

## Diadinoxanthin cycle of the bottom ice algal community during spring in McMurdo Sound, Antarctica

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**Abstract** Using the ice algal community growing at the bottom of the annual sea ice in McMurdo Sound Antarctica, the response of the photoprotective diadinoxanthin (DD)-cycle to exposure to light was investigated. Changes in pigment concentration were detected using high-performance liquid chromatography. A light mixing simulator (LMS) was developed and used to simulate the pigment response to mixing in the upper water column. No DD-cycle was detected under the sea ice under natural light conditions. The DD-cycle was activated after exposure to surface natural light conditions and artificial light conditions. The first-order kinetic rates of the DD-cycle under constant artificial irradiance, natural irradiance and simulations with the LMS were found to be similar to other studies suggesting that ice algae do not vary the rate of deepoxidation depending on light history. Simulations under natural light using the LMS demonstrated that the response of the DD-cycle to static incubations and when subject to vertical mixing was not similar, and that static incubations overestimate DD-cycle activity over the long term. Algae in a simulated vertically mixed environment were able to increase the pool of xanthophyll pigments compared to static conditions where the pool remained the same or decreased. The recovery of DD in the dark or under low light was found to be significantly faster than in temperate algal communities. These results suggest that ice algae at the sea ice bottom can activate the photoprotective DD-cycle to regulate excess thermal energy. Unlike temperate species of diatoms, ice algae can rapidly reconstruct

the pigment pool under low light or in the dark and is likely a particular adaptation to the unique light environment in Antarctica.

**Keywords** Ice algae · Photoprotection · Diadinoxanthin cycle · Light-mixing simulator

### Introduction

Each autumn, the surface of the ocean surrounding the Antarctic continent begins to freeze, forming a layer of sea ice up to 2–3 m thick. As the sea ice forms and ages, distinct micro-habitats are formed in which algal communities flourish (Arrigo and Thomas 2004). The greatest proportion of algae grow in the bottom portion (~20 cm) of the sea ice comprising a shade adapted community composed primarily of diatoms. Although there is strong attenuation of light by overlying ice and snow, there is usually sufficient light for net photosynthesis during austral spring and summer (Arrigo et al. 1991, 2003). Algal biomass can exceed 200 mg Chl *a* m<sup>-2</sup>, potentially contributing up to 50% of the net annual primary productivity of the ice covered areas of the Southern Ocean (Legendre et al. 1992). At the bottom of the sea ice, these shade adapted algal communities are subject to low irradiance of around 0.1% to 0.5% of surface irradiance and rarely experience light intensities sufficient to damage the organisms photosystems (McMinn et al. 1999). Nevertheless, with the melting and thinning of the sea ice during the austral spring and summer, this community can be exposed to significant increases in irradiance. Once released into the water column, the algae are subject to highly variable light conditions as a result of upwelling and mixing of the water column.

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Of current concern is the impact of projected climate-mediated alterations as widely predicted by modellers on the extent and thickness of annual sea ice (Sarmiento et al. 1998; Bopp et al. 2001). A reduction in the thickness of annual sea ice and an earlier seasonal thinning of the sea ice would alter the light regime experienced by the bottom community of sea ice algae. Despite potentially stressful high irradiance which can damage the photosynthetic systems, phytoplankton biomass within the Ross Sea has been shown to respond rapidly to stratification of the water column and increasing surface solar radiation found in austral spring (Smith et al. 2000). For phytoplankton to survive and flourish, the cells must not only be able to collect light as efficiently as possible, but under high-irradiance conditions there must be some mechanism to prevent photo-oxidative damage to the photosynthetic apparatus.

One of the most well studied responses of diatoms to high irradiances is the diadinoxanthin (DD)-cycle in which the deepoxidation of the xanthophyll carotenoid pigment DD to diatoxanthin (DT) dissipates excess light energy into heat to protect the photosynthetic machinery of photosystem II (PSII) (Arsalane et al. 1994; Casper-Lindley and Bjorkman 1998). The DD-cycle has been shown to be activated with increasing irradiance resulting in the rapid interconversion of DD to DT (seconds to minutes) (Oliazola and Yamamoto 1994). Under sustained or intermittent high irradiance, there is a gradual increase in the pool of DD-cycle pigments (minutes–hours) (Oliazola and Yamamoto 1994; Oliazola et al. 1994; Fujiki and Taguchi 2001; Lavaud et al. 2002a). Under dark or very low-light conditions, the reverse interconversion of the pigments occurs with the epoxidation of DT to DD.

To assess the role of the DD-cycle as a photoprotective mechanism in sea ice algae, the algae growing at the bottom of the annual sea ice at Cape Royds in the McMurdo Sound area of the Ross Sea, Antarctica was studied during austral spring 2005. This community is dominated by the diatoms *Entomoneis kjellmanii*, *Berkeleya adeliense* and *Nitzschia stellata* (McMinn and Ashworth 1998; Ryan et al. 2002). Previous studies on sea ice algae in the Ross Sea area have examined the production of Mycosporine-like amino acids (MAAs) in sea ice algae in response to UVB radiation (Ryan et al. 2002), the growth and productivity of sea ice algae under PAR and UV irradiances (McMinn et al. 1999) and the photophysiology of surface pond communities in McMurdo Sound (Robinson et al. 1997).

The aim of this study was twofold. Firstly, to examine the response of the pigments of the DD-cycle of the bottom community of Antarctic sea ice algae to different irradiance conditions and secondly, to determine the rate at which these pigments change under various light regimes.

Assessing the ability of this shade adapted community to adapt to changing light fields is fundamental to improving our understanding of current sea ice biology and for assessing how sea ice algae might respond to climate warming.

## Materials and methods

### Field sampling site

A series of incubation experiments were conducted in the field on first year fast ice at Cape Royds, McMurdo Sound, Antarctica (77°33.80'S, 166°12.23'E) on 27 October, 31 October and 11 November 2005. Sampling was conducted in an area with an ice thickness of 1.8–2 m and surface conditions clear of snow.

### Collection of algal samples

A powered Kovaks ice corer (130 mm diameter core) was used to core 1.5 m through the ice sheet. The last 0.5 m cored with a manual ice auger (75 mm diameter core) to retain as much as possible of the bottom community. On each sampling day, three cores (three replicates) were sampled every 30 min. The cores were withdrawn through a black plastic bag and wrapped in black plastic to minimize any additional light mediated pigment changes. Within less than 5 min, the bottom part of the core was sliced and 15 ml filtered onto Whatman GF/F filter paper (27 mm) using a filtration system adapted from Jeffrey et al. (1997). 300  $\mu$ M dithiothreitol (DTT) was added to each 15 ml of sample prior to filtering to stop xanthophyll cycling (Kashino and Kudoh 2003). Duplicate samples which had not been treated with DTT were also collected. Each filter paper was then folded, placed in a 1.5 ml cryotube, frozen in liquid nitrogen ( $-196^{\circ}\text{C}$ ) and stored until pigment analysis. All these procedures were conducted under very low light. At the end of each sampling day, three additional cores were drilled and retained for laboratory incubation experiments. The cores were placed in black plastic bags, sealed and transported to Scott Base.

### Algal identification

A 20 ml sample of each core section was preserved with 1% acidified Lugol's solution and returned to New Zealand for enumeration and identification of phytoplankton species. Samples were settled in a sedimentation chamber for at least 24 h before counting a minimum of 200 cells using a Nikon inverted microscope. Identifications were made using the methods described in Chang and Gall 1998.

