Wavelength-dependent effects of artificial light at night on phytoplankton growth and community structure

Christina Diamantopoulou [a,b], Eleni Christoforou [b,c], Davide M. Dominoni [c], Eirini Kaiserli [d], Jakub Czyzewski [e], Nosrat Mirzai [e], Sofie Spatharis [b,c*]

[a] University of Ioannina, Department of Biological Applications and Technology, 45110, Ioannina, Greece

[b] University of Glasgow, School of Life Sciences, Glasgow, G128QQ UK

[c] University of Glasgow, Institute of Biodiversity, Animal Health and Comparative Medicine, Glasgow, G128QQ UK

[d] University of Glasgow, Molecular Cell & Systems Biology, Glasgow, G128QQ UK

[e] University of Glasgow, College of Medical, Veterinary & Life Sciences (MVLS), Bioelectronics Unit, G128QQ UK

* Corresponding author.

E-mail address: sofie.spatharis@glasgow.ac.uk

Abstract

Artificial light at night (ALAN) is a disruptive form of pollution, impacting physiological and behavioural processes that may scale up to population and community levels. Evidence from terrestrial habitats show that the severity and type of impact depends on the wavelength and intensity of ALAN; however, research on marine organisms is still limited. Here we experimentally investigated the effect of different ALAN colours on marine primary producers. We tested the effect of green (525 nm), red (624 nm), and broad-spectrum white LED ALAN, compared to a dark control, on the green microalgae *Tetraselmis suesica* and a diatom assemblage. We show that green ALAN boosted chlorophyll production and abundance in *T. suesica*. All ALAN wavelengths affected assemblage biomass and diversity with red and green ALAN having the strongest effects, leading to higher overall abundance and selective dominance of specific diatom species, some known to cause Harmful Algal Blooms. Our findings show that green and red ALAN should be used with caution as alternative LED colours in coastal areas, where there might be a need to strike a balance between the strong effects of green and red light on marine primary producers with the benefit they appear to bring to other organisms.

Keywords: phytoplankton diversity, productivity, artificial light at night, light pollution, species composition, harmful algal blooms, *Tetraselmis suesica*, *Skeletonema* sp.

Introduction

During the last century the use of artificial light at night (ALAN) has increased considerably. Recent analyses have suggested that ALAN, which is strongly associated with the increasing worldwide urbanisation [1], is still currently spreading spatially at a rate between 2 and 6% per year [2,3], with a parallel increase in irradiance at 2.2 % per year [3]. The surge of ALAN has altered natural lightscapes, which in turn may have dramatic effects on wild species and ecosystems [4]. Indeed, the impact of ALAN on wildlife and ecosystems has received a lot of attention in the last two decades [5–8]. In vertebrates, ALAN has been linked to several behavioural and physiological effects, such as disruption of circadian rhythms [9], altered reproductive timing [7,10], poor sleep [11,12], reduced immune function [13] and altered metabolism [14]. Insects are also heavily affected [15–17], particularly because of the strong phototaxis found in many species [18,19].

Despite the surge of interest in the ecological effects of ALAN, most of the evidence collected so far comes from terrestrial habitats, while studies on marine populations and communities are currently limited [20–25]. Although a small number of studies have investigated the effects of monochromatic ALAN on cyanobacteria and microalgae, these focused on benthos and periphyton [2,26–30], while no study so far has investigated the effects of polychromatic LEDs on marine phytoplankton. Due to the continuous expansion of coastal urbanisation [31,32], artificial light at night is a source of pollution that is increasingly relevant for coastal ecosystems [4,21]. Coastal ecosystems globally are also increasingly affected by eutrophication and harmful algal blooms due to nutrient-rich inflows from either agricultural or urban sources [33,34]. Given the ecological importance of light for photoautotrophic phytoplankton species, the potential severity of ripple effects from phytoplankton to higher trophic levels [29], and the existing vulnerability of coastal systems to eutrophication, it is imperative to determine the type and magnitude of the response of marine primary producers to ALAN.

In primary producers, light is a strong modulator of photosynthesis and associated processes driving growth and cell fitness [35]. Light absorption by chlorophyll peaks at approximately 430 nm, although a second, lower peak is also present at longer wavelengths of 670 nm [27,36,37]. Moreover, light serves as an informational cue, regulating the synchronization of diverse intracellular processes ranging from phototactic, photoprotective and physiological responses essential for growth and development [38–40]. For example, green algae have an “eyespot” with which, through a rhodopsin mediated signalling pathway that is sensitive to green light, are able to direct their movement [41,42]. Therefore, disruption of light cycles by ALAN has the potential to impact organism’s physiology and consequently assemblage structure via multiple pathways that are responsive to different light wavelengths. This is a topical question because the spectral composition of ALAN is also changing along its surge in intensity, since many countries are replacing traditional lighting sources with the cost-efficient, energy-saving light-emitting diode (LED) technology [8]. LEDs are very flexible light sources whose colour can be easily modified. Indeed, new light installations use LEDs of different colours. While cool white LEDs (richer in blue/green wavelengths) are the most widespread, warm white (rich in yellow/orange/red wavelengths), green and red LEDs are also in use [8,21].

