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Interactive effects of temperature and light during deep convection: a case study on growth and condition of the diatom *Thalassiosira weissflogii*

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Abstract:
Aim of this study was to expose phytoplankton to growth conditions simulating deep winter convection in the North Atlantic and thereby to assess changes in physiology enabling their survival. Growth rate, biochemical composition, and photosynthetic activity of the diatom *Thalassiosira weissflogii* were determined under two different light scenarios over a temperature range of 5–15°C to simulate conditions experienced by cells during winter deep convection. These metrics were examined under a low light scenario (20 µmol m^{-2} s^{-1}, 12/12 h light/dark), and compared with a scenario of short light pulses of a higher light intensity (120 µmol m^{-2} s^{-1}, 2/22 h light/dark). Both experimental light conditions offered the same daily light dose. No growth was observed at temperatures below 8°C. Above 8°C, growth rates were significantly higher under low light conditions compared with those of short pulsed light exposures, indicating a higher efficiency of light utilization. This could be related to (i) a higher content of Chl *a* per cell in the low light trial and/or (ii) a more efficient transfer of light energy into growth as indicated by constantly low carbohydrate levels. In contrast, pulsed intense light led to an accumulation of carbohydrates, which were catabolized during the longer dark period for maintaining metabolism. Light curves measured via Chl *a* fluorescence indicated low light assimilation for the algae exposed to short pulsed light. We postulate that our trial with short light pluses did not provide sufficient light to reach full light saturation. In general, photosynthesis was more strongly affected by temperature under pulsed light than under low light conditions. Our results indicate that model estimates of primary production in relation to deep convection, which are based on average low light conditions, not considering vertical transportation of algae will lead to an overestimation of *in situ* primary production.

Keywords: deep convection ; Diatoms ; growth rate ; light acclimation ; temperature

Introduction

The spring phytoplankton bloom of the northern North Atlantic is one of the largest biological events on earth influencing biogeochemical cycles and the marine food web (Feng et al., 2009, Platt et al., 2003). A common model for predicting the onset of a phytoplankton spring bloom is the ‚critical depth' model of Sverdrup (1953). This model assumes that net primary production is only possible when the mixed layer depth is shallower than a critical mixing depth, where depth-integrated phytoplankton production equals the loss e.g. by respiration and grazing. Many model and observational studies have supported Sverdrup´s hypothesis (Platt et al., 1991, Obata et al., 1996, Falkowski and Raven, 1997). However, new observations (Townsend et al., 1994, Backhaus et al., 2003, Behrenfeld, 2010) and modeling studies (e.g. Huisman et al., 1999, Nagai et al., 2003, Ross et al,. 2011, Mahadevan et al., 2012) identify flaws in Sverdrup´s critical depth model based on the development of a spring bloom before the onset of stratification.

One of the processes potentially influencing winter light conditions and thus winter production is the occurrence of deep convection (Backhaus et al., 2003). During deep convection, cells can be transported to depths of several hundred meters before potentially being returned to the surface (Marshall and Schott, 1999). Within such a convective cell phytoplankton cells are exposed to short pulses of light and long periods of darkness (MacIntyre et al., 2000).

Individual based model results suggest that phytoplankton cells in a convective cell have the potential to frequently visit the euphotic layer with a return rate of 1-2 days (Backhaus et al., 1999, 2003, D' Asaro, 2008). Furthermore, it has been suggested that neglecting vertical mixing in an ecosystem model can lead to an over estimation of primary production due to the effect of turbulence on the photoadaptive properties (Barkmann and Woods, 1996).

Typically, in the development of light parameterizations for phytoplankton growth models, laboratory and field experiments are carried out to determine growth rates under different

light intensities (e.g. Falkowski and Owen, 1980, Cosper, 1982, Sakshaug and Holm-Hansen, 1986) and temperatures (e.g. Berges et al., 2002) or a combination of both factors (e.g. Fawley, 1984, Bouterfas et al., 2002, Hammer et al., 2002). For the development of parameterizations for winter conditions, studies typically focus on acclimatization to low light (e.g. Post et al., 1984, Cullen and Lewis, 1988, Anning et al., 2000) using light exposures of 8 hours or more. These experimental setups allow algae to acclimatize to low light intensities e.g. by increasing their Chl *a* content. During deep convection phytoplankton may not have sufficient time to acclimatize to the fluctuating light conditions e.g. by an increase in Chl *a* concentration. Other acclimation processes such as the activation of Rusisco can occur in timescales from seconds to few minutes (MacIntyre et al., 2000). Experiments with fluctuating light have shown lower phytoplankton growth rates than under continuous irradiance with the same number of photons during light exposure (Nicklisch, 1998, Shatwell et al., 2012). However, these experiments were mainly carried out under day length of 12 hours. Interestingly, cellular resources i.e. carbohydrate content is affected by a change in light availability due to mixing. The assumption is that more carbohydrates are necessary for the maintenance of the metabolism during prolonged periods of darkness (Raven and Geider, 1988).

A second factor influencing winter phytoplankton growth is temperature. Spring bloom development in the North Atlantic has been related to the survival of the phytoplankton winter stock, which was effected by winter temperatures (Wiltshire et al., 2008). Temperature within the winter mixed layer is relatively constant on a daily temporal scale, with temperature changing seasonally due to the input of solar energy. Future predictions for the North Atlantic suggest an average sea surface temperature (SST) increase of 2 – 4 °C by 2100 due to climate change (Houghton et al., 2001).

It is well known that phytoplankton species have an optimum growth temperature (e.g. Li, 1980). Up to this optimum, temperature increases led to higher enzymatic activity and

photosynthesis rate as well as nutrient uptake. These increases in vital rates in turn lead to higher growth rates (Raven and Geider, 1988, Falkoswki and Raven, 1997). Furthermore, temperature increases can also enhance the kinetics of activation and deactivation of the photosynthetic apparatus and thus influence the acclimation potential (Davison, 1991). Conversely, an increase in temperature can have a negative effect on dark survival of some diatom species (Anita, 1976) and may lead to an increased dark respiration (Verity, 1982, Lombard et al., 2009). The result of the interplay between these processes on growth and survival of phytoplankton cells is unclear with the effect of rising temperatures on the growth rate under short light and long dark periods are present unknown.

Short term pulsed changes in light availability represent a challenge for estimating marine primary production with ecosystem models, a process which is seldom implemented within these models (Ross et al., 2011, Lindemann et al., this issue). However, Lagrangian based individual models can simulate the environmental conditions experienced by phytoplankton cells in the mixed layer and thereby examine the potential importance of changes in light intensity and duration (Woods et al., 2005).

