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Changes in spectral quality of underwater light alter phytoplankton community composition

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Abstract

Light is a fundamental resource for phytoplankton. To utilize the available light, most phytoplankton species possess pigments in taxon-specific combinations and quantities, which in turn result in a specific use of certain wavelengths. This optimizes the light use efficiency, allows for a complementary use of light, and may be an additional driver for community structure. While the effects of light intensity on phytoplankton biomass production and community composition have been intensively studied, here we focused on the effects of specific light spectrum quality (thus light color) on a natural phytoplankton community. In a controlled mesocosm experiment we reduced the supplied wavelength range to its blue, green, or red part of the light spectrum and compared the responses of each treatment to a full spectrum control over 28 d. Highest community growth rates were observed under blue, lowest under red light. Light absorption by the communities showed adaptation toward the supplied wavelength range. Community composition was significantly affected by light quality treatments, driven by Bacillariophyta and Chlorophyta, whereas pigment composition was not. Furthermore, lower species richness but higher evenness occurred when communities were exposed to red light compared to the full spectrum. We expected the response of phytoplankton communities to changes in the light spectrum to be driven by a combination of species sorting and pigment acclimation; however, the effect of species sorting turned out to be stronger. Our study showed that, even if species might acclimate, changes in the available light spectrum affect primary production and phytoplankton community composition.

Introduction

Light is among the most important resources for primary producers and its availability and quality affect phytoplankton biomass as well as its community structure. Within aquatic ecosystems, it exhibits a high spatial heterogeneity in intensity due to, for example, water depth, shading, dissolved organic matter (DOM). Hereby, light intensity is a resource for primary producers which can be exploited, used, and tolerated differently. Hence, a heterogeneity in supply determines competition outcomes, causes community changes, and affects primary productivity which can propagate across the food web and thus even impact higher trophic levels (Boyd 2002; Berger et al. 2006; Karlsson et al. 2009).

In addition to light intensity, the light's spectral quality (hereafter also referred to as "light color") within the range of photosynthetic active radiation (PAR, 400–700 nm) plays a major role for primary producers. Within a water column the spectral range of light shifts from almost full spectrum at the surface toward a limited, predominantly blue spectrum in deeper layers, mainly derived from the absorption by water molecules (Stomp et al. 2007*a*; Kirk 2011; Holtrop et al. 2020). Heterogeneity of the light's spectrum further occurs by presence of light-absorbing substances within the water. In particular, colored DOM (cDOM) by terrestrial matter runoffs can lead to spectral shifts in the ambient light spectrum as so called "brownification." In addition, phytoplankton itself shapes the light environment via light absorption.

Being exposed to various light conditions, phytoplankton species feature a variety of photosynthetic pigments in taxon-specific combinations and quantities in addition to the ubiquitous chlorophyll *a* (Chl *a*). These accessory pigments harvest photons of a specific range within the wavelength spectrum, transport excitation energy, or dissipate excessive energy in parts of the spectrum where Chl *a* cannot effectively absorb (Rowan 2011; Croce and van Amerongen 2014). However, the

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total cellular light harvesting, as combination of individual pigment absorption spectra, does not cover the whole PAR equally. Especially due to the chlorophylls, phytoplankton's photosynthetic light absorption is in general highest in low wavelengths (blue, ~ 460–490 nm) and secondly in high wavelengths (red, ~ 630–700 nm). Whereas a medium range (green, ~ 490–570 nm) is barely covered in phototrophic organisms known as "green gap" that some phytoplankton groups cover via accessory pigments belonging to the carotenoids and phycobilins (Croce and van Amerongen 2014). Thus, the spectrum of light affects phytoplankton primary production in general (Arbones et al. 2000), whereupon it further affects photosynthetic rates in taxon-specific magnitudes due to differences in cellular light-harvesting strategies (e.g., by pigment composition) (Kirk 2011). Previous work showed that phytoplankton light-harvesting strategies differ strongly between but are more similar within phylogenic groups. Thus, phyla of phytoplankton can be used for the definition of functional groups based on these light-harvesting traits (Hood et al. 2006; Behl et al. 2011).

According to the discriminative light harvesting of phytoplankton, light can be considered as a multitude of resources and thus a broad spectral range is expected to promote coexistence and high biodiversity by complementarity of different light-harvesting strategies, whereas a narrowed light spectrum could predictably act as selective factor (Stomp et al. 2007*b*; Luimstra et al. 2019; Tan et al. 2020). Therefore, the ambient light spectrum shapes ecological niche spaces which determine local phytoplankton communities and their spatial distribution by species sorting (Stomp et al. 2007*a*; Hickman et al. 2009). Such a chromatic phylogenetic adaptation theory of phytoplankton communities was early stated by Engelmann (1883) and reported for various regions and depths due to spatial variation in the light climate (Kirk 2011) but changes in spectrum were not experimentally tested so far.

The pigment composition within phytoplankton cells, even though taxon-specific, is not entirely fixed within living individuals. Whereas acclimation to light intensities by biosynthesis or degradation of chlorophylls and accessory pigments is widely demonstrated (Kirk 2011), such an acclimation to certain wavelength as a response to mismatches in the light spectrum is less investigated but has been reported for several species (Grossman et al. 1993). This response by biosynthesis of photosynthetic components is linked to metabolic costs as well as slows growth (Geider et al. 1997) and the degree of pigment plasticity varies across taxonomic groups (Falkowski 1980; Geider et al. 1998). However, prevalence of chromatic acclimation as response to light spectrum differences is still a matter of debate, rarely proven in eukaryotic phytoplankton (Mouget et al. 2004) and predominantly granted to cyanobacteria (Gutu and Kehoe 2012).

