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# THE EFFECT OF IRON LIMITATION ON THE PHOTOPHYSIOLOGY OF *PHAEOCYSTIS ANTARCTICA* (PRYMNESIOPHYCEAE) AND *FRAGILARIOPSIS CYLINDRUS* (BACILLARIOPHYCEAE) UNDER DYNAMIC IRRADIANCE[1]

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**The effects of iron limitation on photoacclimation to dynamic irradiance were studied in *Phaeocystis antarctica* G. Karst. and *Fragilariopsis cylindrus* (Grunow) W. Krieg. in terms of growth rate, photosynthetic parameters, pigment composition, and fluorescence characteristics. Under dynamic light conditions mimicking vertical mixing below the euphotic zone, *P. antarctica* displayed higher growth rates than *F. cylindrus* both under iron (Fe)–replete and Fe-limiting conditions. Both species showed xanthophyll de-epoxidation that was accompanied by low levels of nonphotochemical quenching (NPQ) during the irradiance maximum of the light cycle. The potential for NPQ at light levels corresponding to full sunlight was substantial in both species and increased under Fe limitation in *F. cylindrus*. Although the decline in $F_v/F_m$ under Fe limitation was similar in both species, the accompanying decrease in the maximum rate of photosynthesis and growth rate was much stronger in *F. cylindrus*. Analysis of the electron transport rates through PSII and on to carbon (C) fixation revealed a large potential for photoprotective cyclic electron transport (CET) in *F. cylindrus*, particularly under Fe limitation. Probably, CET aided the photoprotection in *F. cylindrus*, but it also reduced photosynthetic efficiency at higher light intensities. *P. antarctica*, on the other hand, was able to efficiently use electrons flowing through PSII for C fixation at all light levels, particularly under Fe limitation. Thus, Fe limitation enhanced the photophysiological differences between *P. antarctica* and diatoms, supporting field observations where *P. antarctica* is found to dominate deeply mixed water columns, whereas diatoms dominate shallower mixed layers.**

*Key index words:* **Antarctic; diatom; iron; nonphotochemical quenching; *Phaeocystis*; photoinhibition; photoprotection; photosynthesis; Ross Sea; xanthophyll**

***Abbreviations:*** **19′-But, 19′ butanoyloxyfucoxanthin; 19′-Hex, 19′ hexanoyloxyfucoxanthin; *a*, absorption coefficient; C, carbon; CET, cyclic electron transport; DD, diadinoxanthin; DT, diatoxanthin; *E*, irradiance; $e^-$, electron; Fuc, fucoxanthin; $F_v/F_m$, maximum efficiency of PSII; MLD, mixed layer depth; NPQ, nonphotochemical quenching; *P*, photosynthesis; POC, particulate organic carbon; PON, particulate organic nitrogen; *qP*, photochemical quenching; ß-Car, ß-carotene**

Phytoplankton primary productivity in the Southern Ocean plays an important role in modulating the global climate system by anthropogenic $CO_2$ uptake and export to the deep sea (Lovenduski and Gruber 2005). In particular, coastal Antarctic ecosystems can be highly productive (Arrigo and Van Dijken 2003, Arrigo et al. 2008, Smith and Comiso 2008, Vernet et al. 2008). With high levels of unused macronutrients year-round, productivity in the coastal Antarctic is likely to be colimited by Fe and light (Sedwick and DiTullio 1997, Sunda and Huntsman 1997, Arrigo et al. 1998, Boyd 2002). In addition, grazing may have an impact on relatively short spatial and temporal scales (Marrari et al. 2008).

The two phytoplankton taxa that dominate virtually all coastal Antarctic waters are diatoms

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and Prymnesiophyceae, most notably, the species *Phaeocystis antarctica* (Arrigo et al. 1999, 2000, Schoemann et al. 2005, Wright et al. 2010, Kozlowski et al. 2011). These two taxa differ greatly in their ecological and biogeochemical characteristics. Diatoms regulate the silicon cycle in the ocean (Treguer and Jacques 1992) and are important in supporting most krill-based food webs in the Antarctic (Knox 1994). *P. antarctica*, on the other hand, has significantly higher C:P and N:P ratios than diatoms, and hence has the capacity for higher $CO_2$ drawdown per mole phosphate (Arrigo et al. 1999, Sweeney et al. 2000). Moreover, blooms dominated by the colony forming *P. antarctica* are not preferentially grazed by most Antarctic meso- and microzooplankton grazers (Verity and Smetacek 1996, Smith et al. 2003, Nejstgaard et al. 2007). Finally, *P. antarctica* is an important component of the marine sulfur cycle (Liss et al. 1994, DiTullio and Smith 1995) due to its production of dimethylsulfoniopropionate (DMSP). DMSP may be converted to volatile dimethyl sulfide and contribute to the flux of biogenic sulfur to the atmosphere (Rhodes et al. 2009).

The Ross Sea is the largest and, because of the presence of the Ross Sea polynya, the most productive of the Antarctic coastal regions (Arrigo and Van Dijken 2003). Diatoms and *Phaeocystis* blooms dominate the Ross Sea in distinct locations and times: *P. antarctica* blooms early in the more deeply mixed Ross Sea polynya region, whereas diatoms bloom later in the shallow mixed layer of the western continental shelf (Arrigo et al. 1999, Smith et al. 2010). The high correlation reported between species distribution and mixed layer depth (MLD) suggests that diatoms are better adapted to the higher irradiance levels experienced in regions with a shallow MLD, whereas *P. antarctica* is better adapted to low irradiance levels in a deeply mixed water column (Arrigo et al. 2003).

Wind-driven vertical mixing in combination with diurnal changes in solar elevation and cloud cover may result in a highly dynamic in situ light climate. Thus, phytoplankton cells need to adjust their photosynthetic apparatus for optimal use of low irradiance, while minimizing photoinhibitory effects at high irradiance. Field observations in the Ross Sea and elsewhere suggest that taxon-specific differences in photophysiology may govern phytoplankton community composition. A series of physiological studies under different controlled irradiance regimes confirmed that *P. antarctica* has the photosynthetic properties for efficient usage of light when grown under a variable light regime in a deep mixed layer. The Antarctic diatoms *Fragilariopsis cylindrus* and *Chaetoceros brevis*, on the other hand, were better protected from photoinhibition under high light levels and better adapted to grow in a high light environment typical for a shallow MLD (Kropuenske et al. 2009, Arrigo et al. 2010, Mills et al. 2010, Van de Poll et al. 2011).

Low concentrations of Fe limit phytoplankton growth in the Ross Sea in Austral summer (Olson et al. 2000, Sedwick et al. 2000, Arrigo et al. 2003). Since Fe is an integral component of the photosynthetic apparatus, Fe limitation probably affects the photosynthetic properties of the taxa and thus their response to the different irradiance regimes. Various components of the photosynthetic apparatus contain either Fe or require Fe for their synthesis. In eukaryotic phototrophs, the PSII complex incorporates four Fe atoms, whereas the comparatively Fe-rich downstream electron acceptors cytochrome $b_6f$ and the PSI complex require six and 12 Fe atoms, respectively (Raven 1990, Raven et al. 1999). Although the Fe content of the complexes is evolutionarily conserved, there is some variability in Fe requirements because the cellular abundance of the complexes themselves differs between algal taxa (Raven 1990, Greene et al. 1992, Raven et al. 1999, Quigg et al. 2003, Allen et al. 2008) and change in response to growth irradiance and Fe availability (Raven 1990, Sunda and Huntsman 1997, Raven et al. 1999, Allen et al. 2008). The altered cellular abundance and ratios may affect the level of downstream electron acceptors and reoxidation of PSII. The fraction of functional (oxidized) PSII reaction centers at any given time will depend in part on the ambient light level (which determines the rate at which electrons are donated to PSII) and the availability of competent electron acceptors downstream of PSII (Long et al. 1994). Either high irradiance, a limited availability of downstream electron acceptors, or a combination of the two will limit efficient PSII reoxidation, which makes PSII prone to photoinhibition (Adir et al. 2003). Repair of damaged PSII requires the synthesis of new PSII components, and operates on timescales of minutes to hours (Aro et al. 1993, Hazzard et al. 1997).

Both *P. antarctica* and Antarctic diatoms induce xanthophyll-cycle-dependent NPQ as a photoprotection mechanism to prevent overexcitation of PSII. In both taxa, the xanthophyll cycle consists of enzymatic de-epoxidation of the carotenoid diadinoxanthin to diatoxanthin, which thermally dissipates excess energy (Demmig-Adams 1990, Olaizola and Yamamoto 1994, Olaizola et al. 1994, Dimier et al. 2009, Van de Poll and Buma 2009). Xanthophyll de-epoxidation at high irradiance is dependent on the pH gradient ($\Delta$pH) across the thylakoid membrane and reverses at low irradiance on a timescale of minutes, which causes the NPQ to relax. Diatoms show higher levels of fast relaxing NPQ than *P. antarctica* and are therefore better protected from photoinhibition at high irradiance (Kropuenske et al. 2009, Van de Poll et al. 2011).

In addition to xanthophyll cycling, CET around PSII may act as a photoprotection mechanism. CET was reported in the diatom *Phaeodactylum tricornutum* at high irradiance (Lavaud et al. 2002, Feikema et al. 2006). An electron transfer pathway from the

plastoquinone pool or the acceptor side of PSII to the donor side of PSII was rapidly induced at high irradiance. CET persisted as long as the pool of plastoquinone molecules was in the reduced state (Prasil et al. 1996). Thus, while CET was insignificant at low irradiance, it may prevent PSII photoinhibition at high irradiance (Lavaud et al. 2002, Feikema et al. 2006).

