Non-photochemical quenching of chlorophyll fluorescence and xanthophyll cycle responses after excess PAR and UVR in Chaetoceros brevis, Phaeocystis antarctica and coastal Antarctic phytoplankton

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Low iron availability and light limitation due to deep vertical mixing are considered the main factors constraining phytoplankton growth in the open Southern Ocean, leaving high macronutrient concentrations that are not exploited by phytoplankton growth (van Oijen et al. 2004a). Although phytoplankton rarely reaches concentrations in excess of 5 mg chlorophyll a (chl a) m$^{-3}$, there can be considerable variation in chlorophyll...
biodiversity on a temporal and spatial scale. These differences have been primarily attributed to elevated iron concentrations due to natural iron fertilization and to differences in mixed layer depths (De Baar et al. 2005, Blain et al. 2007). Deep mixed layers can extend below the euphotic zone, thereby reducing phytoplankton light exposure. A stabilization of the mixed layer (stratification) can be induced by elevated temperature and by the influx of fresh water.

Limitation of summer time phytoplankton accumulation (~0.2 to 0.4 mg chl a m\(^{-3}\)) due to low iron availability (3.0 nM Fe l\(^{-1}\)) and deep mixed layers (upper mixed layer, UML, at ~80 m) has been observed in the Drake Passage (Hewes et al. 2009, A. C. Alderkamp et al. unpubl.). In contrast, the Bransfield Strait and the waters surrounding the South Shetland Islands (SSI) have relatively high iron concentrations that originate from the Weddell Sea (3.0 nM Fe l\(^{-1}\); Hewes et al. 2008). Here, deep mixed layers (UML at 100 to 40 m) are believed to limit summertime phytoplankton accumulation (0.5 to 1.2 mg chl a m\(^{-3}\)). Seasonally released meltwater (from sea ice, glaciers, and rivers) along the SSI alters the hydrography of coastal waters as in other regions in the West Antarctic Peninsula area (Dierssen et al. 2002). Stratification of the water column provides phytoplankton with a more stable light environment, which allows utilization of the stronger light closer to the water surface, thereby enhancing growth rates. More than 10 yr of monitoring in Potter Cove (King George Island, SSI) has demonstrated increased meltwater input in coastal waters, which decreases surface salinity and increases sediment concentrations (Schloss et al. 2008). Despite enhanced summer stratification, phytoplankton does not reach concentrations in excess of 3.5 mg chl a m\(^{-3}\) in this area (Schloss & Ferreyra 2002, Schloss et al. 2002). Several mesocosm studies indicated that phytoplankton potentially can reach concentrations in excess of 10 mg chl a m\(^{-3}\) (Agusti & Duarte 2000, Helbling et al. 1995). This discrepancy has been attributed to the combination of stratification and high turbidity in the SSI area (Schloss & Ferreyra 2002). Recently, evidence for a role of photoinhibition in controlling phytoplankton productivity in the open Southern Ocean has been found (Alderkamp et al. 2010). Antarctic algal photosynthesis saturates at around 100 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\), but radiation in excess of 1000 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) can be experienced near the water surface. Algae show considerable plasticity in acclimation potential to irradiance changes (Van de Poll et al. 2007, Van de Poll & Buma 2009, Kropuenske et al. 2009). This not only influences the irradiance range that supports growth but also affects the sensitivity of species towards extremes that can occur in their environment such as excess photosynthetically active radiation (PAR) and ultraviolet radiation (UVR: 280 to 400 nm). Genetically fixed differences in photoacclimation potential have been suggested to contribute to the success of individual species under prevailing conditions (Strzepek & Harrison 2004, Lavaud et al. 2007, Dimier et al. 2009). The xanthophyll cycle is believed to be a crucial adaptation to a fluctuating light environment because it responds on a time scale of seconds to minutes to protect Photosystem II (PSII) during periodic overexcitation of photosynthetic electron transport. When exposed to excess light, a photoprotective process (NPQ: non-photochemical quenching of chlorophyll fluorescence) is induced by acidification of the thylakoid lumen. This triggers the xanthophyll cycle (the reversible enzymatic conversion of epoxy-xanthophyll into de-epoxidated xanthophyll), enhancing energy dissipation in the form of heat (Goss & Jakob 2010). As a result, excitation pressure of photosynthetic electron transport is reduced and PSII damage and metabolically costly PSII repair is limited (Van de Poll & Buma 2009). The xanthophyll cycle in diatoms and haptophytes (the diadinoxanthin cycle) differs significantly from the xanthophyll cycle in green plants. Compared to the viola-anthera-zeaxanthin cycle from plants, de-epoxidation of diadinoxanthin (Dt) to diatoxanthin (Dt) occurs at higher pH, and NPQ is directly related to Dt and independent of a proton gradient (Goss et al. 2006). Furthermore, epoxidation of Dt is significantly faster than that of zeaxanthin. However, little is known about the effectiveness of NPQ and the xanthophyll cycle in phytoplankton under natural conditions and how this is influenced by other stressors such as UVR. UVR in the upper part of the water column has been found to enhance photoinhibition in phytoplankton (Fritz et al. 2008). This is of particular importance in the Antarctic where stratospheric ozone depletion increases UVBR (280 to 315 nm) during the austral spring (Arrigo 1994). UVR-induced photoinhibition has been associated with enhanced damage to the D1 reaction centre binding protein (Larkum et al. 2001). In addition, it has been suggested that the activity of the xanthophyll cycle is affected by UVR (Van de Poll & Buma 2009, Buma et al. 2009).