With this background, and a clear need to better understand algal physiology under the influence of exposure to short term light pulses, we conducted laboratory experiments using the marine diatom *Thalassiosira weissflogii* as model organism. The results of the study we propose have the potential to improve primary production models for the testing of deep convection scenario‘s in relation to climate change.

Material and Methods

Algae cultures

Non axenic cultures of the diatom *Thalassiosira weissflogii* (strain CCMP 1336) were obtained from the Provasoli-Guillard National Centre for the Culture of Marine Phytoplankton. Prior to the experiment, a stock culture of algae were maintained in

autoclaved, GF/F filtered and f/2 (Guillard and Ryther, 1962) enriched North Sea water (salinity 32) at a temperature of 15°C. Biolux neon lamps (Osram) were used as a light source providing 160 - 180 µmol m^{-2} s^{-1} light over a 12/12 hours light/dark cycle. Algae were maintained under these conditions for at least three weeks prior to the commencement of the experiments. The stock cultures were continuously bubbled with filtered air to minimize self-shading and sedimentation as well as to ensure a sufficient supply of CO_2 and O_2. Growth rate was determined during the exponential growth phase.

Experimental setup

Experiments were carried out under two different light scenarios offering the same daily light dose, exposed in different light/dark cycles and light intensities combinations. The two different light scenarios were:

- a low light trial (labelled as LL for long exposure and low light intensity) offering a light intensity of 20 µmol m^{-2} s^{-1} over photoperiod of 12/12 hours (light/dark)
- a short light trial (labelled as SH for short exposure and high light intensity) with a light intensity of 120 µmol m^{-2} s^{-1} over a photoperiod of 2/22 hours (light/dark).

The daily light dose in both trials was 0.86 mol m^{-2} d^{-1} (2.16 W m^{-2} converted by the formula of Cloern et al. (1995). Osram Biolux lamps were used as the light source, with intensity controlled by the distance of the sample from the lamps.

Both trials were run in a thermal gradient table (Thomas et al., 1963) with a temperature gradient between 5.5 and 14.6 °C (5.5, 7.8, 10.1, 12.3 and 14.6 ± 0.2°C for LL) and 5 and 12.5 °C (4.9, 6.7, 8.5, 10.3 and 12.5 ± 0.4 °C for SH). Three replicates were performed for each temperature. Algae from the initial stock culture were diluted with autoclaved, GF/C filtered North Sea water enriched with 0.5 mL f/2 stock solution per litre sea water to a final concentration of 6000 to 10000 cells mL^{-1} and put into covered 1 L glass beakers. The

cultures were bubbled with filtered air to ensure a homogeneous cell distribution within the beakers. Samples were brought to experimental temperatures within 24 h.

For each light trial, two identical experiments were carried out. In one experiment all measurements were made at the end of the photophase while in the second experiment samples were taken at the end of the scotophase. Triplicate samples for cell counts were taken after 2, 4 and 6 days. Cell number samples were always taken after 48 h. Aliquots of 30 mL were taken from each beaker with a similar volume of medium added to maintain the sample at a constant volume. Sample aliquots were fixed with Lugol (final concentration of 1%) and measured within three days using a Multisizer 3 (Coulter Counter). Cell number in each sample was determined in triplicate. The specific growth rate was calculated using the equation:

$$\mu=\ln(N_2/N_1)/(t_2-t_1), \quad (1)$$

where μ is the specific growth rate (d^{-1}) and N_1 and N_2 are cell numbers at time 1 (t_1) and time (t_2), respectively. Chl *a* fluorescence emission for each of the triplicates was measured using a Water PAM (WALZ, Germany). As the two different light scenarios were never tested simultaneously with algae coming from the same stock culture an additional experiment was carried out allowing a comparison of low and short light effects to validate the previous results and exclude potential temporal effects. This experiment was only carried out at 15°C in a temperature controlled chamber. In every trial samples for cell number, biochemical analysis and PAM fluorometry were taken from each replicate at the end of the photo- and scotophases after six days of exposure to the experimental conditions. Furthermore, an additional (third) SH experiment was performed under comparable conditions. The aim of this experiment was to - by an increase in the number of samples - reduce the range of variability

of growth rates, and to extend the temperature range examined to 5.6 and 14.6°C, and to evaluate the direct comparison experiment. These experiments were carried out with algae maintained under the same stock culture conditions as the earlier trials.

For a more detailed view of the potential for short term acclimation PAM measurements were carried out during a light/dark cycle of 5 h light and 7 h darkness at a light intensity of 120 µmol $m^{-2}s^{-1}$ at three different temperatures (5, 10 and 15 °C). Algae came from the same stock culture conditions than used for the main experiments and were acclimatized to temperature different exposure temperatures for 24 h in darkness. PAM measurements were carried out in time steps between 5 min and 1 h over a period of about 12 h.

Biochemistry

Samples for biochemical analyses were obtained either at the end of the photo- or scotophase on the last day of the experiment (day 6). Duplicate samples of 70 mL volume were filtered onto pre-combusted Whatman GF/C filters and frozen at -20°C for Chl *a* or -80°C for carbohydrate analyses. Chl *a* was extracted in 90 % acetone and analyzed photometrically after Jeffrey and Humphrey (1975).

Total carbohydrates were determined after Dubois et al. (1956) and Herbert et al. (1971) Furan derivates were formed by adding 96 % sulphuric acid to the sample and pentoses were converted to a-furfurylaldehyde while hexoses are transformed to 5-(hydroxymethyl)-furfurol. These aldehydes react with phenol to produce characteristically coloured products. Measurements of carbohydrates were expressed as glucose equivalents. A D-(+)-glucosemonohydrate solution was used as a primary standard and samples were measured photometrically at 490 nm.

Chl *a* fluorometry

Chl *a* fluorescence was measured with a Water PAM (WALZ, Germany). A light saturation pulse was applied with > 10.000 µmol photons m^{-2} s^{-1} for 0.8 s. Algae were dark adapted for 5 min before estimating rapid light curves (RLC and the maximum quantum yield of PSII (Genty et al., 1989) was determined:

$$F_v/F_m = (F_m - F_o)/F_m \quad (2)$$

Where F_v is the difference of the maximum fluorescence (F_m) measured after a saturating light pulse and the minimum fluorescence (F_o) emitted as a result of the measuring light only. F_v/F_m requires dark-adaptation. Whereas, the effective quantum yield of PSII: $\Phi_{PSII} = \Delta F/F'_m = (F'_m - F)/F'_m$ requires light adaptation.

