Photosynthesis and net primary productivity in three Antarctic diatoms: possible significance for their distribution in the Antarctic marine ecosystem

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Running head: Primary productivity in Antarctic diatoms
Abstract

Photosynthesis and net primary productivity were measured in three Antarctic diatoms, *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp., exposed to rapid changes in temperature and salinity representing a range of conditions found during a seasonal cycle. Measured differences in fluorescence-derived photosynthetic activity and oxygen evolution suggested some alternative electron cycling activity present under high irradiances. *Fragilariopsis cylindrus* displayed the highest rates of relative electron transport and net primary productivity under all salinity and temperature combinations and showed adaptive traits towards the sea ice-like environment. *Pseudo-nitzschia subcurvata* displayed a preference for low saline conditions where production rates were greatest. However, there was evidence of photosynthetic sensitivity to the lowest temperatures and highest salinities, suggesting a lack of adaptation for dealing with sea ice-like conditions. *Chaetoceros* sp. showed high plasticity, acclimating well to all conditions, but performing best under pelagic conditions. The study shows species-specific sensitivities to environmental change highlighting photosynthetic capacity as a potentially important mechanism in ecological niche adaptation. When these data were modelled over different seasons, integrated daily net primary production was greatest under summer pelagic conditions. The findings from this study support the general observations of light control and seasonal development of net primary productivity and species succession in the Antarctic marine ecosystem.
Introduction

Numerous studies have linked phytoplankton physiology with ecological adaptation (Sakshaug et al 1987; Strzepek and Harrison 2004; Bailey et al 2008). Such studies have found that the photosynthetic architecture and physiology of species can differ greatly and adaptive traits can be linked to the species’ ability to inhabit and often dominate a particular ecological niche, including niche occupancy as a function of light (Sakshaug et al 1987; Lavaud et al 2007), iron availability (Strzepek and Harrison 2004; Bailey et al 2008, Mackey et al 2008) and photoprotective capacity (Dimier et al 2009). In particular, the ecological success of diatoms has been linked with a high photosynthetic flexibility, allowing this class of microalgae to outcompete other phytoplankton groups, especially in environments where light is variable (Kashino et al 2002; Wagner et al 2006). Indeed, even within the diatom group, there have been measurable differences in short-term acclimation strategies and photoprotective capacity between individual species from different environments (Dimier et al 2007; Lavaud et al 2007). The large diversity in diatom physiological plasticity and ecological niche selectivity, suggests that Antarctic diatoms in general should be adapted to such variable and extreme conditions and therefore should display high photoprotective capabilities. However, little is known whether differences in photoprotective capacity among Antarctic diatom species can be linked with specific niche environments.

Antarctic marine phytoplankton are exposed to large changes in ecosystem conditions during an annual cycle (Fig. 1). In winter, phytoplankton are rapidly incorporated into the sea ice matrix where they are confined to tiny brine channels (salinities up to 145) at freezing temperatures (Gleitz and Thomas 1992). Initially incoming solar irradiance is very high in the developing sea ice, as a result of being constrained close to the surface, but as the ice thickens, irradiance declines, often to levels of less than 1% surface irradiance (Palmisano et
In the austral spring, the ice begins to melt, and the microalgae are washed out of the brine channels into a surface lens of hyposaline water. The meltwater environment, characterised by low salinities (typically below 33), a stable water column and shallow mixed layer, forms an ideal environment for the development of phytoplankton blooms (Dierssen et al. 2002). In summer, the Southern Ocean mixes phytoplankton from the surface waters to the deep, delivering a moderate and variable light environment, warmer temperatures and moderate salinity (Fig. 1). All of these shifts in light, temperature and salinity, can have a profound effect on phytoplankton growth rates (Aletsee and Jahnke 1992) and carbon assimilation (Thomas and Gleitz 1993). High carbon turnover has been associated with fast growing Antarctic diatoms (Thomas and Gleitz 1993) and growth rates under low temperature/high salinity conditions have been shown to differ between Antarctic diatom species (Aletsee and Jahnke 1992). Therefore it follows that photosynthesis under different environmental conditions will be species-specific, this in turn, would result in differences in species’ contributions to the community net primary productivity.

Traditionally, $^{14}$C-uptake and oxygen evolution were used to estimate primary production and photosynthesis (Juneau and Harrison 2005). However, in recent decades chlorophyll $a$ fluorescence has been a popular technique employed to investigate the photophysiology of plants (Krause and Weiss 1991). Several studies have found a good correlation between fluorescence-derived relative electron transport rates with primary productivity measured as oxygen evolution or carbon fixation under low light conditions (Geel et al. 1997; Prasil et al. 1996; Masojidek et al. 2001). In all cases, linearity disappears beyond light saturation levels, resulting in fluorescence data vastly over-estimating primary production (Gilbert et al. 2000). In diatoms, there have been several alternative electron pathways, not leading to carbon fixation, suggested as possible reasons behind this
discrepancy, including cyclic electron transport around PSII (Prasil et al. 1996; Lavaud et al. 2002), cyclic electron transport around PSI (Bendall and Manesse 1995) and the water-water cycle (Geel et al. 1997).

The physical and biological processes associated with the seasonal formation and decay of sea ice is what shapes Antarctic phytoplankton communities, determining colonisation, growth, succession, grazing and ultimately, productivity. This study investigates short-term acclimation in photosynthesis and net primary production in three Antarctic diatoms exposed to rapid shifts in temperature and salinity simulating transient environmental conditions of seasonal relevance (sea ice, meltwater and pelagic environments). It measures net primary productivity (measured as oxygen evolution) and electron transport efficiency (measured using chlorophyll \(a\) fluorescence), and investigates species-specific physiological sensitivities to rapid changes in temperature and salinity. Furthermore, it discusses how this may influence species-specific contributions to overall primary productivity within the Antarctic marine ecosystem. It is hypothesised that species known to dominate a particular niche environment will show physiological adaptation and rapid acclimation to those conditions. Net primary productivity will vary between species under different environmental conditions and species displaying a low photosynthetic plasticity will express photosynthetic sensitivity to conditions outside their preferred ecological niche.