In the present study we monitored dominant taxonomic groups, using pigment analysis in relation with hydrographic conditions at 3 stations in Potter Cove. These locations are differently affected by meltwater during summer (Schloss et al. 2002). Furthermore, we investigated the potential to recover from excess PAR and UVR for samples from these stations, using NPQ and xanthophyll de-epoxidation characteristics. Supporting data were obtained by studying fast and slow relaxing NPQ and xanthophyll de-epoxidation in high and low light acclimated Chaetoceros brevis and Phaeocystis antarctica cultures. We discuss whether
Photoinhibition provides an explanation for taxon dominance and the moderate phytoplankton biomass that is found in coastal waters of the SSI during summer.

MATERIALS AND METHODS

Field sampling King George Island. Phytoplankton samples were obtained between 15 January and 7 February 2009 from Potter Cove (62°14' S, 58°38' W, King George Island, SSI, Antarctica). A CTD (Seabird SBE 19 plus with a Wetlab wetstar fluorescence sensor and a Licor biospherical PAR sensor was deployed from a Zodiac to a maximum depth of 100 m. CTD data were viewed onboard on a rugged notebook (Getag) and samples were collected with a 20 l Niskin bottle during the upcast. Three stations were sampled (Fig. 1): S1, located in Maxwell Bay at the entrance to Potter Cove (depth >100 m), was sampled 16 times; whereas S2 and S3, positioned inside Potter Cove (depth ~35 m), were sampled 14 and 5 times, respectively. S3 was closest to input sites of sediment-rich water from glaciers that drain into Potter Cove. In contrast, S1 represented water entering Potter Cove after cyclonic circulation in Maxwell Bay, originating from the Bransfield Strait. All stations were sampled in the morning between 9:30 and 11:00 h. The samples were collected from the chl maximum as measured from the CTD fluorescence sensor. Samples from below the chl maximum (depth 40 to 83 m, n = 6) and from the surface (n = 3) were collected on several occasions at S1. The 20 l Niskin samples were transported in plastic bags that were kept dark and cold, and were processed within 2 h after collection for pigment composition, particulate organic carbon (POC), and NPQ experiments. An overview of the experiments is presented in Fig. 2. For pigment analysis, 2 to 10 l of seawater were filtered by vacuum (0.5 mbar) onto 47 mm GF/F filters. Filters were snap frozen in liquid nitrogen and stored at −80°C until analysis. For POC analysis, aliquots of 200 to 300 ml seawater were filtered on 13 mm combusted GF/F filters and stored at −80°C until analysis. For NPQ experiments, ~1 l of sample was used.

Hydrographic data. The attenuation coefficient for PAR ($K_d$) was calculated from linear regression on ln-transformed irradiance data, from which the 1% depth for PAR was calculated. The chl maximum depth was determined from the CTD fluorescence sensor. The depth of the UML was defined as the first depth (from the surface) where density (sigma theta units) increased by 0.05 kg m$^{-3}$.

Field experiments: NPQ and xanthophyll de-epoxidation state. NPQ capacity of field samples (36 samples in total) was determined by exposing aliquots of 400 ml in quartz cuvettes to 20 min excess PAR and PAR+UVR provided from below, in a temperature-controlled setup (1°C). Afterwards, recovery was monitored under low light (5 to 10 µmol photons m$^{-2}$ s$^{-1}$) over 10 min intervals by water PAM fluorometry on 30 ml samples (see ‘Analytical procedures’). The excess PAR and PAR+UVR treatments approximated near-surface irradiance (hereafter referred to as ‘simulated surface irradiance’; see also Van de Poll et al. 2006). Irradiance was provided by a 250 W m$^{-2}$ high-pressure halogen lamp (Philips Powertone) and 2 UVB fluorescent lamps (Philips TL 12, 20 W). A WG 305 glass filter (Schott) was filtered.
used to block unnatural UVBR (280 to 315 nm) and UVR (<280 nm) from the PAR+UVR treatment, whereas a GG 385 filter (Schott) removed UVBR and short wavelength UVAR (315 to 400 nm) from the PAR treatment. Spectral irradiance was measured with a MACAM SR9910 spectroradiometer and a spherical sensor in the cuvettes. Samples subjected to excess PAR+UVR treatment received 410 W m–2 PAR (1900 µmol photons m–2 s–1), 42.6 W m–2 UVAR, and 3.3 W m–2 UVBR. For the PAR treatment the amounts received were 383 W m–2 (1785 µmol photons m–2 s–1) PAR, 21 W m–2 UVAR, and 0.19 W m–2 UVBR.