Immediately after F_v/F_m a RLC was measured as described in Cosgrove and Borowitzka (2006). Each treatment involved nine consecutive, 30 s intervals of actinic light pulses of increasing intensity with an accompanying yield measurement at the end of each actinic interval. Blue light emitting diodes (LEDs) provided the actinic light at levels (PAR) of 0, 86, 124, 190, 281, 399, 556, 922, and 1381 µmol photons m^{-2} s^{-1} and the electron transport rate (ETR) was calculated as:

$$ETR = \Phi_{PSII} * PAR \quad (3)$$

Empirical data for the establishment of photosynthesis (P) at every measured light intensity were fitted to the function of Platt et al. (1980) including photoinhibition and using a Marquardt-Levenberg regression algorithm:

$$P = P_s(1 - e^{-(\alpha Ed/Ps)} * e^{-(\beta Ed/Ps)}), \quad (4)$$

where P_s is a scaling factor defined as the maximum potential rETR, α is the initial slope of the RLC, Ed is the downwellig irradiance (400 - 700 nm) and β characterizes photoinhibition

The two main parameters of the RLC were determined i) the maximum relative electron transport ($rETR_{max}$) and ii) the minimum saturating irradiance (E_k). $rETR_{max}$ is the asymptote of the curve and gives evidence of the ability of the photosystems to utilize the absorbed light energy (Marshall et al., 2000). E_k is determined by the intercept α with the maximum photosynthetic rate (Sakshaug et al., 1997). These parameters were estimated following (Ralph and Gademann, 2005):

$$rETR_{max} = Ps\ (\alpha/(\alpha + \beta)\ (\beta/(\alpha +\beta)\ ^{\beta/\alpha} \quad (5)$$

$$E_k = rETR_{max} / \alpha \quad (6)$$

Data Analysis and Statistics

Growth rate was determined in triplicate, Chl *a* and carbohydrates in duplicates measurements for every independent sample. Mean values were determined and used for plotting and statistic analysis. PAM data was determined only once for each replicate. Data of all parameters were combined into temperature ranges (5°C range: 4.9 – 5.6°C; 8°C range: 6.7 – 8.8°C;10°C range: 10.1 – 10.6°C, 12°C range: 12.1 – 12.5°C and 15°C range: 14.5 – 14.7°C) and used for statistical analysis.

The growth rates from the two experiments (end the photo- and scotophase) of each tested light condition were combined (n=6). Chl *a*, carbohydrates and PAM data were treated separately for each experimental trial (n=3). Significance differences between the two different light conditions (LL and SH) and each determination time (end the photo- and scotophase) were tested for the defined temperature ranges using a One-Way ANOVA. The

effect of temperature was tested using Spearman correlations. Data were assumed to be significantly different at $p < 0.05$. Statistical analyses were carried out using SPSS 15.0 software.

To describe the correlation between growth rate and temperature data were fitted to linear regression equation f = y0+a*x, where is y0 is the intercept and a is the slope of the curve, using Sigma Plot 11. The slopes of the curves were compared using a t-test.

A potential temperature impact on the saturation curves of $rETR_{max}$ during the light period of the experiment exposing a higher temporal resolution was determined using non-linear regression. Logistic models (for each temperature and for all temperatures combined) were compared using a second-order Akaike´s Information criterion (AIC, corrected for small sampling sizes).

Results

Growth

Two concurrent experiments were carried out to investigate the impact of different light regimes (LL: long light exposure of low light intensity and SH: short light exposure of higher light intensity) on the growth of *T. weisflogii* at different temperatures (Fig. 1). At temperatures below 8 °C growth rates were not different from zero under both light conditions. Above this temperature growth rates increased with increasing temperature under both light conditions (Fig. 1).

The temperature growth relation was described by a linear model. For SH the linear model had the best ($r^2 = 0.7524$). The LL treatment had a better fit for the linear model ($r^2 = 0.9317$), however a 3 parameter sigmoid curve had a best fit ($r^2 = 0.9438$). Due to Montage et al. (2003) we choose the linear fitting for both trials. The slopes of the two regressions are significantly different ($p < 0.001$), where the slope of the LL is higher than the slope of the SH trial. These results were confirmed by the statistical tests on the different temperature

ranges (5°C: 4.9 – 5.6°C; 8°C: 6.7 – 8.8°C;10°C: 10.1 – 10.6°C, 12°C: 12.1 – 12.5°C and 15°C: 14.5 – 14.7°C). At all tested temperature ranges above 8°C growth rate was significantly higher under LL than under SH conditions ($p < 0.02$).

The highest measured growth rates of 0.23 ± 0.02 (LL) and 0.10 ± 0.02 (SH) were determined at the highest tested temperature (14.5°C). Growth rates were determined after 48 h for each experiment. Measured growth rate do not give any information about the time of the day when growth occurred.

For logistic reasons all experiment had to be carried out consecutively. To exclude any stock cultural effects on the growth rates, the effect of the different light conditions was directly compared in one follow up experiment at 15°C. The results (shown separately in Fig. 1) confirmed that growth rate was significantly higher ($p < 0.0001$) under the LL than under SH conditions.

Biochemical components

Chl *a* start values from the algae stock cultures used for the different trials varied between 4.5 - 5.4 pg cell^{-1} (shaded area in Fig. 2a). During the LL trial, the Chl *a* content per cell increased during the experimental time of 6 days and was always higher than the initial value. The Chl *a* content per cell during the SH trial remained in the range of the initial values independent of temperature. At every temperature ranges the Chl *a* content under LL conditions was significantly higher than under the comparable SH conditions, for both determination times end of photo- and end of the scotophase, with one exception (10°C; after the scotophase). In the two light trials the Chl *a* content, measured at the end of the photo- or scotophase, was only significantly different at two temperatures under LL conditions (10 and 12°C).

Temperature had a significant effect on the Chl *a* content at the end of the photophase of the SH trial. The lowest Chl *a* content was found at the lowest temperature. The experiment

directly comparing the light treatments at 15° substantiated that the Chl *a* content per cell was significantly higher after the LL than SH conditions.

