Overwintering effects on the spring bloom dynamics of phytoplankton

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Abstract

The influence of winter on the selection of dominant taxa for the phytoplankton spring bloom was studied in batch culture experiments. Different natural phytoplankton assemblages from different phases of the temperate zone winter were exposed to varying periods of darkness (0, 6/7, 13, and 19 weeks) followed by a re-exposure to saturating light intensity for 14 days to experimentally simulate the onset of spring. The results showed that dark incubation plays a strong effect on shaping the phytoplankton community composition. Many taxa disappeared in the absolute darkness. Dark survival ability might be an important contributing factor for the success of diatoms in spring. Different phytoplankton starting assemblages were dominated by the same bloom-forming diatoms, *Skeletonema marinoi* and *Thalassosira* spp., after dark incubation for only 6 weeks, irrespective of the high dissimilarities between phytoplankton communities. The growth capacity of surviving phytoplankton is almost unimpaired by darkness. Similar growth rates as that before darkness could be resumed for the surviving taxa with a potential lag time of 1-7 days dependent on taxon and the duration of darkness.

Introduction

The spring bloom is a renowned feature of the seasonal phytoplankton dynamics in temperate and cold oceans and lakes. Three decades ago, Sommer *et al.* (1986) proposed the plankton ecology group (PEG) model as a standard template to describe the seasonal succession of plankton in common patterns of sequential steps. After that, additional types of interactions driving details in taxonomic and functional group replacements during the growth season were detected by extensive studies (summarized in Sommer *et al.*, 2012b). Nevertheless, relatively little work has been carried out on overwintering dynamic considering it occupies a long period of time in the overall succession process in temperate and cold environments. The widespread lack of attention to the overwintering period has also been noticed in the revised version of the PEG model (Sommer *et al.*, 2012b).

The overwintering period starts from late autumn when the abiotic environment deteriorates, leading to a negative community net production and ends next early spring when a new bloom begins. As the final step mentioned in the original PEG model, a start close to zero of both phytoplankton and zooplankton was assumed during the winter-spring transition (Sommer *et al.*, 1986). However, considerable evidence for some winter growth of phytoplankton (Behrenfeld, 2010)

and for zooplankton overwintering(Campbell, 2008; Hagen *et al.*, 1996) has emerged.

The most obvious characteristic of winter is the low light intensity. Light supply is considered to be the single dominant trigger of the spring bloom in both old and updated PEG models (Sommer *et al.*, 2012b; Sommer *et al.*, 1986). This idea is in agreement with the classical concept of critical depth hypothesis (Sverdrup, 1953). The significance of light in bloom initiation was also confirmed by Siegel *et al.* (2002) who found a notable uniform daily light dose of 1.3 mol photons m^{-2} d^{-1} at the start of the spring bloom for the North Atlantic Ocean. Conversely, lack of light is also seen as the primary explanatory factor for the winter minimum in the cold-temperate and boreal zone, while there is no winter depression of phytoplankton in the more light-rich Mediterranean (Moustaka-Gouni *et al.*, 2014). Thus, different dark survival abilities and strategies between different taxonomic groups in phytoplankton may provide a driving factor for the overwintering dynamics.

Overwintering capabilities of the different phytoplankton taxa might be important for the composition of the subsequent spring bloom because it determines the initial abundance of species for the spring bloom. Long-term survival in darkness has been well studied with isolated phytoplankton strains showing that several diatoms could survive for up to 1 year in the vegetative stage (Antia, 1976), although with interpsecific differences (Antia, 1976; Griffis & Chapman, 1988; Lewis *et al.*, 1999; Peters, 1996; Peters & Thomas, 1996a; Smayda & Mitchell-Innes, 1974). Some bloom-forming diatoms, like *Skeletonema* spp., *Thalassiosira* spp., *and Ditylum brightwellii*, showed strong dark survival ability (Antia, 1976; Griffis & Chapman, 1988; Murphy & Cowles, 1997; Peters, 1996; Peters & Thomas, 1996a). Strains qualified as benthic types usually have longer survival times than pelagic types and the temperature for maximal dark survival could be determined by the temperature regions from which the strains were isolated (Antia, 1976).

Survival of winter darkness is necessary but may not be sufficient for the formation of the spring bloom. Starting growth quickly after the improvement of light conditions and achieving higher exponential growth rates are equally important. It has been shown that diatoms have a higher inherent growth rate than flagellates in the absence of silicate limitation (Egge & Aksnes, 1992). The question is whether this growth rate will be negatively affected by the physiological consequences of prolonged survival in darkness or under low light. Most studies suggest that darkness has no effect on growth rate even after a relatively long period of dark incubation time

(Araujo *et al.*, 2008; Furusato *et al.*, 2004; Murphy & Cowles, 1997; Peters, 1996; Peters & Thomas, 1996a; Peters & Thomas, 1996b; Vermaat & Sand-Jensen, 1987) indicating that species could survive in the dark without physiological impairment. However, a decrease of growth rate with the increase of dark incubation time was reported for several diatom species, such as *Skeletonema costatum*, *Chaetoceros curvisetus*, and *Thalassiosira gravida* (Smayda & Mitchell-Innes, 1974).

Species do not always start exponential growth immediately when re-exposed to the light, but often start after a lag phase. Although growth rate could be resumed at the initial level, the recovery time would increase with the increasing dark incubation time (Peters, 1996; Peters & Thomas, 1996a; Peters & Thomas, 1996b). This might be caused by the gradual decrease of photosynthetic pigments in response to darkness (Lüder, 2003). A lag phase of 1-7 days is common (Araujo *et al.*, 2008; Coughlan, 1977; Peters, 1996; Peters & Thomas, 1996b). It could be longer if the dark incubation time is extended. In the prolonged darkness, it was reported that the lag time of *Thalassiosira antarctica* increased from immediate growth to 13 days when dark incubation time increased from 21 days to 127 days and the lag time of *Thalassiosira tumida* increased from 3 days to 15 days when dark incubation time increased from 148 days to 272 days (Peters & Thomas, 1996a).