Xanthophyll de-epoxidation state during simulated surface irradiance could not be studied in field samples due to long filtration time (>30 min). To increase biomass and reduce filtration time to <5 min, field samples were cultivated under semi-controlled conditions. Samples from the chl maximum of S1 were incubated in 4 transparent 5 L polypropylene bags in a seawater-cooled set-up (1 to 3°C) on shore. Natural radiation was reduced to 9% by 3 layers of black neutral density screen. After 12 d of incubation, 400 ml subsamples were exposed to simulated surface irradiance (PAR and PAR+UVR). After 0, 20, 60 and 120 min of exposure, samples (400 ml) were filtered onto 47 mm GF/F for pigment composition to investigate de-epoxidation of the xanthophyll cycle pigment (Dd). This experiment was repeated once. In another experiment, NPQ and the relaxation of the xanthophyll cycle pigments (epoxidation ofDt) were monitored after 20 min PAR and PAR+UVR exposure in low light for 90 min in aliquots of 400 ml. In this experiment, samples were taken from one bag that received 9% radiation and one bag that was exposed to higher radiation for 2 d (45% of incoming radiation, 1 layer of neutral density screen). There were no replicates in this experiment.

Lab experiments: NPQ and xanthophyll de-epoxidation. Chaetoceros brevis (CCMP 163) and Phaeocystis antarctica (CCMP 1871) were grown in F2-enriched autoclaved seawater at 4 (± 0.5)°C. Constant irradiance was provided by 12 fluorescent light tubes in a U-shaped set-up (Van de Poll et al. 2007) under a 12:12 h light:dark cycle. The cultures were grown under 125 and 20 µmol photons m–2 s–1 (referred to as high and low light, respectively). Irradiance was measured in air in the cultivation vessels with a QSL 100 equipped with a spherical sensor (Biospherical Instruments). The cultures were maintained in semi-continuous batch mode for at least 2 wk prior to the experiments.

Fluorescence measurements (see below) were performed during recovery in low light. NPQ characteristics were determined for low and high light acclimated cultures (100 ml) that were exposed to simulated surface irradiance (PAR, PAR+UVR) for 20 min and placed in low light (10 µmol photons m–2 s–1) for recovery. These NPQ experiments were repeated 3 times.

The de-epoxidation of the xanthophyll cycle pigment Dd during excess PAR and PAR+UVR was studied in low and high light acclimated cultures. Exponentially growing cells were transferred to quartz cuvettes and exposed to simulated surface irradiance as described for the field experiments. Dd de-epoxidation was assessed for 15 ml samples that were obtained after 20, 60, 120, and 200 min of simulated surface irradiance.
Samples were filtered through 25 mm GF/F filters (Whatman) under low vacuum, snap frozen in liquid nitrogen, and stored at −80°C until processing. Sampling took <2 min, thereby avoiding rapid changes in xanthophyll cycle pigments. The experiments were repeated 4 to 6 times for each species and light condition.