Materials and Methods

**Cultures and sampling**

The Antarctic diatom *Fragilariopsis cylindrus* (Grunow) was collected from ice cores (66°S, 147°E) taken in November 2001, *Chaetoceros* sp. was isolated from East Antarctic
coastal waters, and *Pseudo-nitzschia subcurvata* (Hasle) Fryxell PS71/60-1 Plate II B1 was collected from the subpolar South Atlantic ocean (58°S). Diatoms were cultured in natural seawater (salinity 34) enriched with F/2 nutrients in specialised glass bottles (approx. 1 L) under continuous air bubbling and maintained at +4°C (Guillard and Ryther 1962). Light was supplied at 50 μmol photons m⁻² s⁻¹ (Grolux, GMT lighting, Northmead, Australia) on a 16:8 light:dark cycle. For simplicity, one, moderate light level was used in all treatments throughout the experiment to avoid confounding any responses to the salinity and temperature treatments. Once reaching exponential growth phase, measured using in vivo fluorescence (Trilogy, Turner Designs Inc., Sunnyvale CA, United States of America (U.S.A)), cultures were maintained semi-continuously diluting (up to 90% with fresh media) periodically to keep cells in exponential phase for the duration of the experiment. For experimental treatments, cells from independent culture flasks (representing replicates) were gently filter-concentrated above 2 μm polycarbonate membrane filters (Millipore, MA, USA) and resuspended into 250 mL culture flasks. At each temperature treatment (-1.5, +2 and +5°C (± 0.3°C)) cells were transferred into four salinities, 31, 34, 55 and 70 (± 0.5) and were given 72 h in their new conditions before measurements were made. A 3-day acclimation period was chosen in order to investigate short-term acclimation strategies of three diatom species during a rapid freezing or thawing event. Salinities were adjusted either by the addition of MilliQ water or sodium chloride (NaCl) salt (Sigma, USA) and were confirmed by refractometer.

**Chlorophyll a fluorescence**

Fluorescence-based estimates of photosynthesis were measured using Pulse Amplitude Modulated fluorometer (Water-PAM; Walz GmbH, Effeltrich, Germany). A 3 ml
aliquot of each treatment was transferred into a quartz cuvette and maintained under continuous stirring to prevent settling. After 5 min dark-adaptation, minimum (F₀) and maximum (Fₘ) fluorescence were obtained. This was followed by the sequential application of nine actinic light levels (28, 42, 65, 100, 150, 320, 680, 1220 and 2260 µmol photons m⁻² s⁻¹) applied for 5 min each, with saturating pulses of light (pulse duration = 0.6 s; pulse intensity > 3000 µmol photons m⁻² s⁻¹) applied every minute. Relative electron transport rate (rETR) was calculated as the product of effective quantum yield (φ_PSII) and irradiance (µmol photons m⁻² s⁻¹) and normalised to chlorophyll a concentration (mg L⁻¹).

**Oxygen evolution**

Oxygen uptake and evolution were measured using a 24-well plate reader optode system (Pre Sens, Precision Sensing GmbH, Regensburg, Germany). Aliquots (3 mL) of filter-concentrated cultures (3 fold) were transferred into 36 wells of two 24-well plates and left in the dark for 30 min while oxygen consumption was monitored. Cells were then incubated at nine light levels (20, 40, 65, 100, 160, 300, 600, 1100, 2250 µmol photons m⁻² s⁻¹) for 1 h (for each irradiance) and oxygen evolution rate determined. Light levels were attained by covering the well plate lids with neutral density filters and light provided by a non-heat radiating light source (Zeiss KL2500, Zeiss, Germany). Oxygen flux measurements (µmol O₂ L⁻¹ h⁻¹) were used to calculate net primary production rates at each light level and data were normalised to chl a (mg L⁻¹) concentration. All fluorescence and oxygen measurements were conducted at each corresponding treatment temperature.

**Chlorophyll a concentration**

Chlorophyll a concentrations for fluorescence and oxygen measurements were determined by filtering (10 mL) samples onto 25 mm GF/F filters (Whatman, USA),
immediately frozen in liquid nitrogen and stored in the dark at -80°C for later analysis. Pigments were extracted in 90% acetone and left in the dark at 4°C for 24 h. Pigment concentrations were measured on a spectrophotometer (Cary 50, Varian, CA, USA) at wavelengths 750, 664 and 630 nm and calculated according to Ritchie (2006).

Data analysis

Relative ETR and oxygen evolution data were plotted according to the different temperature and salinity conditions experienced in the Antarctic; although not perfectly representative of the natural world, salinity and temperature treatments were combined to correspond to different Antarctic ecological niche environments: sea ice (-1.5°C at 70), meltwater (+2°C at 31) and pelagic (+5°C at 34; Fig. 1). Oxygen evolution and fluorescence data were fitted according to a double exponential function as in Ralph and Gademann (2005). All photosynthetic parameters derived from the oxygen and fluorescence data including: maximum rate of oxygen evolution (O2max), light utilisation efficiency (α), minimum saturating irradiances (Eκ), optimal photosynthetic irradiance (EM), photoinhibitory irradiance (Eθ) and maximum electron transport rate (ETRmax), were obtained from these curves following Ralph and Gademann (2005). Photosynthetic and NPP parameters were tested for statistical significance between species (F. cylindrus, P. subcurvata and Chaetoceros sp.) and environments (sea ice, meltwater and pelagic) using a two-factor ANOVA with Tukey’s post hoc comparisons (α = 0.05). All data were tested for homoscedasticity prior to analysis. The data for the photosynthetic parameters αo2 and EkO2 were log transformed to meet assumptions of ANOVA. Least squares linear regression was used to determine the relationship between rETR and oxygen evolution rates for all data values below and those above minimum saturating irradiance (Eκ). To further investigate
species-specific sensitivity to changes in temperature and salinity, photosynthetic parameters

α_{O2}, E_{K O2} and O_{2max} from oxygen evolution data were plotted as a contour graph using the entire matrix of four salinities (31, 34, 55, and 70) and three temperatures (-1.5, +2, and +5°C) that were tested.