No doubt that these studies provide a valuable reference on the dark survival ability and growth capacity of individual species after winter, the problem is these monoculture studies excluding other species are insufficient to predict if diatoms would still be able to succeed under competition, consumption or infection. Actually, there was one study conducted by Zhang *et al.* (1998) who exposed natural phytoplankton assemblage samples collected from Arctic sea ice to a 6-month dark incubation and found that the dominant species shifted from pennate diatoms to small flagellates after darkness, and flagellates had a higher growth rate than diatoms in the subsequent light culture. These findings are opposite to our expectation derived from the monoculture dark survival experiments.

Therefore, we proposed to fill the knowledge gap by exposing different natural phytoplankton assemblages from different phases of the temperate zone winter immediately and after dark incubation to saturating light intensity to experimentally simulate the onset of the spring bloom at the community level. By analysing the changes in taxon abundance, growth rate and lag phase, we expected to answer three questions:

1. How do different overwintering inocula respond to the darkness?
2. How do growth rates of individual taxa change after dark incubation?
3. How do lag phases of individual taxa change after varying time intervals of dark?

Method

Experimental design

Water samples of different natural phytoplankton assemblages were collected at 5m depth in the early, middle and late winter from Kiel Fjord, Baltic Sea, Germany (54°19'46"N 10°09'18"E). The three communities sampled at different times in winter were called W1, W2, and W3, respectively. In situ, environmental conditions during sampling of the three communities were relatively similar to each other (Table 1). Initial nutrient concentrations were high in all the communities but were slightly lower in the late winter water. Therefore, extra nutrients of 8.42 µmol L^{-1} silicate (Si), 0.47 µmol L^{-1} phosphate (P), and 7.89 µmol L^{-1} nitrate (N) were added in W3 to balance the decline. After filtration by a 250µm mesh to avoid grazing from large zooplankton, water samples were distributed into 2L plastic bottles. Four of the bottles were immediately incubated in light (0 weeks dark incubation = control) while the remaining ones were incubated in darkness for different periods before exposing to light. The dark incubations lasted for 0, 6, 13, and 19 weeks (W1), 0, 7, and 13 weeks (W2), or 0 and 6 weeks (W3). Each treatment was replicated 4 times. Light incubations following darkness lasted for 14d. Light was offered at a saturating level (100 µmol photons m^{-2} s^{-1}; Light/dark cycle= 12:12h). Light and dark incubation temperatures were 4°C. During culturing, bottles were gently shaken every day to assure a homogenous distribution of the plankton and to avoid the growth of benthic microalgae on the wall of bottles. Phytoplankton subsamples (100 mL) were collected three times per week (Monday, Wednesday, and Friday). Subsamples which were counted by inverted microscope were fixed with alkaline Lugol's solution and stored in brown glass bottles. Subsamples for flow cytometric analysis were fixed with 37% formaldehyde, frozen immediately by liquid nitrogen and then stored at -80°C to protect chlorophyll from decomposing.

Phytoplankton >5µm were counted by the inverted microscope method after sedimentation for at least 24 h (Utermöhl, 1958). Phytoplankton were identified to the genus level in most cases. The aim was to count 100 individuals per taxon group in order to obtain 95% confidence limits of ±20%,

except for rare taxa. For log transformations half of the detection limit was used as zero replacement value, if a taxon was undetectable only at a few sampling occasions. Phytoplankton biomass was estimated as carbon biomass converted from cell volumes (Menden-Deuer & Lessard, 2000) which were measured through the calculation of similar geometric standard solids (Hillebrand *et al.*, 1999). Small phytoplankton (<5µm) were counted by a flow cytometer (FACScalibur, Becton Dickinson, San Jose, CA, USA) and distinguished by size and fluorescence of allophycocyanin, chlorophyll a and phycoerythrin without further taxonomic identification. All picoplankton cells detected by flow cytometer were assumed to be spherical and estimated by the volume conversion factor of 0.157 pg C μm^{-3} (Sommer *et al.*, 2012c).

Data analysis

The analysis of the microbial plankton communities focused on phytoplankton and excluded bacteria, heterotrophic flagellates, ciliates, and benthic microalgae. Phytoplankton were categorized into five functional groups by size classification (Sieburth *et al.*, 1978) and the distinction between diatoms and flagellates: picoplankton (<3µm), nanodiatoms (3-20µm), nanoflagellates (3-20µm), dinoflagellates (>20µm), and microdiatoms (>20µm). The dissimilarities between different phytoplankton communities were analysed by SIMPER test (Clarke, 1993) using PRIMER 7, based on the Bray-Curtis dissimilarity coefficient (Bray & Curtis, 1957). Community indexes were calculated without inclusion of picoplankton because of the different level of taxonomic resolution. The Shannon-Wiener index of diversity (H') was calculated from genus abundance data:

$$H' = -\sum_{i=1}^{s} p_i \ log_2 \ p_i \quad (1)$$

where p_i is the relative abundance of taxon i, s is the number of taxa.

