Phytoplankton in deep convection: an experimental approach on the effect of temperature and short light conditions on growth and physiology

Dissertation
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Summary

The phytoplankton spring bloom of the North Atlantic is one of the largest biological events on earth. It strongly affects biogeochemical cycles and the entire marine food web. Previous winter conditions strongly affect the timing and composition of the spring bloom. Low light availability during deep convection was assumed to prevent phytoplankton growth in winter. However, noticeable chlorophyll *a* concentration was determined in a convective mixed layer, where phytoplankton cells are transported via convection through the entire convective cell and thereby visit the euphotic zone for short periods frequently. These short periods of light availability may allow phytoplankton productivity in winter. Yet, very little is known about algae growth and physiology in a deep convection situation.

In this thesis laboratory experiments were carried out to test the growth of the diatom *Thalassiosira weisflogii* under different light and temperature conditions, simulating deep convection. Oxygen development, biochemical compounds and photosynthetic efficiency were measured to study surviving strategies and acclimation processes to low light availability. Furthermore, the effect of different overwintering conditions (complete darkness and deep convection) on the onset of a spring bloom were addressed by comparing differences in surviving strategies and possible competition between two different phytoplankton species (*Thalassiosira weisflogii* and *Rhodomonas* sp.).

T. weissflogii showed positive growth rates under two hours light per day at temperatures above 8 °C. Under comparable experimental conditions positive net primary production was calculated from continuous oxygen measurements explaining these positive growth rates. The comparison of two different light conditions with the same daily light dose indicated much higher growth rates for the low light scenario under non-limiting temperature conditions. This strongly emphasizes that compensation irradiance may not be the correct tool to describe the limit of phytoplankton growth. Furthermore, raising daily light doses applied in different light combinations did not cause a significant increase in net primary production or growth. Only lower light intensities applied over longer periods caused growth supporting acclimation processes such as an increase of chlorophyll *a*. Short light conditions apparently reduced the available time for acclimation processes during the light period and prevented a complete establishment of photosynthetic capacity which was reduced during the long dark periods.

Furthermore, high carbohydrate reserves produced during the short light periods were needed for the maintenance of the metabolism during the prolonged darkness and were thus not available for growth. However, the fact that dark respiration rate was only about 7 % of the photosynthetic rate at optimal temperatures might be a rea-

son for the positive growth even under unfavorable light/dark cycles. Dark respiration was less temperature dependent than photosynthesis. The temperature behavior of photosynthesis and dark respiration indicates that an increase of sea surface temperature due to climate change would increase phytoplankton productivity during winter in higher latitudes. Different winter situations (applied for two weeks) did not affect the growth of *T. weissflogii* and *Rhodomonas* sp. after re-illumination. The diatom could withstand winter conditions much better than the cryptophyte, in terms of (i) lower mortality, less decomposition of chlorophyll *a* and carbohydrates under complete darkness, (ii) positive growth under deep convection conditions and (iii) higher growth rates when being exposed to spring bloom conditions. These finding indicate that due to their high acclimation potential to changing environmental conditions, diatoms have a strong selective advantage over other phytoplankton species during the onset of a spring bloom.

In conclusion, the experiments demonstrate that short light windows provided by convective transport allow phytoplankton growth and might be the reason for relatively high chlorophyll *a* concentrations within the convective mixed layer. The findings of this thesis emphasize the need to include winter productivity into ecosystem models as a dynamic process depending on the light history of a phytoplankton cell. However, new growth rates must be determined for model parametrizations as the calculation of primary production with growth rates derived from experiments with constant low light intensities may lead to an overestimation of primary production of up to 50 % in well mixed water bodies.

Zusammenfassung

Die nordatlantische Phytoplankton-Frühlingsblüte ist eines der größten biologischen Ereignisse der Erde. Sie hat einen starken Einfluss auf biogeochemische Kreisläufe und bildet die Grundlage für das gesamte marine Nahrungsnetz. Der Start und die Zusammensetzung der Frühjahrsblüte hängen von den vorhergehenden Winterbedingungen ab. Lange Zeit wurde angenommen, dass die geringe Lichtverfügbarkeit, hervorgerufen durch die tiefe Durchmischung der Wassersäule im Winter, Phytoplanktonwachstum verhindert. Dahingegen wurden messbare Chlorophyll *a* Konzentrationen in der durchmischten Wasserzelle im Winter gemessen, da Phytoplanktonzellen mit der Konvektion durch die gesamte Zelle transportiert werden und so für kurze Zeit in die euphotische Zone gelangen. Diese kurzen Lichtabschnitte werden für das Auftreten positiver Phytoplankton-Primärproduktion verantwortlich gemacht, wobei nur wenig über das Wachstum und die Physiologie der Algen in Tiefendurchmischung bekannt ist.

Im Rahmen dieser Doktorarbeit wurden Laborexperimente durchgeführt, die das Wachstum der Diatomee *Thalassiosira weissflogii* unter verschiedenen tiefendurchmischungssimulierenden Lichtbedingungen und Temperaturen untersuchten. Um Überlebensstrategien und Akklimatisierungsprozesse an Schwachlicht aufzudecken, wurden die Sauerstoffentwicklung, biochemische Komponenten und die photosynthetische Effizienz gemessen. Weiterhin wurde die Auswirkung verschiedener Überwinterungsbedingungen (vollständige Dunkelheit und Tiefendurchmischung) auf die Entwicklung einer Frühjahrsblüte an zwei verschiedenen Phytoplanktonarten (*Thalassiosira weissflogii* und *Rhodomonas* sp.) untersucht, um unterschiedliche Überlebensstrategien und mögliches Konkurrenzverhalten zu bestimmen.

Bei Temperaturen oberhalb von 8 °C zeigte *T. weissflogii* positives Wachstum, wenn sie zwei Stunden Licht pro Tag ausgesetzt wurden. Positive Nettoprimärproduktion wurde bei vergleichbaren experimentellen Bedingungen anhand von kontinuierlichen Sauerstoffmessungen errechnet. Durch den Vergleich zweier unterschiedlicher Lichtbedingungen bei der gleichen täglichen Lichtmenge konnte gezeigt werden, dass sich der Lichtkompensationspunkt nicht für die Beschreibung eines Wachstumslimits für Phytoplankton eignet. Dies wurde unterstrichen durch die Beobachtung, dass ein Anstieg der täglichen Lichtmenge -vorausgesetzt sie wurde in unterschiedlichen Tag/Nacht Zyklen verabreicht- zu keinem Anstieg der Nettoprimärproduktion führte. Wachstumsfördernde Akklimatisierungsprozesse, wie etwa eine Anreicherung von Chlorophyll *a*, wurden nur unter lang andauernder Schwachlichtbestrahlung beobachtet. Kurztagsbedingungen verkürzen die Zeit für Akklimatisierungsprozesse in der Lichtphase und ließen keine vollständige Entwicklung der photosynthetischen Kapazität zu, die während der langen Dunkelphasen reduziert wurde. Ein Großteil der Speicherstoffe wurde während der

langen Dunkelphase verbraucht. Eine Erklärung für das Auftreten von Wachstum auch unter unvorteilhaften Lichtzyklen könnte sein, dass die Dunkelveratmung nur 7 % der Photosyntheserate entsprach.

Die Dunkelveratmung wurde weniger von der Temperatur beeinflusst als die Photosynthese. Diese Temperaturabhängigkeit lässt vermuten, dass das Phytoplanktonwachstum während eines vom Klimawandel verursachten Anstiegs der Temperatur zunehmen würde. Unterschiedliche Winterbedingungen hatten keinen Effekt auf das Phytoplanktonwachstum nach erneuter Bestrahlung. Die Diatomee konnte die Winterbedingungen besser überdauern als die Flagellate. Sie wies eine geringere Sterblichkeit und einen geringeren Abbau von Chlorophyll und Kohlenhydraten in vollständiger Dunkelheit auf. Weiterhin zeigten sie positives Wachstum unter simulierten Tiefendurchmischungsbedingungen und eine höhere Wachstumsrate nach Belichtung unter simulierten Frühlingsblütenbedingungen als die Flagellate. Auf Grund ihrer hohen Anpassungsfähigkeit an Veränderungen ihrer Umwelt haben Diatomee bei Beginn einer Frühjahrsblüte einen Vorteil gegenüber den meisten Phytoplanktonarten.

Zusammenfassend zeigten die Experimente, dass Lichtintervalle, die durch den Transport von Zellen durch die durchmischte Zelle entstehen, der Grund für die hohen Chlorophyll *a* Werte im Winter in der Wassersäule sind. Die Ergebnisse dieser Doktorarbeit bestärken die Notwendigkeit, die Phytoplankton Produktivität im Winter als einen dynamischen Prozess in Ökosystemmodelle zu integrieren. Für diesen Prozess müssen allerdings neue Wachstumsraten bestimmt werden, da die Verwendung von herkömmlichen Wachstumsraten, die unter konstanten Schwachlichtbedingungen bestimmt wurden, zu einer Überschätzung der Primärproduktion um etwa 50 % führen kann.

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Chapter 1

Abbreviations

α	photosynthetic rate in light-limited region of the RLC
β	photo-inhibition
Chl a	Chlorophyll a
CML	convective mixed layer
d	diameter
E_{d}	down-welling irradiance
E_{k}	minimum saturation irradiance
ETR	electron transport rate
F_0	minimum fluorescence of dark adapted algae
F_{m}	maximum fluorescence of dark adapted algae
$F_{\mathrm{v}}/F_{\mathrm{m}}$	maximum quantum yield of PS II
IBM	individual based model
LEDR	light enhanced dark respiration
LL	long low
NPP	net primary production
PAM	pulse amplitude modulation
PAR	photosynthetic active radiation
P	photosynthesis
PE	photosynthetic-irradiance
P_{s}	photosynthetic scaling factor
PS II	photosystem II
$\mathrm{rETR}_{\mathrm{max}}$	maximal relative electron transport rate
RLC	rapid light curve
SH	short high
SST	sea surface temperature
μ	growth rate

Chapter 2

Introduction

The estimation of ecosystem productivity of the world oceans is one of the biggest challenges for earth system models since phytoplankton primary production is responsible for about 40 % of global carbon fixation (Berger et al., 1989; Falkowski and Woodhead, 1992). Phytoplankton production at higher latitudes follows annual cycles and is influenced by different abiotic and biotic factors. In contrast to terrestrial plants phytoplankton cells are transported passively by currents and convection through the water column. Those kinds of transport can move the cells into unfavorable environmental conditions like darkness.

Light limitation is argued to be the reason that 95 % of the oceans are heterotrophic (Regaudie-de Gioux and Duarte, 2010). Chlorophyll *a* concentration can be determined via satellites at the sea surface and used for the estimation of productivity but this method covers the ocean surface only (e.g. Esaias et al., 1986). For the remaining part of the ocean we only have snapshots of phytoplankton abundances and community compositions investigated during research cruises. Still there is no way to permit *in situ* growth of natural cells nether on sea surface nor in the deeper ocean. The only way to get those kinds of information is the experimental measurement of productivity of either plankton community of single phytoplankton species. Such data exist for several communities and species (e.g. Falkowski and Owens, 1978; Marra, 2004; Gattuso et al., 2006) but data on phytoplankton productivity during winter are scarce. As a consequence, the aim of this thesis is to measure primary production at winter conditions by simulating deep convection conditions in laboratory experiments.

2.1 | Phytoplankton seasonality

In temperate and polar regions phytoplankton shows a typical annual cycle which is influenced by abiotic and biotic factors (Fig. 2.1, Rijkswaterstaat, 1985). The winter period is characterized by low phytoplankton abundances, as phytoplankton growth is

limited by low temperatures and light availability. When growth conditions become favorable due to stratification of the water column in spring, cell numbers strongly increase which can lead to the formation of massive blooms (Lewis, 1989; Siegel et al., 2002; Henson et al., 2009). The North Atlantic spring bloom is one of the largest annual events in the world and visible from space (Feng et al., 2009). The biomass produced during spring bloom builds the basis for the entire food web of the region (Platt et al., 2003) and plays a key role in the global biochemical cycle (Ducklow, 1989; Behrenfeld et al., 2013). The high peak of phytoplankton abundance developed during spring collapses due to sinking for the cells and zooplankton grazing (Smetacek et al., 1978; Townsend et al., 1994). Furthermore, most of the essential nutrients in the upper water column were assimilated during spring so growth is nutrient limited in summer. Small bloom events can occur when storm events in autumn brake up stratification, and mixing transports nutrients into the euphotic zone (Cushing, 1989). In general, autumn and winter in northern areas are mainly characterized by low phytoplankton concentration due to unfavorable growth conditions. However, a phytoplankton winter stock is still necessary to initiate the spring bloom (Drinkwater et al., 2003).

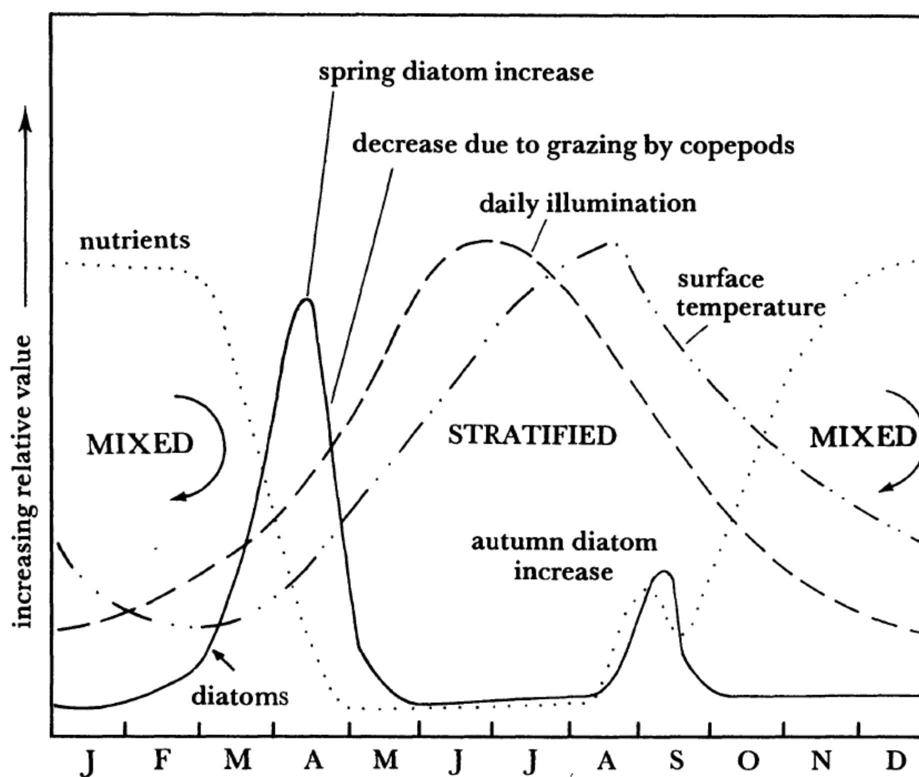

Figure 2.1: Seasonal cycle of phytoplankton and nutrient concentration in the northern North Sea (Rijkswaterstaat, 1985).

In shallow and coastal areas phytoplankton cells survive the winter months as inactive

cells or resting spores in the sediment. When storm events in spring induce mixing of the water column down to the sea bottom those cells are transported back into the water column and seed the spring bloom (Smetacek, 1985; Eilertsen et al., 1995). In the open ocean where water depth is greater than mixing, re-suspension is no option for spring bloom seeding. Here some cells have to survive in the water column to seed the proximate spring bloom (Platt et al., 1991). The winter situation of the North Atlantic is characterized by deep convection (Gordon, 1982; Marshall and Schott, 1999).

2.2 | Deep convection and the critical depth theory

Water cooling and high wind intensity in autumn in the northern North Atlantic forms the convective mixed layer (CML) with a depth of up to 800 m (Marshall and Schott, 1999). The CML comprises one water mass of equal temperature, salinity, and nutrient concentration. The phytoplankton cells within the mixed layer are transported by convection , (Fig. 2.2, Backhaus et al., 2003). Due to the fact that mixed layer depth is deeper than the euphotic zone and day lengths during winter are short, light is the most limiting factor of phytoplankton growth in the winter month of the North Atlantic (Ross et al., 2008). Based on Sverdrup's critical depth theory (1953) for a long time

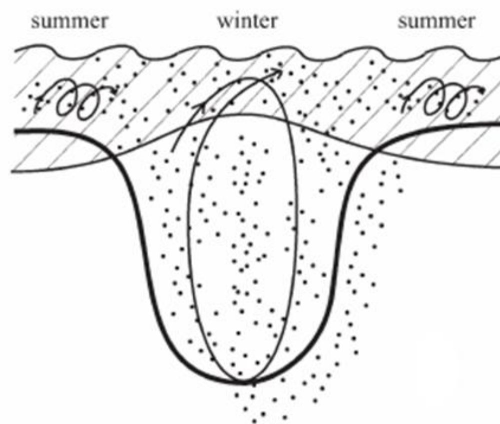

Figure 2.2: Annual evolution of plankton dispersed within the convective mixed layer (CML). Looped arrows indicate orbits of convection. The CML (thick line) in winter is deeper than the euphotic zone (diagonally striped) (Backhaus et al., 2003).

it was assumed that no appreciable primary production can occur in deep convection. Sverdrup (1953) defined the critical depth (Fig. 2.3) as depth where primary production, integrated through the whole water column equals the daily loss, and claimed that no positive phytoplankton production is possible as long as the mixed layer depth is deeper than the critical depth. For his calculations Sverdrup (1953) assumed a constant decrease of light intensity with depth, equal phytoplankton distribution and constant loss by respiration over the entire water column. The losses included in the critical

depth model were later expended by zooplankton grazing and cell sinking out of the euphotic zone (Smetacek and Passow, 1990). Although the mixed layer depth in this area is assumed to be always deeper than the critical depth (Follows and Dutkiewicz, 2002; Siegel et al., 2002) phytoplankton concentrations comparable to a spring bloom concentration distributed over the whole mixed water column were observed during winter in the northern North Atlantic (Backhaus et al., 2003).

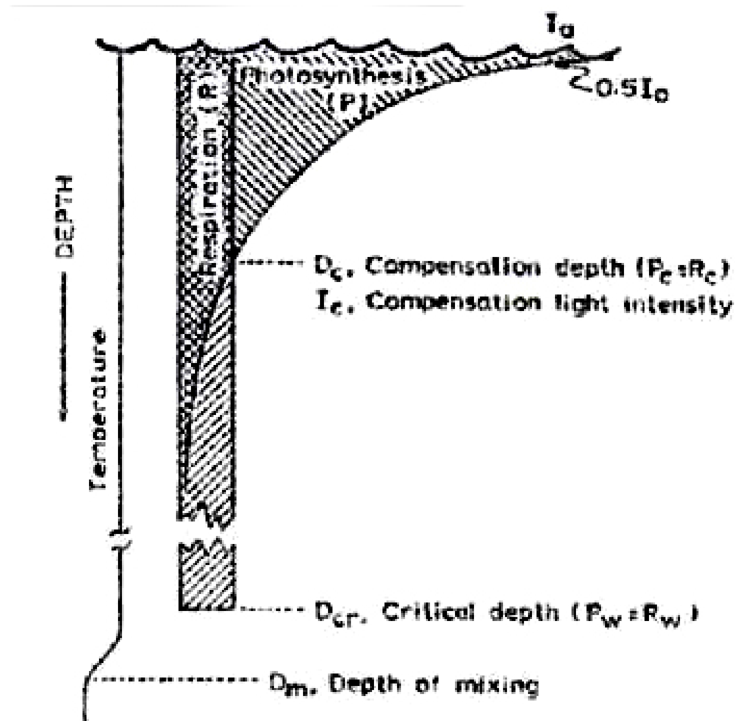

Figure 2.3: The critical depth model by Sverdrup (1953).

2.3 | Phytoplankton production

A possible explanation for the appearance of comparatively high chlorophyll *a* concentration during winter is the hypothesis of phyto-convection (Backhaus et al., 1999). This hypothesis argues that cells within the CML are transported by convection through the whole mixed water body and therefore frequently visit the euphotic zone (Backhaus et al., 2003; Lindemann et al., viwe). Model calculations predict an interval of this short but frequently visits in the euphotic layer being between one and two days (Backhaus et al., 1999, 2003; D'Asaro, 2008). All cells within the CML would be exposed to a statistically same amount of light depending on the mixed layer depth. As there is no possibility to determine the transport of phytoplankton particles by convection the only way to describe the environmental conditions phytoplankton particles might be exposed to is an individual based model (IBM). Lindemann et al. (viwe) used a

Lagrangian approach of an IBM to follow certain phytoplankton cells (tracers) within the mixed layer. For carbon budget calculations the model assumes that phytoplankton gain carbon in light and loose it during the dark period. The calculation bases on the theory that phytoplankton growth occurs at positive net production (Langdon, 1987). Ecosystem models are evaluated based on phytoplankton growth rates mainly originating from laboratory experiments. Most of these experiments were carried out under light/dark cycles of 12/12 h or longer, which exceed light availability during deep convection.

Growth rate and productivity of many different phytoplankton species were carried out under different growth conditions in laboratories all over the world. There are three main focuses laboratory experimental are designed for: (i) to determine the maximal growth rate of algae e.g. for their use in aquaculture (Sandnes et al., 2005; Bouterfas et al., 2006), (ii) to test their acclimatization potential to non-optimal conditions (Post et al., 1984; Falkowski and LaRoche, 1991) and (iii) to determine the growth rate under ambient conditions (Kromkamp and Limbeek, 1993; Hammer et al., 2002; van der Grinten et al., 2005), whereat it is always hard to define ambient conditions for the open ocean. During winter and early spring in the North Atlantic nutrient availability is still high, thus that light and temperature are the factors dictating phytoplankton growth (Ross et al., 2008).

