of investments in the biosynthesis of the carrier protein itself and those necessary to energize the transport process (Raven and Lucas, 1985; Raven and Johnston, 1991). On the other hand costs involved with operation of a CCM might be compensated by the avoidance of photorespiration and/or the need for smaller amounts of Rubisco.

Another concept of a CCM involves indirect utilization of the bicarbonate pool via the enzyme external carbonic anhydrase (eCA). External CA is known to significantly accelerate the interconversion of CO_2 and HCO_3^- . Located at the outside of the cell the enzyme is commonly regarded to facilitate the production and consequently uptake of CO_2 at intermediate values of pH (e.g. Falkowski and Raven, 1997; Elzenga et al., 2000). An alternative view on the functioning of eCA at high pH suggests, that eCA serves the purpose of a CO_2 recycling system by rapidly converting CO_2 which leaked out of the cell back to HCO_3^- (Martin and Tortell, 2008; Trimborn et al., 2008). Within this 'pump and leak' process eCA could serve as part of an energy dissipation mechanism to protect phytoplankton from excess light energy and hence photodamage (Tchernov et al., 1997; Kaplan and Reinhold, 1999). Such a mechanism could be especially relevant under low Fe and/or low CO_2 conditions (Young and Beardall, 2005).

Although laboratory studies have been very useful in studying the functionality of CCMs, there are several problems that may hamper extrapolation to the real world. Several studies have shown that inorganic carbon acquisition differs between species (Rost et al., 2003, 2006; Trimborn et al., 2009) and even between strains of the same species (Elzenga et al., 2000). This inter- and intraspecific variability and the diversity in strategies for the uptake of DIC complicate realistic projections of global phytoplanktonic uptake of DIC and underline the necessity to characterize carbon uptake for natural phytoplankton assemblages in different regions of the oceans and how these respond to changes in the DIC system.

The Southern Ocean is considered to be one of the key regions with respect to oceanic carbon cycling and has received much attention in recent years (Kohfeld et al., 2005; Arrigo et al., 2008; Takahashi et al., 2009). It is firmly established that carbon draw-down in Antarctic coastal seas is driven by the *in-situ* biological production (Lo Monaco et al., 2005; Arrigo et al., 2008). Phytoplankton assemblages in the Southern Ocean are able to form blooms, which have the potential to export photosynthetically fixed carbon into the deep ocean, a process referred to as the biological pump (Volk and Hoffert, 1985; de Baar et al., 1995; Arrigo et al., 1999). Several studies have shown that phytoplanktonic production in this region is primarily controlled by the inadequate availability of dissolved iron (de Baar et al., 1990; Buma et al., 1991; Boyd et al., 2007) and light (Boyd, 2002). Iron deficiency directly decreases pigment content and consequently light harvesting efficiency of the photosynthetic systems of microalgae (van Leeuwe and de Baar, 2000; Timmermans et al., 2001; van de Poll et al., 2009) as well as decreases the photosynthetic electron transport capacity (the effects of iron-limitation were reviewed by Geider and La Roche, 1994). Ultimately the production of reductant in the form of NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) and chemical energy in the form of adenosine-5'-triphosphate (ATP) is reduced. Low availability of light in the Southern Ocean additionally enhances this energy deficit (Raven, 1990; Sunda and Huntsman, 1997; Boyd, 2002).

Whether the supply of DIC could pose another co-limiting factor to Southern Ocean phytoplankton has received only limited attention. However, accurate estimates of the relative contribution of CO_2 and HCO_3^- are essential for the parameterisation of physiology-based phytoplankton growth models and ultimately global carbon models. Due to the small number of studies, however, it is difficult to judge whether these figures might be valid for marine microalgae assemblages in general and whether these parameters are related to changes in the concentration of CO_2 .

In this study, we present the results of assessments of DIC uptake by natural phytoplankton assemblages from the Atlantic section of the Southern Ocean. The acquisition of DIC was measured along three different transects: on the Prime Meridian, in the Weddell Sea and in the Drake Passage. Due to the extent of the study area, different oceanographic regimes (open ocean upwelling, High Nutrient Low Chlorophyll (HNLC) open ocean, coastal shelf, ice covered shelf) characterized by distinct chemical and physical parameters were sampled. Ambient variations in the carbon chemistry of seawater resulted in a natural experiment. These data are discussed in the perspective of rising atmospheric CO_2 and plasticity of Southern Ocean phytoplankton, defined as the ability to physiologically modify inorganic carbon uptake in response to future high CO_2 levels.

2. Material and methods

To assess inorganic C uptake of natural phytoplankton assemblages, 22 stations were sampled in the Atlantic sector of the Southern Ocean (R.V. Polarstern, ANT XXIV-3, February–April 2008) (Fig. 1).

2.1. Sampling and filtration

Seawater samples were collected from the deep chlorophyll maximum when present. In case of a uniform surface mixed layer, the samples were taken at 40 m depth. To minimize stress on phytoplanktonic cells, the subsequent handling took place under dim light in a temperature-controlled (4 °C) laboratory container.

Depending on the natural cell abundance, 6 to 12 L seawater were vacuum filtered (< 0.1 bar, 100–200-fold concentrated) onto 2 µm pore size polycarbonate filters (diameter 47 mm, GE Water & Process Technologies, Belgium). The concentrated sample was then subdivided into samples for short-term $^{14}\text{CO}_2/\text{H}^{14}\text{CO}_3^-$ disequilibrium experiments, analysis of phytoplanktonic pigments and microscopic analysis.

During filtration, cells were gently kept in suspension using a plastic pipette. In order to check for physiological damage due to the filtration procedure, the efficiency of photosystem II (Fv/Fm) was measured using chlorophyll fluorometry (Phyto PAM, Heinz Walz GmbH, Germany), before and after filtration at station 104. No significant change following filtration could be observed (Fv/Fm = 0.42 ± 0.05 before and after filtration, E. Freijling, personal communication, 2008). For future experimental designs it would be desirable to perform Fv/Fm measurements before and after the filtration step and quantify the amount of biomass lost as routine control measurements. Comparison of concentrated and directly filtered HPLC subsamples revealed that biomass losses, possibly as large as 30%, could have occurred.