## Surface incubations of sea ice algae

At the beginning of each field sampling day, the sea ice algae attached to the bottom of the first ice core was retained for experiments on the ice surface to simulate the influence of the higher natural irradiance at the surface on the xanthophyll pigments. The bottom 20 cm of each ice core was shaved into small ice pieces under very low light and diluted to 1.5 l (dilution factor  $> 5\times$ ) to prevent salinity shock with filtered seawater from the Scott Base wet laboratory. The samples were melted in the dark for 1 h to form an ice slurry between  $0^\circ$  and  $-1.5^\circ\text{C}$ . The samples were placed in 2 l beakers in an aluminium frame with circulating sea water around the beakers keeping temperatures close to the natural sea water temperature of  $-1.8^\circ\text{C}$ . Every 30 min, three replicate samples of 15 ml treated with  $300\ \mu\text{M}$  DTT and three replicate samples without DTT were filtered under low light and stored in liquid nitrogen at  $-196^\circ\text{C}$ .

## Irradiance measurements

Photosynthetically Active Radiation (PAR, 400–700 nm wavelengths) below the sea ice and within the sea ice algae bottom community was measured with a Li-Cor LI-192SA underwater quantum sensor (calibrated in January 2005) attached to a Li-Cor LI-1000 data logger. Prior to the commencement of sampling on each day, three separate ice cores were drilled with the manual ice auger in the centre of the sampling area. The underwater quantum sensor was embedded into the bottom of the core and the core then placed back in the hole with an ice screw placed in the top of the core to allow later retrieval. Two additional submersible Odyssey photosynthetic irradiance loggers were placed in a similar fashion in the remaining two ice holes. Surface PAR measurements were made with three Odyssey photosynthetic irradiance loggers placed in the frame holding the surface sea ice algal samples. Additional PAR data (400–600 nm) was obtained from the National Science Foundation (NSF) UVR monitoring program at McMurdo Sound ( $77^\circ50'\text{S}$ ,  $166^\circ40'\text{E}$ ). The NSF monitoring program utilizes a Biospherical Instruments scanning SUV-100 spectroradiometer (280–600 nm) calibrated fortnightly with irradiance standard lamps.

## Laboratory incubations

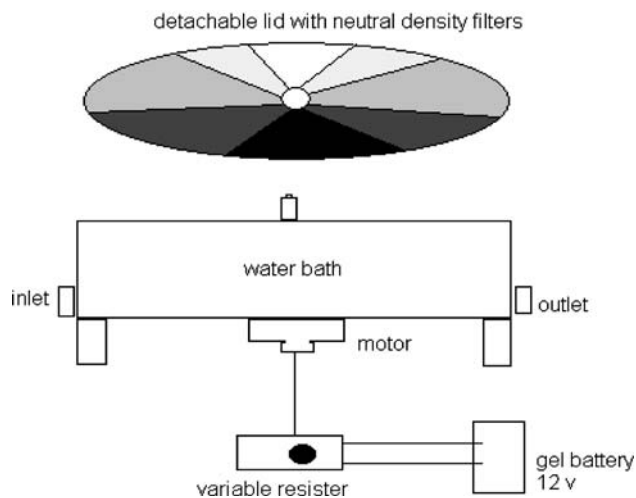
The sea ice algal samples collected at Cape Royds were stored and kept frozen in black plastic bags in a circulating water bath ( $-1.9^\circ\text{C}$ ) outside the wet laboratory at Scott Base for no longer than 12 h. The bottom 20 cm of each ice core was sliced off and then thinly sliced into 2 l open topped beakers under low light ( $<2\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ )

containing 1.5 l of filtered sea water and allowed to melt in the dark prior to the start of the experiments. The diluted algal samples were transferred to 2 l wide-bottom glass beakers and placed in a circulating sea water bath ( $-1.8^\circ\text{C}$ ) and stirred at regular intervals. Three separate incubation experiments were conducted under artificial light provided by two FS20 lamps (General Electric, Schenectady, NY, USA). The output of the artificial light source was measured with a LiCor Li-1800UW spectroradiometer. The open top flasks were then screened with Lee neutral density filters to provide three irradiance treatments of 50, 100, and  $200\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ . Sub samples were removed at various time intervals, one set of samples were treated with  $300\ \mu\text{M l}^{-1}$  dithiothreitol (DTT) and one set were left untreated. All the samples were then filtered under very low light on to Whatman GF/F filter paper (27 mm) and stored in liquid nitrogen ( $-196^\circ\text{C}$ ) until analysis.

To confirm the effectiveness of DTT on stopping the deepoxidation activity of the DD cycle in Antarctic sea ice algae, three samples were incubated in the dark for 6 h and then exposed to  $200\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$  for 30 min. Subsamples were then treated to an exposure of 0, 100, 300 and  $500\ \mu\text{M l}^{-1}$  of DTT (final concentration). On exposure to light, the relative amount of DT to Chl *a* increased significantly in the first 30 min without exposure to DTT. In algae exposed to DTT concentrations greater than  $300\ \mu\text{M l}^{-1}$  there was no change in the amount of DT demonstrating the effectiveness of DTT in completely stopping deepoxidase activity. These results are consistent with those reported by Kudoh et al. (2003) who have demonstrated that  $300\ \mu\text{M l}^{-1}$  of DTT was effective in completely stopping deepoxidase activity in ice algae from northern Japan. The results reported in this paper are for samples treated with DTT.

## Simulation of light and mixing

A simulator which we have termed a light-mixing simulator (LMS) was developed to conduct field experiments. The LMS is constructed of a sealed circular aluminium water bath with an inner diameter of 62 cm and a depth of 12 cm mounted on 6 cm legs (Fig. 1). A removable rotating transparent lid made of 'whiteglass' is screwed into a spindle in the centre of the water bath. The transparent lid can be fitted with up to 11 pie shaped sections of light filters to simulate various light regimes. The LMS can be used with natural daylight or artificial light sources. The spindle is rotated by an electric motor attached to a variable resistor and powered by a 12 V gel battery. The speed of rotation can be varied from 10 s to 60 min. The simulator is light and easily handled and can be used in the laboratory, on-board ship, land and in polar conditions. An optionally attached modified marine bilge pump allows



**Fig. 1** Diagram of the light-mixing simulator (LMS) showing the water bath in which samples can be placed. The detachable rotating lid with attached neutral density filters (up to 11) is screwed into a central spindle attached to a 12 V electric motor and powered by a gel battery. The variable resistor controls the rotation speed of the lightweight lid

water to be continuously fed into the water bath maintaining a constant water temperature. In polar conditions, an attached pump action bottle can be used to feed small amounts of ethylene glycol into the water bath to prevent freezing. The outlet for the water bath can be attached to waste bottles when glycol is used. Sample beakers or bottles up to 2 l each containing algal samples can be placed in the water bath. It is recommended that at least three samples for each experiment be placed in opposite positions within the water bath.

#### Simulation of light attenuation

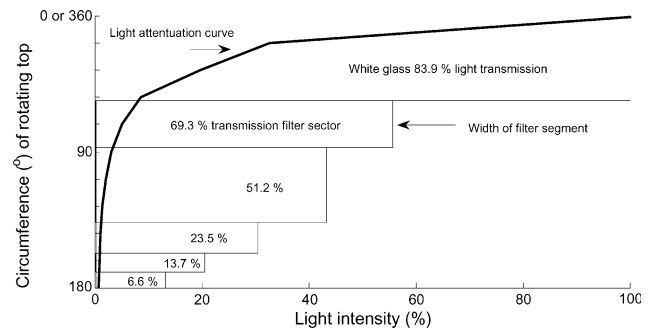
The LMS is designed to simulate light attenuation in a hypothetical euphotic zone using a set of different neutral density filters which can be attached to the rotating lid. The neutral density filters (Lee Filters, Burbank, CA, USA) can be arranged in decreasing transparency to simulate the exponential decrease of irradiance with depth. To simulate the natural environment, the light attenuation coefficient and mean water column irradiance needs to be determined. The total light attenuation coefficient of the water column  $k$  ( $m^{-1}$ ) can be computed by:

$$k = (\ln I_o - \ln I_z) / z \quad (1)$$

and the mean water column irradiance  $I_x$  can be determined from

$$I_x = I_o [1 - \exp(-kz)] / kz \quad (2)$$

where  $z$  is the water column depth (m),  $I_o$  is surface irradiance, and  $I_z$  is irradiance at depth  $z$  (Mallin and Paerl 1992).