Growth rate and lag time were taken as the main indicators of growth capacity. The growth rate was calculated as the slope of a linear regression fitted through a semi-logarithmic plot of abundance on time (log N – time) during the exponential phase, i.e. the linear portion of the growth curve. Lag time was calculated by the intercept between the fitted regression line and the baseline which was the abundance of taxa at the beginning. Negative values of lag time implying that no lag phase was found were adjusted to zero. If the growth curve did not show the typical batch culture pattern (lag phase, exponential phase, stationary phase), an average growth rate ($\overline{\mu}_{growth}$) from day 0 to 14 was calculated instead. Differences between metrics of growth capacity (i.e., growth rate and lag time) were evaluated for statistical significance using analysis of variance. One-way ANOVA

was conducted to analyse the impact of dark incubation on growth rate and lag phase. Two-way ANOVA was used to examine the combined effect of dark incubation and community. Normality was checked by Shapiro-Wilk test and homogeneity of variance was checked by Fligner-Killeen test. If the assumptions of normality and homogeneity could not be satisfied even after transformations, an alternative non-parametric test was used instead (i.e. Welch's t-test).

Results

Phytoplankton community

The initial phytoplankton community compositions differed strongly between different sampling periods, but were uniform between replicates of the same community (Table 2). The dissimilarities of initial composition were 77% between W1 and W2, 54% between W1 and W3, and 77% between W2 and W3. The total biomass ranged from 18 to 22 µg C L^{-1} in W1 and W3 but was lower in W2 with only 6 µg C L^{-1}. In W1, initial phytoplankton biomass was dominated by microdiatoms (75.5±4.2%, SD). W2 was dominated by nanoflagellates (63.8±18.9%). W3 was dominated by microdiatoms (53.8±5.1%) followed by picoplankton (19.9±3.5%) and nanoflagellates (19.3±5.8%). Dinoflagellates together with nanodiatoms formed less than 10% of the total biomass in each community. In all communities, picoplankton were represented by the same two clusters distinguished by differences in fluorescence of phycoerythrin and chlorophyll a. The abundances of heterotrophic plankton are listed in Table 3 as a reference. Nauplii and copepods were discovered in W2 and W3 but not in W1, while, microzooplankton (ciliates and heterotrophic flagellates) were more abundant in W1 than in W2 and W3.

Dark survival capability

Most of the phytoplankton taxa did not survive 6 weeks of dark incubation in the natural assemblage communities. The diversity of communities decreased dramatically during that time. The diversity index (H') decreased from 1.46 to 0.38, 0.92 to 0.25, and 2.31 to 0.81 for W1, W2, and W3, respectively. The richness of detectable surviving taxa was also low, with 8 taxa in W1, 4 taxa in W2, and 5 taxa in W3. Among the surviving phytoplankton, some taxa were unable to resume measurable cell division after re-illumination, few other taxa could grow again (Table 4). Several heterotrophic zooplankton could also survive in the dark, such as *Protoperidinium*, *Gyrodinium*, *Strobilidium*, and copepods. No resting spores or cysts were observed during the dark

incubation experiment. Picoplankton became undetectable during dark incubation but re-appearance of both clusters after re-illumination suggests that they had not disappeared. The abundance ratio between heterotrophs and phytoplankton increased from less than 0.01 before dark incubation in all communities to about 0.02 in W1 and W3 and to even more than 1.0 in W2 after darkness.

Taxa that survived the first 6 weeks of dark incubation normally persisted during prolonged darkness although the abundance gradually decreased as the dark incubation time increased. The survivorship patterns varied among different taxa in W1 (Fig. 1). *Skeletonema* displayed the typical type I survivorship curve ($p<0.001$), whereas *Thalassiosira* with a steady mortality rate followed the type II survivorship curve ($p<0.001$). For both taxa, only about 5% of the initial biomass of each taxon survived after 19 weeks of darkness. All other phytoplankton, which had much lower abundance after dark incubation and were calculated together as "all other species", showed the type III survivorship curve ($p<0.001$). The surviving "all other species" formed only about 1% of their initial biomass after 19 weeks of darkness.

Pseudo-nitzschia from W1 and W3 communities behaved differently from each other. *Pseudo-nitzschia* from W1 experienced a catastrophic population decline already after 6 weeks in the darkness with no ability to grow after re-illumination, while, the *Pseudo-nitzschia* from W3 not only exhibited a much higher survival rate but could also regrow in the subsequent growth experiment. A morphological analysis based on the density of striae showed that these were two different types presented in the two communities (W1: 17 striae in 10 μm; W3: 23 striae in 10 μm; assessed with empty frustules under phase contrast). Contrary to *Pseudo-nitzschia,* the dark survival abilities of other phytoplankton taxa showed only minor change between the different experimental communities.

Growth after dark incubation

Skeletonema marinoi and *Thalassiosira* were the winners in the light culture after varying periods of dark incubations, comprising more than 98%, 94%, and 85% of the total biomass at the end of culture in W1, W2, and W3, respectively. However, the three phytoplankton communities showed clearly different compositions in the light culture without prior dark incubation. W1 was dominated by a *S. marinoi* alone which contributed more than 80% to the total biomass after 14-day light incubation, while, W2 and W3 were co-dominated by several taxa. Specifically, W2 was dominated by the diatoms *Proboscia alata* (19±3%), *Skeletonema marinoi* (15±7%), *Chaetoceros*

(24±6%), *Coscinodiscus* (20±12%), and the cryptophyte *Teleaulax* (10±13%) equally. W3 was dominated by *Thalassiosira* (48±20%) followed by *Skeletonema marinoi* (26±19%) and *Pseudo-nitzschia* (12±3%).

Only few taxa could resume growth after dark incubation (Table 4). Growth rates (Fig. 2; Supplementary Document 1) could be calculated for *Skeletonema marinoi* and *Thalassiosira* in all the communities and the sum of "all other species" for W1 and W3, while for W3 growth rates could also be calculated for *Pseudo-nitzschia*. The growth rates of picoplankton and *Attheya septentrionalis* could not be calculated.