2.2. Carbon acquisition mechanisms

2.2.1. Short-term $^{14}\text{CO}_2/\text{H}^{14}\text{CO}_3^-$ disequilibrium experiments

The contribution of direct HCO_3^- , CO_2 and eCA-mediated uptake of inorganic C was studied using the isotopic disequilibrium

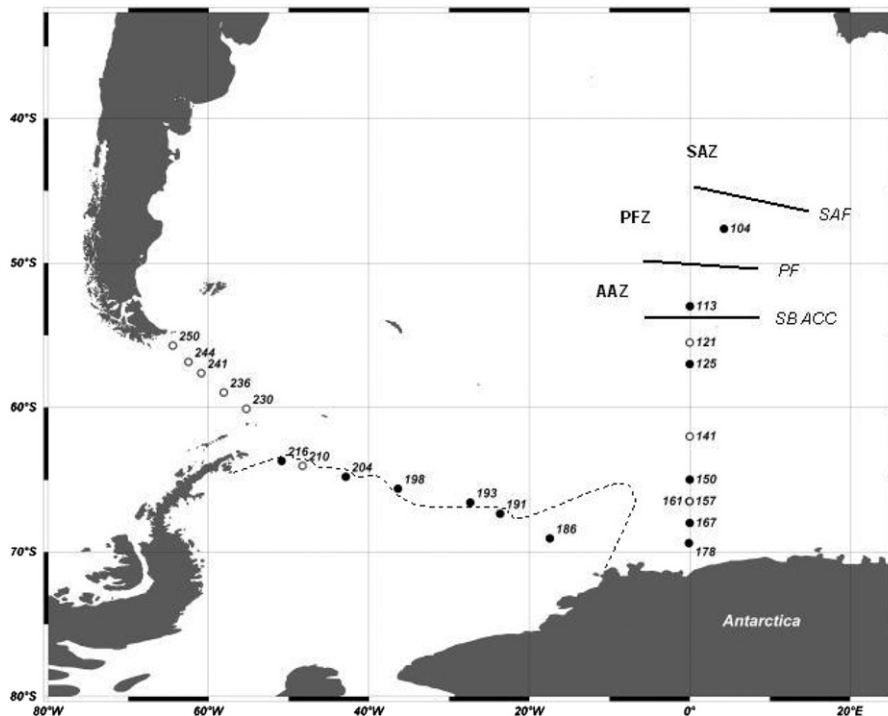


Fig. 1. Study area and sampling stations in the Atlantic sector of the Southern Ocean during ANT XXIV/3 with R.V. Polarstern (February–April 2008). Black dots represent stations for which DIC uptake data is available. Note that station 157 and 161 is the same location sampled twice. The locations of fronts are taken from Middag et al. (2011). (Abbreviations in alphabetical order: AAZ: Antarctic Zone, PF: Polar Front, PFZ: Polar Frontal Zone, SAZ: Subantarctic Zone, SAF: Subantarctic Front, SB ACC: Southern Boundary of the Antarctic Circumpolar Current.) The dashed line represents the approximate position of the ice edge in March 2008.

technique (Espie and Colman, 1986) following the protocol of Elzenga et al. (2000) with minor adjustments.

The method makes use of a relatively slow equilibration of DIC species. Upon a $^{14}\text{CO}_2$ spike (at pH 7), the ^{14}DIC uptake of phytoplankton is followed until equilibrium is re-established (at pH 8.5). At the low experimental temperature (2°C), it takes approximately 2.5 min until the surplus of the $^{14}\text{CO}_2$ has subsided, whereas in the presence of eCA, the imposed disequilibrium of carbon species is broken down more quickly. By conducting the experiment with and without an inhibitor of eCA, it is possible to quantify uptake rates of CO_2 and HCO_3^- and semi-quantitatively determine the relative contribution of eCA facilitated DIC uptake to total C acquisition.

Immediately after filtration, 4 ml of the concentrated phytoplankton samples were buffered at pH 8.5 (final concentration 2 mM bis-Tris-propane-HCl buffer, pH was measured each time before and after buffer addition) and transferred into a temperature- (2°C) and light- ($100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$, KL 1500 electronics, Schott, Germany) controlled glass cuvette. Cells were left for 5 min before the start of the experiment to allow steady state photosynthesis to be reached.

To initiate the experiment, a spike of $10\ \mu\text{L}$ radioactively labelled sodium bicarbonate ($740\ \text{kBq}$ ($20\ \mu\text{Ci}$) $\text{NaH}^{14}\text{CO}_3$, CFA.3 GE Healthcare, Germany) buffered at pH 7 in 50 mM BTP-HCl buffer was added to the concentrated phytoplankton sample. This resulted in a $\text{SA}_{\text{DIC}} = 6.73 \times 10^{12}\ \text{DPM mol}^{-1}$. Subsequently, subsamples of $200\ \mu\text{L}$ were drawn in short time intervals and mixed directly into $1.5\ \text{mL}$ 6 N HCl. As a consequence, photosynthetic carbon fixing activity is stopped and unfixed inorganic ^{14}C is converted into CO_2 , which then will degas from the solution. The same experiment was repeated after the addition of an inhibitor of eCA, Acetazolamide (Sigma, The Netherlands) in the final concentration of $20\ \mu\text{M}$. The inhibitor was added to the phytoplankton sample at least 15 min before the experiment.

After degassing overnight, samples were neutralized by the addition of 1.4 mL of 6 N NaOH. Subsequently, 10 mL scintillation cocktail (Ultima Gold AB, Packard) was added and samples were measured in a Liquid Scintillation Counter (Tri-Carb[®] 2900 TR, Packard) at least 6 h later to avoid possible quenching effects. To account for residual ^{14}C , $0.2\ \mu\text{m}$ filtered seawater blanks were treated in the same way as phytoplankton samples and background counts were subtracted from experimental counts.

2.2.2. Statistics and fitting procedure

By following the incorporation of acid stable fixed ^{14}C during the time course of equilibration, it is possible to evaluate which C species is taken up (Elzenga et al., 2000; Rost et al., 2007). The measured radioactivity at time t (DPM_t in dpm s^{-1}) is the sum of incorporated $^{14}\text{CO}_2$ and/or $\text{H}^{14}\text{CO}_3^-$ and can be calculated using the following formula (after Elzenga et al., 2000):

$$\text{DPM}_t = V_{\text{CO}_2} \left[\alpha_1 t + \frac{\Delta \text{SA}_{\text{CO}_2}}{\text{SA}_{\text{DIC}}} \times (1 - e^{-\alpha_1 t}) \right] / \alpha_1 + V_{\text{HCO}_3^-} \left[\alpha_2 t + \frac{\Delta \text{SA}_{\text{HCO}_3^-}}{\text{SA}_{\text{DIC}}} \times (1 - e^{-\alpha_2 t}) \right] / \alpha_2$$

where V_{CO_2} and $V_{\text{HCO}_3^-}$ (in dpm s^{-1}) represent the uptake rates of $^{14}\text{CO}_2$ and $\text{H}^{14}\text{CO}_3^-$. The remainder of the term describes the changes in specific activity of $^{14}\text{CO}_2$ and $\text{H}^{14}\text{CO}_3^-$ in time. The parameters α_1 and α_2 are rate constants for the equilibration of CO_2 and HCO_3^- , which change as a function of temperature, salinity and pH. They were calculated using equilibrium constants ($\text{p}K_1 = 6.08$, $\text{p}K_3 = 9.33$, measured at 2°C , salinity = 34.78) of Dickson and Millero (1987). Values for the dissociation constants ($k_1 = 0.003\ \text{s}^{-1}$ and $k_3 = 1900\ \text{kg mol}^{-1}\ \text{s}^{-1}$) were taken from Zeebe and Wolf-Gladrow (2001, figure 2.3.6).