**Fig. 2** Matching of different neutral density filters to a simulated light attenuation curve. In this example the light coefficient  $k = 0.048 m^{-1}$  based on a surface irradiance of 1,020 and  $8.40 \mu mol photons m^{-2} s^{-1}$  at 100 m depth. Based on concept of Glocke and Lenz 2004. The kinks in the curve represent the thin tubes on the lid which retain the filters

The width and % transmission of the filter segments can be matched to the calculated light curve based on the appropriate total light attenuation coefficient to approximate the natural irradiance regime (Fig. 2). The filters can be arranged in a circle forming pie shaped sectors of varying width and light transmission. The lid rotating from  $0^\circ$  to  $180^\circ$  will expose a sample to a light field simulating the passage of a phytoplankton cell from the surface to the designated light level at depth and back up to the surface as the lid rotates back to  $360^\circ$ . The filter segments approximate the water column light curve with a phytoplankton cell receiving an average irradiance for the entire circle depending on the arrangement of neutral density filters.

The rotation of the top was designed to approximate the variable light regime experienced by an algal cell during a full vertical cycle through the euphotic zone, with a variable rotation speed to simulate different mixing rates. The rotation speed is infinitely variable between 10 s and 60 min for a full rotation. At the highest rotation speed of 10 s, an algal cell would be transported up and down at an average speed of  $19.66 cm s^{-1}$ , while at the slowest rotation speed of 60 min the circulation speed is  $0.05 cm s^{-1}$ .

#### Field experiments

Field experiments were conducted with the LMS outside the wet laboratory at Scott Base, McMurdo Sound Antarctica during austral spring 2005. Two submersible Odyssey photosynthetic irradiance loggers were placed opposite each other in the water bath to measure the incident and mean Photosynthetically Available Radiation (PAR, 400–700 nm wavelengths) received by the samples under the rotating lid. A Li-Cor LI-192SA underwater quantum sensor measured the PAR irradiance outside of the LMS. Seawater ( $-1.8^\circ C$ ) was circulated through the water bath from the seawater intake of the wet laboratory at

Scott base to maintain a constant temperature. Sea ice algal samples previously collected (within 24 h) from Cape Royds were dark incubated for 12 h prior to the start of experiments. Two groups of three open topped beakers containing the samples were placed opposite each other at 0° (360°) and 180° in the water bath. As sea ice covered the Ross Sea during the study period, it was not possible to calculate the light attenuation through the water column. Instead a light attenuation  $k$  ( $\text{m}^{-1}$ ) of  $0.048 \text{ m}^{-1}$  calculated from Southern Ocean water (G. Griffith, unpublished data) was used to determine the water-column irradiance curve. Neutral density filters with a light transmission of 69.3, 51.2, 23.5, 13.7, and 6.6% were matched to the light curve and fitted to the rotating top of the LMS. During one rotation, the sea-ice algal samples were circulated through a light regime analogous to a passage in the water column from 69.3% of incident irradiance at the surface to a 6.6% light level and back to the surface again.

#### Sample preparation and high-performance liquid chromatography (HPLC)

All the samples were filtered immediately on to Whatman GF/F filter paper (27 mm) stored in liquid nitrogen at  $-196^\circ\text{C}$ , returned to New Zealand stored in dry ice ( $-78.5^\circ\text{C}$ ) and re-stored in liquid nitrogen at  $-196^\circ\text{C}$  prior to analysis. Filtering was conducted in the dark and took less than 1 min to minimize any additional light mediated deepoxidation of DD to DT. The pigment concentrations of DD and DT were determined using high-performance liquid chromatography (HPLC). To extract the pigments, the filters were cut into small pieces and placed in 2.5 ml microcentrifuge tubes with 100% methanol. The samples were placed in an ice bath and sonicated in the dark for 15 min. The samples were then placed in the dark and extracted at  $-20^\circ\text{C}$  for 24 h. After extraction, the samples were centrifuged, filtered through a  $0.45 \mu\text{m}$  PTFE filter and analysed using a Shimadzu LC-10AD with a SCL-10aVP system controller. Pigments were separated with a Phenomenex Onyx Monolithic C8 column  $100 \times 4.6 \text{ mm}$ . Just prior to the HPLC run an ion pairing (IP) solution (1.0 M ammonium acetate) was added to each sample vial in a ratio of three part extract: one part ammonium acetate to improve peak resolution. The pigments were separated using an analytical gradient protocol specifically developed for chlorophyll and carotenoid pigments with methanol: acetonitrile: aqueous pyridine (50:25:25 v: v: v) as Solvent A and acetonitrile: acetone (80:20 v: v) as solvent B at a flow rate of  $0.8 \text{ ml min}^{-1}$  over 50 min (Zapata et al. 2000). The separated pigments were detected at 436 nm and identified using Shimadzu Class-VP version 5.032 software. Each sample was analysed three times and the resulting concentrations averaged. Standard pigments of DD and DT were purchased from DHI Water

and Environment in Denmark and the concentration of each pigment was determined against these standards. Chlorophyll *a* was determined fluorometrically using a Turner Designs Trilogy laboratory fluorometer.

## Results

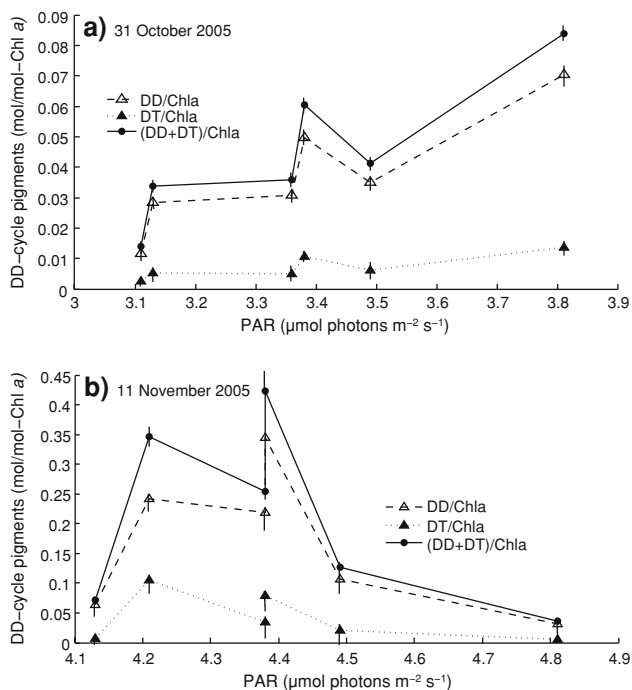
#### Response of pigments to natural under ice and surface irradiance

The DD-cycle of the bottom community of ice algae was not active with the low natural under ice irradiance experienced during the day at Cape Royds (Fig. 3). Daily average PAR in the bottom community was  $3.38 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  on 31 October and  $4.4 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  on 11 November representing an attenuation of light through 1.9 m of fast ice of up to 99.7% of surface irradiance. The ratio of DT/Chl *a* from samples collected within the sea ice remained relatively constant over the range of ambient under ice irradiance experienced on both sampling days. The DD/Chl *a* ratio increased with increasing irradiance on 31 October, however, this trend is not evident with the higher under ice irradiances experienced on 11 November. However, by 11 November the cellular content of the photoprotective carotenoids to Chl *a* of the bottom community had increased substantially. The DD pigment pool evaluated by the relative amount of (DD + DT) to Chl *a* averaged 0.043 in the bottom community in October and had increased to 0.2 by early November indicating a three to fourfold increase of the pigment pool.