To uncouple the effects of changes in light intensity and light spectrum that naturally co-occur, for example, with increasing water depth or changes by brownification or massive algal growth, and solely focus on the effect of changes in the available light spectrum on phytoplankton, we investigated a natural coastal North Sea community and reduced the available light to distinct sections of its spectrum. Therefore, we provided the same integrated light intensities of different "colors": red at 721 nm (maximum of transmission peak), green at 520 nm (maximum of transmission peak) and blue at 456 nm (maximum of transmission peak) as well as a combined full spectrum as control.

We hypothesized that:

(1) The limitation in the light spectrum to a certain range would affect the community growth and biomass yield. Due to strong absorption of blue light in phytoplankton cells we expected high community growth under these conditions compared to other treatments, whereas the green light treatment was expected to reveal lowest growth due to the "green gap."

(2) Due to the limitation in the light spectrum to a certain range, the phytoplankton community absorption characteristics were expected to change. We expected the community light absorption to change toward an enhanced use of the supplied light range. This adaption was expected to be mediated by:

(2a) acclimation on cellular level by changes in the pigment composition, as well as (2b) changes in taxonomical composition and biodiversity of the phytoplankton communities.

(3) We expected the response of phytoplankton communities to different light quality treatments to be driven by a combination of species sorting and pigment acclimation, with stronger changes in taxonomic than pigment composition.

Methods

Experimental setup

The experiment was performed in 12 mesocosm units called "Planktotrons" of 600 liter each (Gall et al. 2017) filled with natural seawater originating from the German Jade Bight (lat. 53.513, long. 8.153). The experiment was conducted in 2–30 April 2019 and lasted over a period of 28 d. The water, including the natural phytoplankton community, was prefiltered over a 100-μm mesh to remove macrozooplankton. To prevent early nutrient limitations but assure close to natural starting conditions, the water was amended with nutrients according to the 90% percentile in Helgoland Roads data of April 1962–2008: 50.5 μmol N L^{-1}, 1.7 μmol P L^{-1}, 30 μmol Si L^{-1}, respectively. During the experiment, 10% of the initial nutrient concentration was supplied continuously per day. The medium was supplemented with Vitamins B1, B7, B12 at day 13 and with trace metals at day 14, both according to the concentrations used in F/2 media (Guillard and Ryther 1962) to avoid (co-)limitations. The temperature was held constant at an average value of 11°C similar to the German Jade Bight water temperature in April. Thermal stratification within the mesocosms was prevented by thermal convection, and to

assure homogeneous distribution of phytoplankton within the water column, mixing was conducted every 2 h for a duration of 5 min. Light was supplied via two light-emitting diode (LED) modules per mesocosm (Evergrow IT2040; Shenzhen Sanxinbao Semiconductor Lightning Co. Ltd) in a day : night cycle of 14 : 10 h. The light spectrum treatments of red, green, and blue light as well as white-light control were obtained by covering the mesocosms with colored foils of relatively narrow (i.e., half-peak width) thus distinct transmission spectra (LEE and Chris James Lighting filters, *see* Supporting Information Fig. S1). Red treatment peaking at 660 nm, half-peak width approx. 39 nm; green treatment peaking at 520 nm, half-peak width approx. 51 nm; and blue treatment peaking at 456 nm, half-peak width approx. 31 nm. The control was intensity adjusted without affecting the LED spectrum (using a "dark gray" foil). Treatments and control were set up in triplicates and light intensity was adjusted coequally based on in situ photosynthetically usable radiation, following calculation by Morel et al. (1987) and Orefice et al. (2016) to values of averaged 8.144 μmol photons m^{-2} s^{-1} (SE = 1.030, *see* Supporting Information for details).

Sample analyses

Samples for bulk phytoplankton pigment analysis and particulate organic carbon (POC) were taken every other day, filtered on precombusted, acid-washed glass-fiber filters (GF/C, Whatman) and stored at −80°C until analysis. In addition to these samples, pigment concentrations at days 13 and 27 were analyzed. Filters for POC were dried and analyzed via elemental analyzer (Flash EA 1112, Thermo Scientific). Filters for pigment analysis were extracted in 1.8 mL of EtOH (90 vol%) via sonication for 30 min on ice and subsequently for 20 h in the dark at 6°C. Absorption spectra of the extracted samples were analyzed photometrically (Synergy H1, Biotek), and pigment concentrations were calculated according to Thrane et al. (2015). All absorption spectra were measured at a wavelength range of 350–750 nm in steps of 1 nm and calculated as absorbance per wavelength.

The phytoplankton community composition was microscopically identified after fixation with Lugol's Iodine solution (1 vol% final concentration) of samples at days 0, 13, and 27. Microscopically identification (Axiovert 10, Zeiss) of the samples was performed with at least 400 counted individuals per sample after cell settlement using Utermöhl's method (Utermöhl 1958). Biovolume was calculated by measuring cellular dimensions of at least 20 individuals per species and defining geometric shapes according to Olenina et al. (2006). For rare species, literature data derived from the HELCOM PEG-QA biovolume list of 2019 were used (Olenina et al. 2006).