Net primary production estimates

To place these results in a broader and more ecologically relevant context, the parameters derived from the light response curves were used to estimate daily integrated net primary production [µmol O₂ (mg chl a)^{-1} d^{-1}] for each species under each environmental condition. Daily surface irradiance was computed as a bell shaped curve with a noon maximum irradiance and a photoperiod typical of each environment derived from literature values (Table 1; Fig. 2). Data on the monthly photoperiod for each environmental condition was taken from www.esrl.noaa.gov/gmd/grad/solcalc which are based on the astronomical algorithms of Jean Meeus (Earth System Research Laboratory, National Oceanic & Atmospheric Administration) using latitude (065°S) and longitude (145°E) of the east Antarctic seasonal sea ice zone south of the polar front. The photoperiods chosen were for the months of August, November, January and March to represent winter sea ice, spring meltwater, summer pelagic and autumn new ice conditions, respectively (Table 1).

Approximations of maximum daily irradiance values for the different months were derived from the measurements of Lizotte and Sullivan (1991) that were taken at a similar (approx. 064°S) latitude (Table 1). Given missing data for January, summer maximum irradiance was estimated to be 2000 µmol photons m^{-2} s^{-1} (Rozema et al 2001) assuming no cloud cover.

I_Z = nI_O e^{-kz}         \quad (1)

Irradiance within the water column or sea ice was estimated using Beer’s Law:
where \( I_z \) is the irradiance at depth \( z \), \( I_0 \) is incident irradiance, \( k \) is the light attenuation coefficient and \( n \) is the transmissivity of the interface between air and water or air and ice.

The attenuation coefficients differed between environments (Table 1). For the meltwater and pelagic environments, \( k \) values were taken from Rasmus et al (2004) who measured attenuation from the marginal ice zone to the polar front in the range of 0.03 to 0.09 m\(^{-1}\). The higher attenuation values were found at the ice edge and polar front due to increased phytoplankton biomass, with the lowest attenuation measured in intermediate pelagic waters (Rasmus et al 2004). Therefore, \( k \) values of 0.09 and 0.03 m\(^{-1}\) were used for the meltwater and pelagic environments, respectively (Table 1). For the sea ice environment a \( k \) value of 1.5 m\(^{-1}\) was used (Maykut 1985). To account for reflectance at the air-sea interface in the meltwater and pelagic environments, \( n \) was assumed equal to 0.95 (Gregg and Carder 1990, valid for solar zenith angles less than 45 degrees). To account for irradiance loss due to high albedo of snow and ice, \( n \) was set to 0.2 and 0.5 in the presence and absence of snow, respectively (Maykut 1985).

To better simulate fluctuating light conditions that result from strong vertical mixing, pelagic irradiance was determined at two depths (15 and 60 m) and values obtained from each were used interchangeably on a 1:1 h (high light: low light) cycle for the entire photoperiod (Fig. 2). It is recognised that the irradiance values are only model estimates for in situ irradiance that use over-simplified coefficients of extinction and albedo and that these modelled data fail to account for variations in absorption, reflection and scattering properties of snow, ice and water of differing composition.
Chlorophyll a fluorescence

Relative electron transport rates (rETR\textsubscript{Chl}) varied between all three species and all environments (Fig. 3). There was more than a two-fold difference in rETR\textsubscript{Chl} for \textit{F. cylindrus} under the pelagic and sea ice conditions when compared with the other two species (Fig. 3). \textit{Pseudo-nitzschia subcurvata} showed minimal rETR\textsubscript{Chl} under sea ice-like conditions and maximal rates under the low saline (meltwater) conditions (Fig. 3A, B). In contrast, \textit{Chaetoceros} sp. showed the reverse, with maximal rETR\textsubscript{Chl} in the sea ice and minimal rates under pelagic conditions (Fig. 3A, C). Maximum rETR (rETR\textsubscript{max}) was significantly different between all three species across all three salinity and temperature treatments ($P < 0.001$). It was consistently higher in \textit{F. cylindrus} in all environments with highest values measured under the conditions similar to sea ice (Table 2). For \textit{P. subcurvata} values of rETR\textsubscript{max} were lowest under the sea ice conditions and highest in meltwater conditions (Table 2). A decreasing trend from sea ice to the pelagic environment was measured in \textit{Chaetoceros} sp..

