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EXPOSURE TO SUDDEN LIGHT BURST AFTER PROLONGED DARKNESS – A CASE STUDY ON BENTHIC DIATOMS IN ANTARCTICA

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In polar areas, benthic diatoms are regarded to play a major role in supplying energy to the benthic fauna, particularly prior to the release of microalgae from sea ice and the phytoplankton bloom. As phototrophs, benthic polar diatoms have to contend not only with dark polar nights but also with darkness due to sea-ice and snow cover that can prevail in the littoral zone for additional months. Upon sea ice break-up the autotrophs are suddenly exposed to high light intensities including ultraviolet radiation. The aim of our study was to mimic a sudden spring-time sea ice break-up, focusing on the ultraviolet part of the solar spectrum. We therefore exposed a semi-natural community of benthic diatoms to light burst after a period of total darkness. We studied the effects of different spectral qualities: photosynthetically active radiation (PAR, 400–700 nm; P treatment), PAR+ UV-A (UV-A 320–400 nm; PA treatment), and PAR+UV-A+UV-B (UV-B 280–320 nm; PAB treatment) on cell number (growth), species composition and optimum quantum yield (F_v/F_m) in 2 separate experiments where diatoms were kept in darkness for 15 and 64 days, respectively. In both experiments, the most frequently (>50%) observed species were *Gyrosigma fasciola* and *G. obscurum*. No growth was observed and no resting spores were found. In both experiments, the initial optimum quantum yield of the PSII prior to dark treatment was comparable (F_v/F_m = 0.70). The F_v/F_m was not affected after 15 days dark incubation but a significant decrease in photosynthetic efficiency was observed after 64 days in the dark (F_v/F_m = 0.39). Exposure to different light treatments (P, PA, PAB) immediately after different dark incubation periods showed higher reduction in F_v/F_m (PAB > PA > P) after the longer dark period. Estimated P-E curve parameters showed an efficient light

harvesting and photosynthetic conversion capacity (α= 0.20; $rETR_{max}$=14) that was significantly reduced after 64 days in the dark (α= 0.06; $rETR_{max}$=8). The reduction in these photo-physiological indices (α and $rETR_{max}$) after dark incubation was compensated with higher saturating irradiance (E_k), which we suspect to be a mechanism to optimize photochemical processes. But the PSII antenna was relatively light-sensitive because photosynthesis was already photoinhibited at half the photon flux density ($\geq$ 585 µmol photons m^{-2} s^{-1}) relative to light-adapted ($\geq$ 972 µmol photons m^{-2} s^{-1}) diatoms. We conclude that the benthic diatoms in our study were able to resume photosynthetic activity after 64 days in darkness and they were able to cope with relatively high intensities of UV radiation compared with their natural habitat.

INTRODUCTION

Polar microalgae have to relate to constant low temperatures and prolonged darkness. In addition to the dark winter months, darkness can prevail in the littoral zone due to sea ice and the overlaying snow cover. Thus, pelagic and benthic organisms underneath can experience either twilight illumination or complete darkness, depending on the total thickness of the snow-covered sea ice, for up to 10 months each (Lüder *et al.* 2002). Of course some cells need to survive these dark periods in order to provide seed cells when the light returns. It is not clear, however, if they form resting spores or survive as vegetative cells, or both. Dark survival of diatoms is not a new phenomenon (see Table 1 for an overview) and it has been speculated that their ability to withstand dark conditions was a possible reason for surviving the large extinction during the Cretaceous period (Kitchell *et al.* 1986). Several diatom species have been found to survive dark periods up to a year or more (Table 1). However, these studies concern planktonic and ice-living diatoms and, to our knowledge, no studies have been published on dark survival of sediment-living Antarctic diatoms.

Upon ice-break during the Antarctic spring, phototrophic organisms are exposed to a burst of high light intensities including ultraviolet radiation (UVR; UV-A, 320–400 nm, and UV-B, 280–320 nm). In our study area at King George Island, UVR can penetrate to considerable depth into the water column (19 m, 1% of incident light levels, Zacher *et al.* 2007a), and could thereby also affect subtidal organisms. Although the sediment has been considered to be a refuge to escape harmful radiation, UV radiation has been shown to penetrate ca 1 mm into sandy sediment (Garcia-Pichel & Bebout 1996, Wulff *et al.* 1999).

Our aim was to mimic a sudden spring-time sea ice break-up, focusing on the ultraviolet part of the solar spectrum, exposing benthic diatoms to light burst after a period of total darkness. We therefore studied the effects of different spectral qualities (PAB, PA, P) on cell number (growth), species composition and optimum photosynthetic efficiency (F_v/F_m) in 2 separate experiments where diatoms were kept in darkness for 15 and 64 days, respectively.