There are strong interactions between Fe limitation and photoinhibition because Fe limitation decreases the synthesis of photosynthetic proteins such as the D1 reaction center protein, which is the first protein to become damaged by excessive irradiance (Greene et al. 1992, Vassiliev et al. 1995). On the other hand, Fe-limited cells generally contain less chl *a*, which decreases the potential to absorb excess irradiance (Greene et al. 1992, Van Leeuwe and Stefels 1998, 2007, Van de Poll et al. 2005). Moreover, reports on the effect of Fe limitation on photoprotective mechanisms such as NPQ vary (Strzepek and Harrison 2004, Allen et al. 2008, Van de Poll et al. 2009). Thus, the net effect of Fe limitation on photoinhibition remains unclear.

The aim of this study was to compare the effect of Fe limitation on photophysiological properties of a key Antarctic diatom and *P. antarctica* growing under a dynamic light regime. In addition, we evaluated how Fe limitation affects protection from photoinhibition in the two taxa. To this end, cultures of the diatom *F. cylindrus* and *P. antarctica* were grown under dynamic light conditions, and their photosynthetic properties were compared under either Fe-replete or Fe-limited conditions. Finally, we interpret our findings with respect to the distribution of *P. antarctica* and diatoms in the Ross Sea.

## MATERIALS AND METHODS

*Strains and culture conditions:* Experiments were performed with the Antarctic prymnesiophyte *P. antarctica* (strain CCMP #1871) and the diatom *F. cylindrus* (strain CCMP #1102) obtained from the Center for Culture of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Science, West Boothbay Harbor, Maine, USA. The cultures were grown at 2°C under dynamic irradiance consisting of a 2 h light cycle where phytoplankton reside in the dark for 1 h followed by sinusoidal irradiance from 0 to 250 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ for 1 h. Irradiance was provided by Sylvania DuLux L 55W (FT55DL/841) lamps (Sylvania, Danvers, MA, USA) with dimmable ballasts (Sylvania Quicktronic Pho-Dim) that were regulated via a Velleman K8000 computer interface board (Velleman N.V., Gavere, Belgium). The treatment was designed to simulate an upper mixed layer with mixing below the euphotic zone. Semicontinuous cultures (1,000 mL) were grown in polycarbonate Erlenmeyer flasks in the artificial seawater medium Aquil (Price et al. 1989 as modified from the original by Morel et al. 1979). All media and nutrient solutions were passed over a Chelex column to remove trace metals. Nutrients were added to the media after passing over the Chelex column to a final concentration of 100 μM $NO_3$, 10 μM $PO_4$, and 100 μM $Si(OH)_4$. Trace metals were added dissolved in a stock solution containing EDTA to give a final concentration of 100 μM EDTA. Fe was added to obtain Fe-replete cultures at a final concentration of 1 μM. Trace-metal-clean techniques were followed throughout the experiment, and sampling was performed in a Class 100 laminar flow hood (Envirco Corporation, Sanford, NC, USA). All labware was soaked in 1% HCl and rinsed three times with Milli-Q water (Millipore, Billerica, MA, USA) prior to use. Fe limitation of the cultures was achieved by repeated transfer of cultures in medium with no added Fe while monitoring the $F_v/F_m$ (see below). We aimed for our cultures to achieve photosynthetic properties similar to those observed for diatoms and *P. antarctica* in the Ross Sea (Peloquin and Smith 2007, Rose et al. 2009). No additional Fe was added to the Fe-deplete cultures of *P. antarctica* #1871, while Fe was added to a final concentration of 1 nM to the Fe-deplete cultures of *F. cylindrus* #1102 to achieve values of $F_v/F_m$ that approximated the mean $F_v/F_m$ of 0.28 of a diatom-dominated bloom in the Ross Sea that was presumably Fe limited (Peloquin and Smith 2007). Initial attempts to grow *F. cylindrus* #1102 in media without adding Fe resulted in $F_v/F_m$ values below 0.15 and eventual death of the cultures.

Cultures were grown for ~10 generations in semicontinuous culture until acclimated to the treatment irradiance and Fe conditions. Replicate cultures were then inoculated in a one in 10 dilution from acclimated precultures and growth rates were monitored by measuring chl *a* concentration every other day. Cultures were grown until midexponential phase, at which point a sample of the culture was collected during the dark phase of the light cycle and processed for all measurements outlined below. In a subset of cultures (duplicates), acclimation of pigment composition within the light cycle was studied by taking an additional sample at the irradiance maximum of the light cycle.

To confirm the Fe-limitation status in the Fe-depleted cultures, 1 μM Fe (final concentration) was added to the cultures after sampling and $F_v/F_m$ (see below) was monitored for 48 h. The increase in $F_v/F_m$ was linear over time in all cultures, and the rates of $F_v/F_m$ increase were similar for all cultures at 0.07 $d^{-1}$.

*Cell counts:* Aliquots (5 mL) of culture were preserved by adding 2% (final concentration) glutaraldehyde and stored in the dark at 4°C. Slides for cell counts were prepared by filtering 1 or 2 mL of preserved sample onto 0.8 μm black polycarbonate filters under low vacuum pressure (<5 kPa). The filter was then mounted on a glass slide using immersion oil. At least 400 cells or 20 fields of view were counted under ×100 magnification on a Leitz Laborlux 11 epifluorescence microscope (Leica, Wetzlar, Germany).

*Analysis of pigments:* Duplicate aliquots for pigment analysis by HPLC were collected during the dark phase and at maximum irradiance by filtering 20 mL of culture onto 25 mm glass fiber filters (GF/F, Whatman, Maidstone, UK). The filters were snap-frozen in liquid $N_2$ and stored at −80°C until analysis. The filters were freeze-dried (48 h) and extracted in 90% acetone (48 h). Pigments were separated on a HPLC system (Waters 2690 separation module, 996 photodiode array detector) using a $C_{18}$ 5 μm DeltaPak reverse-phase column (Kraay et al. 1992, Van Leeuwe et al. 2006). Quantification was done using standards obtained by DHI Lab products (Hoersholm, Denmark). After linearity was established, single point calibrations were used of the following standards: chl *a*, chl $c_3$, 19′ butanoyloxyfucoxanthin (19′-But), fucoxanthin (Fuc), 19′ hexanoyloxyfucoxanthin (19′-Hex), diadinoxanthin (DD), diatoxanthin (DT), and ß-carotene, (ß-Car). The chl *a* breakdown/intermediate product, chlorophyllide *a*, was analyzed, but detected in very low concentrations and is not presented here.

*Particulate organic carbon and nitrogen (POC and PON):* Duplicate aliquots for POC and PON analysis were collected during the dark phase and at maximum irradiance by filtering 15–25 mL of culture onto precombusted (450°C for 4 h)

25 mm GF/F filters. The filters were subsequently dried at 60°C and stored until analysis on a Carlo-Erba NA-1500 elemental analyzer (Thermo Scientific, Waltham, MA, USA) using acetanilide as a calibration standard.

*Photosynthesis versus irradiance curves (*P-E*):* The *P-E* relationships were determined using the $^{14}$C- bicarbonate incorporation technique by incubation of 2 mL aliquots in a photosynthetron for 1 h over a range of 20 different light intensities ranging from 3 to 542 µmol photons · $m^{-2}$ · $s^{-1}$ at 2°C (Lewis and Smith 1983, as modified by Arrigo et al. 2010). Inorganic C uptake, normalized by chl *a* and POC concentration, was calculated from radioisotope incorporation, and the data were fit by least squares nonlinear regression to the (Webb et al. 1974) model in which the $P^*_o$ term was added representing inorganic C uptake at $E = 0$ µmol photons · $m^{-2}$ · $s^{-1}$, or $CO_2$ release in case $P^*_o$ is negative (Arrigo et al. 2010):

$$P^* = P^*_m\left(1 - exp\left(-\alpha^* \frac{E}{P^*_m}\right)\right) - P^*_o \quad (1)$$

$P^*_m$ is the maximum rate of inorganic C uptake and $\alpha^*$ is the initial slope of the *P-E* curve (g C · $g^{-1}$ · chl *a* · $h^{-1}$ [µmol photons · $m^{-2}$ · $s^{-1}$]$^{-1}$). The photoacclimation parameter $E_k$ was calculated as $P^*_m/\alpha^*$. The radioisotope incorporation was also fitted according to the model of (Platt et al. 1980), which contains the photoinhibition parameter ß* (g C · $g^{-1}$ chl *a* · $h^{-1}$ [µmol photons · $m^{-2}$ · $s^{-1}$]$^{-1}$). However, there was no significant fit of the ß* parameter in any of the *P-E* curves, and therefore this model was disregarded.

The corresponding POC normalized photosynthetic parameters ($P^C_m$, $\alpha^C$, $P^C_o$) were calculated by normalizing the inorganic C uptake rates by POC instead of chl *a* and refitting the data.

