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Photophysiological responses of Southern Ocean phytoplankton to changes in CO_2 concentrations: Short-term versus acclimation effects ☆

Scarlett Trimborn [a,*], Silke Thoms [a], Katherina Petrou [b], Sven A. Kranz [c], Björn Rost [a]

[a] Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[b] University of Technology, Plant Functional Biology and Climate Change Cluster, Department of Environmental Sciences, PO Box 123, Broadway, NSW 2007, Sydney, Australia
[c] Princeton University, Department of Geosciences, Princeton, NJ 08544, USA

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ABSTRACT

The present study examines how different pCO_2 acclimations affect the CO_2- and light-dependence of photophysiological processes and O_2 fluxes in four Southern Ocean (SO) key phytoplankton species. We grew *Chaetoceros debilis* (Cleve), *Pseudo-nitzschia subcurvata* (Hasle), *Fragilariopsis kerguelensis* (O'Meara) and *Phaeocystis antarctica* (Karsten) under low (160 μatm) and high (1000 μatm) pCO_2. The CO_2- and light-dependence of fluorescence parameters of photosystem II (PSII) were determined by means of a fluorescence induction relaxation system (FIRe). In all tested species, nonphotochemical quenching (NPQ) is the primary photoprotection strategy in response to short-term exposure to high light or low CO_2 concentrations. In *C. debilis* and *P. subcurvata*, PSII connectivity (p) and functional absorption cross-sections of PSII in ambient light (σ'_{PSII}) also contributed to photoprotection while changes in re-oxidation times of Q_a acceptor (τ_{Qa}) were more significant in *F. kerguelensis*. The latter was also the only species being responsive to high acclimation pCO_2, as these cells had enhanced relative electron transport rates (rETRs) and σ'_{PSII} while τ_{Qa} and p were reduced under short-term exposure to high irradiance. Low CO_2-acclimated cells of *F. kerguelensis* and all pCO_2 acclimations of *C. debilis* and *P. subcurvata* showed dynamic photoinhibition with increasing irradiance. To test for the role and presence of the Mehler reaction in *C. debilis* and *P. subcurvata*, the light-dependence of O_2 fluxes was estimated using membrane inlet mass spectrometry (MIMS). Our results show that the Mehler reaction is absent in both species under the tested conditions. We also observed that dark respiration was strongly reduced under high pCO_2 in *C. debilis* while it remained unaltered in *P. subcurvata*. Our study revealed species-specific differences in the photophysiological responses to pCO_2, both on the acclimation as well as the short-term level.

Abbreviations: a^*_{PSII}, optical cross section for photosystem II; α, maximum light-use efficiency; ATP, adenosine triphosphate; CCM, carbon concentrating mechanism; cf, conversion factor; Chl *a*, chlorophyll *a*; C_i, inorganic carbon; CO_2, carbon dioxide; DBS, dextran-bound sulfonamide (inhibitor for eCA); DIC, dissolved inorganic carbon; eCA, extracellular carbonic anhydrase; ETR, electron transport rate; FIRe, fluorescence induction relaxation system; F_0, minimum fluorescence; F'_0, light-adapted minimum fluorescence; F_m, maximum fluorescence; F'_m, light-adapted maximum fluorescence; F_v/F_m, maximum quantum yield of photochemistry in photosystem II according to Genty et al. (1989); F'_q/F'_m, effective quantum yield of photochemistry in photosystem II; HCO_3^-, bicarbonate; HEPES, 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; I, irradiance; I_k, light acclimation index; J, connectivity parameter according to Lavergne and Trissl (1995); MIMS, membrane inlet mass spectrometry; MTF, multiple turnover flash; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; $NaHCO_3$, sodium bicarbonate; NPQ, non-photochemical quenching; O_2, oxygen; p, connectivity between photosystem II according to Joliot and Joliot (1964); p', connectivity between photosystem II in ambient light; pCO_2, carbon dioxide partial pressure; Φ_{PSII}, quantum yield of photochemistry in photosystem II according to Lavergne and Trissl (1995); Φ^m_{PSII}, maximum quantum yield of photochemistry in photosystem II according to Lavergne and Trissl (1995); PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; q, overall fraction of open photosystem II units; rETR, relative electron transport rate; RuBisCO, ribulose-1,5-bisphosphate carboxylase–oxygenase; σ_{PSII}, functional absorption cross section of photosystem II; σ'_{PSII}, functional absorption cross section of photosystem II in ambient light; SO, Southern Ocean; STF, single turnover flash; τ_{Qa}, re-oxidation time of the Q_a acceptor; τ'_{Qa}, re-oxidation time of the Q_a acceptor in ambient light; V_{max}, light-saturated net rate of photosynthesis.