Samples of the bottom community, incubated on the surface showed an exponential increase in the DT/Chl *a* ratio and an exponential decrease in DD/Chl *a* ratio with increasing PAR consistent with DD-cycle activity (Fig. 4) The pigment pool (DD + DT)/Chl *a* remained relatively constant with daily increasing irradiance. On both 31 October and 11 November the initial exposure of ice algae to surface irradiance of between 110 and  $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  resulted in a substantial drop in the DD/Chl *a* ratio (Fig. 4a, b).

#### Response of pigments to artificial irradiance

When the ice algal cells which had been dark adapted for 6 h were exposed to three different artificial light regimes ( $50, 100, 200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) the DD-cycle pigments showed dynamic changes within 10 min on exposure to the artificial light source (Fig. 5). The relative amounts of DT/Chl *a* increased and DD/Chl *a* decreased rapidly during the first 10 min for cells exposed to 50 and  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . At a higher irradiance of

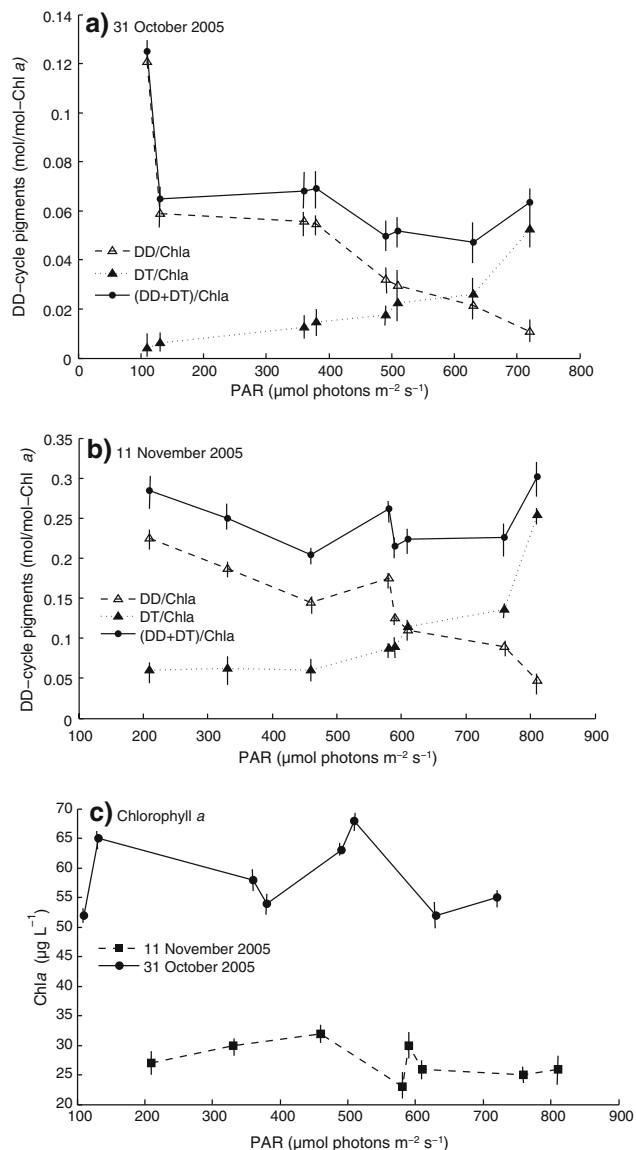


**Fig. 3** Change in DD-cycle pigments on **a** 31 October 9AM-2PM NZDT; **b** 11 November 9AM-2PM NZDT in the bottom community of sea ice algae as a function of increasing under ice irradiance. Mean values with standard error of the mean ( $n = 3$ )

200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  DT/Chl *a* increased gradually over 60 min, while DD/Chl *a* decreased rapidly in the first 10 min. The relative amount of DT/Chl *a* increased substantially with higher levels of illumination with an increase of 119% at 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and 322% at 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The total pigment pool (DD + DT)/Chl *a* decreased by nearly 50% over the incubation period.

When the algal suspensions from the 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  incubation were placed in the dark, DT/Chl *a* decreased and DD/Chl *a* increased (Fig. 6). Over the next 60 min in the dark, the pigment pool doubled, reaching values close to those observed at the start of the light incubation.

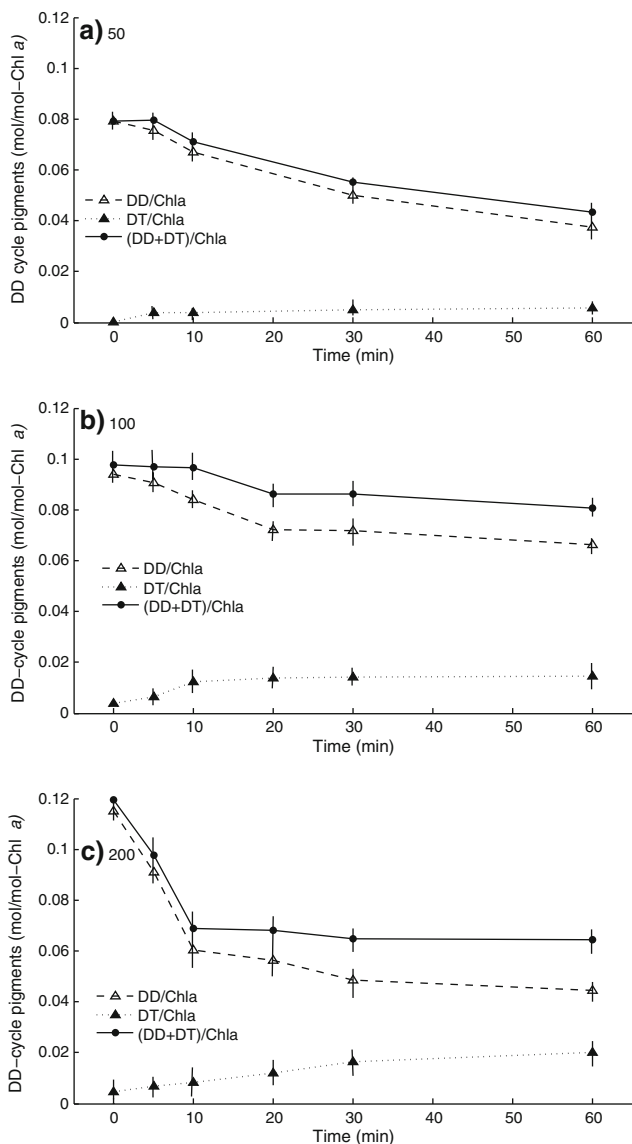
When samples from the bottom community of sea ice algae at Cape Royds are exposed to long term static incubations of 12 h under various artificial light regimes the diadinoxanthin pigment pool (DD + DT/Chl *a*) responds differently depending on the irradiance intensity (Fig. 7). Under a constant 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  there was no statistically significant decrease in the pigment pool. Under 12 h of constant exposure to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , the DD pigment pool declined gradually for the first 4 h and the remained relatively constant. The pool of DD-cycle pigments is significantly reduced during the first 2 h of exposure to a constant 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , with no subsequent loss of pool pigments.



