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1 FREEZING, MELTING AND LIGHT STRESS ON THE PHOTOPHYSIOLOGY OF
2 ICE ALGAE: *EX SITU* INCUBATION OF THE ICE ALGAL DIATOM
3 *FRAGILARIOPSIS CYLINDRUS* (BACILLARIOPHYCEAE) USING AN ICE TANK¹

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RUNNING HEAD: Ice algal physiology in an ice tank

32

33 KEYWORDS: active chl *a* fluorescence; algal pigments; ice tank incubation;

34 photoprotection; *psbA*; *rbcL*; sea ice

35

36 ABBREVIATIONS:

37 cDNA, complementary DNA; Chl *a*, chlorophyll *a*; ChlF, active chlorophyll *a*

38 fluorescence; DD, diadinoxanthin; DT, diatoxanthin; DES, de-epoxidation state index

39 of DD-DD xanthophyll cycle; ETR, electron transport rate; Fe³⁺, inorganic Fe species;

40 FRRf, fast repetition rate fluorometry; HL, high light; LL, low light; NPQ, non-

41 photochemical quenching; PQ, plastoquinone; *PP*, primary productivity; PPC,

42 photoprotective carotenoid; PSC, photosynthetic carotenoid; Q_A, the first quinone

43 electron acceptor; Q_B, secondary quinone electron acceptor; Tchl *a*, total chl *a*;

44 UHPLC, ultra-high performance liquid chromatography

45

46 ABSTRACT

Sea-ice algae contribute up to 25% of the primary productivity of polar seas and seed large-scale ice-edge blooms. Fluctuations in temperature, salinity, and light associated with the freeze/thaw cycle can significantly impact the photophysiology of ice-associated taxa. The effects of multiple co-stressors (i.e., freezing temperature and high brine salinity or sudden high light exposure) on the photophysiology of ice algae were investigated in a series of ice tank experiments with the polar diatom *Fragilariopsis cylindrus* under different light intensities. When algal cells were frozen into the ice, the maximum quantum yield of photosystem II photochemistry (PSII) (F_v/F_m) decreased possibly due to the damage of PSII reaction centres and/or high brine salinity stress suppressing the reduction capacity downstream of PSII. Expression of the *rbcL* gene was highly upregulated, suggesting that cells initiated strategies to enhance survival upon freezing in. Algae contained within the ice-matrix displayed similar levels of F_v/F_m regardless of the light treatments. Upon melting out, cells were exposed to high light ($800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), resulting in a rapid decline in F_v/F_m and significant upregulation of non-photochemical quenching (NPQ). These results suggest that ice algae employed safety valves (i.e., NPQ) to maintain their photosynthetic capability during the sudden environmental changes. Our results infer that sea ice algae are highly adaptable when exposed to multiple co-stressors and that their success can, in part, be

- 65 explained by the ability to rapidly modify their photosynthetic competence; a key factor
- 66 contributing to algal bloom formation in the polar seas.

INTRODUCTION

Sea ice algae are significant primary producers in sea ice zones. They contribute 5–25% of the total primary production (*PP*) in seasonally ice-covered regions (Legendre et al. 1992, Lizotte 2001, Michel et al. 2006, Loose et al. 2011, Arrigo 2017) and ~50% of the annual *PP* in perennially ice-covered regions (Sato et al. 1989, McMinn et al. 2010, Fernandez-Mendez et al. 2015). Ice-associated algae are of high ecological importance as not only the primary energy source for sea ice biota such as zooplankton, krill, larval fish and benthos (Lizotte 2001, Boetius et al. 2013, 2015, Kohlbach et al. 2017, Moteki et al. 2017, Bernard et al. 2018) but also as seed biomass for ice-edge blooms in marginal sea ice zones (e.g., Smith and Nelson 1986, Syvertsen 1991, Arrigo and van Dijken 2003, Arrigo 2014). Ice-edge blooms, which ice algae may seed, contribute ~50% of the total *PP* in marginal sea ice zone of the Southern Ocean (Smith and Nelson 1986, Sakshaug and Slagstad 1991, Deppeler and Davidson 2017), although it is still controversial which ice algae or phytoplankton actually contribute to the blooms (see review of van Leeuwe et al. 2018). Upon freezing in, algae can form aggregates within microstructures of the ice, which channel excluded liquid brine from sea ice to under-ice seawater when ice grows (i.e., brine channels) (Melnikov and Bondarchuk 1987, Assmy et al. 2013, Boetius et al. 2013, Katlein et al. 2015,

Fernandez-Mendez et al. 2014, Boetius et al. 2015). During the sea ice melt, these algal aggregates can rapidly sink and transport carbon down to the sea floor. This process contributes to the biological carbon pump (Riebesell et al. 1991, Taguchi et al. 1997, Boetius et al. 2013, Katlein et al. 2015) and underlines the important contribution of ice algae to both the ecology and biogeochemistry of sea ice zones. It is thus important to understand how ice algae survive in the dynamic and heterogeneous sea ice environment. Sea ice environments are subject to large fluctuations in temperature (~ -20 °C to -1.8 °C; Petrich and Eicken 2017), salinity (~ 25 to 170; Arrigo et al. 2010) and light availability (~ 0 to $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$; Galindo et al. 2017). Variation in these abiotic parameters can influence the photosynthetic efficiency of ice algae, however, they are not independent (i.e. multiple co-stressors; McMinn et al. 2017). Few studies have addressed the influence of multiple co-stressors on the photophysiology of sea ice algae (Ralph et al. 2005, 2007, Ryan et al. 2011, Martin et al. 2011, McMinn et al. 2014; see review of McMinn 2017), and little is known about how co-stressors influence ice algal photosynthesis and production during freeze/thaw cycles (McMinn 2017). Despite their significant ecological and biogeochemical importance, it remains difficult to directly obtain the photophysiological properties of sea ice taxa *in situ*. Kennedy et al. (2012) were the first to use sea ice tanks to undertake short-term

incubation of the haptophyte *Phaeocystis antarctica* within the laboratory. This method has the advantage that ice algae can be incubated in an environment mimicking pack ice conditions. Although many studies have addressed ice algal photosynthesis and production using isolates resuspended in water (e.g., Yan et al. 2019), it remains difficult to incubate them in the laboratory (e.g. in a culturing flask), while simulating realistic environmental conditions. Using a previous ice tank model, Kennedy et al. (2012) found that a freezing event suppressed the photochemical efficiency of PSII. Although these experiments were able to reveal the effects of freezing on the photophysiology of ice algae, their short-term nature did not address the longer-term mechanism of freezing stress. Understanding how ice algae maintain photosynthetic capacity and survive during freeze/thaw cycles is of considerable importance to better assess primary productivity in sea ice environments. In this study, a novel 70 L ice tank was constructed for the long-term incubation of ice algae under ecologically realistic conditions. It was used to investigate the effects of multiple co-stressors on ice algal photophysiology:

- (1) freezing stress with low temperature and high brine salinity;
- (2) chronic low light availability under low temperature and high salinity;

(3) melting stress with exposure to less saline meltwaters and sudden increase in light.