Recent findings have demonstrated that ALAN from warm white High Pressure Sodium (HPS) lamps can affect multiple signalling events and metabolic pathways essential for photosynthesis in freshwater cyanobacteria [43]. ALAN from cool white LEDs can increase the photosynthetic biomass of microphytobenthos [29] and its temporal variability [30], alter periphyton composition [44] and modify community structure of freshwater benthic microorganisms [26]. However, the aforementioned studies experimented with a single ALAN wavelength, while it is increasingly recognised that different wavelengths can cause profoundly different responses in wild organisms [45–51]. This can potentially lead to competing conservation goals [51]. Compared to the open ocean where short (blue) wavelengths propagate best, in coastal waters, green-yellow wavelengths, including those

produced by ALAN sources [52] are more dominant [53,54]. In coastal areas, red LED light has been recommended as a source of illumination because it doesn't interfere with sea turtle nesting and hatching [55], as well as with coral biology [56], whereas green light was suggested to minimise the impact of ALAN on seabird navigation [57]. To understand and therefore inform the ecological management of coastal areas, it is also essential to establish the effects of different wavelengths of ALAN on important aspects of microalgae assemblages, ranging from single species growth to community level properties such as diversity and species composition.

In the present study we investigate experimentally the response of marine phytoplankton to three ALAN wavelengths [white 4500K, green (525 nm) and red (624 nm)] compared to dark nights. Our first objective is to determine whether different wavelengths of ALAN can have different impact on the growth of a single phytoplankton species. Furthermore, we aim to assess whether different wavelengths can have different impacts on the diversity and species composition of a phytoplankton assemblage. We hypothesize that white ALAN might stimulate growth compared to the dark, as it partly overlaps in wavelength with the first absorption peak of chlorophyll-a, an abundant pigment in all microalgae, at approximately 465 nm [36]. Conversely, we predict that the green and red ALAN should have a weaker, if any, effect as its spectral properties have a minimal overlap with the light absorption range of chlorophyll-a (Figure S1). Finally, we predict that the effect on single species growth could cascade to the community level, as phytoplankton species' competitive ability has been shown to shift with water colour [36].

Materials and methods

Experimental set up and light sources

To test the effect of ALAN wavelengths on single species growth and assemblage biomass and diversity we run two concurrent experiments from 23/11/2020 and for a period of 18 days:

Experiment 1 tested the effects of ALAN on the green microalgae *Tetraselmis suesica* and experiment 2 on a natural coastal assemblage dominated by diatom species. The experimental design comprised of four treatments: control (12:12 Light-Dark), and three different ALAN wavelengths, green, red and white (12:12 Light-ALAN). Each of the two experiments comprised of five replicated cultures within each of the four treatments for a total of 40 experimental units (Erlenmeyer flasks of 200ml each).

Since algae use light as a source of both information and energy [58,59], light treatments were standardised to levels of irradiance (i.e. energy content) rather than illuminance (i.e. luminous flux incident on a surface). Daytime light irradiance was 6.5 Watt m^{-2} and was provided by a 10W flood light (Prolite, Ritelite Systems Ltd, UK) equipped with two arrays of high-power LEDs (6,000K). Each ALAN source consisted of a strip of 3 LED diodes. The green ALAN wavelength was 525 nm (MULTICOMP), the red was 624 nm (MULTICOMP) and the broad-spectrum white LED light contained a higher peak at 470 nm and lower peaks between 550-600 nm (BROADCOM) (for full spectral characteristics of LED lights see Fig. S1). The emission spectra were measured by a spectrometer (AvaSpec-2048L, Avantes, Apeldoorn, The Netherlands). Night-time light irradiance was measured with a LI-200R pyranometer (LI-COR, USA) and was standardised at 0.023 Watt m^{-2} for all three ALAN treatments. This irradiance level is within the range of values reported in previous studies on ALAN [26,28,60,61]. With respect to illuminance, our standardised irradiance level corresponds to 8.51 lux for the white light, to 3.4 lux for the red light and 12.81 lux for the green light. These values are ecologically relevant and within plausible ranges of ALAN observed in near shore epifaunal invertebrate assemblages (0.005-21.6 lux) [23,62,63].

The distance between the surface of the water in the flasks and the LED lights was approximately 40 cm. Each light treatment was applied inside a light-proof box (55x62x62cm), where the experimental replicates were introduced (see below for details on how these were produced in each experiment). The replicates were partially submerged (by 1/3 of the flask height) into water

baths (44x41x22cm). Light irradiance was measured at mid flask height at six different locations within each water bath and was not statistically different between the ALAN treatments both during the day (linear mixed model, $\chi = 3.9$, $p = 0.41$) and during the night (linear mixed model, $\chi = 4.1$, $p = 0.13$). Temperature in the water baths was maintained at a temperature of 14-15°C chosen to reflect the mean annual sea surface temperature of mid-latitude seas. To minimise box effects, water in the baths had identical temperature as it was fed from a central tank where temperature was regulated. Cultures were mixed once a day when their position inside each treatment box was also randomised.

Experimental procedure for experiment 1: single species response

The green microalgae *T. suesica* was selected for the single species response experiment because of its use as a model species in studies using continuous illumination with monochromatic LEDs [64–66], because of the industrial potential of the species as a high-lipid content strain [67], and its importance as fish and shellfish aquaculture feed [68].

Inoculum from our *T. suesica* culture (sourced by CCAP 66/4) was grown in F/2 medium (Guillard 1975) made by ultrapure artificial seawater at 35ppm salinity (V= 200mL). All cultures were initiated at a concentration of 5,000 cells/mL. Every second day, 5ml samples were taken from each replicated culture, two hours after the onset of day light in the morning, to calculate cell numbers and growth rate. Cells were counted using Fast-Read® 102 counting chambers under a light microscope. *T. suesica* growth showed a lag phase of 8 days due to the acclimation of cells from 20°C to 15°C and thereafter growth entered the exponential phase. Maximum growth rate for each replicate culture was determined based on the formula $\mu = \ln(N_2/N_1)/(t_2 - t_1)$, where μ is the specific growth rate, and N1 and N2 are the cell number at time 1 (day 8) and time 2 (day 18), respectively. At day 18 of the experiment, 50ml samples were also taken to determine chlorophyll-a concentration according to Parsons et al. (1984).