**Fig. 4** Change in DD-cycle pigments on **a** 31 October 9AM-2PM NZDT; **b** 11 November 9AM-2PM NZDT in the surface incubated samples of sea ice algae as a function of increasing surface irradiance and **c** Change in Chl *a*. Mean values with standard error of the mean ( $n = 3$ )

#### First-order kinetics

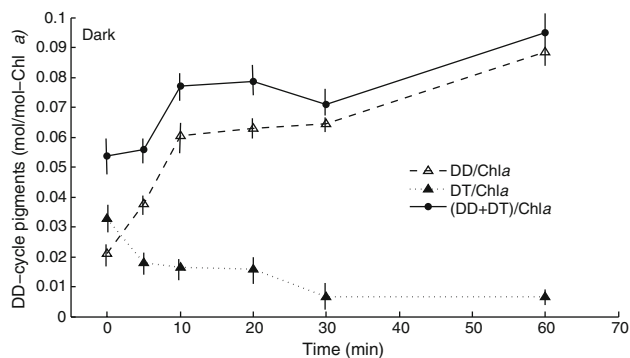
The time-course studies of the changes in the pigments shown in Figs. 4, 5 and 6 suggest that the changes in the pigments can be approximated by first-order kinetics as a function of changes in light intensity, under conditions of constant artificial irradiance and natural daytime irradiance and for the recovery of diadinoxanthin in the dark. The first order kinetic model of Oliazola and Yamamoto (1994) can be applied to determine the approximate first order rate constant  $k$  for the pigment changes:



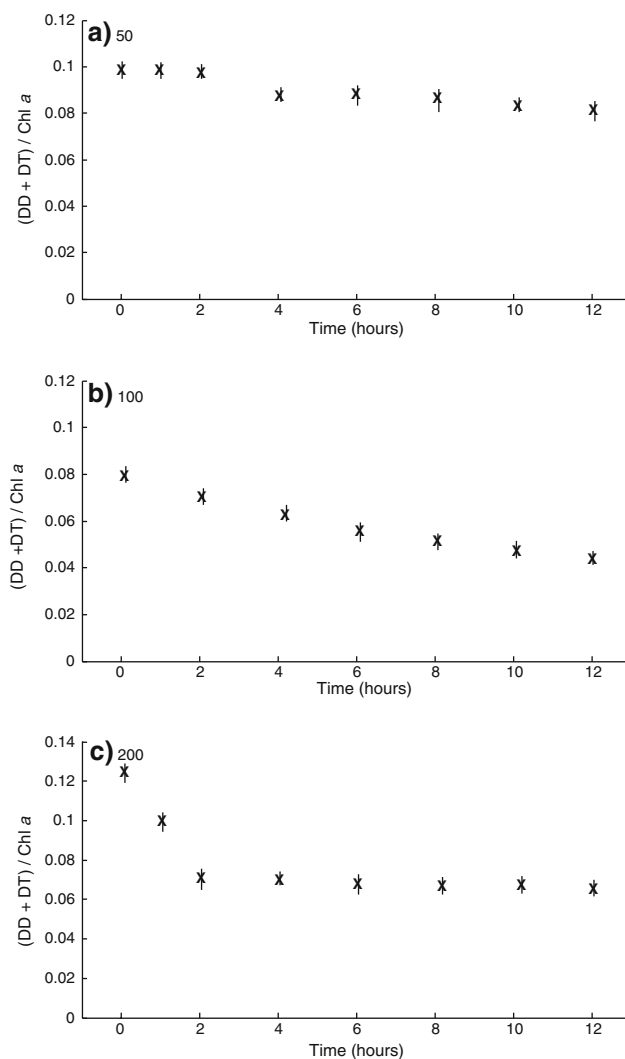
**Fig. 5** Changes in DD-cycle pigments after exposure to **a** 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , **b** 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and **c** 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  of artificial irradiance. Mean values with standard error of the mean ( $n = 3$ )

$$-kt = \ln[(P_t - P_\infty)/(P_0 - P_\infty)] \quad (3)$$

where,  $P_0$  was the initial value of the pigment and  $P_t$  was the relative amount of the pigment at time  $t$ .  $P_\infty$  was assumed to be the time at which the pigment changes became near constant (zero-order kinetics). Clearly, the  $P_\infty$  value cannot be accurately determined as it does not reach an asymptotic value. This is a shortcoming of the application of first-order kinetics to photadaptive processes (Cullen and Lewis 1988). Nevertheless, the results of the time-course experiments are in close agreement with Eq. 3, suggesting that the pigment changes may follow first-order kinetics. The other point worth noting is that the pigment



**Fig. 6** Changes in the relative amounts of the DD-cycle pigments when placed in the dark after 60 min of artificial irradiance of 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Mean values with standard error of the mean ( $n = 3$ )



**Fig. 7** Changes in the pool of DD-cycle pigments under artificial irradiance of **a** 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , **b** 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and **c** 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Each sample point is the average of three replicates. Mean values with standard error of the mean ( $n = 3$ )

**Table 1** First-order rate constants  $k$  ( $\text{min}^{-1}$ ) of deepoxidation and epoxidation of the relative pigment changes under constant artificial irradiance (Treatments 1–3) and increasing daytime irradiance in the

field at Cape Royds for 31 October (Treatment 5) and 11 November (Treatment 6) Treatment 4 refers to the rate constant in the dark after samples had been exposed to light during treatment 3

Experiment	PAR ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ )	DD/Chl $a \rightarrow$ DT/Chl $a$	DT/Chl $a \rightarrow$ DD/Chl $a$
Treatment 1	50	1.07	–
Treatment 2	100	1.6	–
Treatment 3	200	1.3	–
Treatment 4	Dark	–	0.87
Treatment 5	110–730	0.43	–
Treatment 6	200–800	0.21	–

Mean values with standard deviation ( $n = 3$ )

change follows two different first-order kinetic changes with an initial pigment change on a time scale of minutes and the longer term pigment change on a time scale of minutes to hours. As this initial pigment change is very rapid and is defined by only a few data points, the first-order kinetic rate constant is calculated on the basis of the entire duration of the experiment. Here, it is assumed that this will be more reliable and indicative of the sea-ice algal community as a whole.

The first-order rate constants for the deepoxidation of DD/Chl  $a$  to DT/Chl  $a$  varied between 1.07 and 1.6  $\text{min}^{-1}$  for the ice algal samples exposed to constant artificial irradiance of 50, 100 and 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Table 1). The rate constant  $k$  did not increase with increasing irradiance with the fastest rate constant (1.6  $\text{min}^{-1}$ ) for samples exposed to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . For the ice algal samples exposed to increasing daytime irradiance on the surface at Cape Royds (Treatments 5 and 6), the first-order rate constants were

slower than those calculated under constant artificial irradiance (Treatments 1, 2, and 3) varying between 0.21 and 0.43  $\text{min}^{-1}$ . For the three samples exposed to artificial irradiance of 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and then placed in the dark, the rate constant for the relative epoxidation of DT to DD in the dark was 0.87  $\text{min}^{-1}$ .