No significant changes of growth rates were discovered after dark incubation by the analysis with three different taxa. *Skeletonema* and *Thalassiosira* maintained consistent growth rates in W1 and W2 during 19 and 13 weeks dark incubation. Growth rates of the three taxa analysed in W3 decreased, but not significantly. In all the experiments, *Skeletonema* and *Thalassiosira* showed similar growth rates ranging from 0.50 to 0.88 day^{-1} which was higher than that of *Pseudo-nitzschia* with 0.36 day^{-1}. The growth rate of "all other species" was even lower which ranged from 0.08 to 0.27 day^{-1} even before dark incubation. There were almost no biomass increases of "all other species" in the cultures after darkness, except for W3 (Fig. 2; Supplementary Document 1).

A two-factor ANOVA showed no significant effect of the duration of darkness on the growth rates of *Skeletonema* and *Thalassiosira*, while there was a significant effect of community of origin and (*Skeletonema*: $F_{2,18}$=8.8, P<0.01; *Thalassiosira*: $F_{2,18}$=34.5, P<0.001) and a significant interaction effect of darkness and community (*Skeletonema*: $F_{2,18}$=8.0, P<0.01; *Thalassiosira*.: $F_{2,18}$=5.7, P<0.05). To balance the design of experimental duration, the two-way ANOVA only included dark incubation times of 0 and 6 weeks.

The responses of lag time to the duration of dark incubation varied among taxa. *Skeletonema* was not negatively influenced by darkness and maintained a similar lag time after 19 weeks dark incubation and the lag time of *Skeletonema* in W2 even decreased after darkness ($F_{1,10}$=11.58, P<0.01). In contrast, *Thalassiosira*, from both W1 and W3, displayed a significant increase in lag time of 2-3 days (W1: $F_{1,14}$=15.12, P<0.01; W3: $F_{1,6}$=12.01, P<0.05). *Pseudo-nitzschia* from W3 showed the longest lag time of 5 days after 6 weeks incubation (Welch's test: p<0.01). The following two-way ANOVA tests with *Skeletonema* and *Thalassiosira* confirmed that the lag times of *Thalassiosira* were significantly influenced by darkness ($F_{1,18}$=7.1, P<0.05), while the lag time of

Skeletonema was significantly affected by the factor community and its interaction with darkness ($F_{2,18}$=4.6, P<0.05).The lag time of the three taxa and "all other species" from different communities were only minor (if at all detectable) and ranged from 1-7 days in the culture before and after dark incubation.

Discussion

Our experiments focused on the two most important traits which enable phytoplankton to dominate the spring bloom in cold-temperate and boreal latitudes, survival of an extended low-light period and the ability to resume growth thereafter. The dark incubations indicated a strong selection pressure by the combination of lack of an essential growth resource (light) and continued losses to heterotrophic consumers. All phytoplankton taxa under study substantially lost biomass and the majority became undetectable leading to low diversity of the surviving communities. Only few diatoms and mixotrophic flagellates together with taxonomically unidentified picoplankton formed the residual biomass after dark incubation. The comparison between the three experimental communities shows a unifying effect of dark incubation on phytoplankton community composition. Irrespective of the initial composition, the three different communities were dominated by the same bloom-forming diatoms, *Skeletonema* and *Thalassiosira*, when cultured in the light again. Interspecific differences in growth rate after re-illumination reinforced the survival effect because *Skeletonema* and *Thalassiosira* outperformed the rest of the taxa.

According to the long-term observations in the Kiel Bight, three diatom genera, *Skeletonema*, *Thalassiosira*, and *Chaetoceros*, are the most important components in the spring phytoplankton biomass (Smetacek, 1985; Wasmund *et al.*, 2008; Wasmund *et al.*, 1998). Unlike the other two diatoms, *Chaetoceros* is more likely to become dominant when there is a later spring bloom (Smetacek, 1985; Wasmund *et al.*, 2008). Considering this difference, the mechanism promoting the dominance of *Chaetoceros* might be slightly different from the other two genera. Photographs taken during the course of the experiment suggest that most of the *Chaetoceros* in our samples were *C. decipiens.*

The successful survival of diatoms is in agreement with dark survival studies of individual species (Antia, 1976; Griffis & Chapman, 1988; Murphy & Cowles, 1997; Peters, 1996; Peters & Thomas, 1996a). Similarly, the ability of diatoms to start growth after darkness either immediately

283 or after a short delay (<1 week) has been demonstrated by several single species culture studies
284 (Araujo *et al.*, 2008; Furusato *et al.*, 2004; Murphy & Cowles, 1997; Peters, 1996; Peters & Thomas,
285 1996a; Peters & Thomas, 1996b; Vermaat & Sand-Jensen, 1987). However, one study conducted
286 with a natural phytoplankton assemblages resulted in the dominance of flagellates after dark
287 incubation (Zhang *et al.*, 1998). A possible explanation for the important difference between both
288 studies lies in the fact that Zhang *et al.* (1998) obtained their experimental community from melting
289 ice which means phytoplankton had been frozen before the study. Freezing has been shown to
290 strongly influence survival and growth ability of microalgae (Vermaat & Sand-Jensen, 1987), but
291 possibly with different taxon specific effects than darkness.

292 While the temperature conditions and nutrient concentrations of our study are representative of
293 present-day conditions in the Baltic Sea, further climate warming might change survival and
294 re-growth capabilities of overwintering phytoplankton. Reeves *et al.* (2011) suggested that
295 increasing temperature during Antarctic winter will have little effect on diatoms since the dark
296 survival of Antarctic diatoms is temperature insensitive, only significantly impacted at an unrealistic
297 temperature of 10°C. However, increasing food demand of heterotrophs and mixotrophic flagellates
298 is likely to increase mortality rates of phytoplankton in darkness as indicated by the continued
299 decrease in abundance of surviving taxa during 19 weeks of darkness.