There were significant differences in light utilisation efficiency ($\alpha_{ETR}$) between environmental conditions ($P = 0.001$) and between all three species ($P < 0.001$), with the highest $\alpha_{ETR}$ measured in both \textit{F. cylindrus} and \textit{Chaetoceros} sp. acclimated to sea ice conditions (Table 2), while in \textit{P. subcurvata} the highest value was measured under meltwater conditions (Table 2). Minimum saturating irradiances (E\textsubscript{KETR}) varied significantly between species ($P < 0.001$) and across the three environments ($P = 0.001$). In all three environments, E\textsubscript{KETR} was greatest in \textit{F. cylindrus} increasing from the sea ice to the pelagic environment. Minimum saturating irradiance levels were relatively similar in the other two species. There was an increasing trend from the sea ice to the pelagic environment for \textit{P. subcurvata}, but no difference in E\textsubscript{KETR} for \textit{Chaetoceros} sp. between the three environments (Fig. 3; Table 2).
Oxygen evolution

The oxygen data showed very similar patterns to the rETR_{Chl} light response curves with clear variations between all three species under all three environments (Fig. 4). Again, there was more than a two-fold difference in net primary production for *F. cylindrus* under the pelagic and sea ice conditions when compared with the other two species (Fig. 4). As with the fluorescence data, *P. subcurvata* showed minimal net primary production under sea ice conditions and maximal rates under meltwater conditions (Fig. 4A, B). In contrast, *Chaetoceros* sp. showed a decreasing trend in oxygen evolution rates from the sea ice to the pelagic environment (Fig. 4). Maximum oxygen evolution (O_{2max}) was significantly different between all three species across all three treatments (P < 0.001) and was consistently higher in *F. cylindrus* under all environmental conditions (Table 2). There were significant differences in light utilisation efficiency (α_{O2}) between environmental conditions across the three species (P < 0.001), with the highest α_{O2} measured in both *F. cylindrus* and *Chaetoceros* sp. acclimated to sea ice conditions (Table 2). As with the fluorescence data, the highest α_{O2} value was measured under meltwater conditions for *P. subcurvata* (Table 2).

Minimum saturating irradiances (E_{K O2}) varied significantly between species across the three environments (P < 0.001). In all three environments, E_{K O2} was greatest in *F. cylindrus* and increased from the sea ice to the pelagic environments. In the other two species, E_{K O2} was lowest under sea ice conditions and maximal in the meltwater environment (Fig. 4; Table 2).

Optimal irradiance and photoinhibition

Optimal photosynthetic irradiance (E_{M O2}) in *F. cylindrus* was approximately double the minimum saturating irradiance values under each environmental condition and like E_{K O2}
(Table 2), increased from the sea ice to the pelagic environmental conditions (Table 3). In *P. subcurvata* there was no difference in $E_{M, O2}$ between environmental conditions, whereas for *Chaetoceros* sp., optimal irradiance was greatest under meltwater and lowest in the sea ice conditions (Table 3). Photoinhibitory irradiance ($E_{B, O2}$) revealed photoinhibition in all species under all environmental conditions (Fig. 4; Table 3). The greatest photoinhibition was measured in the meltwater and lowest under sea ice conditions for *F. cylindrus*. It increased from the sea ice to the pelagic environment in *P. subcurvata* and was lowest under meltwater conditions for *Chaetoceros* sp. (Table 3).

**Oxygen versus fluorescence based net primary production**

Oxygen evolution and rETR plotted together as a function of light showed good correlation at irradiances below $E_K$, but considerable differences in shape of the curve at higher irradiances (Fig. 5). The pooled oxygen and fluorescence data for all species under all environmental conditions showed a significant correlation between the linear component of the fluorescence and oxygen data ($R^2 = 0.8392, P = 0.0001$; Fig. 6, regression A). However, the remaining data for irradiance levels well above $E_K$ for each species was not significant (Fig 6, regression B).

**Salinity and temperature responses**

The complete matrix of photosynthetic parameters determined from the oxygen data, $\alpha_{O2}$, $O_{2\text{max}}$, and $E_{K, O2}$ in response to changes in salinity and temperature, revealed differences between the three diatom species (Fig. 7). *Fragilariopsis cylindrus* and *Chaetoceros* sp. showed high light utilisation efficiency ($\alpha_{O2}$) in the low to mid-range temperatures and mid-
to high salinities (Fig. 7A, C), whereas *P. subcurvata* showed a preference for the intermediate to high temperatures and lower salinities (Fig. 7B). Maximum photosynthesis ($O_{2\text{max}}$) varied across the three species, with *F. cylindrus* yielding maximum values at mid- to high salinities and warmer temperatures (Fig. 7D). *Fragilariopsis cylindrus* showed a salinity threshold in which high rates of photosynthesis began to decline unless temperature also decreased (Fig. 7D). In *P. subcurvata* photosynthetic activity ($O_{2\text{max}}$) was optimal at lower salinities and warmer conditions (Fig. 7E), while for *Chaetoceros* sp., maximum photosynthesis occurred at higher salinities and mid-range temperatures (Fig. 7F). Minimum light saturation values ($E_{K O_{2}}$) were highest in warmer temperatures for all three species; however there were some differences in salinity sensitivity between species. *Fragilariopsis cylindrus* showed high values across a range of salinities (Fig. 7G), whereas in *P. subcurvata* the lowest salinities yielded the highest saturating irradiances (Fig. 7H). *Chaetoceros* sp. showed higher minimum light saturation values at the higher salinities (Fig. 7I).

**Estimated daily net primary production: species, environment and light climate**

Daily estimates of integrated net primary production determined from the modelled integrated PAR for each environment (Fig. 2) showed *F. cylindrus* with the highest productivity of all three species under all environments (Fig. 8). *Fragilariopsis cylindrus* showed a light-dependent response, with low values in the winter sea ice and very high values in the meltwater, pelagic and newly formed ice environments (Fig. 8). Net primary production was strongly inhibited in *P. subcurvata* under sea ice conditions at both high and low irradiances, however rates of NPP were highest under the higher irradiances combined with lower salinities and warmer temperatures of the meltwater and pelagic environments (Fig. 8). Daily estimates of net primary production for *Chaetoceros* sp. were much lower in
the meltwater environment compared with the other two species, but followed the same light-dependent pattern as *F. cylindrus* with the highest rates estimated for the pelagic environment (Fig. 8).