#### Response of DD cycle to short-term constant artificial irradiance and simulations with the LMS

Table 2 shows the results from a series of six experiments comparing the short-term (minutes) change in the relative amount of DD/Chl  $a$  and DT/Chl  $a$  of samples of the bottom community of sea ice algae exposed to constant artificial irradiance 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and simulations under natural irradiance with the LMS. The filters were arranged on the LMS to match the simulated light attenuation curve (Fig. 2) so that a samples moving from 0° to 180° would experience a light field corresponding to the

**Table 2** Comparison of the change in the relative amount of the pigments of the DD cycle exposed to 20 min of constant artificial irradiance (Static) and natural irradiance with the light-mixing simulator (LMS)

Date	Method	DD/Chl $a$	% Diff DD/Chl $a$	DT/Chl $a$	% Diff DT/Chl $a$	Sig.
28 October	Static	– 0.073	27	0.0163	43	**
	LMS	– 0.02		0.007		
29 October	Static	– 0.021	47	0.014	50	**
	LMS	– 0.01		0.007		
1 November	Static	– 0.083	15	0.019	26	NS
	LMS	– 0.013		0.005		
2 November	Static	– 0.234	47	0.0237	65	**
	LMS	– 0.126		0.0155		
13 November	Static	– 0.047	77	0.017	29	**
	LMS	– 0.035		0.005		
14 November	Static	– 0.054	22	0.011	36	**
	LMS	– 0.012		0.004		

NS not significant

% Difference in DD/Chl  $a$  and DT/Chl  $a$  is shown as the proportion of the static results (LMS/Static)\* Statistical relationships from Student's  $t$  test are significant at \*\*  $P < 0.01$ . Ratios are the mean of three replicates within 1 SE of the mean

algal cells moving from the surface to a light depth of 6.6% and from there back up to the surface again. At the simulated surface the cells receive 69.3% of the surface irradiance. The rotation speed of the LMS was set to an average speed of  $0.05 \text{ cm s}^{-1}$  which is within the estimated vertical velocity rates recorded during austral summer in the open waters of the Antarctic Peninsula (Moline 1998).

The light regime characteristics were similar for both the static and LMS incubations with the static samples exposed to a mean irradiance of  $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and the LMS samples received mean irradiance varying between 190 and  $265 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of the surface irradiance during the duration of the experiment. Samples for the LMS experiments were initially placed under the 6.6% filter of surface irradiance at the start of each experiment. Although the mean irradiance was similar for both methods, the LMS simulated samples resulted in a smaller reduction in the relative amounts of DD and a smaller increase in the relative amounts of DT. The static samples incubated for 20 min under the constant artificial irradiance yielded between 26 and 65% higher relative amounts of DT/Chl *a* and 15–77% less relative amounts of DD/Chl *a* (Table 2). The Student's *t* test was used to compare the results between the two methods with the difference in relationships statistically significant ( $P < 0.01$ ) in five out of the six experiments. These observations suggest that static incubations stimulate higher DD-cycle activity relative to the variable light regime of the LMS.

Varying the rotation speed of the LMS with the same set and width of neutral filters does not alter the mean irradiance that the samples are exposed to, but will result in greater simulated turbulent motion or a deepening of the euphotic zone (Table 3). If sea ice algal cells are moved up and down in a euphotic zone at a faster velocity defined in this case by the 6.6% light depth, the first-order kinetic rate ( $k \text{ min}^{-1}$ ) is higher for the initial increase in the relative amount of DT and initial decrease in DD. The changes in the first order kinetic rate shown in Table 3 indicate that the first order

kinetic rate changes for the pigments are independent of the rotation speed of the LMS. This suggests that under the different rates of mixing in the surface mixed layer, the first order kinetic rate of the DD-cycle in sea ice algae is the same and does not alter when the cells are transported at a faster rate up and down in the euphotic zone.

Figure 8 shows one of the three LMS simulations of the changes in the DD pigment pool using the same experimental protocol of Table 2. The samples in the LMS received 26% of the incident PAR. The changes in the DD-cycle pigment pool followed the changes in incident PAR, with the pool decreasing as incident PAR increased and increasing as incident PAR decreased. This trend was evident in all LMS simulations. The reconstruction of the pigment pool occurs when incident PAR is below  $230 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . This reconstruction at lower light intensities was not evident in the static incubations under artificial irradiance shown in Fig. 7.

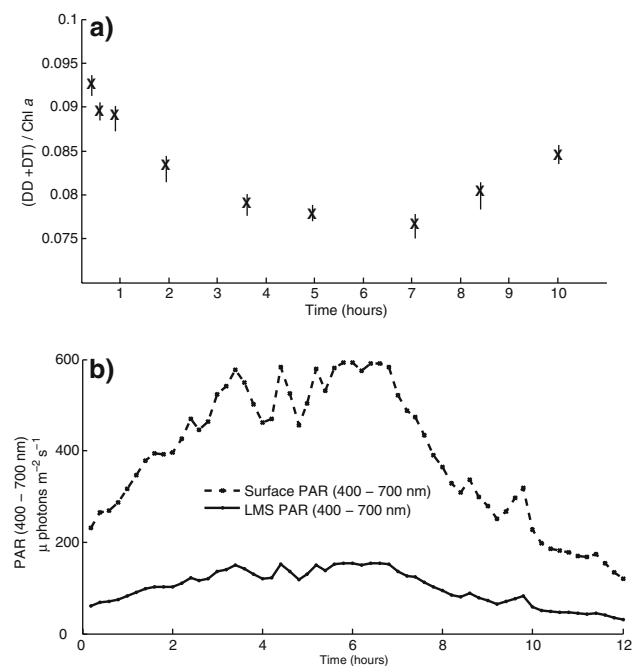
## Discussion

Under the 1.7–1.9 m of annual sea ice, during austral spring the bottom infiltration algal communities at Cape Royds were subject to low irradiance conditions of between 3 and  $5 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . This is similar to the low irradiance fields reported further south at Cape Evans over the same period as this study (McMinn et al. 1999). Under these

**Table 3** First-order kinetic coefficients ( $k$ ,  $\text{min}^{-1}$ ) for DT/Chl *a* and DD/Chl *a* for the simulated variations in turbulent motion with the LMS under natural irradiance

Rotation speed ( $\text{cm s}^{-1}$ )	LMS $I_s$	DT/Chl <i>a</i>	DD/Chl <i>a</i>
0.05	22	$0.12 \pm 0.017$	$0.2 \pm 0.016$
1.0	26	$0.19 \pm 0.021$	$0.23 \pm 0.025$
2.0	28	$0.19 \pm 0.027$	$0.31 \pm 0.004$
4.0	26	$0.23 \pm 0.028$	$0.35 \pm 0.029$
8.0	24	$0.25 \pm 0.046$	$0.22 \pm 0.07$
12.0	27	$0.25 \pm 0.09$	$0.27 \pm 0.051$

LMS  $I_s$ —mean irradiance under the rotating LMS as a percent of surface irradiance. Differences between rotation speeds were not statistically significant (Student's *t* test at  $p < 0.01$ )



**Fig. 8** **a** Changes in the DD pigment pool with LMS simulation under natural daylight. **b** The change in incident PAR (400–700 nm) at the surface and the incident PAR, the samples were exposed to in the LMS. All three simulations showed a similar trend

low irradiance conditions, indicating a high shade environment there was no evidence of DD-cycle activity.