300 Compared to natural conditions, the darkness incubation was an extreme treatment, because
301 winter phytoplankton experience low light, but not complete darkness, except for the polar night
302 and of ice covered water bodies with a thick layer of snow. This difference might explain why some
303 of the taxa unable to survive darkness were found in the mid- and late winter field samples, e.g. the
304 diatom *Proboscia alata* and the cryptophyte *Teleaulax*.

305 The incubation in 2 L bottles might have caused some artifacts, e.g. the reduction of loss rates
306 relative to in situ conditions due to sinking or to grazers present in situ at abundances of less than a
307 1 Ind L^{-1}. However, these losses are considered low: (1) sedimentary losses play a negligible role
308 during the high turbulence regime of an ice-free winter, (2) except for excluding mesozooplankton
309 by sieving with 250 µm mesh size, grazer densities at the start of the experiment conformed to the
310 natural situation. The decline of abundance during dark incubation affected all autotrophic,
311 mixotrophic and heterotrophic protists, therefore competitive, allelopathic and predatory
312 interactions will have declined, but this is a community wide effect of darkness (with indirect

ramifications through biotic interactions) which was within the target of a study at the community level and not an artifact. The tendency of increasing heterotroph to autotroph ratios during darkness might have led to increasing grazing losses affecting mostly picoplankton, while diatoms are less likely to be grazed by the microzooplankton. Similarly, increases of microzooplankton because of the removal of copepods will have mainly affected picoplankton. The absence of sediment in the bottles might have excluded resting stages and discriminated against taxa relying on resting stages for overwintering.

The observed lag-phases were short (less than 1 week) and confirm the ability of phytoplankton to quickly resume growth when light availability reaches a sufficient level. Increases in lag as a consequence of increasingly long dark incubation were found in some cases, but not in all cases (Fig. 3; Supplementary Document 1). The increase in lag time by dark incubation was also found with monospecific cultures (Peters, 1996; Peters & Thomas, 1996a; Peters & Thomas, 1996b). However, the duration of the lag phase after darkness is only a minor effect on the timing of the spring bloom compared to the one-month delay that could be caused by the low light (Sommer *et al.*, 2012a). Therefore, changes in lag time introduced by variability in exposure to darkness does not explain the time shift of the spring bloom from April to March in Kiel Bight at the beginning of 21st century (Wasmund *et al.*, 2008). Future research should focus, inter alia, on the effect of warming, which is expected to increase respiration rates both of auto- and heterotrophs while seasonal light availability will not increase during the period before the onset of thermal stratification. However, an earlier onset of stratification will not only improve light supply to phytoplankton (Sverdrup, 1963), it will also lead to an earlier onset of nutrient limitation, as opposed to the nutrient-replete conditions in our experiment.

Conclusions

Darkness in winter is a very unfavorable environment for phytoplankton and many taxa cannot survive in the absolute dark for few weeks in the natural assemblage community. However, despite its strong impact on the survival of phytoplankton, the growth capacity of surviving phytoplankton is almost unimpaired. Surviving taxa could still resume a similar growth rate as that before darkness with a potential lag phase of only a few days. Dark survival ability might be the contributing factor for the success of diatoms in the spring bloom and seems the most plausible explanation for the

annually repeating pattern of the phytoplankton spring bloom. Three different communities were dominated by the same bloom-forming diatoms in the culture after dark incubation.

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Data Archiving

Upon acceptance of the manuscript, data will be made publicly available in PANGAEA (www.pangaea.de)

References

Antia, N. J. (1976) Effects of temperature on the darkness survival of marine microplanktonic algae. *Microb. Ecol.,* **3,** 41-54.

Araujo, C. V., Diz, F. R., Moreno-Garrido, I., Lubian, L. M. and Blasco, J. (2008) Effects of cold-dark storage on growth of Cylindrotheca closterium and its sensitivity to copper. *Chemosphere,* **72,** 1366-72.

Behrenfeld, M. J. (2010) Abandoning Sverdrup's critical depth hypothesis on phytoplankton blooms. *Ecology,* **91,** 977-989.

Bray, J. R. and Curtis, J. T. (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.,* **27,** 325-349.

Campbell, R. W. (2008) Overwintering habitat of Calanus finmarchicus in the North Atlantic inferred from autonomous profiling floats. *Deep Sea Res., Part I,* **55,** 630-645.

Clarke, K. R. (1993) Non-parametric multivariate analyses of changes in community structure. *Aust.*