Statistical examination

All graphs and statistical tests were performed in R (version 3.6.1, The R Foundation for Statistical Computing Platform). A level of $\alpha = 0.05$ was considered for statistical significance in all analyses.

General treatment effects on biomass over time were estimated using linear mixed-effect model (LMM). Therefore, POC was used as proxy for phytoplankton biomass and log-transformed to fit normal distribution. The LMM considered the *treatment* and *time* as fixed effects, whereas *mesocosm units* as well as *sampling day* were included as random effects. By treating *sampling day* as a categorical variable in the random component, we accounted for the sampling's timing and allowed for nonlinear dynamics within a mesocosm.

A logistic growth curve for carbon concentrations over time was used to determine maximum growth rate (μ_{max}) (Eq. 1, with *time* expressed as experimental day, POC_0 as initial biomass concentration, and K as carrying capacity). Significance of treatment effects at μ_{max} and final biomass were estimated using one-way analysis of variance (ANOVA) and Tukey multiple pairwise comparison.

$$\mathrm{POC(time)} = \frac{K \times \mathrm{POC}_0}{\mathrm{POC}_0 + (K - \mathrm{POC}_0) \times e^{(-\mu_{max} \times \mathrm{time})}} \quad (1)$$

To qualitatively analyze the overall phytoplankton community light harvesting at day 27, the absorption spectra of the pigment extract were compared. This was established in a single quantile regression generalized additive model (QGAM), in order to account for proper 50%-quantile calculation across the replicates as well as dealing with heteroscedasticity (Fasiolo et al. 2017). Prior blank subtracted absorption spectra (expressed as absorbance per wavelength) of extracted pigments were normalized to their respective filtration volume and POC content, as the shape of the absorption spectrum is a function of biovolume per unit area of the illuminating beam (Kirk 2011). As POC data for day 27 were not available, interpolated concentrations of days before and after were used, respectively. To enable comparability in between treatments and sampling days, the absorption spectra were z-transformed (Eq. 2, μ = population mean, σ = population SD), contrariwise to a usual normalization at a specific wavelength, as we did not know either the absorption spectrum of every single species nor its proportion to the overall community spectrum or the degree of pigment plasticity. This allowed a qualitative examination of absorption spectra independent of the amount of biomass during filtration and extraction. A comparison of fitted spectra was performed to investigate significant differences of treatments effects to control conditions at a confidence level of 0.95 (n = 3 per treatment) within the treatment's wavelength ranges.

$$z = \frac{x - \mu}{\sigma}. \quad (2)$$

Pigment concentrations were calculated as weighted sums of Gaussian functions according to Thrane et al. (2015) and normalized by their respective extraction and filtration volume as well as sample POC concentration. Comparison

of either pigment or community composition (based on genus-level biovolume) was performed via analysis of similarity tests (ANOSIM; Clarke 1993) using Bray–Curtis index with 999 permutations. As taxonomic phytoplankton phyla can be used to distinguish functional groups by their light harvesting (Hood et al. 2006; Behl et al. 2011), all asserted genera were additionally consolidated and compared by phylum-level ANOSIM. Significant ANOSIM results were further investigated via similarities percentage test (SIMPER) to identify the most influential specimens (Clarke 1993). A comparison based on biovolume was preferred over abundancies as the extent of light absorption per cell depends on the cell's dimension. Treatment effects at single phyla and pigments were investigated by calculating their proportion to the total sample composition and subsequent tested via one-way ANOVA and, if significant, further by Tukey multiple pairwise comparison. Using the relative shares allowed a comparison within and between treatments despite different community biomass. Treatment effects at biodiversity metrics were calculated as species richness, Shannon diversity and Pielou's evenness (Magurran and McGill 2011) based on species abundances. If individuals could not be identified down to species level, they were accounted as distinguishable species based on morphological categories. To test for significant treatment effects, one-way ANOVA and, if significant, a Tukey multiple pairwise comparison was performed for each metric.

Results

Phytoplankton growth

Phytoplankton biomass (POC-based) revealed significant treatments effects over time (LMM $F_{3,154} = 48.444$, $p < 0.001$; Fig. 1a). The maximum growth of the phytoplankton community significantly differed due to the treatments, and higher growth rates were observed under blue light compared to red and green light (Fig. 1b; Table 1). Final community biomass (at the end of the experiment) also tended to be higher under blue light compared to red and green light (Fig. 1c); however, these differences were statistically not significant.

Phytoplankton community light absorption

The absorption spectra of the phytoplankton communities revealed significant differences according to the treatments among several wavelength ranges at the end of the