MATERIAL AND METHODS

The experiments were performed between December 2004 and February 2005 at Dallmann Laboratory, Potter Cove, King George Island, Antarctica (62° 15'S, 58° 41'W). Fine grained sandy to silty sediment with brown mats of benthic diatoms were collected from 5 to 7 m water depth (SCUBA diving). The top layer (1 cm) was scraped off and the sediment was brought to the laboratory, gently shaken and sieved (mesh size 500 µm) using filtered surface seawater. The sediment was stirred and the overlying water containing suspended microalgae was transferred to a glass beaker gently bubbled with air and left to grow for ca 3 weeks under dim white light (ca 10 µmol photons m^{-2} s^{-1}). The overlying water was enriched once a week with macronutrients and micronutrients, corresponding to f/2 medium (Guillard 1975).

Experimental set-up and treatments

Two separate experiments were carried out (Expt 1 and Expt 2) in a temperature-controlled laboratory container at 4–6°C. For each experiment, 20 Petri dishes (55 mm, n = 5) were prepared with ca 0.5 mm layer of acid-cleaned sand (5 g), respectively, and they were carefully submerged into the bottom of a filtered seawater-filled plastic container with minimal disruption of the sand cover inside each dish. A microalgal suspension was evenly poured into the water surface of the basin. The suspended diatoms in the water column were allowed to settle into the sand-containing Petri dishes for ca 12 hours. After settlement, Petri dishes were slowly removed from the bottom of the basin and transferred to the experimental workbench under dim white light (< 10 μmol photons m^{-2} s^{-1}) and the initial optimum quantum yield (see details below) was measured. Then all samples were individually transferred to 20 ml plastic vials (n=20 for each experiment), the vials were sealed inside a cardboard box wrapped with black plastic bags and kept in total darkness under temperature controlled conditions (4°C) for 15 (Expt 1) and 64 days (Expt 2), respectively.

After each dark period treatment, F_v/F_m and rapid light curves (P-E curves) of the diatoms were measured. Care was taken not to expose the diatom cells to any light before the photosynthetic measurements. After measuring the optimum quantum yield, the diatom suspensions were transferred to their respective Petri dishes and, in both experiments, the diatoms were exposed for 4 h to the different radiation treatments followed by recovery radiation (ca 4 μmol photons m^{-2} s^{-1}) for 1 h. Before the treatments started, 5 Petri dishes were put in weak PAR (ca 4 μmol photons m^{-2} s^{-1}) and served as control. After 4 h of radiation treatments (described below), 5 replicates of each treatment were sampled to measure F_v/F_m. Then the diatom suspensions were transferred to the Petri dishes and left under recovery light for 1 h after which another F_v/F_m measurement was performed. To allow for a maximal UV effect, samples were taken just before the UV lamps were turned off. Radiation treatments were PAR+UV-A+UV-B (PAB, 280 to 700 nm), PAR+UV-A (PA, 320 to 700 nm) and PAR (P, 400 to 700 nm). The PAR intensity was 44 μmol photons m^{-2} s^{-1}, UV-A 9.8 W m^{-2}, and UV-B 0.9 W m^{-2} (unfiltered measurements).

Light was provided by white fluorescent lamps (Osram GmbH, L65 Watt/25S, Munich, Germany), emitting background PAR and UV lamps (Q-Panel UV-A-340, 40 Watt, Cleveland, Ohio, USA), emitting a spectrum qualitatively similar to solar radiation in the range of 295 to 340 nm. Three kinds of filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent lamps: (i) Ultraphan transparent (Digefra GmbH, Munich, Germany), (ii) Folanorm 320 (Folex GmbH, Cologne, Germany), and (iii) Ultraphan URUV farblos (Digefra), corresponding to the PAB, PA and P treatments, respectively. Ultraphan filters are acetate based and the Folanorm filter is a polyester filter. The available filters cut off wavelengths were slightly differing from the definition of CIE (Commission Internationale De l'Éclairage, UV-B = 280–315 nm, UV-A = 315–400 nm). Irradiation in the laboratory was measured below the cut-off filters using a Solar Light PMA 2100 radiometer (Solar Light, Philadelphia, USA) equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar Light, Philadelphia, USA). As the spectral range of the UV-A sensor extends into the UV-B region of the spectrum, UV-A radiation measurements were always made using a Schott WG320 filter (Schott, Mainz, Germany) to exclude wavelengths below 320 nm. The UV-B measurements recorded were obtained by subtracting the reading with the WG320 filter from the reading without the filter. PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1400 datalogger (LI-COR Bioscience, Lincoln, USA). Light intensities (PAR, UV-A and UV-B) under the different cut-off filters corresponding to different treatments are shown in Table 2.

Table 1. An overview of diatom survival times and viability after being kept in darkness. Viability has been defined as the ability to grow in the light after a period in darkness, with or without a lag phase from days to weeks. For comparison, dark exposure times have been transferred to number of days. The species names are shown according to cited references but when species have been transferred to a different genus, the new names are shown between brackets.