Experimental procedure for experiment 2: diatom assemblage response

We used a natural diatom-dominated marine sample in our assemblage response experiment. Specifically, we assorted at equal volume (200ml) across the 20 experimental replicates, an inoculum of unfiltered marine surface water collected at 50cm depth from the shore of Largs, Scotland (55.794659, -4867615) on 22/11/2020, 11:00 am. The initial inoculum had a chlorophyll-a concentration of 0.7 µg/L and salinity 30 psu which is lower than the salinity of the open sea as the area receives freshwater inflows.

The culture medium consisted of the collected marine sample and added nutrients commensurate with F/2 medium concentration [70]. Species identities, cell counts and chlorophyll concentration were determined on day 12 of the experiment when cultures just entered the stationary phase as determined by the cell counts of selected replicates. Specifically, a 5 ml sample was collected for species identification and was preserved with Lugol's iodine solution. Samples were subsequently filtered through a SartoriusTM Cellulose Nitrate Membrane Filters (0.45µm pore size, 25mm diameter) and dried in an incubator at 40^{o}C for an hour. The filter was made transparent by the addition of a drop of immersion oil and was observed under a light microscope (40x/0.65) where 15 randomly-selected fields of view were used to identify and enumerate the different species. The volume of sample examined was equal across all samples thus species' cell counts as well as total assemblage cell counts are directly comparable across replicates and reported as counts. Chlorophyll concentration was determined from 50 ml samples as in the case of the *T. suesica* experiment.

Data analysis

For the *T. suesica* single species response, we used three Gaussian linear models to determine the effect of ALAN treatment (4 levels: green, red and white ALAN and the dark control) on each of three

response variables: the growth rate, and the cell number and chlorophyll-a measured on the final day of the experiment (day 18).

For the diatom assemblage response, we used four linear models to test the effect of ALAN treatment on each of four response variables: assemblage total cell count, chlorophyll-a, Menhinick richness [71] and Pielou's evenness [72]. The Menhinick species richness index, is defined as the number of species in the sample divided by the square root of the total abundance of individuals in the sample and was used to enable standardisation of species richness across samples based on the total cell abundance. Pielou's evenness index is defined as the Shannon diversity divided by the maximum possible value of Shannon (if all species had equal abundance in the sample) and was used to provide a measure of dominance in cell counts by specific species in a sample. The Menhinick and Evenness indices were sensitive in expressing changes in phytoplankton diversity in previous studies comparing multiple diversity indices using phytoplankton species abundance data [73]. Assemblage total cell count, chlorophyll-a and Menhinick richness were modelled with Gaussian models. Evenness was modelled with a beta distribution model as its values were confined between 0 and 1 and the effect of treatment was tested using likelihood ratio test (LRT) between the null model (not containing treatment) and the full model (which contained the factor treatment).

To test the effect of ALAN treatment on assemblage composition, we performed analysis of similarity between all pairwise combinations of the 20 replicates using the Bray-Curtis similarity index [74] on non-transformed species-abundance data. We visualised these similarities using cluster analysis to check the grouping of samples based on the different treatments. We fitted additional linear models to test for the effect of treatment on the abundance of specific diatom species. Finally, the percentage changes we report in the first paragraph of the discussion eg for the cell number were calculated according the formula: [(Cell number of ALAN treatment - Cell number of dark control)/ Cell number of dark control]*100.

Model selection was carried out based on the least squares approach apart for the evenness model where we used the LRT test. We also conducted post-hoc pair-wise t-tests to assess differences between the four treatment levels. All statistical analyses was carried out in R v.3.5.0 (RStudio Team, 2016). The packages ggplot2 v.3.3.0 [75], ggpubr v.0.2.5 [76], ggdendro v0.1-20 [77] and dendextend v.1.13.4 [78] were employed for plot generation and data visualisation. The package emmeans was used for pairwise comparisons between treatment levels [79]. For data manipulation, reshape2 v.1.4.3 [80] , plyr v.1.8.6 [81]. For modelling the beta distribution, we used the function glmmTMB and family function beta_family(link = "logit") in the package glmmTMB [82]. We used the *vegan* v.2.5-6 R [83] and cluster v.2.1.0 packages [84] to perform the pairwise similarity of species-abundance data and related cluster analysis.

Results

Green and red ALAN promote growth of the green microalgae Tetraselmis suesica.

The ALAN treatments had a statistically significant effect on the growth rate of *T. suesica* cells ($F_{3,16}$ = 6.64, p =0.004) and this was shown to be wavelength specific. Specifically, a significantly higher growth rate was observed under the green ALAN treatment compared to white ALAN and the dark treatment. Furthermore, the red ALAN was also higher than the white ALAN treatment (Fig. 1A and Supplementary Table S1).

The *T. suesica* cell concentration was significantly affected by the ALAN treatment ($F_{3,16}$ = 7.691, p<0.002). Specifically, on day 18 of the experiment, the cell number was significantly higher in response to the green ALAN treatment compared to the white ALAN and dark treatments and was also higher in the red ALAN compared to white. No difference was observed between the white and dark treatments (Fig. 1B and Supplementary Table S1). These results are comparable to those obtained in a pilot experiment where LED colours were allocated to different experimental boxes and

light intensity was standardised at 20 lux (for details of this pilot experiment and relative results, see Supplementary Fig. S2).