**Analytical procedures.** 

**NPQ measurements:** In the field experiments, 30 ml samples were collected from the cuvettes by a syringe and injected into the flow-through measuring cuvette of a pulse amplitude modulated fluorometer (WATER-PAM, Walz). For the lab cultures, 5 ml samples were used. Measurements were performed before and after 20 min PAR and PAR+UVR, and during recovery (90 min) in low light over 10 min intervals. Terminology of fluorescence parameters is according to Maxwell & Johnson (2000): $F'_{m}$ was determined after a saturating light pulse (0.8 s, 4000 μmol photons m$^{-2}$ s$^{-1}$) and $F'/F_m$ was calculated from the basal fluorescence ($F_0$) and $F_m$: $F_v/F_m = (F'_v × F'_0)/F_m$. A new sample was used for each measurement. The instrument settings were not changed during the experiment and filtered sea water was used to zero the instrument. A 120 ml phytoplankton sample was dark-adapted in the same set-up for 90 min to determine $F'_m$ and $F_m$, from which the maximum dark-adapted quantum yield ($F_v/F_m$) was determined. NPQ of chlorophyll fluorescence was calculated from the measurements after dark adaptation and from those during recovery after excess radiation according to Bilger & Björkman (1990): $NPQ = (F'_0 - F'_m)/F'_m$. We distinguished between slow (>40 min) and fast (0 to 40 min) relaxing NPQ components (Walters & Horton 1991). The slow component ($NPQ_s$) is assumed to represent repair of PSII damage, since slow relaxing zeaxanthin quenching is not present in the investigated algal species. The fast component ($NPQ_f$) is assumed to represent relaxation of xanthophyll cycle quenching. State transitions that also have a short relaxation time have not been reported for the investigated species. $NPQ_s$ and $NPQ_f$ were calculated according to Maxwell & Johnson (2000): $NPQ_s = (F'_0 - F'_m)/F'_m$, and $NPQ_f = (F'_m/F'_m) - (F'_0/F'_m)$. Because significant $F_0$ quenching was observed in most samples, $F'_m$ was calculated from $F_v (= F'_m - F'_0)$ of the last part of the relaxation curve, which is more accurate than $F'_m$ (Roháček 2010). Log-transformed $F'_v$ from 40 to 90 min recovery was plotted against time. $F'_v$ was extrapolated by linear regression back to the time when simulated surface irradiance exposure ended. By adding $F_0$ to this value the $F'_v$ was calculated. The $F'_m$ represented $F'_m$ that would have been measured directly after exposure if only the slow relaxing component was present. For this calculation it is assumed that $F_0$ quenching is attributed to $NPQ_f$. Total NPQ was calculated as $NPQ_t$, which equals the NPQ directly after excess irradiance: $(F'_0 - F'_m)/F'_m$.

**Pigments:** Filters were freeze dried for 48 h and extracted in 5 ml aceton (90% v/v) for 48 h in darkness at 4°C. Pigments were separated on a Waters 960 HPLC system with a C$_{18}$ 5 μm DeltaPak reverse phase column (Waters) and identified using retention time and diode array detection at 436 nm. The HPLC was calibrated against standards obtained from DHI LAB Products. The contribution of dinoflagellates, cryptophytes, chlorophytes (chlorophytes and prasinophytes were not distinguished), diatoms, haptophytes (haptophyte types 6 and 8 were pooled), and cyanobacteria was estimated using the CHEMTAX 1.95 program (Mackey et al. 1996). Using pigment ratios relative to chl a for the Southern Ocean phytoplankton, this program applies a steepest descent algorithm to estimate the contribution of taxonomic groups to the chl a concentration. The same pigment ratios were applied for samples from the chl maximum and below the chl maximum. The pigments applied in CHEMTAX were chl c$_1$, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, peridinin, alloxanthin, violaxanthin, zeaxanthin, antheraxanthin, lutein, chl a and chl b.

Xanthophyll de-epoxidation state was determined as Dt relative to the sum of Dd and Dt. The photoprotective pigment ratio was calculated as the sum of Dd and Dt relative to chl a.

**POC:** Filters were acidified for 4 h by fuming HCl (37% v/v), dried at 60°C overnight, folded in tin capsules, and measured on a nitrogen and carbon analyzer (Flash EA™ 1112, Interscience).

**Statistics:** Total NPQ, and contributions of NPQs and NPQf after PAR and PAR+UVR from different sampling stations were compared with a multi factor analysis of variance (ANOVA, Statistica 8.0). Hydrographic parameters (chl maximum depth, PAR attenuation, salinity in the upper water column) from S1 and S2 were compared using a single factor ANOVA. These parameters were also tested separately for shared sampling days of S1, S2, and S3 (n = 5). The same was done for CHEMTAX composition of taxonomic groups, photoprotective pigment ratios and NPQ characteristics after excess PAR and UVR. S1 samples from 2 depths (chl maximum and below chl maximum) were compared for 6 shared sampling days (n = 6). The maximum quantum yield of PSII from dark-adapted samples ($F_v/F_m$) was compared by a single factor ANOVA for S1, S2, and S3. For *Chaetoceros brevis* and *Phaeocystis antarctica* cultures, differences in total NPQ, contributions of NPQs and NPQf, and xanthophyll cycle pigment de-epoxidation state were tested with a multi factor ANOVA (species, PAR, PAR+UVR). Differences were considered significant at p < 0.05.
RESULTS

Hydrographic conditions, phytoplankton distribution, and photoacclimation of field samples