Species	Temperature	Dark exposure times (days)	Reference
Achnanthes brevipes Agardh	20°C	56^{*}	Antia & Cheng 1970
Achnanthes brevipes Agardh	2, 20°C	364, 126	Antia 1976
Amphiprora paludosa Smith	20°C	56^{*}	Antia & Cheng 1970
Amphiprora paludosa var. *duplex* Donkin	2, 20°C	189, 133	Antia 1976
Amphora coffeaeformis (Ag.) Kütz.	7°C	28^{*}	Anderson 1975b
Anaulus australis Drebes *et* Schulz	18°C (±2)	62^{*}	du Preez & Bate
Araphid, pennate diatom ca 10 µm	-2°C	$310\ (364)^{a}$	Palmisano & Sullivan 1982
Asterionella japonica Cleve (*Asterionellopsis glacialis* (Castracane) Round)	15°C	90^{*}	Smayda & Mitchell-Innes 1974
Bacteriastrum sp.	18°C	17^{*}	Jochem 1999
Bellerochea polymorpha Hargraves & Guillard (*Minutocellus polymorphus* (Hargraves & Guillard) Hasle, von Stosch & Syvertsen)	2, 10, 20°C	63, >140, 84	Antia 1976
Chaetoceros curvisetus Cleve	15°C	90^{*}	Smayda & Mitchell-Innes 1974
Chaetoceros didymus Ehrenberg	15°C	$90d^{*}$	Smayda & Mitchell-Innes 1974
Chaetoceros fragile Meunier	-1.8°C	93^{*}	Bunt & Lee 1972
Chaetoceros gracilis Schütt	20°C	56^{*}	Antia & Cheng 1970
Chaetoceros gracilis Schütt	2, 10, 20°C	112, >210*, 133	Antia 1976
Cyclotella cryptica Reimann, Lewin & Guillard	20°C	56^{*}	Antia & Cheng 1970
Cyclotella cryptica Reimann, Lewin & Guillard	2, 20°C	189, 105	Antia 1976
Cyclotella nana Hustedt	20°C	49	Antia & Cheng 1970
Cylindrotheca fusiformis Reimann & Lewin	20°C	56^{*}	Antia & Cheng 1970
Cylindrotheca fusiformis Reimann & Lewin	2, 20°C	364	Antia 1976
Ditylum brightwellii (West) Grunow	8, 15°C	35^{**}, 30	Peters 1996
Ditylum brightwellii (West) Grunow	15°C	90^{*}	Smayda & Mitchell-Innes 1974
Fragilaria pinnata Ehrenberg (*Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round)	2, 20°C	280, 364	Antia 1976
Fragilaria sublinearis van Heurck	-1.8°C	93^{*}	Bunt & Lee 1972
Fragilariopsis kerguelensis (O'Meara) Hustedt	0°C (±1)	127	Peters & Thomas 1996

Lithodesmium undulatum Ehrenberg	15°C	90*	Smayda & Mitchell-Innes 1974
Melosira nummuloides Agardh	20°C	56*	Antia & Cheng 1970
Melosira nummuloides Agardh	2, 20°C	336, 105	Antia 1976
Navicula incerta Grunow ex Van Heurck	20°C	56*	Antia & Cheng 1970
Navicula incerta Grunow ex Van Heurck	2, 20°C	364 (1092)[a], 364 wks	Antia 1976
Nitzschia angularis Smith	20°C	56*	Antia & Cheng 1970
Nitzschia angularis var. *affinis* (Grunow) Grunow	2, 20°C	364 (1092)[a], 364	Antia 1976
Nitzschia cylindrus (Grunow) Hasle, size 4 µm	-2°C	155 (364)[b]	Palmisano & Sullivan 1982
Nitzschia cylindrus (Grunow) Hasle, size 6 µm	-2°C	155 (364)[b]	Palmisano & Sullivan 1982
Nitzschia-like	18°C	17*	Jochem 1999
Phaeodactylum tricornutum Bohlin	20°C	168*	Antia & Cheng 1970
Phaeodactylum tricornutum Bohlin	2, 20°C	364, 364	Antia 1976
Phaeodactylum tricornutum Bohlin	5°C	93	Umebayashi 1972 cited in Anderson 1975b
Porosira pseudodenticulata (Hustedt) Jousé	0°C (±1)	272	Peters & Thomas 1996
Proboscia inermis (Castracane) Jordan & Ligowski	0°C (±1)	214	Peters & Thomas 1996
Rhizosolenia fragilissima Bergon (*Dactyliosolen fragilissimus* (Bergon) Hasle)	18°C	23*	Ignatiades & Smayda
Rhizosolenia setigera Brightwell	8°C	21	Peters 1996
Skeletonema costatum (Greville) Cleve	20°C	7	Antia & Cheng 1970
Skeletonema costatum (Greville) Cleve	2, 10, 20°C	168, 63, 28 wks	Antia 1976
Skeletonema costatum (Greville) Cleve	15°C	49	Smayda & Mitchell-Innes 1974
Thalassiosira antarctica Comber	0°C (±1)	214	Peters & Thomas 1996
Thalassiosira fluviatilis Hustedt	20°C	56*	Antia & Cheng 1970
Thalassiosira fluviatilis Hustedt	2, 20°C	140, 105	Antia 1976
Thalassiosira gravida Cleve	15°C	90*	Smayda & Mitchell-Innes 1974
Thalassiosira pseudonana Hasle & Heimdal	2, 20°C	161, 105	Antia 1976
Thalassiosira punctigera (Castracane) Hasle	8°C	35**	Peters 1996
Thalassiosira sp.	15°C	90*	Smayda & Mitchell-Innes 1974