The chlorophyll-a concentration of *Tetraselmis* cultures on day 18 was also significantly affected by the ALAN wavelength ($F_{3,16}$ = 20.584, p<0.001). Specifically, chlorophyll-a content was significantly higher in the red and green ALAN treatments compared to the dark and white treatments, whereas no difference was observed between the white and dark treatments (Fig. 1C and Supplementary Table S1).

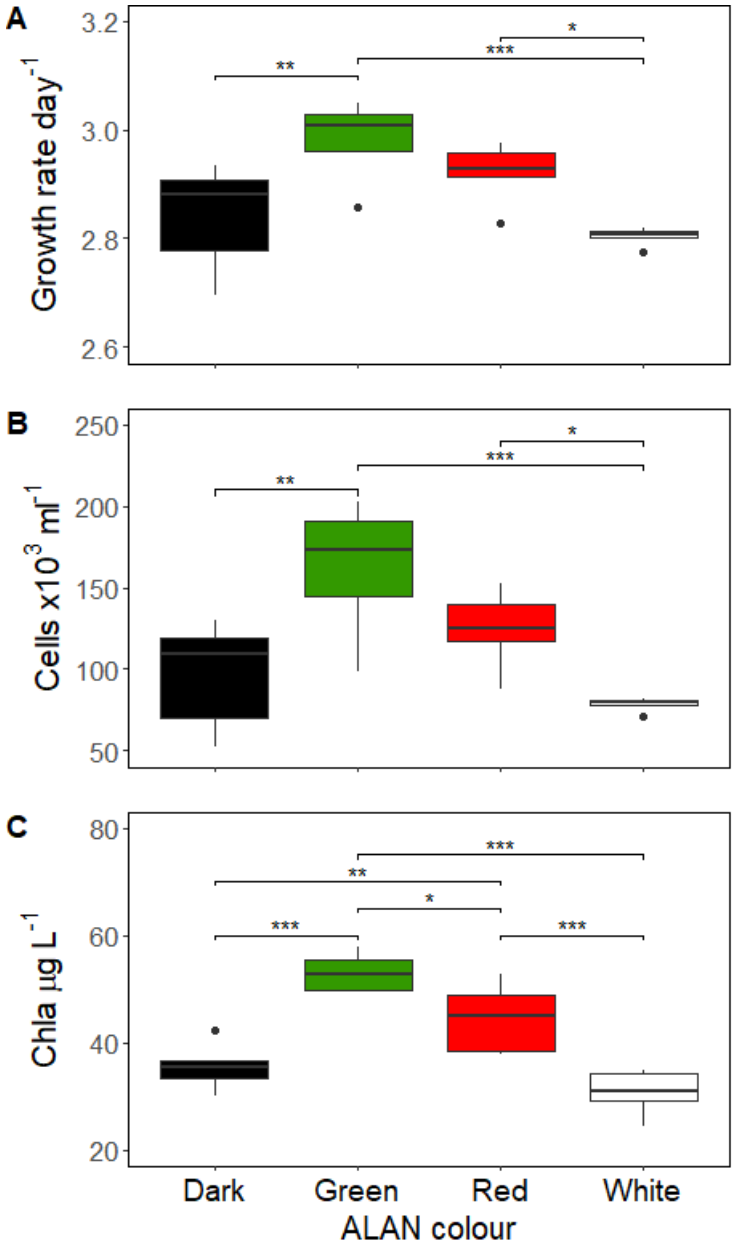


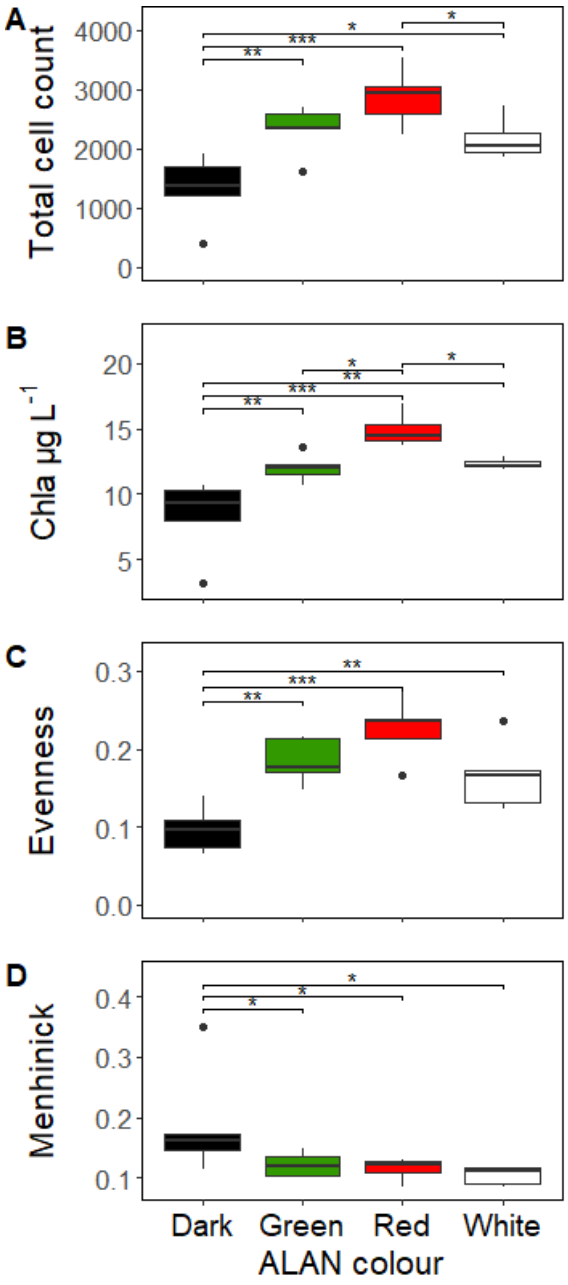
Figure 1. **ALAN affects growth rate, cell and chlorophyll-a concentration of *Tetraselmis suesica* in a colour-dependent manner.** Effect of ALAN treatments (dark, green, red and white) on the growth rate calculated during the exponential growth phase (days 8-18) (panel A), on the cell concentration at day 18 (panel B) and chlorophyll-a concentration at day 18 (panel C). Pairwise comparisons show differences between treatments (not shown: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001).