The SA_{DIC} denotes the specific activity of total dissolved inorganic carbon, thus of the initial ^{14}C spike (accordingly SA_{DIC} does not

change during the experiment). The ratios $\Delta SA_{CO_2}/SA_{DIC}$ and $\Delta SA_{HCO_3^-}/SA_{DIC}$ are the changes in specific activities of $^{14}CO_2$ and $H^{14}CO_3^-$ over time. At 2 °C, salinity 34 and pH 8.5 the values of α_1 and α_2 (per second) are 0.0203 and 0.0233, respectively, while $\Delta SA_{CO_2}/SA_{DIC} = 31.83$ and $\Delta SA_{HCO_3^-}/SA_{DIC} = 0.0238$.

Eq. (1) was fitted to the data using the non-linear fitting function of Prism software version 4.03 for Windows (Graph Pad Software, San Diego, California, USA). The uptake rates for HCO_3^- and CO_2 were free-running parameters, but constrained to >0 . Akaike's Information Criterion was used to evaluate whether a free running intercept as a second parameter should be introduced to improve the goodness of fit. In 67% of the cases the simple model (intercept=0) fitted the data better than the more complex model (free running intercept), hence the simple model was used to fit data in all cases.

The contribution of eCA to total carbon uptake was calculated by subtracting the percentage of HCO_3^- uptake after eCA was inhibited from the percentage of HCO_3^- uptake in the control experiments. When the fitted uptake curves for control and AZ treated cells differed significantly from each other the contribution of eCA was considered to be significant.

2.2.3. Steady state inorganic carbon fixation

The uptake of ^{14}C was followed for approximately 2 more minutes after the new equilibrium of C species had been reached. The slope of this segment of the DIC uptake curves represents total DIC uptake at steady state photosynthesis after the carbon species have equilibrated and is therefore linear. Based on this data DIC fixation rates per 1 m⁻³ seawater (P_t) were calculated (after Nielsen and Bresta, 1984):

$$P_t = \frac{V_{DI^{14}C}[DIC] \times 12 \times 1.05}{TC} \times \frac{1}{FE} \times 1000$$

V_{DIC} (in dpm h⁻¹) corresponds to the rate at which phytoplanktonic cells are taking up $DI^{14}C$. $[DIC]$ is the concentration of DIC measured in situ at the same station and is given in mmol L⁻¹. 12 is the atomic weight of carbon. 1.05 is a correction factor for the effect of ^{14}C discrimination, which is 5% slower than the uptake of the ^{12}C isotope. TC stands for 'total counts', hence represents the total amount of ^{14}C added to the experiment. The remainder of the term (FE=filtration equivalent) is necessary for the conversion to m⁻³ seawater.

Due to the short incubation time, P_t is indicative of gross production at the specified conditions.

2.3. Photopigment analysis

Known volumes of concentrated samples were filtered through Whatman GF/F glass-fibre filters (diameter 25 mm), immediately snap-frozen in liquid nitrogen, wrapped in aluminium foil and stored at -80 °C until analysis. Filters were freeze-dried, extracted in 90% acetone and measured with a Waters liquid chromatography HPLC system (Model 2690) following the protocol of van Leeuwe et al. (2006).

2.4. Microscope samples

For microscopic counts 5 mL of concentrated seawater were fixed immediately with 50 μ L Lugol's iodine glutaraldehyde mixture and stored at 4 °C until analysis. Samples were allowed to settle overnight. Species were identified according to Tomas and Hasle (1997) and Scott and Thomas (2005) and counted in horizontal transects with an inverted microscope (Olympus IMT-2). Cell sizes (length or diameter) were measured ($n=20$) under the microscope and used to calculate biovolumes using approximations of the cell geometry (Count Manager 6.2a, Koeman &

Bijkerk B.V., The Netherlands). When $n < 10$ default cell sizes of the programme were used. Subsequently phytoplankton was classified in the size groups: small: $< 1000 \mu m^3$, medium: $1001-5000 \mu m^3$ and large: $> 5000 \mu m^3$.

2.5. Chemical analyses

Samples for measurements of DIC and total alkalinity (A_T) were analysed on a VINDTA 3C instrument (Versatile Instrument for Determination of Titration Alkalinity, MARIANDA, Kiel, Germany) in general accordance with Dickson et al. (2007). For a detailed description of the method the reader is referred to van Heuven et al. (2011). Nutrients were analysed spectrophotometrically after Grasshoff and Ehrhardt (1983) using a TrAAcs autoanalyzer 800+ (Bran and Luebbe, Hamburg, Germany). Samples for measurements of dFe were measured on board following the method of de Baar et al. (2008) with minor adjustments using a flow injection-chemiluminescence method (Klunder et al., 2011).

3. Results

3.1. General description of different transects

Clear latitudinal trends in temperature and concentrations of nutrients (N, P, Si) were observed in the near-surface waters (0–60 m) through the Prime Meridian, Weddell Sea and Drake Passage transects (Table 1).

Stations north of 60°S along the Prime Meridian were characterized by water temperature > 0 °C and nutrient concentrations representative of the pelagic ocean (Knox, 2007 and references therein). South of 60°S water temperature dropped below 0 °C and nutrients concentrations increased to typical values for HNLC regions. As is characteristic for the Southern Ocean, concentrations of dissolved iron (dFe) were very low, decreasing from 0.21 nmol L⁻¹ north of 60°S to approximately 0.12 nmol L⁻¹ close to the Antarctic continent. A detailed description of dFe profiles is provided by Klunder et al. (2011). The sea surface concentration of CO_2 varied from 20.19 to 26.83 μ mol kg⁻¹. A full description of the parameters of the carbonate system during ANT XXIV-3 is presented by van Heuven et al. (2011).

The Weddell Sea transect was characterized by early and strong ice coverage (with the exception of station 198). This coincided with high nutrient concentrations and supersaturated pCO_2 values (van Heuven et al., 2011). Concentrations of dFe were approximating 0.05 nmol L⁻¹ (Klunder et al., 2011).

Finally when crossing the Drake Passage, the temperature rose above 0 °C towards the South American continent and macronutrient concentrations decreased again.

3.2. Description of the phytoplanktonic community

Direct Chl *a* measurements sometimes deviated from our measurements derived from concentrated samples, but overall trends are comparable (Alderkamp et al., 2010). Distinct differences in the distribution of calculated Chl *a* were present, ranging from 6.7 to 212 ng Chl *a* L⁻¹ (Table 2). Within this range, calculated Chl *a* was low on the Prime Meridian (47.1 ± 17.5 ng L⁻¹ average of stations 104, 121, 125, 141) with exception of the Antarctic zone (station 113, approximately 161 ng L⁻¹) and coastal currents (Fig. 2A). In the majority of cases, biomass close to the ice edge and in the Weddell Sea ranged between 140 and 200 ng Chl *a* L⁻¹. The lowest biomass was observed in the Drake Passage with calculated Chl *a* concentrations lower than 30 ng L⁻¹ (average: 14.28 ± 8.76 ng L⁻¹).