* maximum test period

** cell number constant over 63 days

[a] retention of viability after 3 years darkness exposure

[b] undiluted cultures were left for 12 mo and growth was resumed after a lag phase of 3-6 mo

Table 2. Light intensities under cut-off filter foils used in the different treatments. For a complete description of filter foils see Material and Methods.

Treatment	Filter foils used	PAR (µmol photons m^{-2} s^{-1})	UV-A (W m^{-2})	UV-B (W m^{-2})
PAB	Ultraphan transparent	40	9.8	0.7
PA	Folanorm 320	40	9.05	0.06
P	Ultraphan URUV farblos	40	0.08	0.01

Diatom density and species composition

Diatom density and species composition (n=2) before and after the 64d dark period was determined while only the initial data were collected for the 15d dark treatment. The sample was vigorously shaken by hand for 30 seconds and after ca 30 seconds (to allow sand grains to settle), a minimum of two individual subsamples (40 µl) of the algal suspension were pipetted onto a light microscope slide (20×, Zeiss, Axiolab, Germany) and cells with and without intact chloroplasts were counted. Naphrax mounted slides were prepared for diatom species identification. Samples were washed with distilled water to remove the salts and then boiled with 30% H_2O_2 to remove organic matter. 1–2 drops of 50% HCl were added to remove carbonates and to eliminate H_2O_2. After washing, diatom suspensions were allowed to settle on a cover slip and left to dry before being mounted. For species identification, differential interference contrast and phase contrast microscopy (1000× magnification) were used (Axioplan 2 imaging, Zeiss, Germany). Diatoms were identified following Hustedt (1961–1966), Krammer & Lange-Bertalot (1986, 1988), Hendey (1952, 1964) & Witkowski *et al.* (2000). The nomenclature was updated with the help of Round *et al.* (1990).

Diatom photosynthesis

The effects of UV radiation on optimum quantum yield of the diatom suspension was determined by measuring the variable chlorophyll fluorescence of PSII by use of a pulse-amplitude modulated fluorometer (Water-PAM, connected to a PC with WIN CONTROL Software, Walz GmbH, Effeltrich, Germany). The content of the whole Petri dish was sampled in a 20 ml vial, the bottle was shaken 30 sec, the sand grains left to settle for 30 sec, and 4 ml of the microalgal suspension was filled into 5 ml Quartz cuvettes for measurements with the Water-PAM. F_v/F_m was measured after 3 min dark adaptation to determine changes in the photosynthetic efficiency. Prior to the dark adaptation the samples were exposed for 5 sec to weak far-red light. For measurements done immediately after the dark periods, no additional dark adaptation was performed. To check if the handling of the diatoms had any effect on the F_v/F_m, a control experiment was set-up where F_v/F_m was measured repeatedly (6 times) over 28 h. For each measurement, the F_v/F_m decreased by 5–10% and compared with the undisturbed diatoms (measured after 28 h) the F_v/F_m of the third measurement was ca 8% lower. After 28 h (sixth measurements) the F_v/F_m had decreased by 22%. The data were corrected for the disturbance effect by adding 10% to the resulting ratio for each measurement after the initial.

Photoadaptive index was estimated from photosynthesis (in terms of relative electron transport rate, rETR = PFR x ΔF/Fm') versus irradiance curve (P-E curve). P-E curves were measured two times in every replicate (n=3, chosen at random from the 5 replicates) using low and high actinic light intensities making up 12 points (17, 26, 38, 58, 87, 128, 198, 294, 419, 585, 972, 1458 µmol photons m^{-2} s^{-1}) as described by Roleda *et al.* (2006). The hyperbolic tangent model of Jassby & Platt (1976) was used to estimate P-E curve parameters described as:

$$rETR = rETR_{max} * \tanh (\alpha * E_{PAR} * rETR_{max}^{-1})$$

where $rETR_{max}$ is the maximum relative electron transport rate, tanh is the hyperbolic tangent function, α is the electron transport efficiency and E is the photon fluence rate of PAR. The saturation irradiance for electron transport (E_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote ($rETR_{max}$). Curve fit was calculated with the Solver Module of MS-Excel using the least squares method comparing differences between measured and calculated data. The saturating photosynthetic photon flux density (PPDF) value at which photosynthesis is at 95% of the maximum value ($E_{0.95}$) is directly proportional to E_k and can be derived using the equation $E_{0.95} = \tanh^{-1}(0.95)\, E_k$ (Chalker *et al.* 1983).