ALAN affects assemblage biomass and diversity

Analysis of the initial inoculum upon collection from the sea showed that the assemblage was comprised of 14 species of which 11 species were Diatomophyceae, two were Dinophyceae and one was Dictyochophyceae. Most dominant species were *Skeletonema* sp. (28% dominance), *Cyclotella* sp.1 (19% dominance), *Cyclotella* sp.2 (17% dominance), *Ceratium lineatum* (15% dominance) and *Navicula* sp.1 (6% dominance) whereas all other species were subdominant with relative abundance <2%. On day 12 of the experiment, overall biomass had considerably increased and stabilised across treatments and assemblages. Experimental units on day 12 comprised of 4-7 species of diatoms (13 species overall across all treatments). The planktic colonial diatoms *Skeletonema* sp., *Thalassiosira nordenskioeldii*, and to a lesser extend *T. eccentrica* were more dominant across treatments. However, the absolute and relative abundance of *Skeletonema* sp., *T. nordenskioeldii* presented differences between treatments as discussed below.

ALAN affected assemblage cell counts and diversity independent of colour whereas chlorophyll-a concentration was affected in a wavelength specific manner. Specifically, the total diatom assemblage cell count (i.e., cells summed across all species in the assemblage) was significantly affected by every ALAN treatment tested ($F_{3,16}$=9.589, p< 0.001). Cell count was statistically higher under all ALAN wavelength conditions compared to the dark but no differences were observed between the ALAN wavelengths (Fig. 2A and Supplementary Table S2). The

chlorophyll-a concentration of the diatom assemblage was also significantly affected by the variable treatment ($F_{3,16}$=12.393, p< 0.001), with all ALAN wavelengths having a higher concentration compared to the dark control whereas the red wavelength was also higher from the green and white (Fig. 2B and Supplementary Table S2). A significant effect of treatment was also observed on the assemblage evenness (LRT, DF=3, p<0.001), whereby the assemblages under all ALAN wavelengths had significantly higher evenness (more evenly distributed species' populations) compared to the dark control (Fig. 2C and Supplementary Table 2 for results of post-hoc tests). A significant effect of ALAN was observed on the Menhinick richness ($F_{3,16}$=3.260, p=0.049), with the dark treatment showing significantly higher richness compared to all ALAN wavelengths tested (Fig. 2D and Supplementary Table S2).



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277 **Figure 2. ALAN affects assemblage biomass and diversity.** Effect of treatment (dark, green, red and

278 white) on the total cell counts (panel A), chlorophyll-a (panel B), evenness (panel C) and Menhinick

279 richness (panel D) measured on day 12 of the diatom assemblage experiment. Pairwise comparisons

280 show differences between treatments (not shown: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <=

281 0.001).

282

283 ***ALAN affects species' relative abundances***

ALAN did not lead to a shift in the species' identities comprising the assemblages. However, ALAN affected the absolute and relative abundance of species (measured as standardised cell counts) within treatments in a wavelength specific manner. In particular, assemblages growing under the green and red ALAN were 80% similar and were 44% dissimilar from the assemblages under the dark control and white ALAN conditions, respectively, with the exception of two replicates which grouped with the dark/white cluster mainly due to lower numbers in *Skeletonema* sp. (Fig. 3A). This was due to a significant increase in the species *Skeletonema* sp. and *T. nordenskioeldii* relative to the subdominant species in the assemblage (i.e. all species excluding *Skeletonema* sp., *T. nordenskioeldii* and *T. eccentrica*). Specifically, *Skeletonema* sp. had a significantly higher abundance in all ALAN colours compared to the control ($F_{3,16}$=7.708, p=0.002) (Fig. 3B). *T. nordenskioeldii* was significantly higher in response to the red ALAN treatment compared to the white and the dark control ($F_{3,16}$=8.8574, p=0.001) (Fig. 3C). No differences between the treatments were observed in the abundance of the subdominant species ($F_{3,16}$=0.833, p=0.495) (Fig. 3D). These differences in relative abundances between dominant and subdominant species (Fig. 3B,C,D) suggest that the increased evenness in ALAN treatments compared to the dark control (Fig. 2C) was likely due to increased evenness of the dominant species in the assemblage (Fig. 3B,C) rather than an increase in evenness across dominant and subdominant species.