The sea ice diatom *Fragilariopsis cylindrus* was maintained in the ice tank for 20 days followed by ice melt and light exposure experiments to reproduce the environment that released ice algae experience. This study aims to investigate the effects of multiple co-stressors on ice algal photophysiology in an artificial sea ice environment during a freeze/thaw cycle. It provides new insights into how ice algae are able to tolerate environmental fluctuations that contribute to their dominance of ice-edge blooms.

MATERIALS AND METHODS

Ice tank incubation

The polar pennate diatom *Fragilariopsis cylindrus* (isolated from Antarctic sea ice in 2015, Davis station, East Antarctica; Kennedy et al. 2019), was incubated in a purpose designed ice tank (Island Research, Tasmania). The algal culture was less than two years old at the start of the experiments. The ice tank was contained within a freezer (–20 °C), and the ice thickness and temperature gradient of the ice were controlled by interactions between a basal heater and the adjustable ambient freezer temperature (Fig. 1). This enabled an ice thickness of approximately 5.5 cm to be maintained during the experiment. *F. cylindrus* was incubated in Aquil media (Price et al. 1989), buffered with

ethylenediaminetetraacetic acid (EDTA) (final concentration 5.00 μM) at a salinity of 35, and a Fe concentration of 400 nM. The concentration of total inorganic forms of Fe (Fe') was 1.54 nM, this being continuously supplied from the Fe-EDTA complexes to the medium calculated using the software Visual MINTEQ, ver. 3.1 (<https://vminteq.lwr.kth.se>). Light intensities beneath the ice, measured with a spherical photosynthetically active radiation (PAR) sensor (QSL-100, Biospherical Instrument Inc.), were set at 150 and 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (White LED, PANEL-300-18W, LED Lighting Products, Australia; Fig. S1) as high and low light (HL and LL) treatments, respectively. Two discrete ice tank runs were conducted for each light treatment. The diatom *F. cylindrus* was inoculated with low cell densities (HL: $4011 \pm 151 \text{ cells mL}^{-1}$; LL: $3933 \pm 132 \text{ cells mL}^{-1}$) to avoid the possibilities of self-shading and nutrient starvation during the incubation experiments. They were maintained in the ice tank for 3 days for acclimation where seawater temperature was maintained at 2.5 °C. Prior to freezing in, a sample was taken to assess the original physiological state of the algae (day -05, hereafter). The temperature lowered to -1.8 °C to initiate ice formation. Once ice had formed after 3 days, the under-ice water was partially replaced with ultrapure water to reduce the salinity down to 35; as the salinity of the underlying water had increased (to ~38) as a result of brine rejection. Two-day acclimation period

was used prior to sampling. Ice sampling was conducted every 5 days for 20 days (days 00, 05, 10, 15, and 20). Brine salinity and brine fraction were estimated following Cox and Weeks (1983) and Eicken (2009). To minimize heterogeneity among ice cores, ice samples were randomly collected from the tank chamber (pseudo-replicate) with a trace metal-free hand drill (2 cm in diameter) from randomly annotated grids on the ice surface, following normal random sampling numbers generated by the software R (<https://www.r-project.org/>). To assess both the effects of melting and high light exposure, ice samples were melted at 2.5 °C and salinity 25 for 2 days (melt samples, hereafter). The melt samples were then exposed to intense light (light samples, hereafter) ($800 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) to mimick the typical summer surface (MODIS/Aqua).

Fast repetition rate (FRR) fluorometry

To monitor the photophysiology of *F. cylindrus* during the freezing and melting processes, variable chlorophyll *a* fluorescence (ChlF) measurements were conducted using a bench-top Fast Repetition Rate fluorometer (FRRf) (FastOcean Act2Run Systems, Chelsea Technologies) with Act2Run software (Chelsea Technologies). Thawed ice samples were buffered with filtered seawater (ice:seawater ratio = 1: 1) at

2.5 °C in the dark for 30 min, and the slushy melted ice samples were placed in a quartz cuvette to measure fluorescence. A single turnover protocol was applied for ChlF measurement; 100 flashlets with 1 µs duration at a wavelength 450 nm and 2 µs intervals for excitation of reaction centres of PSII and 20 flashlets with 1 µs duration and 20 flashlets with 1 µs duration and 100 µs intervals for relaxation. Eighteen light steps were applied to generate a rapid light curve (RLC) from 0 to 800 µmol photons m⁻² s⁻¹, taking <5 min to complete one RLC. At each light step (~15 s), at least five induction and relaxation curves were averaged to obtain ChlF yields, described in Table 1, after calibrating the ChlF yields with filtered seawater. According to the models proposed by Kolber et al. (1998), photosynthetic parameters of chlorophyll *a* (chl *a*) induction curves were calculated based on the ChlF yields as shown in Table 1. Electron transport rate through the reaction centres of PSII (RCII) (ETR_{RCII}) was calculated as follows:

$$ETR_{RCII} = E \times \sigma_{PSII} \times \frac{F'_q/F'_m}{F_v/F_m} \times \Phi_{RCII} \times 6.022 \times 10^{-1}$$

following Suggett et al. (2011) and Schuback et al. (2016), an alternative approach to calculating ETR_{RCII} of Kolber et al. (1998), wherein Φ_{RCII} is the quantum yield of RCII

assumed as 1, and the following number is a conversion factor to mol quanta mol
 $\text{RCII}^{-1} \text{ s}^{-1}$. The calculated ETR_{RCII} at each light step were fitted to the model of Platt et
 al. (1980) to obtain photosynthesis–irradiance ($\text{ETR}_{\text{RCII}}-E$) parameters (Table 1). To
 assess the heat dissipation of algal cells, non-photochemical quenching based on the
 normalized Stern-Volmer (S-V) coefficient (NPQ_{NSV}') under the actinic light equivalent
 to the incubation light intensities was also calculated following McKew et al. (2013):

$$\text{NPQ}_{\text{NSV}}' = F_o'/F_v'$$

where F_o' is a minimum ChlF yield after a relaxation sequence calculated following
 Oxborough and Baker (1997) (Table 1).

Validation experiments of melting procedures of ice samples for ChlF measurement

While ChlF has been widely used on seawater samples (e.g., Suggett et al. 2011), it
 has not been broadly applied to ice algae research (Miller et al. 2015) because of the
 need for the ice samples to be melted. Melting the ice samples changes the
 photophysiology of the algal cells (e.g., Garrison and Buck 1986, Mikkelsen and
 Witkowski 2010, Rintala et al. 2014, Campbell et al. 2019). Here, multiple comparisons

were conducted on melt procedures of an ice sample from the ice tank. Measurements of ChlF were performed on three treatments of ice samples; (1) intact ice, (2) slushy melted ice suspended in filtered seawater (ice: seawater ratio=1: 1), (3) slowly (24 h) and directly melted samples. The blank calibration for F_o was conducted with filtered seawater as described, however, an empty quartz tube was used for the calibration because it was impossible to prepare a blank ice sample for the treatment 1. It was thus assumed that the ice samples for the treatment had a high enough biomass without F_o calibration. All samples were stored or melted in the dark for 30 min for the treatments 1 and 2, whereas the other samples (i.e. the treatment 3) were kept at 2.5 °C during the overnight melting. The FRRf was used for the ice samples from the ice tank as described above.