The carbohydrate contents per cell under SH conditions measured at the end of the photo- was always higher than at the end of the scotophase (maximum 0.94 pg cell $^{-1}$ at 6.7 °C at the end of the photophase; minimum 0.33 ± 0.06 at 5 °C at the end of the scotophase) (Fig. 2b). During the LL trial, the values were constantly low (about 0.5 pg cell $^{-1}$) with no significant effect of sampling time (end of the photo- or scotophase) with one exception at 10°C. These results were substantiated by the direct comparison experiment where the carbohydrate content was also significantly higher under SH conditions. Temperature had a significant effect on the carbohydrate content at all tested treatments beside the once measured at the end of the photophase of the SH trial.

Chlorophyll *a* fluorometry

The following metrics of phytoplankton photosynthetic status were determined via Chl *a* fluorescence: the maximal quantum yield (F_v/F_m, Fig. 3a) and two different parameters inferred from Rapid Light Curves (RLC): $rETR_{max}$ and E_k, (Fig. 3 b and c). The algae stock cultures in 2011 did not differ significantly in their PAM parameters (F_v/F_m: 0.63 ± 0.02 relative values; $rETR_{max}$: 58.2 ± 4.4 relative values and E_k: 107 ± 12 $\mu molm^{-2}s^{-1}$).

Parameters of the RLC from the direct comparison experiment performed in 2012 were significantly different to start values ($p < 0.05$) and can therefore not be compared directly to the measurements of 2011.

During the LL trial, F_v/F_m values increased up to the highest observed value of 0.73 ± 0.01 at 14.7°C (Fig. 3a), whereas during the SH experiment F_v/F_m never increased above the initial value. All values measured during the LL were higher than during SH trial. But only at temperatures below 12°C the differences were significant ($p < 0.005$).

Temperature had a significant effect on F_v/F_m during the SH trial ($p < 0.03$). The value decreased with decreasing temperature especially at the end of the 22 h dark period. During this trial we also determined the lowest value of 0.35 ± 0.03 at 5°C. During the LL trial temperature only affected F_v/F_m measured at the end of the photophase ($p < 0.001$). Differences between the F_v/F_m measured at the end of the photo-or the scotophase were only evident for the SH trial at 5°C.

$rETR_{max}$ ranged between a maximum of 94.5 ± 7.8 at 14.7°C at the end of the photophase at LL conditions and 4.6 ± 0.8 at 5°C at the end of the scotophase at SH conditions (Fig. 3b). RLCs were more strongly influenced by the different light conditions than F_v/F_m. $rETR_{max}$ decreased during the scotophase. Under both light conditions, at every tested temperature $rETR_{max}$ was significantly higher at the end of the photo- than scotophase (LL: $p < 0.01$; SH: $p < 0.0001$). Furthermore, all $rETR_{max}$ values measured under LL conditions were significantly higher than the corresponding SH data (end of phot- $p < 0.02$ and scotophase $p < 0.0001$). Temperature had a significant effect on $rETR_{max}$ during SH ($p < 0.02$) but not during LL conditions. Delta $rETR_{max}$ at the end of photo- and scotophase (of about 40) was similar for both light scenarios.

$rETR_{max}$ values of the two light scenarios of the ~~"d~~irect comparison"-experiment were not significantly different. However, the value at the end of the photo- and scotophase for each scenario was significantly different.

E_k is ${}_rETR_{max}$ / α, where data of α are not shown. Comparable to the results of ${}_rETR_{max}$ and E_k α was higher under LL than under SH conditions, whereas during the LL trial delta at the end of the photo- and scotophase was less extended than during the SH trial. Treatment behaviour of α was comparable to the one of E_k. Due to the higher physiological relevance of E_k we decided to only show these values.

E_k values were always higher at the end of the photo- than at the end of the scotophase. This effect was always significant for the results of the SH trial ($p < 0.008$) but only significant at

10°C ($p = 0.003$) and 15°C ($p = 0.002$) during the LL trial. Temperature only had a significant effect on the E_k values at the end of the scotophase of the SH trail.

The values described relative to the experimental light intensity give important physiological information (experimental light intensities are marked as lines in Fig. 3c). Under LL conditions E_k was always higher than the experimental light condition of 20 µmol $m^{-2}s^{-2}$ with the lowest measured value of 87 ± 27 µmol $m^{-2}s^{1}$ at the end of the scotophyse. In contrast at the end of the long dark period of the SH trial E_k was always lower than the experimental light intensity of 120 µmol $m^{-2}s^{-1}$ (between 29.8 ± 5.1 and 90.1 ± 8.8 µmol $m^{-2}s^{-1}$).

The temporal evolution of $rETR_{max}$ measured during a light/dark cycle of 5 h light and 7 h darkness (Fig. 4) was investigated for three temperatures. All results show a continuous increase of $rETR_{max}$ from the beginning of the light phase. The increase was stronger at higher temperatures. After 5 h of irradiance with a light intensity of 120 µmol m^{-2} s^{-1}, saturation was not reached as indicated by the E_k values (results not shown). During the subsequent dark period $rETR_{max}$ decreased and stabilized after about 4 h. Temperature had a significant positive effect on $rETR_{max}$ levels based on the lower corrected AIC ($\Delta AIC_{corr}=34$) for the model including a temperature impact. The slopes of the decrease during darkness were low comparable to the increase in light and not significantly different. After an initial decrease during the first 10 h, the values remained more or less constant for the following 20 h (data not shown).

Methodological issues

Due to logistics and equipment availability each experiment had to be carried out separately.

To separate between treatment effects and a potential bias of different start cultures two additional experiments were carried out. The complete SH experiment was repeated at this time (with sampling only for growth rate at the end of the photophase) plus an experiment to test the direct effect of the two light treatments at one temperature. To account for potential

setup effects, data from the –direct comparison"-experiment are always presented separately and were not used for curve fitting of the temperature growth curve. These validation experiments show that observed differences in growth rate and biological composition were caused by light treatment and not by differences in start conditions. Chl *a* fluorescence measurements on the other hand deviated from the pattern observed in the previous experiments. Start values of the RLC parameters were higher in later experiment and light conditions effected fluorescence parameters less than in former.

Chl *a* fluorescence is generally a good tool for showing photo-physiological differences. Furthermore many studies have shown a high correlation with primary production (Morris and Kromkamp, 2003, Goto et al., 2008) although this approach can not be used as a direct measure for primary production.