Table 1
Overview of non-biological properties of sampling stations during ANT XXIV/3.

| Station | Date | Sampling depth (m) | Temperature (°C) | Nitrate ($\mu\text{mol kg}^{-1}$) | Phosphate ($\mu\text{mol kg}^{-1}$) | Silicate ($\mu\text{mol kg}^{-1}$) | CO ₂ ($\mu\text{mol kg}^{-1}$) | CO ₂ (μatm) |
|---------|-------------|--------------------|------------------|-------------------------------------|---------------------------------------|--------------------------------------|---|-------------------------------------|
| 104* | 17 Feb 2008 | 60.8 | 6.5 | 20.37 | 1.42 | 5.67 | 20.19 | 346 |
| 113* | 20 Feb 2008 | 73.6 | 1.2 | 26.06 | 1.75 | 35.55 | 22.32 | 382 |
| 121 | 22 Feb 2008 | 60.1 | 0.4 | 25.82 | 1.77 | 57.04 | 22.79 | 396 |
| 125* | 23 Feb 2008 | 50.3 | 0.5 | 26.01 | 1.79 | 63.38 | 21.74 | 373 |
| 141 | 27 Feb 2008 | 49.8 | 0.3 | 25.96 | 1.90 | 59.96 | 25.20 | 407 |
| 150* | 29 Feb 2008 | 39.5 | -0.7 | 25.54 | 1.70 | 64.94 | 21.25 | 364 |
| 157 | 07 Mar 2008 | 45.1 | -1.7 | 28.89 | 2.04 | 64.44 | 22.15 | 380 |
| 161* | 09 Mar 2008 | 40.5 | -1.7 | 29.75 | 2.09 | 62.68 | 25.81 | 437 |
| 167* | 10 Mar 2008 | 39.1 | -0.7 | 26.20 | 1.75 | 63.96 | 21.67 | 382 |
| 178* | 12 Mar 2008 | 20.0 | -1.5 | n/a | n/a | n/a | 20.21 | 333 |
| 186* | 15 Mar 2008 | 25.0 | -1.8 | 27.45 | 1.92 | 65.24 | 23.21 | 398 |
| 191* | 17 Mar 2008 | 55.9 | -1.7 | 28.26 | 1.93 | 73.42 | 24.11 | 414 |
| 193* | 19 Mar 2008 | 60.7 | -1.6 | 28.95 | 2.00 | 77.12 | 24.60 | 423 |
| 198* | 21 Mar 2008 | 50.2 | -1.5 | n/a | n/a | n/a | 21.09 | 353 |
| 204* | 23 Mar 2008 | 50.4 | -1.7 | 29.26 | 2.04 | 79.85 | 25.99 | 447 |
| 210 | 25 Mar 2008 | 45.5 | -1.6 | 30.73 | 2.11 | 85.91 | 25.02 | 428 |
| 216* | 27 Mar 2008 | 29.9 | -1.8 | 28.92 | 2.05 | 73.04 | 23.90 | 410 |
| 230 | 02 Apr 2008 | 65.1 | 0.7 | 28.90 | 2.05 | 75.15 | 26.47 | 422 |
| 236 | 05 Apr 2008 | 60.0 | 2.5 | 26.59 | 1.84 | 39.88 | n/a | n/a |
| 241 | 07 Apr 2008 | 75.0 | 2.7 | 25.64 | 1.78 | 10.44 | n/a | n/a |
| 244 | 09 Apr 2008 | 61.0 | 5.3 | 22.56 | 1.59 | 4.23 | n/a | n/a |
| 250 | 11 Apr 2008 | 73.4 | 5.1 | 23.01 | 1.60 | 4.18 | n/a | n/a |

Asterisk indicates stations for which DIC uptake data is available (n/a=not analysed).

Table 2
Overview of biological properties of the concentrated samples used in this study.

| Station | Date | Chl <i>a</i> (ng L ⁻¹) | Size classes (%) | | | Dominant phytoplankton (microscopic analysis) |
|---------|-------------|------------------------------------|------------------|--------|-------|---|
| | | | Small | Medium | Large | |
| 104* | 17 Feb 2008 | 57.3 | 48 | 2 | 50 | Dinoflagellates, small flagellates |
| 113* | 20 Feb 2008 | 161.4 | 10 | 1 | 89 | Large diatoms |
| 121 | 22 Feb 2008 | 64.7 | n/a | n/a | n/a | n/a |
| 125* | 23 Feb 2008 | 40.9 | 9 | 20 | 71 | Large diatoms |
| 141 | 27 Feb 2008 | 25.6 | n/a | n/a | n/a | n/a |
| 150* | 29 Feb 2008 | 193.3 | 5 | 17 | 78 | Large diatoms |
| 157 | 07 Mar 2008 | 148.2 | n/a | n/a | n/a | n/a |
| 161* | 09 Mar 2008 | 144.3 | 2 | 11 | 87 | Large diatoms |
| 167* | 10 Mar 2008 | 212.1 | 3 | 10 | 87 | Large diatoms |
| 178* | 12 Mar 2008 | 183.3 | 3 | 15 | 82 | Large diatoms |
| 186* | 15-Mar 2008 | 23.8 | n/a | n/a | n/a | n/a |
| 191* | 17 Mar 2008 | 89.7 | 5 | 2 | 93 | Large diatoms |
| 193* | 19 Mar 2008 | 160.2 | 5 | 20 | 75 | large diatoms |
| 198* | 21 Mar 2008 | 163.7 | 7 | 44 | 49 | Large and medium sized diatoms |
| 204* | 23 Mar 2008 | 92.7 | 7 | 17 | 76 | Large diatoms |
| 210 | 25 Mar 2008 | 70.1 | 12 | 32 | 56 | Large and medium sized diatoms |
| 216* | 27 Mar 2008 | 29.1 | 20 | 13 | 67 | Large diatoms |
| 230 | 02 Apr 2008 | 6.7 | 38 | 62 | 0 | Small and medium sized diatoms |
| 236 | 05 Apr 2008 | 27.2 | n/a | n/a | n/a | n/a |
| 241 | 07 Apr 2008 | 19.3 | n/a | n/a | n/a | n/a |
| 244 | 09 Apr 2008 | 8.1 | n/a | n/a | n/a | n/a |
| 250 | 11 Apr 2008 | 10.0 | n/a | n/a | n/a | n/a |

Asterisk indicates stations for which DIC uptake data is available. Presented Chl *a* data reflect the ambient seawater concentration assuming no loss during filtration step. Phytoplanktonic size classes are given in % based on biovolumes (small= < 1000 μm^3 , medium= 1000–5000 μm^3 , large= > 5000 μm^3).

Analysis of photopigments and microscopic examination revealed that the phytoplanktonic community south of 60°S consisted mainly of large Southern Ocean diatoms (Table 2 and Fig. 2B), such as: *Corethron*, *Leptocylindrus*, *Proboscia*, *Dactyliosolen*, *Chaetoceros*, *Navicula* and *Rhizosolenia*. In most samples, only small numbers of dino- and silicoflagellates and few prymnesio-, chloro- and cryptophytes were present. However, the contribution of 19'hexanoyloxyfucoxanthin to overall pigment composition increased towards the Northern stations (station 104, 241, 244, 250), indicating an increase in the fraction of prymnesiophytes of the phytoplanktonic community.