*Phytoplankton absorption:* Aliquots (15 mL) were filtered onto 25 mm GF/F filters for determination of algal absorption spectra ($a_{ph}$, 300–800 nm), as measured on a Perkin-Elmer Lambda 35 spectrophotometer equipped with an integrating sphere (Labsphere) using the filter pad method and optical corrections in (Mitchell and Kiefer 1988) and the coefficients of Bricaud and Stramski (1990). Detrital absorption ($a_{det}$, 300–800 nm) was assayed after methanol extraction according to the method of (Kishino et al. 1985). Chl *a*–specific absorption coefficients at each wavelength [$a^*_{ph}(\lambda)$] were calculated as

$$\frac{a^*_{ph}(\lambda) = a_p(\lambda) - a_{det}(\lambda)}{[\text{Chl } a]} \quad (2)$$

where [chl *a*] is the chl *a* concentration of the sample.

Spectrally weighted mean chl *a*–specific absorption coefficients ($\bar{a}^*$, $m^2$ · $mg^{-1}$ chl *a*) were calculated using the equation

$$\bar{a}^* = \frac{\sum_{400}^{700} a^*_{ph} E(\lambda)}{\sum_{400}^{700} E(\lambda)} \quad (3)$$

where $E(\lambda)$ (µmol photons · $m^{-2}$ · $s^{-1}$) is the spectral irradiance of the incubator.

As the assumption that the maximum quantum yield of photosynthesis ($\Phi_m$) is achieved at the lowest irradiance does not always hold (Johnson and Barber 2003), $\Phi_m$ was determined from $\bar{a}^*$ (calculated with $E(\lambda)$ of the photosynthetron incubator) and the chl *a*–normalized C uptake (*P**) measured at each photosynthetron light level, as outlined in Johnson and Barber (2003).

*Chl fluorescence:* Energy absorbed by photosystems can be used in photochemical and nonphotochemical energy pathways, or be reemitted as fluorescence (reviewed by Maxwell and Johnson 2000). Chl fluorescence analysis uses this energy balance to provide information about the efficiency of photochemistry on the basis of changes in PSII fluorescence under a range of different light treatments. To this end, chl fluorescence was measured with a WATER-PAM fluorometer and WinControl Software (Heinz Walz GmbH, Effeltrich, Germany), according to (Maxwell and Johnson 2000). Phytoplankton samples were dark-acclimated for 20 min at 2°C prior to each fluorescence measurement. When the measuring light (non-photochemistry-inducing) of the PAM was turned on, the minimum fluorescence ($F_o$) of the sample was measured. The maximum fluorescence ($F_m$) was then measured by applying a saturating light pulse of 4,000 µmol photons · $m^{-2}$ · $s^{-1}$ for 0.8 ms to close all PSII reaction centers. The maximum dark-acclimated efficiency of PSII ($F_v/F_m$) was calculated as (Krause and Weis 1991)

$$F_v/F_m = \frac{F_m - F_o}{F_m} \quad (4)$$

After a 12 s delay, the actinic (photochemistry-inducing) light source was turned on for 200 s at one of four different intensities (27, 90, 206, and 2,243 µmol photons · $m^{-2}$ · $s^{-1}$), allowing the fluorescence signal to reach a steady-state balance (defined as $F_s$) between fluorescence, photochemical, and nonphotochemical energy pathways. The actinic light levels were chosen to represent the low (27), mid (90), and high (206) light levels during the dynamic light cycle, as well as the maximum light level (2,243 µmol photons · $m^{-2}$ · $s^{-1}$) of in situ PAR on a sunny day. The actinic light period was followed by another saturating irradiance pulse to determine $F'_m$, defined as the maximum PSII fluorescence in light-acclimated cells. Directly after this second saturating light pulse following the actinic light period, the minimum fluorescence in the light-acclimated cells ($F'_o$) was measured. All fluorescence parameters were calculated by standard equations (Genty et al. 1989, Campbell et al. 1998, Maxwell and Johnson 2000). The light-acclimated photochemical efficiency of PSII (ΦPSII) at a specific actinic irradiance level was calculated as:

$$\Phi\text{PSII} = \frac{F'_m - F_s}{F'_m} \quad (5)$$

The fraction of oxidized reaction centers (*qP*) at a specific actinic irradiance was calculated as:

$$qP = \frac{F'_m - F_s}{F'_m - F'_o} \quad (6)$$

The NPQ at a specific actinic irradiance was calculated as (Bilger and Björkman 1990):

$$\text{NPQ} = \frac{F_m - F'_m}{F'_m} \quad (7)$$

*Calculated rates of electron transport and carbon fixation over the light cycle:* During the course of a light cycle, we evaluated the light intensity at which the rate of electron ($e^-$) flow through PSII became restricted when compared to the maximum rate based on $F_v/F_m$ and the light intensity at which alternative electron sinks may have been operational in the dynamic light cycle. The $F_v/F_m$ represents the maximum rate at which electrons may be released in dark-acclimated PSII. Thus, the maximum electron transport rate through PSII for dark-acclimated cells normalized to chl *a* ($ETR_{dark}$, µmol $e^-$ · $mg^{-1}$ chl *a* · $s^{-1}$) was approximated for each light level (*E*) in the dynamic light cycle at one minute intervals based on $F_v/F_m$:

$$\text{ETR}_{\text{dark}} = 0.5 * F_v/F_m * E * \bar{a}^* \quad (8)$$

In reality, ETR through PSII depends on the absorption cross section of PSII, which may be obtained from the effective cross-section of PSII corrected to the spectrum of the ambient irradiance that drives photosynthesis (Suggett et al. 2009). This

requires assessment of the cross-section of PSII (σPSII) and requires knowledge of the concentration of PSII reaction centers, which is difficult to quantify (Suggett et al. 2009). Here, we used the spectrally weighted mean chl *a*–specific absorption coefficient, weighted to the spectra (400–700 nm) of the light source from the growth incubator ($\bar{a}^*$), and the factor 0.5 to correct for the partitioning of photons between PSI and PSII, assuming that excitation energy is distributed evenly between the two photosystems (Maxwell and Johnson 2000, Wagner et al. 2006).

At increasing light intensity, the $F_v/F_m$ decreases to the light-acclimated or variable photochemical efficiency of PSII (ΦPSII) by the photochemical processes of increasing NPQ and decreasing *qP* at increased light intensity. Thus, the rate of electron transport in light-acclimated PSII ($ETR_{light}$; μmol $e^- \cdot mg^{-1}$ chl $a \cdot s^{-1}$) was calculated for each light level in the light cycle at one-minute intervals based on the ΦPSII that was interpolated between the actinic light levels at which it was measured to match *E*.

$$ETR_{light} = 0.5 * \Phi PSII * E * \bar{a}^* \quad (9)$$

Finally, the number of electrons used for C fixation (μmol $e^- \cdot mg^{-1}$ chl $a \cdot s^{-1}$) was calculated for each light level (*E*) in the light cycle at 1 min intervals using the parameters derived from the *P-E* curves based on the assumption that four electrons are needed for the fixation of one $CO_2$ molecule.

*Statistics:* The effect of taxa and Fe limitation on physiological parameters was tested with a nonparametric Mann–Whitney *U* test. The effects of taxon and Fe limitation on fluorescence parameters were tested with a repeated measures analysis of variance (ANOVA) using Statistica software (release 7, StatSoft Inc., Tulsa, OK, USA). Differences were considered significant at $P < 0.05$.

## RESULTS

*Physiological characteristics.* Physiological responses to Fe limitation were evident in cultures of both *P. antarctica* #1871 and *F. cylindrus* #1102 as a reduction in growth rate, $F_v/F_m$, and cellular chl *a* and carbon content (Table 1). In Fe-limited cultures of *P. antarctica* #1871, growth rates were reduced by 51% and $F_v/F_m$ by 41% relative to the Fe-replete cultures (Table 1). As the cellular content of both chl *a* and C were similarly reduced under Fe limitation, the C:chl *a* ratio was unchanged. Similarly, $\bar{a}^*$ was not affected by Fe limitation. However, the C:N ratio showed a modest decline of 8% under Fe limitation.

For most parameters, the response of the Fe-limited cultures of *F. cylindrus* #1102 resembled that of *P. antarctica* #1871 (Table 1). Due to the addition of 1 nmol $\cdot L^{-1}$ of Fe to the Fe-limited cultures of *F. cylindrus*, the reduction in the $F_v/F_m$ was similar in the two species. However, this resulted in a stronger reduction in growth rate (67%) in *F. cylindrus* #1102. Also, the cellular content of C and chl *a* decreased in Fe-limited *F. cylindrus* #1102 cultures, similar to *P. antarctica* #1871, resulting in no change in the C:chl *a* ratio. In contrast, the C:N ratios decreased by 32% in Fe-limited *F. cylindrus* #1102, to 3.96.

*Pigment composition.* The main pigments of *P. antarctica* #1871 were chl *a*, chl $c_3$, fucoxanthins (19′-But, Fuc, and 19′-Hex), and xanthophyll-cycle pigments (DD and DT) (Table 2). In general, the cellular content of pigments decreased under Fe limitation in *P. antarctica* #1871, with the exception of the xanthophyll-cycle pigments, which remained constant. Interestingly, the ratio of both total fucoxanthin and xanthophyll-cycle pigments to chl *a* increased under Fe limitation in *P. antarctica* #1871, due to a proportionally larger drop in chl *a* per cell. However, not all fucoxanthins exhibited similar changes with Fe limitation; cellular contents of 19′-Hex changed very little at low Fe, whereas 19′-But dropped by 60%, and Fuc declined to undetectable levels.