**Discussion**

Microalgae exposed to variable environments generally possess the capacity for rapid acclimation and a highly plastic photosynthetic apparatus (Kolber et al 1988). All three species of Antarctic diatoms exhibited photophysiological plasticity by their ability to acclimate to the rapid shift in environmental condition. Each diatom showed different physiological responses that correlated well with their known distribution, displaying physiological preferences for one particular niche environment over another. The photosynthetic parameters obtained from the oxygen measurements showed similarity in the patterns to those obtained from the fluorescence data (Table 2). However, there were some differences between the fluorescence and oxygen measurements including maximum photosynthesis in *F. cylindrus* (Table 2). According to oxygen measurements, O$_{2\text{max}}$ occurred under pelagic conditions, yet rETR$_{\text{max}}$, based on fluorescence data, occurred under sea ice conditions, which could be due to the presence of alternative electron cycling (AEC).

The variability in accessory pigments and physiological properties between species means that there is considerable heterogeneity in light utilisation efficiency within a phytoplankton community (Wilhelm et al 1990). The higher rates of light utilisation efficiency ($\alpha$) in *F. cylindrus* and *Chaetoceros* sp. under sea ice conditions, suggest an increase in light capturing capacity by the antenna of PSII, which would explain light saturation occurring at lower irradiances in these two species. In contrast, the small $\alpha$ under
sea ice conditions measured in *P. subcurvata* suggests a smaller PSII antenna, and explains
the high irradiance required to reach photosynthetic saturation compared with the other
environmental conditions (Juneau and Harrison 2005). The lower α values measured in *P. subcurvata* under all three environmental conditions is likely due to it being a much larger
cell (approx. 50 µm vs 5 µm) and therefore having much higher chlorophyll a content,
leading to increased packaging effect and lower photoprotective capacity (Dimier et al 2009).
This suggests that this species would display maximum efficiency under stable light climates,
such as those provided by the meltwater environment (Dierssen et al 2002), which was
indeed observed in this study.

Values of $E_{K_o2}$ ranged between 37 and 163 $\mu$mol photons m$^{-2}$ s$^{-1}$ which are consistent
with Antarctic phytoplankton both from the pack ice (Lizotte and Sullivan 1991; Dower et al
1996) and pelagic environments (Tilzer et al 1986; Brightman and Smith 1989). Saturating
irradiance is an indicator of photoadaptation, changing as a result of light history and thereby
helping to identify shade- vs light-adapted species. The increase in $E_K$ from the sea ice to the
open ocean in *F. cylindrus* suggests that this species is well-adapted to sea ice conditions in
which light levels are rarely as high in comparison to the meltwater or pelagic environments
(Palmisano et al 1987). By maximising light utilisation efficiency and not light absorption
capacity—since light levels are rarely excessive—*F. cylindrus* can optimise photosynthesis in
the sea ice environment. Additionally, *F. cylindrus* is well-adapted to the other environments,
whereby reducing light captured by the PSII antenna, the photosystem can tolerate higher
irradiances such as the levels occurring in the meltwater and pelagic environments. Light
saturation did not vary greatly between environments for *P. subcurvata*, but was greatest in
the meltwater environment. A higher $E_K$ at lower salinities is indicative of a reduced capacity
for low light acclimation (Arrigo and Sullivan 1992), which makes sense for *P. subcurvata*
given the reduced light utilisation efficiency observed. Similarly, both oxygen and
fluorescence data revealed *Chaetoceros* sp. to exhibit the highest light saturation under
meltwater conditions and not pelagic conditions, as was found in *F. cylindrus*. The
seemingly contradictory higher O$_2$ evolution (O$_{2\text{max}}$) under sea ice conditions may be the
result of greater light respiration at the warmer temperatures. Previous work on another
Antarctic *Chaetoceros* sp. showed that lower temperatures (-1.5°C) resulted in more efficient
carbon assimilation and less respiratory losses (Thomas et al 1992). Although *Chaetoceros*
sp. showed similar light utilisation efficiency ($\alpha$) under sea ice conditions as *F. cylindrus*, $E_K$
was much lower, suggesting greater sensitivity to sea ice conditions and different light
harvesting capacity.

The trends in $E_K$ were the same in the fluorescence and oxygen measurements, but
there were large differences in the actual values, where $E_K$ determined from rETR were much
higher than those obtained from the oxygen measurements (Fig. 4; Table 2). Such differences
have been measured, with PSII electron transport determined $E_K$ exceeding values determined
by oxygen evolution rates (Prasial et al 1996; Gilbert et al 2000; Wagner et al 2006). This is
due to the fact that oxygen evolution measures net photosynthesis, whereas fluorescence-
based rETR represents gross PSII-dependent electron transport, failing to differentiate
between electrons derived from water-splitting with electrons being re-cycled through
photosystem II (Genty et al 1989). While no direct measurement of cyclic electron transport
of PSII were made in this study, it seems highly likely that the difference in the shape of the
light response curves and $E_K$ values based on fluorescence and oxygen measurements can be
attributed to the non-oxygen consuming process of cyclic electron transport around PSII
(Prasial et al 1996; Lavaud et al 2002). The advantage to cyclic electron transport is that it can
be switched on faster than heat dissipation via non-photochemical quenching (Onno Feikema
et al 2006) and allows the cell to maintain maximum photosynthetic capacity while keeping energy-dependent quenching minimal (Lavaud 2007).

Relative ETR and $\alpha$ are temperature-dependent processes (Palmisano et al 1987; Arrigo and Sullivan 1992) however, only Chaetoceros sp. expressed such a response in this study (Table 2). Instead, a salinity trend was detected. Using the $^{14}$C method, Arrigo and Sullivan (1992) found rETR and $\alpha$ parameters to increase with increasing salinity up to 50 before declining again in Antarctic sea ice algal populations. A similar salinity trend was detected in this study, where rETR and $\alpha$ were highest in the sea ice and lowest in the meltwater environments for F. cylindrus (Table 2). This same trend was observed in $\alpha$, determined from the oxygen data, in Chaetoceros sp. (Table 2). However, for P. subcurvata, maximum values of all parameters were detected under meltwater conditions, further implying adaptation to lower salinities. This response correlates well with a previous study where increased salinity retarded growth more than lowered temperatures (Aletsee and Jahnke 1992) assuming photosynthetic activity as a proxy for growth.