3.3. DIC uptake of natural phytoplankton assemblages

Of the 22 stations where isotopic disequilibrium experiments were performed, the data of 13 stations were of sufficient quality to allow determination of the contribution of CO₂ and HCO₃⁻ uptake. Of these 13 stations 7 were on the zero meridian and 6 in the Weddell Sea. Steady state inorganic carbon fixation, *P_i*, an indicator for primary productivity could be determined from all stations.

Two typical examples of short-term ¹⁴CO₂/H¹⁴CO₃⁻ disequilibrium experiments are shown in Fig. 3. The differences in curvature indicate differences in the fractions of CO₂ uptake

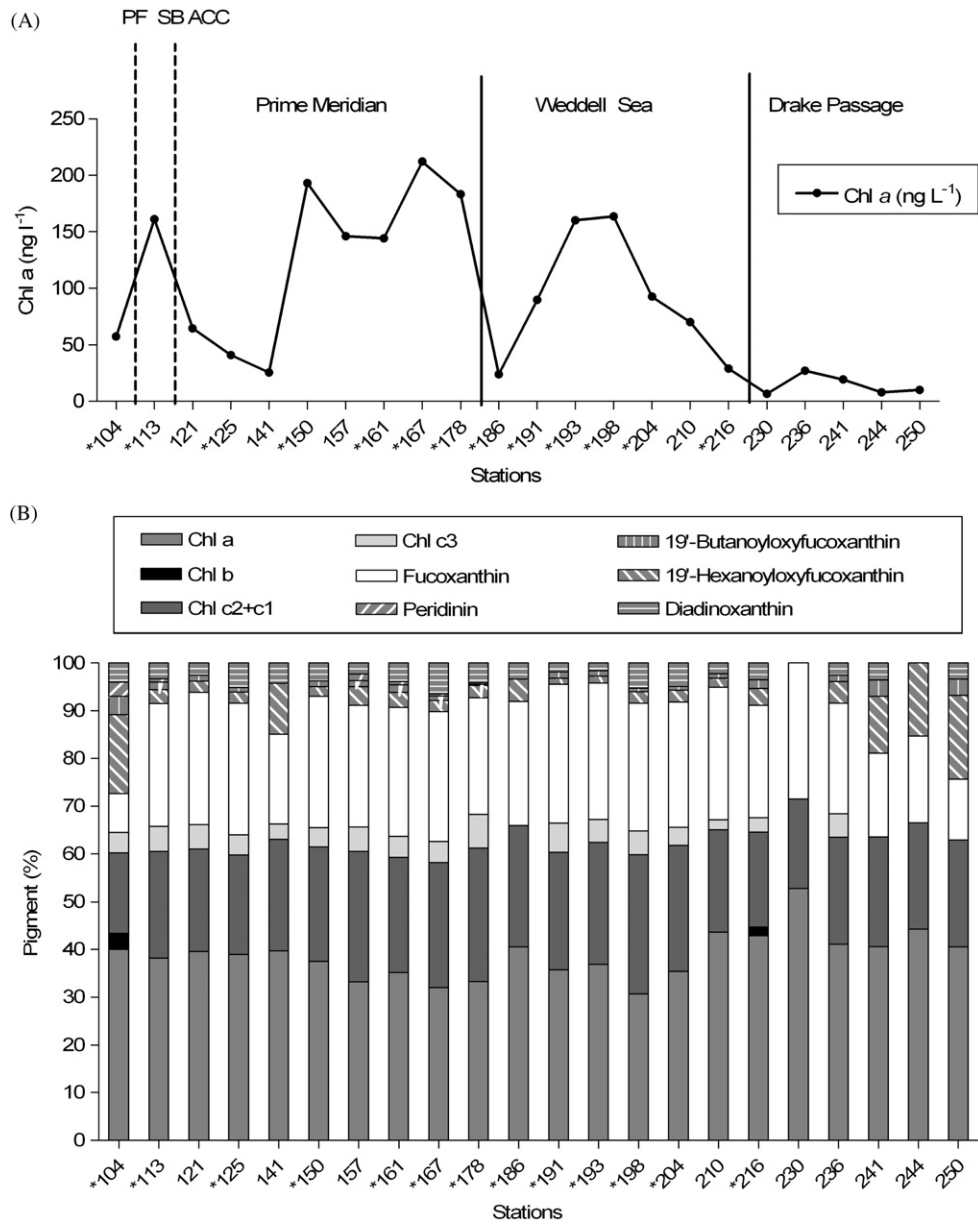


Fig. 2. (A) Overview of Chl *a* distribution (in ng L⁻¹) of sampling stations during ANT XXIV-3. (B) Overview of phytoplankton marker pigment distribution (in %) of sampling stations during ANT XXIV-3.

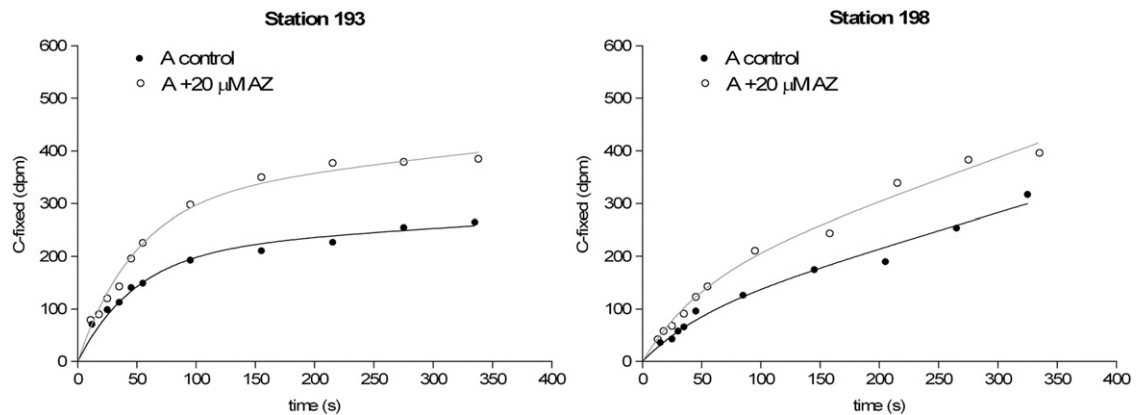


Fig. 3. Examples of short-term ¹⁴CO₂/H¹⁴CO₃⁻ disequilibrium experiments for 2 stations from the Weddell Sea. Station 193 is dominated by CO₂ uptake. Station 198 is dominated by direct HCO₃⁻ uptake (AZ: acetazolamide).