The main pigments of *F. cylindrus* #1102 were chl *a*, Fuc, and xanthophyll-cycle pigments (Table 2). Under Fe limitation, the cellular pigment content decreased, including the xanthophyll-cycle pigments. When pigments were normalized by chl *a*, the Fuc:chl *a* ratio increased under Fe limitation. The ratio of xanthophyll-cycle pigments to chl *a*, however, decreased under Fe limitation, opposite to that of *P. antarctica* #1871.

*Pigment dynamics during the light cycle.* To study the photoprotective effect of alterations in pigment composition, the cellular pigment content during the dark part of the light cycle was compared to that at the irradiance maximum of the light cycle. The cellular chl *a* content remained unchanged over the light cycle in all cultures (Mann–Whitney

TABLE 1. Mean (±SD) of physiological parameters of *Phaeocystis antarctica* #1871 and *Fragilariopsis cylindrus* #1102 for Fe-replete (+Fe) and Fe-limited (−Fe) cells. The differences between taxa are significant (Mann–Whitney *U* test, $P < 0.05$) unless connected by ~, and between Fe treatment unless they are connected by the same letter.

| | *P. antarctica* | | *F. cylindrus* | |
|---|---|---|---|---|
| | +Fe ($n = 5$) | −Fe ($n = 3$) | +Fe ($n = 5$) | −Fe ($n = 3$) |
| $\mu_{cell}$ ($d^{-1}$) | 0.38 (0.025) | 0.19 (0.006) | 0.16 (0.046) | 0.05 (0.001) |
| Chl *a* (fg $\cdot$ $cell^{-1}$) | 165.1 (24.1) | 110.3 (13.1) | 121.6 (12.8) | 83.0 (3.4) |
| POC (pg $\cdot$ $cell^{-1}$) | 27.17 (4.61) | 18.04 (1.54) | 21.12 (2.18) | 13.33 (1.50) |
| C:N [atm:atm] | 6.01~ (0.19) | 5.49 (0.15) | 5.83~ (0.31) | 3.96 (0.09) |
| C:chl *a* [wt:wt] | 164.83~[a] (15.87) | 166.15[a] (5.87) | 176.35~[b] (7.28) | 160.53[b] (16.16) |
| $F_v/F_m$ | 0.57 (0.02) | 0.34 (0.03) | 0.53 (0.02) | 0.30 (0.02) |
| $\bar{a}^*$ ($m^2 \cdot mg^{-1}$ chl *a*) | 0.0293~[a] (0.0041) | 0.0242[a] (0.0021) | 0.0308~[b] (0.0080) | 0.0269[b] (0.0027) |

TABLE 2. Mean (±SD) of pigment composition expressed as cellular contents and ratio to chl *a* for *Phaeocystis antarctica* #1871 and *Fragilariopsis cylindrus* #1102 for Fe-replete (+Fe) and Fe-limited (−Fe) cells. The differences between taxa are significant (Mann–Whitney *U* test, $P < 0.05$) unless connected by ~, and between Fe treatment unless they are connected by the same letter.

| | Cellular pigment content (fg · cell$^{-1}$) | | | | Pigment ratio to chl *a* (wt:wt) | | | |
|---|---|---|---|---|---|---|---|---|
| | *P. antarctica* | | *F. cylindrus* | | *P. antarctica* | | *F. cylindrus* | |
| | +Fe ($n = 5$) | −Fe ($n = 3$) | +Fe ($n = 5$) | −Fe ($n = 3$) | +Fe ($n = 5$) | −Fe ($n = 3$) | +Fe ($n = 5$) | −Fe ($n = 3$) |
| Chl $c_3$ | 38.3 (5.5) | 29.9 (1.7) | – | – | 0.232 (0.006) | 0.292 (0.015) | – | – |
| 19′-But | 9.1 (1.5) | 3.7 (0.7) | – | – | 0.055 (0.002) | 0.036 (0.0061) | – | – |
| Fuc | 9.9 (1.4) | 0.0 (0.0) | 73.1 (7.6) | 60.9 (2.7) | 0.060 (0.004) | 0.0 (0.0) | 0.597 (0.010) | 0.733 (0.015) |
| 19′-Hex | 119.5$^{a}$ (17.1) | 103.6$^{a}$ (4.1) | – | – | 0.724 (0.003) | 1.014 (0.004) | – | – |
| Total fucoxanthins | 138.6 (19.7) | 107.3 (4.6) | 73.1 (7.6) | 60.9 (2.7) | 0.840 (0.004) | 1.050 (0.002) | 0.597 (0.010) | 0.733 (0.015) |
| DD | 24.2$^{\sim a}$ (3.6) | 22.9$^{a}$ (3.1) | 22.2$^{\sim}$ (5.6) | 11.8 (0.5) | 0.147$^{\sim}$ (0.007) | 0.224 (0.028) | 0.173$^{\sim b}$ (0.028) | 0.143$^{b}$ (0.002) |
| DT | 1.5 (0.2) | 0.0 (0.0) | 3.7$^{\sim b}$ (3.8) | 0.4$^{6}$ (0.8) | 0.006$^{\sim}$ (0.010) | 0.0 (0.0) | 0.051$^{\sim b}$ (0.052) | 0.006$^{b}$ (0.010) |
| DD + DT | 25.7$^{\sim a}$ (3.7) | 22.9$^{a}$ (3.1) | 25.9$^{\sim}$ (4.9) | 12.3 (0.3) | 0.156 (0.008) | 0.224 (0.028) | 0.224 (0.036) | 0.148 (0.009) |
| β-Car | 3.6 (0.8) | 0.1 (0.2) | 2.3 (0.3) | 1.1 (0.6) | 0.022$^{\sim}$ (0.002) | 0.011 (0.005) | 0.020$^{\sim}$ (0.002) | 0.001 (0.002) |

19′-But, 19′ butanoyloxyfucoxanthin; Fuc, fucoxanthin; 19′-Hex, 19′ hexanoyloxyfucoxanthin; DD, diadinoxanthin; DT, diatoxanthin; β-Car, β-carotene.

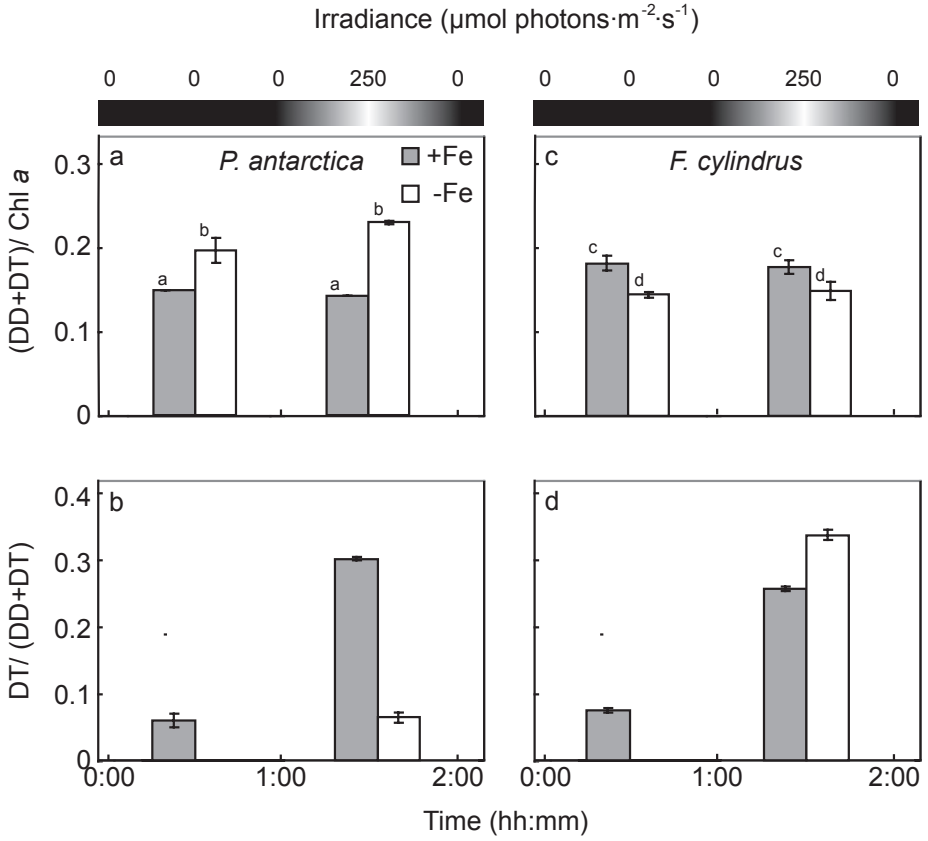


FIG. 1. Ratio of (DD + DT)/chl *a* (a and c) and DT:(DD + DT) (b and d) in the middle of the dark period and at the highest irradiance level of the sinusoidal irradiance curve in Fe-replete (+Fe) and Fe-limited (−Fe) cultures of *Phaeocystis antarctica* #1871 (a and b) and *Fragilariopsis cylindrus* #1102 (c and d). No differences within the light cycle were observed for the (DD + DT):chl *a* ratio (Mann–Whitney *U* test, $P < 0.05$, indicated by the same letter), but the differences in pigment ratios between phase of the light cycle and Fe treatment were significant (Mann–Whitney *U* test, $P < 0.05$) for the DT:(DD + DT) ratios (b and d). DD, diadinoxanthin; DT, diatoxanthin.

*U* test, $P > 0.05$), and no changes were observed in the ratio of any of the accessory pigments to chl *a*, including the (DD + DT):chl *a* ratio (Fig. 1, a and c). However, DD was converted to DT during the irradiance maximum in all cultures, albeit to differing degrees in *P. antarctica* #1871 and *F. cylindrus* #1102 (Fig. 1, b and d).