As photoautotroph organisms, phytoplankton carry out photosynthesis to fixate energy. The photochemical reaction it self is temperature independent whereas all growth-related processes depend on temperature (Davison, 1991). Phytoplankton growth increases with increasing temperatures up to a species-specific optimal growth temperature (Eppley, 1972; Montagnes and Franklin, 2001). This temperature dependence is mainly driven by enzymatic activity and membrane fluidity (Raven and Geider, 1988; Davison, 1991). Since temperature range during this time of the year is supposed to be located below the temperature optimum of the occurring phytoplankton species, growth is limited by temperature. Light saturation is reduced at cold temperatures (Davison, 1991). The relation between irradiance and photosynthesis is described by the so call photosynthetic irradiance curve (PE curve). Phytoplankton growth increases with increasing light intensity until light saturation. Above this intensity photoinhibition occurs (Platt et al., 1977; Falkowski and Raven, 1997). Temperature and irradiance also have an interactive effect on phytoplankton growth (Harris, 1978; Raven and Geider, 1988). For phytoplankton growth a certain threshold of temperature and irradiance must be passed. The minimal necessary irradiance for phytoplankton growth is usually determined as the compensation irradiance, defined as the daily light dose where net primary production is zero (Marra, 2004). The compensation irradiance includes both, light intensity and duration in one parameter (Sommer, 1994), whereas a daily light dose applied in different light/dark cycle intensity combinations could cause different growth rates (Nicklisch et al., 2008). This for example could be caused by the temporal variability of acclimation processes to changes of the environment (MacIntyre et al., 2000).

Algae have a high potential to acclimate to changing light availabilities. Especially

at low light intensity cells can better their light saturation capacity by an increase of chlorophyll *a* content (Post et al., 1984). Many experiments were carried out to investigate low light behavior and acclimation of phytoplankton under constant low light intensities applied in natural daily variations (Falkowski and Owens, 1980; Anning et al., 2000; MacIntyre et al., 2002), whereas pelagic phytoplankton are seldom exposed to constant low light conditions. In the open ocean and especially under deep convection, light exposure is highly variable (MacIntyre et al., 2000). Those mixing processes have already been simulated in laboratory experiments by the application of fluctuating light. Most of those laboratory experiments so far simulated surface radiation and small scale mixing processes with peaks of high light intensity focusing on photoinhibition (Fietz and Nicklisch, 2002; Milligan et al., 2012). There are only very few investigations focusing on phytoplankton growth and physiology under short light exposure as they are expected to occur under deep convection (Foy, 1983; Thompson, 1999; Bouterfas et al., 2006).

In this thesis laboratory experiments were carried out to investigate the effect of short light windows on phytoplankton growth, physiology, and acclimation as well as the effect on different winter conditions on the development of a spring bloom.

2.4 | Objectives

The aim of this work is to elucidate phytoplankton growth and physiology under deep convection situation of the North Atlantic. The main questions underlying this thesis are:

1. Is positive primary production possible under short light conditions as expected to occur during North Atlantic winter situation?
2. What are the survival strategies and the acclimation processes of phytoplankton under such conditions?
3. How does overwintering under deep convection influence the spring bloom development?
4. How do changes in the environment due to climate change influence the productivity and species composition of a phytoplankton winter stock and the spring bloom seeding?
5. Is the interaction of temperature, light intensity and light duration relevant for the estimation of *in situ* productivity or for ecosystem modelling?

To answer these questions different laboratory experiments were carried out. For this mainly the diatom *Thalassiosira weissflogii* was investigated under different light availabilities and temperatures to simulate a deep convection situation in the North Atlantic. As a first approach the potential of growth under short light windows (2 h

light per day) was tested and compared to a rather classical low light approach offering light of the same daily light dose divided as lower light intensity over 12 h light per day **(manuscript 1)**. Chlorophyll *a* and carbohydrate content were determined to expose the physiological mechanisms underlying the growth of phytoplankton under those unfavorable growth conditions. Furthermore, the acclimation of the photosynthetic apparatus was investigated.

Continuously oxygen measurements were carried out at different low light conditions and temperatures to look more precise on how productivity under growth unfavorable conditions like short light and long dark periods is possible **(manuscript 2)**. Photosynthetic and respiratory rate of dark respiration were analyzed according to their dependence on temperature and light availabilities. These parameters were used for the calculation of net primary production which was related to phytoplankton growth. In respect to the good correlation between net primary production and growth we would recommend the calculation of net primary production for model calculations of ecosystem productivity. Finally, the effect of different winter conditions on the onset of a phytoplankton spring bloom was tested **(manuscript 3)**. Here for, two different phytoplankton species, the diatom *T. weissflogii* and the flagellate *Rhodomonas* sp. were exposed to two possible winter situations - complete darkness and short low light intervals simulating deep convection - to test their survival strategies on possible winter situations. The effect of raising temperatures on the winter survival of the two species was tested as well as the effect of winter temperatures and light conditions on the phytoplankton growth after re-illumination. Algae preconditioned at different winter situations and temperatures were exposed to spring bloom simulating growth conditions to test the effect of different winter condition on their re-illumination behavior and on the competitive potential of these two different phytoplankton species.

Figure 2.4: *Thalassiosira weissflogii* (a) and *Rhodomonas* sp. (b).

All experiments **(manuscript 1, 2 and 3)** were carried out at different temperatures to test the effect of temperature on algae behavior and acclimation mechanism with regard on rising sea surface temperature due to climate change. To determine possible acclimation processes of the photosynthesis to the different light availabilities or the complete absence of light during winter chlorophyll *a* fluorometry with a Water PAM

(Walz Germyn) was carried out (**manuscript 1 and 3**). chlorophyll *a* fluorometry is a rapid, invasive method to expose photosynthetic activity, physiological state of the algae as well as possible acclimation processes.

The experiments were mainly carried out using the diatom *Thalassiosira weissflogii* as a model organism (**manuscript 1, 2 and 3**). The cryptophyte *Rhodomonas* sp. was used for the competition experiment (**manuscript 3**). Both phytoplankton species are well studied and already used for many laboratory experiments.

Thalassiosira weissflogii (Grunow) Fryxell and Hastle 1977 (Fig. 2.4 a) is a non chain building, cylindrical diatom of middle size (5 - 32 μm in diameter). It is a widely distributed marine species that can also survive in brackish or freshwater environments (Guiry and Guiry, 2013). As mainly other *Thalassiosira* species as well it was found to be part of the North Atlantic winter stock and during spring blooms (Dickson et al., 1988). *Rhodomonas* sp. (Wislouch) Hill and Wetherbee 1989 (Fig. 2.4 b) is a small flagellate (5 - 10 μm) widely distributed in marine brackish and freshwater environment often use as food source in aquaculture. As flagellates they are motile (Guiry and Guiry, 2013).

Both phytoplankton species suite very well for basic investigation, due to the high comparability to other investigation already carried out with these species. Furthermore, they are representative for many other phytoplankton species.

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Chapter 3

Interactive effects of temperature and variable light on growth and biochemical composition of the diatom *Thalassiosira weissflogii*

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Abstract

Growth rate, biochemical composition and photosynthetic activity of *Thalassiosira weissflogii* were determined under two different light scenarios and 5 different temperatures to simulate conditions experienced by cells during winter deep convection to identify possible acclimation mechanisms to varying light conditions. A low light scenario ($20\,\mu\mathrm{mol\,m^{-2}s^{-1}}$, 12/12 hours light/dark), was compared to a scenario exposing short light pulses of a higher light intensity ($120\,\mu\mathrm{mol\,m^{-2}s^{-1}}$, 2/22 h light/dark). No growth occurred at temperatures below 8 °C. Above 8 °C, growth rates were significantly higher under low light than under short pulsed light conditions, indicating a higher degree of efficiency. This was related to (i) higher concentrations of chlorophyll *a* per cell in the low light treatment and/or (ii) a more efficient transfer of gained 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 the metabolism. Photosynthetic parameters measured as chlorophyll *a* fluorescence (using PAM fluorometry) showed a typical low light behavior for the algae exposed to short light. Short light pluses were not sufficient to reach full light saturation. Photosynthesis was more strongly affected by temperature under pulsed light than under low light conditions. Our results indicate that model estimation of primary production in relation to deep convection, which are based on average low light conditions and do not consider vertical transportation of algae, will lead to an overestimation of *in situ* primary production.

3.1 | Introduction

Marine primary production is a major component of the global carbon budget and production patterns being largely determined by the availability of limiting nutrients and light (Falkowski et al., 1998). During winter deep convection and in spring prior to the spring bloom in temperate and polar regions nutrient levels are high and the availability of light limits marine primary production (Ross et al., 2008). Light availability for primary production varies on temporal scales from seconds to month as a result of surface mixing or seasonal cycles (MacIntyre et al., 2000). Previously, many investigations have focused on the onset of spring phytoplankton blooms in temperate regions such as the North Atlantic, because of their importance for the entire food web as well as their role in the carbon cycle (e.g., Li et al., 1993; Bury et al., 2001; Henson et al., 2009).

A common theoretical model for the development of a phytoplankton spring bloom is the critical depth model of Sverdrup (1953). This model is based on the assumption that net primary production is only possible when the mixed layer depth is shallower than a critical depth, where depth-integrated phytoplankton production equals the loss by respiration or grazing. Many studies have provided support for the Sverdrup hypothesis based on model studies or observations (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 tools (Huisman et al., 1999; Nagai et al., 2003; Ross et al., 2011) challenge Sverdups critical depth model identifying the development of a spring bloom well before the onset of stratification. Sverdrups model assumes exponentially decreasing light and homogenously distributed phytoplankton cells within the mixed layer. The response to changing abiotic conditions such as light and any loss (e.g. grazing or respiration) is kept constant.

During deep convection, phytoplankton cells can be transported due to vertical velocities to hundred meters of depth before potentially being returned to the surface (Marshall and Schott, 1999). Observational support for the importance of deep convection comes from Backhaus et al. (2003) who found a homogenous chlorophyll *a* distribution in the deep mixed layer of the North Atlantic during winter with a total integrated biomass comparable to spring bloom conditions. Within a convective cell phytoplankton cells are exposed to short, rapidly changing pulses of light and long periods of darkness (MacIntyre et al., 2000). Individual based model (IBM) 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). Both, the critical depth model of Sverdrup and the IBM model of Backhaus et al. (2003) do not consider the implication of rapid changes of light conditions on phytoplankton growth. However, neglecting vertical mixing in an ecosystem model can lead to an overestimation of primary production, due to an up to 40 % higher photosynthetic rate of non mixed cells caused by the effect of mixing on the photo adaptation potential of the cells (Barkmann and Woods, 1996).

In the development of light parameterizations for modeling phytoplankton growth,

typically laboratory and field experiments have been carried out to determine growth rate of different algae species under different light intensities (Falkowski and Owens, 1980; Cosper, 1982; Sakshaug and Holm-Hansen, 1986), temperatures (Berges et al., 2002) and the combination of both factors (Fawley, 1984; Bouterfas et al., 2002; Hammer et al., 2002). Light experiments often focus on low light acclimation (Post et al., 1984; Cullen and Lewis, 1988; Anning et al., 2000) with most low light experiments carried out with light durations of 8 hours and more. These experimental setups allow algae to acclimate to low irradiances e.g. by increasing chlorophyll *a* content. However, during deep convection phytoplankton may not have enough time to acclimate to ambient light conditions (MacIntyre et al., 2000). Experiments with fluctuating light have shown lower phytoplankton growth rates than under continuous irradiance with the same number of photons of light exposure (Nicklisch, 1998; Shatwell et al., 2012). However, these experiments were carried out under day length of 12 h. Also the balance of cellular resources as e.g. carbohydrates is affected by a change in light availability due to mixing. Most likely more carbohydrates are necessary for the maintenance of the metabolism during the longer dark periods (Raven and Geider, 1988). The development of a spring bloom in the North Atlantic depends on the survival of the phytoplankton winter stock and is affected by winter temperature (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 increase leads to higher enzymatic activity and photosynthesis rate as well as nutrient uptake and thus in turn to a higher growth rate (Raven and Geider, 1988; Falkowski and Raven, 1997). Temperature increases can also enhance the kinetics of activation and deactivation of the photosynthetic apparatus and thus influence the acclimation potential (Davison, 1991). An increase in temperature has a negative effect on dark survival of some diatom species (Antia, 1976) and may lead to an increased dark respiration (Verity, 1982; Lombard et al., 2009). However, the interacting effect of rising temperatures on the growth rate under short light and long dark periods is at present unknown. Short-time changes in light availability represent a challenge for estimating marine primary production with ecosystem models and this process is seldom implemented within these models (Ross et al., 2011; Lindemann et al., viwe). However, individual based models (IBMs) can track phytoplankton cells in the mixed layer and record 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 with the marine diatom *T. weissflogii* comparing a classical (12/12 hour light/dark cycle) low light scenario and a scenario with short intervals of higher light intensity and long dark periods (2/22 hour light/dark simulating a simplified deep convection scenario). Furthermore, the effect of temperature on these two light treat-

ments was tested as well as the interaction between these two limiting abiotic factors. Growth rates were determined as the major parameter describing primary production, chlorophyll *a* content and fluorescence in order to describe acclimation processes of the photosystem and carbohydrate content for an estimate of the energy budget. The results will help to improve IBM based primary production models for the testing of deep convection scenarios in relation to climate change.

3.2 | Material and Methods

3.2.1 | 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. Algae were grown 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 producing 160 - 180 μmol m^{-2}s^{-1}light in a 12/12 h light/dark cycle. Algae were cultured under these conditions for at least three weeks prior to the commencement of experiments. Cultures were continuously bubbled with filtered air to minimize self shading and sedimentation and ensure sufficient supply of CO_2 and O_2. Growth rate of the stock culture in the exponential phase was 0.87 ± 0.01 day^{-1}.

3.2.2 | Experimental setup

Growth experiments were carried out under two different light scenarios with the same amount of photons per day, but with different light/dark cycles and different light intensities. The low light setup (labeled as LL for long and low light) had a light intensity of 20 μmol m^{-2}s^{-1}and a photoperiod of 12/12 h light/dark. The short light setup (labeled as SH for short and high light) had a light intensity of 120 μmol m^{-2}s^{-1}and a photoperiod of 2/22 h light/dark. The total number of photons amounted in both cases to 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 light source, where the light intensity was adjusted by numbers and distance of the lamps.

Both setups 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 at each temperature. Algae from the initial culture were diluted with autoclaved, GF/F filtered North Sea water enriched with 0.5 mL f/2 stock solution per liter 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. For each light exposure setup, two identical experiments were carried out. In one experiment all measurements were made

at the end of the light period while in the second experiment samples were taken at the end of the dark period. These four experiments were carried out between May and August 2011. Triplicate samples for cell counts were taken after 2, 4 and 6 days. 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 at a final concentration of 1 % and measured within three days with a Multisizer 3 (Coulter Counter). Cell number was determined in triplicate for each beaker via the performance of three Coulter Counter runs for each of the triplicates. Growth rate was calculated according to:

$$\mu = (\ln(\mathrm{C}_{\mathrm{dayx}}) - \ln(\mathrm{C}_{\mathrm{day0}}))/\mathrm{days} \tag{3.1}$$

Chlorophyll *a* fluorescence emission for each of the triplicates modulate was measured with a Water PAM (WALZ, Germany). Due to the variability in the growth rates under SH conditions a third experiment was performed in August 2012 under comparable conditions within a temperature gradient between 5.6 and 14.6 °C (5.6, 7.8, 10.1, 12.3 and 14.6 °C). Algae were maintained under the same stock culture conditions as in 2011. As the two different light scenarios were never tested with algae coming from exactly the same stock culture and to validate the results and exclude potential temporal effects an additional experiment was carried out directly comparing low and short light effects of algae coming from the same cultural preconditions. This experiment was carried out at only one temperature (15 °C) in a temperature controlled chamber. After six days, samples for cell abundance, biochemical analysis and PAM fluorometry were taken from each replicate at the end of the light and at the end of the dark period. Henceforth it will be named as the "direct comparison".

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

3.2.3 | **Biochemistry**

Samples for biochemical analyses were filtered either at the end of the light or dark period at 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 chlorophyll *a* or -80 °C for carbohydrate analyses.

Chlorophyll *a* was extracted in 90 % acetone and analyzed photometrically as describe in the method of Jeffrey and Humphrey (1975). Changes in relation of to start values were calculated for each treatment.

Total carbohydrates were determined after Herbert et al. (1971) and Dubois et al. (1956). 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 colored 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.

3.2.4 | Chlorophyll *a* flourescence

Chlorophyll *a* fluorescence was measured with a Water PAM (WALZ, Germany). Light saturation pulse was applied with about 10.000 μmol m^{-2}s^{-1}for 0.8 s. Algae were dark adapted for 5 min before measuring $F_{\mathrm{v}}/F_{\mathrm{m}}$ and rapid light curves (RLC). Whereby first the maximum quantum yield of PSII (Genty et al., 1989) was determined:

$$F_{\mathrm{v}}/F_{\mathrm{m}} = (F_{\mathrm{m}} - F_0)/F_{\mathrm{m}} \tag{3.2}$$

F_{v} is the difference of the maximum fluorescence (F_{m}) measured after a saturating light pulse and the minimum fluorescence (F_0) emitted as a result of the measuring light only. $F_{\mathrm{v}}/F_{\mathrm{m}}$ is the photosynthetic efficiency and can be derived from the minimum and maximum values. Immediately after $F_{\mathrm{v}}/F_{\mathrm{m}}$ 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 m^{-2}s^{-1}and the electron transport rate (ETR) was calculated as:

$$\mathrm{ETR} = \mathrm{yield} * \mathrm{PAR} \tag{3.3}$$

The RLC was fitted to the empirical equation of Platt et al. (1980) using Matlab (R2008a), just like a PE curve. Platt's equation including photoinhibition was selected as saturation or inhibition was observed for every RLC:

$$P = P_{\mathrm{s}}(1 - e^{\alpha E_{\mathrm{d}}/P_{\mathrm{s}}}) * e^{\beta E_{\mathrm{d}}/P_{\mathrm{s}}} \tag{3.4}$$

Two main parameters of the RLC were determined: the maximum relative electron transport ($rETR_{\mathrm{max}}$) and the minimum saturating irradiance (E_{k}). $rETR_{\mathrm{max}}$ 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 alpha with the maximum photosynthetic rate (Sakshaug et al., 1997). α is the slope of the curve, $rETR_{\mathrm{max}}$ and E_{k} were estimated using the following equations (Ralph and Gademann, 2005):

$$\mathrm{rETR_{max}} = \mathrm{P_s}\frac{\alpha}{\alpha+\beta}\left(\frac{\beta}{\alpha+\beta}\right)^{\beta/\alpha} \tag{3.5}$$

$$E_{\mathrm{k}} = rETR_{max}/\alpha \tag{3.6}$$

3.2.5 | Data Analysis and Statistics

For determination of the average growth rate of the LL and the SH experiment a three parameter sigmoid curve was fitted using the Marquarat-Levenberg algorithm (least sum of squares) in Sigma Plot 11.0. Statistical analyses were carried out using SPSS 15.0 software. Growth rate and chlorophyll *a* values data from the two different experiments (end light and end dark) were combined, while for carbohydrates and PAM data, each treatment was processed separately. Data were divided into 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 plus the -direct comparison- experiment) and tested by a Mann-Whitney-U-test. Data were assumed to be significantly different at $p < 0.05$. The effect of temperature on PAM parameters was tested using Spearman correlations. A potential temperature impact on the saturation curves of $rETR_{\max}$ during the light period was determined using non-linear regression. Logistic models (for each temperature and for all temperature combinded) were compared using a second-order Akaike's Information criterion (AIC, corrected for small sampling sizes).

3.3 | Results

Growth rates of each experiment increased with increasing temperature (Fig. 3.1). At temperatures below 8 °C the growth rates were low for both light scenarios (< 0.05 d^{-1}) and not significantly different. In all tested temperature ranges above 8 °C (10, 12 and 15 °C) growth rates of the LL experiment were significantly higher than of the SH experiment. These treatment effects were validated by the results of the direct comparison, where algae from the same precondition were tested at one temperature (Fig. 3.1). Temperature had a significant effect on the growth rate of *T. weissflogii* for both light scenarios. Data variance was greater under SH conditions. Comparison of the sigmoid curve for both light setups showed that the slope of the temperature curve was lower under SH than under LL conditions and a saturation was reached at a growth rate of 0.10 ± 0.02 and 0.23 ± 0.02 at SH and LL, respectively.