A strong density (calculated as sigma theta) gradient was a persistent feature in CTD profiles from Potter Cove (example Fig. 3). A shallow mixed layer (2 to 28 m) was calculated at all stations (Table 1). Chlorophyll fluorescence profiles in Potter Cove showed a distinct subsurface maximum below the estimated mixed layer. Although S1, S2, and S3 were only a few kilometers apart (Fig. 1), differences in hydrographic conditions were noted. On average, the depth of the chl maximum was significantly shallower in S2 and S3 (10.3 ± 3.2, 9.2 ± 3.9 m) than in S1 (13.9 ± 4.7 m, p = 0.011) (Table 1). The PAR attenuation coefficient for S1 and S2 was on average 0.22 and 0.3 m–1, respectively, and the chl maximum was well above the 1% PAR depth. High $K_d$ values (average 0.86 m–1) were found for S3, where the chl maximum was frequently below the 1% PAR depth. There was a significant negative correlation between near-surface salinity (salinity binned for the first 1 to 4 m) and the attenuation coefficient for PAR when comparing data from all stations (n = 35, p = 0.0008, $R^2 = 0.77$, results not shown). The chl a concentration in the chl maximum increased over time, reaching a maximum of 3.2 mg m–3 at the beginning of February (Table 1). No clear differences in average chl a concentration of the chl maximum were found between sampling locations (p = 0.07). Near the water surface, fluorescence suggested lower chl than determined by HPLC (Fig. 3). POC (mg l–1) correlated significantly (p < 0.001) with chl a concentration ($R^2 = 0.8$, n = 41, data from all stations, results not shown). The C:N ratio was on average 4.982 ± 0.436 and did not change significantly over time (data from all stations, results not shown). Differences in the photoprotective pigment ratio were noted between samples from different depths. The photoprotective pigment ratio was significantly lower in S1 samples from below the chl maximum than those from the chl maximum (p < 0.0001, Fig. 4C). The photoprotective ratio was not different in S1, S2, and S3 on shared sampling days (Fig. 4C).

Table 1. Hydrographic data (depth of the euphotic zone, 1% PAR, upper mixed layer, UML, and chl maximum, chl $Z_{max}$) and the chl a concentration in the chl maximum at sampling stations S1, S2 and S3 in Potter Cove, King George Island, Antarctica. nd = not determined

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Fig. 3. Chlorophyll fluorescence and density (sigma theta units) profile from a CTD cast in Potter Cove at Stn S1 on 19 January 2009. HPLC determined chl a (symbols) is included for 3 depths. Note the deviation between chl a and the signal from the fluorescence sensor near the surface.
Taxonomic composition based on CHEMTAX

Samples (n = 44) from the chl maximum were dominated by diatoms (Fig. 5). Microscopy revealed the presence of species from the genera *Thalassiosira*, *Chaetoceros*, *Corethron*, and *Coscinodiscus*. Diatom abundance increased over time from 66% in January to 78% in the beginning of February, whereas haptophytes (including *Phaeocystis antarctica* flagellates) decreased from 18 to 10% over the same period. This coincided with an increase in 19'-butanoyloxyfucoxanthin at the expense of 19'-hexanoyloxyfucoxanthin. Below the chl maximum (S1), haptophytes (37 ± 14%) were significantly more abundant, whereas diatoms (55 ± 11%) were lower, compared to samples from the chl maximum (p = 0.001, results not shown). Chlorophytes decreased in abundance below the chl maximum from 12 to 3%, whereas dinoflagellates and cryptophytes were present in low relative amounts (3 to 1%). Taxonomic composition in the chl maximum as calculated by CHEMTAX was not significantly different between S1, S2 and S3 on shared sampling days (p = 0.5).

Maximum quantum yield of PSII

Average dark-adapted maximum quantum yield of PSII (Fv/Fm) in the chl maximum samples decreased over time from 0.684 (±0.06) to 0.599 (±0.03). The highest values were recorded in samples from below the chl maximum from S1 (average Fv/Fm: 0.706 ± 0.09) (results not shown).

Field experiments

NPQ of field samples after simulated surface irradiance

Exposure for 20 min to PAR and PAR+UVR caused a strong reduction of the photosynthetic yield of PSII (Fig. 6) and a strong induction of NPQ. Total NPQ (on average 3.3 ± 0.7, n = 62) was significantly lower after PAR+UVR (3.2 ± 0.7) than after PAR (3.5 ± 0.7, p = 0.002). This effect was caused by stronger F0 quenching after PAR than after PAR+UVR. Total NPQ (2.9 ± 0.6) was significantly lower for S1 samples from below the chl maximum compared to S1 chl maximum samples. NPQp was always dominant in samples from S1, S2, and S3, but was significantly (p < 0.0001) lower after PAR+UVR (72.9 ± 9.2%, n = 31) than after PAR (85.8 ± 6.6%) (Fig. 4). Conversely, the slow relaxing component comprised a significantly larger part of total NPQ after PAR+UVR (27.1 ± 0.7%), compared to PAR (14.2 ± 0.7%) (Fig. 4). The contribution of NPQS was significantly higher (p < 0.0001) for S1 samples from below the chl maximum (40 to 83 m depth) (38.1 ± 11.0 and 53.4 ± 9.5% for PAR and PAR+UVR, respectively, n = 6) than that from the chl maximum of S1 and S2 on shared sampling days. No significant differences in the contribution of slow and fast NPQ components were found between S1, S2, and S3 on shared sampling days (n = 4 to 14, p = 0.4).
Bag experiments: NPQ

The bags that received 9% radiation for 12 d showed a higher contribution of NPQ$_S$ (PAR: 24%; PAR+UVR: 49%) to total NPQ than those that received 45% radiation for 2 additional days (PAR: 7.1%; PAR+UVR: 15%), when challenged by 20 min simulated surface irradiance (data not shown).