372 *J. Ecol.,* **18,** 117-143.
373 Coughlan, S. (1977) The effect of organic substrates on the growth, photosynthesis and dark
374 survival of marine algae. *Br. phycol. J.,* **12,** 155-162.
375 Egge, J. K. and Aksnes, D. L. (1992) Silicate as regulating nutrient in phytoplankton competition.
376 *Mar. Ecol. Prog. Ser.,* **83,** 281-289.
377 Furusato, E., Asaeda, T. and Manatunge, J. (2004) Tolerance for prolonged darkness of three
378 phytoplankton species, Microcystis aeruginosa (Cyanophyceae), Scenedesmus quadricauda
379 (Chlorophyceae), and Melosira ambigua (Bacillariophyceae). *Hydrobiologia,* **527,** 153-162.
380 Griffis, K. and Chapman, D. J. (1988) Survival of phytoplankton under prolonged darkness:
381 implications for the Cretaceous-Tertiary boundary darkness hypothesis. *Palaeogeogr.,*
382 *Palaeoclimatol., Palaeoecol.,* **67,** 305-314.
383 Hagen, W., Van Vleet, E. and Kattner, G. (1996) Seasonal lipid storage as overwintering strategy of
384 Antarctic krill. *Mar. Ecol. Prog. Ser.,* **134,** 85-89.
385 Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U. and Zohary, T. (1999) Biovolume
386 calculation for pelagic and benthic microalgae. *J. Phycol.,* **35,** 403-424.
387 Lewis, J., Harris, A., Jones, K. and Edmonds, R. (1999) Long-term survival of marine planktonic
388 diatoms and dinoflagellates in stored sediment samples. *J. Plankton Res.,* **21,** 343-354.
389 Lüder, U. (2003) Acclimation of the photosynthetic apparatus of the endemic Antarctic red
390 macroalga *Palmaria decipiens* to seasonally changing light conditions. *Ber. Polarforsch.*
391 *Meeresforsch.,* **469,** 141.
392 Menden-Deuer, S. and Lessard, E. J. (2000) Carbon to volume relationships for dinoflagellates,
393 diatoms, and other protist plankton. *Limnol. Oceanogr.,* **45,** 569-579.
394 Moustaka-Gouni, M., Michaloudi, E. and Sommer, U. (2014) Modifying the PEG model for
395 Mediterranean lakes - no biological winter and strong fish predation. *Freshwater Biol.,* **59,**
396 1136-1144.
397 Murphy, A. M. and Cowles, T. J. (1997) Effects of darkness on multi- excitation in vivo
398 fluorescence and survival in a marine diatom. *Limnol. Oceanogr.,* **42,** 1444-1453.
399 Peters, E. (1996) Prolonged darkness and diatom mortality: II. Marine temperate species. *J. Exp.*
400 *Mar. Biol. Ecol.,* **207,** 43-58.
401 Peters, E. and Thomas, D. (1996a) Prolonged darkness and diatom mortality I: Marine Antarctic

species. *J. Exp. Mar. Biol. Ecol.*, **207,** 25-41.

Peters, E. and Thomas, D. (1996b) Prolonged nitrate exhaustion and diatom mortality: a comparison of polar and temperate Thalassiosira species. *J. Plankton Res.*, **18,** 953-968.

Reeves, S., Mcminn, A. and Martin, A. (2011) The effect of prolonged darkness on the growth, recovery and survival of Antarctic sea ice diatoms. *Polar Biol.*, **34,** 1019-1032.

Riley, G. A. (1957) Phytoplankton of the North Central Sargasso Sea. *Limnol. Oceanogr.*, **2,** 252-270.

Sieburth, J. M., Smetacek, V. and Lenz, J. (1978) Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.*, **23,** 1256-1263.

Siegel, D. A., Doney, S. C. and Yoder, J. A. (2002) The North Atlantic spring phytoplankton bloom and Sverdrup's critical depth hypothesis. *Science,* **296,** 730-3.

Smayda, T. and Mitchell-Innes, B. (1974) Dark survival of autotrophic, planktonic marine diatoms. *Mar. Biol.*, **25,** 195-202.

Smetacek, V. (1985) The annual cycle of Kiel Bight plankton: a long-term analysis. *Estuaries,* **8,** 145-157.

Sommer, U., Aberle, N., Lengfellner, K. and Lewandowska, A. (2012a) The Baltic Sea spring phytoplankton bloom in a changing climate: an experimental approach. *Mar. Biol.*, **159,** 2479-2490.

Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., Jeppesen, E., Lürling, M., and et al. (2012b) Beyond the Plankton Ecology Group (PEG) model: mechanisms driving plankton succession. *Annu. Rev. Ecol. Evol. Syst.*, **43,** 429-448.

Sommer, U., Gliwicz, Z. M., Lampert, W. and Duncan, A. (1986) The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.*, **106,** 433-471.

Sommer, U., Lengfellner, K. and Lewandowska, A. (2012c) Experimental induction of a coastal spring bloom early in the year by intermittent high-light episodes. *Mar. Ecol. Prog. Ser.*, **446,** 61-71.

Sverdrup, H. (1953) On conditions for the vernal blooming of phytoplankton. *J. Cons. Int. Explor. Mer,* **18,** 287-295.

Utermöhl, H. (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. *Mitt. int. Ver. theor. angew. Limnol.,* **9,** 1-38.

Vermaat, J. E. and Sand-Jensen, K. (1987) Survival, metabolism and growth of Ulva lactuca under winter conditions: a laboratory study of bottlenecks in the life cycle. *Mar. Biol.,* **95,** 55-61.

Wasmund, N., Göbel, J. and Bodungen, B. V. (2008) 100-years-changes in the phytoplankton community of Kiel Bight (Baltic Sea). *J. Mar. Syst.,* **73,** 300-322.

Wasmund, N., Nausch, G. and Matthäus, W. (1998) Phytoplankton spring blooms in the southern Baltic Sea—spatio-temporal development and long-term trends. *J. Plankton Res.,* **20,** 1099-1117.

Zhang, Q., Gradinger, R. and Spindler, M. (1998) Dark survival of marine microalgae in the high Arctic (Greenland Sea). *Polarforschung,* **65,** 111-116.