Discussion

Winter deep convection plays an important role on the seasonal dynamics of phytoplankton by maintaining a viable community in the water column and thereby sustaining the spring seed population while its retreat the pre-spring bloom particle fluxes seen in sediment traps. (e.g. Honjo et al., 1988, D´Asaro, 2008). In our study we examined the effect of different light scenarios presumed to be experienced by phytoplankton cells during deep winter convection on phytoplankton growth and physiology. The basis for our experimental design comes from a model study of Lindeman et al. (this issue) who simulated an average exposure time of 2.1 h for cells experiencing deep convection in early spring in the North Atlantic with a mixed layer depth of 500 m. The light conditions we tested included a classical low light scenario (12/12 hour light/dark cycle of 20 µmol $m^{-2}s^{-1}$) assuming the retention of cells in a subsurface chlorophyll *a* maximum layer and a scenario with short intervals of higher light intensity (2/22 hour light/dark of 120 µmol $m^{-2}s^{-1}$) simulating a simplified deep convection situation. Both scenarios offered the same daily light dose of 0.86 mol $m^{-2}d^{-1}$.

The temperature range of 5 to 15°C was chosen around the observed winter temperature of 9.4°C in the North Atlantic.

Growth rates

In this study, above a critical temperature of 8°C growth rates of *T. weissflogii* increased with increasing temperature under all experiential conditions. However, in the long exposure low light trial (LL) growth rates were always higher than those observed during the short exposure high light trial (SH). Notably, all experimental conditions above 8°C elicited a positive growth response in our experimental algae. The growth response was best fitted using a linear model comparable to Eppley (1972) and Montagnes et al. (2003). Although a sigmoid model would describe the biological processes including a temperature limitation below 8°C and an anticipated maximal growth rate at a certain temperature. The maximum growth rates obtained in our study (< 0.23 d^{-1}), were below those observed by Montagnes and Franklin (2001) for *T. weissflogii* who observed growth rates of 0.5 – 0.6 d^{-1}, when exposed to at 50 µmol m^{-2} s^{-1} over a day/night cycle of 14/10 h at a temperature range between 12 - 20°C. Our temperature growth behavior does not show a clear temperature optimum under either light condition. Hence an optimal growth temperature under our experimental conditions can not be determined.

Furthermore, based on our two levels of light exposure we found a clear effect of exposure duration on growth rates especially at higher temperatures. Thompson (1999) also tested the effect of different daily light doses and temperature on *Thalassiosira pseudonana.* He found in contrast to our findings no temperature effect on the initial slope of a daily light dose growth relationship. In addition, he found that daily light doses below 1 mol $m^{-2}d^{-1}$ day length did not affect growth, when testing light cycles within a range of continuous light and the shortest light period of 4 h. Despite the daily light dose in our experiment being lower than

Thompson (1999) (0.86 mol m^{-2} d^{-1}) we did find an effect of exposure time. We suggest that a shorter light period of 2 h does not allow the cell to use the available light.

Similar to our findings, Verity (1982) showed an effect of day length on growth only at higher temperatures. The shortest day length examined during this study was 9/15 h light/dark. To our knowledge our study represents the only examination of the effects of light exposure at a period of 2h light as 3/21 h light/dark cycle has been the shortest light period tested to date (Foy, 1983, Bouterfas et al., 2006).

Acclimatization

The algae in our experiment were not acclimated to the experimental light or temperature conditions. Rather, the study was designed to examine the ability of a cell to function during transition from a fall stratified situation to a regime of deep convection. Allowing the cells time to acclimatize due to prolonged exposure to specific light and temperature conditions will select for different genotypes and as well as allowing the cell to optimize its internal environment (e.g. Chlorophyll *a* content) and as a result will lead to different physiological responses. This change can be considered to be an adaptation, a change which occurs over generations as compared to acclimation which is the response of the individual. Hence our focus was on the cells short term ability to acclimatize due to changes in Chl *a* metabolism (periods of 10^4 to 10^3 sec) and Rubisco activity (MacIntrey, 2000).

The potential of phytoplankton species to adapt to changing environmental temperatures is still not well understood. For example, a correlation between changing environmental temperature and optimal growth temperature was found (Boyd et al., 2012) while conversely no clear relationship has been observed between (Thomas et al., 2012). In order to address the issue of adaptation a longer term study would be required examining changes in response over generations. This is beyond the scope of this study but represents a key issue for

understanding the evolution of the phytoplankton community as influenced by climate change.

Biochemical compounds

Light availability has a clear effect on phytoplankton biochemistry. For example, lower light intensities induce an increase in Chl *a* content to optimize light harvesting capacity (Post et al., 1984, Cullen and Lewis, 1988, Anning et al., 2000, Wagner et al., 2006, Dimier et al., 2009, Milligan et al., 2012). Our findings support this as an increased Chl *a* content was observed under the LL but not under SH conditions. In contrast to other studies (Post et al., 1984, Fábregas et al. 2002) we did not observe diel changes of the Chl *a* content. Chl *a* content during the SH scenario was comparable to the initial value independent of temperature. Hence, light limitation due to a short period light dose did not cause an increase of the Chl *a* content. Changes in Chl *a* content have been observed under fluctuating light experiments. Most likely the duration of low light intensities was more important than the period of high light intensities (e.g. Fietz and Nicklisch, 2002, van Leeuwe et al., 2005, Dimier et al., 2009). During these experiments the increased Chl *a* content did not affect growth rate (Fietz and Nicklisch, 2002, Dimier et al., 2009). In contrast our findings showed higher growth rates at higher Chl *a* concentrations.

Carbohydrates are the main energy reserve in marine algae and can be accumulated under high light irradiances (Granum et al., 2002). Temperature has a two-fold influence on carbohydrate dynamics influencing anabolism during periods of light (Varum et al., 1986) and catabolism during dark respiration (Raven and Geider, 1988, Falkowski and Raven, 1997). In our study, *T. weissflogii* accumulated high amounts of carbohydrates during the short light intervals in the SH trial. Interestingly, given the low growth rates observed, this surplus energy was not transformed into growth but consumed during dark respiration. The constant

carbohydrate content during the LL experiment over the day/night cycle may reflect more balanced conditions supporting higher growth rates. Halsey et al (2011, 2013) observed higher polysaccharide accumulation at higher growth rates. In these studies nutrients were the growth limiting factor. Growth rates during our experiment were generally low and light limited. The high carbohydrate accumulation we observed during the light phase of the SH trial did not result into higher growth rates, but could potentially enabled a longer period of survival during dark periods as survival during darkness depends on the reserve carbon availability (Furusato and Asaeda, 2009, Talmy et al., 2014).

Metrics of Photobiology

PAM metrics such as the maximal quantum yield (F_v/F_m), photosynthetic capacity (relative maximum electron transport rate; $rETR_{max}$), and minimum saturation irradiance (E_k) are often used as indicators of the fitness or to describe photoacclimation of algae (Cullen and Davis, 2003, Franklin et al., 2009, McMinn et al., 2010).