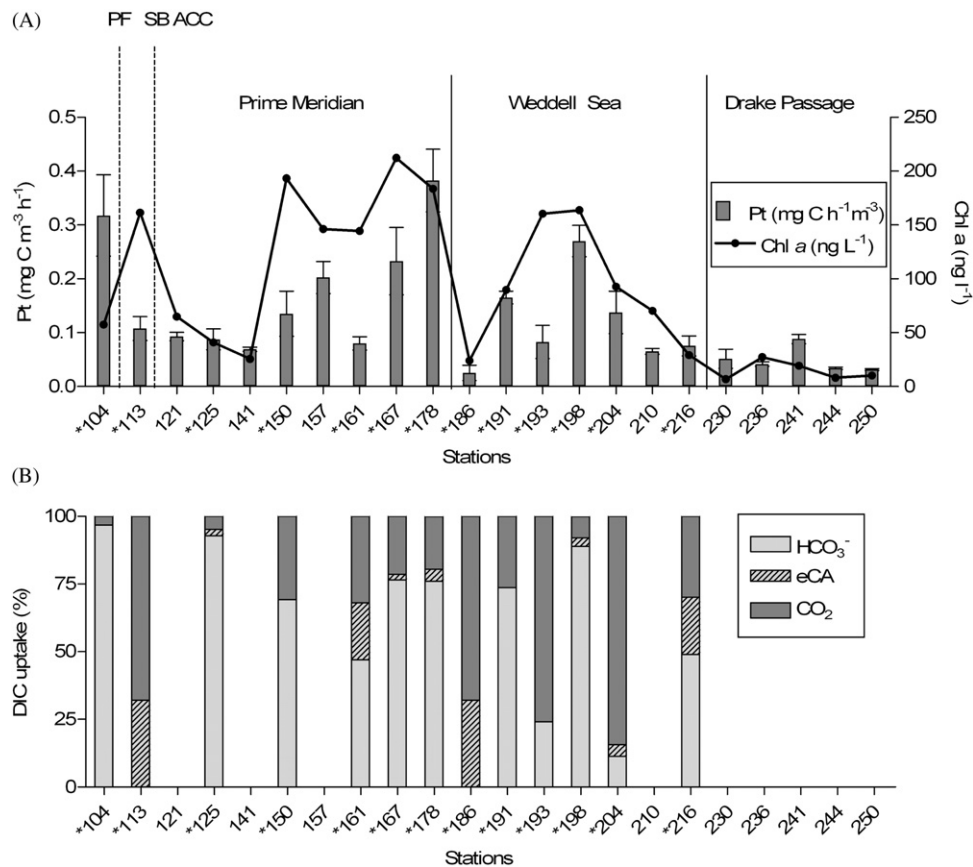


Fig. 4. (A) Steady state inorganic carbon uptake (P_t in mg C m⁻³ h⁻¹) and Chl *a* (in ng L⁻¹) of sampling stations during ANT XXIV-3. (B) Contribution of CO₂, HCO₃⁻ and eCA-mediated conversion of HCO₃⁻ (in %) to total DIC uptake of the phytoplanktonic community is shown for 13 stations during ANT XXIV-3.

Table 3

Contribution of different C species (in % ± SE) and eCA to phytoplanktonic DIC uptake.

| Station | HCO ₃ ⁻ | CO ₂ | eCA |
|---------|-------------------------------|-----------------|---------------|
| 104 | 96.82 ± 0.51 | 3.18 ± 1.89 | 0.00 ± 0.55 |
| 113* | 0.00 ± 12.18 | 67.87 ± 0.67 | 32.13 ± 14.09 |
| 125* | 92.85 ± 1.06 | 4.79 ± 1.92 | 2.36 ± 1.50 |
| 150 | 69.28 ± 1.59 | 30.72 ± 1.78 | 0.00 ± 8.79 |
| 161* | 47.05 ± 1.96 | 31.85 ± 1.96 | 21.10 ± 8.27 |
| 167* | 76.52 ± 6.41 | 21.47 ± 0.98 | 2.01 ± 7.93 |
| 178* | 76.04 ± 5.52 | 19.56 ± 1.20 | 4.40 ± 6.36 |
| 186* | 0.00 ± 11.77 | 67.87 ± 1.66 | 32.13 ± 19.21 |
| 191 | 73.74 ± 4.70 | 26.26 ± 1.21 | 0.00 ± 7.07 |
| 193 | 24.13 ± 15.63 | 75.87 ± 0.99 | 0.00 ± 19.03 |
| 198* | 88.97 ± 0.89 | 7.95 ± 0.96 | 3.08 ± 1.14 |
| 204* | 11.37 ± 9.47 | 84.33 ± 1.17 | 4.30 ± 14.65 |
| 216* | 48.96 ± 2.92 | 29.85 ± 2.92 | 21.19 ± 11.68 |

Asterisk indicates stations for which eCA contribution was found to be significant. The SE's for the contribution of eCA were calculated from the SE's for HCO₃⁻ contributions determined from control and eCA inhibited experiments using SE propagation.

versus bicarbonate uptake, while the final slope represents steady state DIC uptake (P_t). Station 193 illustrates a community where CO₂ is the preferred carbon species taken up. In contrast station 198 is dominated by direct bicarbonate uptake. Pollock and Colman (2001) reported that the commonly used CA inhibitors AZ and dextrane-bound sulphonamide (DBS) inhibited active HCO₃⁻ transport in *Chlorella saccharophila* and attributed this finding to a CA-like transporter. If this is the case, HCO₃⁻ contribution to total carbon acquisition will be underestimated. An inhibitory effect can be perceived as a reduced steady state ¹⁴C

uptake rate, relative to the control. Comparing Control and AZ-treated experiments we observed no general trends, indicating that photosynthesis was not constrained by the inhibitor treatment.

P_t averaged 0.14 ± 0.07 mg C m⁻³ seawater h⁻¹ (Fig. 4A) and was strongly correlated to concentrations of Chl *a* (Pearson correlation, (one-tailed) $p=0.001$, $r=0.72$). However, the absolute values for P_t should be treated with caution as P_t has been obtained under experimental (high pH (8.5), high light) and not in situ conditions. On the other hand, using these data to calculate specific growth rates, yielded rates of 0.37 (± 0.28) per day, indicating that the cells in the experimental set-up were performing well.

The proportions of DIC uptake due to CO₂ and HCO₃⁻ of phytoplankton assemblages in the Weddell Sea and on the Prime Meridian were highly variable (Table 3 and Fig. 4B). On average, bicarbonate (direct and indirect) uptake accounted for 64 (± 28)% of the total DIC uptake, ranging from 0% to 98%. In 9 out of 13 samples overall DIC uptake was dominated by HCO₃⁻ uptake. At the other 4 stations the phytoplanktonic community consisted of predominant CO₂ users, with 68–85% of DIC uptake due to CO₂.

In a total of 9 samples, the presence of external Carbonic Anhydrase (eCA) was demonstrated, although in 5 of those stations the contribution of eCA to overall DIC uptake was minimal (2–5%). In the remaining 4 cases (station 113, 161, 186 and 216), utilization of HCO₃⁻ via eCA contributed 21–32% to the total DIC uptake.