Statistical analyses

One-way ANOVA was used to test for the effects of UVR on F_v/F_m for each exposure time ($p < 0.05$). Prior to analysis data were tested for homogeneity of variances (Cochran's test, Winer *et al.* 1991).

RESULTS

Species and cell numbers

In both experiments, the diatom assemblages were dominated (> 50%) by *Gyrosigma fasciola* (Ehrenberg) Griffith & Henfrey and *G. obscurum* (Smith) Griffith & Henfrey. Less frequently occurring (10–50%) taxa were *Amphora* sp., *Navicula cancellata* Donkin, *N. directa* (Smith) Ralfs, *Odontella aurita* var. *aurita* (Lyngbye) Agardh, *Odontella aurita* var. *obtusa* (Kützing) Hustedt, *Pinnularia quadratarea* (A. Schmidt) Cleve and *Trachyneis aspera* var. *aspera* (Ehrenberg) Cleve. The initial cell concentrations were 1.93×10^7 (Expt 1) and 8.58×10^7 cells m^{-2} (Expt 2). In Expt 1, after the initial cell counting no further counting was performed. Over the 64 days in darkness, no growth was observed (Expt 2) and the proportion of empty frustules was on average 51–65%, for all frequently-occurring species. Because very few empty frustules were observed in the initial samples apparently several cells had died. No resting spores were found but several cells had contracted chloroplasts, indicating that they were in physiologically resting stages (Sicko-Goad *et al.* 1989).

Diatom photosynthesis

In both experiments, the initial F_v/F_m was 0.7 (Fig. 1a). In Expt 1, after 15 days in darkness F_v/F_m was still 0.7. After 4 h radiation treatments, the F_v/F_m decreased to 0.29 (PAB), 0.36 (PA) and 0.65 (P) (Fig. 1a). The recovery under weak PAR, resulted in increased F_v/F_m (all treatments) but the recovery was most pronounced in the PAB treatment (56%, $p<0.001$) followed by PA (47%, $p<0.001$) and P (13%, $p<0.001$). In Expt 2, the diatoms were to a larger extent affected by the prolonged darkness. After 64 days in total darkness, the initial F_v/F_m was 0.39. After 4 h radiation treatments, the F_v/F_m decreased to 0.06 (PAB), 0.06 (PA) and 0.28 (P) (Fig. 1b). After 1 h recovery under weak PAR, F_v/F_m increased in all treatments but the recovery was most pronounced in the PA treatment (48%, $p<0.01$) followed by PAB (36%, $p=0.08$) and P (23%, $p<0.05$).

Photosynthetic parameter estimates derived from the P-E curves showed an efficient light harvesting and photosynthetic conversion capacity in diatom communities before dark treatment. This was exemplified by the steep increase ($\alpha = 0.2$) of $rETR_{max}$ at low light intensities (Fig. 2). All parameters including the saturating irradiance (E_k) were comparable between the two separate experiments conducted (Table 3). No depression of photosynthesis was observed in the P-E curves up to a photon fluence rate (PFR) of 800 μmol photons $m^{-2}\, s^{-1}$ (11 times more than the E_K). After 64 days in the dark, the decrease in light harvesting

capacity and photosynthetic conversion efficiency (α = 0.06) led to the corresponding decrease in photosynthetic capacity ($rETR_{max}$) by 45%. Saturating irradiance (E_k) however increased by 47% after the 64 days dark treatment. Photoinhibition of photosynthesis was observed already at a PFR of 800 µmol photons $m^{-2} s^{-1}$.