Chlorophyll *a* start values of the algae used for each treatment were significantly different, but varied between 4.5 - 5.4 pg cell^{-1}. To identify chlorophyll *a* anabolism or catabolism during the different light treatments changes to the start values were calculated (Fig. 3.2 a). Chlorophyll *a* contents measured at the end of the light or dark period were combined for statistic analysis. After six days under different experimental conditions chlorophyll *a* content was significantly higher for LL than SH conditions except for those at the lowest temperature. A similar result was found for total chlorophyll *a* contents per cell. Start conditions had no effect on the acclimatization potential of the algae. During the SH experiment the content was comparable or lower than the start value, while under LL conditions all values increased. At temperatures higher than 5 °C chlorophyll *a* content was not affected by temperature. Maximum chlorophyll *a* concentration was found at 10 °C LL treatment (7.33 ± 0.5 pg cell^{-1}equivalent

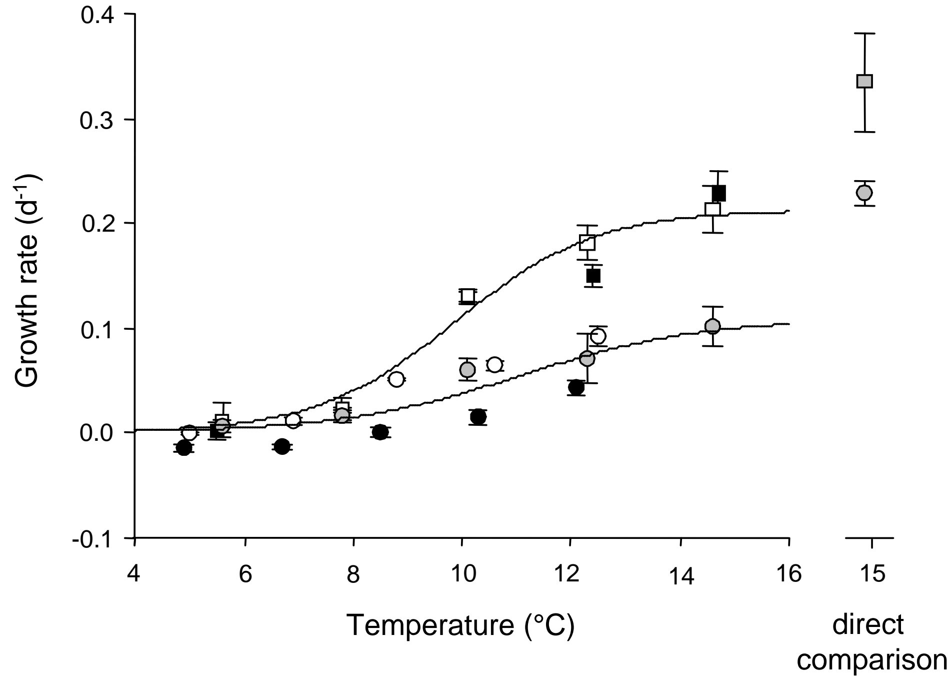

Figure 3.1: Averaged growth rate of *T. weisflogii* under different temperatures and light scenarios: 12/12 h light/dark cycles with 20 μmol m^{-2}s^{-1}(square) and 2/22 h light/dark with 120 μmol m^{-2}s^{-1}(circle). White: measured at the end of the light phase, black: measured at the end of the dark phase (first experiment run 2011). Grey: second experiment run 2012 end of light phase. Mean values with error bars of standard deviation (n = 3).

to an increase of 1.85 pg cell^{-1}), the minimum content was found in the SH treatment of the direct comparison experiment at 15 °C (3.44 $\pm$ 0.6 pg cell^{-1}). For this experiment the largest difference between the tested light treatments was found (2.5 pg cell^{-1}).

Under SH conditions carbohydrate contents per cell (Fig. 3.2 b) was significantly higher at the end of the light (maximum 0.94 pg cell^{-1} -1 at 6.7 °C) than at the end of the dark phase (minimum 0.33 $\pm$ 0.06 at 5 °C), whereas under LL treatment they were constantly low (about 0.5 pg cell^{-1}) and independent of measuremental time. The highest anabolism during the light phase was found again during the SH setup of the direct comparison experiment: 0.25 pg carbohydrates were produced per cell and hour. In this case the delta values reflected the true consumption, as samples were taken from the same replicate.

Two different kinds of chlorophyll *a* fluorescence measurements were carried out: the maximal quantum yield F_v/F_m (Fig. 3.3 a) and the determination of a Rapid Light Curves (RLC) whereof $rETR_{max}$ and E_k were used for interpretation (Fig. 3.3 b and c). The start cultures in 2011 did not differ significantly in their PAM parameters (F_v/F_m: 0.63 $\pm$ 0.02 relative values; $rETR_{max}$: 58.2 $\pm$ 4.4 relative values and E_k: 107 $\pm$ 12 μmol m^{-2}s^{-1}). F_v/F_m values for every tested temperature range were significantly

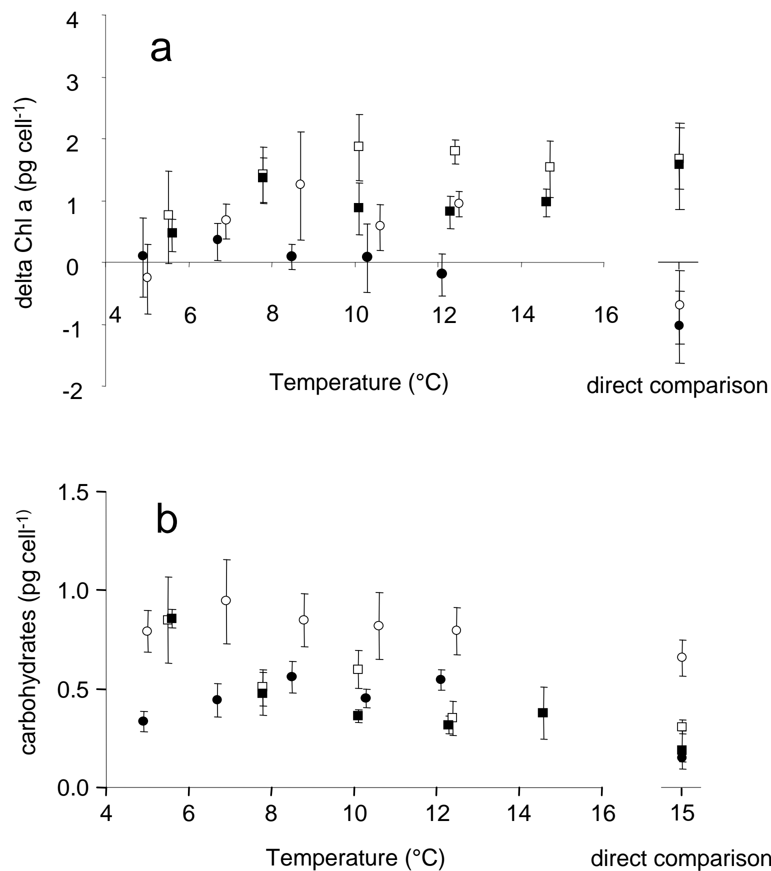

Figure 3.2: Cell components (pg cell^{-1}) under different temperatures and light scenarios: chlorophyll *a* (a), carbohydrate (b). Squares: 12/12 h light/dark cycles with 20 μmol m^{-2}s^{-1}and cycles: 2/22 h light/dark with 120 μmol m^{-2}s^{-1}. Open symbols: measured at the end of the light phase, closed symbols: measured at the end of the dark phase. Mean values with error bars of standard deviation (n = 6).

higher under LL than SH conditions at the end of light and dark phase, respectively. All values measured after six days of the 2011 LL treatment were higher than the start value. Values increased up to the highest value of 0.73 ± 0.01 at 14.7 °C. During the SH experiment F_v/F_m was never higher than the start value and decreased with decreasing temperature especially at the end of the 22 h dark period, where the lowest value was measured at 5 °C (0.35 ± 0.03). Under SH conditions temperature had a significant effect on F_v/F_m. PAM values from the direct comparison experiment taken in 2012 had significantly different start values and can therefore not be compared directly to the measurements of 2011. During this experiment F_v/F_m was significantly lower at the end of the light period of the SH than the LL treatment. All F_v/F_m, except for the lowest temperature of the SH treatment at the end of darkness, were quite high and indicate that algae were in a good physiological state.

RLCs were stronger affected from the different treatments than F_v/F_m. All values

decreased during the dark period, especially $rETR_{\max}$. Under both light treatments $rETR_{\max}$ was significantly higher at the end of the light period than after darkness, at every tested temperature. $rETR_{\max}$ under LL conditions was always significantly higher than of the corresponding SH treatment. Temperature had a significant effect in the SH experiment but not in the LL treatment. Values at the end of the dark period varied between 31.7 ± 0.6 at the highest and 4.6 ± 0.8 at the lowest temperature and between 74.7 ± 2.8 and 44 ± 2.8 at the end of the light period, respectively. The increase between light and dark were similar for both light scenarios. The highest $rETR_{\max}$ for the temperature table experiment of 2011 was 94.5 ± 7.8 (14.7 °C at the end of light). The start values of $rETR_{\max}$ were higher in 2012 than 2011. $rETR_{\max}$ of the "direct comparison" were significantly different for the time of the measurement (end light or darkness), but there were no differences between the two experimental setups.

Data variance of E_k (Fig. 3.3 c) was higher than for F_v/F_m or $rETR_{\max}$. The sampling time (end light or dark) had only an effect during the SH setup. Under the LL treatment data were only significantly different at 10 and 15 °C with at each temperature the value at the end of the dark period being lower than at the end of the light period. Temperature had only a significant effect on E_k values measured at the end of the dark period of the SH setup. The difference between the light and dark measurements during this setup increased with decreasing temperature. Even if E_k values are not always significantly different the proportion of the values to the experimental light intensity gives important physiological information. During the LL experiment E_k was always higher than the experimental light condition of 20 μmol m^{-2}s^{-1}(end of dark between 87 ± 27 and 110 ± 6 and the maximum of 164 ± 35). While especially at the end of the long dark period of the SH setup 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). Light saturation values comparable to the experimental light intensity were just reached at the end of the 2 h light period (between 107 ± 7.1 and 133 ± 6.6). The E_k values measured in 2012 showed less effect of light cycle and sampling time than during the temperature table experiment. None of the data are significantly different.

The temporal resolution of $rETR_{\max}$ values measured during light and darkness (Fig. 3.4) showed a continuous increase of $rETR_{\max}$ after the beginning of the light phase for all three tested temperatures. After 5 h of light phase, with an intensity of 120 μmol m^{-2}s^{-1}, no saturation was reached. In the 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 (Δ AIC com = 34) for the model including a temperature impact. The slopes of the decrease during darkness were lower comparable to the increase in light and not significantly different. The values for all temperatures stood stable during the subsequent 20 h in darkness.

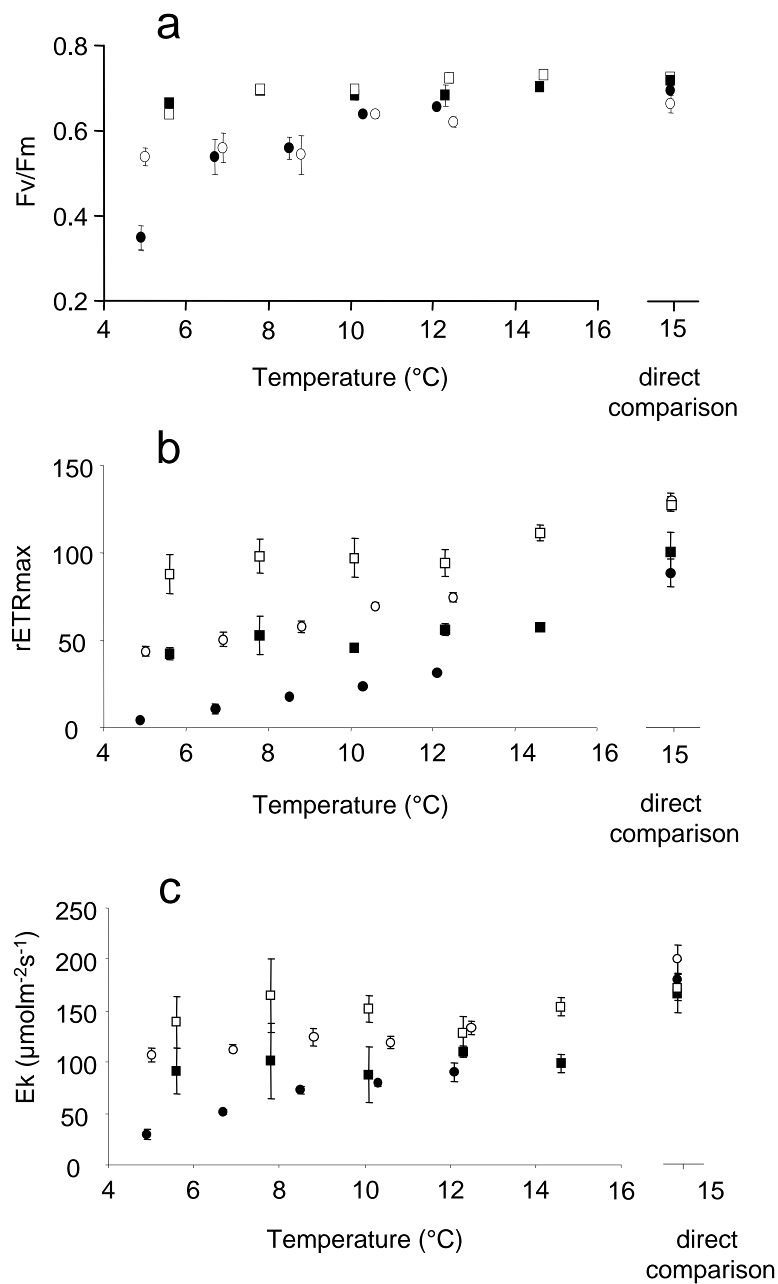

Figure 3.3: Effect of temperature and light availability on PAM data. Squares: 12/12 h light/dark cycles, $20\,\mu\mathrm{mol\,m^{-2}s^{-1}}$and cycles: 2/22 h light/dark, $120\,\mu\mathrm{mol\,m^{-2}s^{-1}}$. $F_\mathrm{v}/F_\mathrm{m}$ (a), $rETR_\mathrm{max}$ (b) and E_k (c) at experimental day 6. Open symbols: measured at the end of the light phase, closed symbols: measured at the end of the dark phase. Mean values with error bars of standard deviation (n = 3).

3.4 | Methodological issues

Every different light treatment had to be carried out separately because of logistic issues. To separate between treatment effects and a potential bias of different start cultures two additional experiments were carried out in 2012. The complete SH experiment was repeated (with sampling only for growth rate at the end of the light period) plus an experiment to test the direct effect of the two light treatments at one temperature with algae coming from the same start conditions, where all measurements from 2011 were carries out. To account for potential setup effects, data from the "direct comparison" were 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. Chlorophyll *a* fluorescence measurements on the other hand deviated from the pattern observed in 2011. Light treatment had less effect on the fluorescence parameters than in 2011. This may be caused by the higher temperatures but also by general differences in photo-physiology of the algae. Start values of the RLC parameters were already higher in 2012. Chlorophyll *a* fluorescence is a good tool for showing photophysiological differences. However, although many studies showed a high correlation with primary production (Morris and Kromkamp, 2003; Goto et al., 2008) it can not be used as a direct measure for primary production. For predictions of photo-acclimation stages it seems to be necessary to use algae coming from the same batch.

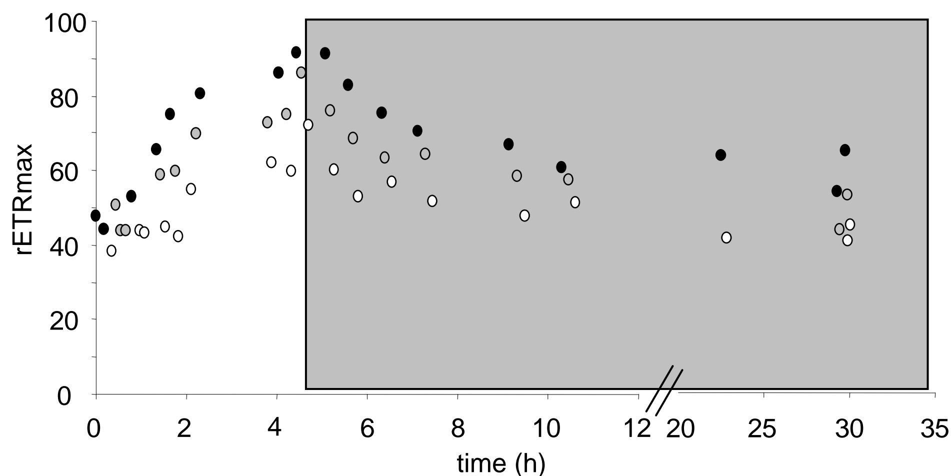

Figure 3.4: $rETR_{\max}$ over time in light and darkness (grey box) at three different temperature (white: 5°C; grey: 10°C and black: 15°C).

3.5 | Discussion

There is a growing recognition of the importance of winter conditions on the dynamics of marine phytoplankton communities both due to their influence of winter production and particle flux as well as their function as a seed population for the spring phytoplankton blooms (Honjo et al., 1988; D'Asaro, 2008). To contribute to our ability to model phytoplankton dynamics during this period we tested the potential of *T. weissflogii* to grow under short term exposure to light similar to the conditions algae would be exposed to in deep convection regime. Furthermore, we describe the acclimation processes to this special environmental condition relative to low light intensity acclimation.

In our study, growth rates of *T. weissflogii* under both light scenarios increased with increasing temperature, whereas the slope of the temperature curve was higher for the LL than the SH treatment. It is well established that phytoplankton growth rates depend on temperature (e.g. Li, 1980). Maximum growth rates for *T. weissflogii* have been found in a wide temperature range of 12 and 20 °C (Montagnes and Franklin, 2001). In our experiment *T. weissflogii* did not grow below 8 °C independent of the light treatment suggesting that this temperature represents a lower boundary on growth for this species. However, our analyses of chlorophyll *a* and carbohydrate content and growth indicate that cells were still active and had not turned into resting stages as often observed in bad growth conditions (McQuoid and Hobson, 1996). While the parameters of chlorophyll *a* fluorescence measurements (F_v/F_m, $rETR_{max}$ and E_k) of the LL treatment did not show any temperature effect, low temperature had a negative effect on the physiological state (F_v/F_m) and the light saturation ($rETR_{max}$) under SH conditions. These observations are comparable to the results of Falkowski and LaRoche (1991) with algae having a lower light saturation level at lower temperatures, hence less energy is used for photosynthesis. This lower light saturation lever leads to an additional problem under low temperature. As light reaction of photosynthesis is temperature independent but the enzymatic reactions of the photophosphorylation and the electron transport depends on temperature, low temperatures can lead to an imbalance of energy absorption and carbon fixation (Davison, 1991). If the dark reaction is slower due to low temperatures, algae exposed to higher light intensities can not utilize all of the light absorbed by the light reaction. Light absorbed in excess potentially causes photo-oxidative damage manly of the PS II (Demmig-Adams and Adams III, 1992). Higher chlorophyll *a* fluorescence parameter under LL condition let assume that the applied light intensity did not induce damage.

Our experiment showed that a similar daily light dose but delivered over different periods did not lead to the same growth rate of *T. weissflogii* at temperatures where growth was not generally limited by low temperature. These light dependent differences in growth efficiency could either be caused by higher energy loss in the SH scenario, by higher energy gain in the LL scenario or a mixture of both. Carbohydrates are the main energy reserve in marine algae and their metabolism is temperature dependent. Temperature has a supporting effect on the anabolism during light (Varum et al., 1986) but

it also increases carbohydrate catabolism during dark respiration (Raven and Geider, 1988; Falkowski and Raven, 1997). Carbohydrate reserves in the SH treatment were relatively high after the light period, reflecting surplus energy that has not been transferred into growth and that was potentially used for dark respiration. The divergence in growth between the two light treatments increased with increasing temperature, which most likely is caused by higher respiration rates at higher temperatures which was not compensated during the short light intervals. During the LL experiment carbohydrate content was constant over the day/night cycle in accordance with constant growth. As previously identified, the accumulation of any cellular component is equal to the rate of population growth under steady state growth (Post et al., 1984). Thus a more stable environment offers better growth conditions even with a lower light intensity per unit time. Our results differ from the results of Thompson (1999) who found a constant relation between growth and irradiance independent of day length for *Thalassiosira pseudonana*, where even 20 hours of darkness did not seem to have a significant effect on growth. In contrast, in our experiment, 22 h of darkness did affect growth rate especially at higher temperatures. Similarly, Verity (1982) showed an effect of day length on growth only at higher temperature, with the shortest tested day length during his experiment being 9/15 h light/dark. To our knowledge the 3/21 h dark/light cycle has been the shortest light period tested so far (Foy, 1983; Bouterfas et al., 2006). Recent studies focus mainly on the effect of fluctuating light intensity on acclimation and growth by simulating surface mixing (Nicklisch, 1998; Wagner et al., 2006; Su et al., 2012). However, simulations of short ephemeral visits of phytoplankton cells in the euphotic zone during deep convection including also longer periods of darkness as predicted by Backhaus et al. (2003) or Lindemann et al. (viwe) are still lacking.

Low light intensities can induce an increase of chlorophyll *a* content to optimize the light harvesting capacity (Post et al., 1984; Cullen and Lewis, 1988; Anning et al., 2000) as was also observed under LL conditions in this study. A similar acclimation did not occur in the SH treatment. Chlorophyll *a* anabolism as an acclimation mechanism only occurred, when light intensity was limiting, with no direct effect of cumulative light dose nor light duration observed. Chlorophyll *a* content significantly increased with increasing amplitude of sinusoid light exposure which was always linked with a lower daily light dose and low light intensities (Wagner et al., 2006; Dimier et al., 2009; Milligan et al., 2012). This supports our finding that chlorophyll *a* accumulation seems to be initiated by low light intensities, whereby this process was independent of periods of high light intensities in the fluctuating light investigations. However, also no increase of chlorophyll *a* was observed during comparable fluctuating setups (Fietz and Nicklisch, 2002; Van Leeuwe et al., 2005). Chlorophyll *a* anabolism is assumed to be a rather slow acclimation process, on the hourly scale (MacIntyre et al., 2000) while PAM fluorometry detects quick acclimation processes like photochemical efficiency of the photosystem II (PSII) and the electron transport over the electron transport chain (Cullen and Davis, 2003; Franklin et al., 2009; McMinn et al., 2010). Despite the higher light intensity PAM measurement results of the SH treatment, algae showed a typical low light behavior in terms of lower $rETR_{\mathrm{max}}$ and E_{k} values. Several previous studies

have identified lower $rETR_{\max}$ and E_k values for algae exposed to lower light intensity (Ralph and Gademann, 2005). The values decreased during the long dark periods and the short light windows seamed not to be sufficient for an increase of the photosynthetic capacity up to a maximal light saturation.