In our study, the F_v/F_m was generally high especially under LL conditions indicating a good physiological state of the algae. Only long dark periods in combination with low temperatures negatively affected F_v/F_m. Low temperatures also had a negative effect on $rETR_{max}$ under SH conditions. In contrast, these parameters in the LL trial were not affected by temperature. A decrease of temperatures reduces light saturation due to the fact that the light reaction of photosynthesis is temperature independent in contrast to the dark reaction (Davison, 1991). A lower light saturation level most likely leads to an earlier appearance of photodamage (Falkoswki and La Roche, 1991, Demming-Adams and Adams, 1992). The lower light intensities during the LL trial did not seem to induce damage at any of the tested temperatures.

In contrast to our study several previous studies have identified lower $rETR_{max}$ and E_k values for algae exposed to lower light intensity (e.g. Ralph and Gademann, 2005). In our study,

algae exposed to higher light intensity (SH) showed a typical low light response with lower $rETR_{max}$ and E_k values. Values decreased during the long dark periods and the short pulsed light exposures were not sufficient to induce an increase of the photosynthetic capacity. E_k results from $_rETR_{max}$ and the slope of the RLC α (data not shown). In our case temperature and light dependence of α was more strongly related to E_k than $_rETR_{max}$.

The minimum saturation irradiance (E_k) is the most representative parameter to explain photosynthetic efficiency and potential damage. This index, in combination with the ambient light intensity gives important information about light utilization potential (Behrenfeld et al., 2004). During the long dark phase of the SH scenario E_k decreased below the experimental light intensity of 120 $\mu molm^{-2}s^{-1}$. Thus, especially at the beginning of the light period photons could not be completely used for photosynthesis. Excess energy during this period could potentially lead to photo-damage thus necessitating the activation of cell apparatus for repair and thereby reducing growth efficiency, causing the decreased values of F_v/F_m and $rETR_{max}$, especially at low temperatures.

At the end of the short light period the minimum saturation irradiance was comparable to the experimental light intensity, which suggests high photosynthetic capacity but at a level which could still induce more damage than lower intensities (Aro et al., 1993). In contrast, under LL conditions E_k was always higher than the experimental light intensity. Hence, the available light could immediately be used completely for photosynthesis and most likely caused less damage.

Photoacclimation is a time dependent process. Moore et al. (2006) found a strong correlation between E_k and the ambient light conditions in stratified waters, in contrast to deep mixed water, potentially due to the fact that mixing rate was faster than acclimation time. During this study algae showed comparable values to surface populations with a high light acclimation. In our study algae exposed to conditions that simulated deep mixed conditions are low light acclimated. Both observations might be a result of insufficient acclimation time.

In our study, characterization of the time dependence of photoacclimation, was performed by following the temporal evolution of $rETR_{max}$ during the changes between light and darkness at three different temperatures. $rETR_{max}$ followed a light and dark rhythm as been described for benthic diatoms in field (Seródio et al., 2005). For *T. weisflogii* in our study, $rETR_{max}$ increased continuously during the first 5 h of the photophase. No saturation could be observed within the tested light period. During darkness the decrease of $rETR_{max}$ stabilized after about 4 h. Hence, the decrease of $rETR_{max}$ was similar during both light scenarios. This supports our finding that 2 h light are just not sufficient to reach full photosynthetic capacity. In contrast, Nymark et al. (2013) found after 48 h of darkness that the diatom *Phaeodactylum tricornutum* found that of $rETR_{max}$ recovered to a value even higher than the start value during the first 30 min, with no further increase during the following 24 h of light. Thus we postulate that recovery of the $rETR_{max}$ after dark incubation may be species specific.

Implications

The goal of this study was to assess the potential for individual phytoplankton cells to remain viable in the deep convective circulations occurring during winter in the North Atlantic. Our results clearly identify that cells can maintain positive albeit low growth rates in conditions simulating those they would experience. Our study showed 50 % higher growth rate under constant low light than under pulsed light simulating conditions. The analyses of, carbohydrate content and Chl *a* fluorescence indicate that cells experiencing periods of short pulses of light can use those light windows for growth if temperature is not limiting even if growth rates are very low. Furthermore accumulated carbohydrates could potentially allow the cells to survive for longer periods due these reserves. These findings are particularly important for understanding the role of deep convection in maintaining an integrated phytoplankton biomass of the same magnitude as it was found in the spring bloom (Li et al.,

1980; Backhaus et al., 2003) as well as providing the seed population for the spring bloom in the North Atlantic.

In conclusion, our experiment showed that a calculation of primary production with growth rates coming from laboratory experiments with constant low light intensities, as often assumed in ecosystem models, could lead to an overestimation of primary production in well mixed water bodies. Earlier studies have focused on the difference between constant and fluctuating light (Marra 1978, Barkmann and Woods, 1996, Anning et al., 2000, Ross et al., 2011). These results have been inconclusive with an underestimation of primary production of 87 % (Marra, 1978) to an overestimation of 40 % (Barkmann and Woods, 1996) when comparing fluctuating and fixed light incubations. The latter model calculation did not take into account that particles within the whole mixed layer have the same ability to short ephemeral visits in the euphotic zone to use the short available light windows for growth as shown by Lindemann et al. (this issue).

Rapidly changing light exposures are a challenge for autotrophic organisms. Many studies focus on the photo-protection mechanism activated due to quickly rising light intensities (Dimier et al., 2009, Alderkamp et al., 2011). However, a rapid rise in light saturation after long dark periods, as occurring during deep convection, is rarely explored. Studies focusing on photo-acclimation under unsaturated conditions, including this study have illustrated that low light intensity primarily triggers processes such as Chl *a* anabolism, changes in enzyme activity or light harvesting complexes to increase growth. Algae exposed to short light intervals have a limited potential to raise their capacity to use the incoming light, and have higher losses due to dark respiration thus leading to lower growth rates. Our experiments have for the first time demonstrated positive growth under short temporal periods of only two hours light per day in the laboratory. This scenario was taken from model calculations by Lindemann et al (this issue) and illustrates that, if temperature limitation does not occur, in

contrast to Sverdrup´s assumption primary production in the convective mixed layer of the North Atlantic is possible during winter, even if the mixed layer depth is much deeper than the critical depth (Backhaus et al., 2003, Beherenfeld et al., 2010). Furthermore our findings bring into question the use of daily light doses for phytoplankton growth or critical light boundaries. In a next step our findings should be implemented into an IBM thereby allowing for phytoplankton growth even under unfavorable winter conditions. We suggest that future laboratory studies should focus on more fluctuating light conditions including photoinhibition under short light term light exposure. Finally, in order to understand the future evolution of the North Atlantic phytoplankton community and their contributions to biogeochemical and ecosystem services, understanding future changes winter deep convection and the response of the phytoplankton community is critical