In Fe-replete cultures of *P. antarctica* #1871, 30% of the xanthophyll-cycle pigment was measured as DT during the irradiance maximum of the light cycle, while only 6% existed as DT during the dark period. The (DD + DT):chl *a* ratio increased under Fe limitation in *P. antarctica* #1871, while xanthophyll de-epoxidation decreased. Only 6% of the xanthophyll-cycle pigment was measured as DT during the irradiance maximum of the light cycle, while no DT was found in the dark.

Under Fe-replete conditions, xanthophyll pigment cycling in *F. cylindrus* #1102 was similar to that of *P. antarctica* #1871. During the irradiance maximum, 25% of the xanthophyll cycle pigment was measured as DT in *F. cylindrus*, while 7% was found as DT during the dark. Under Fe limitation, the ratio (DD + DT):chl *a* decreased slightly, whereas de-epoxidation levels at the irradiance maximum increased; 33% of xanthophyll-cycle pigment was found as DT during the irradiance maximum. This de-epoxidation level was much higher than that under nutrient-replete conditions and higher than that of Fe-limited *P. antarctica* #1871. No DT was observed during the dark period in Fe-limited cultures of *F. cylindrus* #1102.

*Photosynthesis versus irradiance responses.* The *P-E* characteristics of *P. antarctica* #1871 and *F. cylindrus* #1102 responded similarly to Fe limitation (Table 3, Fig. 2). In *P. antarctica,* the maximum C-fixation rates per chl *a* ($P_m^*$) decreased by 28% in the Fe-limited cultures (Fig. 2a). The same pattern was observed for the initial slope of the *P-E* curve ($\alpha^*$), which decreased by 39% in the Fe-limited cultures of *P. antarctica* #1871 (Fig. 2b). Consistent with these results was the decrease in the maximum quantum yield of photosynthesis ($\Phi_m$) (Fig. 2d). The photoacclimation parameter ($E_k$) increased by 34% in Fe-limited cultures of *P. antarctica* #1871 (Fig. 2c), indicating that $\alpha^*$ dropped more substantially under Fe-limitation than $P_m^*$.

In Fe-replete cultures of *F. cylindrus* #1102, $P_m^*$ was lower than in Fe-replete cultures of *P. antarctica* #1871, although the relatively large spread in the $P_m^*$ of *F. cylindrus* #1102 caused the differences to be nonsignificant. Since $\alpha^*$ was similar in the two strains, the $E_k$ of *F. cylindrus* #1102 was lower than that of *P. antarctica* #1871. Under Fe-limitation, $P_m^*$ of *F. cylindrus* #1102 was reduced by 80% (Fig. 2a), much stronger than seen in *P. antarctica* #1871. The decrease in $\alpha^*$ of 63% was similar to that of *P. antarctica* #1871. This resulted in a decrease in $E_k$ by 50% under Fe limitation to 21.1 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ (Fig. 2c), which is much lower than the average irradiance level of the dynamic light cycle of 73 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$. The $\Phi_m$ in *F. cylindrus* was similar to that of *P. antarctica* #1871, for Fe-replete as well as Fe-limited cultures (Fig. 2d).

When inorganic C uptake was normalized to POC rather than chl *a*, the photophysiological responses to Fe limitation of the two phytoplankton species were similar (Table 3), due to their similar C:chl *a* response to Fe limitation. The POC normalized respiration ($P_o^C$) showed no differences between the species or between the Fe treatments (Mann–Whitney *U* test, $P > 0.05$). However, when $P_o^C$ was expressed as fraction of the $P_m^C$, interesting differences became apparent. Both species devoted 8.5% of their $P_m^C$ to respiration under Fe-replete conditions. In *P. antarctica* #1871, this was reduced under Fe-limiting conditions (2.9%), whereas in *F. cylindrus* #1102, respiration under Fe-limiting conditions was as high as 35% of the $P_m^C$.

*Chl fluorescence quenching.* At actinic light levels similar to those experienced during the dynamic light cycle (27, 90, and 206 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$), Fe-replete *P. antarctica* #1871 cultures exhibited low levels of NPQ (<0.1) (Fig. 3a). The levels of *qP* were high (>0.83) at these actinic light levels (Fig. 3b), resulting in close to maximum levels of ΦPSII (>0.44) (Fig. 3c). The levels of NPQ that were measured at the maximum actinic light level of 2,243 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ were high (1.05) (Fig. 3a), indicating that *P. antarctica* #1871 has the potential to use NPQ as a strategy for coping with supraoptimal irradiance. At the maximum actinic light level, both *qP* and ΦPSII were not significantly different from zero (Fig. 3, b and c).

Fe limitation did not affect NPQ in *P. antarctica* #1871 at any of the analyzed actinic light levels (Fig. 3a) (repeated measures ANOVA, $P > 0.05$]). Fe limitation resulted in a reduction in *qP* (repeated measures ANOVA, $P < 0.05$), particularly at actinic light levels corresponding to the irradiance

TABLE 3. Mean (±SD) of chl *a*–normalized and C-normalized coefficients from the *P-E* curves of *Phaeocystis antarctica* #1871 and *Fragilariopsis cylindrus* #1102 for Fe-replete (+Fe) and Fe-limited (−Fe) cells. The differences between taxa are significant (Mann–Whitney *U* test, $P < 0.05$) unless connected by ~, and between Fe treatment unless they are connected by the same letter.

| | *P. antarctica* | | *F. cylindrus* | |
|---|---|---|---|---|
| | +Fe ($n = 5$) | −Fe ($n = 3$) | +Fe ($n = 4$) | −Fe ($n = 2$) |
| $P_m^*$ (g C $\cdot$ $g^{-1}$ chl *a* $\cdot$ $h^{-1}$) | 7.77~ (0.71) | 4.63 (0.43) | 5.84~ (1.89) | 1.23 (0.29) |
| $\alpha^*$ (g C $\cdot$ $g^{-1}$ chl *a* $\cdot$ $h^{-1}$ [μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$]$^{-1}$) | 0.136* (0.010) | 0.062 (0.010) | 0.149*[a] (0.066) | 0.058[a] (0.009) |
| $P_o^*$ (g C $\cdot$ $g^{-1}$ chl *a* $\cdot$ $h^{-1}$) | 0.47 (0.31)~[a] | 0.22 (0.30)[a] | 0.50 (0.14)~[b] | 0.44 (0.06)[b] |
| $E_k$ (μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) | 57.6 (9.4) | 74.9 (7.9) | 41.1 (7.1) | 21.1 (1.8) |
| $P_m^C$ ($d^{-1}$) | 1.15 (0.20) | 0.63 (0.01) | 0.79 (0.22) | 0.20 (0.06) |
| $\alpha^C$ ($d^{-1}$ [μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$]$^{-1}$) | 1.99 $10^{-2}$~ (0.02 $10^{-2}$) | 0.85 $10^{-2}$ (0.09 $10^{-2}$) | 2.01 $10^{-2}$~[a] (0.08 $10^{-2}$) | 0.92 $10^{-2}$ [a] (0.19 $10^{-2}$) |
| $P_o^C$ ($d^{-1}$) | 0.066~[a] (0.046) | 0.032[a] (0.045) | 0.069~[b] (0.006) | 0.067[b] (0.018) |
| $\phi_m$ (mol C $\cdot$ $mol^{-1}$ photons) | 0.105~ (0.018) | 0.061 (0.004) | 0.095~[b] (0.025) | 0.055[b] (0.003) |

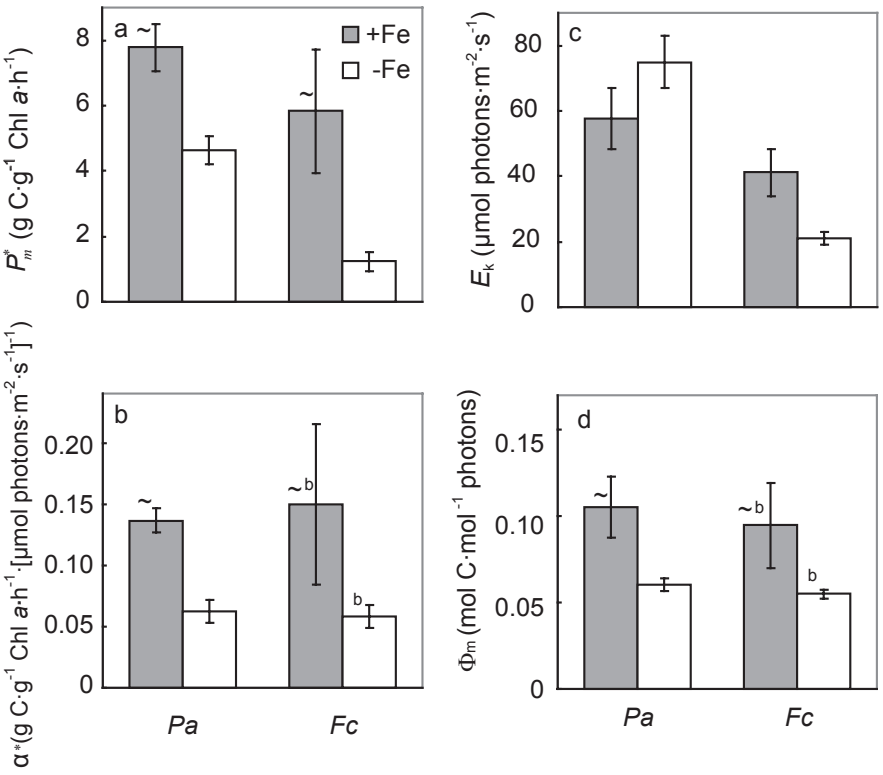


FIG. 2. Photosynthesis versus irradiance characteristics of *Phaeocystis antarctica* #1871 (*Pa*) and *Fragilariopsis cylindrus* #1102 (*Fc*) in Fe-replete (+Fe) and Fe-limited (−Fe) cultures. (a) Maximum rate of C fixation normalized to chl *a* ($P^*_m$), (b) the initial slope of the *P-E* curve ($\alpha^*$), (c) the photoacclimation parameter ($E_k$), and (d) the maximum quantum yield of photosynthesis ($\Phi_m$). The differences between taxa are significant (Mann–Whitney *U* test, $P < 0.05$) unless connected by ∼, and between Fe treatment unless they are connected by the same letter.

maximum of the dynamic light cycle (Fig. 3b). This resulted in a reduction in ΦPSII that was strongest at the actinic light level of 206 µmol photons · $m^{-2}$ · $s^{-1}$ (Fig. 3c).