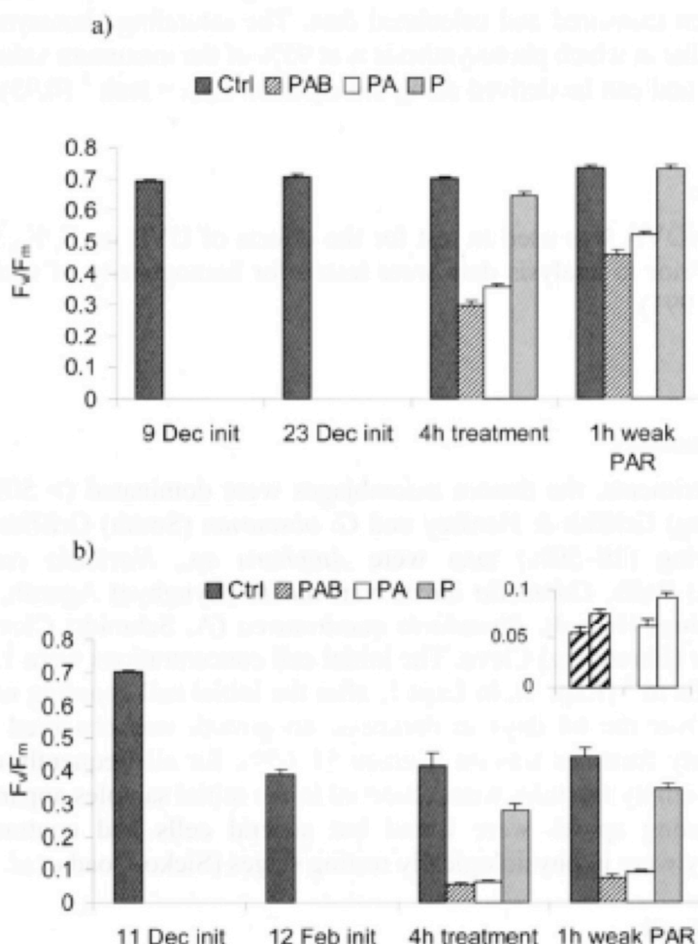

Fig. 1. Experiment 1 (a; 15 days dark treatment), and Expt 2 (b; 64 days dark treatment) (initial samples (init) n=20, 4 h treatments and recovery n=5). The bars show optimum PSII maximum efficiency (F_v/F_m) after exposure to different light treatments (± SE), PAR+UV-A+UV-B (PAB), PAR+UV-A (PA) and PAR (P), respectively. Ctrl means control treatment exposed to weak PAR (4 µmol photons $m^{-2} s^{-1}$).

DISCUSSION

The novelty of our study is the use of a semi-natural community (not monocultures) and the experimental exposure of the diatoms to different light qualities (P, PA and PAB) immediately after a long period in complete darkness to simulate sea-ice break up which may occur at different times of the day in the presence or absence of UVR. In our experiments, the diatoms were exposed to a UV-B radiation of ca 0.7 W m^{-2}, an intensity they will most likely never experience at 5 m water depth at the study site. Between December 2004 and February 2005, for example, maximum UV-B irradiance at 2 m depth was 0.26 W m^{-2} (Zacher *et al.* 2007a). However, we altered the natural ratios between PAR, UV-A and UV-B and therefore our approach should be considered mechanistic.

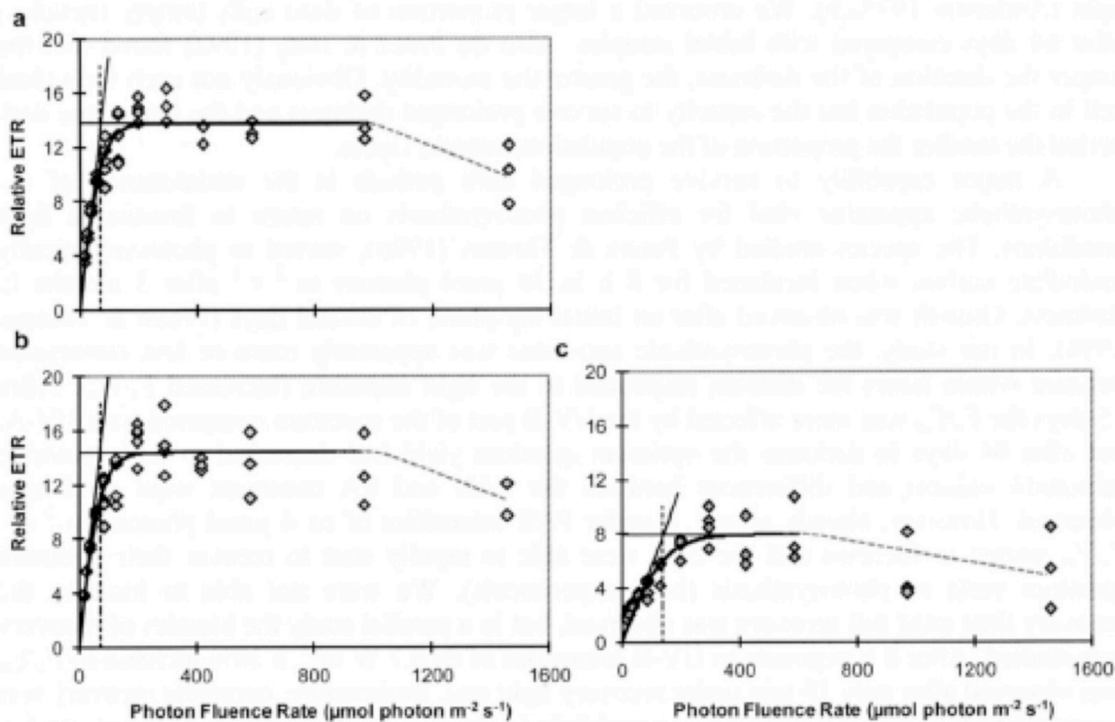

Fig. 2. Photosynthetic performance (P-E curve) of the benthic diatom community before 15 or 64 days dark treatment (a, b) and after 64 days in the dark (c). PFR is the fluence rate of actinic white light and rETR is the relative electron transport rate. Saturating irradiance (E_k, vertical broken lines) is the point at which the initial slope (α, alpha) crosses maximum photosynthesis ($rETR_{max}$) using a hyperbolic tangent model.