Cell abundance of ice algae

Triplicated microscopic samples were fixed with neutralized formaldehyde solution (final concentration 2%). Cell abundance of *F. cylindrus* was determined with an inverted microscope (Axiovert 25, Zeiss) and a Sedwick-Rafter counting chamber (Pyser-SGI Lmd.). At least 500 cells were enumerated to determine cell abundance (LeGresley and McDermott 2010). Growth rate was calculated following Wood et al.

(2005):

$$\mu = \frac{\ln(N_t/N_0)}{t - t_o}$$

Pigment composition

Pigment concentrations were determined to quantify chlorophylls, photosynthetic and photoprotective carotenoids, and chlorophyll derivatives with a Ultra-High Performance Liquid Chromatography (UHPLC) (Suzuki et al. 2015). On each sampling day, triplicated ice or seawater samples were filtered onto 25 mm GF/F filters (Whatman) using gentle vacuum (<0.013MPa) via a 25 mm polypropylene in-line filter holder (Swinnex, Merck). Rintala et al. (2014) confirmed that fast and direct melting was a reliable method for pigment analysis in ice algae. The filter was flash frozen in liquid nitrogen and stored in a deep freezer (−80 °C). After thawing, the filter was blotted dry with a filter paper, and the pigments were extracted using the *N, N*-dimethylformamide (DMF) bead-beating method (Suzuki et al. 2015). The extracted pigments were suspended in DMF and then injected into an UHPLC for pigment determination. The ratio of diadinoxanthin (DD) and diatoxanthin (DT) was calculated to assess the xanthophyll epoxidation-de-epoxidation cycle and photoprotective strategies of algae (Katayama et al. 2017), assessed as a de-epoxidation state (DES; DT/(DD+DT)). Total

chl *a* (Tchl *a*: sum of chl *a*, chlorophyllide *a*, chl *a*-allomer, and chl *a*-epimer)
concentrations were calculated as an indicator of algal biomass. Contributions of
chlorophyllide (chlide *a*) to Tchl *a* were also calculated as an index of the breakdown
of chl *a*. Following Schuback et al. (2016) and Yan et al. (2019), ratios of
photoprotective carotenoids (PPC: DD, DT, and β,β -carotene) to photosynthetic
carotenoids (PSC: only fucoxanthin here) in diatoms and the xanthophyll pool size
((DD+DT)/Tchl *a*) were calculated to quantify photoprotective potentials of the diatom
F. cylindrus.

Gene expression of photosynthesis-related genes; psbA and rbcL

To investigate the mechanisms of freezing and melting stress on cells of *F. cylindrus*
in water and in the ice, gene expression of the photosynthesis-related genes, *psbA* and
rbcL, was measured. To stabilize the RNA, 250 μ L of RNAlater (Sigma) was
immediately added to the ice core after collection. Triplicated melted ice and seawater
samples were filtered onto two 25 mm, 2 μ m polycarbonate Isopore membrane filters
(Millipore) with gentle vacuum (<0.013MPa) passing through a 25 mm polypropylene
in-line filter holder (Swinnex, Merck) for DNA and RNA samples. The DNA samples
were placed in a cryotube and flash frozen in liquid nitrogen and stored at -80°C . RNA

samples were suspended in 600 μ L RLT buffer (Qiagen) in a cryotube, to which 10 μ L of β -mercaptoethanol (Sigma-Aldrich) was added. The RNA sample then was flash frozen in liquid nitrogen prior to storage at -80°C until further analysis. DNA and RNA were extracted following Endo et al. (2013) and Endo et al. (2015), respectively. The extracted RNA was reverse-transcribed to complementary DNA (cDNA) with the PrimeScriptTM RT Master Mix (RR036, Takara) reagent according to the manufacturer's specification. DNA and cDNA copy numbers of the *psbA* and *rbcL* genes were quantified by quantitative PCR (qPCR). The primer sets and PCR conditions used in this study are shown in Table S1 in the Supporting Information. Gene expression was defined as the ratio of copy numbers of cDNA to DNA.

Statistical analysis

Statistical analyses were conducted using the SigmaPlot software program ver. 11.0 (SystStat Software, Inc.). One-way ANOVA with Tukey's test was performed on the individual sampling days in each light treatment to identify variations in ChlF parameters, pigment concentrations and gene expression. The differences in each parameter were considered significant when $p < 0.05$. Two-way ANOVA with Tukey's

tests was performed on the individual sampling days and light treatment to identify the differences in ChlF and pigment data to assess the effects of differences in photosynthetic ability and pigment composition between light treatments. Data was transformed for normalization prior to the ANOVA tests with the R statistical software using the R package MASS. Normality and variance of the data were checked by performing Shapiro-Wilk's test and Levene's test using the SigmaPlot software program after the data transformation, respectively.

RESULTS

Ice physics and ice algal growth

During the experiments, ice thickness remained stable at 5.5 cm with little basal melting or sublimation. Temperature within the ice increased with depth (ice surface: $-22.5\text{ }^{\circ}\text{C}$, ice-water interface: $-2.2\text{ }^{\circ}\text{C}$) (Fig. 2) and the underlying seawater was stable at ca. $-1.8\text{ }^{\circ}\text{C}$ throughout the incubations. Brine salinity decreased with depth from 293.7 ± 0.0 to 39.4 ± 0.5 , whereas the brine fraction increased ($3.7 \pm 0.0\%$ to $34 \pm 0\%$, assuming no gas bubbles were present). Nutrient concentrations did not significantly change during the incubations (data not shown). The ice formation concentrated *F. cylindrus* cells in the ice (HL: $8883 \pm 224\text{ cells mL}^{-1}$; LL: $8600 \pm 573\text{ cells mL}^{-1}$)

compared with under-ice seawater (3551 ± 95 cells mL^{-1} ; LL: 3639 ± 135 cells mL^{-1}).

Cells of *F. cylindrus* were thus successfully incorporated into the ice matrix where slow but positive growth in both treatments was observed (0.020 ± 0.001 and 0.020 ± 0.002 under HL and LL, respectively); there was no significant difference in growth rate between the treatments ($t=0.00$, $p>0.05$, Welch's t -test).

Variable chl a fluorescence

Dark values (F_v/F_m and σ_{PSII})

At the beginning of the incubations (day -05), initial F_v/F_m of *F. cylindrus* showed comparable and relatively high values in both high light (HL: 0.47 ± 0.05) and low light (LL: 0.45 ± 0.06) conditions ($t=0.63$, $p>0.05$, Welch's t -test) (Fig. 3A, B). Upon freezing in (day 00), F_v/F_m decreased significantly to 0.2–0.3 in both HL and LL (HL: $F_{(7,40)}=143.27$, $q=31.26$, $p<0.001$; LL: $F_{(7,40)}=501.93$, $q=35.77$, $p<0.001$, one-way ANOVA, Tukey's test) (Fig. 3A, B), while σ_{PSII} did not change (HL: $F_{(7,40)}=10.59$, $q=2.01$, $p>0.05$; LL: $F_{(7,40)}=12.97$, $q=3.65$, $p>0.05$, one-way ANOVA) (Fig. 3C, D).