3.4. Environmental and taxonomical effects on phytoplanktonic DIC uptake

The dataset was examined for potential correlations between DIC uptake mechanisms and ambient concentrations of CO₂ in

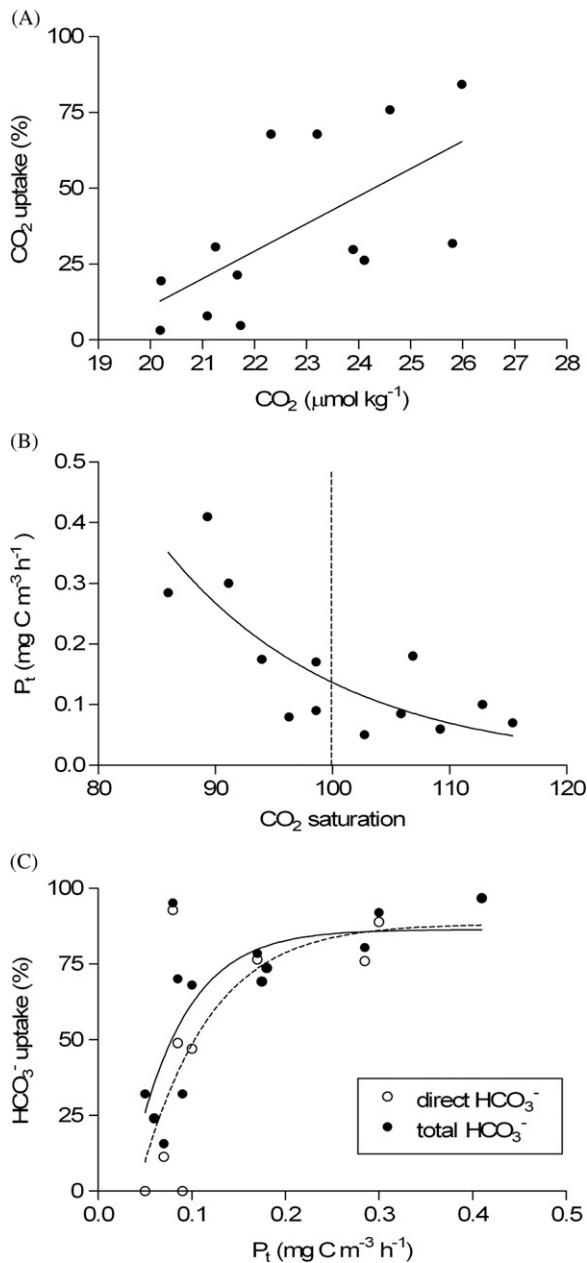


Fig. 5. (A) Influence of ambient CO₂ concentration (in μmol kg⁻¹) on phytoplankton uptake of CO₂ (in %). (B) Ambient CO₂ saturation of seawater expressed as percentage of atmospheric CO₂ concentration decreases with increasing DIC uptake at steady state photosynthesis (P_t). (C) Fraction of direct and total (direct and eCA mediated) uptake of HCO₃⁻ increases with increasing DIC uptake at steady state photosynthesis (P_t).

seawater as well as the taxonomic composition and size distribution of the concentrated phytoplankton samples.

The contribution of CO₂ to total DIC uptake was significantly correlated to ambient concentrations of CO₂ in seawater (Pearson correlation, (one-tailed) $p=0.009$, $r=0.64$), i.e. natural phytoplankton assemblages take up a larger fraction in the form of CO₂ under high ambient CO₂ conditions than under lower CO₂ conditions (Fig. 5A). The CO₂ saturation state of seawater expressed as percentage of atmospheric CO₂ concentration (385.57 ppm, www.co2now.org) was inversely correlated to steady state inorganic carbon fixation (exponential decrease, $R_2=0.64$, Fig. 5B) indicating a role for biology in the draw-down of CO₂. Direct bicarbonate uptake was significantly correlated to P_t (one phase exponential association, $R^2=0.61$, Fig. 5C). Also total

bicarbonate uptake significantly increased (one phase exponential association, $R^2=0.56$, Fig. 5C) with increasing P_t.

Twelve of the 13 stations of which DIC uptake data is available consisted mainly of large diatom genera. No correlation could be observed between the DIC acquisition mechanism and any of the observed genera.

Also, when phytoplankton samples were grouped in 3 different size classes (based on biovolumes: small: < 1000 μm³, medium: 1001–5000 μm³ and large: > 5000 μm³; Table 2) no correlation between size and mode of DIC uptake was observed.

4. Discussion

In the present study, the acquisition of inorganic carbon by natural phytoplankton assemblages from the Atlantic sector of the Southern Ocean was investigated. Using the isotopic disequilibrium technique, the contribution of different carbon species (CO₂ and bicarbonate) to the overall DIC uptake and the extent to which eCA plays a role in facilitating DIC uptake was estimated. Simultaneous uptake of CO₂ and HCO₃⁻ was observed in all cases, but the proportions in which different DIC species were taken up varied considerably between stations (Fig. 4B). It was shown that bicarbonate as well as CO₂ could be the major DIC source for local phytoplankton assemblages. There was a positive correlation between the contribution of CO₂ to total DIC uptake and ambient concentrations of CO₂ in seawater (Fig. 5A).

4.1. Methodology

In order to determine, which DIC species crosses the cell membrane, the isotopic disequilibrium technique was used. Although it has been successfully used in several field studies over the past years, its biggest obstacle in High Nutrient Low Chlorophyll (HNLC) regions such as the Southern Ocean is that a concentration step is needed to obtain sufficient biomass. Several studies circumvented this problem by applying gravity filtration (Martin and Tortell, 2006; Tortell et al., 2008a) or vacuum filtration (Cassar et al., 2004, this study). Although none of the authors mentioned artefacts, filtration always presents a stress for phytoplanktonic cells and smaller cells will be lost from the filtrate. This might be particularly relevant for studies performed in the Southern Ocean where a large and variable proportion of the phytoplanktonic community can consist of pico- (< 2 μm) and/or nanoplankton (2–30 μm) (Knox, 2007). When interpreting the results of the present and similar studies, one should be aware that an unknown fraction of the natural community is lost.

This might be particularly relevant when estimating P_t from isotopic disequilibrium experiments. Future research should account for filtration losses as well as directly compare standard P_t protocols with the method used in this study.

4.2. High plasticity of DIC uptake by natural assemblages of the Southern Ocean

The few recent field studies on Southern Ocean microalgae assemblages presented a consistent picture of predominant direct HCO₃⁻ uptake (60–98%) for assemblages from the Ross Sea (Tortell et al., 2008a, 2008b). A somewhat lower average bicarbonate uptake (52%) for assemblages from the Polar Frontal Zone with values ranging from 22% to 67% was reported by Cassar et al. (2004). Despite the broad range of the latter study, no correlation between ambient concentrations of CO₂ and the uptake of CO₂ versus HCO₃⁻ was observed. Our study confirms that natural assemblages from the Atlantic sector of the Southern Ocean are often able to take up HCO₃⁻ and CO₂ simultaneously and

frequently rely on direct HCO_3^- uptake. However, in accordance with Cassar et al. (2004) our data also indicate that CO_2 often constitutes the major DIC source.

More importantly, this work is the first to show that DIC substrate preference of Southern Ocean phytoplankton communities is correlated to the concentration of CO_2 . We found that differences in the fractions of HCO_3^- versus CO_2 uptake are correlated to ambient concentrations of CO_2 (Fig. 5A). This implies that phytoplankton adjust DIC uptake in response to changing CO_2 conditions by changing DIC substrate preference. Although we examined taxonomic composition of our samples microscopically and by analysing phytoplanktonic pigments, our data do not reveal whether the observed correlation is the result of shifts in community structure (as we did not follow a local assemblage over time) or of a physiological response.