Bag experiments: xanthophyll de-epoxidation state during simulated surface irradiance

After 2 wk under 9% light, the chl $a$ concentration in the bags (46.5 ± 3.7 mg m$^{-3}$, 3 bags, 42 samples in total) allowed the investigation of the xanthophyll cycle during simulated surface irradiance. The algal assemblages showed significant Dd de-epoxidation during simulated surface irradiance (Fig. 7C). De-epoxidation of Dd continued during 120 min exposure. Significant differences between PAR and PAR+UVR were not observed with respect to Dd de-epoxidation. The algal assemblages from the bags that were exposed to 45% of ambient light for 2 d showed significantly higher de-epoxidation compared to the 9% light exposed assemblages (Fig. 8). The relaxation of the xanthophyll cycle (Dt epoxidation) in low light was completed within 40 min after simulated surface irradiance (Fig. 8). The photoprotective pigment ratio increased significantly from 0.06 ± 0.008 to 0.18 ± 0.003 after transition from 9 to 45% light, respectively (p < 0.0001) (results not shown). Violaxanthin, antheraxanthin, and zeaxanthin were detected in low amounts (18 times lower concentration than Dd and Dt) and were not further considered.

Lab experiments

Maximum quantum yield of PSII, $F_v/F_m$, was higher after low light compared to high light acclimation for both species (low light: 0.659 ± 0.002 and 0.702 ± 0.01; high light: 0.495 ± 0.004 and 0.573 ± 0.04; for Chaetoceros brevis and Phaeocystis antarctica, respectively, n = 5 to 8). $F_v/F_m$ was significantly higher in P. antarctica for both light conditions.

NPQ after simulated surface irradiance

Total NPQ after 20 min simulated surface irradiance was significantly (multi factor ANOVA p < 0.0001) higher for the diatom Chaetoceros brevis compared to the haptophyte Phaeocystis antarctica (Fig. 4). High light acclimated cultures of both species showed significantly more NPQ than low light acclimated cultures (p < 0.0001). NPQ total after PAR+UVR and PAR was not different for P. antarctica and C. brevis. Significant differences were found in slow and fast relaxing components of total NPQ due to low and high light acclimation and between Chaetoceros brevis and Phaeocystis antarctica. Low light acclimated C. brevis displayed significantly more NPQ$_F$ than low light acclimated P. antarctica (p < 0.0001). NPQ$_F$ was significantly higher in high light than in low light.
acclimated cultures of both species ($p < 0.0001$). After low light acclimation NPQ_{S} in \textit{P. antarctica} contributed on average 94.1 ± 4.0% to total NPQ, whereas this was 20.9 ± 4.2% for \textit{C. brevis}. Relative contributions of NPQ_{S} were 6.6 ± 1.9 and 13.5 ± 2.9% for \textit{C. brevis} and \textit{P. antarctica} respectively after high light acclimation. Relative contributions of NPQ_{F} and NPQ_{S} were not different after PAR+UVR and PAR for both species.

Simulated surface irradiance for 20 min caused significant Dd de-epoxidation in \textit{Chaetoceros brevis} and \textit{Phaeocystis antarctica} (Fig. 7A,B). Dd de-epoxidation characteristics in low and high light \textit{C. brevis} were comparable, whereas clear differences were observed between low and high light acclimated \textit{P. antarctica}. Low light acclimated \textit{P. antarctica} showed reduced Dd de-epoxidation in simulated surface irradiance. After 200 min PAR+UVR pigments could not be detected in
low light acclimated *P. antarctica* cultures. High light acclimated *P. antarctica* showed similar Dd de-epoxidation characteristics compared to *C. brevis*, but initial de-epoxidation was higher. UVR effects on Dd de-epoxidation were not found during 200 min simulated surface irradiance.