Phytoplankton photosynthetic efficiency is influenced by both temperature and salinity, as both factors affect rates of photosynthesis. Factors can act independently (one has an effect, while the other doesn’t), synergistically (the combined affect is greater than the individual) or antagonistically (where one stress offsets the damage of the other). Interactive effects between temperature and salinity were detected in F. cylindrus, where elevated salinity mitigated the effects of lower temperatures (Fig. 7). This demonstrates a situation in which multiple stressors, rather than causing a synergistic effect, can actually be less stressful than the individual stress. These data suggest that this mitigative response in F. cylindrus is linked with a greater adaptability to the sea ice environment (which naturally has simultaneously low temperatures and high salinities), supporting the observed dominance of
this species in the Antarctic sea ice. In contrast, the same combination of low temperature and high salinity in \textit{P. subcurvata} yielded low, \(\alpha_{O2}\), \(O_{2\text{max}}\), and \(E_{K\text{O}_2}\) values having more of a negative effect. At low salinities and warmer temperatures, \textit{P. subcurvata} showed less photosynthetic stress, indicating temperature and salinity sensitivity and suggesting that its preferred ecological niche is somewhere between meltwater and pelagic conditions (Fig. 7). \textit{Chaetoceros} sp. responded in a similar manner as \textit{F. cylindrus}, but showed greater sensitivity towards low salinities, a situation in which an individual factor is responsible for negative responses alone.

It should be noted that this study compared acclimation capacity after only 3 days, and therefore cannot rule out the possibility that all species could in fact acclimate successfully to all conditions given more time. However, what this study does highlight is that there are species-specific differences in rapid acclimation capacity, which could have implications for an ecological competitive advantage. In addition, it must be noted that the short-term (72 h) acclimation period used in this study may have led to the experimental conditions slightly over-estimated the stress of the sea ice environment, as the shift from growth environment (+4°C at 34) to the low temperature/high salinity sea ice conditions (-1.5°C at 70) would likely take longer to reach acclimation than to the meltwater conditions (+2°C at 32). However, this would not have affected differences in the short-term acclimation capacity between the three species.

Daily integrated NPP was greatest in the summer pelagic environment and lowest under the winter sea ice conditions for \textit{F. cylindrus} and \textit{Chaetoceros} sp., while maximal NPP was calculated for \textit{P. subcurvata} under meltwater conditions (Fig. 8). The light-dependent response of \textit{F. cylindrus} and \textit{Chaetoceros} sp. highlights the strong link between light availability and NPP. For these two species, low irradiances limited the amount of active
photosynthesis. In contrast, *P. subcurvata* showed an interactive effect of salinity and
temperature with light, where the low temperature and high salinities of the sea ice
environments drastically reduced NPP regardless of available irradiance. It is important to
note that the growth irradiance in this experiment was not representative of field conditions
and was instead kept constant throughout the salinity and temperature manipulations,
potentially influencing the photoacclimation potential of the phytoplankton. Furthermore, the
irradiance level used was below light saturation for photosynthesis in all species under all
environmental conditions. Thus, it is possible that when acclimated to different light
intensities, the diatoms may show entirely different photosynthesis versus irradiance
characteristics, particularly with respect to photochemical efficiency at low irradiances and
photoinhibition at higher light levels. Future studies should aim to combine light, temperature
and salinity shifts to provide greater insight into the true photoacclimation potential and NPP
of each species. Photoinhibition was not great enough to affect the light-dependent response
of NPP in this study, but this could be an artefact of the constant photon fluence rates used.
The greatest average daily irradiance of 232 $\mu$mol photons m$^{-2}$ s$^{-1}$ in the pelagic environment
resulted in the highest NPP regardless of high photoinhibition, with the exception of *P.
subcurvata*, where salinity concentrations and constant high light had a greater influence over
NPP (Fig. 8). Even with the fluctuations in light, the total irradiance was still greater than in
the meltwater environment. Photoinhibition was present under all conditions with the
exception of the winter sea ice scenario, which had a calculated daily mean irradiance of just
3.4 $\mu$mol photons m$^{-2}$ s$^{-1}$. Therefore, the contribution of photoinhibition can be entirely
ignored in the winter sea ice scenario. However, the low NPP values for *P. subcurvata* in
both (autumn/early spring) sea ice environments is indicative of sensitivity to the high
salinity and low temperatures, but also shows high light adaptation and limited
photosynthetic plasticity for low light conditions. The higher NPP values of *P. subcurvata*
compared to *Chaetoceros* sp. in the high light meltwater conditions is largely due to higher photoinhibition in *Chaetoceros* sp.. Whereas values are relatively similar in the pelagic waters as a result of the intermittent low light that drastically lowers NPP in *P. subcurvata*.

While there are many recognised limitations to these modelled data, the derived values of NPP are consistent with the fluorescence and oxygen physiological data and all tell a similar story. *Fragilariopsis cylindrus* possesses a highly plastic photosystem and while well-adapted for the sea ice environment, remains the most productive species under all three conditions. Based on this study, the results show that *F. cylindrus* is able to acclimate to the new salinity and temperature conditions more rapidly than the other two species, potentially giving it a competitive advantage in the marine environment. This strongly supports its wide distribution as a generalist species and upholds its prevalence throughout the Antarctic marine ecosystem (Lizotte, 2001; Kopczynska et al., 2007; Roberts et al., 2007; Beans et al., 2008). In contrast, *P. subcurvata* showed a particular adaptation to meltwater characteristics with a much lower level of photosynthetic plasticity for changes in salinity, temperature and light, perfectly matching its geographical environment of known greatest abundance (Almandoz et al., 2008). Finally, *Chaetoceros* sp. expressed an ability to adjust to sea ice conditions, but displayed a clear preference toward the more pelagic environment with sensitivity to low saline conditions, correlating with its known distribution in the Antarctic coastal pelagic environment.