Once incorporated into the ice matrix the F_v/F_m of *F. cylindrus* cells remained stable from day 00 to day 20 regardless of the light availability (HL: 0.20 ± 0.03 ; LL: 0.16 ± 0.05 , $t=1.68$, $p>0.05$, Welch's t -test), until initiation of the melt cycle. The lowest F_v/F_m

was observed on day 15 in both HL and LL (HL: 0.18 ± 0.02 ; LL: 0.09 ± 0.02) (Fig. 3A, B). The σ_{PSII} values at both light levels were relatively stable throughout the incubations with slight decreases with time (Fig. 3C, D). Once the ice had melted, F_v/F_m values seemed to be recovered from the values at day 20 in LL (HL: 0.18 ± 0.02 ; LL: 0.19 ± 0.02) to almost equivalent levels on day -05 (see above), respectively. There were, however, significant differences in F_v/F_m values between day -05 and melt samples both in HL and LL (HL: $q=35.57$, $p<0.001$; $q=35.77$, $p<0.001$, one-way ANOVA, Tukey's test) ($39 \pm 1\%$ lower and $15 \pm 1\%$ higher, respectively) (Fig. 3A, B). The responses of σ_{PSII} differed to F_v/F_m , which was significantly different on day -05, but similar to σ_{PSII} values on day 20 regardless of the light availability (HL: $q=1.07$, $p>0.05$; LL: $q=1.08$, $p>0.05$, one-way ANOVA) (Fig. 3C, D). Immediately after light exposing the melted samples at $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, F_v/F_m significantly decreased in both light treatments (HL: $q=13.09$, $p<0.001$; LL: $q=53.11$, $p<0.001$, one-way ANOVA, Tukey's test) ($39 \pm 3\%$ and $29 \pm 2\%$ for HL and LL treatments, respectively). Despite the large decreases in F_v/F_m , σ_{PSII} showed minimal variation.

Non-photochemical quenching (NPQ_{NSV})

Values of non-photochemical quenching (NPQ_{NSV}) in both light treatments were low

on day -05 prior to freezing in. Upon ice incorporation, NPQ_{NSV}' significantly increased in both HL and LL treatments (HL: $F_{(7,40)} = 52.55$, $q=44.54$, $p<0.001$; LL: $F_{(7,40)} = 149.81$, $q=10.10$, $p<0.001$, one-way ANOVA, Tukey's test) (Fig. 4). NPQ_{NSV}' in HL plateaued immediately after ice formation (i.e., day 00), while NPQ_{NSV}' in LL was observed to gradually increase and reach a maximum on day 05 (Fig. 4). Post ice melt, NPQ_{NSV}' in the LL tank decreased to the identical levels as those observed on day -05 ($q=1.36$, $p>0.05$, one-way ANOVA), whereas the HL treatment showed a significant increase in NPQ_{NSV}' ($q=6.676$, $p<0.001$, one-way ANOVA, Tukey's test). The light exposure after the melting event significantly enhanced NPQ_{NSV}' (almost doubled) in both HL and LL treatments (HL: $q=11.39$, $p<0.001$; LL: $q=5.22$, $p=0.012$, one-way ANOVA) (Fig. 4).

Photosynthesis-irradiance (ETR_{RCH}-E) curve

The initial slopes of ETR_{RCH}-E curve, α (regarded as light utilization efficiency), dropped significantly when the algae were frozen into the ice (HL: $F_{(7,40)} = 8.20$, $q=6.84$, $p<0.001$; LL: $F_{(7,40)} = 11.27$, $q=8.24$, $p<0.001$, one-way ANOVA, Tukey's test) but did not significantly differ thereafter in HL treatment ($p>0.05$, one-way ANOVA) (Fig. 5A, B). A similar trend was observed in the LL treatment, although α increased significantly

towards the end of the incubation ($p<0.05$, one-way ANOVA, Tukey's test). After the ice melted, α values did not significantly change in HL ($q=0.97$, $p>0.05$, one-way ANOVA), while, in LL, α returned the initial levels at day -05. Maximum electron transport rates (ETR_{max}) in both treatments significantly decreased upon freezing in (HL: $F_{(7,40)}=7.63$, $q=11.85$, $p<0.001$; LL: $F_{(7,40)}=86.30$, $q=18.52$, $p<0.001$, one-way ANOVA, Tukey's test) (from 281 ± 37 and 300 ± 67 to 111 ± 53 and 38 ± 13 in HL and LL treatments, respectively). Upon melting out, ETR_{max} in LL returned (335 ± 57) to the initial levels at day -05 ($q=2.40$, $p>0.05$, one-way ANOVA), whereas, in HL, ETR_{max} was not recovered ($q=14.41$, $p<0.001$, one-way ANOVA, Tukey's test) (Fig. 5C, D). Light saturation indices, E_k , gradually decreased during the course of the incubation experiments without any conspicuous variation during the frozen period (Fig. 5E, F). The HL treatment showed higher E_k throughout the frozen period from day 05 to day 20 compared to LL ($F_{(1,40)}=9.02$, $p=0.004$, two-way ANOVA, Tukey's test). When melted out, E_k values returned to the initial levels on day -05 in both treatments although the LL treatment show a higher E_k (HL: $F_{(7,40)}=7.63$, $q=0.63$, $p>0.05$; LL: $F_{(7,40)}=66.51$, $q=12.30$, $p<0.001$, one-way ANOVA, Tukey's test). On the other hand, E_k significantly decreased in both HL and LL treatments (HL: $q=8.20$, $p<0.001$; LL: $q=7.91$, $p<0.001$, one-way ANOVA, Tukey's test) (Fig. 5E, F).

369

370 *Validation experiments for sample melt procedures*

371 Variable ChlF parameters varied among the different melt procedures of the ice sample
372 (Fig. S2 in the Supporting Information). When the intact crushed ice sample was packed
373 directly into a quartz tube, F_v/F_m values were lower, followed by that of the slushy
374 melted sample. These values were almost the same and did not show any significant
375 difference ($F_{(2,7)}=10.76$, $q=2.90$, $p>0.05$, one-way ANOVA), whereas the slow-direct
376 melt sample showed a significantly higher value compared to its counterparts ($q=4.94$,
377 $p=0.024$, one-way ANOVA, Tukey's test). Unlike F_v/F_m values, σ_{PSII} varied little
378 regardless of the melt methods and did not show any significant enhancement or
379 suppression ($F_{(2,7)}=3.31$, $p>0.05$, one-way ANOVA) (Fig. S2 in the Supporting
380 Information).