Tables

Table 1. Summary of the environmental conditions

Sample	Day	I_{mix}	pH	Salinity	T	Si	PO_4	NO_3	NH_4
W1	Dec. 7, 2015	18.4	7.86	21.4	8.55	19.79	1.21	13.44	4.76
W2	Jan. 18, 2016	43.7	7.96	20.8	2.95	17.17	0.94	12.26	3.06
W3[a]	Mar. 7, 2016	186.1	7.96	20.4	4.18	22.17	1.20	19.28	2.24

I_{mix} is the 24 h mean of the integrated mixed water column (12m) irradiance, calculated from surface irradiance according to Riley (1957), assuming an attenuation coefficient of 0.3 $m^{-}1$ (µmol photons PAR m^{-2} d^{-1}), T is the temperature measured in situ (°C); dissolved nutrients are the concentration in the bottles (µmol L^{-1})

[a] includes the extra nutrients

452 **Table 2.** The biomass of functional groups of phytoplankton before the culture

Phytoplankton group	Mean biomass (μg C L^{-1}±SD)		
	W1	W2	W3
Microdiatoms			
Chaetoceros	-	+	3.08±0.34
Coscinodiscus	-	-	4.08±1.88
Ditylum brightwellii	0.15±0.15	-	-
Guinardia flaccida	+	-	+
Guinardia	+	-	0.26±0.16
Proboscia alata	0.45±0.16	0.86±0.15	+
Pseudo-nitzschia	12.3±2.6	-	2.4±0.3
Rhizosolenia	0.64±0.35	-	-
Thalassionema	+	-	-
Thalassiosira	1.92±0.42	-	0.21±0.06
Dinoflagellates			
Ceratium lineatum	+	+	0.83±0.29
Dinophysis	0.14±0.14	0.19±0.2	0.14±0.1
Prorocentrum	+	+	+
Ceratium fusus	0.23±0.02	+	+
Ceratium tripos	-	+	-
Other microplanktonic flagellates			
Eutreptiella braarudii	-	+	-
Nanodiatoms			
Chaetoceros minimus	+	-	-
Leptocylindrus minimus	+	-	+
Skeletonema	1.67±0.44	+	0.28±0.2
Nanoflagellates			
Dictyocha	+	+	+
Eutreptiella gymnastica	-	+	2.97±0.61
Plagioselmis	-	1.18±0.52	+
Teleaulax	2.34±0.08	3.26±3.08	0.43±0.34
Picoplankton			
pico A	0.16±0.03	+	+
pico B	0.16±0.01	0.53±0.03	3.57±0.52

453 + means the rare taxa with biomass less than 0.1 μg C L^{-1}

454 - means the absence of taxa

455 **Table 3.** The abundance of heterotrophs before the culture

Heterotrophic group	Mean abundance (N L^{-1}±SD)		
	W1	W2	W3
Ciliates	170±50	340±190	160±100
Gyrodinium	140±120	120±50	-
Protoperidinium	370±170	110±40	80±40
Katodinium	910±1230	-	-
Protoperidinium bipes	480±560	440±620	-
Nauplii	-	5±10	20±23
Copepods	-	-	40±32

456 - means the absence of taxa

457 **Table 4.** The survival abilities of phytoplankton after dark incubation for 6 weeks

No survival	Survival without growth [a]	Survival and regrowth [a]
Ceratium fusus	*Chaetoceros*	*Attheya septentrionalis*
Ceratium lineatum	*Coscinodiscus*	Picoplankton [b]
Ceratium tripos	*Ditylum brightwellii*	*Pseudo-nitzschia* [c]
Chaetoceros minimus	*Dinophysis*	*Skeletonema*
Dactyliosolen fragillissimus	*Prorocentrum*	*Thalassiosira*
Dictyocha	*Pseudo-nitzschia* [c]	
Eutreptiella braarudii		
Eutreptiella gymnastica		
Heterocapsa rotundata		
Guinardia flaccida		
Guinardia		
Leptocylindrus minimus		
Picoplankton [b]		
Plagioselmis		
Proboscia alata		
Rhizosolenia		
Teleaulax		
Thalassionema		

458 [a] survival means the taxa showed relatively consistent presence in the subsequent light culture

459 [b] the picoplankton discovered before darkness had disappeared, while, new picoplankton were identified to grow

460 [c] *Pseudo-nitzschia* behaved differently for the species from different communities

461

Figure Legends

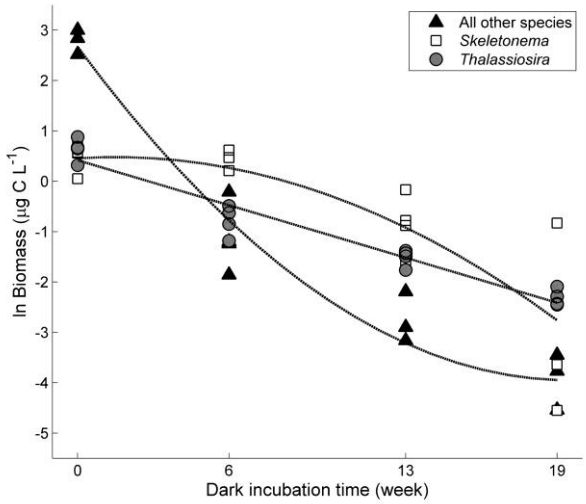


Fig. 1 Dark survival rates of species from W1, the lines show the regressions fitted to the data: all other species: $Y=0.017X^2-0.679X+2.671$, $R^2=0.961$; *Skeletonema*: $Y=-0.011X^2+0.032X+0.4556$, $R^2=0.721$; *Thalassiosira*: $Y=-0.149X+0.418$, $R^2=0.938$