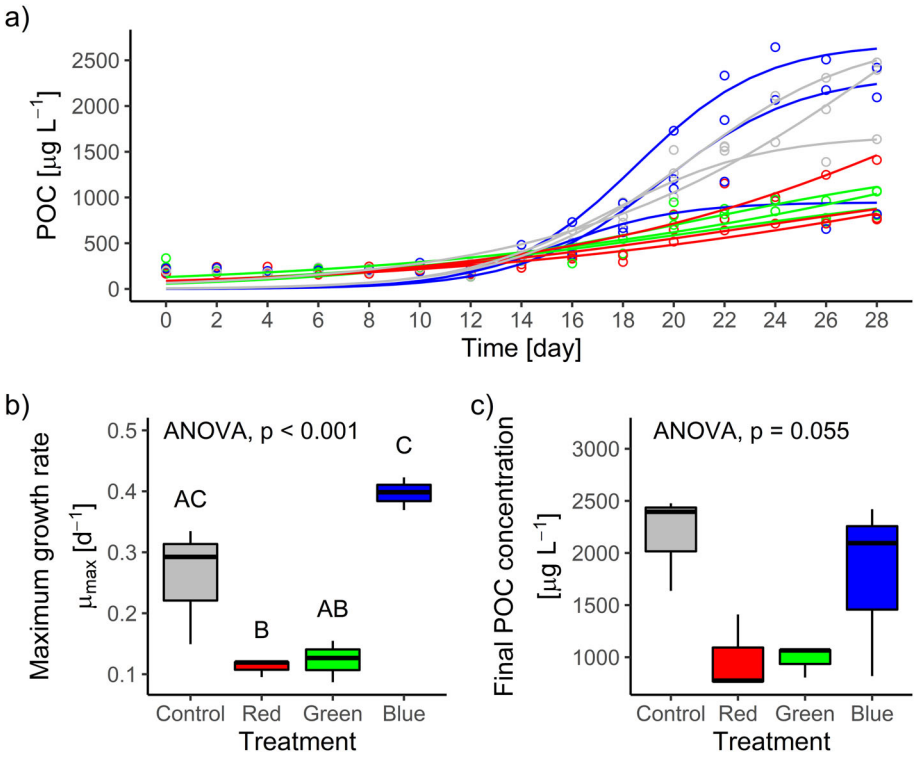

Fig. 1. Phytoplankton biomass (POC in μg L^{-1}) over time, colors indicate the respective light treatments and gray the control. (**a**) Curves were fitted using logistic growth functions for each experimental unit separately. (**b**) Maximum growth rate μmax and (**c**) final concentrations at day 28, as Tukey's boxplot. ANOVA results are stated and if significant ($p < 0.05$), Tukey multiple pairwise-comparisons are indicated by dissenting letter combination ($p < 0.05$, $n = 12$).

Table 1. Treatment effects on phytoplankton community growth: maximum growth rate and final biomass (POC in μg L^{-1}) concentration at day 28. ANOVA $F_{3,8}$ and p values (in brackets) are presented. Comparisons results are presented by differences in mean and p values (in brackets). Significant results ($p < 0.05$) are highlighted in bold.

Growth parameter	$F_{3,8}$ (p)	Tukey multiple pairwise comparison	
		Treatment	**Difference in mean (p)**
Maximum growth rate (μ_{max})	**18.74 (< 0.001)**	Control–blue	−0.138 (0.054)
		Green–blue	**−0.274 (0.001)**
		Red–blue	**−0.285 (0.001)**
		Green–control	−0.136 (0.057)
		Red–control	**−0.147 (0.040)**
		Red–green	−0.011 (0.994)
Final biomass concentration	3.89 (0.055)	Control–blue	−391.7 (0.796)
		Green–blue	796.9 (0.312)
		Red–blue	796.1 (0.313)
		Green–control	−1188.5 (0.081)
		Red–control	−1187.7 (0.090)
		Red–green	−0.8 (1.000)