381

382 *Pigment composition*

383 Initial Tchl *a* concentrations were at the same level between incubations at day-05
384 ($F_{(1,31)}=2.29$, $p>0.05$, Two-way ANOVA) (Fig. 6A, B). After *F. cylindrus* cells were
385 incorporated into the ice, ice algae in the HL treatment maintained stable Tchl *a*
386 biomass ($F_{(7,16)}=2.27$, $q=0.09$, $p>0.05$, one-way ANOVA) although Tchl *a* in the LL

treatment showed a gradual increase. Contributions of chl *a* to Tchl *a* were slightly
 higher in the HL treatment than in the LL tank throughout the incubations
 ($F_{(1,31)}=59.96$, $p<0.001$, Two-way ANOVA, Tukey's test; 48.0–62.5% and 34.0–54.9%,
 respectively) (Fig. 6A, B). The cellular DD-DT pool size in both the HL and LL
 treatments at the beginning of the incubations was comparable ($F_{(1,31)}=73.27$, $q=1.76$,
 $p>0.05$, Two-way ANOVA), whereas, during the frozen period the cellular pool size
 grew faster was significantly larger in the HL ice tank than those in the LL tank
 ($F_{(7,16)}=73.27$, $q=12.11$, $p<0.001$, Two-way ANOVA, Tukey's test; 17.3–25.3% and
 8.8–14.7% to Tchl *a*, respectively) (Fig. 6C, D). During the frozen period from day 05
 to day 20, DES was also higher in the HL ice tank (0.293–0.552) compared to the LL
 ice tank (0.107–0.231) ($F_{(1,31)}=35.65$, $p=0.027$, Two-way ANOVA, Tukey's test) (Fig.
 6C, D). Light exposure enhanced the DES in both treatments (4.20- and 5.23-fold
 increases in the HL and LL ice tank, respectively). Higher PPC/PSC ratios were present
 in the HL ice tank than in LL throughout the incubations ($F_{(1,31)}=69.22$, $p<0.001$, Two-
 way ANOVA, Tukey's test) (Fig. 6E, F). The PPC/PSC ratios showed little variation
 before and after light exposure in both light treatments (HL: $F_{(7,16)}=3.82$, $q=0.04$,
 $p>0.05$; LL: $F_{(7,16)}=3.86$, $q=0.85$, $p>0.05$, one-way ANOVA).

Gene expression of photosynthesis-related genes

rbcL

Prior to incubation there was no significant difference in the expression of the *rbcL* gene in either HL or LL ($t=0.33$, $p>0.05$, Welch's t -test) (Fig. 7A, B). Transcriptional activity of the *rbcL* gene was highly upregulated when algae were frozen into the ice (HL: $F_{(7,16)}=7.65$, $q=5.30$, $p=0.03$; LL: $F_{(7,16)}=7.39$, $q=6.89$, $p=0.003$, one-way ANOVA, Tukey's test) in both HL and LL (Fig. 7A, B). After day 05 and day 10, gene expression of the *rbcL* gene decreased significantly in the HL and LL treatments (HL: $q=7.290$, $p=0.002$; LL: $q=6.79$, $p=0.040$, one-way ANOVA, Tukey's test), respectively (Fig. 7A, B). This low level was sustained during the frozen period. Comparing the results of melt and light periods, the *rbcL* gene expression did not show any conspicuous change in either treatment (HL: $q=2.173$, $p>0.05$; LL: $q=0.31$, $p>0.05$, one-way ANOVA).

psbA

Unlike the *rbcL* gene, the expression of the *psbA* gene differed with respect to light availability (Fig. 7C, D). The initial value in the HL treatment was ~2-fold higher than that in LL. In HL, transcription of the *psbA* gene was highly upregulated during freezing

($F_{(7,16)}=19.90$, $q=10.28$, $p<0.001$, one-way ANOVA, Tukey's test); which was similar to the *rbcL* gene. In LL, upregulation of *psbA* was not evident ($F_{(7,16)}=2.02$, $p>0.05$, one-way ANOVA). Expression of *psbA* in HL showed a similar trend to the *rbcL* gene, whereas, under LL, the *psbA* gene expression did not change throughout the experiment (Fig. 7C, D).

DISCUSSION

Ice tank incubation

We here, for the first time, successfully demonstrated the use of a purpose designed ice tank to study the photophysiology of ice algae for a prolonged period (i.e. 20 days) under environmentally relevant conditions. When ice formed, cooling of the water body from the surface in the ice tank, in a -20°C freezer, realistically mimicked *in situ* ice formation events in wintertime, with cells of *F. cylindrus* successfully incorporated into the ice. The temperature profile of the artificial sea ice indicates that the ice properties simulated winter ice environments, inferred from a sharp decrease in temperature with depth (Fig. 2) (Petrich and Eicken 2017). It suggests that the ice tank was well suited to investigate survival strategies of ice algae under chronic multiple stress in wintertime.

The identical initial F_v/F_m values at day-05 (Fig. 3A, B) indicate that the two discrete ice tank runs (i.e. HL and LL treatments) started with the similar physiological states of *F. cylindrus*.

Freezing event

The freezing events in the ice tank experiments reported herein suppressed photochemical quantum efficiency of PSII (Fig. 3A, B). Drawdowns in F_v/F_m were also observed in the previous ice tank study using *Phaeocystis antarctica* (Kennedy et al. 2012) during ice formation. This suppression has also been noted in natural sea ice, inferred from lower F_v/F_m of ice algae than pelagic phytoplankton (McMinn et al. 2008, Yamamoto et al. 2014).

A significant amount of chl *a* breakdown products has been reported in ice algae of both landfast and pack-ice in the Arctic and Antarctic (e.g., Horner 1985, Alou-Font et al. 2013). One might interpret the here observed increase in chl *a* (Fig. 6A, B) as inactivation of PSII reaction centres. Chl *a*, lacking the phytol chain, has a less pigment aggregation potential (Fiedor et al. 2003), leading to reduced coupling of excitons (inefficient energy transfer between pigments) (Rosenbach-Belkin et al. 1991).

However, the stable amount of chl *a* during the freezing events (Fig. 6A, B) indicates that the existence of chl *a* had only a minor effect on the drawdown in F_v/F_m . Also, not all inactive pigments (i.e. chl *a*) are involved in reaction centres but function as photosynthetic antennae pigments.

Suggett et al. (2009) demonstrated that chronic stress decreases F_v/F_m with a concomitant enhancement of σ_{PSII} because of the absorbed light energy being funnelled to fewer active PSII reaction centres if some reaction centres are damaged/inactive (Suggett et al. 2009, 2011, Falkowski and Raven 2013). In this study, however, little variation in σ_{PSII} was observed during the freezing event (Fig. 3C, D). This suggests that photoinactivation could not be significant during ice formation.