Knowledge of species-specific photosynthetic capacity is essential to obtain good estimates of primary productivity (Juneau and Harrison 2005). This study has shown that photosynthesis in three Antarctic diatoms is sensitive to rapid changes in temperature and salinity that occur in the Antarctic ecosystem during an annual cycle. Better understanding the influence these changes can have on photosynthesis and NPP may shed some light on the
physiological mechanism controlling the distribution of phytoplankton in the Antarctic marine environment. The species-specific sensitivities to changes in salinity and temperature uncovered in this study have strengthened the link between phytoplankton photosynthetic capacity and ecological niche occupancy.
Acknowledgements

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References

Aletsee L, Jahnke J (1992) Growth and productivity of the psychrophilic marine diatoms *Thalassiosira antarctica* Comber and *Nitzschia frigida* Grunow in batch cultures at temperatures below the freezing point of sea water. Pol Biol 11:643-647


Table 1. Summary of data used to model seasonal daily solar irradiance in the winter sea ice, meltwater, pelagic and new sea ice environments for estimations of NPP [$\mu$mol O$_2$ (mg chl $a$)$^{-1}$ d$^{-1}$] for *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp..

<table>
<thead>
<tr>
<th>Environment</th>
<th>Month</th>
<th>Photoperiod (h)$^a$</th>
<th>Maximum I$_O$$^b$</th>
<th>k (m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea ice</td>
<td>August</td>
<td>8</td>
<td>150</td>
<td>1.5$^d$</td>
</tr>
<tr>
<td>Meltwater</td>
<td>November</td>
<td>18.5</td>
<td>1500</td>
<td>0.09$^e$</td>
</tr>
<tr>
<td>Pelagic</td>
<td>January</td>
<td>20</td>
<td>2000$^c$</td>
<td>0.03$^e$</td>
</tr>
<tr>
<td>New sea ice</td>
<td>March</td>
<td>13</td>
<td>1800</td>
<td>1.5$^d$</td>
</tr>
</tbody>
</table>

$^a$ Obtained from www.esrl.noaa.gov/gmd/grad/solcalc for latitude 065°S and longitude 145°E

$^b$ Values are in $\mu$mol photons m$^{-2}$ s$^{-1}$ and were derived from Lizotte and Sullivan (1991)

$^c$ Estimated values assuming no cloud cover

$^d$ Light attenuation coefficient (k) for sea ice was taken from Maykut (1985)

$^d$ Light attenuation coefficients (k) obtained from Rasmus et al (2004)
Table 2. Photosynthetic parameters maximum electron transport rate (ETR$_{\text{max}}$), maximum oxygen evolution (O$_{2\text{max}}$), light utilisation efficiency ($\alpha$) and minimum saturating irradiance (E$_{\text{K}}$) calculated from light response curves of relative electron transport or oxygen evolution according to Ralph and Gademann (2005). Data represent means for *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp., ± SD (n=4).

<table>
<thead>
<tr>
<th>Ecosystem</th>
<th>Species</th>
<th>rETR$_{\text{max}}$ [µmol e$^{-}$ (mg chl a)$^{-1}$ h$^{-1}$]</th>
<th>$\alpha_{\text{ETR}}$</th>
<th>E$_{\text{KETR}}$ [µmol photons m$^{-2}$ s$^{-1}$]</th>
<th>O$<em>{2\text{max}}$ [µmol O$</em>{2}$ (mg chl a)$^{-1}$ h$^{-1}$]</th>
<th>$\alpha_{\text{O2}}$</th>
<th>E$_{\text{K02}}$ [µmol photons m$^{-2}$ s$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea ice</td>
<td><em>F. cylindrus</em></td>
<td>3105 ± 403</td>
<td>10.4 ± 3.7</td>
<td>315 ± 81</td>
<td>780 ± 105</td>
<td>12.8 ± 3.9</td>
<td>61 ± 3.6</td>
</tr>
<tr>
<td></td>
<td><em>P. subcurvata</em></td>
<td>603 ± 76</td>
<td>4.6 ± 0.3</td>
<td>132 ± 24</td>
<td>332 ± 39.9</td>
<td>5.1 ± 0.7</td>
<td>44 ± 6.8</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros</em> sp.</td>
<td>1577 ± 241</td>
<td>10.9 ± 1.6</td>
<td>144 ± 17</td>
<td>373 ± 61.9</td>
<td>10.0 ± 2.5</td>
<td>37 ± 2.3</td>
</tr>
<tr>
<td>Meltwater</td>
<td><em>F. cylindrus</em></td>
<td>1929 ± 526</td>
<td>4.73 ± 0.85</td>
<td>406 ± 66</td>
<td>470 ± 62</td>
<td>5.22 ± 1.3</td>
<td>89 ± 8.3</td>
</tr>
<tr>
<td></td>
<td><em>P. subcurvata</em></td>
<td>1163 ± 149</td>
<td>6.38 ± 0.55</td>
<td>183 ± 28</td>
<td>449 ± 133</td>
<td>7.8 ± 1.4</td>
<td>57 ± 6.5</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros</em> sp.</td>
<td>1265 ± 232</td>
<td>7.07 ± 0.31</td>
<td>178 ± 25</td>
<td>276 ± 90</td>
<td>4.1 ± 1.8</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>Pelagic</td>
<td><em>F. cylindrus</em></td>
<td>2382 ± 307</td>
<td>5.70 ± 1.20</td>
<td>420 ± 106</td>
<td>1087 ± 106</td>
<td>6.07 ± 0.43</td>
<td>163 ± 3.6</td>
</tr>
<tr>
<td></td>
<td><em>P. subcurvata</em></td>
<td>877 ± 79</td>
<td>3.95 ± 0.62</td>
<td>225 ± 35</td>
<td>346 ± 33</td>
<td>4.8 ± 1.7</td>
<td>50 ± 4</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros</em> sp.</td>
<td>643 ± 55</td>
<td>4.44 ± 0.53</td>
<td>145 ± 73</td>
<td>270 ± 36</td>
<td>8.01 ± 0.93</td>
<td>56 ± 15</td>
</tr>
</tbody>
</table>
Table 3. Photosynthetic parameters optimal photosynthetic irradiance (E_M) and photoinhibitory irradiance (E_B) calculated from light response curves of oxygen evolution according to Ralph and Gademann (2005). Data represent means of *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp. ± SD (n=4).