**DISCUSSION**

The Dd-Dt cycle was the major xanthophyll cycle in field samples as well as in *Chaetoceros brevis* and *Phaeocystis antarctica*. Because the abundance of these pigments depends on the experienced light, comparing the ratio of xanthophyll cycle pigments relative to chl a (the photoprotective pigment ratio) could provide information on phytoplankton photoacclimation. The photoprotective pigment ratios of chl maximum samples were in the range of high light (125 µmol photons m⁻² s⁻¹) acclimated *C. brevis* and *P. antarctica*. Due to strong light attenuation in these coastal waters (average *Kₜ* for S1, S2, S3 was 0.22, 0.30, and 0.86 m⁻¹, respectively) the chl maximum experienced on average 4.9, 4.6, and 0% of the incoming light for S1, S2 and S3, respectively. For S1 and S2 this corresponded with a maximum light intensity of around 140 µmol photons m⁻² s⁻¹ at noon. The stabilized water column due to the observed strong density gradient allowed phytoplankton to grow close to the water surface, where it acclimated to relatively high light, explaining the resemblance with high light acclimated cultures.

Not surprisingly, NPQₗ dominated in all chl maximum samples when exposed to excess light, comprising on average 80% of total NPQ. NPQₗ and NPQₘ of field samples were in the range of low light (20 µmol photons m⁻² s⁻¹) acclimated *Chaetoceros brevis* and high light (125 µmol photons m⁻² s⁻¹) acclimated *Phaeocystis antarctica*. Nevertheless, the maximum quantum yield (*Fₚ/Fₚₘ*) of the field samples was higher than that of high light acclimated *C. brevis* and *P. antarctica*. Thus, the chl maximum samples shared characteristics of high and low light acclimation. These differences may be attributed to the light fluctuations experienced in the field (MacIntyre et al. 1996, Van de Poll et al. 2007, Kropuenske et al. 2009), or alternatively, may be caused by species-specific differences in photoacclimation.

Although NPQₘ dominated, a significant part (on average 20%) of NPQ from the chl maximum samples was in the slowly relaxing form after 20 min simulated surface irradiance, whereas significant NPQₘ was also induced in high light acclimated *Phaeocystis antarctica* and *Chaetoceros brevis*. Excess PAR and UVR effects on photosynthesis are minimized but not entirely prevented by high light acclimation (Sobrino et al. 2005, Van de Poll et al. 2010). Relaxation of NPQₗ has been attributed to repair of damaged PSII reaction centers. In the present study, Dt epoxidation (relaxation of NPQₘ) in field samples from the chl maximum appeared to be completed in 40 min, whereas complete PSII recovery took 1 h or longer. NPQₘ increased due to UVR exposure in field samples. This is in line with the observation that UVR increases PSII damage (Hazzard et al. 1997, Bouchard et al. 2005, 2006). The applied UVR was relatively high, and particularly UVBR can be considered as the upper exposure limit for phytoplankton near the water surface. However, significant UVR effects on xanthophyll cycle de-epoxidation state and NPQ were not found over a 200 min exposure period for lab cultures, which was sufficient to induce complete photodegradation of pigments in low light acclimated *P. antarctica*. Total NPQ from the field samples was slightly reduced in response to PAR+UVR, whereas this was not found for the lab cultures. UVBR causes reduced xanthophyll cycle activity in the diatom *Phaeodactylum tricornutum* (Mewes & Richter 2002). Because NPQ total and the contribution of NPQₘ and NPQₗ of field samples and cultures responded differently to UVR, this issue remains unresolved.

We further showed differences in the capacity to deal with periodic excess PAR and UVR between *Phaeocystis antarctica* and *Chaetoceros brevis*. The diatom showed an increased capacity for NPQₗ compared to *P. antarctica*, in concert with higher xanthophyll cycle pigments. Differences between *C. brevis* and *P. antarctica* were most pronounced in low light acclimated cultures. Low light acclimated *P. antarctica* contained less xanthophyll cycle pigments per chl a than low light acclimated *C. brevis*, and showed a highly reduced capacity for NPQₗ. Comparable results were found for NPQ in *P. antarctica* and the diatom *Fragilariopsis cylindrus* (Kropuenske et al. 2009). Using a protein synthesis inhibitor, this study also showed that *P. antarctica* relied more strongly on chloroplast protein synthesis to maintain photosynthesis than the diatom *F. cylindrus* (Kropuenske et al. 2009, Mills et al. 2010). In the Ross Sea, *P. antarctica* is abundant in deeply mixed waters, whereas diatom abundance is associated with a stratified water column (Arrigo et al. 1999). Similarly, flagellates dominated over diatoms in deeply mixed stations in Admiralty Bay, King George Island (Kopczynska 1992). The observed differences in photoprotective capacity between diatoms and haptophytes may have caused the diatom dominance during the stratified late summer water column conditions in Potter Cove. Moreover, samples from below the chl maximum were enriched in haptophytes. When exposed to simulated surface irradiance, these samples induced significantly
less total NPQ, with a stronger NPQ$_{5}$ component compared to those from the chl maximum of S1 and S2. This coincided with a lower photoprotective pigment ratio for the deep samples than those from the chl maximum, comparable to that of low light acclimated _P. antarctica_.