For our study the most useful index for the explaining differences in photobiology between the two different light treatments is the minimum saturation irradiance (E_k). This index, in combination with the ambient light intensity gives important information about light utilization potential (Behrenfeld et al., 2004). During the long dark period of the SH setup E_k decreased below the experimental light intensity of $120\,\mu\mathrm{mol\,m^{-2}s^{-1}}$. Especially at the beginning of the light period photons could not be completely used for photosynthesis. Excess energy could even lead to photodamage so that repairing mechanisms inhibit growth by consuming necessary resource additionally to the already consuming long dark periods. Even at the end of the short light period the minimum saturation irradiance was comparable to the experimental light intensity. Algae could most likely use more of the provided energy than at the beginning of the light period while an expose to E_k equivalent light intensities still occur more damage e.g. of the vulnerable D1 protein than lower intensities (Aro et al., 1993). Under LL conditions in contrast E_k was always higher than the experimental light intensity. The available light could immediately used completely for photosynthesis and most likely initiated less damage.

For a better characterization of the short-term acclimation process an additional experiment was carried out following $rETR_{\max}$ during the changes between light and darkness at three different temperatures. $rETR_{\max}$ followed a light and dark rhythm as it was already described for benthic diatoms in field (Serôdio et al., 2005). For *T. weisflogii* $rETR_{\max}$ increased continuously during the first five hours of light. No saturation could be observed within the tested light time. In darkness the decrease of $rETR_{\max}$ seemed to remain constant 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 complete full photosynthetic capacity. However, Nymark et al. (2013) found a recovery of $rETR_{\max}$ 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 after 48 h of dark exposure.

PAM data are only a snapshot of the photosynhtic parameters (Glud et al., 2002) and even if many studies show a close relation to oxygen production (Henley and Ramus, 1993; Hanelt et al., 1995; Hartig et al., 1998) it can not be used as a real estimate of primary production. During the temperature table experiment PAM data fitted well with growth rate however, interestingly measurements in 2012 were not in agreement. Electrons that involved in PSII electron transport could also been used for cycle electron transport or other metabolic processes (Behrenfeld et al., 2004). Thus, for interpretation of PAM data it is advisable to compare data from algae coming from the same batch. Rapidly changing light exposure is 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

fast rise of light saturation after long dark periods, as it is necessary for algae to survive 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 chlorophyll *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.

To simulate winter and early spring conditions in the North Atlantic our experiments were carried out within a temperature range between 5 °C and 15 °C in order to examine the low temperatures *T. weissflogii* would experience during winter in North Atlantic deep convective cells and a close to growth saturating temperature. The optimal growth temperature for diatoms in general is assumed to be between 10 and 20 °C (Fott, 1971). However, species dominated at Polar Regions can have much lower growth optima (Fiala and Oriol, 1990). Temperature growth rate correlations are species-specific and depend on the acclimatization to environmental conditions (Morris and Clover, 1974; Staehr and Birkeland, 2006). Typical spring bloom diatoms as *Chaetoceros*, *Nitzschia* or *Rhizosolenia* (Sieracki et al., 1993; Backhaus et al., 2003) have an optimal growth temperature according to the environmental conditions (*C. lacinious* e.g. 9 °C; (Schöne, 1977)). Our study indicates that above temperature limitation up to the temperature optimum light duration will strongly affect diatom growth, and this effect increases with increasing temperature. An increase of temperature due to climate change in this area (SST increase of about 2 - 4 °C till 2100 (Houghton et al., 2001)) would most likely benefit phytoplankton growth in two ways: by the positive effect of temperature increase in a rather cold environment and an increase of stratification (Li, 2002) thus reducing light limitation. Higher temperature and longer light exposure times, independent of the light intensity, would lead to an increased primary production.

In conclusion, our experiment showed that a calculation of primary production with growth rates coming from experiments with constant low light intensities, as often assumed in model calculations, leads to an overestimation of primary production in well mixed water bodies. In our study growth rate was about 50 % higher under constant low light than under pulsed light. Some model calculations and experiments have focused on the difference between constant and fluctuating light (Marra, 1978; Barkmann and Woods, 1996; Anning et al., 2000; Ross et al., 2011). The 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. Whereby this 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 it is expected to be in Lindemann et al. (viwe).

Our experiments demonstrated for the first time positive growth under short temporal periods of only two hours light per day in the laboratory. Our study illustrates that, if temperature limitation does not occur, primary production in the whole mixed layer is possible if particles are introduced to the euphotic zone due to convective mix-

ing. Depending on the relation between mixed layer depth and compensation depth a consideration of positive growth within the whole mixed layer depth production during winter would be higher than for the critical depth model. Determining primary production under variable environmental conditions is one of the major goals of biological oceanography (Barber and Hilting, 2002). Even if laboratory experiments can never mimic natural conditions our experiments do give important information about physiological processes and acclimatization during different light dark cycles necessary to improve model calculations.

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Chapter 4

The influence of temperature and light on photosynthesis and respiration of *Thalassiosira weissflogii* exposed to short light conditions

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Abstract

Continuous oxygen measurements were carried out at six different temperature/light combinations on *Thalassiosira weissflogii* to analyze the effect of temperature and light on photosynthesis, respiration and growth at low light conditions. The conditions comprised short light applications of 3 hours light per day at a light intensity of 120 μmol m^{-2}s^{-1}and a temperature range from 4 to 15 °C. In addition, two light/dark cycles of 6/18 (80 μmol m^{-2}s^{-1}) and 12/12 (20 μmol m^{-2}s^{-1}) at 10 and 15°C were tested. At all light conditions growth rates were below 0.2 d^{-1} and we found a good correlation between growth rate and net primary production. Dark respiration rates were less temperature dependent than photosynthesis and an increase in temperature supported NPP and growth rate even at unbalanced light/dark conditions. Dark respiration as well as photosynthesis did not increase linear with temperature. Dark respiration had its temperature optimum at 7 °C and a further increase in temperature did not increase dark respiration. Oxygen consumption in darkness was affected by previous light conditions and an increase in light intensity and daily light dose led to an increase in dark respiration. At the 4 and 7 °C treatments we could observe light enhanced dark respiration (LEDR). NPP and growth was not affected by the daily light dose and hence, a calculation of the compensation irradiance was not possible. Continuous oxygen measurements are a power full tool to describe algae physiology and productivity. Furthermore, we recommend NPP for ecosystem productivity calculations due to its good correlation to growth rate independent on temperature or light application and avoid against the use of compensation irradiance especially for the prediction of plankton community productivity especially for mixed water bodies.

4.1 | Introduction

Phytoplankton growth in the world oceans is the result of exploiting favourable growth conditions and losses like respiration, grazing, sinking or cell death. Positive net ecosystem production is only possible when photosynthetic production exceeds all these losses (Staehr et al., 2011). The balance between photosynthetic production of organic matter and its degradation plays a key role for ecosystem dynamics and impacting the carbon cycle (Duarte and Agusti, 1998; Marra, 2004). Ecosystems where productivity exceeds losses are net autotrophic and act as CO_2 sink (Regaudie-de Gioux and Duarte, 2010).

The estimation of ecosystem productivity is one of the main challenges in marine science especially in higher latitudes where strong seasonally variable environmental conditions dictate phytoplankton growth. During winter and early spring light is the limiting factor for phytoplankton growth (Ross et al., 2008). Phytoplankton needs adequate light levels such that growth can exceed losses. A possible way to describe this minimal production is the compensation irradiance, which is a daily light dose in mol $m^{-2}d^{-1}$ where positive phytoplankton growth occurs. It can ether be determined for single phytoplankton species in the laboratory by an extrapolation to zero growth from a series of cultures grown at different light irradiances (Falkowski and Owens, 1978; Langdon, 1987) or in the field as compensation irradiance of community, which is the daily light dose where planktonic primary production equals the loss of the entire community (Gattuso et al., 2006; Marra, 2004). Laboratory experiments found values in a range of 0.016 - 2.4 mol $m^{-2}d^{-1}$ for several different phytoplankton species mainly diatoms (Langdon, 1987; Eilertsen and Degerlund, 2010). The high variability of compensation irradiance derives from the differences of the respiratory rate. This falls even more into account when plankton communities are exposed to longer dark periods due to deep mixing or deep chlorophyll maxima. As respiratory rates are highly variable and difficult to determine many calculations assumed respiratory rate of the order of 12 % of gross photosynthesis at light saturation (Falkowski and Owens, 1980) even if it is known to be highly temperature dependent (Verity, 1982b; Atkin et al., 2005). Further more dark respiration is effect by previous light conditions (Graham et al., 1996). Higher metabolic rates during the light phase increase respiration, especially during the first hours after darkening. This phenomenon of higher post-illuminated O_2 consumptions is termed light enhanced dark respiration (LEDR) (Weger, 1989; Beardall et al., 1994; Ekelund, 2000) and occurs most likely due to an increase in the amount of substrates produced during photosynthesis available for respiration (Falkowski et al., 1985) or to the reparation of photodamage (Beardall et al., 1994).

Based on photosynthetic productivity and dark consumption net primary production (NPP) can be calculated. A good correlation between NPP and growth rate was found for diatoms growing under different light intensities at a fixed temperature and day/night cycle (Langdon, 1987). Many ecosystem models calculate plankton community production based on the above principles. But calculations are complex in particular in unstratified waters where light conditions are fluctuating. Lagrangian models can follow phytoplankton particles on their way through mixed water bodies and show

their individual light/dark exposure over a time period (D'Asaro, 2008; Lindemann et al., viwe). Phytoplankton growth under unfavourable light/dark conditions as they can occur during deep mixed waters has received little investigated so far (Thompson, 1999; Bouterfas et al., 2006, manuscript 1). Disregarding unfavourable light/dark cycles can lead to an overestimate of primary production as some studies could show that similar daily light doses applied in varying light/dark cycles resulted different growth rate (Bouterfas et al., 2006; Nicklisch et al., 2008, manuscript 1).

To further our understanding of phytoplankton growth in deep mixed water bodies we continuously measured oxygen evolution of the diatom *Thalassiosira weissflogii* under light/dark cycles of 3/21 h at different temperatures. From these measurements we determined photosynthetic and dark respiration rate and calculated NPP to test Langdon (1987) hypothesis that NPP and growth rates correlate under unfavorable light conditions. To uncouple the correlation from light/dark cycle two additional daily light doses applied in different light/dark cycles were carried out. The results will increase the scientific basis for previous model studies to calculate phytoplankton growth via the determination of net productivity.

4.2 | Material and Methods

4.2.1 | Algae cultures

Non axenic cultures of the diatom *Thalassiosira weissflogii* (strain CCMP 1336) obtained from the Provasoli-Guillard National Centre for the Culture of Marine Phytoplankton were cultured in autoclaved, GF/F filtered and f/2 (Guillard and Ryther, 1962) enriched North Sea water (salinity 33) at a temperature of 15°C. Biolux neon lamps (Osram) were use as a light source producing 160 - 180 μmol m^{-2}s^{-1}light in a 12/12 h light/dark cycle. Algae were cultured under these conditions for at least three weeks prior to the commencement of the experiments. Cultures were continuously bubbled with filtered air to minimize self-shading and sedimentation and ensure sufficient supply of CO_2 and O_2.

4.2.2 | Oxygen measurements

For the different experiments the stock culture algae were diluted with autoclaved GF/F filtered North Sea water (salinity 33) of a nutrient concentration containing half of the stock culture concentration to guarantee nutrient saturation under low cell densities and expected growth rates. The cell concentration at the beginning of the experiment was between 9000 - 17000 cells ml^{-1} depending on the temperature and light conditions. Oxygen measurements were carried out in temperature controlled and gas tight bioreactors (Blue Biotech) consist of 4 2 l Schott glass bottles. Temperature was constant within a range of < 0.1°C and logged continuously. The Schott bottles were filled completely with algae suspension to avoid gas exchange via a head space.

Table 4.1: Experimental light and temperature conditions for continuous oxygen measurements.

temperature (°C)	light/dark cycle	light intensity (μmol m^{-2}s^{-1})	daily light dose (mol d^{-1})	abbr.
4	3/21	120	1.296	4°3h
7	3/21	120	1.296	7°3h
10	3/21	120	1.296	10°3h
15	3/21	120	1.296	15°3h
10	6/18	80	1.728	10°6h
15	12/12	20	0.864	15°12h

A magnetic stirrer prevented sedimentation and guaranteed mixing of O_2.

Algae were brought from the stock culture conditions into the different experimental conditions and temperatures by decreasing temperature with 0.2°C per hour in darkness. When experimental temperature was reached light cycles were started with the dark phase. The light source was the same as during stock culture conditions and light intensity was adjusted by placing the bottles in different distances from the light source.

Measurements were carried out for 4 days at 6 different temperature and light treatments listed in Tab. 4.1. The first day was not used for analysis and served as an acclimation period to experimental conditions. During the following 3 days oxygen concentration and temperature were logged every minute. Oxygen measurements were carried out with an Oxy-10-Mini (PreSens) with Optodes. The probe was inserted into the bioreactor at the upper part of the bottle and tightened with a rubber seal. The Oxy-Mini system uses optodes to determine pro cent oxygen saturation assuming constant temperatures. Temperature adjustment was already carried out by the PreSens program for a defined temperature. As oxygen saturation also varies with salinity measured oxygen saturation was adjust to a salinity of 33 (e.g. Grasshoff et al., 1983).

Growth rate was determined every second day for the experiments carried out at 7, 10 and 15°C and over the entire experimental time at 4°C as growth rates were expected to be close to zero. Cell number was determined by taking 40 mL of algae suspension and refilling the head space with autoclaved GF/F filtered sea water. Cell number was determined with a Multisizer 3 (Coulter Counter). The 40 mL sample was split into 3 subsamples and three Coulter Counter runs were carried out for each of the triplicates. Growth rate was calculated according to:

$$\mu = (\ln(C_{\mathrm{dayx}}) - \ln(C_{\mathrm{day0}}))/\mathrm{days} \tag{4.1}$$

O_2 concentration was measured every minute continuously over the entire four days. Average photosynthetic and dark respiratory rate were calculated for the entire light and dark periods in pmol O_2 cell^{-1}h^{-1}using the last cell number determination. Furthermore the entire production and consumption over the entire light or dark period

was calculated. These rates were taken for the calculation of NPP.

We identified possible changes of the metabolic rate during the photosynthetic and the respiratory period by identifying break points in the oxygen curves with a piecewise general linear model with three automatically estimated break points (Muggeo, 2003). The first and the third break point were defined by the beginning or end of the light period, and the middle break point identifying a change in the metabolic rate (Fig. 4.1). However, due to the bootstrap restarting algorithm, here using 100 bootstrap samples, as described in (Wood, 2001), the break point estimation turned out to be not sensitive to the choice of initial values. Due to the fact that the number of break points is fixed in this model, break points will always be identified irrespective of whether the slope shows a clear change or not. The resulting models were validated by only accepting break points with a standard deviation of > 0.3. In cases of clear changes the ratio of the slope before and after the break point was investigated and assumed to be of physiological relevance if it was larger then 2. All calculations were performed using R as language for statistical computing (R Core Team 2012) and the package segmented was used for broken-line regressions (Muggeo, 2008).

4.2.3 | Statistics

The differences between the treatments as well as the effect of temperature and daily light dose for the calculated values were tested via one way ANOVA using SPSS (15.0).

4.3 | Results

Continuous O_2 evolution of *T. weissflogii* was determined under 6 different temperature and light combinations. Fig. 4.1 exemplifies O_2 evolutions determined at 4°C (a) and 15°C (b) with a light/dark cycle of 3/21 h of 120 μmol m^{-2}s^{-1}light intensity and documents the rapid response of the metabolisms to changes in the light availability. O_2 production started immediately after re-illumination and consumption started immediately when light was turned off. At 4°3h O_2 consumption was enhanced during the first hour after darkening. After a clear break point (BP) dark respiration decreased and remained nearly constant over the following 20 h. At the 15°3h treatment we could also observe a small decrease in dark respiration after 9.5 h but the difference between the slope of the respiration before and after the break point was smaller than 2 hence it was not of physiological relevance. Photosynthesis during both applications increased nearly constant over the entire light period.

Additionally, the average photosynthetic and dark respiration rate for the entire light and dark period was calculated (Fig. 4.2). Photosynthetic rate of the 3/21 h light/dark cycle treatments offering 120 μmol m^{-2}s^{-1}increased within the temperature range between 4 and 10°C from 0.33 ± 0.04 at 4°C to the highest value of 0.9 ± 0.08 pmol O_2 cell^{-1}h^{-1}at 10°C. At 15°C the photosynthetic rate was significantly lower than at 10°C. The photosynthetic rate was also affected by light intensity. At 10 and

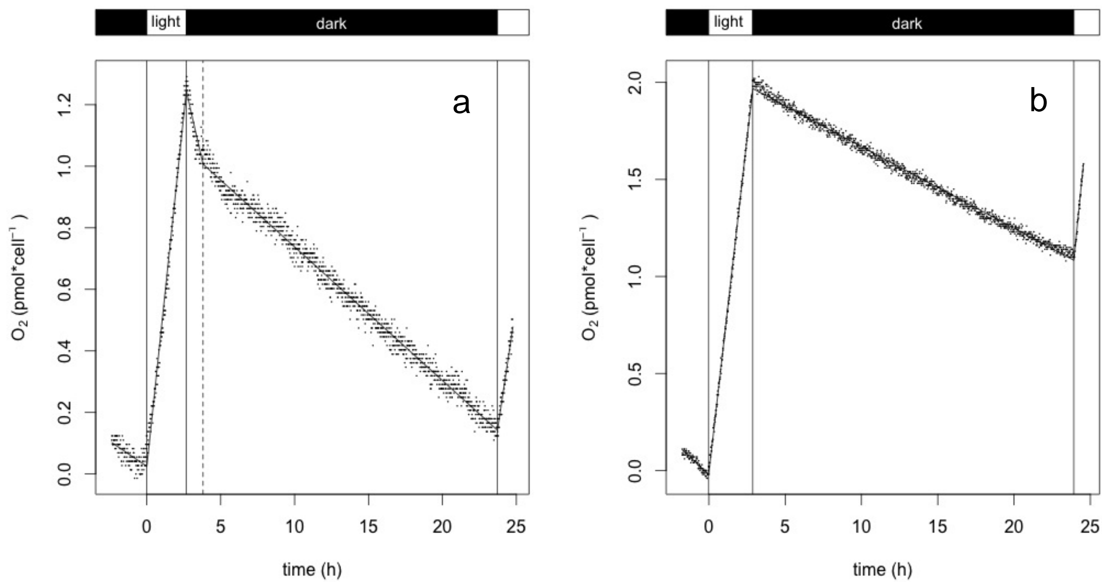

Figure 4.1: Oxygen evolution over one exemplary light/dark cycle of 3/21h of a light intensity of 120 μmol m^{-2}s^{-1}at 4°C (a) and 15°C (b) temperature.

15°C light intensity had a stronger effect on photosynthesis than temperature. The photosynthetic rate at 15°12h (20 μmol m^{-2}s^{-1}) was significantly lower than the rate at 4°3h (120 μmol m^{-2}s^{-1}). A decrease of light intensity from 120 to 80 μmol m^{-2}s^{-1}led to a significantly lower photosynthetic rate at the same temperature. The rate at 10°6h (80 μmol m^{-2}s^{-1}) was not significantly different from the photosynthetic rate at 7°3h (120 μmol m^{-2}s^{-1}). The calculated average respiratory rate for the 3/21 h light/dark treatments tested at different temperatures increased only within the temperature range from 4 to 7°C. The highest respiration rate (0.09 ± 0.01 pmol O_2 cell^{-1}h^{-1}) was found at 7°C. With a further increase of temperature the rate decreased slightly where the rate was only significantly lower at 15°C. The Q10 values were calculated for photosynthesis and respiration of the 3/21 h light/dark cycle treatment for a temperature range of 4 to 10°C. It was determined to be more than twice as high (4.4) for photosynthesis than for respiration (2.0).