Table 3. Photosynthesis–irradiance (P-E) curve parameter estimates using the hyperbolic tangent equation of Jassby & Platt (1976) and Chalker *et al.* (1983).

	Short dark treatment (15 days)		Long dark treatment (64 days)	
	Before dark	After dark	Before dark	After dark
E_K (µmol photons m^{-2} s^{-1})	71	n.d.	73	139
$E_{0.95}$ (µmol photons m^{-2} s^{-1})	130	n.d.	134	255
ETR_{max}	13.78	n.d.	14.44	7.96
Alpha	0.19	n.d.	0.20	0.06
R^2	0.99	n.d.	0.96	0.96

E_k is the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote, the maximum relative electron transport rate ($rETR_{max}$). $E_{0.95}$ is the saturating photosynthetic photon flux density (PPDF) value at which photosynthesis is at 95% of the maximum value [$E_{0.95}$ = tan h^{-1} (0.95) E_k]. R^2 is the fitness of the hyperbolic tangent curve.

Based on the rapid increase in F_v/F_m, we safely draw the conclusion that although the cells probably were physiologically resting in the dark they were not forming resting stages such as spores or cysts. Physiologically resting cells are morphologically similar to the vegetative cells, but are physiologically dormant and can be induced when cells are transferred to cold and dark conditions (Anderson 1975a). Like in our study, these cells have condensed protoplasts which are transformed back to the former state (within hours) upon re-exposure to

light (Anderson 1975a,b). We observed a larger proportion of dead cells (empty frustules) after 64 days compared with initial samples. Also du Preez & Bate (1992) found that the longer the duration of the darkness, the greater the mortality. Obviously not each individual cell in the population has the capacity to survive prolonged darkness and the longer the dark period the smaller the proportion of the populations remain viable.

A major capability to survive prolonged dark periods is the maintenance of the photosynthetic apparatus vital for efficient photosynthesis on return to favourable light conditions. The species studied by Peters & Thomas (1996), started to photosynthetically assimilate carbon when incubated for 8 h in 30 μmol photons m^{-2} s^{-1} after 3 months in darkness. Growth was observed after an initial lag phase of several days (Peters & Thomas 1996). In our study, the photosynthetic apparatus was apparently more or less maintained because within hours the diatoms responded to the light exposure (increased F_v/F_m). After 15 days the F_v/F_m was more affected by the UV-B part of the spectrum compared with UV-A, but after 64 days in darkness the optimum quantum yield had decreased to 0.06 (possible minimum values) and differences between the PAB and PA treatment were no longer observed. However, already after 1 h under PAR intensities of ca 4 μmol photons m^{-2} s^{-1}, F_v/F_m started to increase and the cells were able to rapidly start to recover their optimum quantum yield of photosynthesis (both experiments). We were not able to increase the recovery time until full recovery was observed, but in a parallel study the kinetics of recovery was studied. After 8 h exposure to UV-B intensities of ca 0.7 W m^{-2}, a 30% increase in F_v/F_m was observed after only 10 min under recovery light and, furthermore, complete recovery was accomplished in 6 h (Wulff *et al.* unpublished). In the PAB treatment (present study), recovery of photosynthetic capacity was observed to be dependent on the dark exposure period. Prolonged period in the dark delayed photosynthetic recovery in PAB-treated samples compared to shorter dark treatment with corresponding 36% and 56% recovery in F_v/F_m after 1-hour recovery under low white light, respectively. In PA-treated samples, recovery of photosynthetic capacity was independent of the length of dark treatment. The UV-B part of the spectrum had a larger impact on sudden exposure of dark-acclimated diatoms to light compared with UV-A. A similar observation was reported by Roleda *et al.* (2006) on the kinetics of photosynthetic recovery among different species of brown macroalgal zoospores exposed to different light qualities. Photosynthetic recovery was delayed in PAB-treated spores compared with PA- and P-treated zoospores.

The approximately 2-fold increase in E_k (139 μmol photons m^{-2} s^{-1}) after dark treatment observed in this study remains unreported in the literature and can be interpreted as a compensating light-harvesting mechanism. We hypothesize that this mechanism enables the cells to effectively fix new photosynthate after a prolonged period in the dark, in lieu of the stored carbon consumed in the dark for maintaining essential metabolic processes. The increase in E_k for photosynthesis is not improbable since photoinhibition of photosynthesis before and after dark treatment occurred only at photon fluence rate higher than 500 μmol photons m^{-2} s^{-1}.