<table>
<thead>
<tr>
<th>Environment</th>
<th>Species</th>
<th>E_M O₂ [µmol photons m⁻² s⁻¹]</th>
<th>E_B O₂ [µmol photons m⁻² s⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea ice</td>
<td><em>F. cylindrus</em></td>
<td>124 ± 10</td>
<td>138 ± 21</td>
</tr>
<tr>
<td></td>
<td><em>P. subcurvata</em></td>
<td>62 ± 17</td>
<td>155 ± 35</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros</em> sp.</td>
<td>36 ± 4.4</td>
<td>403 ± 192</td>
</tr>
<tr>
<td>Meltwater</td>
<td><em>F. cylindrus</em></td>
<td>185 ± 21</td>
<td>969 ± 77</td>
</tr>
<tr>
<td></td>
<td><em>P. subcurvata</em></td>
<td>64 ± 38</td>
<td>554 ± 156</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros</em> sp.</td>
<td>132 ± 31</td>
<td>201 ± 28</td>
</tr>
<tr>
<td>Pelagic</td>
<td><em>F. cylindrus</em></td>
<td>366 ± 9.8</td>
<td>367 ± 9.9</td>
</tr>
<tr>
<td></td>
<td><em>P. subcurvata</em></td>
<td>48 ± 11</td>
<td>1077 ± 477</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros</em> sp.</td>
<td>86 ± 38</td>
<td>756 ± 86</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Spatial and temporal evolution and decay of sea ice in the Antarctic marine ecosystem. Mixed layer depth and light attenuation are depicted. The seasonal pathway of phytoplankton from the winter sea ice to the meltwater and pelagic environments are shown. Temperature, salinity and irradiance properties for each environment are described in the table below. Figure adapted from Eicken (1992).

Figure 2. Modelled daily in situ solar irradiance for microalgae in the sea ice (August - late winter), meltwater (November - spring), pelagic (January - summer) and new sea ice (March - autumn) environments at 065°S and 145°E. Fluctuations in irradiance are shown by the 1:1 h mixing pattern for PAR at 15 and 60 m.

Figure 3. Relative electron transport rates (rETR) as a function of irradiance in the Antarctic diatoms *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp. under A) sea ice B) meltwater and C) pelagic conditions. Maximum electron transport rate (ETR$_{max}$), and saturating irradiance (E$_{KETR}$) are shown (dotted lines). Data represent mean ± SD ($n = 4$).

Figure 4. Net primary production (measured as oxygen evolution) as a function of irradiance in the Antarctic diatoms *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp. exposed to A) sea ice B) meltwater and C) pelagic conditions. Maximum
oxygen evolution rates ($O_{2\text{max}}$), and saturating irradiance ($E_{K_{O2}}$) are shown (dotted lines). Data represent mean ± SD ($n = 4$).

Figure 5. Comparison of light response curves for oxygen and fluorescence-based photosynthesis in *Fragilariopsis cylindrus* under sea ice-like conditions. Light curves were fitted according to Ralph and Gademann (2005). The relationship between rETR and $O_2$ evolution at low irradiance (initial points of light curve) are fitted with a linear regression (inset). Data represent means ± SD ($n = 4$).

Figure 6. Correlation between pooled means of oxygen and fluorescence-based photosynthesis of A) the values in the light curve below $E_K$ (black circles) and B) remaining points above $E_K$ (white circles) after which the oxygen and fluorescence deviate. Data are fitted with a linear regression. Data represent the mean of all species under sea ice meltwater and pelagic conditions ($n = 4$).

Figure 7. Contour plots of the oxygen derived parameters $\alpha$, $O_{2\text{max}}$ [$\mu$mol O$_2$ (mg chl a)$^{-1}$ h$^{-1}$] and $E_K$ [$\mu$mol photons m$^{-2}$ s$^{-1}$] in *Fragilariopsis cylindrus* (A, D, G), *Pseudo-nitzschia subcurvata* (B, E, H) and *Chaetoceros* sp. (C, F, I) exposed to 31, 34, 55, and 70 at -1.5, +2 and +5°C.

Figure 8. Daily integrated net primary production as a function of modelled seasonal light climate (see Fig. 2) in the sea ice (late winter), meltwater (spring), pelagic (summer) and new
sea ice (autumn) environments for Antarctic diatoms *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata* and *Chaetoceros* sp. exposed to altered salinity and temperature.
Fig. 2

[Graph showing irradiance (µmol photons m⁻² s⁻¹) over time of day (h) with different categories: Sea Ice, Meltwater, Pelagic, and New Sea Ice.]
Fig. 3
Fig. 4

Net primary production [μmol O₂ (mg chl.a)⁻¹ h⁻¹]

Irradiance (μmol photons m⁻² s⁻¹)
Fig. 5
Fig. 6
Fig. 8