Previous light conditions had a significant effect on the respiratory rate of the treatments exposed to the same temperature (10°3h vs. 10°6h and 15°3h vs. 15°12h). An increase of the daily light dose led to an increase of dark respiration and a decrease reduced respiration, at 10 and 15°C, respectively (Fig. 4.2). Within this temperature range the effect of the previous daily light dose was bigger than the effect of temperature. The lowest dark respiration was found at 15°12h (0.03 ± 0.001 pmol O_2 cell^{-1}h^{-1}) being lower than the rate of 4°3h. To further explore the relation between dark respiration and previous production we plotted the respiration against the photosynthetic production. Within the applied light period we found a strong correlation between productivity and following dark respiratory rate (Fig. 4.3). Especially at higher tem-

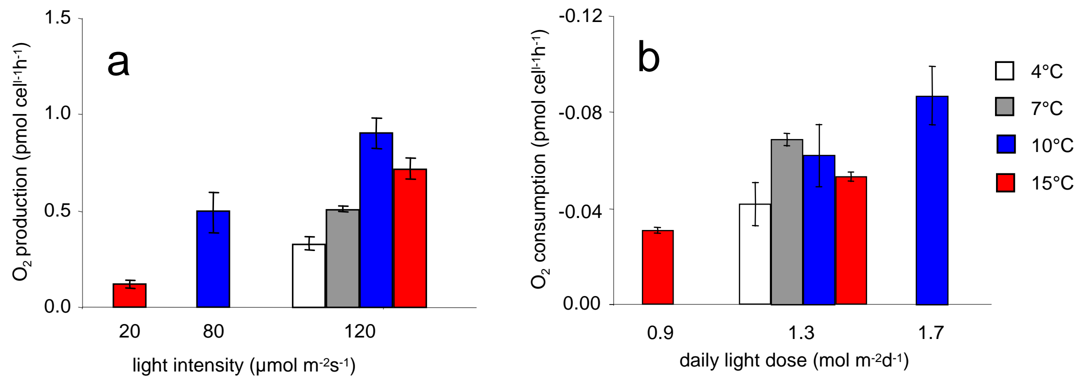

Figure 4.2: Mean values of photosynthetic (a); and dark respiration rate (b) for the entire experiment under different growth conditions. n = 12 for all treatments beside 10°6h (n = 9).

peratures (10 and 15°C) the correlation was almost linear. The two treatments at 4 and 7°C showed a higher respiratory rate at comparable productivities. During these treatments we could observe a pronounced dark respiration during the first hours after darkening at almost every tested dark period. This LEDR is described by a higher ratio of the slope before than after the detected break point (Tab. 4.2). The ratios were plotted as single measurements separated for the different days (Fig. 4.4).

For the two coldest treatments (4 and 7°C) the BP was mostly found within the first 2.5 h after darkening but after 12.3 ± 3.7 h of darkness on the third day of the 4°3h treatment. The early BPs were mainly accompanied by high ratios $<$ 2 of the slope before and after the BP (Fig. 4.4). These ratios were assumed to be of physiological relevance. For all other treatments we could not observe LEDR as the ratio of the slopes was below 2. Due to the long duration of the dark period, the LEDR observed during the 2nd and 4th day of 4°3h in contrast to the 3rd day did not lead to a significant increase of respiratory rate over the entire dark period. BP analysis was also carried out for photosynthesis and the relation of the slopes before and after the BP was never above 2 hence it was not of physiological relevance.

Fig. 4.3 shows the relation between the daily production and respiration. The line where both rates are equal marks zero NPP and data points above this line have a positive NPP. NPP of the 4°3h and 7°3h treatments are located slightly above the zero NPP line showing that consumption and production are nearly balanced. The calculated NPP for 4°3h and 7°3h were not significantly different from each other but to all other treatments. At 10°C and 15°C a positive NPP was found. Photosynthetic and respiratory rates differed significantly between all treatments at 10 and 15°C. Although production and respiration rates covered a wide range depending on the

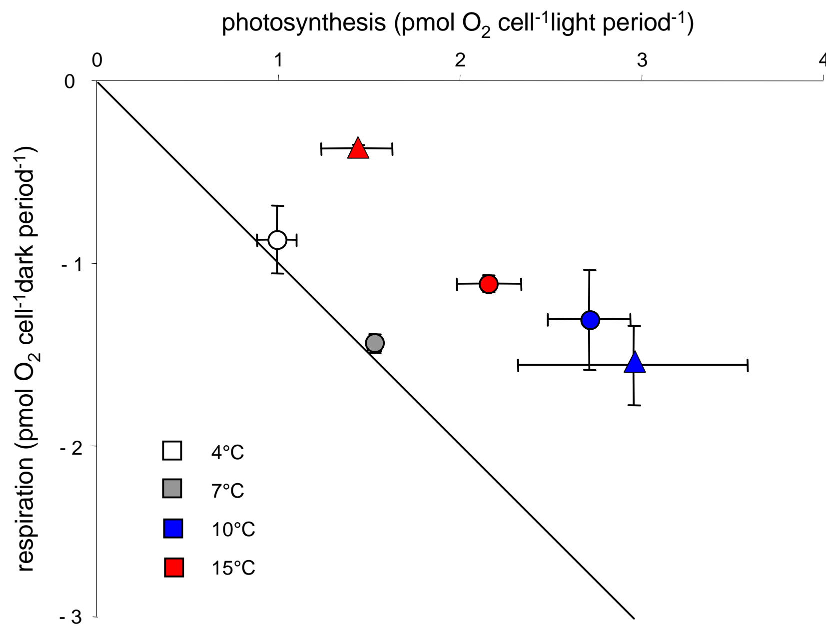

Figure 4.3: Dark respiration of the entire dark period in relation to the photosynthetic production of the entire light period of different light and temperature combination: 3/21 h light/dark cycle (cycles), 6/18 h at 10°C and 12/12 h at 15°C (triangle). n = 12 for all treatments beside 10/6 (n = 9).

applied light conditions, the NPP was quite similar as indicated by similar distances to the zero NPP line. The calculated NPP varied between 1.05 ± 0.13 (15°3h) and 1.54 ± 0.25 pmol O_2 cell^{-1}h^{-1}(10°6h) and were not significantly different. We could not find a correlation between NPP and daily light dose.

By bringing growth rates and calculated NPP into relation (Fig. 4.5) we could show that a NPP around zero caused slightly positive growth rates and a NPP between 1 and 2 pmol O_2 cell^{-1}h^{-1}caused growth rates between 0.1 and 0.2 d^{-1}. Treatments with no significantly different NPP did also not differ in growth rates.

Table 4.2: The mean value (± SE) of the break point (BP) in hours after light turned off and slope of dark respiration for each period before and after the BP of different experimental treatments at each day of the experiment. n = 4 for all treatments beside 10°6h (n = 3).

		4°3h	7°3h	10°3h	15°3h	10°6h
day 2	BP	1.1 ± 3.7	2.1 ± 1	13.6 ± 1.3	10.6 ± 2	3.1 ± 1.2
	before	-0.4 ± 0.06	-0.3 ± 0.24	-0.08 ± 0.02	-0.08 ± 0.01	-0.1 ± 0.01
	after	-0.03 ± 0.01	-0.05 ± 0.02	-0.05 ± 0.02	-0.06 ± 0.01	-0.1 ± 0.01
day 3	BP	12.2 ± 3.7	2.5 ± 1.0	13 ± 1.3	7.9 ± 2.0	4 ± 1.2
	before	-0.04 ± 0.01	-0.1 ± 0.03	-0.08 ± 0.02	-0.06 ± 0.0	-0.16 ± 0.03
	after	-0.03 ± 0.01	-0.06 ± 0.01	-0.05 ± 0.01	-0.04 ± 0.0	-0.07 ± 0.01
day 4	BP	1.6 ± 1.2	0.9 ± 1.2	3.6 ± 3.3	6.3 ± 5.9	
	before	-0.17 ± 0.12	-0.71 ± 0.63	-0.11 ± 0.05	-0.05 ± 0.01	
	after	-0.04 ± 0.01	-0.07 ± 0.02	-0.05 ± 0.00	-0.04 ± 0.00	

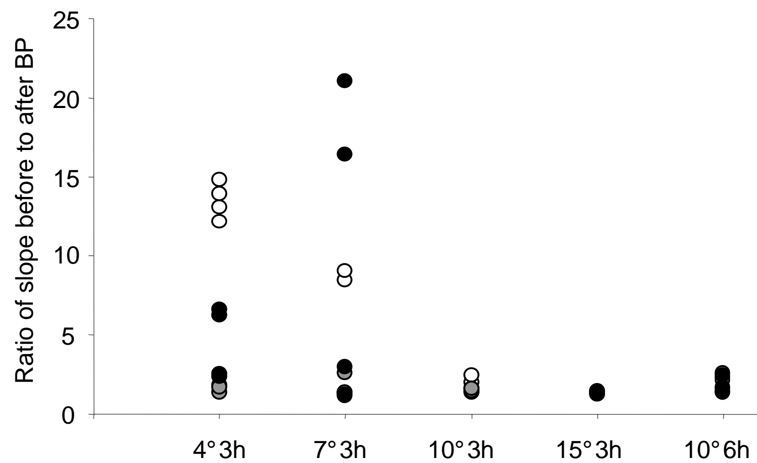

Figure 4.4: Ratio of respiration slope before to after the break point for each single measurement of day 2 (white), 3 (gray) and 4 (black). Single measurements.

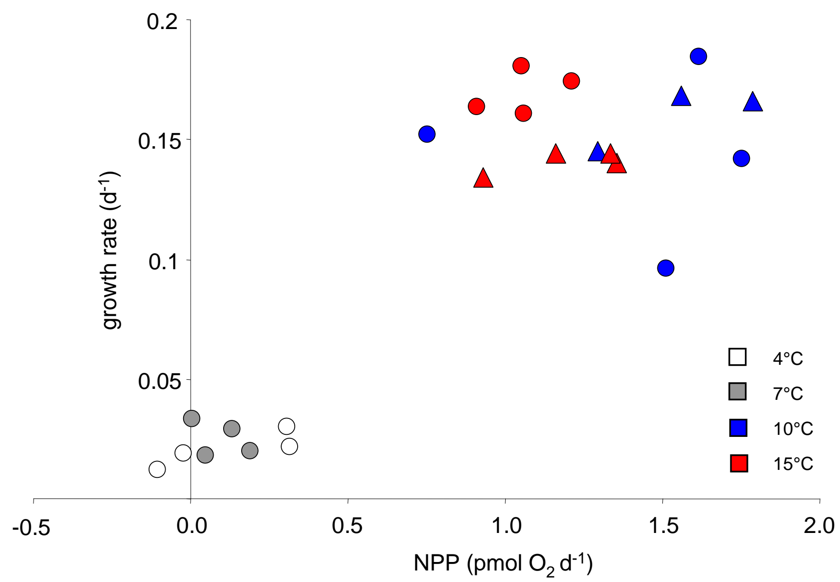

Figure 4.5: Correlation of growth rate and net primary production measured under different experimental conditions: 3/21 h light/dark cycle (cycles), 6/18 h at 10°C) and 12/12 h at 15°C (triangle) plotted as single measurements.

4.4 | Discussion

To further our understanding of phytoplankton dynamics and physiology during deep ocean convection we carried out a set of experiments with the diatom *T. weissflogii* under low light conditions and short light periods. O_2 evolution was determined under 3/21 h light/dark cycles (120 μmol m^{-2}s^{-1}) within a temperature range of 4 to 15°C and used for the calculation of net primary production. For a precise view on how differences in light application effect NPP and phytoplankton growth two additional light set ups were carried out one offering light of 80 μmol m^{-2}s^{-1}within a light/dark cycle of 6/18 h (10°C) and one 20 μmol m^{-2}s^{-1}in 12/12 h (15°C). We observed a complex interaction between ambient temperature, light intensity and light duration and respiration, photosynthesis rates and net primary production. A correlation between NPP and growth rate was found within the tested range of low light intensity causing low algae growth independent on temperature or light application.

4.4.1 | Photosynthesis and respiration

Continuous O_2 measurements give valuable insight in algae physiology and in the oxygen balance of plankton communities. For *T. weissflogii* we could show a rapid reaction of the metabolism. The photosynthetic rate was affected by light intensity and temperature as it was already shown many times before (Graham et al., 1996; El-Sabaawi and Harrison, 2006). Even if we only tested a limited combination of temperature and light our data suggest that light intensity has a stronger effect on photosynthesis than temperature with a Q10 of about 4.4. No significant changes in O_2 production rates were observed during the light period.

In contrast to photosynthesis dark respiration was clearly affected by daily light dose but not by light intensity. A higher productivity during the pervious light period led to higher dark respiration rates. Graham et al. (1996) also observed an increase of dark respiration with increasing temperature and light intensity at constant light/dark cycles of 18/6 h. He observed an increase of dark respiration with temperature. For photosynthesis an optimal temperature could be observed and rate decreased above this temperature. In contrast to our findings a temperature increase led to a reduction of NPP but it should be noted that our chosen temperature range was not above the optimal growth temperature for *T. weissflogii* (Montagnes and Franklin, 2001).

Photosynthetic rates increased from 4°C to 10°C but decreased from 10 to 15°C. The suppression of photosynthetic rates at higher temperatures can be explained by a higher temperature dependence of light respiration in contrast to photosynthetic O_2 production, due to the higher affinity of Rubisco to O_2 than CO_2 at higher temperatures (Berry and Raison, 1981; Raven and Geider, 1988). By measuring the O_2 concentration in the water we could only detect gross photosynthesis and the O_2 consumed during light respiration cannot be separated from the overall production. The only evidence we have of light respiration is dark respiration especially directly after darkening as it is assumed that light respiration is comparable to dark reaction immediately after

darkening (Weger, 1989). Our observation did not support this hypothesis as dark respiration was lower at 15 than 10°C at the same light intensity. If gross photosynthesis decreased at higher temperatures due to a stronger increase of light respiration, light respiration must be stronger affected by temperature than dark respiration as it was observed for some species (Grande et al., 1989).

Dark respiration was less affected by temperature than photosynthesis. Q10 values within a temperature range between 4 and 10°C was less than half of the Q10 of photosynthesis (2.0 and 4.4 respectively). The optimal temperature for respiration was already reached at 7°C. Our findings disagree with studies describing a continuous increase of dark respiration with temperature up to damaging temperature range (Verity, 1982b; Atkin et al., 2005). This suggests that models based on this assumption potentially are wrong. The observed temperature response of respiration is rather unusual but was observed for *Rhodomonas salina* being exposed to low light conditions before (Hammer et al., 2002).

4.4.2 | LEDR

We observed LEDR at most of the dark periods but only at low temperatures up to 7°C. O_2 consumption was in many cases strongly enhanced during the first 1 to 2.5 h after darkening. The phenomena of LEDR is known to occur after higher previous light dose and is assumed to be a consequence of a photosynthesis derived increase of the amount of substrates for respiration (Xue et al., 1996) or a high reparation effort due to photodamage (Beardall et al., 1994). Our findings support those hypotheses as we found LEDR only at lower temperatures (4 and 7°C) where photodamage is known to appear already at lower light intensities due to the imbalance between temperature independent light reaction and the temperature depending dark reaction (Davison, 1991). This imbalance can cause an extended production of harmful reactive oxygen species (Asada and Takahashi, 1987). Surprisingly this LEDR was not found at the third day of the measurements at 4°h3. This could be interpreted as an acclimation process to low temperature or the ambient light conditions, but LEDR occurred again at the fourth day of the measurements. We do not have any reasonable explication for the absence of LEDR at the third day of the 4°3h experiment.

LEDR may explain the higher respiratory rate of 4°3h and 7°3h compared to similar production levels observed at 15°12. In a plot of dark respiration versus production, respiration in the lower temperatures treatments were shifted to higher rates in comparison to those at higher temperatures which show a linear relation between production and respiration (Fig. 4.4). At 10 and 15°C we observed a linear relation between daily respiration and photosynthesis but with equal distances from the zero productivity line implying comparable NPP. Although light intensity and duration were very different with a daily light dose between 0.86 and 1.72 $mol\,m^{-2}d^{-1}$ this did not significantly affect NPP and growth. Difference in the photosynthetic and respiratory rate due to different light intensities where compensated by more favorable day/night cycle. The effect of lower light intensities or daily light doses on respiratory rate at 4 and 7°C

remains to be investigated as only one light condition was tested.

4.4.3 | **Compensation irradiance**

An effect of the daily light dose was mainly found when only light intensity or light duration caused the variation in daily light dose (Verity, 1982a; Hammer et al., 2002; Bouterfas et al., 2006). A linear correlation between NPP or growth and the daily light dose would be necessary for the calculation of the compensation irradiance via an extrapolation to zero (Falkowski and Owens, 1978; Langdon, 1987). Compensation irradiance is a common tool to determine the minimal needed light for growth expressing the light availability as an integrated daily value in $\mathrm{mol\,m^{-2}d^{-1}}$ and removing the influence of day length by combining the two dimensions of the light resource into one (Sommer, 1994). Many ecosystem models did not allow phytoplankton growth below a certain compensation irradiance (e.g. Bissett et al., 1999; Siegel et al., 2002). As experiments showed that the daily light dose if applied in different light intensity and duration combination did not caused equal growth rates (Bouterfas et al., 2006, manuscript 1). In the present study we suggest that the compensation irradiance is not an appropriate metric describing the threshold for plankton community productivity. Especially in mixed water bodies the photoperiod needs to be taken into account as it was already suggested for lakes (Nicklisch et al., 2008).

4.4.4 | **Effect of temperature**

When testing an equal light/dark cycle Foy (1983) observed a decrease of growth at the highest tested temperature (23°C) and a lower optimal growth temperature than at continuous light for the cyanobacteria *Oscillatoria redekei* and *O. agardhii*. There are two hypotheses how temperature can affect growth under short light conditions: (i) growth rates decrease due to raised metabolism during the long dark periods or (ii) growth rates increase because photosynthesis depends stronger on temperature than respiration. Dark respiration is generally assumed to be 12 % of gross photosynthesis at light saturation independent on temperature (Falkowski and Owens, 1980) and this is used in model calculation (Sakshaug et al., 1989). According to this assumption NPP would not be positive at light/dark cycle below 2/22 h. The light intensity of $120\,\mu\mathrm{mol\,m^{-2}s^{-1}}$used during the 3/21 h experiments was close to optimal growth irradiance for *T. weissflogii* (Costello and Chisholm, 1981). In our experiments dark respiratory rate was found to be about 13 % of the photosynthetic rate at 4 and 7°C and 7 % at 10 and 15°C. The more favorable relation at higher temperatures support growth and therefore supports the second hypothesis. In turbid systems where a large part of the phytoplankton population is exposed to low light or darkness plankton communities would benefit from an increase in temperature due to climate change through a higher production levels. The higher temperature dependence of photosynthesis in respect to respiration would lead to a positive NPP even at lower light periods. A warming of the sea surface temperature would lead to a decrease of mixing depth and

longer exposure times or more frequent visits in the euphotic zone (Li, 2002) both supporting a higher phytoplankton growth rate.

4.4.5 | Correlation NPP and growth

For the tested range of suboptimal growth conditions we found a relation between NPP and growth rate. Langdon (1987) observed a strong correlation of these two parameters by testing three different phytoplankton species at a fixed temperature and light/dark cycle with changing light irradiances. In relation to (Langdon, 1987) our experiments covered only about 10 % of his used range of irradiance and caused growth rates. We found mainly two areas where NPP and growth rates can not be distinguish statistically. Due to our finding we suggest that the correlation between NPP and growth is independent of temperature and light application. We observed slightly positive growth even if NPP was zero or negative. We explain this phenomenon by the fact that algae coming from good cultural conditions can use stored resources for producing some cells even under growth unfavorable conditions.

Many ecosystem models ignore the effect of photoperiod (Moisan et al., 2002) although laboratory experiments have shown that photoperiod specific influences growth (Gibson and Foy, 1983; Nicklisch and Kohl, 1989; Thompson, 1999). A realistic way to predict phytoplankton production in strongly mixed water bodies is to use a Lagragian model that can follow particular phytoplankton particles on their way during deep convection as used by (Lindemann et al., viwe). Particles gain carbon during their time in light and respire it during darkness. In contrast to the model where particles only produce biomass as cell volume, positive production in nature is only possible if gained energy could be used for cell division. Our results from this and a previous study (manuscript 1) showed for the first time that the energy gained during very short light windows is sufficient to exceed metabolic losses of the following long dark periods. Our finding further support the use of NPP calculated from photosynthetic and respiratory rates to predict the growth of phytoplankton especially for mixed water bodies where light availability is highly variable.

Both parameters photosynthesis and respiration are highly variable to changes in environmental conditions and furthermore, species specific (Eppley, 1972; Fiala and Oriol, 1990). However, our experiments provide important data of algae physiology under deep mixing necessary to improve ecosystem models. The correlation between NPP and growth rate need to be extended with further light/dark cycles within a higher range of light intensity and fluctuating light conditions. Continuous O_2 measurements are a useful tool for this kind of experiments.

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Chapter 5

The effect of overwintering conditions on the onset of a phytoplankton spring bloom: an experimentally comparison of two different phytoplankton species

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Abstract

Here we tested the effect of two different wintering conditions (complete darkness and short light intervals of low light intensity simulating deep convection) on the survival and physiology of two phytoplankton species (the diatom *Thalassiosira weissflogii* and the cryptophyte *Rhodomonas* sp.) under 3 different temperatures (4, 7 and 10 °C) over 12 days. Both species could withstand unfavorable conditions without large cell loss or physiological damage. *T. weissflogii* reduced its metabolic rate. Cell division stopped after transfer into winter conditions, carbohydrates were used especially during complete darkness but not below a critical value. Additionally, chlorophyll *a* content and photosynthetic capacity was only slightly affected. In contrast *Rhodomonas* sp. continue to grow after transfer to winter conditions during the first 6 days after which cell number started to decrease. More than 70 % of the carbohydrate reserves were used independent on the treatment and the chlorophyll *a* content decreased. The maximum quantum yield was not affected by the winter conditions. Short light intervals (3 h, 20 μmol m^{-2}s^{-1}) had a positive effect on *T. weissflogii* enabling growth at 10 °C. The effect of different wintering conditions on the growth after re-illumination (10 h, 120 μmol m^{-2}s^{-1}) at 10 °C simulating the transition to spring bloom conditions was tested. During the first 3 days the growth rate of *T. weissflogii* was significantly higher than of *Rhodomonas* sp.. *T. weissflogii* benefited from better start conditions and would outcompete *Rhodomonas* sp. in the competition for light and nutrients during spring bloom.