When the ice algae were removed from the low light under ice environment and incubated on the surface under conditions of increasing natural irradiance without the blocking effect of sea ice, the ratio DT/Chl *a* increased while DD/Chl *a* decreased consistent with the light mediated deepoxidation of DD to DT. The DD cycle was also active when ice algae from the bottom algal community was exposed to continuous irradiance of 50, 100 and 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  provided by white lamps. Under these constant irradiance conditions, the conversion of DD to DT occurred in two phases. In the first phase, there is a rapid increase in the relative amount of DT against Chl *a* and a rapid decrease in the relative amount of DD against Chl *a* in the first 10 min of dark adapted algae being exposed to light. This change, which provides evidence of DD-cycle activity, occurred even at the lowest light exposure of 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . These results do not show the light intensity at which the DD cycle is active but must be less than 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The initial rapid rise in DT and concurrent decrease in DD has also been observed in laboratory cultures of northern hemisphere ice algae incubated under 50 and 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Kudoh et al. 2003). As a comparison, no DT was present in five temperate species of diatoms exposed to 40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Lavaud et al. 2004). In the second phase, after the initial fast rise, the relative amount of DT did not change for algae exposed to 50 and 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , but did increase slightly for 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The close relationship between DD-cycle activity and the onset of nonphotochemical fluorescence quenching (NPQ) or thermal dissipation of excess light energy suggests that the DD cycle plays an effective role to protect the phytoplankton cells photosystem against excess irradiance (Demers et al. 1991; Oliazola and Yamamoto 1994; Lavaud et al. 2002a, b, 2003). In the results reported here, there were no direct measurements of NPQ and it can only be inferred from these previous studies that the DD-cycle activity observed is a function of photoprotective activity within the natural population of sea ice algae.

In the results from both field and laboratory incubations, there was an incomplete deepoxidation of DD relative to Chl *a* (30–60% in vivo). In all of the laboratory incubations, the relative amount of DD continued to fall without an increase in DT beyond the first 10–15 min. Oliazola and Yamamoto (1994) noted a similar result in the temperate diatom *Chaetoceros muelleri*, suggesting the presence of two functionally distinct pools of DD. Some of the pool of DD is thought to be bound to the pigment-protein complexes in the light-harvesting complex (LHC) of PSII and not available for deepoxidation (Lohr and Wilhelm 2001). Lavaud et al. (2004) found DD present in all the LHC

fractions. A major LHC fraction of DD was located at the distal antennae readily available for deepoxidation, while two smaller fractions were associated with photosystem centres. Lohr and Wilhelm (1999) have suggested that under conditions of low light, some of the DD can be converted to fucoxanthin (FX); the major light harvesting pigment of diatoms while under conditions of high light, DD can be converted to DT dissipating excess thermal energy from photosystem II.

There was a three to fourfold increase in the relative amount of DD to Chl *a* in this community under conditions of very low light (3–5  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) between late-October and early-November. The temporal increase in DD potentially provides greater amounts of DD that can be converted to FX to enhance the algal community's capacity to photosynthesize at low-light levels. The larger amounts of DD present in November compared to October, also increases the photoprotective capacity of the community.

The most likely explanation for this temporal increase in DD is a species succession to species possessing a higher cellular content of DD. The cellular content of DD has been shown to vary among species (Jeffrey et al. 1997). There was a higher percentage of the strand forming *Berkelaya adeliense* in November (25%) compared to October (4%). Ryan and Beaglehole (1994) recorded a shift in the bottom community of sea ice algae in McMurdo Sound from a community dominated by *Nitzschia stellata* and *Entomoneis kjellmannii* towards one dominated by *Berkelaya adeliense* as austral spring progressed. McMinn and Ashworth (1998) suggested that exposure of ice algae to additional PAR and higher ultraviolet-B radiation (UVB) resulting from stratospheric ozone depletion over Antarctica during austral spring may invoke a species succession with *Berkelaya adeliense* dominating under clear blue ice. Another possible but untested explanation is an increase in the cellular content of DD as an intermediary between the conversion of the carotenoid violaxanthin (VX) to FX. Under this hypothetical pathway, an increase in FX would also increase the cells ability to harvest light under the low-irradiance conditions experienced under the ice (Lohr and Wilhelm 1999). Intermittent light or continuous high light has been shown to cause a doubling of the DD pool in the diatom *Phaeodactylum tricorutum* (Lavaud et al. 2002a) and also in the centric diatom *Chaetoceros gracilis* (Kashino and Kudoh 2003). However, this occurs after exposure to much higher irradiance (>200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) than the under ice irradiance (<10  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) experienced here.

DT was present in the cells of the bottom community of sea ice algae even under low irradiance conditions when it is clear that there was no DD-cycle activity. The accumulation of DT without the deepoxidation step has been observed and may correspond to a direct conversion of VX

to DT, without DD as the intermediate (Lohr and Wilhelm 2001). The photoprotective dissipation of excess energy by nonphotochemical fluorescence quenching (NPQ) is linearly related to DT and if there is no DT then NPQ cannot form (Lavaud et al. 2002b). However, Lavaud et al. (2003) noted no DT present in a number of diatom species under low-light intensity.

In the dark, the DD-cycle pigments showed changes in reverse to those observed in the light with DT decreasing and DD increasing. During the first 10 min in the dark, there was a directly inverse relationship between the two pigments consistent with the other reports (Lohr and Wilhelm 1999; Kashino and Kudoh 2003). However, substantial amounts of DD were then accumulated, greater than what would be available from the epoxidation of DT. This implies that the reconstruction of the DD-cycle pool is not only from DT but via another pigment conversion. The conversion of VX to DD is the most likely candidate. Unlike previous studies, the reconstruction of the DD-cycle pool in the dark occurs quite rapidly with the DD-cycle pool fully recovered within 60 min suggesting that sea ice algae bottom community can rapidly reconstruct these xanthophyll pigments under low light or dark conditions.

The cellular concentration of the pool (DD + DT)/Chl *a* is within the range (up to 0.12) reported by Kudoh et al. (2003) for sea ice algae in northern Japan and by Moline (1998) for an open water diatom bloom during summer at Palmer Station on the Antarctic Peninsula. They are substantially less than values (up to 0.50) reported for surface ice algae in McMurdo Sound which were found to be supersaturated with respect to incident PAR over most of the 24 h light period (Robinson et al. 1997). A high cellular concentration of DD-cycle pigments under high light can compete with other light harvesting pigments for the absorption of light, diminishing the light energy available for photosynthesis (Fujiki and Taguchi 2001). The low-cellular concentration of DD-cycle pigments in the bottom community of sea ice algae reported here is unlikely to impact on their photosynthetic capacity but sufficient pigments are available to dissipate absorbed excess light energy despite the low temperatures and low irradiance.

#### Comparison between static and LMS simulations

The LMS was developed to simulate the light regime that algae would be exposed to when released into the water column as the sea ice thins over the Austral summer. Additionally, the LMS can be used to test if the artificial light incubations carried out may lead to higher light stress on the ice algae resulting in an overestimation of natural light mediated DD-cycle activity.

Our observations suggest that static incubations stimulate higher DD-cycle activity relative to the variable light

regime of the LMS. The light regime characteristics were similar for both the static and LMS incubations with the static samples exposed to a mean irradiance of 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and the LMS samples receiving mean irradiance varying between 190 and 265  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  of the surface irradiance during the duration of the experiments. Although the mean irradiance was similar for both methods, the LMS simulated samples resulted in a smaller reduction in the relative amounts of DD and a smaller increase in the relative amounts of DT. The static samples incubated under the constant artificial irradiance yielded between 26 and 65% higher relative amounts of DT/Chl *a* and 15–77% less relative amounts of DD/Chl *a* with the difference in relationships statistically significant ( $P < 0.01$ ) in five out of the six experiments.