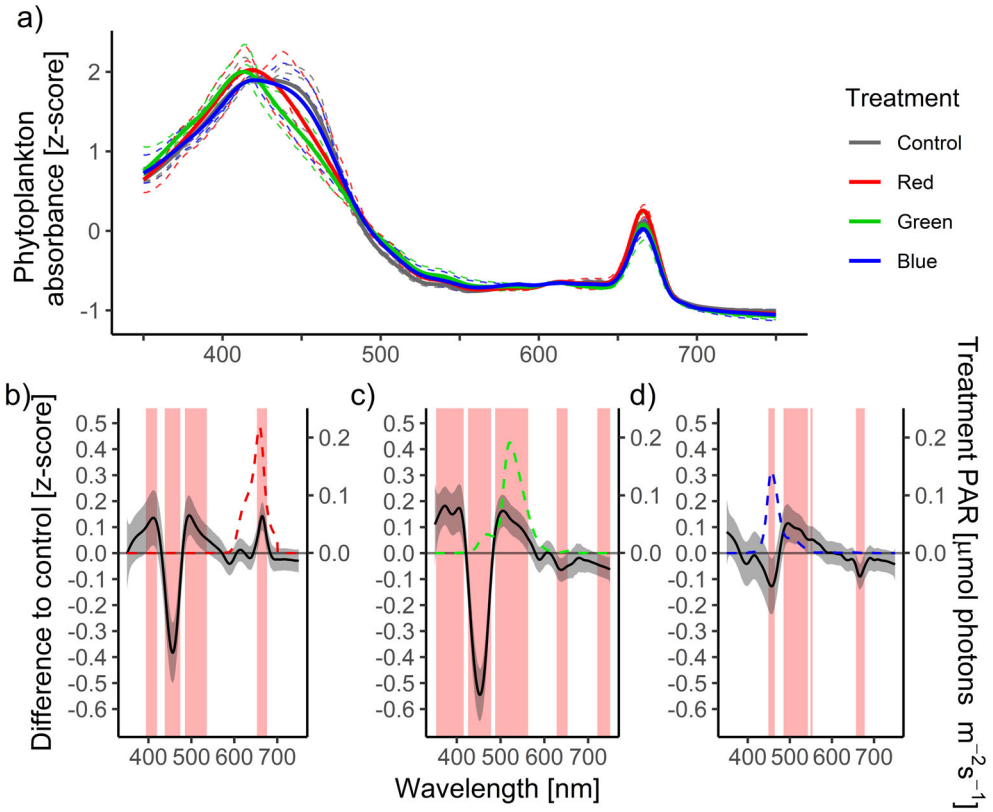

Fig. 2. Phytoplankton light-harvesting properties based on total pigment absorption spectra. (**a**) QGAM modeled median ($n = 3$) absorption spectra of each community at the end of the experiment (day 27) as solid lines; dashed lines display individual unfitted absorption spectra input. Absorption spectra were analyzed and expressed as absorbance per wavelength. Colors indicate the respective light treatments and gray the control. (**b–d)** Difference plots of communities exposed to (**b**) red, (**c**) green, (**d**) blue light toward the control displayed as black lines. A positive difference indicates a higher light absorbance per wavelength as shaped by the control community. Gray ribbons indicate the level of confidence of 0.95. Ranges of significant differences in absorption spectra of the treatments compared to control are shaded in red. Colored dashed lines indicate each treatments incident light spectra (note secondary y-axis) for comparison.

experiment (Fig. 2, significant ranges are highlighted in red; Table 2). A positive difference of fitted community absorption spectra toward the control indicates a higher light absorption in the treatment compared to the control. The communities exposed to red light showed positive differences within the red wavelength range (Fig. 2b) while all other communities revealed negative differences compared to the control in this range. This indicated higher absorption of red light after the incubation under red light compared to the control or the treatments. The community exposed to green light exhibited a positive difference in the green wavelength range (Fig. 2c). Although the other communities revealed positive differences in this wavelength range as well, the difference was highest, and the significant wavelength range was the broadest in the communities exposed to green light. This indicated a higher absorption of green light after the treatment with green light compared to the other treatments, while the control communities absorbed the least light within this range. The community exposed to blue light showed a negative difference in the blue wavelength range (Fig. 2d). As the communities of the control had very high absorption of blue light all treatments showed a comparatively strong

Table 2. Maximum significant differences (peak height) and range of difference (peak-base width) in phytoplankton light-harvesting properties within the range of respective treatment (QGAM fit). Significance was defined by the level of confidence of 0.95.

Treatment	Maximum significant difference	Range of significant difference (nm)
Red	0.143 (at 665 nm)	653–676
Green	0.163 (at 506 nm)	488–563
Blue	−0.129 (at 457 nm)	449–464

Table 3. Treatment effects on relative pigment composition (individual pigment per total concentration) at the end of the experiment (day 27). ANOVA $F_{3,8}$ values and p values (in brackets) are presented.

Pigment	$F_{3,8}$ (p)
Chlorophyll *a*	2.047 (0.186)
Pheophytin *a*	0.862 (0.499)
Fucoxanthin	1.835 (0.219)
Chlorophyll *b*	2.789 (0.109)
Chlorophyll *c2*	1.316 (9.335)
trans-Echinenone	0.451 (0.723)
Diatoxanthin	3.968 (0.053)
9′-*cis*-Neoxanthin	0.954 (0.46)
trans-Canthaxanthin	2.060 (0.184)
all-*trans*-Zeaxanthin	1.000 (0.441)

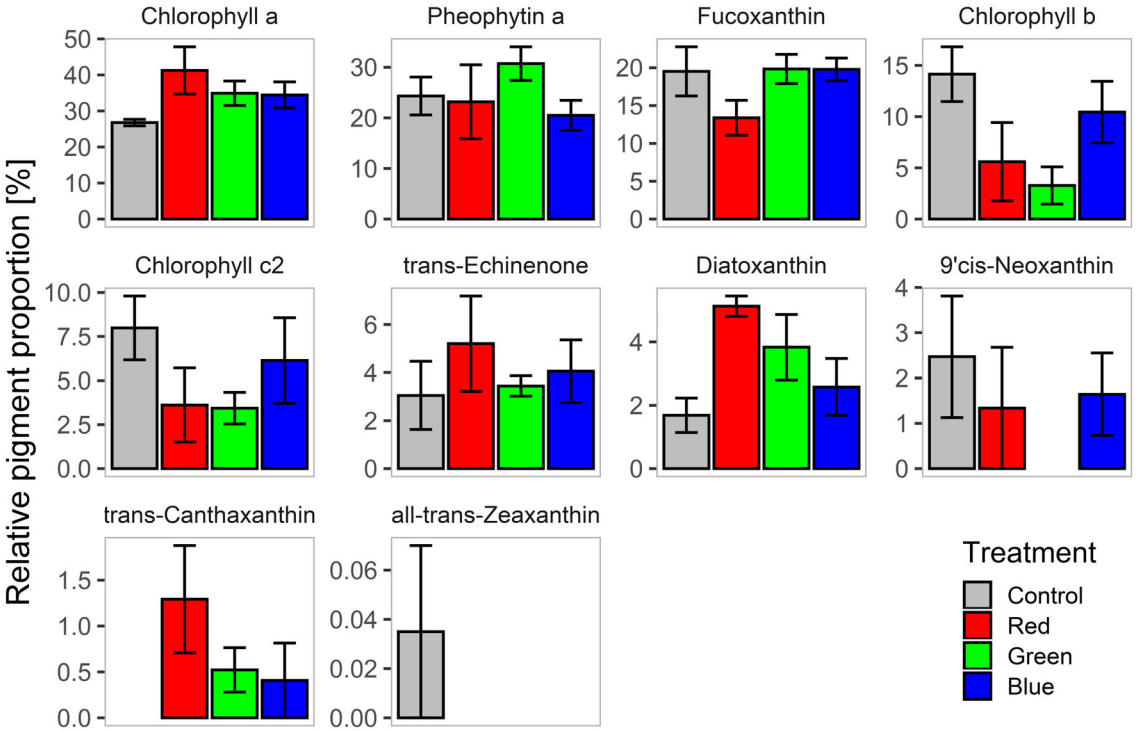

Fig. 3. Relative pigment composition (individual pigment per total concentration) at the end of the experiment (day 27), displayed as mean ± *SE* ($n = 3$).