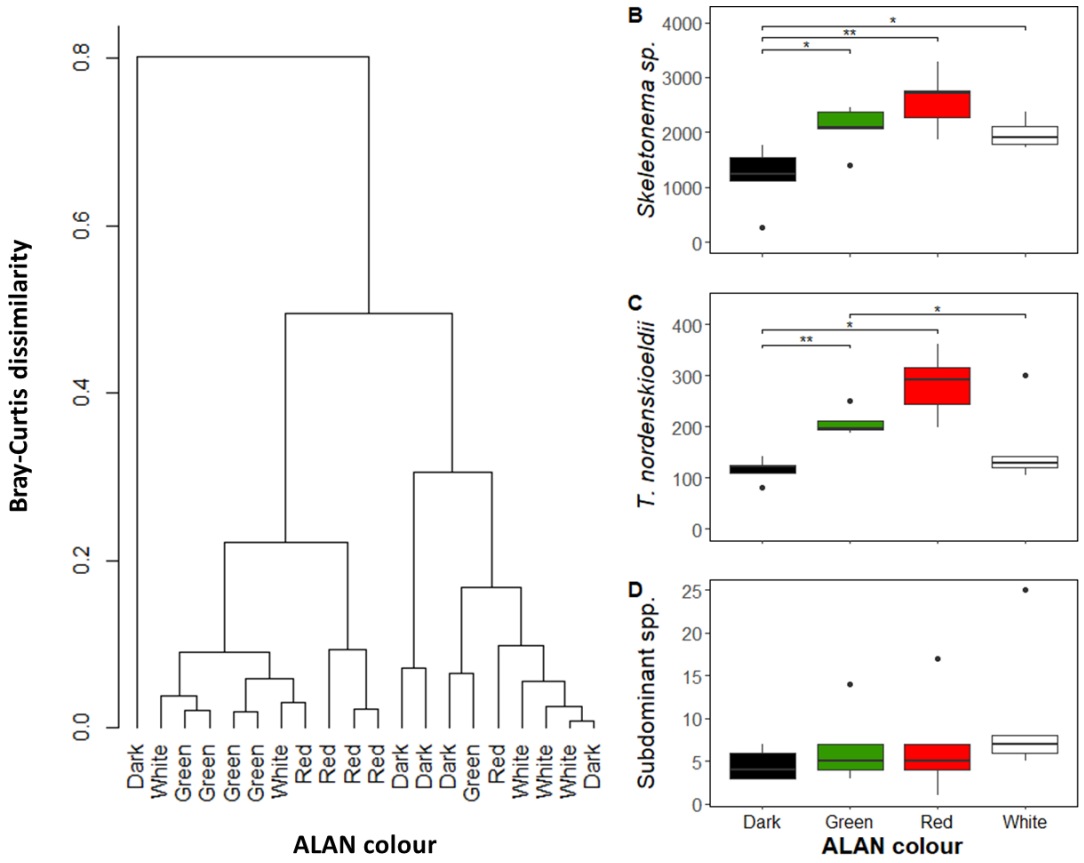

Figure 3. Red and green ALAN lead to similar responses in assemblage composition. Cluster showing the pairwise similarities between the replicate assemblages based on the Bray-Curtis similarity index calculated on non-transformed species-abundance data (panel A). Pairwise comparisons of abundances of *Skeletonema* sp. (panel B)*, Thalassiosira nordenskioeldii* (panel C), the sum of all subdominant species in the assemblage (panel D) (not shown: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001).

Discussion

In this study, we tested the effect of different ALAN wavelengths on phytoplankton growth, assemblage diversity and species composition. We predicted that the effect on single species and assemblage level would be more pronounced under white ALAN, compared to dark and red and green ALAN, as it partly overlaps in wavelength with the first absorption peak of chlorophyll-a, an

abundant pigment in all microalgae [36]. Contrary to our expectations, our findings suggest that red and green ALAN have more pervasive impact on phytoplankton growth and assemblage structure compared to the white ALAN. More specifically, our experiments showed that exposure of the green microalgae *Tetraselmis suesica* to green ALAN led to a 5% increase in growth rate, 67% in cell number and 49% in chlorophyll-a concentration, compared to the dark night condition. Exposure to red ALAN led to a similar response to the green ALAN, as it resulted in higher chlorophyll-a, but it did not affect growth rate and total cell numbers compared to the dark treatment. Red and green ALAN treatments also affected the diatom-dominated phytoplankton assemblage. For example, red ALAN led to higher total cell count and chlorophyll-a concentration by 118% and 80% respectively compared to the dark control. More interestingly, red and green ALAN led to a similar assemblage response by balancing the biomass of the most abundant species (thus leading to higher evenness), but also by enhancing the biomass of the most abundant species relative to the subdominant species. These effects were less pronounced in response to the white ALAN treatment although it had a significant impact on assemblage richness.

Previous studies on the effect of white ALAN on freshwater primary producers have reported longer-term (6 week experiments) increases in the abundance of benthic microalgae [26] but also shorter-term (3 weeks) decreases in periphyton abundance, as well as community composition shifts [27]. Our experimental findings provide additional insights into ALAN effects by offering comparative information on different LED colours. Our findings show that although exposure to white ALAN can lead to changes in diatom assemblage diversity, species' relative abundances and biomass increase within 12 days of exposure, this effect was less pronounced compared to the red and green LED, and our white ALAN treatment had no effect on the growth rate of the green microalgae *Tetraselmis*.