Freezing stress, combined with low temperatures and high brine salinity, could be the main cause of the reduction in the photochemistry of PSII and the capacity of electron sinks (Ensminger et al. 2006, Ralph et al. 2007). Low temperatures reduce membrane viscosity, which suppresses photochemical reactions in the electron transport chain (Morgan-Kiss et al. 2006). At the same time, high brine salinity might suppress the reduction capability of electron donors around PSII (i.e., the first quinone electron acceptor Q_A and the plastoquinone (PQ) pool; Ralph et al. 2007). Indeed, the here

observed high brine salinity (55 on average and >80 in the upper part of the ice) was high enough to suppress the downstream components of PSII (Ralph et al. 2007). This combined stress of low temperature and high salinity has been found to stagnate electron transfer along the electron transport chain down to ferredoxin and cause “electron clogging” (e.g., Lazár et al. 2005). This “electron clogging” consequently can lead to (a) the low F_v/F_m (e.g., Schwarz et al. 2017), (b) overreduction of the PQ pool (Maxwell et al. 1995, Allen and Nilson 1997, Pfannschmidt 2003) and (c) an imbalance of the photochemical electron transport (“photostasis”; Öquist and Huner 2003). We interpret the here observed increase in NPQ_{NSV}’ (Fig. 4) as a measure for alleviating excess excitation pressure (e.g. Caron et al. 1987, Ting and Owens 1993, Goss and Jakob 2010).

The HL treatment exhibited a larger DD-DT pool and higher PPC/PSC compared with the LL counterpart (Fig. 6C, D, E, F). These light responses can be interpreted as up-front protection to mitigate the production of reactive oxygen species under high light (McMinn and Hegseth 2004, Katayama and Taguchi 2013, Kuczynska et al. 2015). This notion is supported by field observations of (Kropuenske et al. 2009, Schuback et al. 2016, Galindo et al. 2017) and emphasized light-photoprotection relationships (Lavaud et al. 2002, Domingues et al. 2012).

Photosynthetic performance, assessed with $ETR_{RCII}-E$ curves, showed rapid photoacclimation responses during freezing; a greater decrease in ETR_{max} than that of α led to apparent dark acclimation with a lower E_k after freezing (Fig. 5). However, the concomitant decrease of α does not coincide with classical shade photoacclimatisation strategies (i.e. higher α and lower E_k under lower light would be expected; MacIntyre et al. 2002). The decrease in α suggests a decrease in n_{PSII} , the active fraction of PSII to chl *a* (e.g. Falkowski and Raven 2013). Values of α are represented as a product of σ_{PSII} and n_{PSII} ; however, σ_{PSII} values were stable during the freezing event, suggesting photoinactivation of RCII (i.e., lowered n_{PSII}). It is thus suggested that both the combination of direct photoinactivation and electron clogging deactivated the photochemical reactions at RCII. The freezing event, therefore, suppressed RCII photochemistry both directly and indirectly.

The strong upregulation of expression of the *psbA* gene in the HL treatment accelerated repair of damaged PSII under overexcitation pressure in the HL condition and vice versa (Fig. 7C, D) (Petrou et al. 2010, Galindo et al. 2017). The significant upregulation of the *rbcL* gene (Fig. 7A, B) could be evidence of a cold acclimation strategy to increase cellular RuBisCO and compensate for the low catalytic activity of the temperature-sensitive enzyme by increasing its concentration (Devos et al. 1998,

Lyon and Mock 2014, Young et al. 2015). However, Mock and Hoch (2005) reported downregulation of *rbcL* transcription in *F. cylindrus* under low temperatures.

During the frozen period

ChlF parameters remained relatively stable during the frozen period and little variation in F_v/F_m , σ_{PSII} , and NPQ_{NSV} values were observed within the ice (Figs. 3 and 4), indicating that sea ice is a stable platform for algal photosynthesis (Maccario et al. 2015, Arrigo 2017). The comparable levels of F_v/F_m and σ_{PSII} in both ice tanks suggest that the *F. cylindrus* cells sustained or optimized their photosynthesis within the ice regardless of light availability (Fig. 3). This physiological acclimation to ice environments may play a key role in the successful survival and proliferation of ice algae within and at the bottom of sea ice (Kropuenske et al. 2010, Lacour et al. 2018). McMinn and Hegseth (2004) suggested that ice diatoms can grow out in sea ice if F_v/F_m is >0.125 . In this study, the observed F_v/F_m values during the frozen period were high enough to actively grow in the ice. Indeed, this study, for the first time, reported positive growth in the ice tank. Although the growth rates in both treatments ($\sim 0.02\text{ d}^{-1}$) were lower than other reported values from culture experiments (e.g., Mock and Hoch 2008, Kropuenske et al. 2010). Interestingly, the growth rates in both the HL and LL

treatments were comparable regardless of the under-ice light intensities. This indicates
 that light availability was not a limiting factor for algal growth in the artificial sea ice.
 Evidently, photosynthetic and photoprotective abilities between the HL and LL ice
 tanks were comparable during the frozen periods, as inferred from the identical levels of
 F_v/F_m and NPQ_{NSV} (Fig. 3A, B; Fig. 4). As discussed above, the electron clogging
 caused by multiple co-stressors could be the major determinant of algal growth. The
 low temperature lowered the enzymatic activity of RuBisCO (Young et al. 2015), which
 could be another possible cause of the identical growth rates observed in both HL and
 LL treatments. The gradual increase in light utilization index α in the LL ice tank (Fig.
 5A, B) was consistent with the classical concept of photoacclimation (MacIntyre et al.
 2002). As noted above, σ_{PSII} was stable in the LL treatment (Fig. 3C, D). Cells under
 LL may increase the proportion of active RCII relative to pigmentation (chl *a*) in order
 to enhance light capture during freezing in (Fig. 5E, F). Only algae in the LL ice tank
 showed a gradual increase in Tchl *a* concentration, in spite of the same algal growth rate
 in both HL and LL treatments; this could be further evidence of dark acclimation (e.g.,
 Falkowski and Owens 1980). The smaller PPC/PSC in the LL ice tank also supports this
 suggestion by indicating preferential synthesis of photosynthetic chlorophylls and
 carotenoids rather than PPC (Fig. 6E, F). Cells in the HL treatment strengthened their

photoprotective capability, reflected in higher values of PPC/PSC and DES compared to cells in LL (Fig. 6C, D, E, F) (Kropuenske et al. 2009, 2010). However, both HL and LL treatments showed increased their DD-DT pool and DES, suggesting again a highly reduced PQ pool due to the electron clogging (i.e., the development of ΔpH) (Leptit et al. 2010, 2013). Interestingly, NPQ_{NSV}' levels were comparable between the light treatments regardless of the difference in DES (Figs. 4 and 5C, D). Nymark et al. (2013) suggested that low-light acclimated cells have more *LHCX* proteins, which enlarges their NPQ capacity (Bailleul et al. 2010). Interestingly, the transcriptional activity of the *rbcL* gene greatly decreased after the sharp increase on day 00 (onset of freezing), suggesting that cells had used the high abundance of RuBisCO (or its mRNA) that was synthesized during the freezing event (Fig. 7A, B). The constant but lower expression of *psbA* could keep the repair of PSII to a 'business as usual' rate (Fig. 7C, D), whereas the downregulation of the gene after initial significant upregulation in the HL treatment might be caused to alleviate the photochemical imbalance in the electron transport chain due to the upregulation when shocked by freezing stress (e.g. changes in PSII to PSI ratio).