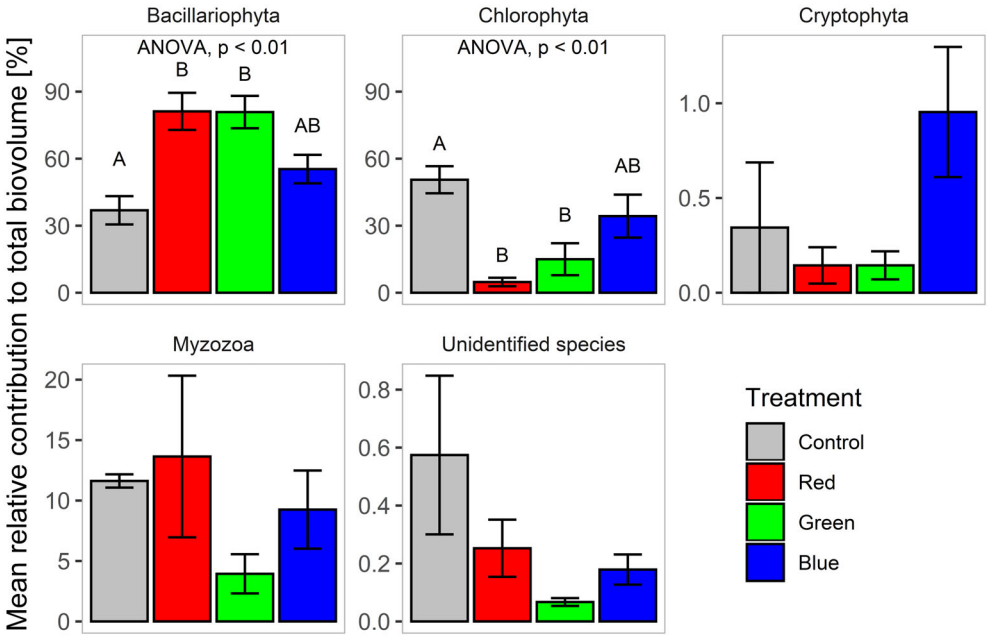

Fig. 4. Relative phytoplankton phylogenic biovolume (per total biovolume) per treatment at the end of the experiment (day 27), displayed as mean ± *SE* ($n = 3$). Cells which could not be identified otherwise due to low size (< 2 μm) were accounted as unidentified species. ANOVA results are stated if significant ($p < 0.05$) and significant Tukey multiple pairwise-comparisons are indicated by dissenting letter combination.

Table 4. Treatment effects on relative phytoplankton phylogenic biovolume (per total biovolume) at the end of the experiment (day 27). ANOVA $F_{3,8}$ values are presented, as well as Tukey multiple pairwise-comparison by differences in mean (p values in brackets). Significant results are highlighted in bold. Cells which could not be identified otherwise due to low size (< 2 μm) were accounted as unidentified species.

Phylum	$F_{3,8}$ (p)	Tukey multiple pairwise-comparison	
		Treatment	Difference in mean (p)
Bacillariophyta	**9.149 (0.006)**	Control–blue	−18.4 (0.326)
		Green–blue	25.5 (0.128)
		Red–blue	25.8 (0.122)
		Green–control	**44.0 (0.010)**
		Red–control	**44.3 (0.011)**
		Red–green	−31.9 (1.000)
Chlorophyta	**9.000 (0.006)**	Control–blue	16.3 (0.384)
		Green–blue	−19.3 (0.261)
		Red–blue	−29.5 (0.060)
		Green–control	**−35.6 (0.025)**
		Red–control	**−45.8 (< 0.01)**
		Red–green	−10.2 (0.72)
Cryptophyta	2.340 (0.150)		
Myzozoa	1.207 (0.368)		
Unidentified species	2.166 (0.170)		

negative difference in this range. Thus, the communities exposed to blue light revealed the least pronounced negative differences and narrowest significant wavelength range compared to other treatments, indicating a higher absorbance of blue in comparison to the other treatments.

Phytoplankton communities' pigment and taxonomic composition

The phytoplankton bulk pigment composition at the end of the experiment revealed low and non-significant differences in between the treatments ($ANOSIM_{Day27}$ $R = 0.096$, $p = 0.272$).