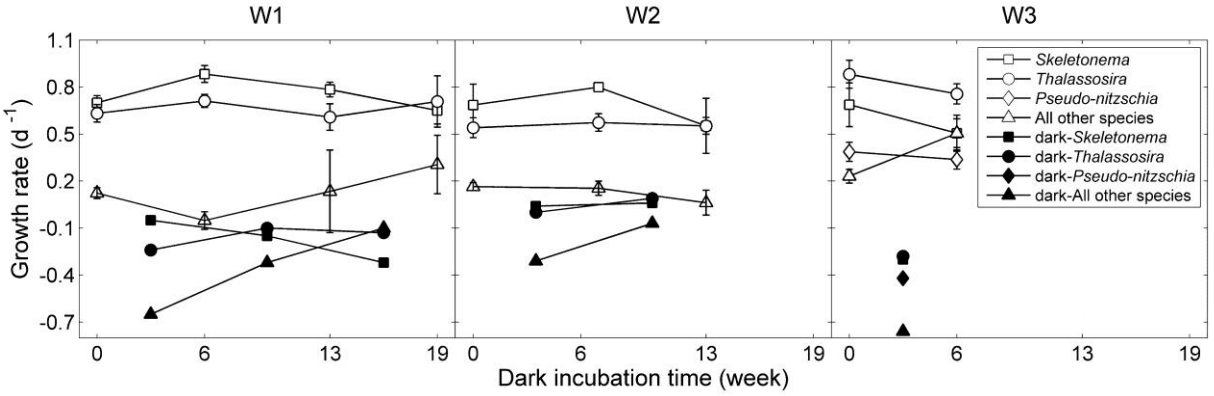
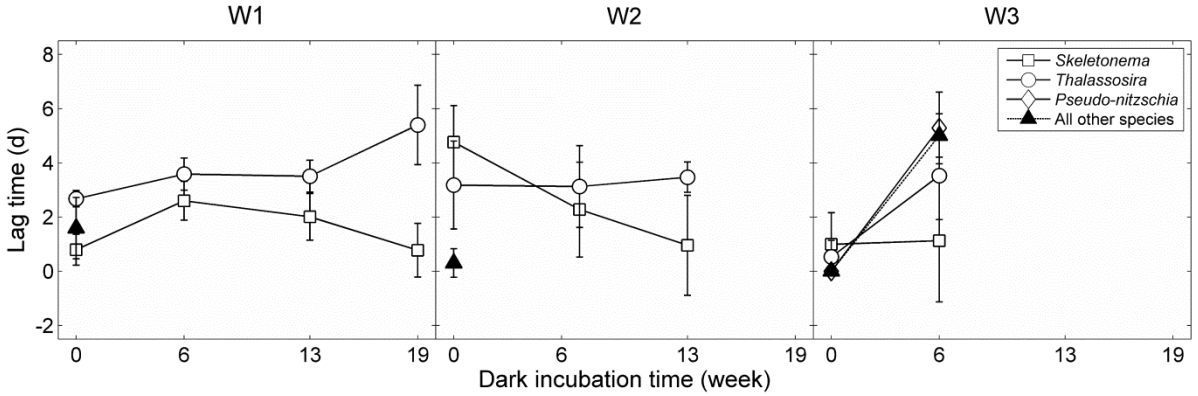


Fig. 2 Growth rates as a function of the length of dark incubation prior to re-illumination, after varying periods of darkness and average growth rates during dark incubation, error bars mean ± 1 SD. Growth rates in light culture: open squares: *Skeletonema*, open circles: *Thalassiosira*; open diamonds: *Pseudo-nitzschia*, open triangles: all other species. Growth rates in dark incubation: full squares: *Skeletonema*, full circles: *Thalassiosira*; full diamonds: *Pseudo-nitzschia*, full triangles: all other species.



477

478 **Fig. 3** Lag times as a function of the length of dark incubation prior to re-illumination, after varying
479 periods of darkness, error bars mean ± 1 SD. Open squares: *Skeletonema*, open circles:
480 *Thalassiosira*; open diamonds: *Pseudo-nitzschia*, full triangles: all other species.

481

Supplementary Document 1. Growth rate and lag time after varying periods of darkness

Community	Species	Darkness	μ_{growth} ± SD (d^{-1})	Lag time ± SD (d)	$\overline{\mu}_{growth}$ ± SD (d^{-1})
W1	*Skeletonema*	0 week	0.70±0.05	0.80±0.57	-
		6 week	0.88±0.05	2.60±0.72	-
		13 week	0.78±0.05	2.01±0.86	-
		19 week	0.65±0.09	0.78±0.99	-
	Thalassiosira	0 week	0.63±0.06	2.68±0.30	-
		6 week	0.71±0.04	3.58±0.59	-
		13 week	0.61±0.09	3.50±0.59	-
		19 week	0.71±0.16	5.40±1.46	-
	All other species	0 week	0.12±0.04	1.59±1.13	-
		6 week	-	-	-0.05±0.06
		13 week	-	-	0.13±0.26
		19 week	-	-	0.31±0.19
W2	*Skeletonema*	0 week	0.69±0.13	4.76±1.35	-
		7 week	0.80±0.03	2.28±1.75	-
		13 week	0.55±0.05	0.96±1.84	-
	Thalassiosira	0 week	0.54±0.06	3.18±1.62	-
		7 week	0.57±0.06	3.12±1.51	-
		13 week	0.55±0.18	3.47±0.56	-
	All other species	0 week	0.17±0.03	2.00±3.42	-
		7 week	-	-	0.15±0.05
		13 week	-	-	0.06±0.08
W3	*Skeletonema*	0 week	0.69±0.14	0.99±1.17	-
		6 week	0.51±0.12	1.13±2.57	-
	Thalassiosira	0 week	0.88±0.09	0.53±0.61	-
		6 week	0.76±0.06	3.53±1.62	-
	Pseudo-nitzschia	0 week	0.39±0.06	0	-
		6 week	0.34±0.06	5.29±1.33	-
	All other species	0 week	0.23±0.04	0	-
		6 week	0.51±0.09	5.00±0.80	-

Supplementary Figure