* Corresponding author at: Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12 (E-2010), 27570 Bremerhaven, Germany. Tel.: +49 471 4831 1038; fax: +49 471 4831 2020.
E-mail address: scarlett.trimborn@awi.de (S. Trimborn).

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1. Introduction

By the end of 2100, the ongoing anthropogenic emissions of carbon dioxide (CO_2) will likely have increased atmospheric CO_2 concentrations from ~390 μatm to >750 μatm (IPCC report I, 2007). Biogeochemical models for the ocean indicate that the rise in atmospheric CO_2 levels will affect seawater carbonate chemistry by decreasing the current seawater pH of ~8.1 by 0.3 units (Feely et al., 2009). Next to this reduction in pH, higher CO_2 concentrations will also lead to reduced carbonate ion concentrations and saturation states, a phenomenon commonly referred to as 'ocean acidification' (Orr et al., 2005). Due to the high solubility of CO_2 in cold waters, these changes in carbonate chemistry will be most pronounced in polar waters. The Southern Ocean (SO) ecosystem strongly influences the marine carbon cycle and has a great potential to affect atmospheric CO_2 concentrations (Sigman et al., 2010). Although SO phytoplankton are major drivers of global carbon cycling accounting for ~2 Pg C of annual primary production (Arrigo et al., 2008), the potential effects of ocean acidification on the physiology and ecology of SO phytoplankton are still not well understood. There is evidence that high pCO_2 differently affects SO phytoplankton growth (Boelen et al., 2011; Hoogstraten et al., 2012a, 2012b; Ihnken et al., 2011a; Trimborn et al., 2013) potentially causing changes in the community structure (Feng et al., 2010; Tortell et al., 2008). For natural phytoplankton assemblages of the China Sea, it was observed that high pCO_2 in conjunction with high light exposure can reduce their primary productivity and increase light stress (Gao et al., 2012). A higher susceptibility for photoinhibition at high pCO_2 was also indicated in different temperate phytoplankton species (McCarthy et al., 2012; Wu et al., 2010; Yang and Gao, 2012). In this context, it is particularly surprising that most central processes like the photophysiology of SO phytoplankton have hardly been studied in the context of ocean acidification research.

Thus far, studies on the photophysiology of SO phytoplankton mainly focused on the effect of different light levels (Arrigo et al., 2010; Kropuenske et al., 2009; Robinson et al., 1997; Van Leuwe et al., 2005). Next to the adjustment of cellular pigment composition and concentration (Arrigo et al., 2010; Van Leuwe et al., 2005), the photoacclimation strategy of polar phytoplankton is to adjust photosystem II (PSII) reaction centers through changing the size of the effective absorption cross-section (σ_{PSII}) rather than the number of PSII reaction centers per cell (Kropuenske et al., 2010; Robinson et al., 1997). Phytoplankton can also alter the PSII connectivity (p) allowing the redistribution of excitons from closed to open PSII providing a more or less efficient use of light (Ihnken et al., 2011b). Regarding the impact of pCO_2 on photophysiology, Spalding et al. (1984) reported an increase in σ_{PSII} in *Chlamydomonas reinhardtii* when grown at 5% CO_2. In the Antarctic diatom *Chaetoceros brevis*, however, Boelen et al. (2011) observed no effect by elevated pCO_2 (750 μatm) on either pigment content and composition or the activity of the carbon-fixing enzyme Ribulose-1,5-bisphosphate carboxylase–oxygenase (RubisCO).

Next to light, photosynthesis requires CO_2 as the substrate of RubisCO and its availability may also affect the photophysiology of the cells. To avoid limitations arising from low CO_2 supply and the low CO_2 affinities of RubisCO, most phytoplankton operate so-called carbon concentrating mechanisms (CCMs; Reinfelder, 2011). Trimborn et al. (2013) demonstrated that SO phytoplankton species have diverse and highly efficient CCMs, which were often constitutively expressed independent of the acclimation pCO_2. The operation of a CCM is an energy-requiring process and is therefore strongly dependent on light (Raven and Lucas, 1985). In fact, carbon acquisition and subsequent fixation consume the largest share of the ATP and NADPH produced in the light reaction of photosynthesis. As photosynthesis cannot go faster than either the carboxylase activity or the electron transport rate and considering the large variability in irradiance and CO_2 in the natural environment, phytoplankton require high flexibility to adjust their CCM as well as their photosynthetic apparatus for optimal use. It has been suggested that under low CO_2 conditions, not only the cycling of electrons around photosystem I (PSI; Spalding et al., 1984), but also indirectly the Mehler reaction, the photoreduction of O_2, supplies ATP required for the operation of the CCM (Raven and Beardall, 1981; Sültemeyer et al., 1993). High pCO_2, on the other hand, may reduce costs for CCM activity and thus the energy demand (Kranz et al., 2010), all of which may feedback on photophysiology. Until now, information on these processes and their sensitivity to ocean acidification is lacking for SO phytoplankton.