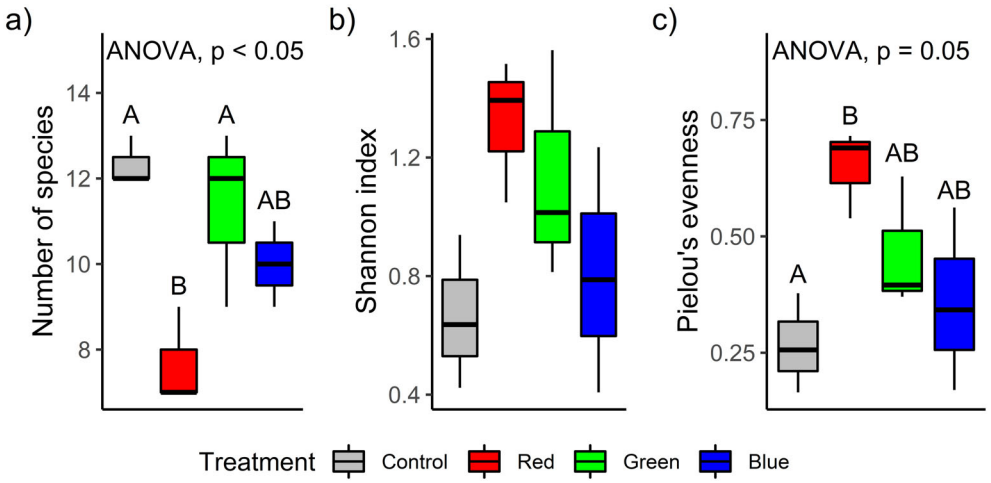

Fig. 5. Phytoplankton species biodiversity metrics at the end of the experiment (day 27) displayed as Tukey's boxplots of, (**a**) richness by the total number of species. (**b**) Diversity by the Shannon index and (**c**) Pielou's evenness. Colors indicate the respective light treatments and gray the control. ANOVA results are stated if significant ($p < 0.05$) and significant Tukey multiple pairwise-comparisons are indicated by dissenting letter combination ($p < 0.05$, $n = 12$).

Table 5. Treatment effects on biodiversity metrics at the end of the experiment (day 27). ANOVA $F_{3,8}$ and p (in brackets) values, and if significant ($p < 0.05$), Tukey multiple pairwise-comparison by differences in mean are presented. Significant results ($p < 0.05$) are highlighted in bold.

Biodiversity metric	$F_{3,8}$ (p)	Tukey multiple pairwise comparison	
		Treatment	Difference in mean (p)
Species richness	**6.984 (0.013)**	Control–blue	2.333 (0.214)
		Green–blue	1.333 (0.624)
		Red–blue	−2.333 (0.214)
		Green–control	−1.000 (0.792)
		Red–control	**−4.667 (0.010)**
		Red–green	**−3.667 (0.038)**
Shannon index	2.369 (0.147)		
Pielou's evenness	**4.066 (0.050)**	Control–blue	−0.091 (0.854)
		Green–blue	0.107 (0.791)
		Red–blue	0.290 (0.130)
		Green–control	0.199 (0.372)
		Red–control	**0.382 (0.042)**
		Red–green	0.183 (0.433)

Comparing the relative pigment composition between the treatments revealed no significant treatment effects (Fig. 3; Table 3). As expected, Chl *a* concentrations were highest among all treatments for each sample. However, Chl *b* and Chl *c2* tended to be higher under blue and control conditions than under red and green (Fig. 3; for changes over time, *see* Supporting Information Fig. S2).

Phytoplankton taxonomic composition (based on biovolume) based on genus level did not differ between the treatments at the end of the experiment ($ANOSIM_{Day27}$ $R = 0.161$, $p = 0.116$). However, summarized into functional phylogenic groups, phytoplankton composition changed. Consequently, at experimental end (Fig. 4; Table 4) significant treatment effects existed ($ANOSIM_{Day27}$ $R = 0.3148$, $p < 0.05$) based on Bacillariophyta and Chlorophyta (SIMPER test, Supporting Information Table S1). Bacillariophyta dominated when the communities experienced red and green light, whereas Chlorophyta contributed similar as Bacillariophyta to the control and almost similar under blue light (Fig. 4; for changes over time, *see* Supporting Information Fig. S3).

Species richness at the end of the experiment was significantly affected by the treatment, thus communities exposed to red light showed lower species richness compared to the control (Fig. 5a; Table 5). Shannon's diversity was not

significantly affected by the treatment, but slightly higher in the red treatment compared to the control (Fig. 5b; Table 5). Species evenness was significantly affected by the treatments, highest evenness occurred in communities exposed to red light and lowest evenness in the control (Fig. 5c; Table 5).

Discussion

Effect of light spectrum on phytoplankton performance

Phytoplankton community growth and final biomass differed due to the applied light treatments. The blue light treatment did result in highest biomass and faster growth rates comparable to the control. Communities exposed to red or green light had significantly lower growth rates which highlights the importance of the natural blue light in aquatic environments, to which the absorption properties of Chl *a* match. Thus, we can accept our first hypothesis that reduced and specific light spectra (despite providing the same light intensity) affected phytoplankton community growth. Other studies, manipulating a natural light spectrum via brownification in lakes (Urrutia-Cordero et al. 2017; Lebret et al. 2018), could not show effects on biomass but changes in community composition in artificially red-colored lake waters. However, these experiments manipulated the light spectrum and light intensity at the same time while we focused on the solely effect of differing wavelength ranges.

Absorption spectra of phytoplankton communities

The differences in light absorption indicated a specific chromatic adaptation of the communities, as communities showed an increase in absorption within the wavelength ranges of their respective treatments. In open waters, such community adaption was found due to the spectral shift with depth (Hickman et al. 2009). However, our analysis was based on absorption measurement of extracted pigments and thus neglected the packaging effect due to homogenization of pigments which are packed in cells. Furthermore, in vivo light-harvesting characteristics might have differed slightly compared to measurements of extracted samples due to shifted absorption of pigment–protein complexes and cell tissues. For example, nonphotosynthetic carotenoids in thylakoid membranes act as photoprotectants or sunscreens blocking UV radiation and thus would impact in vivo light-harvesting characteristics (Gao and Garcia-Pichel 2011). Overall, phytoplankton absorption spectra in our experiment were significantly affected by the light color treatments and showed a treatment specific chromatic adaption supporting our second hypothesis.