Light exposure after ice melt

Light exposure to released algal cells in spring time suppressed photosynthetic activities. Exposure to irradiance following melting decreased F_v/F_m due to the high excitation pressure (Fig. 3A, B), despite ice melt before light exposure increasing photochemical activity. The increase in F_v/F_m after ice melt was consistent with results of the validation experiments (Fig. S2 in the Supporting Information). The degree of drawdown in F_v/F_m was comparable regardless of the incubation light intensity after light exposure (Fig. 3A, B). NPQ_{NSV} was downregulated when the ice melted but was enhanced after light exposure (Fig. 4). This reverse response to light exposure between F_v/F_m and NPQ_{NSV} might be evidence for fast activation of photoprotection (Figs. 3A, B and 4) especially in the HL treatment with the higher DES (Fig. 6C, D). Damage of PSII, however, was likely because of the considerable increase in the ratio of chl *a* to Tchl *a* after light exposure in both ice tanks (Fig. 6A, B). One might suspect that the melting process prior to sample filtration caused the significant breakdown of chl *a* with activation of chlorophyllase (Jeffery and Hallegraeff 1987); however, a stable level of chl *a* to Tchl *a* observed throughout the incubation period even from day -05 before ice formation (Fig. 5A, B) suggests the melting process (osmotic stress) could not be responsible for the high concentrations of chl *a* and other degraded pigments. However, the significant difference in chl *a* contributions between HL and LL

throughout the incubations might relate to the breakdown of chl *a* because high light to
F. cylindrus leads a high qI (non-photochemical quenching by photoinactivation)
 (Kropuenske et al. 2009). After ice melt, PPC/PSC decreased while F_v/F_m increased
 (Figs. 3A, B and 6E, F). This suggests a re-optimization of photosynthetic performance
 to a water environment after release from freezing stress. Light exposure, however,
 activated the DD-DT xanthophyll cycle (i.e., de-epoxidation) (Fig. 6C, D) (Petrrou et al.
 2010), although the size of the DD-DT pool was little changed (Fig. 6C, D). Activation
 of the DD-DT cycle is the fastest photoprotective strategy and occurs at a scale of
 seconds to minutes (Goss and Jakob 2010), whereas the size of the pool actually
 changes much slower. Interestingly, NPQ_{NSV}' in the HL treatment was much higher
 than that of the LL treatment with differences in their DD-DT pool size (Figs. 4 and 6C,
 D), suggesting that pigmentation and light history were largely responsible for their
 potential photoprotective ability (Szechyńska- Hebda et al. 2010, Galindo et al. 2017). It
 seems to be unusual that E_k values significantly decreased (i.e. dark acclimation) upon
 light exposure, contrary to the expected response from high-light acclimation (Fig. 5E,
 F). Transcriptional activity of the *psbA* gene was stable in both treatments after light
 exposure (Fig. 7C, D). It is suggested that algal cells did not immediately start to
 synthesize mRNA for repair of the damaged RCII, possibly because post-transcriptional

regulation is significant for D1 protein synthesis (Malnoë et al. 1988, Danon and Mayfield 1991) with a large mRNA pool (Mulo et al. 2012). The dark reaction might also be independent or to have been slow to respond to the light exposure event because the gene expression of the *rbcL* gene was invariant before and after light exposure (Fig. 6A, B).

CONCLUSIONS

This study demonstrates how the combined effects of ice formation and melting together with light exposure affect the photophysiology of *F. cylindrus*. This study is the first attempt to conduct the *ex situ* incubation of the ice algal *F. cylindrus* in artificial sea ice to quantify the effects of multiple co-stressors in sea ice and to provide new insights into the fate of ice algae. The ice tank successfully mimicked ice formation events as experienced in the field (Figs. 1 and 2). This enabled investigation into the effects of multiple co-stressors on the capacity of *F. cylindrus* to survive this ephemeral habitat. We successfully observed, for the first time, positive growth of ice algal cells in artificial sea ice. Ice formation events suppressed the photochemical process of RCII, despite optimal irradiance prior to freezing in (Fig. 3A, B). This was likely a result of high brine salinity and low temperature, which led to electron clogging in the electron

transport chain. Ice algal cells rapidly optimized their photophysiology to the dark environment within the ice, inferred from decreases in E_k (Fig. 3 and 5E, F). Light availability could affect the photoacclimative ability of ice algae, possibly by increasing active RCII. Further investigation on the connectivity of photosynthetic antennae to RCII would be needed to address the efficiency of energy transfer to RCII (e.g., D’Haene et al. 2015, Yoshida et al. 2018). Although ice melt stimulated algal photosynthesis, the subsequent light exposure resulted in photoinhibition (Fig. 3A, B) with significant chl *a* accumulation and NPQ_{NSV}’ enhancement. However, photoprotection was rapidly activated. We conclude that the diatom *F. cylindrus* possesses a high photosynthetic plasticity that contributes to the ecological success of this species in both pelagic and sea-ice habitats.

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635 Scientific Research (JP18H03352, JP17H00775).

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Table 1. Terminology and definition of chlorophyll *a* fluorescence yields obtained from FRRf fluorometry

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Figure 1. A schematic (A) and a photograph (B) of the 70 L ice tank in a $-20\text{ }^{\circ}\text{C}$ freezer.

Figure 2. Vertical profiles of ice temperature (T_{ice} ; closed circle), brine salinity (S_{br} : open circle), and the fraction of brine volume (V_{br} : closed square) in artificial sea ice in the ice tank.

Error bars indicate 1 standard deviation ($n=3$).

Figure 3. Maximum photochemical quantum yield of PSII (F_v/F_m) (A and B) and functional absorption cross-section of PSII (σ_{PSII}) (C and D) during the ice tank incubation experiments.

Left (A and C) and right (B and D) panels indicate data from the HL and LL treatment, respectively. Open, closed and shaded bars indicate values of seawater samples before freezing, ice and seawater samples after melting, respectively. Different letters above bars in a panel indicate significant differences in values between sampling days with one-way ANOVA with Tukey's test; there is no significant difference between values if a given letter is shown in the combination of letters of the counterparts. The D stands for 'day', while Melt and Light indicate values after melting and light exposure experiments, respectively. Error bars show 1 standard deviation ($n \geq 5$).

Figure 4. Non-photochemical quenching based on the normalized Stern-Volmer quenching coefficient under the actinic light (150 and $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for HL and LL, respectively) (NPQ_{NSV}) during the ice tank incubation experiments. Panels A and B indicate data from the HL and LL treatments, respectively. Different letters above bars in a panel indicate significant differences in values between sampling days with one-way ANOVA with Tukey's test; there is no significant difference between values if a given letter is shown in the combination of letters of the counterparts. The D stands for 'day', while Melt and Light indicate values after melting and light exposure experiments, respectively. Error bars show 1 standard deviation ($n \geq 5$).