Our findings also suggest that the red and green ALAN colours have the potential to enhance the growth of Harmful Algal Bloom (HAB) species such as the diatom *Skeletonema* sp.. This species is

commonly known for forming dense blooms causing mortality to other organisms through physical damage (e.g. fish gill lesions) or anoxia, consequently impacting trophic interactions, biodiversity and overall ecosystem health [85]. This finding is supported by Oh et al. (2008) who showed that green LEDs can selectively stimulate the growth of the diatom *Skeletonema costatum* compared to other species in the assemblage. In addition, the biomass of planktic colonial diatoms such as *Skeletonema* and *Thalassiosira* was enhanced under red and green ALAN compared to epiphytic (i.e., growing on macroalgae and rocks) and epipsamic (i.e., growing on sand) diatom genera. Given that maximal transmission of light in coastal systems is around 550 nm (green/yellow) [53] and that coastal seafloors are susceptible to ALAN, particularly within the green range (495–560) [52], we could anticipate impacts of ALAN on phytoplankton biomass and assemblage structure in coastal ecosystems.

A key question is why green and to some extent also red ALAN had a stronger effect on the growth and photosynthetic biomass of *Tetraselmis* compared to white light. A first insight stems from comparisons with previous studies that focused on maximizing the growth and biochemical composition of *Tetraselmis* to fully exploit the industrial potential of this algae. Unlike our 12:12 light:dark period, these studies used continuous (24 h) high intensity LED illumination of different monochromatic LEDs [64–66]. Abiusi et al. (2014) reported maximum growth and biomass concentration under red and white continuous light, whereas these traits were less pronounced under green light (all light conditions were standardised at 160 µmol m^{-2} s^{-1}). Aidar et al. (1994) also reported increased growth under continuous red and white light compared to the blue-green light (all light conditions were standardised at 25 µmol m^{-2} s^{-1}). These results contrast with the higher growth rate under green, dim night-time illumination found in our experiment. This discrepancy raises the question of whether dim artificial light at night has the potential to induce different responses to LED wavelengths compared to higher intensity light at night.

Stimulated growth and photosynthetic activity in our green algae monoculture and diatom assemblage under the red light could be explained by the partial overlap of chlorophyll a absorption spectra with the red emission range [87] (see also Fig. S1). Absorption of other photosynthetic pigments present in diatoms (chlorophyll c, β-carotene, Zeaxhanthin, Diatoxanthin, Diadinoxanthin, Fucoxanthin) and green algae (chlorophyll b, β-carotene, Zeaxhanthin, Violaxanthin, Neoxanthin, Loroxanthin) [66] typically peak in the 420-480 nm range [see datasets from 78] and although they partially overlap with our green ALAN spectrum this does not justify why higher growth and biomass was not also observed also in the white ALAN treatment with which they overlap significantly more (see Fig. S1 for our ALAN light spectra).

Although pigments such as chlorophylls within chloroplasts absorb light energy to fix inorganic CO_2 towards biomass production and growth, other photopigments act as photoreceptors and are involved in functions that regulate circadian clocks and phototaxis [54,59]. Some of these, such as phytochromes, are sensitive to specific wavelengths of light. One conceivable hypothesis to explain increased growth under red and green, but not white ALAN, is that the white ALAN has disrupted the natural photocycle of *Tetraselmis* sp., with downstream consequences on photosynthesis, cell division and growth [59,88,89]. This hypothesis could be tested by simultaneously monitoring chlorophyll, growth and clock gene expression under different ALAN colours.

Nevertheless, the strong effects of green ALAN on abundance and photosynthetic biomass seen in our study and are still puzzling considering that a green light receptor has never been found and that cell division is typically stimulated by blue light. A photoreceptor that may have played a role in our study could be rhodopsin, which is sensitive to light in the mid-range of the visible spectrum, peaking at ~ 500 nm. Rhodopsins are known from all algae groups and are associated with phototactic responses [54,90]. Although the activity of such photoreceptors can benefit microalgae

386 growth in the marine environment where light is variable and often limiting, it is unclear how this
387 mechanism led to growth stimulation in our controlled experiment where light conditions were more
388 homogeneous. Nevertheless, this rhodopsin-mediated effect cannot be precluded since Tetraselmis
389 is a flagellated microalgae and capable of movement to more optimal positions for capturing light
390 (eg water surface in flaks). This would merit further testing with appropriate experiments that would
391 track phototaxis in flagellated algae. Finally, although cryptochromes are the primary receptors of
392 UV-A and blue light, it has been reported that green light affects cryptochrome photochemistry and
393 activity as green light reverts cryptochromes to their inactive state [54,91]. In particular,
394 cryptochromes integrate green light signals into the circadian system as well as modulating plant
395 growth and architecture in response to an increase in green/blue light ratio under a canopy [92–94].
396 Our data show that there is a significant impact of green and red ALAN on phytoplankton that
397 should be taken into account when planning nocturnal illumination in marine environments. In fact,
398 these results may lead to conservation dilemmas, as both red and green LED lights have been
399 suggested as alternative ALAN sources for public illumination. Specifically, red light illumination has
400 been recommended in coastal areas because it interferes less with sea turtle nesting and hatching
401 compared to broad-spectrum white light [55]. Similarly, the use of green light has been
402 recommended to minimise the impact of light pollution on migratory birds [57]. In general, shifting
403 spectral signatures towards longer wavelengths than blue light seems to be less harmful to many
404 organisms, including insects [15], bats [95] and songbirds [96]. However, our study shows that the
405 use of green and red ALAN LEDs can impact aquatic primary producers by enhancing the growth of
406 different taxonomic groups (green algae and diatoms) indicating a potential to encourage
407 eutrophication phenomena in marine coastal (but potentially also freshwater) systems where these
408 taxonomic groups are also present. Although the batch culture set-up used in our study is more
409 representative of coastal systems affected by pulsed nutrient inputs [97], it would be interesting to

410 also simulate systems that show less pronounced fluctuations using continuous or semi-continuous
411 nutrient supply setups.