*F. cylindrus* #1102 showed similar levels of NPQ under Fe-replete conditions as *P. antarctica* #1871 at all analyzed actinic light levels (Fig. 3d). The *qP* at the actinic light levels corresponding to those in the dynamic light cycle, however, were lower in Fe-replete *F. cylindrus* #1102 than in *P. antarctica* #1871 (Fig. 3e). This was apparent at the actinic light level of 27 µmol photons · $m^{-2}$ · $s^{-1}$, and *qP* decreased with increasing actinic light. Significant levels of *qP* were still measured at the highest actinic light, in contrast to *P. antarctica* #1871. ΦPSII showed a similar trend to that of *qP* over the range of actinic light used here (Fig. 3f).

Fe-limitation did not affect NPQ in *F. cylindrus* #1102 at actinic light levels similar to those of the dynamic light cycle (Fig. 3d). However, the NPQ measured at 2,243 µmol photons $m^{-2}$ · $s^{-1}$ increased under Fe limitation, resulting in an overall significant effect of Fe limitation (repeated measures ANOVA, $P < 0.05$). Fe limitation caused a stronger reduction of *qP* in *F. cylindrus* #1102 than in *P. antarctica*, particularly at actinic light levels of 90 and 206 µmol photons · $m^{-2}$ · $s^{-1}$ (Fig. 3e). This resulted in low ΦPSII at all light levels in Fe-limited cultures of *F. cylindrus* #1102 (Fig. 3f).

*Calculated rates of electron transport and carbon fixation over the light cycle.* The ETRs based on the $ETR_{dark}$, $ETR_{light}$, and *P-E* characteristics were similar at low light levels, but diverged at higher light levels for both *P. antarctica* #1871 and *F. cylindrus* #1102 under both Fe-replete and Fe-limiting conditions (Fig. 4). The difference between the potential $ETR_{dark}$ and variable $ETR_{light}$ corresponds to an increase in NPQ at higher light intensity, which limits the inflow of quanta to PSII and a decrease of *qP* that indicates limited electron flow downstream of PSII. The divergence of $ETR_{light}$ and the electron flow to C fixation suggests the presence of alternative electron pathways such as CET that do not result in C fixation. In Fe-replete cultures of *P. antarctica* #1871, $ETR_{dark}$ and $ETR_{light}$ diverged when light levels exceeded 100 µmol photons · $m^{-2}$ · $s^{-1}$ during the dynamic light cycle (Fig. 4a). Correspondingly, actinic light exceeding 90 µmol photons · $m^{-2}$ · $s^{-1}$ induced some NPQ and a decrease in *qP* (Fig. 3, a and b). The $ETR_{light}$ and rate of electron flow to C fixation diverged when light exceeded the $E_k$ value derived from the *P-E* curves (∼50 µmol photons · $m^{-2}$ · $s^{-1}$). Thus, at light levels exceeding $E_k$, there is increased potential for CET.

Fe limitation in *P. antarctica* #1871 resulted in a decrease in both $ETR_{dark}$ and $ETR_{light}$ (Fig. 4b). $ETR_{light}$ decreased more strongly than $ETR_{dark}$, corresponding to a decrease in *qP* levels under Fe limitation. The difference between the $ETR_{light}$ and the rate of electron flow to C fixation was negligible under Fe limitation. This indicates that Fe-limited *P. antarctica* #1871 was able to efficiently channel electrons from PSII to C fixation with little loss to alternative electron pathways like CET, consistent

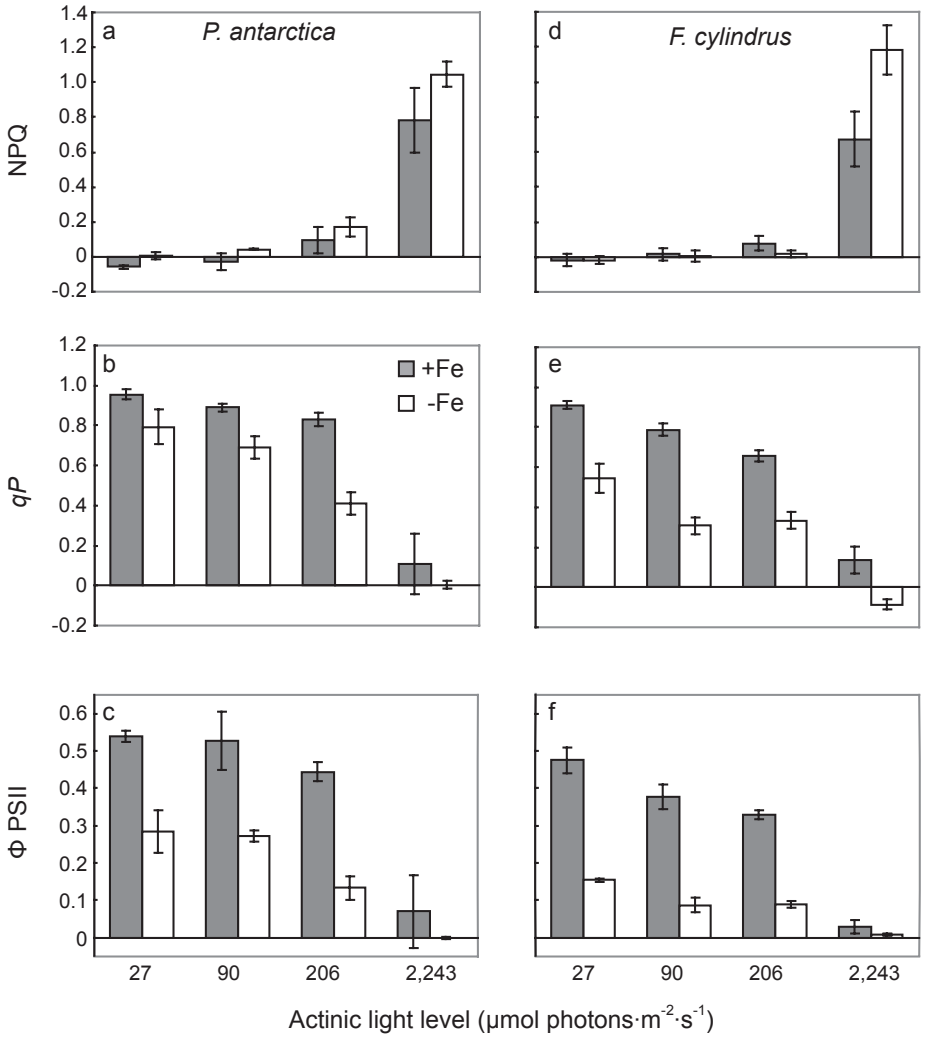


FIG. 3. Fluorescence parameters (dimensionless) of Fe-replete (+Fe) and Fe-limited (−Fe) cultures of *Phaeocystis antarctica* #1871 (a,b,c) and *Fragilariopsis cylindrus* #1102 (d,e,f). (a and d) Nonphotochemical quenching (NPQ), (b and e) photochemical quenching (*qP*), and (c and f) light-acclimated photochemical efficiency of PSII (ΦPSII). Differences between taxa and Fe treatment were significant (repeated measures analysis of variance [ANOVA], $P < 0.05$), except for the NPQ levels of Fe-replete *P. antarctica* and *F. cylindrus* that were similar (repeated measures ANOVA, $P > 0.05$) and in *P. antarctica* where no effect of Fe-limitation was observed (repeated measures ANOVA, $P > 0.05$).

with their high growth rates and $P^*_m$ under Fe limitation.

In Fe-replete cultures of *F. cylindrus* #1102, the $ETR_{dark}$ over the light cycle was similar to that of Fe-replete *P. antarctica* #1871 (Fig. 4c). The $ETR_{light}$, however, decreased more strongly at the peak of the light cycle, and the rates of $ETR_{dark}$ and $ETR_{light}$ diverged at light levels as low as 20 µmol photons $\cdot$ $m^{-2} \cdot s^{-1}$, which was lower than in *P. antarctica* #1871. The low $ETR_{dark}$ corresponded to reduced *qP* at intermediate and high actinic light levels in *F. cylindrus* #1102 (Fig. 3e). Since the $E_k$ of *F. cylindrus* #1102 was lower than that of *P. antarctica* #1871, the rate of electron flow to C-fixation leveled off at lower light intensities.