Within our dataset, the concentration of CO_2 in seawater was significantly anti-correlated with P_t , indicating that when low CO_2 conditions were observed, they were the result of biological activity (Fig. 5B). When seawater was found to be CO_2 supersaturated, local phytoplankton assemblages yielded low values for steady state inorganic carbon fixation as an indicator for primary productivity and consisted mainly of CO_2 users. During the transition from high to low CO_2 concentrations, the relative contribution of HCO_3^- uptake to DIC uptake increased (Fig. 5C). These results are in agreement with several laboratory studies where significant differences in the capacity for HCO_3^- transport as well as the ability to regulate CCM activity in response to changes in the concentration of CO_2 have been observed among marine microalgae (Elzenga et al., 2000; Burkhardt et al., 2001; Trimbom et al., 2009). Considering that most laboratory experiments employed a much broader $p\text{CO}_2$ range in comparison to the approximately 100 μatm variation observed in this study, this finding is even more ecologically relevant.

We interpret these results as a means of Southern Ocean phytoplankton to adjust their carbon uptake mechanisms according to available energy. Under conditions of low CO_2 concentrations one would expect CCM activity to be induced. If, however, CCM induction is impeded by cellular energy shortage at the same time, carbon uptake normalized to chlorophyll *a* (in the following: carbon uptake efficiency) will become reduced. In our data no trend in carbon uptake efficiency was observed (data not shown). This can be related to the fact that Fe limitation is known to result in a reduction in cellular Chl *a* content (Greene et al., 1992), which counteracts a reduction in Chl *a* normalized DIC uptake. Ambient dFe concentrations were generally low (0.1–0.3 nM) during our cruise (Klunder et al., 2011), which has been shown to be indicative for Fe-limited phytoplankton production (de Baar et al., 1990; Buma et al., 1991).

In contrast to our findings, Tortell et al. (2008b) reported that assemblages from the Ross Sea (directly from the field and after several days of incubation) upregulated their chlorophyll *a*-normalized maximum carbon uptake rate (V_{max}) in response to decreasing CO_2 concentrations. Since these authors could not find a correlation between CO_2 concentration and relative contribution of CO_2 uptake, this observation could not be explained with a shift in carbon uptake mechanism. Differences in approach and methods employed might explain the discrepancy between the results of Tortell et al. (2008b) and the present study. With field samples the authors performed 10-min ^{14}C uptake experiments and derived substrate-saturated (maximal) DIC uptake rates, whereas the DIC uptake rates published in our study were calculated from short-term isotopic disequilibrium experiments. The latter represent steady state DIC uptake under certain conditions (depending on e.g., pH, DIC and light), but are not necessarily maximal. In addition, the authors conducted isotopic disequilibrium experiments with phytoplankton samples incubated for several days

under a wide $p\text{CO}_2$ range (100–800 ppm) and with Fe added. These conditions affect the physiology of the cells and are not comparable to field conditions.

4.3. Taxonomy

Several laboratory studies have shown that carbon acquisition strategies of microalgae can range from one extreme to the other. Cassar et al. (2002) reported that a strain of the diatom *Phaeodactylum tricornutum* is not able to take up HCO_3^- even under severe CO_2 limitation. The diatom *Thalassiosira punctigera* is another example of an obligatory CO_2 user, whereas a strain of *Thalassiosira pseudonana* was shown to be an obligatory HCO_3^- user over a large range of CO_2 concentrations (Elzenga et al., 2000). Also Trimbom et al. (2009) showed that species of the same genus might differ in their mode of carbon uptake. Elzenga et al. (2000) demonstrated that DIC uptake might not only differ between species of the same genus but even within different strains of the same species. Although none of the above mentioned species occur in the Southern Ocean, it can be anticipated that species with a comparable range of carbon acquisition mechanisms exist also in the study area.

No correlation between the taxonomical composition of samples at the level of genera, families, orders or classes and uptake strategy of DIC could be established in this study. This result is in accordance with findings of Tortell et al. (2008a), who did not find a correlation between direct HCO_3^- uptake and taxonomic composition of the natural community at class level. However, the latter study did observe significantly higher eCA levels in diatom-dominated assemblages compared to *Phaeocystis*-dominated samples. Our study does not support this observation. With the exception of 4 stations, the eCA levels were low, whereas diatoms dominated all samples.

4.4. Specific ecology of the study area

In large areas of the Southern Ocean, microalgae are suffering from a co-limitation of Fe and light (de Baar et al., 2005). Both stress factors result in a severely reduced cellular energy budget and it can be anticipated that phytoplankton cells will optimize all energy requiring processes (van Leeuwe and de Baar, 2000). Under such conditions, a low CO_2 concentration at the carboxylation site of Rubisco can exert additional stress to the cell, as the resulting increased photooxidative activity of Rubisco will further limit the energy availability. Consequently, there will be a trade-off between energy investment in a CCM and loss of energy due to reduced Calvin cycle activity (Raven, 1990, 1991).

As a consequence, it is an advantage for energy stressed phytoplankton communities to take up a larger fraction of DIC in the form of CO_2 , when CO_2 is available, thereby decreasing costs related to DIC uptake. Our data indicate that Southern Ocean plankton communities are able to adapt their CCMs according to environmental conditions. We hypothesize that this ability partly alleviates energy stress due to Fe and light limitation. Whether or not this is the result of shifts in species composition or shifts in carbon acquisition of the algae themselves cannot be concluded from this study.

4.5. Implications for models and conclusions

A quantitative understanding of the processes governing the uptake of DIC from the ocean by phytoplankton could provide a basis for predicting primary productivity in a world of rising atmospheric CO_2 concentrations. However, the proportions at which CO_2 and HCO_3^- are taken up in natural phytoplankton assemblages and the degree to which overall DIC uptake of the

community is affected by changes in CO₂ supply are still inadequately quantified.

If bicarbonate was strictly the main carbon species used by phytoplankton and microalgae were unable to regulate their uptake mechanisms, then it would be plausible that primary productivity is insensitive to rising CO₂. From an evolutionary point of view, and as several laboratory studies suggest, it is unlikely that phytoplankton communities are unable to adjust HCO₃⁻ utilization in response to ambient DIC conditions.

Our study indicates that Southern Ocean natural microalgal assemblages are flexible in the regulation of carbon acquisition. Under high seawater CO₂ conditions, a larger fraction of DIC uptake can be assigned to CO₂, potentially enabling phytoplankton to cut metabolic cost related to carbon acquisition. Our study also indicates that Southern Ocean phytoplankton is well adapted to highly energy-efficient use of carbon. We observed that areas of high CO₂ were associated with low primary productivity. This indicates that CO₂ was not limiting primary productivity and other parameters were more important, with light conditions and Fe-limitation as potential candidates. We presume that in areas where such growth-limiting parameters were sufficiently available, an increased primary productivity resulted in a draw-down of DIC with subsequent reduced CO₂ concentration.

These results indicate that a higher CO₂ concentration due to anthropogenic input per se will not lead to increased primary productivity, since Southern Ocean phytoplankton is mainly energy limited. However, under conditions that energy resources are elevated, DIC will be quickly taken up.

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