5.1 | Introduction

The phytoplankton spring bloom in northern and temperate regions is one of the largest global annual biological events building the base of the whole food web of these regions (Falkowski, 1994). The spring bloom is seeded by a small amount of cells that manage to survive unfavorable winter situation (Backhaus et al., 2003). Phytoplankton concentration is rather low during autumn and reduced mixing due to warming of the surface water in spring increases light availability due increasing day length and high nutrient concentration support an explosively development of phytoplankton (Henson et al., 2009). Two different strategies for overwintering and seeding of a spring bloom can be considered: cells sink out of the euphotic layer and overwinter as resting cells in the sediment and are dispersed back into the water column by strong mixing events in spring (Smetacek, 1985; Dehning and Tilzer, 1989; Eilertsen et al., 1995) or cells survive winter in the water column, as the so called winter stock, and bloom develops due to reduced mixing (stratification) and increased light availability (Backhaus et al., 1999; D'Asaro, 2008). Winter conditions and the ensuing spring bloom depend on the area. In coastal regions the re-suspension from resting spores and cells from the sediment may occur, but in the open ocean, where water depth is higher than mixing depth, the winter stocks provide the seeding stocks. Backhaus et al. (2003) observed in the North Atlantic in winter 1999 that chlorophyll *a* was distributed over the whole mixed layer with a biomass comparable to a surface water spring bloom.
Studies focusing on the survival of different phytoplankton species under darkness could demonstrate maximum surviving times from days to several months (Antia, 1976; Jochem, 1999; Popels et al., 2007) and a negative effect of temperature (Antia, 1976; Dehning and Tilzer, 1989). Phytoplankton developed different mechanisms to survive prolonged darkness like the formation of resting cysts or cells (Durbin, 1978; Smetacek, 1985; McQuoid and Hobson, 1996), reducing their physiological activity (Antia, 1976; Peters, 1996; Furusato et al., 2004) or heterotrophic nutrition (Harris, 1978; Deventer and Heckman, 1996). Surviving strategies and the adaptation potential are species specific (Peters, 1996). *Thalassiosira puntigera* lost about 65 % of its cellular chlorophyll *a* content during 35 days in darkness while it remained constant for *Rhizosolenia setigera* (Peters, 1996). Murphy and Cowles (1997) observed that *T. weissflogii* could withstand 2 months of complete darkness without a noticeable loss of chlorophyll *a*. For metabolism during darkness resources as carbohydrates are used. Dehning and Tilzer (1989) and Popels et al. (2007) observed a decrease of carbohydrates of 70 - 90 % for Chlorophyceae and Pelagophyceae within two weeks.
Only few studies on open ocean species exist (Peters, 1996; Murphy and Cowles, 1997) and little is known about the winter survival of phytoplankton during deep convection. Individual based models of phyto-convection predict short ephemeral visit in the euphotic layer with returning rates of 1 - 2 days (Backhaus et al., 2003; D'Asaro, 2008). Nymark et al. (2013) recently focused on the survival of diatoms in turbulent mixed water bodies by re-illuminating *Phaeodactylum tricornutum* after 48 hours of darkness. They found that algae could immediately recover after re-illumination. Unfortunately,

the study only focused on time after re-illumination and did not report on survival of algae under short light pulses or rapidly changing environments. In manuscript 1 we observed positive growth of *T. weissflogii* under 2 h of daily light at temperatures above 8 °C. Growth rate was slower than during the comparative approach with a similar daily light dose applied of 0.864 mol $m^{-2}d^{-1}$ in lower light intensities over 12 h of light per day.
The onset, extent and species composition of a spring bloom depend on the pre-bloom phytoplankton stock (Waniek, 2003) as well as the winter conditions (Irigoien et al., 2000). Diatoms are typically the first species to bloom in temperate and cold regions (Margalef, 1978; Barlow et al., 1993; Wiltshire et al., 2008) nevertheless, they are rarely present in North Atlantic winter population (Backhaus et al., 2003). In a competition on limited source the species with the lowest critical level for the limiting sources most likely out competes all other species (Tilman, 1980). Different phytoplankton species build out different life forms to prevent competition by observing different ecological niches. Diatoms are well adapted to turbulent waters with high nutrient concentrations. Flagellates in contrast have higher buoyancy and are better adapted to lower nutrient concentrations which is of benefit for growing in stratified waters (Margalef, 1978). Especially in highly turbulent waters light is often the most limiting factor. Under constant low light conditions, species with a low critical light intensity are the better competitors (Huisman et al., 2004) but the classical competition theory may not work for deep convection because of the non homogenous distribution of light (Huisman and Weissing, 1994). Being adapted to turbulent water requires a high physiological flexibility allow diatoms to be dominant especially in the highly variable environment of the open ocean (Kashino et al., 2002; Wagner et al., 2006). Only little is known about the effect of different winter conditions on the onset of a spring bloom and the competitive potential of different phytoplankton species. The key-questions underlying our experiments are:
How do different wintering conditions affect the survival of phytoplankton? And how do these different wintering conditions affect the onset of a spring bloom?

We carried out laboratory experiments testing the survival of different winter conditions of two phytoplankton species and their potential to react on spring bloom conditions. *Thalassiosira weissflogii* (Bacillariophyceae) and *Rhodomonas* sp. (Cryptophyceae) were cultured for 12 days under complete darkness or two pulsed, low light set-ups simulating deep convection at different temperatures. Growth rate, photosynthetic activity and the biochemical compounds chlorophyll *a* and carbohydrates were determined at the end of the overwintering part of the experiment. In a second part of the experiment preconditioned algae were exposed to spring bloom conditions, where only the initial growth rate was measured.

5.2 | Material and Methods

5.2.1 | Algae cultures

Thalassiosira weissflogii (Bacillariophyceae) (strain CCMP 1336) obtained from the Provasoli-Guillard National Centre for the Culture of Marine Phytoplankton and *Rhodomonas sp.*(Cryptophyceae) (strain CCAC 0083) obstained from Culture Collection of Algae at the University of Cologne were cultured as non axenic cultures in the Laboratory of the Institute of Hydrobiology and Fisheries Science in Hamburg (Germany). Algae were grown in autoclaved, GF/F filtered North Sea water (salinity 32) at a temperature of 15 °C. For the diatoms f/2 medium (Guillard and Ryther, 1962) was used as nutrients source while a Walne's medium (McVej, 1993) enriched with biotin (Stottrup and Jensen, 1990) was used for the cryptophytes. Biolux neon lamps (Osram) were used as a light source spending 120 μmol m^{-2}s^{-1}light in a 12/12 h light/dark cycle. Cultures were continuously mixed with filtered air for minimizing sedimentation, self shading and for an adequate supply of CO_2 and O_2.

5.2.2 | Experimental set up

The experiment was split into to different parts, an overwintering and a spring bloom simulating part as schematically described in Fig. 5.1.

Overwintering set up Algae from the stock cultures were exposed for 12 days to 6 different overwintering conditions. Stock cultures were diluted with autoclaved GF/F filtered North Sea water to a final cell concentration of 7000 and 10000 per mL for *Thalassiosira weisflogii* and *Rhodomonas* sp., respectively and filled into 620 mL Greiner culture bottles. Nutrient concentration were half of the stock culture concentration, leading to nutrient sufficient conditions at respective cell concentrations and low expected growth rates.

Light intensity during the short, 3 h light intervals simulating deep convection was 20 μmol m^{-2}s^{-1}. Every light and temperature combination was carried out in triplicates. After an adaptation period of three days cell number and PAM fluorometry were measured every third day of the experiment (day 6, 9 and 12). 30 mL were taken out of the bottles and refilled with culture medium. Samples were always taken at the end of the light phase, for the treatments simulating deep convection light cycle. Samples for cell counts were fixed with Lugol solution to a final concentration of 1 % and stored in cold and dark no more than two days before analyzing with a Coulter Counter (Multisizer 3). Two Coulter Counter runs were carried out for each of the triplicates. Size fraction was configured by hand between 8 to 14 μm diameters for *Thalassiosira weissflogii* and 5 to 9 μm for *Rhodomonas* sp.. The growth rate was calculated by the following equation:

$$\mu = (\ln(\mathrm{C_{dayx}}) - \ln(\mathrm{C_{day0}}))/\mathrm{days} \tag{5.1}$$

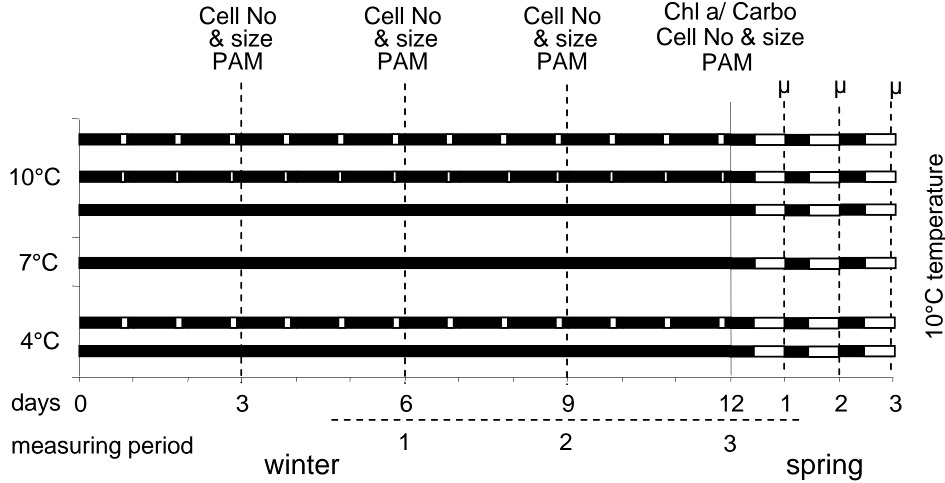

Figure 5.1: Experimental set up.

Coulter Counter runs were also used to determine changes in cell size. The overall size fraction was split into 2 μm steps for *T. weissflogii* and 1 μm steps for *Rhodomonas* sp. and mean diameter size was calculated. Biovolume of both species was calculated according to (Olenina et al., 2006). For *T. weisflogii* the geometric shape of a cylinder with the volume of

$$V = p/4 * d^2 * h \tag{5.2}$$

was used for calculations. The mean diameter (d) was taken form the Coulter Counter calculations and cell height was assumed to be d/1.3 as it is described for cells with a diameter of 12-15 μm.
The volume of *Rhodomonas* sp. was calculated assuming that geometrical shape of the cell is most similar to a cone plus a half sphere. The formula for the volume calculations of this shape is

$$V = p/12 * d^2 * (h + d/2) \tag{5.3}$$

with cell height taken from the Coulter Counter calculations and the cell diameter assumed to be half of the height.
After 12 days at the end of the overwintering simulation cell volume was calculated as percent difference from the start volume, final samples for cell number and volume and PAM fluorometry and samples for the analysis of chlorophyll *a* and carbohydrates were taken by filtering 50 mL of algae suspension onto pre-combusted GF/C filter and stored at -80 °C until analysis.

5.2.3 | **Spring bloom experiment**

Algae suspensions remaining from each incubation described above were used for the spring bloom set up. 400 mL of the incubated algae were transferred into 1 L glass beaker and filled up with autoclaved GF/F filter North Sea water containing the same

nutrients concentration as at the start of the incubation experiment and brought into a 14/10 h light/dark cycle of 120 μmol m^{-2}s^{-1}at 10 °C. The beakers were covered with Petri dishes, fixed with Parafilm, bubbled with filtered air for an equal cell distribution within the beaker and randomly distributed in a temperature controlled table. Growth rate was determined every day for three days as described before.

5.2.4 | Biochemical analyses

Duplicate samples of 50 mL volume were filtered onto Whatman GF/C filters.

Chlorophyll *a* was extracted in 90 % acetone and analyzed photometrically after Jeffrey and Humphrey (1975).

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

5.2.5 | PAM fluorometry

Chlorophyll *a* fluorescence was measured by a Water PAM (WALZ Germany). Samples were taken at the end of the light pulses for the deep convection simulating set ups and at a comparable day time for the samples incubated in complete darkness. Photosynthetic efficiency ($F_{\mathrm{v}}/F_{\mathrm{m}}$) was determined after 5 min of dark adaptation and subsequently a rapid light curve (RLC) was measured while stirring the sample for 20 s during the 30 s light incubation as it is described in Cosgrove and Borowitzka (2006).

Each treatment involved 9 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 of 0, 99, 143, 219, 324, 460, 641, 1063, and 1529 μmol m^{-2}s^{-1}.

5.2.6 | Data analysis and Statistics

Chlorophyll *a* and carbohydrate contents were determined as pg cell volume^{-1} and calculated as percent different from the start value. PAM data are relative values and were also calculated in relation to the start value. Difference between measurements on *T. weissflogii* and *Rhodomonas* sp. as well as the difference between the treatments were tested by T-test for independent variables. Because of a multiple use of the data the significant was adjust via the Dunn-Sidak method (Ury, 1976). Significance was accepted with a p value of < 0.05. Statistic analyses were carried out with SPSS 15.0 soft ware.

5.3 | Results

5.3.1 | Growth (during winter scenarios: day 0-12)

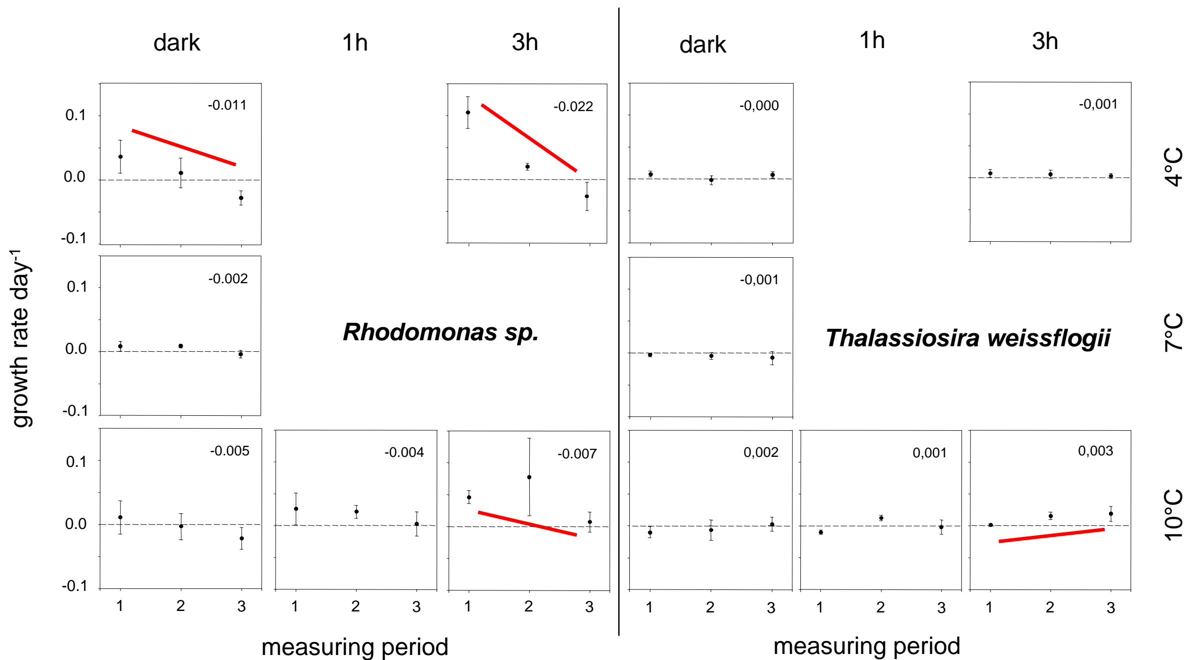

Figure 5.2: Growth rate day^{-1} at the different the overwintering condition 4, 7 and 10 °C under complete darkness, 1 and 3 h light plotted over experimental time. Mean value (n = 3) plus standard deviation. Red line marks the treatments were time had a statistically significant effect ($p < 0.05$) on the growth rate. Number given in each box is the slope of the growth rate over time.

Both *Rhodomonas* sp. and *T. weissflogii* could withstand 12 days of all different simulated winter conditions without a large cell loss. The growth rate of *Rhodomonas* sp. was relatively high after 6 days of incubation (e.g. 0.105 ± 0.025 day^{-1} at 4 °C 3 h light treatment) and an decreased for all treatments during the next 6 days (Fig. 5.2). For the 4 °C dark and 3 h light treatment as well as for the 10 °C 3 h light treatment the difference between day 6 and day 12 growth rates were significantly lower. *T. weissflogii* in contrast had growth rates close to zero except for the 10 °C 3 h light treatment where growth rates were significantly higher at the end of the overwintering experimental time (day 12: 0.019 ± 0.01 day^{-1}) and did significantly increase from day 6 to 12. After 12 days of incubation the growth rate of *Rhodomonas* sp. was only significantly lower than *T. weissflogii* at 4 °C darkness. No further significant differences were found.

Overall growth rates were very low especially for *T. weissflogii*. Average growth rate (Fig. 5.3 a) of *T. weissflogii* was significantly higher under the 3 light treatment than under the dark incubation as well as under the 1 h light treatment at 10 °C. Short light pulses always had a positive effect on the survival of *Rhodomonas* sp. at 10 °C.

The average growth rate for the 1 and 3 h light pulses treatment were significantly higher than of the dark treatment. In the case of *Rhodomonas* sp. this was due to higher growth rates during the first 6 days of incubation whereas the increase of *T. weissflogii* occurred during the later incubation phase. Although the differences in growth rate during the low light and dark setting were rather small we could show that *T. weissflogii* can withstand long periods of winter conditions better than *Rhodomonas* sp.. Furthermore, we could show a significantly positive effect of short light intervals on growth of *T. weissflogii* at least at 10 °C.

5.3.2 | **Biochemistry (winter)**

The biochemical parameters (chlorophyll *a* and carbohydrates) and chlorophyll *a* fluorescence measurements elucidate the different strategies of *T. weissflogii* and *Rhodomonas* sp. to cope with dark and low light conditions. Furthermore, these parameters characterize the start conditions affecting the growth after re-illumination from day 12 onward.

Values from now on are given as percent difference from the initial value. The cell volume decreased for both species and all treatments (data not shown). Cell volume of *T. weissflogii* decreased about 20 % compared to the initial value under all treatments. The cell volume of *Rhodomonas* sp. was reduced by about 20 % at 4 °C in contrast to about 10 % at all other temperatures (7 and 10 °C). Because of the rather large differences in cell volume at the end of the overwintering period carbohydrate and chlorophyll *a* content were calculated as pg volume^{-1} and again given in percent of the start value.

The carbohydrate content of *Rhodomonas* sp. strongly decreased (70 - 80 % to the initial value) during the 12 days in all treatments (Fig. 5.3 b). Under all treatments the carbohydrate content of *Rhodomonas* sp. decreased significantly stronger than of *T. weissflogii*. The treatments (complete darkness or deep convection) had no significant effect on the carbohydrate content of *Rhodomonas* sp.. Carbohydrate contents of *T. weissflogii* in contrast decreased only for all dark treatments, while temperature had no significant effect on the contents. Light had a significant positive effect on the carbohydrate content of *T. weissflogii* especially at 4 °C. *T. weissflogii* accumulated carbohydrates during the short light periods. The content is significantly higher than the dark treatment at 4 °C. At 10 °C the carbohydrate decrease in darkness was significantly higher than under the 1 and 3 h light pulses. *T. weissflogii* did not accumulate carbohydrates during the 10 °C 3 h light treatment. Instead, we could find a significantly positive growth rate. 1 h light per day was not sufficient for carbohydrate accumulation or growth.

Also the chlorophyll *a* content of *Rhodomonas* sp. decreased during all tested overwintering treatments within the incubation period of 12 days (Fig. 5.3 c). The values decreased from 11.9 ± 0.6 ng Chl *a* μm $^{-3}$ to a minimum of 6.3 ± 0.8 ng Chl *a* μm $^{-3}$ during 12 days of an 3 h light incubation at 4 °C. Chlorophyll *a* contents of *T. weissflogii* did only increased during 12 days of dark incubation at 10 °C (from a start value

of 5.8 ± 0.7 to 5.3 ± 0.7 ng Chl *a* μm cell volume^{-1}). In 4 of the 6 tested treatments the chlorophyll *a* content of *Rhodomonas* sp. decreased significantly stronger than the content of *T. weissflogii* (except the dark treatment at 7 and 10°C). In the dark incubation temperature had a negative effect on chlorophyll *a* content of *T. weissflogii.* After 12 days the content at 4 °C it was significantly higher than at 7 and 10 °C. Chlorophyll *a* per volume decreased significantly stronger at 4 °C incubation than at 10 °C. 3 h light pulses had a positive effect on the chlorophyll *a* content of *T. weissflogii* at 10 °C the content increased to 6.9 ± 0.7 ng Chl *a* μm $^{-3}$. Chlorophyll *a* per volume was significantly higher for the 3 h treatment than after dark incubation.

5.3.3 | Chloropyll *a* fluorescence during the winter scenarios: day 0 - 12

The maximum quantum yield F_v/F_m was only slightly affected by the incubation under low light or darkness (Fig. 5.4 a). The values of all treatments fluctuated about ± 5 % comparable to the start value. Only the combination of light and low temperature had a negative effect on the maximum quantum yield. During the 4 °C 3 h light treatment F_v/F_m of both species decreased about 10 % compared to the initial value and was significantly lower than the dark treatment at the same temperature. The maximum quantum yield after 12 days was surprisingly high in all treatments. Minimum values were found at the end of the 3 h light period at 4 °C were F_v/F_m was 0.65 ± 0.03 and 0.63 ± 0.01 for *Rhodomonas* sp. and *T. weissflogii* respectively in comparison to a initial value of 0.72 ± 0.006 and 0.68 ± 0.004. These values still suggest a good physiological fitness of the algae.