Fe limitation in *F. cylindrus* #1102 resulted in a decrease in both $ETR_{dark}$ and $ETR_{light}$. Similar to *P. antarctica* #1871, the $ETR_{light}$ decreased proportionally more than the $ETR_{dark}$. This finding corresponded to decreased levels of *qP* measured at all actinic light levels (Fig. 3e). The rate of electron flow to C fixation was low over the entire light cycle, corresponding to the low $E_k$ and $P^*_m$ of Fe-limited *F. cylindrus* #1102 (Table 3). The relative difference between the $ETR_{light}$ and electron flow to C fixation over the light cycle was largest in Fe-limited cultures of *F. cylindrus* #1102, indicating a strong potential for CET in Fe-limited *F. cylindrus* #1102. Thus, at light levels exceeding the $E_k$, a large fraction of the electrons passing through PSII were not used for C fixation. This may explain the stronger decrease in growth rate under Fe limitation in *F. cylindrus* #1102 than in *P. antarctica* #1871, despite a similar decrease in $F_v/F_m$.

## DISCUSSION

In general, phytoplankton cells exposed to dynamic irradiance need to adjust their photosynthetic apparatus for optimal use of low irradiance, while minimizing photoinhibitory effects at high irradiance. Our study provides evidence for species-specific responses to Fe limitation under dynamic irradiance. *P. antarctica* #1871 managed to sustain high growth rates under Fe limitation, although no Fe was added to the cultures in our experiments. In

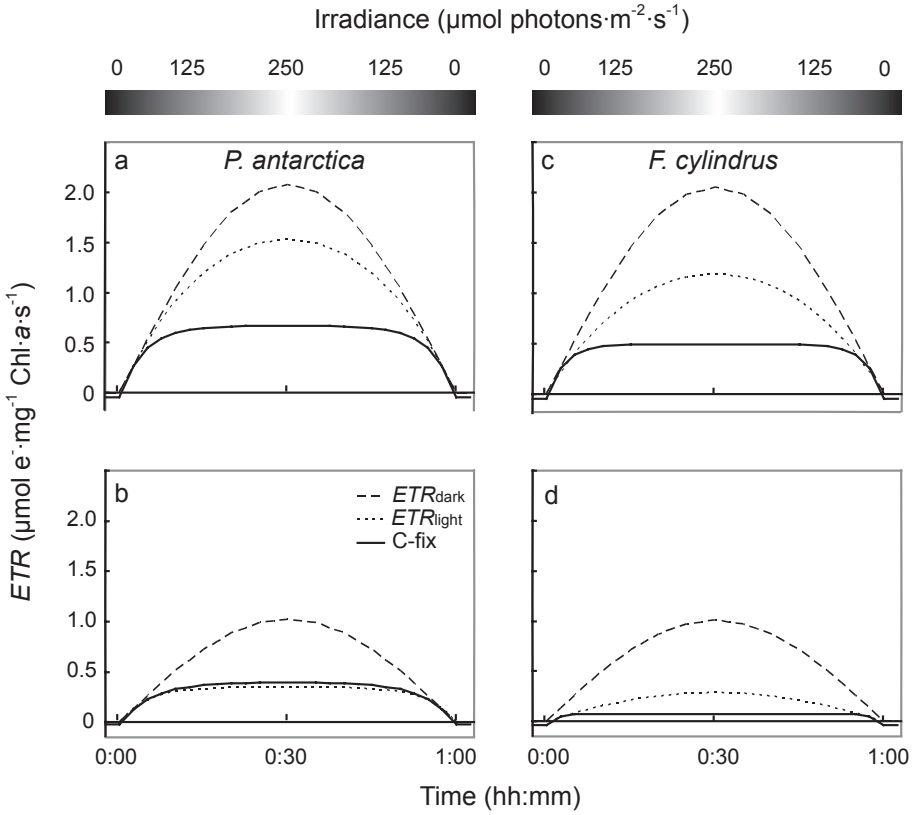


FIG. 4. Comparison of potential electron transport rate in the dark acclimated PSII ($ETR_{dark}$), electron transport rate in light acclimated PSII ($ETR_{light}$), and electrons needed for C fixation based on the photosynthesis versus irradiance relations over the light cycle in *Phaeocystis antarctica* #1871 (a and b) and *Fragilariopsis cylindrus* #1102 (c and d) in Fe-replete cultures (a and c) and under Fe limitation (b and d).

contrast, a small amount of Fe had to be added to the *F. cylindrus* #1102 cultures to avoid viability loss. To achieve Fe-limiting conditions for both phytoplankton taxa, we used EDTA to complex the trace amounts of Fe that may have entered the cultures despite working under trace-metal-clean conditions, similar to previous studies (Van Leeuwe and Stefels 2007, Van de Poll et al. 2009). Despite the notion that *P. antarctica* as well as several Antarctic diatoms are able to access EDTA-complexed Fe (Strzepek et al. 2011), all Fe-limited cultures showed clear signs of Fe-limitation. The diminished $F_v/F_m$ and growth rates of both species were relieved by the addition Fe to the Fe-limited cultures at the end of the experiment, suggesting that Fe contamination was minor. Because of potential differences between *P. antarctica* #1871 and *F. cylindrus* #1102 in the ability to access EDTA-complexed Fe, we cannot conclude that *P. antarctica* has a lower Fe requirement than *F. cylindrus*. However, there are suggestions from field studies for higher Fe requirements for Antarctic diatoms than *P. antarctica* in the Ross Sea. The areas with deep MLDs dominated by *P. antarctica* are also regions with lower Fe concentrations, whereas Fe input from melting sea ice increases Fe concentrations in areas with shallow MLDs where diatoms dominate (Sedwick et al. 2000). Moreover, Feng et al. (2010) reported that the addition of Fe to Fe-depleted surface waters in bioassay experiments in the Ross Sea resulted in an increase in relative diatom abundance. On the other hand, culture studies revealed higher cellular Fe:C ratios in *P. antarctica* than several Antarctic diatoms, under both Fe-replete and Fe-deplete conditions (Strzepek et al. 2011).

Blooms of *P. antarctica* in the Southern Ocean often consist of both colonies and single cells (Peloquin and Smith 2007, Wright et al. 2010), whereas single cell *Phaeocystis* may be abundant in mixed species communities (Schoemann et al. 2005, Kozlowski et al. 2011). In our study, both Fe-replete and Fe-limited cultures of *P. antarctica* #1871 were primarily in the single cell state, although small colonies were observed in both treatments. The photophysiological results in this study can be extrapolated to colonial cells as well, since the mucous matrix in which cells are embedded contributes little to the carbon biomass of *P. antarctica* colonies (Mathot et al. 2000, Alderkamp et al. 2007), and has thus little effect on the elemental composition of *P. antarctica* and C to pigment ratio. In addition, no differences in photosynthetic properties were reported between *P. antarctica* colonies and single cells in the Ross Sea (Shields and Smith 2009). In general, the single cell state has a higher surface to volume ratio, which is advantageous under nutrient-limiting conditions, and a higher percentage of *P. antarctica* cells were shown to be in the single cell state under Fe limitation (Van Leeuwe and Stefels 2007). Moreover, additions of Fe in

bioassays in the Ross Sea lead to a higher percentage of *P. antarctica* in the colonial morphotype (Feng et al. 2010) and high Fe requirements were suggested for colonial *P. antarctica* (Sedwick et al. 2007).

The pigment content and composition of both *P. antarctica* #1871 and *F. cylindrus* #1102 changed in response to Fe limitation, similar to other reports on various strains of *P. antarctica* (DiTullio et al. 2007, Van Leeuwe and Stefels 2007) and other Antarctic diatom species (Kosakowska et al. 2004, Van Oijen et al. 2004). In *P. antarctica* #1871, Fe limitation decreased cellular Fuc content, whereas cellular 19′Hex remained unaffected, similar to what was reported by Van Leeuwe and Stefels (2007). Also, the Fuc content of *F. cylindrus* #1102 decreased under Fe limitation similar to reports of other Antarctic diatom species (Kosakowska et al. 2004, Van Oijen et al. 2004). The ratio Fuc:19′-Hex has occasionally been used to determine the relative abundance of diatoms and *P. antarctica* within Antarctic phytoplankton populations (e.g., Smith and Asper 2001, Feng et al. 2010). However, since the decrease in Fuc content under Fe limitation was quantitatively much greater in Antarctic diatoms, this approach may lead to an underestimation of the diatom contribution under low Fe concentrations. The low Fuc:19′-Hex pigment ratios of the Fe-limited *P. antarctica* #1871 resemble those of field measurements in the Southern Ocean (Wright et al. 2010). These authors used a pigment signature including the chl $c_3$:chl *a* ratio in the CHEMTAX analysis to distinguish a Fe-limited population of *P. antarctica*. Based on our data and those of Van Leeuwe and Stefels (2007), 19′-But could also be incorporated into this signature, with 19′-But content decreasing under Fe limitation. Incorporation of an additional pigment into these algorithms would improve our ability to distinguish between nutrient replete and Fe-limited *P. antarctica* and diatoms in Antarctic waters using CHEMTAX or other pigment-based methods.