Pigment and taxonomic composition

Although the community absorption differed due to the applied treatments in our experiment, the relative pigment compositions within the communities did not. The pigment compositions were mostly determined by the relative contributions of Chl *a* and Chl *b* + *c2*. In the communities exposed to blue light and the control, the relative contribution of Chl *b* and Chl *c*2 was higher compared to those exposed to red and green light, contrariwise to Chl *a*. This trend might indicate an adaption of the phytoplankton communities to the light treatments: The incident light in the blue treatment as well as in the control peaked at 456 nm (Supporting Information Fig. S1), where the Chl *b* absorption spectrum peaks at approx. 460 nm, while Chl *a* peaks around 440 nm. The spectrum of the red treatment peaked at 660 nm (Supporting Information Fig. S1), close to the second maximum of Chl *a* that peaks at ~ 660 nm, but unlike the Chl *b* second peak at ~ 640 nm (Kirk 2011). In addition, Chl *c* shows a strong absorption under blue light but almost none at higher wavelengths, hence its appearance (as Chl *c*2) under blue light and in the control might have been a consequence of chromatic acclimation. Thus, even though apparent changes in pigment content could be linked to the wavelength ranges and peaks of the light treatments, the light treatment effects on pigment composition were not significant. Hence, we did not have sufficient evidence to support hypothesis 2a.

Those apparent changes in pigment composition could be explained by the contribution of Chlorophyta to the total biovolume of the communities under blue light and the control, as Chl *b* is predominantly related to this phylum, and further by the decrease in Bacillariophyta biovolume in the control which resulted in very low proportions of fucoxanthin, a characteristic carotenoid of this phylum (Stauber and Jeffrey 1988; Kirk 2011). This furthermore reflected potential trends in chromatic adaption in our experiment, as the ecological disadvantage of chlorophytes under green and red light due to their pigmentation was also discussed by Kirk (2011) who suspected the relative low abundance of chlorophytes in North European waters based on their low harvesting of the predominantly green and brownish spectra.

The community composition as well as diversity metrics indicated that the initial community composition was adapted to white light (full spectrum) in their natural environment. Exposing this community to red light resulted in a restriction of the light spectrum that did not fit the demands of the species within the community, growth rates were reduced, and some species excluded. The reduction in species richness under red light exposure further highlights the impact of brownification on the light spectrum. This was also shown in other mesocosm studies, however, coupled with either light intensity decrease (Rasconi et al. 2015) or nutrient input (Urrutia-Cordero et al. 2017). Obtaining highest species richness in the control might indicate, yet not prove, a coexistence of species by the availability of multiple colors as predicted by Burson et al. (2019). Hence, this supports our hypothesis 2b, as we showed general treatment effects on the community composition (in terms of the phylogenic groups: Bacillariophyta and Chlorophyta) and further significant effects of different light colors on determined biodiversity metrics (Species richness and Pielou's evenness).

Comparing effects on community composition with those on pigment composition revealed higher effects at the level of taxonomy during our experiment (*see* Supporting Information Fig. S4 for a direct comparison). Regarding our third hypothesis that response of phytoplankton communities to different light quality treatments would be driven by a combination of

species sorting and pigment acclimation, we could particularly support the change in taxonomic composition. A likely reason for that might have been the similarity of cellular pigment composition in different phytoplankton species, primarily dominated by high proportions of Chl *a*. Hence, a complete replacement of species would not be reflected to the same degree in their pigments, as the most abundant pigments would only slightly change in their concentration. However, for investigating the effect on pigment concentrations and composition in detail, more temporally resolved investigation at species and community level are needed.

Impact of light quality in the North Sea

The phytoplankton community analyzed in our study originated from the North Sea, a relative shallow sea which has encountered severe changes in nutrient concentrations affecting algae blooms (Philippart et al. 2000) and loads of organic matter (Opdal et al. 2019). With our treatments we estimated the impact light color changes, such as brownification due to cDOM, greening due to phytoplankton, bluish at deep-water layers, could have in such an ecosystem. This highlights that light spectrum shifts of brownification or greening scenarios may lead to phytoplankton community alterations and subsequently affect higher trophic levels.

Conclusion

While previous studies focused on light spectrum effects either based on monocultures or artificial mixtures of few phytoplankton species, we investigated the effect of light quality on a natural phytoplankton community and constrained the supplied light to the red, green, and blue part of the spectrum, yet with the same intensity. Thus, this study provides insight into spectral light quality effects on phytoplankton performance and community composition: while community growth rates and biomass were highest under blue, compared to red and green light, phytoplankton performance was not reduced if the full light spectrum was limited to the blue wavelength range. Even though spectral mismatches might have been tolerated or compensated by pigment plasticity of some species, our results indicated that, over the timespan of days to weeks, response of phytoplankton communities to different light quality treatments were mainly driven by species sorting. Thus, investigating the combined effect of light intensity and changes in light spectrum on phytoplankton is highly relevant for determining light effects on aquatic primary production under current and future scenarios.

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Conflict of Interest

None declared.

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