Biomass reached 25-fold higher concentrations when cultivated in transparent plastic bags than in the shallow chl maximum. The bags received 9% of the incoming light, which was higher than the average light experienced at the chl maximum depth (4.6% for S1 and S2). Nevertheless, the algal samples from the bags showed characteristics of low light acclimation, in contrast to those from the chl maximum, possibly caused by shading from high biomass and from sediments in the set-up. Luxurious phytoplankton growth in cultured water samples from the SSI area has previously been described (Schloss et al. 2002, Helbling et al. 1995, Agusti & Duarte 2000) and demonstrates the enormous potential for phytoplankton growth of these waters in terms of available nutrients. Although we did not work in a trace metal free environment and iron was not measured in this study, iron availability is believed not to limit phytoplankton growth in the coastal waters of the SSI (Hewes et al. 2008). This was confirmed by the high maximal quantum yield of PSII ($F_{v}/F_{m}$) of the field samples, because $F_{v}/F_{m}$ is one of the first parameters that is affected by iron limitation, before growth rates start to decrease (Greene et al. 1992, van Oijen et al. 2004b).

Since there was no evidence for resource limitation apart from light, phytoplankton growth was constrained either by physical (unfavorable hydrographic conditions) or biological factors (grazing). With regard to photoinhibition, we showed that this is likely to occur near the water surface. The phytoplankton in the shallow chl maximum was dominated by diatoms, which were shown to have a high photoprotective capacity. This does not prevent photoinhibition during excess irradiance, but enhances recovery under favorable irradiance conditions and survival after prolonged excess irradiance exposure. This flexibility is obviously an advantage during frequent excess irradiance exposure. HPLC determined chl $a$ from the chl maximum and occasional surface samples were not much different, whereas the CTD fluorescence sensor indicated 5-fold lower concentrations. PAM fluorescence measurements showed that 20 min simulated surface irradiance reduced background fluorescence ($F_{b}$) on average to 56% of the maximum value. Assuming that depressed fluorescence of the CTD sensor can be attributed to NPQ, algal excess radiation effects on photosynthesis were detectable in the upper 10 m of the water column. Furthermore, direct $F_{v}/F_{m}$ measurements (no dark adaptation) on board revealed low $F_{v}/F_{m}$ (~0.2) near the surface, increasing to near maximal values (0.6) around 10 m depth (results not shown). A strong gradient in PSII fluorescence parameters in the upper part of the water column was previously reported for oligotrophic open ocean stations (Raateoja et al. 2009).

Due to the stabilized water column, wind mediated vertical mixing appeared to be confined to the upper meters during the late summer conditions in Potter Cove. Because the depth of the mixed layer affects phytoplankton photoacclimation, photoinhibition will be more pronounced in low light acclimated phytoplankton from deeper mixed layers than those observed for Potter Cove. Accordingly, it was found that UVBR effects were more pronounced in open ocean phytoplankton samples than in samples from coastal Antarctic waters (Fritz et al. 2008). However, such differences are most likely explained by a combination of factors governed by nutrient and light conditions: species composition, photoacclimation (present study), and also the size of the phytoplankton species (Key et al. 2010).

Despite the small spatial scale, considerable variation was observed in salinity, temperature, and PAR attenuation in the upper water layer between sampling stations. PAR attenuation was variable ($K_{p}$ PAR range: 0.1 to 1.2 m$^{-1}$) due to periodically high sediment concentrations, caused by glacial meltwater influx. However, significant differences were not found in photo-protective pigment ratios and NPQ between samples from S1, S2, and S3, and no significant differences in taxonomic composition were noted between stations in Potter Cove. Therefore, the variable light conditions at S3 did not cause consistent differences in phytoplankton photoacclimation. Furthermore, the residence time under low irradiance conditions at S3 was not enough to create regional differences in dominant taxonomic groups. The elevated temperature during the past decades has increased meltwater influx and sediment loads in the West Antarctic Peninsula area and also in Potter Cove (Schloss et al. 2008, Schofield et al. 2010). In this respect, and in agreement with previous work, our research shows that 2 effects can be distinguished: stratification of the water column, which potentially enhances phytoplankton growth, and locally high sediment loads that reduce phytoplankton growth due to strong light attenuation (S3). The hydrographic conditions in S1 and S2 favored phytoplankton growth, although this was probably also moderated by the influx of sediment and by photoinhibition near the surface. Currents continuously transport phytoplankton from Potter Cove into the Bransfield Strait, where suspended sediments are lower and the density gradient is diluted. How far the stabilizing influence of fresh water from the SSI extends into Bransfield Strait in
unknown. Assessment of this effect would require collection of phytoplankton and hydrographic data on a broader spatial and temporal scale.

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