669

670 Figure 5. Photosynthesis–irradiance ($ETR_{RCII}-E$) parameters during the ice tank
671 incubation experiments. Top panels (A and B) show light utilization efficiency under
672 dim light (α) as initial slopes of the $ETR_{RCII}-E$ curves, middle panels (C and D) indicate
673 maximum electron transport rate (ETR_{max}), and bottom panels (E and F) show light
674 saturation index (E_k). Left (A, C and E) and right (B, D, and F) panels show data from
675 the HL and LL treatments. Different letters above bars in a panel indicate significant
676 differences in values between sampling days with one-way ANOVA with Tukey’s test;
677 there is no significant difference between values if a given letter is shown in the
678 combination of letters of the counterparts. The D stands for ‘day’, while Melt and Light
679 indicate values after melting and light exposure experiments, respectively. Error bars
680 show 1 standard deviation ($n \geq 5$).

681

682 Figure 6. Variations in pigment concentrations during the ice tank incubation
683 experiments. Top panels (A and B) show total chl *a* concentration (Tchl *a*) [$mg\ m^{-3}$]
684 and contribution of chlorophyllide *a* to Tchl *a*, middle panels (C and D) indicate the size
685 of DD-DT pool ($(DD+DT)/Tchl\ a$) and de-epoxidation state of xanthophyll pigments

(DES; DT/(DD+DT)), and bottom panels (E and F) show ratio of photoprotective carotenoids (PPC) to the photosynthetic carotenoid (PSC) quantified with UHPLC. Left (A, C, and E) and right (B, D, and F) show data from the HL and LL treatments, respectively. The D stands for ‘day’, while Melt and Light indicate values after melting and light exposure experiments, respectively. Results of one-way and two-way ANOVA tests performed on the pigment data were described in the text. Error bars show 1 standard deviation ($n=3$).

Figure 7. Gene expression [cDNA/DNA] of photosynthesis-related genes. Top (A and B) and bottom (C and D) panels show gene expression of the *rbcL* and *psbA* genes during the ice tank incubation experiments. Left (A and C) and right (B and D) show data from the HL and LL treatments, respectively. Different letters above bars in a panel indicate significant differences in values between sampling days with one-way ANOVA with Tukey’s test; there is no significant difference between values if a given letter is shown in the combination of letters of the counterparts. The D stands for ‘day’, while Melt and Light indicate values after melting and light exposure experiments, respectively. Error bars show 1 standard deviation ($n=3$).

703

704 **LIST OF SUPPLEMENTAL MATERIALS**

705 Table S1. Primer sets and PCR conditions of qPCR and qRT-PCR for the *psbA* and
706 *rbcL* genes

707

708 Figure S1. A photo of the ice tank with a light panel.

709 The light panel has a white LED with light diffuser to homogenize the light field over
710 the ice.

711

712 Figure S2. Chlorophyll *a* fluorescence parameters with different melting procedures.

713 (a): Maximum quantum yield of PSII photochemistry (F_v/F_m); (b): functional

714 absorption cross-section (σ_{PSII}), determined with FRR fluorometry.

715 Intact ice: intact ice placed into the cuvette for ChlF measurement; slush; ice sample

716 partially melted in filtered seawater; Direct slow: melted ice sample overnight at 2.5 °C.

717 Different letters indicate significant differences between the given values. Error bars

718 indicate 1 standard deviation. $n=6$.

Table 1.

Yield and parameter		Unit	Derivation or reference
Fluorescence yield			
F	Fluorescence yield	Unitless	
F_o	Minimum fluorescence yield of dark regulated cells	Unitless	
F_m	Maximum fluorescence yield of dark regulated cells	Unitless	
F_v	Maximum variable fluorescence yield	Unitless	$F_m - F_o$
F'	Fluorescence yield under actinic light	Unitless	
F_o'	Minimum fluorescence yield of actinic light-acclimated cells	Unitless	Oxborough and Baker (1997)
F_m'	Maximum fluorescence yield of actinic light-acclimated cells	Unitless	
F_v'	Variable fluorescence yield under actinic light	Unitless	$F_m' - F_o'$
F_q'	Difference in fluorescence yields between F' and F_m'	Unitless	$F_m' - F'$
Fluorescence parameters in the dark			
σ_{PSII}	Functional absorption cross-section of PSII in the dark	$\text{nm}^2 \text{RCII}^{-1}$	Kolber et al. (1998)
F_v/F_m	Maximum quantum yield of PSII photochemistry	Unitless	$(F_m - F_o)/F_m$
τ	Time constant for Q_A reoxidation	ms	Kolber et al. (1998)
Fluorescence parameters under actinic light			
F_q'/F_m'	Effective photochemical efficiency of PSII under actinic light	Unitless	$(F_m' - F')/F_m'$
NPQ_{NSV}'	Non-photochemical quenching based on the S-V approach	Unitless	McKew et al. (2013)
ETR_{RCII}-E curve parameters			
ETR _{RCII}	Absolute electron transport rate through RCII	$\text{mol e}^{-1} \text{s}^{-1}$	Schuback et al. (2016)
E	Actinic light irradiance	$\text{mol photons m}^{-2} \text{s}^{-1}$	
α	Light utilization index under dim light	$(\text{mol e}^{-1} \text{s}^{-1}) (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$	Platt et al. (1980)
β	Light inhibition index under high light caused PSII photoinhibition	$(\text{mol e}^{-1} \text{s}^{-1}) (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$	Platt et al. (1980)
ETR _{max}	Maximum electron transport rate through RCII	$\text{mol e}^{-1} \text{s}^{-1}$	Platt et al. (1980)
E_k	Light saturation index	$\mu\text{mol photons m}^{-2} \text{s}^{-1}$	Platt et al. (1980)

Table S1

psbA

Forward primer	5'-AGAACCACCAAATACACCAGCAA-3'
Reverse primer	5'-TCCAAGCTGAGCACAAACATCTT-3'
Amplicon size	71
PCR condition	94 °C for 60 s; 40 cycles under 98 °C for 10 s, 62 °C for 60 s, and 72 °C for 60s
Baseline gene	Gene fragment of <i>Fragilariopsis cylindrus</i> from Antarctic sea ice ^{*1}
Reference	Krell et al. (2007)

rbcL

Forward primer	5'-GATGATGARAAAYATTAACWCW-3'
Reverse primer	5'-TAWGAACCTTTWACTTCWCC-3'
Amplicon size	113
PCR condition	94 °C for 60 s; 40 cycles under 98 °C for 10 s, 55 °C for 60 s, and 72 °C for 60s
Baseline gene	Gene fragment of <i>Thalassiosira weissflogii</i> (CCMP1336)
Reference	John et al. (2007); Endo et al. (2015)

720 *1: The isolate of *F.cylindrus* was obtained from Antarctic sea ice in the Weddel Sea in 1991

721 (Krell et al., 2007).

Fig. 1

724

725 Fig.2

Fig. 3

Fig. 4

731

732 Fig. 5

Fig. 6

Fig. 7

738 Figure S2

739

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