In the dark or under low light, it has been well documented in laboratory studies on single species of temperate diatoms that the reverse and up to ten times slower conversion of DT back to DD occurs (Oliozola et al. 1994; Lohr and Wilhelm 1999). During the recovery of DD in the dark, the pool size of the DD-cycle pigments is generally reconstructed back to the same pool size of pigments due to an increase in DD as at the start of the previous light period (Welschmeyer and Hoepffner 1986). If the diadinoxanthin cycle requires dark or very low-light conditions to reconstruct the pigment pool, then how is the pigment pool of sea ice algae able to be reconstructed under the 24 h daylight of the Antarctic summer? During austral summer, the sea ice thins exposing the bottom community of sea ice algae to higher and longer periods of irradiance. As the annual sea ice disappears the mixed layer of the increasing patches of open water tends to be stable with enhanced stratification (Arrigo and Thomas 2004). Under these conditions it can be hypothesized that the sea ice algae can reconstruct the pool of DD-cycle pigments during daylight compared to temperate species of diatoms which require darkness or very low-light conditions. This hypothesis was tested by comparing the changes in the pigment pool over 12 h when exposed to static incubations under three different artificial light regimes and when exposed to a series of six LMS simulations under natural light.

When samples from the bottom community of sea ice algae at Cape Royds are exposed to long-term static incubations of 12 h under various artificial light regimes and under natural irradiance with the LMS, the pigment pool responds differently depending on the irradiance intensity and light history. Under continuous artificial irradiance conditions, we anticipated that the pigment pool would continue to decline over the 12 h of the experiment. After 12 h exposure to a constant 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  there was no statistically significant loss in the pigment pool. Under a constant exposure to both 100 and 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , the DD pigment pool

declined over the first few hours but then remained relatively constant.

With the LMS simulations under natural irradiance the changes in the pigment pool followed the changes in incident PAR shown with the pool decreasing as incident PAR increased and increasing as incident PAR decreased. This trend was evident in all the LMS simulations. The reconstruction of the pigment pool occurs when incident PAR is below  $230 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Kudoh et al. (2003) sampling from within the natural ice algal of an ice algal community in Northern Japan under low natural light observed a similar increase in the pool size with the pool size gradually increasing during the daytime with a maximum at sunset. When these ice algae were incubated under  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in the laboratory, the pigment pool declined with a similar trend to the observations reported here.

Our results from both the artificial irradiance experiments and with the LMS have some interesting ecological implications. They suggest that Antarctic ice algae have the ability to reduce the loss of critical photoprotective pigments when exposed to continuous high-irradiance conditions and to reconstruct the pigment pool under lower irradiance conditions during the daytime.

#### Determination of the rate kinetics

The second aim of this study was to accurately determine the rate at which these pigments change under various light regimes. While the rate kinetics for some individual diatom species are known from controlled laboratory experiments, the rates for an Antarctic sea ice algal community consisting predominately of diatoms under natural light are unknown. Knowledge of these rates is crucial to understanding if the Antarctic ice algal community could survive exposure to both sustained periods of higher light intensity or the sudden exposure to higher levels of ambient light.

The initial rate constants  $k$  for the rapid rise in DT during the first 10 min varied between  $1.07$  and  $1.6 \text{ min}^{-1}$  for the cells exposed to sustained artificial light at  $50$ ,  $100$  and  $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The initial rate constants were lower in the range of  $0.19$ – $0.55 \text{ min}^{-1}$  for the sea ice algae exposed to natural daylight on the surface of the sea ice at Cape Royds. These rate constants are within the range reported in previous laboratory studies with temperate species of diatoms. Using the same first order kinetic model Oliazola et al. (1994) calculated rate constants of  $0.089$ – $0.131 \text{ min}^{-1}$  at  $394 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and  $1.6 \text{ min}^{-1}$  at  $1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for *Chaetoceros muelleri* Lemmerman. While for *Chaetoceros gracilis* the first order rate constant under a continuous light intensity of  $50$  and  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  has been shown to vary between  $0.1$  and  $0.2 \text{ min}^{-1}$  (Kudoh et al. 2003). At a

light intensity of  $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and by fitting an exponential regression curve, Lavaud et al. (2004) found the rate of deepoxidation to vary between  $1.2$  and  $2.2 \text{ min}^{-1}$  for five temperate species of diatoms. The rate constants of the laboratory incubations were significantly higher than under natural irradiance. The mean overestimate of  $k$  obtained from the laboratory incubations relative to the sea ice surface in situ measurements was 310%.

Using the rate constant  $k$  of  $1.3 \text{ min}^{-1}$  determined from the incubations under  $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , an initial starting ratio of  $0.1 \text{ DT/Chl } a$  and a finishing amount of  $0.001$ , the amount of DD will be essentially exhausted in about 11 h, assuming no replenishment of DD. Obviously, increasing the initial value of DD will extend the time for the complete conversion to DT. This demonstrates that there is sufficient DD in the bottom community algae in austral spring to provide effective photoprotection for extended periods of sustained light. However, later in the summer with ice melt, sea ice algae can be exposed to incident PAR for at least 24 h at an intensity which would activate the DD cycle. Further study is required on the DD cycle over extended periods of sustained high-light intensity

#### The light-mixing simulator (LMS)

Two previous simulators on which this design has been based were used to assess phytoplankton productivity in relation to mixing and light attenuation. Mallin and Paerl (1992) devised a light-field simulator (LFS) designed to rotate a 4-m diameter wheel horizontally, driven by a variable-speed electric motor. The rotating wheel consisted of 12 pie-shaped sections of neutral density screens which could be adjusted to rotate from 6 min to >24 h. The LFS was designed to provide a reasonable approximation of one cycle of phytoplankton cells down to the bottom of the euphotic zone and up to the surface again. Glocke and Lenz (2004) independently devised a similar type of simulator without being aware of the previous development of the LFS. Their design consists of a perforated water bath containing a circular sample holder with eight 30 ml and eight 10 ml polycarbonate centrifuge tubes. An electric motor attached to a reduction gearbox rotates the water bath at a constant 12 min per revolution under a lid consisting of 11 neutral filter segments. The incubator simulates an algal cell moving up and down in a mixed euphotic zone of 180 cm. This 'turbulence incubator' is especially suitable for vertically mixed waters such as shallow bays, tidal estuaries and rivers.

The current LMS has a number of significant advantages over these previous simulators, particularly for oceanographic and polar research. Firstly, the filters and segments of the lid can be easily adjusted to simulate various light

regimes. By choosing the neutral density filters and calculating a suitable light attenuation coefficient, it is possible to adapt the LMS to specific field conditions. As the lid is light and rotates, it can be driven by a small electric motor. The design is light, easily handled and transportable. The attached gel battery allows the LMS to function in polar environments away from other power sources. The LMS has been tested at temperatures lower than  $-20^{\circ}\text{C}$  in Antarctica during austral spring. In comparison to previous designs the speed of rotation is variable which allows an adjustment not only of the light regimes using the light filter segments on the lid but also allows the vertical mixing rate to be varied through the simulated light regime. Previous designs were also limited by the size of samples which could only be from 10 to 125 ml, while this design can handle sample bottles up to 2,000 ml. This is particularly important in Southern Ocean research where larger samples are required to obtain sufficient filtered phytoplankton for HPLC. A current limitation of the LMS is the possible sedimentation and self-shading effect of algae in the sampling bottles. One possible solution would be to attach the sample bottles to a geared central spindle. This would, however, reduce the size of the sample bottles. In the experiments conducted here it is assumed that sedimentation and self-shading had a minimum effect as the samples were mixed every 30 min and the results averaged by analysing triplicate samples at each time period. In open ocean and polar sea ice research, a significant problem with in situ incubations at depth of the DD-cycle is the period of time that it takes for the samples to be returned to the surface. The LMS can be directly sampled allowing an estimate of the pigment concentrations closer to that of the natural in situ values.

While the LMS was designed as a proof of concept to solve a particular experimental of conducting in situ incubations on the photoprotective DD cycle of sea ice algae as the nearest approximation of natural field conditions in Antarctica, its use could also be extended simulate any physiological or photosynthetic response.

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