The exposure of phytoplankton cells to high irradiances requires the dissipation of excess energy to prevent damage of PSII. To this end, phytoplankton possess photoprotective mechanisms such as non-photochemical quenching (NPQ) and the electron cycling around the PSI and/or PSII (Prasil et al., 1996) that respond within time scales of seconds to minutes. Xanthophyll-cycle-dependent NPQ has been observed in both *Phaeocystis antarctica* and SO diatoms (Boelen et al., 2011; Kropuenske et al., 2009; Mills et al., 2010; Petrou et al., 2011) and involves the enzymatic removal of the epoxy group of the carotenoid diadinoxanthin to diatoxanthin. This process is triggered by a decrease in the pH of the thylakoid lumen and represents a central mechanism to prevent photoinhibition under excessive light. The Mehler reaction can also act as a photoprotective mechanism in phytoplankton (Raven and Beardall, 1981). Kranz et al. (2010) observed Mehler activity in the cyanobacterium *Trichdesmium*, which was acclimated to low pCO_2 combined with high light, but this process was absent under high pCO_2. In the temperate diatom *Chaetoceros muelleri*, increasing pCO_2 was found to enhance relative electron transport rates (rETRs) under saturating light, suggesting that higher rETRs were enabled because of elevated CO_2 fixation rates by RubisCO (Ihnken et al., 2011a, 2011b). McCarthy et al. (2012) also observed higher carboxylation rates with increasing pCO_2 in two temperate diatom strains of *Thalassiosira pseudonana*. In the same study, the tested species were nonetheless found to be more susceptible to photoinhibition and to have a higher capacity for PSII repair at elevated pCO_2. Whether Antarctic phytoplankton may respond in a similar way is not yet resolved.

The present study examines how the acclimation pCO_2 affects photophysiological processes and O_2 fluxes of four SO key phytoplankton species in response to short-term changes in CO_2 or irradiance. To this end, *Chaetoceros debilis*, *Pseudo-nitzschia subcurvata*, *Fragilariopsis kerguelensis* and *P. antarctica* were acclimated to 160 and 1000 μatm pCO_2. The CO_2- and light-dependence of chlorophyll *a* fluorescence were assessed using a fluorescence induction relaxation system (FIRe; Satlantic, Canada). Also, the light-dependence of O_2 fluxes (gross and net photosynthesis as well as O_2 uptake in the light and in the dark) was determined in *C. debilis* and *P. subcurvata* according to the method of Peltier and Thibault (1985) by means of membrane inlet mass spectrometry (MIMS).

2. Material and methods

2.1. Culture conditions and carbonate chemistry

Semi-continuous cultures of the diatom species *C. debilis* (Polarstern expedition 'EIFEX' ANT-XXI/3, In-Patch, 2004, 49°36 S Lat, 02°05 E Long, isolated by Philipp Assmy), *P. subcurvata* (Polarstern expedition ANT-XXI/4 in April 2004 at 49° S Lat, 02° E Long, isolated by Philipp Assmy) and *F. kerguelensis* (Polarstern expedition ANT-XXIV/2 in 2008 at 64° S Lat, 0° E Long, isolated by Philipp Assmy) and the flagellate *P. antarctica* (solitary cells isolated by P. Pendoley in March 1992 at 68°39 S, 72°21) were grown at 3 °C in sterile-filtered (0.2 μm) unbuffered Antarctic seawater (salinity 33.9 psu). The seawater was enriched with trace metals and vitamins according to F/2 medium (Guillard and Ryther, 1962). Nitrate and phosphate were added in concentrations of 100 and 6.25 μmol L^{-1}, respectively, reflecting the Redfield N:P ratio of 16:1 (Redfield, 1958). Experiments were carried out using a light:dark cycle of 16:8 h at an incident light intensity of