*The effect of Fe limitation on photoprotection.* The low values of *qP* at high actinic light levels and under Fe limitation show a small fraction of oxidized ''open'' PSII reaction centers under Fe limitation, particularly in *F. cylindrus* #1102. This finding could be explained by lower cellular content of the Fe-rich downstream electron acceptors cytochrome $b_6f$ and PSI that would decrease the efficiency of the electron flow downstream of PSII (Greene et al. 1992, Strzepek and Harrison 2004). Certain phytoplankton species, most notably coastal diatoms, are known to lower the cellular content of cytochrome $b_6f$ and PSI under Fe limitation (Greene et al. 1992, Geider et al. 1993). On the other hand, phytoplankton species growing in environments with constant low Fe concentrations have constitutively low cellular contents of cytochrome $b_6f$ and PSI (Strzepek and Harrison 2004, Lavaud et al. 2007). Since cytochrome $b_6f$ complexes are crucial in the build-up of the ΔpH across the thylakoid membrane that drives NPQ by xanthophyll cycling, it was suggested that Fe limitation lowers photoprotection by NPQ (Strzepek and Harrison 2004). Moreover, it was suggested that oceanic diatoms with constitutively low cellular cytochrome $b_6f$ were less able to cope with rapid irradiance fluctuations (Strzepek and Harrison 2004, Lavaud et al. 2007). However, the relatively high NPQ at the highest actinic light level under both Fe-replete and Fe-limiting conditions suggests that this is not the case for *F. cylindrus* #1102 or *P. antarctica* #1871. Thus, although xanthophyll cycling during the dynamic light cycle was reduced under Fe limitation in *P. antarctica* #1871, corroborating results by Van Leeuwe and Stefels (2007), the potential for photoprotection by NPQ at irradiance levels that approximate exposure to direct sunlight was not affected. Moreover, the maximum NPQ in *F. cylindrus* #1102 increased under Fe limitation, despite a decreased (DD + DT):chl *a* ratio and cellular DD + DT content. High NPQ levels that were independent of either xanthophyll-cycle pigment content or (DD + DT):chl *a* ratio have been previously reported in Antarctic diatoms, even when acclimated to relatively low light levels (Kropuenske et al. 2009, Van de Poll et al. 2011). Moreover, an increase in NPQ under Fe limitation is consistent with similar observations made in the diatom *P. tricornutum* (Allen et al. 2008). Our *P-E* results show no indication of photoinhibition at irradiance levels up to 540 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ in any of our *P. antarctica* or *F. cylindrus* cultures. Thus, although the higher fraction of reduced PSII centers under Fe limitation are more prone to photodamage (Long et al. 1994, Adir et al. 2003), both *P. antarctica* #1871 and *F. cylindrus* #1102 were well protected from photoinhibition.

*Calculated rates of electron transport and carbon fixation over the light cycle.* Under Fe-replete conditions, *P. antarctica* #1871 was able to maintain high growth rates under the dynamic light regime in these experiments. The growth rate of Fe-replete cultures of *F. cylindrus* #1102 was 42% of those of *P. antarctica* #1871, similar to what was observed by (Mills et al. 2010) under the same culture conditions. In *P. antarctica* #1871, Fe limitation reduced $F_v/F_m$ (−43%), $P^*_m$ (−40%), and growth rate (−51%) to a similar degree. Fe limitation in *F. cylindrus* #1102 resulted in a similar decease in $F_v/F_m$ (−44%), although the accompanying decreases in $P^*_m$ (−79%) and growth rate (−67%) were much stronger. In linear photosynthetic electron flow, a decrease in $F_v/F_m$ due to Fe limitation would result in a decrease in oxygen evolution and C fixation (Long et al. 1994). However, these parameters would not necessarily covary if alternative PSII photoacclimation strategies such as CET were involved (Oquist et al. 1992). Thus, the similar reduction in $F_v/F_m$, $P^*_m$, and growth rate under Fe

limitation in *P. antarctica* #1871 suggests that this species reduces the electron flow through the various steps of the photosynthetic process to a similar degree. In *F. cylindrus* #1102, on the other hand, electron flow may be limited downstream of PSII or alternative electron sinks may become more important under Fe-limitation.

The difference between the potential $ETR_{dark}$ and variable $ETR_{light}$ corresponds to an increase in NPQ at higher light intensity, which limits the inflow of quanta to PSII and a decrease of *qP* that indicates limited electron flow downstream of PSII. The divergence of $ETR_{light}$ and the electron flow to C fixation only at high light levels resembles the activity of photoprotective CET that was observed in *P. tricornutum* (Lavaud et al. 2002, Feikema et al. 2006, Wagner et al. 2006, Suggett et al. 2009), which drives electron transport through PSII, but does not lead to C fixation. Therefore, a photoprotective mechanism resembling CET seems to be active in both *P. antarctica* and *F. cylindrus*. In addition to CET, the disparity between $ETR_{light}$ and C fixation may be due to processes such as mitochondrial respiration (Fernie et al. 2004), the Mehler reaction whereby oxygen is reduced at the acceptor side of PSI (Mehler 1951, Mehler and Brown 1952, Asada et al. 1974), and photorespiration. However, these latter processes do not chiefly operate at high light levels, and therefore CET is likely the main contributor to the divergence of $ETR_{light}$ and the electron flow to C fixation.

In our calculations, we approximated the absorption cross-section of PSII by using the spectrally weighed absorption cross-section of chl *a* ($\bar{a}^*$) and assumed an equal partitioning between PSII and PSI (Maxwell and Johnson 2000, Wagner et al. 2006). This may have overestimated $ETR_{dark}$ and $ETR_{light}$ since we assumed that $\bar{a}^*$ was constant during the light cycle. However, the effective absorption cross section of both PSII and PSI are highly variable, being affected by nutrient status, photoacclimation, and photodamage (Suggett et al. 2007, 2009, Ragni et al. 2008). A decrease in absorption during photoacclimation to high light would reduce the amount of quanta absorbed and, thus, the ETR. Based on the comparison between ETR and electrons flowing to C fixation, the reduction of PSII cross-section under high irradiance is likely strongest in Fe-limited *F. cylindrus* #1102 and weakest in Fe-limited *P. antarctica* #1871. On the other hand, the factor of 0.5 to represent the partitioning of quanta between PSII and PSI may be too low, since the PSII:PSI ratio is higher in diatoms adapted to growth under low Fe conditions, such as observed in *Thalassiosira oceanica* (Strzepek and Harrison 2004, Peers and Price 2006). Moreover, in other diatom species, such as *T. weissflogii* and *P. tricornutum*, the PSII:PSI ratio was flexible and increased by a factor of 2.8 under Fe-limitation (Allen et al. 2008). Based on the comparison between ETR and electron flow to C fixation, an increase in PSII:PSI under Fe limitation would most likely be seen in Fe-limited *P. antarctica* #1871, whereas *F. cylindrus* #1102 may have a constitutively expressed PSII:PSI ratio. Finally, photosynthetic state transition is a process whereby the balance between excitation energy flow into PSII and PSI is altered. Governed by the redox state of the plastoquinone pool, a subpopulation of light-harvesting complex II may migrate from PSII to PSI (Ruban and Johnson 2009). As a result, the PSII cross-section decreases, whereas the PSI cross-section increases, meaning that our calculations would overestimate the ETR. Based on the comparison between the ETR and electrons flowing to C fixation, state transitions would be unlikely in Fe-limited *P. antarctica*, whereas they may be present in Fe-replete *P. antarctica*. State transitions are pronounced in cyanobacteria and green algae, but have never been documented in diatoms (Ruban and Johnson 2009).

*The effect of Fe limitation on distribution of* P. antarctica *and* F. cylindrus *in the Ross Sea.* A series of physiological studies under different controlled irradiance regimes confirmed that the high correlation between distributions of *P. antarctica* and diatoms and MLD in the Ross Sea was governed by the photophysiological adaptations in the two taxa (Mills et al. 2010). When grown under high irradiance, Antarctic diatoms such as *F. cylindrus* and *C. brevis* showed higher levels of photoprotection, such as NPQ, than *P. antarctica* (Kropuenske et al. 2009, Van de Poll et al. 2011). Although we did not address potential intraspecific differences by studying only one strain from each taxa, our results suggest that Fe limitation increases photophysiological differences between the two taxa. Fe limitation increased the levels of photoprotection in *F. cylindrus* #1102, expressed as higher *NPQ* and a greater fraction of electrons flowing through alternative electron pathways such as CET. Thus, in areas where ice melt results in high Fe concentrations (Sedwick et al. 2000) and irradiance is relatively high under constant irradiance levels due to a shallow mixed layer, diatoms will outcompete *P. antarctica* (Arrigo et al. 2010, Mills et al. 2010). Later in the season when Fe is depleted, our results show that diatoms like *F. cylindrus* #1102 remain well protected from photoinhibition in areas with a shallow MLD. On the other hand, *P. antarctica* under Fe-replete conditions was able to efficiently use the wider range of light levels characteristic of a deep MLD (Arrigo et al. 2010, Mills et al. 2010). Our results show that *P. antarctica* #1871 growing under dynamic irradiance under Fe limitation was extremely efficient in channeling electrons from PSII to C fixation at all light levels. Therefore, it was still able to use the wide range of light levels for C fixation under Fe limitation, whereas in *F. cylindrus* #1102 electrons were channeled to alternative electron pathways like CET at light levels exceeding

20 μmol photons · $m^{-2}$ · $s^{-1}$. Thus, by assuming our two strains to be model species for *P. antarctica* and Antarctic diatoms under Fe limitation in the field, *P. antarctica* will outcompete diatoms in areas with a deep mixed layer to a higher degree than under Fe-replete conditions.

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