Maximal relative electron transport rate ($rETR_{\mathrm{max}}$) was stronger affected by different winter conditions than F_v/F_m. $rETR_{\mathrm{max}}$ of *Rhodomonas* sp. decreased after 12 days in the dark at 10 °C to 71 ± 3.4 % of the initial value of 141 ± 4.03 (Fig. 5.4 b). The lowest value for *T. weissflogii* was determined at 4 °C and darkness (36.8 ± 4.7 equaling a decrease of 68 ± 3.8 % of the initial value). Temperature had a significant negative effect on $rETR_{\mathrm{max}}$ of *Rhodomonas* sp.. Values decrease with increasing temperatures, while the opposite was observed for *T. weissflogii.* Both species could recover their $rETR_{\mathrm{max}}$ during the 3 light period to almost 100 % of the initial value. Cold temperature had a negative effect on the recovery of *Rhodomonas* sp.. At low light and 4 °C $rETR_{\mathrm{max}}$ of *Rhodomonas* sp. was significantly lower than the initial value and the values of *T. weissflogii.* Time had a significant negative effect on $rETR_{\mathrm{max}}$ of *Rhodomonas* sp. in all treatments. In contrast, for $rETR_{\mathrm{max}}$ of *T. weissflogii* only decreased significantly under the dark treatments at 4 °C temperature (data not shown).

5.3.4 | Growth (spring conditions)

After 12 days of simulating different winter conditions, algae were transferred to spring bloom growth conditions including 10 °C temperature and a light exposure of 120

μmol m^{-2}s^{-1}applied over a 14/10 h light/dark cycle. During the first day under spring bloom simulating conditions growth was low for both species coming from all different treatments (Fig. 5.5 a). There were neither significant differences between the two tested species nor between the treatments for any of the species. After three days of spring bloom conditions the average growth rate (Fig. 5.5 b) of *T. weissflogii* was significantly higher than of *Rhodomonas* sp. at all treatments but at 10 °C dark. The only noticeable effect of winter conditions on growth during spring was found for the dark treatment of *T. weissflogii*. Growth rate after re-illumination was significantly higher for the algae coming from the 4 °C incubation than from 7 and 10 °C.

In summary, we observed two different overwintering strategies for the two species. *Rhodomonas* sp. continued to grow during the first days after transfer to winter conditions, probably as long as their carbohydrates supply lasted. After that, growth rates decreased and algae started to die. In contrast, *T. weissflogii* growth stopped when brought into unfavorable conditions and withstood these conditions without cell losses or chlorophyll degradation. *T. weissflogii* was able to produce carbohydrates during the short light intervals already at 4 °C. Chlorophyll *a* content of *Rhodomonas* sp. decreased for all tested treatments. Surprisingly the photosynthetic capacity was almost unchanged during the whole experimental time and the maximal electron transport rate was comparable to the start value in contrast to the dark incubation values which decrease more than 60 % to the start value. Short light windows were sufficient to maintain photosynthetic activity even if algae did not benefit of this nether during overwintering times nor after re-illumination.

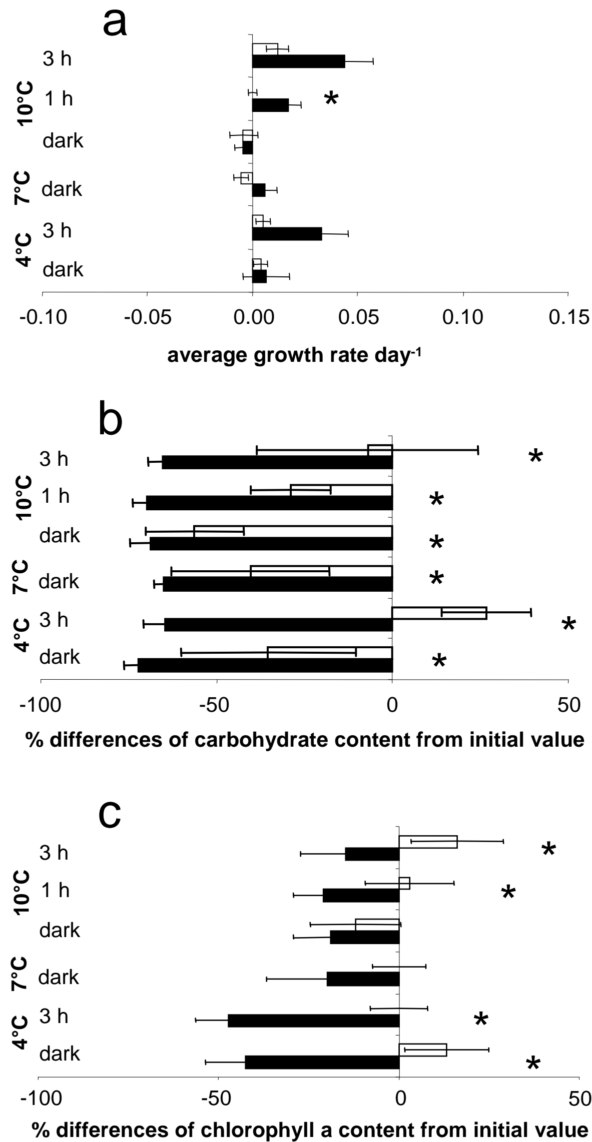

Figure 5.3: (a) Average growth rate (d^{-1}) during the entire 12 days under different winter conditions as mean values plus standard deviation (n = 3). (b) Carbohydrate and (c) chlorophyll *a* content as percent differences from the initial value of *T. weissflogii* (white) and *Rhodomonas* sp. (black bars). Statistically significantly difference ($p < 0.05$) between the two species are marked with an asterisk.

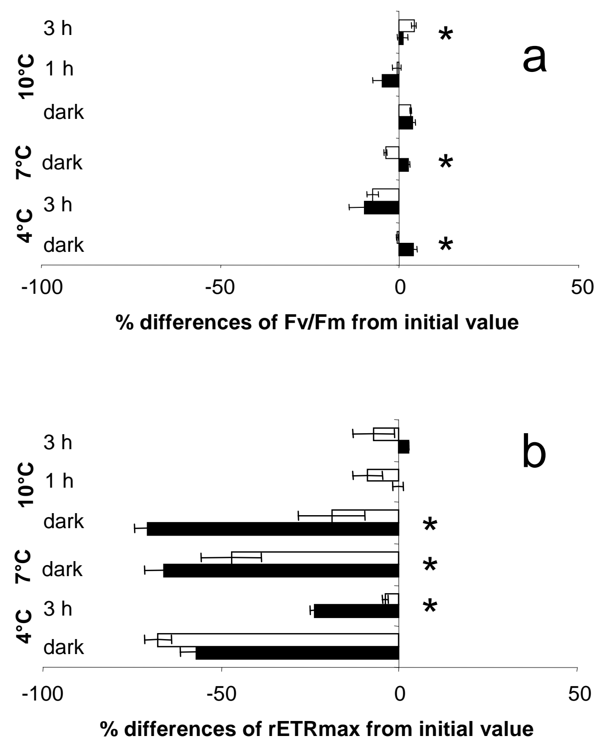

Figure 5.4: PAM measurements after 12 days of different winter conditions (a) maximal quantum yield ($F_\mathrm{v}/F_\mathrm{m}$) and (b) maximal electron transport rate ($rETR_\mathrm{max}$) as percent differences from the initial value of *T. weissflogii* (white) and *Rhodomonas* sp. (black bars). Mean values (n = 3) plus standard deviation. Statistically significantly difference ($p < 0.05$) between the two species are marked with an asterisk.

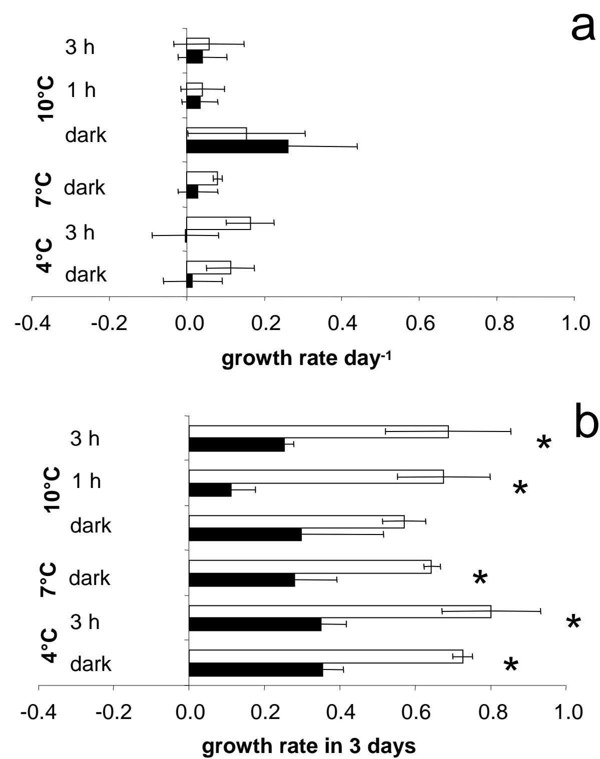

Figure 5.5: Growth rate (d^{-1}) after 1 day (a) and all over growth rate ($3d^{-1}$) of 3 days (b) spring bloom simulating illumination at 10 °C and 120 $\mu mol\, m^{-2} s^{-1}$in a 14/10 light/dark cycle of *T. weissflogii* (white) and *Rhodomonas* sp. (black bars). Mean values (n = 3) plus standard deviation. Statistically significantly difference ($p < 0.05$) between the two species are marked with an asterisk.

5.4 | Discussion

To test the effect of different winter conditions on the survival, physiology and bloom building capacity of two phytoplankton species (*Rhodomonas* sp. and *T.weissflogii*) we carried out laboratory experiment simulating different winter conditions at three different temperatures. The simulated conditions were: (i) complete darkness as algae would be exposed to when sinking to the sediment and (ii) short low-light intervals per days as algae might be exposed to during deep mixing in the northern North Atlantic. Growth rate, carbohydrates and chlorophyll *a* as well as the photosynthetic activity were determined. Algae coming from different winter conditions transferred to spring bloom conditions to test the effect of different winter conditions on their growth and competitive potential after re-illumination.

5.4.1 | Impact of darkness and low light conditions on the survival and physiology of algae

Both species could survive two weeks of simulated winter conditions without appreciable cell loss or great physiological damage. We found a slightly positive effect of short light intervals simulating deep mixing on the survival of both tested phytoplankton species when compared with complete darkness. Only recently studies were carried out investigate growth under short light pluses simulating phytoplankton convection within a deep mixed layer (Nicklisch et al., 2008, manuscript 1). In manuscript 1 we showed that at temperatures above 8 °C *T. weissflogii* could grow with a daily light dose of $0.864\,\mathrm{mol\,m^{-2}\,d^{-1}}$ applied as 2 h of $120\,\mu\mathrm{mol\,m^{-2}s^{-1}}$. In the present study the available daily light dose was even lower ($0.216\,\mathrm{mol\,m^{-2}\,d^{-1}}$) enabling growth at 10 °C. Growth is only possible if net production is positive. Even low positive values of net production lead to positive growth rates (manuscript 2). The compensation irradiance, where net production is zero, differs between diatom species in a range from 0.016 and $2.4\,\mathrm{Wm^{-2}}$, but the experiments were carried out under longer light periods (Eilertsen and Degerlund, 2010).

In our experiments, deep convection simulating conditions of very low light availability were compared to complete darkness and both species could withstand 12 days of darkness without great cell loss nor losing the potential of growth after re-illumination. Maximal dark survival times of different phytoplankton species were determined to be between 7 days and several months with species specific difference in mortality (Antia, 1976; Peters, 1996; Jochem, 1999). Many diatom species survive dark periods without great damage and show rapid growth after re-illumination (Jochem, 1999). In our experiment (4 - 10 °C) temperature had a negligible effect on the dark survival of both species. By testing a larger temperature ranges of 2 to 20 °C Antia (1976) found a strong negative effect of rising temperature on the maximum dark survival time.

The ability of diatoms to survive prolonged darkness is assumed to be due to the reduction of the metabolism (French and Hargraves, 1980), the formation of resting spores (Durbin, 1978; Doucette and Fryxell, 1983) or resting cells (Anderson, 1975;

Hargraves and French, 1983). There are two possibilities how an interruption of darkness with short light intervals can affect the overwintering of algae. They can either shorten the survival time of a species or extend it by giving the possibility to produce needed storage resources (French and Hargraves, 1980). Carbohydrates act as a buffer between photosynthesis and growth and allow growth also during darkness (Lancelot and Mathot, 1985; Granum and Myklestad, 1999). *T. weissflogii* did accumulate carbohydrates during the short light windows. The content at the end of the winter experiment was comparable (10 °C) or even higher than the start value (4 °C). The latter can be explained by the fact that no growth occurred at 4 °C and carbohydrates were stored, whereas at 10 °C growth occurred. *T. weissflogii* stopped cell division when brought into darkness. Diatoms are known to be well adapted to turbulent waters. In a deep mixed water body they benefit from their high flexibility and their potential to survive prolonged darkness. In contrast to *T. weissflogii*, *Rhodomonas* sp. benefited from short light interval but only during the first days of the experiment. At day 12 the growth rate was zero or negative. The strong carbohydrate decrease showed that the light intervals were not sufficient to accumulate carbohydrates or other components needed for cell division. As small flagellates are more adapted to less turbulent conditions they possibly are less flexible to quickly respond to changing light conditions and as their buoyancy usually prevents them from sinking they did not developed surviving strategies for the surviving of prolonged darkness and continue to grow within the first days after darkening. The low carbohydrate content of *Rhodomonas* sp. suggests that the buffer was used during the rather high growth rates at the beginning of the dark periods leaving only carbohydrates essential for maintaining the cell structure were left (Varum et al., 1986).

The chlorophyll *a* content gives information about the physiological state of algae during survival of unfavorable conditions and about the growth potential when light availability increased. During prolonged darkness algae sometimes reduce their chlorophyll content (Peters and Thomas, 1996; Katayama et al., 2011). After consuming their resources they start to metabolize the pigments for the maintaining dark metabolism (Katayama et al., 2011). The chlorophyll *a* content of *Rhodomonas* sp. was reduced down to almost 50 % of the initial value per μm $^{-3}$ cell volume for the 4 °C treatment and 25 % for the other treatments. The low carbohydrate content may have lead to the delayed growth after re-illumination. *T. weissflogii* in contrast had not significantly reduced chlorophyll *a* during the 12 incubation days. At 10 °C 3 h of 20 μmol m^{-2}s^{-1}even allowed *T. weissflogii* to accumulate chlorophyll *a* perhaps as a low light acclimation. Also at 4 °C and darkness, the chlorophyll *a* increased.

Light intensity was lower under deep convection simulating light periods than during stock culture conditions. Many studies showed an increase of chlorophyll *a* content under lower light intensities (Platt et al., 1984; Hammer et al., 2002). However, the light intervals tested so far were always higher than 6 h per day. The time needed for low light acclimation by an increase of chlorophyll *a* content is assumed to be within hours (MacIntyre et al., 2000). In manuscript 1 we could show that 2 h light pulses of 120 μmol m^{-2}s^{-1}did not lead to an increase in chlorophyll *a* content in contrast to

the low light set up of 20 μmol m^{-2}s^{-1}applied for 12 h. Light limitation as obviously detected by *T. weissflogii* only by a reduction of light intensity.

Chlorophyll *a* fluorometry is a power full tool to determine the photosynthetic capacity and the acclimation state of the photosynthetic apparatus to ambient light availability by determining the maximum quantum yield and rapid light curves (RLC) (Maxwell and Johnson, 2000; Cullen and Davis, 2003; Schreiber, 2004; Franklin et al., 2009). The maximum quantum yield (F_v/F_m) was constantly high during all treatments, demonstrating that photosynthetic performance was healthy even after 12 days of complete darkness and low temperatures. As F_v/F_m per se is species specific (Suggett et al., 2004; Moore et al., 2006) reaction of the photosynthetic performance to different environmental conditions can be very different between species. Franklin and Berges (2004) demonstrated the complete collapse of F_v/F_m within 3 days dark incubation for a dinoflagellate, whereas an ice algae community showed no reduction of F_v/F_m after 15 days of dark incubation (Wulff et al., 2008). Although *Rhodomonas* sp. was not very well acclimated to overwintering conditions as indicated by a starting cell and chlorophyll *a* decline and the very low carbohydrates contents, the healthiness of the photosynthetic apparatus was unexpected.

For RLC provide the maximum relative electron transport rate ($rETR_{max}$), which is a measure of the ability of the photosystems to utilize the absorbed light energy thus reflecting the acclimation to light conditions can be calculated (Marshall et al., 2000). $rETR_{max}$ of *Rhodomonas* sp. decreased about 70 % during the dark treatments at 10 °C. In opposite to the chlorophyll *a* content higher temperatures had a negative effect on $rETR_{max}$. Even if the transport rate is not necessarily related to the chlorophyll *a* content, a stronger decrease of chlorophyll *a* due to higher stress may also favor a stronger decrease of $rETR_{max}$ (Lüder et al., 2002). Photosynthetic activity of both species was positively affected by short light intervals being sufficient for a complete recovery of $rETR_{max}$ to values comparable to the start values. This is in agreement with the findings by Nymark et al. (2013) who tested the re-illumination behavior of diatoms after 48 h darkness and found a higher $rETR_{max}$ after 0.5 h of re-illumination than before the dark incubation. $rETR_{max}$ did not further increase during the next 6 or 24 h. Our investigations on *T. weissflogii* showed a continuous increase of $rETR_{max}$ during the first 5 h of re-illumination (manuscript 1). A decline of $rETR_{max}$ as it was found for all dark treatments indicates the onset of degradation of the photosynthetic apparatus, whereby even after a collapse of $rETR_{max}$ a full recovery could be observed (Lüder et al., 2002; Reeves et al., 2011).

Most chlorophyll *a* fluorometry measurements under dark conditions were conducted with ice algae (Meiners et al., 2009; Ralph et al., 2002), or macroalgae of polar regions (Lüder et al., 2002). Data for temperate pelagic algae are rare (Murphy and Cowles, 1997; Popels and Hutchins, 2002; Nymark et al., 2013). Even less is known about the effect of short light intervals on the photosynthetic capacity. Our experiments provide unique data showing the physiological responds of pelagic algae exposed to deep convection simulating conditions. We could show that short, low intensity light periods kept the photosynthetic apparatus of *T. weissflogii* and *Rhodomonas* sp. active.

T. weissflogii could even use the windows to refill the carbohydrate pool and grow at 10°C.

T.weissflogii and *Rhodomonas* sp. seem to have different overwintering strategies. *Rhodomonas* sp. reacted especially to low temperature (4°C) with an increase of chlorophyll *a* content and cells size. *Rhodomonas* sp. divided during the first days of the new conditions leading to an almost complete use of storage carbohydrates and a subsequent degradation of cells. *Rhodomonas* sp. can be described as type II algae Jochem (1999) where most phytoflagellates smaller than 10 μm belong to. This type does not adjust to darkness by changing their metabolism and therefore decrease cell number. A similar behavior was e.g. observed for *Anaulus australia* (duPreez and Bate, 1992). This lack of adaptive potential may be due to their motility which gives them the possibility to escape darkness rather than the need to withstand (Margalef, 1978).

T. weissflogii cell in contrast seem to be rather a type I algae. Species belonging to this type recognize the problem and react by an increase of metabolism. *T. weissflogii* did not divide when brought into changed environmental conditions and used less carbohydrate during complete darkness which could be due to a reduced metabolism. Furthermore, *T. weissflogii* could even use short light intervals of low light availability carbohydrate accumulation and even low positive growth. Our data support the theory that diatoms are adapted to strong mixing (Margalef, 1978), lower light conditions (Cushing, 1989) and sudden periods of low light whereas *Rhodomonas* sp. is more adapted to constant conditions.

5.4.2 | Effect of winter conditions on an experimental spring bloom

Winter survival of phytoplankton determines the biomass and species composition initiating the spring bloom. Some cells have to overcome extended periods of reduced light availability before new population can develop (Smetacek, 1985; Backhaus et al., 1999). To test for such effects, algae coming from the different overwintering conditions were brought into a spring bloom simulating experimental set up with 14 hours daily light of an intensity of 120 μmol m^{-2}s^{-1}and an incubation temperature of 10 °C and growth rate was observed during the first 3 days after re-illumination. Growth after started with a short lag phase of about one day where no differences between the tested species and no treatment effect could be observed. Overall, *T. weissflogii* grew significantly faster during the first three days than *Rhodomonas* sp. for all treatments beside the 10 °C dark treatment. Growth rate of the stock culture was higher of *T. weissflogii* (0.87 ± 0.1 day^{-1}) than of *Rhodomonas* sp. (0.66 ± 0.014 day^{-1}) before the experiment starts but not significantly different. In case of a competition for light and nutrients at the onset of a spring bloom, the species with the highest initial growth will rate most likely out compete other species (Ryabov and Bernd, 2011). Diatoms are the most abundant species of a North Atlantic spring bloom (Barlow et al., 1993; Cetinic et al., 2012) possibly because of their high reaction potential to chancing environments

(Margalef, 1978). Although our experiment was no real competition experiment it supports the assumption that diatoms would out compete flagellates. Huisman (1999) showed in his competition experiment that the species with the higher initial growth rate out-competed the slower growing one when light was the limiting factor.