Most often, change in photosynthetic parameters are reported in response to *in situ* light environment where high alpha and low E_k was observed in benthic diatom mats before ice breakout with low *in situ* under-ice irradiance. After ice breakout, the increase *in situ* irradiance effectively decreased the alpha and increased the E_k of the benthic diatom mats (McMinn *et al.* 2004). At high irradiance, the decrease in light harvesting and photosynthetic conversion efficiency (low alpha) can be interpreted as a photo-protective mechanism to regulate energy flow required for photosynthesis. Among light-adapted phototrophs, photoinhibition of photosynthesis usually occurs at irradiance higher than the estimated E_k, the minimum saturating irradiance at which the initial slope crosses maximum photosynthesis ($rETR_{max}$) or $E_{0.95}$, the saturating photosynthetic photon flux density value at which

photosynthesis is at 95% of the optimum. For example, the E_k of sea-ice algae is less than the daily average PAR reaching the algal communities while photoinhibition of photosynthesis occurs at light intensities 4–8 fold higher than the measured E_k (Robinson *et al.* 1997). The E_k is therefore not a good measure to characterize the acclimation capacity of the organism to their high-irradiance environment as implied by Robinson and co-workers (1997) but the photon flux density at which photosynthesis is photoinhibited. In our study, we observed that dark-adapted diatoms were photoinhibited already at half the photon flux density ($\geq$ 585 µmol photons m^{-2} s^{-1}) compared to light-adapted diatoms ($\geq$ 972 µmol photons m^{-2} s^{-1}). *Trachyneis aspera*, one of the most frequently occurring species in our study, was reported to be low-light adapted where saturating irradiance for photosynthesis was observed already at 11 µmol photons m^{-2} s^{-1}. Cells were however observed to maintain photosynthesis at higher light intensities; no photoinhibition was observed at 300 µmol photons m^{-2} s^{-1} (Palmisano *et al.* 1985).

From earlier studies on UV effects on benthic microalgae (soft bottom communities), it seems like benthic diatoms are very tolerant of UV-B radiation (Peletier 1996, Wulff 1999 and references therein). The main variable to some extent affected in these communities was primary productivity while structural variables such as the overall biomass and microalgal pigments appeared unaffected (Odmark *et al.* 1998, Sundbäck *et al.* 1997, Wulff *et al.* 1999, 2000). When UV-B effects on optimum quantum yield of photosynthesis was measured, negative UV-B effects were found, however, the effects were transient over the experimental period (Wulff *et al.* 2008). Over a 4-month field experiment in Antarctica, no negative UV effects on hard bottom benthic diatoms were observed (Zacher *et al.* 2007b). Thus, benthic diatoms are very tolerant to UV exposure.

Reductions in metabolism and in particular respiratory activity have been proposed as major factors enabling microalgae to survive periods of darkness (Anderson 1975a,b). In our study, the diatoms were transferred to 20 ml vials with ca 15 ml f/2 medium without further addition of nutrients over the treatment periods. To overcome a possible nutrient depletion it is possible organic nutrients were used for a reduced metabolism. Bunt & Lee (1972) found that the carbon content of *Fragilaria sublinearis* van Heurck increased in the dark coinciding with a decline in cell concentrations although no nutrients were added over the 3-month dark period. They suggested a re-utilization of organics derived from senescent members of the population – a scenario possible also in our case. Facultative heterotrophy is not a new observation for diatoms and was reported as early as 1953 (Lewin 1953). Although heterotrophic growth is more common among pennate, benthic forms (Hellebust & Lewin 1977, Rivkin & Putt 1987), the centric diatoms *Cyclotella cryptica* and *Coscinodiscus* sp. both grew well over a 1 year period without any major reduction in cellular carbon and nitrogen, chlorophylls *a* and *c*, or in photosynthetic ability, compared with light grown cells (White 1974).

Temperature seems to be important for the ability to withstand darkness and the temperature dependency appears to be related to the actual temperature range at the region of occurrence. Therefore it is not surprising that at least some cold water species do not survive dark periods when the temperatures at their natural habitats are not considered (cf Table 1).

Over the first 15 days, it appears diatoms relied on cellular reserves to maintain their photosynthetic apparatus. A similar result was reported by Popels *et al.* (2007) for the planktonic *Aureococcus anophagefferens*. In their study, no changes in chl *a* or ribulose bisphosphate carboxylase (rubisco) were found during 14 days of darkness. After our dark treatment of 64 days, however, a degradation of the photosynthetic apparatus had possibly started (very low F_v/F_m) but the diatoms were still able to start to recover upon light exposure. Half-time of D1 protein turnover can be 30 min (Aro *et al.* 1993) and we exposed the diatoms for at least 4 h radiation.

We conclude that the benthic diatoms in our study were able to resume photosynthetic activity after 64 days in darkness and they were able to cope with relatively high intensities of UVR compared with their natural habitat. However, although the cells surviving the darkness were able to cope with the applied UVR, each individual cell in the population did not survive 64 days in the dark.

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