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Figures

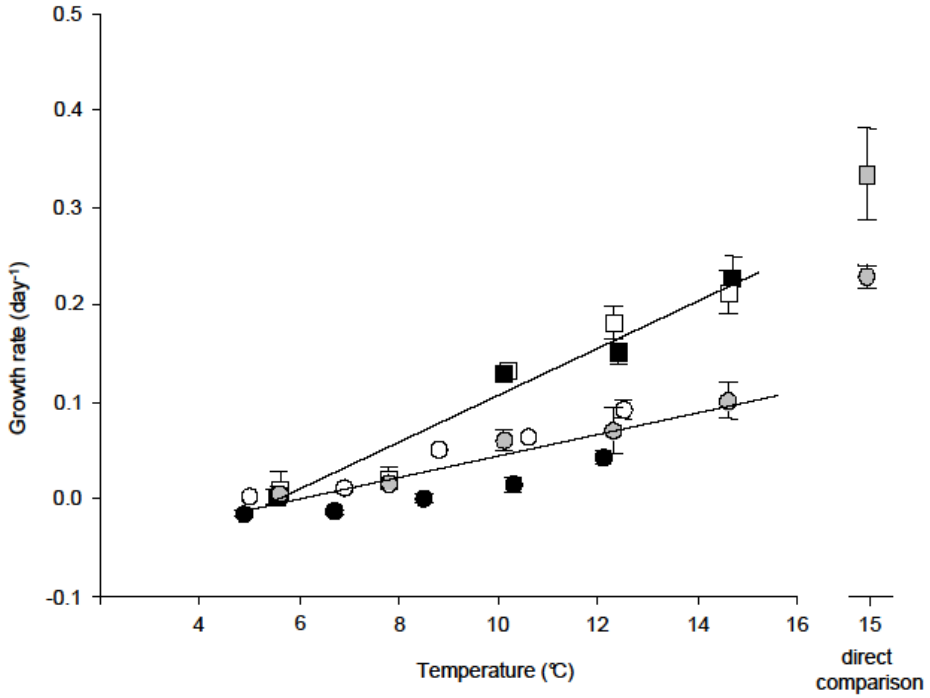

Fig. 1: Averaged growth rate of *T. weissflogii* under different temperatures and light scenarios: 12/12 h light/dark cycles with 20 µmol $m^{-2}s^{-1}$ (squares) and 2/22 h light/dark with 120 µmol $m^{-2}s^{-1}$ (circles). White: measured at the end of the light phase; black: end of the dark phase (first experiment). Grey: second experiment end of light phase. Mean values with error bars of standard deviation (n = 3). Lines describe the linear model fitting the data.

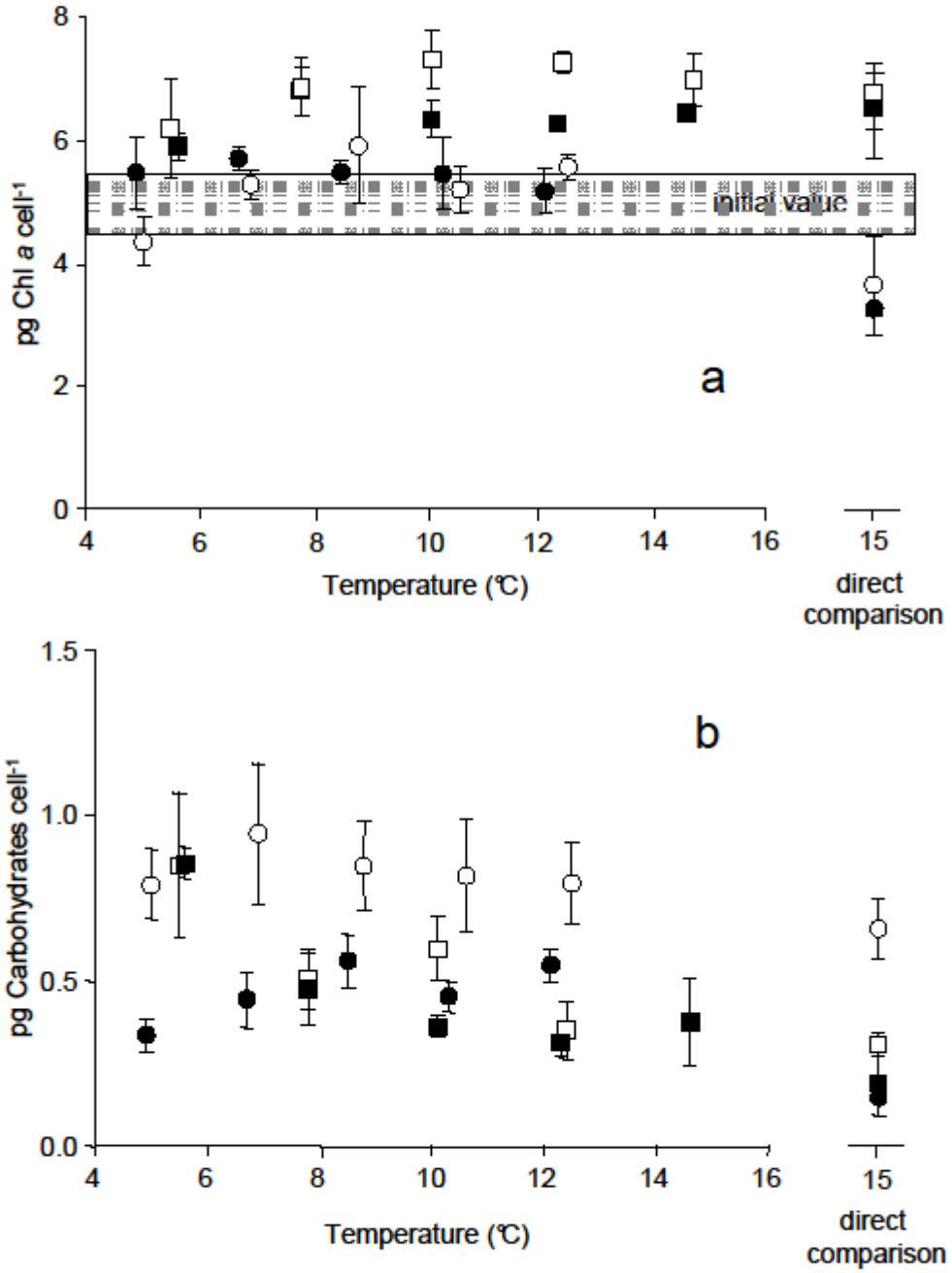

Fig. 2: Cell components (pg cell^{-1}) under different temperatures and light scenarios: Chl *a* (a), carbohydrate (b). Squares: 12/12 h light/dark cycles with 20 µmol m^{-2}s^{-1} and circles:

2/22 h light/dark with 120 µmol $m^{-2}s^{-1}$. White: measured at the end of the light phase; black: end of the dark phase. Mean values with error bars of standard deviation (n = 3).