Lower winter temperature had a significant effect on the growth after re-illumination of *T. weissflogii* after incubation complete darkness. Algae coming from 4 °C had a significantly higher growth rate than algae coming from 7 or 10 °C, and algae coming from 4 °C had significantly higher chlorophyll *a* concentration. Better start conditions as a higher chlorophyll *a* and carbohydrate content apparently support higher growth rates after light conditions improved (Furusato et al., 2004).

In summery, our data show the positive effect of short periods of light on the growth of *T. weissflogii* under winter conditions.The diatom was better adapted to winter conditions (darkness or short, low light periods) than the flagellate *Rhodomonas* sp.. In comparison to the real winter conditions our experiments were short. But also in the field the winter is not characterized by constant conditions for several. The wintering conditions in many cases depend on the area, but also on environmental conditions e.g. when strong storm events mix up algae from the sediment or change the mixed layer depth.

Our incubations, however, do support that different winter conditions affect the survival as well as the competitive behavior of algae species leading to different initial physiological conditions before the onset of a spring bloom. An increase of the incubation time would most likely lead to even higher differences and therefore also stronger effect the growth after re-illumination.

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Chapter 6

General discussion

Phytoplankton growth during deep convection is one of the factors influencing the spring bloom dynamics in the North Atlantic, but many of the processes involved are not well understood and appropriately quantified during short light periods. This knowledge is prerequisite for ecosystem models aiming at understanding the impact of global change on the North Atlantic bloom dynamics. This thesis addresses the growth performance as well as the physiological mechanisms and acclimation processes underlying growth under the unfavorable light and temperature conditions prevailing during deep convection. *Thalassiosira weissflogii* and *Rhodomonas* sp. were exposed to different simulated winter scenarios. Major parameters describing phytoplankton productivity as growth, net primary production, biochemical components as chlorophyll *a* and carbohydrates as well as the photosynthetic activity were determined in order to address the following five questions.

1. Is positive primary production possible under short light conditions as expected to occur during North Atlantic winter situation?

2. What are the survival strategies and the acclimation processes of phytoplankton under such conditions?

3. How does overwintering under deep convection influence the spring bloom development?

4. How do changes in the environment due to climate change influence the productivity and species composition of a phytoplankton winter stock and the spring bloom seeding?

5. Is the interaction of temperature, light intensity and light duration relevant for the estimation of in situ productivity or for ecosystem modelling?

1. Is positive primary production possible under short light conditions as expected to occur during North Atlantic winter situation?

This study showed for the first time that under non temperature limiting conditions (here $> 8\,^{\circ}$C) an interruption of darkness with only 2 hours of light per day is sufficient for diatom growth such as *T. weissflogii*. This contradicts the critical depth theory of Sverdrup (1953) in some respects, saying that bloom building phytoplankton production was only possible in stratified water bodies. Positive growth rates were observed under saturating light intensity (2 h of 120 μmol m^{-2}s^{-1}- equates a daily light does of 0.864 mol m^{-2}d^{-1}; **manuscript 1**) and even under lower light intensities (3 h of 20 μmol m^{-2}s^{-1}- equates a daily light dose of 0.216 mol m^{-2}d^{-1}; **manuscript 3**). Also positive net primary production (NPP), determined by continuous oxygen measurements, occurred under low light conditions at 10 and 15 °C **(manuscript 2)**. However, daily light doses of 0.072 mol m^{-2}d^{-1} were not sufficient for growth. The low light set up was additionally tested with *Rhodomonas* sp. but in contrast to *T. weissflogii* even a daily light dose of 0.216 mol m^{-2}d^{-1} did not initiate positive growth at the tested temperatures. These findings support the hypothesis that diatoms are better adapted to changing environmental conditions than flagellates (Margalef, 1978). At temperatures below 8 °C no growth occurred independent on light conditions or species. This indicates that temperature may have a stronger potential to limit growth during deep convection than light availability.

In order to test whether light availability is better characterised by light intensity or daily light dose, NPP was determined under daily light doses between 0.864 and 1.728 mol m^{-2}d^{-1} with different light/dark cycles **(manuscript 2)**. Within this light range no significant effect of the daily light dose on NPP was found. A linear correlation between NPP or growth and the daily light dose would be necessary to determine the compensation irradiance, which describes the minimal light dose required for positive productivity (Falkowski and Owens, 1978; Langdon, 1987). These results differ from findings of Verity (1982), who showed that photoperiod did not affect the growth efficiency of phytoplankton, and Thompson (1999) who showed comparable results at least for low light doses. Bouterfas et al. (2006) in contrast showed a growth increase with increasing day length of three freshwater algae under constant light intensities as well as constant daily light doses.

Compensation irradiance is an established tool to calculate ecosystem productivity as it describes the lower border for positive production of autotrophic organisms or an entire plankton community including loss due to zooplankton and bacteria respiration and grazing (Gattuso et al., 2006; Marra, 2004). It combines light intensity and duration into one parameter (Sommer, 1994). Our findings of no correlation between daily light dose and NPP for the tested low light range support the statement of Nicklisch et al. (2008) that one parameter is not sufficient to describe phytoplankton growth, especially in strongly mixed water bodies. Growth was strongly affected by the light duration as shown by experiments using different light/dark cycles with the same daily light dose **(manuscript 1)**. This also questions the use of the compensation depth based

on daily light dose alone as the parameter of choice for the estimate of phytoplankton growth.

When growth occurred at temperatures above 8 °C the growth rate was always higher for the set up offering lower light intensity over a longer light period. At optimal temperatures the growth rate of this set up was about twice the growth rate of the deep convection simulating set up. These light dependent differences in growth efficiency could either be caused by higher energy loss in the short light scenario, by higher energy gain during the longer photoperiods of lower light intensity or a mixture of both. This leads to the second question.

2. What are the survival strategies and the acclimation mechanisms of phytoplankton to such conditions?

For a more precise view on how growth can occur under short light conditions and in order to reveal potential acclimation processes biochemical parameters and photosynthetic capacity were measured at the end of the light and the dark period **(manuscript 1)**. These results demonstrate the main problems occurring under those unfavorable light/dark cycles mainly the resource consuming maintenance of the metabolism during the long dark periods. Carbohydrate content of the 2/22 h light/dark cycle was high at the end of the light period indicating a high production during the short period of saturating light intensity but also a high consumption during the long dark period. During the 12/12 h light/dark the content did not vary with the light period hence gained energy was used continuously for growth. Carbohydrates act as a buffer between photosynthesis and growth and allow growth also during darkness (Lancelot and Mathot, 1985; Granum and Myklestad, 1999).

The increasing divergence with raising temperature between growth rates of the two light treatments can have two reasons: (i) higher dark respiration at raised temperatures reduces growth under short light conditions or (ii) higher photosynthetic production due to a higher acclimation potential to low light intensity supports growth during the longer light periods of low light intensity.

The continuous oxygen measurements contradicted possibility (i), since dark respiration did not increase with raising temperature **(manuscript 2)**. Since, photosynthesis was affected more strongly by temperature than dark respiration the positive effect of longer light periods is stronger than the negative effect of long dark periods. An extension of the light period further allows better acclimation to low light intensities.

Acclimation processes, such as the increase of the main light harvesting pigment chlorophyll *a*, were only observed under low light intensity **(manuscript 1 and 3)**. An increase of the chlorophyll *a* content during low light intensity is universally accepted (Post et al. 1984). Investigations on fluctuated light showed that acclimation processes to lower light availability only react on a decrease of light intensity not on a decrease of the daily light dose (Wagner et al., 2006; Dimier et al., 2009; Milligan et al., 2012). Also short windows of low light intensity were sufficient to initiate this process with a rather long reaction time (MacIntyre et al., 2000). *T. weissflogii* also slightly increased

their chlorophyll *a* content during the 3 h light windows of lower light intensity than the initial culturing intensity during the over-wintering experiment **(manuscript 3)**. Light limitation caused by short light duration did not lead to an increase in chlorophyll *a* content **(manuscript 1)**.

Algae have different strategies to adapt to changing light availabilities as adjustments of the photosynthetic apparatus or of the electron transport chain, which can be demonstrated by the use of chlorophyll *a* fluorometry. The parameters from a rapid light curve (RLC) showed that during the short light windows the available light could not be used completely **(manuscript 1)**, since the minimum saturation irradiance(E_k) was lower than the ambient light intensity (Behrenfeld et al., 2004). E_k increased over the light period but the time was not sufficient to counteract the decrease during prolonged dark periods. This could be shown even better by a small scale release of $rETR_{max}$ during a change between light and darkness at three different temperatures. Nymark et al. (2013) found a rapid recovery of $rETR_{max}$ after 30 min of re-illumination to a value even higher than the initial value when exposing algae to darkness for 48 h. In contrast in this study $rETR_{max}$ continuously increased during the first 5 h after re-illumination **(manuscript 1)**. For the low light intensity set up E_k was always higher than the experimental light intensity. The available light could be used entirely for photosynthesis and most likely initiated less damage. Light intensities above the saturation irradiance can induce photoinhibition or even damage and therefore limit growth additionally (Davison, 1991). Overall, chlorophyll *a* fluorometry proved to be a powerful tool, since it revealed the mechanisms underlying phytoplankton photophysiology under different light/dark exposure times.

Different survival strategies were also demonstrated by comparing the survival under different winter conditions of the diatom *T. weissflogii* and the cryptophyte *Rhodomonas* sp. **(manuscript 3)**. These two phytoplankton groups are known to occupy two different ecological niches. Diatoms have a high flexibility to fast changing environmental conditions but have a low tolerance against low nutrient concentrations. Flagellates in contrast are motile and can move through the water column and are adapted to low nutrient concentrations. This characterization leads to a lower flexibility against changing environmental conditions and dark survival (Margalef, 1978). The results from the overwintering experiment support this hypothesis as *Rhodomonas* sp. initially continued to grow when brought into darkness. The flagellates used most resources within the first days, which led to negative growth rates occurring after a certain experimental time. At the end of the experiment carbohydrates were completely depleted. *T. weissflogii* in contrast stopped growth after the transfer into darkness, withstood two weeks of darkness without great losses and could even use short light windows to produce carbohydrates and grow at 10 °C **(manuscript 3)**. There are at least two possibilities how short light intervals could affect dark survival: they may (i) prevent the appearance of resting stages or reduced metabolism and therefore shorten the survival time of a species or (ii) provide the possibility for photosynthesis and refill of energy storages and therefore extend the survival time (French and Hargraves, 1980). Short light intervals during winter positively affected the survival of *T. weissflogii*, while no effect

was observed on the survival of *Rhodomonas* sp.. A possible explanation of the high potential of *T. weissflogii* to withstand longer dark periods could be the relatively low dark respiration rates especially at higher temperatures **(manuscript 2)**. At 15 °C respiration was only about 7 % of the photosynthetic rate, where it is mostly expected to be 12 % (Falkowski and Owens, 1980). This profitable relation allows growth also at short light windows.

Dark respiration was furthermore affected by the previous light conditions. Lower daily light doses led to a decrease of dark respiration rate. Additionally, we observed enhanced respiration rates immediately after darkening. Light enhanced dark respiration (LEDR) is known to occur after strong light exposure and is related to higher catabolic rates because of a higher production of e.g. carbohydrates during light or reparation of photodamage (Falkowski et al., 1985; Beardall et al., 1994). Our light applications did only cause LEDR at lower temperatures, as the same light intensity is often already harmful at lower temperatures (Davison, 1991).

3. How does over-wintering under deep convection influence the spring bloom development?

Some cells need to survive harsh winter conditions to seed the subsequent spring bloom (Smetacek, 1985; Backhaus et al., 1999) and previous winter conditions may influence bloom development and composition (Waniek, 2003; Irigoien et al., 2000). To test the effect of different winter conditions on spring bloom development two phytoplankton species coming from different light and temperature scenarios were exposed to spring bloom conditions **(manuscript 3)**. After two weeks of winter conditions *Rhodomonas* sp. were in a bad physiological state since chlorophyll *a* content was reduced and carbohydrates were almost completely consumed, while *T. weissfloggi* was generally in a better condition. This difference was reflected in the respective growth behavior after re-illumination. Both species did not grow well during the first day after re-illumination. After this short lag phase *T. weissflogii* had a higher growth rate than *Rhodomonas* sp.. With their better strategy of surviving unfavorable growth conditions according to light availability the diatom could out compete the flagellates due to a higher and more rapid growth when environmental conditions become more favorable in spring. The short light windows during winter had no verifiable effect on the growth after re-illumination as in a spring situation. The relatively high chlorophyll *a* content and short lag phase after re-illumination did not indicate formation of any resting stages during winter conditions, in contrast to other findings (Durbin, 1978; Doucette and Fryxell, 1983). The measurements of chlorophyll *a* fluorometry showed that algae were constantly in a good physiological state. Although complete darkness led to a strong decrease of the maximal relative electron transport in some cases, which could indicate a degradation of the photosystems. During the short light intervals both species could increase there $rETR_{\max}$ but *Rhodomonas* sp. did not profited from this in terms of growth rates after re-illumination.

4. How may changes in the environment due to climate change influence the productivity and species composition of a phytoplankton winter stock and the spring bloom seeding?

All experiments were carried out over a temperature range including limiting, ambient and increased temperatures to test the lower temperature limit of growth as well as the effect of a rising sea surface temperature on phytoplankton survival, physiology and composition. Since temperature during winter or early spring in the North Atlantic is located at the lower side of the temperature growth curve of most of the occurring phytoplankton species an increase of temperature will most likely support phytoplankton growth. Growth of *T. weissflogii* increased with raising temperature at every tested light condition **(manuscript 1, 2 and 3)** and even prolonged darkness and increasing temperature did not reduce growth.

A temperature rise due to climate change is predicted to be about 2 - 4 °C till 2100 for the northern North Atlantic (Houghton et al., 2001). A temperature increase within this area would not only affect phytoplankton growth directly by higher temperatures but also indirectly by a decrease of mixed layer depth (Li, 2002). The decrease of mixed layer depth would additionally support growth as it prolonged the time each particle spends in the euphotic layer. Since dark respiration is less affected by temperature than photosynthesis, NPP benefits from a temperature increase even if phytoplankton is exposed to long dark periods or located in a deep chlorophyll *a* maximum **(manuscript 2)**.

Dark survival of many phytoplankton species is reduced by raising temperatures (Antia, 1976). This is especially of relevance for cells, which sink down to the bottom and have to survive several month in complete darkness (Smetacek, 1985). These experiments were carried out under wide temperature ranges and long periods of darkness (Antia, 1976). Temperature did not affect the dark survival of *T. weissflogii* or *Rhodomonas* sp.. Colder winter situations had a significantly positive effect on the growth of *T. weissflogii* after re-illumination **(manuscript 3)**. An increase of SST might have a negative effect on those cells that have to survive winter in the sediment. Contrasting this prediction is the observation of a general decrease of chlorophyll *a* concentration over the last 60 years in the worlds oceans, which was related to a SST increase (Boyce et al., 2010). The concentration also decreased over the North Atlantic, although the positive effect of a decrease of the mixed layer depth was taken into account. Based on the hypothesis that positive population growth during deep convection is only possible due to low zooplankton grazing pressure (Behrenfeld et al., 2013), the observed reduction of chlorophyll could also be explained by an increase of zooplankton activity rather than by a decrease of growth rates due to raising winter temperatures. A higher grazing rate due to an increase of temperature could exceed the gain of phytoplankton production due to higher temperatures and light availability (Peters and Downing, 1984; Aberle et al., 2006). Since this study focused only on phytoplankton net primary production a prediction of in situ community productivity in future scenarios remains unresolved. However, it provide essential informations needed

for solid productivity estimates using ecosystem models. This leads to the final question.

5. Is the interaction of temperature, light intensity and light duration relevant for the estimation of in situ productivity or for ecosystem modelling?

This study illustrates that estimations of primary production based on growth rates coming from experiments with constant low irradiances may lead to an overestimation of primary production in well mixed water bodies. The application of the same daily light dose in two different light/dark cycles and irradiances led to differences of growth rates by a factor of two under optimal temperature conditions **(manuscript 1)**. Some model calculations and experiments have focused on the difference between constant and fluctuating light (Marra, 1978; Barkmann and Woods, 1996; Anning et al., 2000; Ross et al., 2011) with rather inconclusive results between an underestimation of primary production of 87 % (Marra, 1978) to an overestimation of 40 % (Barkmann and Woods, 1996). This thesis showed that using growth rates from constant low light conditions applied in an e.g.12/12 h day/night cycle would lead to an overestimate of growth of about 50 %, if no temperature limitation occurs.

Sverdrup's (1953) critical depth theory still builds the basis for many ecosystem models which accept phytoplankton productivities to be zero in deep mixed water bodies (Bissett et al., 1999; Siegel et al., 2002). The biggest drawback of the critical depth model, which also includes a compensation depth (comparable to the compensation irradiance) is the assumption of a static system: photosynthesis only takes place within the euphotic layer, vertical transport is ignored and respiration is assumed to be constant independent on previous light conditions or temperature. These kinds of models do not take into account that particles within the whole mixed layer ephemerally visit the euphotic zone and can use these short light windows for growth. Depending on the mixed layer depth positive productivity during winter can be high enough to exceed all losses. An alternative option to predict phytoplankton production during deep convection is a Lagragian model, which follows individual phytoplankton particles on their way through deep convection as e.g. used by (Lindemann et al., viwe). Particles gain carbon during their time in light and losses it in darkness. In contrast to the model, where particles produce only biomass in terms of cell volume, positive production in nature includes also population growth, where gained energy is used for cell division. Gained energy during the short light windows exceeded the metabolic losses of the following long dark periods **(manuscript 2)** and the NPP correlated with growth rates. The results of this thesis showes that NPP calculated from photosynthetic and respiratory rates can be used to predict growth of phytoplankton, especially for mixed water bodies, where light availability is highly variable.

The different overwintering conditions did not significantly affect the onset of the spring bloom. However, there were clear taxon-specific differences in the strategies to outlast unfavorable growth conditions **(manuscript 3)**. These findings highlight the necessity to include different phytoplankton groups and thus physiological strategies

into models. The composition of the phytoplankton community is also of high interest for the productivity according to carbon fixation and grazing behavior.

The high variability of the parameters included in phytoplankton productivity makes it difficult to select a solid parametrisation for ecosystem models. Due to the strong temperature dependence of photosynthesis, knowledge on the general temperature-growth relationships of the over-wintering populations will be essential to evaluate whether light conditions may have a significant effect on growth during deep convection, or not. As discussed in the next chapter, more studies will be needed to assess the relevance of this effect for in situ productivity in winter and the subsequent spring bloom development, those will be addressed in the next chapter.

Perspectives

This study was designed as a first step in understanding the interactive effects of temperature and light during deep convection. It focused mainly on one diatom species as model organism and simplified deep convection by simulating different light and temperature scenarios. It thus provided first mechanistic insights into the physiological processes involved in phytoplankton growth during winter in the North Atlantic. However, it can only provide a small piece of the complex ecosystem interactions. More studies will be needed to look specifically at the following aspects.

(i) Taxon specific responses: There are plenty of possible light and dark combinations to be tested on different phytoplankton species. Growth behavior and acclimation potential are species-specific and depend among others on cell size. To verify the hypothesis that short light intervals allow phytoplankton growth under deep convection investigations on additional phytoplankton species of different functional groups and sizes should be carried out. Since temperature and light dependence of photosynthesis and dark respiration is also highly variable, additional rates should be determined specifically focusing on representatives of early and late bloom forming groups, such as diatoms, coccolithophorids and dinoflagellates, also including cold adapted species. These investigations will show different strategies and possible competitions important for spring bloom development and composition and can also be used to confirm the correlation between NPP and growth rate. Furthermore, the correlations should be extended with measurements of fluctuating light conditions. However, the experimental effort should be related to the benefit models gain from these investigations.

(ii) Acclimation processes and impact of light history: The biggest challenge for model calculations as well as for laboratory studies is to describe acclimation processes to changes of the environment. A powerful tool to describe acclimation processes of the photosynthesis is the photosynthesis irradiance curve. Further PE curves must be determined for different possible positions within a convective mixed layer. The chlorophyll *a* fluorometry is of great benefit for these kinds of measurements as it is a non invasive method that can be used with high temporal resolution. Light saturation values coming from a rapid light curve give important information about photosynthetic

efficiency under ambient light conditions. Dark respiration also depends on previous light conditions and temperature. To describe this relation a dark respiration post-irradiance curves should be developed to be used in the same way as a PE curve. Continuous O_2 measurements are very useful for these approaches as they give the possibility to directly compare productivity and consumption even under fluctuating light conditions.

So far it is still not possible to determine the transport of phytoplankton cells in a convective cell. A possible way to support the phyto-convection hypothesis in the field is to measure photo-acclimation of phytoplankton from different depth of a convective cell by chlorophyll *a* fluorometry and pigment analysis. These methods can give information about the time, when phytoplankton cells were photosynthetically active for the last time. Furthermore, rations of chlorophyll *a* to carbon and to carbohydrates determined in different depth give information about the light history of the algae. However, the auto- or heterotrophy of an ecosystem is caused by the interaction of all trophic levels. Since phytoplankton primary production builds the basis of the food web it is of high relevance for model calculations.

Phytoplankton during winter should be included into model concepts as a dynamic process, which allows phytoplankton cells to grow according to their individual light history and not to instantaneous light conditions. For the parametrization of the winter productivity additional growth rates and net primary productions must be determined under experimental conditions which match better to deep convection environmental conditions than convenient growth experiments.

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