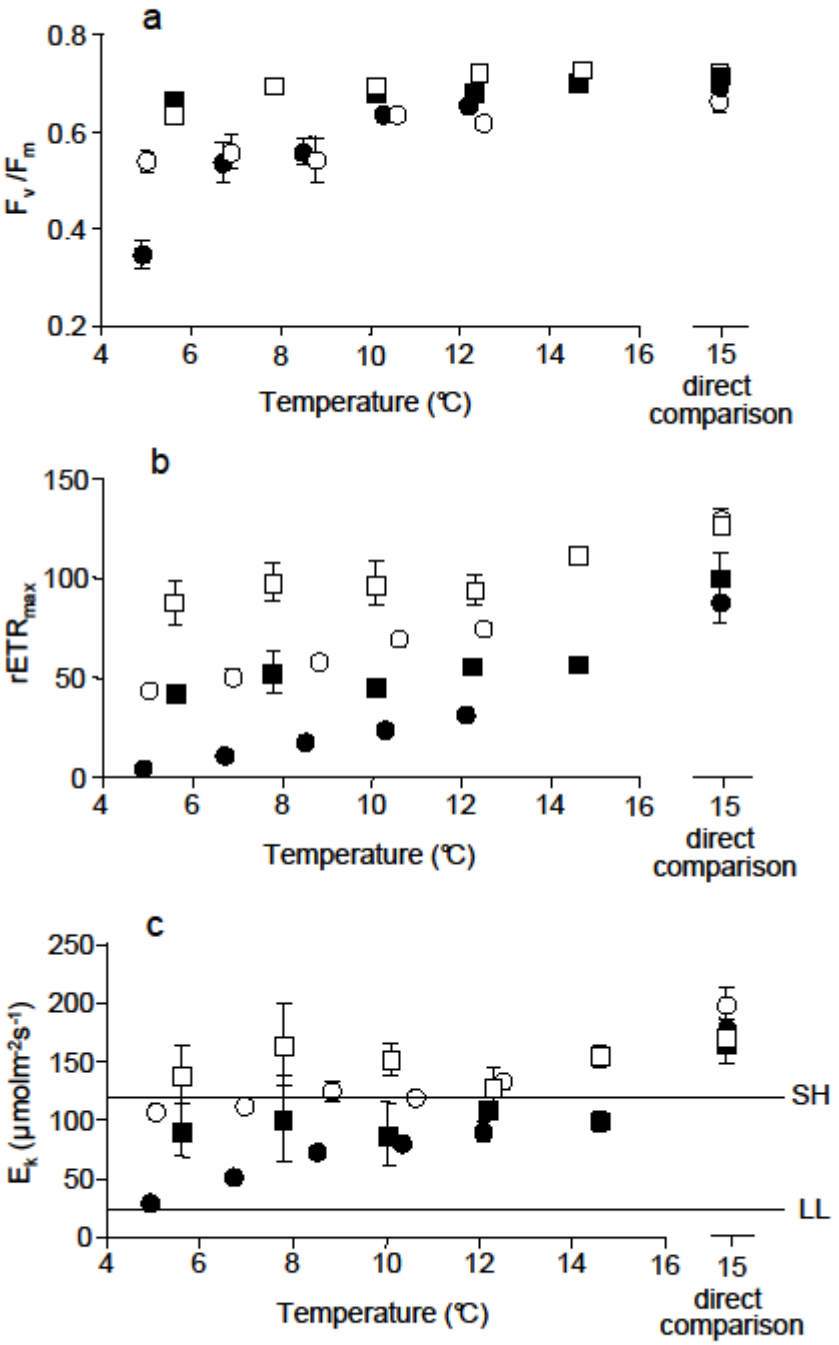

Fig. 3: Effect of temperature and light availability on PAM data. Squares: 12/12 h light/dark cycles; 20 µmol $m^{-2}s^{-1}$ and circles: 2/22 h light/dark; 120 µmol $m^{-2}s^{-1}$. F_v/F_m (a), $rETR_{max}$ (b) and E_k (c) at experimental day 6. White: measured at the end of the light phase; black: end of the dark phase. Mean values with error bars of standard deviation (n = 3). Lines (c) mark the experimental light intensity (µmol $m^{-2}s^{-1}$)

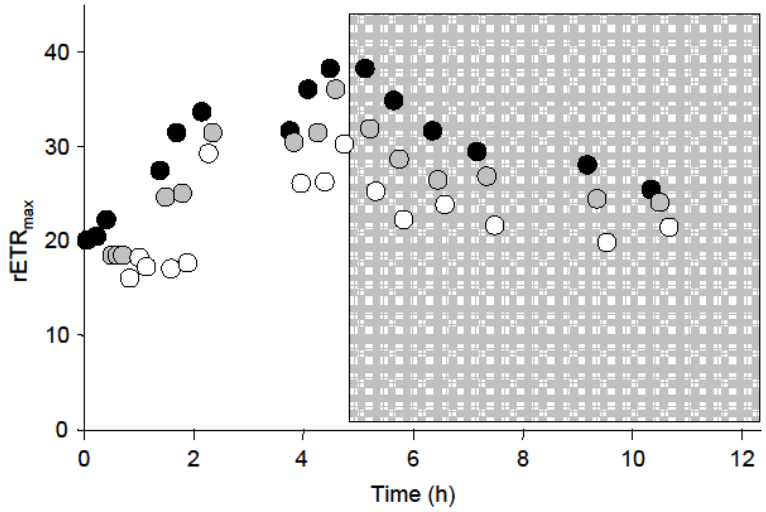

Fig. 4: $rETR_{max}$ over time in light and darkness (grey box) at three different temperature (white: 5°C; grey: 10°C and black: 15°C).