412 The equivalent illuminance to the standardised irradiance used in our experiment ranged
413 between 3.4 and 12.8 lux (depending on the colour). This is within the range of illuminance measured
414 in coastal systems near ALAN affected areas by previous studies (0.005-21.6 lux) [23,62,63]. We thus
415 conclude that effects on marine microalgae can be expected in coastal ecosystems and particularly
416 in the proximity to shoreline illuminations, heavily urbanised environments and ports. In addition,
417 given that both green algae and diatoms are also found in freshwater systems, we anticipate that our
418 results may be relevant also for slowly moving riverine systems or lake systems also affected by ALAN.

419

420 Our pre-print: [98]

421

422 ## Acknowledgements

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427 ## Author contributions

428 CD, EC, DMD and SS designed the study. JC and NM designed the light system. CD, EC, DMD and SS
429 performed the experiment. CD, EC and SS performed the lab analyses. SS and EC performed the
430 statistical analyses. EK contributed to the interpretation of the results. CD, DMD and SS wrote the
431 paper. All other authors read and commented on multiple drafts of the manuscripts, and all authors
432 approved the final submitted version.

References

1. Seto KC, Güneralp B, Hutyra LR. 2012 Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 16083–16088. (doi:10.1073/pnas.1211658109)
2. Hölker F *et al.* 2010 The dark side of light: A transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* **15**, 13. (doi:10.5751/ES-03685-150413)
3. Kyba CCM *et al.* 2017 Artificially lit surface of Earth at night increasing in radiance and extent. *Sci. Adv.* **3**, 1–9. (doi:10.1126/sciadv.1701528)
4. Zapata MJ, Sullivan SMP, Gray SM. 2019 Artificial Lighting at Night in Estuaries—Implications from Individuals to Ecosystems. *Estuaries and Coasts*. **42**, 309–330. (doi:10.1007/s12237-018-0479-3)
5. Navara KJ, Nelson RJ. 2007 The dark side of light at night: Physiological, epidemiological, and ecological consequences. *J. Pineal Res.* **43**, 215–224. (doi:10.1111/j.1600-079X.2007.00473.x)
6. Longcore T, Rich C. 2004 Ecological light pollution. *Front. Ecol. Environ.* **2**, 191–198. (doi:10.1890/1540-9295(2004)002[0191:ELP]2.0.CO;2)
7. Dominoni DM, de Jong M, Bellingham M, O'Shaughnessy P, van Oers K, Robinson J, Smith B, Visser ME, Helm B. 2018 Dose-response effects of light at night on the reproductive physiology of great tits (Parus major): Integrating morphological analyses with candidate gene expression. *J. Exp. Zool. Part A Ecol. Integr. Physiol.* **329**, 473–487. (doi:10.1002/jez.2214)
8. Gaston KJ, Davies TW, Bennie J, Hopkins J. 2012 Reducing the ecological consequences of night-time light pollution: Options and developments. *J. Appl. Ecol.* **49**, 1256–1266. (doi:10.1111/j.1365-2664.2012.02212.x)
9. Gaston KJ, Davies TW, Nedelec SL, Holt LA. 2017 Impacts of artificial light at night on biological timings. *Annu. Rev. Ecol. Evol. Syst.* **48**, 49–68. (doi:10.1146/annurev-ecolsys-110316-022745)
10. Robert KA, Lesku JA, Partecke J, Chambers B. 2015 Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. *Proc. R. Soc. B Biol. Sci.* **282**. (doi:10.1098/rspb.2015.1745)
11. Raap T, Pinxten R, Eens M. 2015 Light pollution disrupts sleep in free-living animals. *Sci. Rep.* **5**, 1–8. (doi:10.1038/srep13557)
12. Aulsebrook AE, Connelly F, Johnsson RD, Jones TM, Mulder RA, Hall ML, Vyssotski AL, Lesku JA. 2020 White and amber light at night disrupt sleep physiology in birds. *Curr. Biol.* **30**, 3657-3663.e5. (doi:10.1016/j.cub.2020.06.085)
13. Kernbach ME *et al.* 2019 Light pollution increases West Nile virus competence of a ubiquitous passerine reservoir species. *Proc. R. Soc. B Biol. Sci.* **286**. (doi:10.1098/rspb.2019.1051)
14. Pulgar J *et al.* 2019 Endogenous cycles, activity patterns and energy expenditure of an intertidal fish is modified by artificial light pollution at night (ALAN). *Environ. Pollut.* **244**, 361–366. (doi:10.1016/j.envpol.2018.10.063)
15. Owens ACS, Cochard P, Durrant J, Farnworth B, Perkin EK, Seymoure B. 2020 Light pollution is a driver of insect declines. *Biol. Conserv.* **241**, 108259. (doi:10.1016/j.biocon.2019.108259)
16. Knop E, Zoller L, Ryser R, Gerpe C, Hörler M, Fontaine C. 2017 Artificial light at night as a new threat to pollination. *Nature* **548**, 206–209. (doi:10.1038/nature23288)
17. van Grunsven RHA, van Deijk JR, Donners M, Berendse F, Visser ME, Veenendaal E, Spoelstra K. 2020 Experimental light at night has a negative long-term impact on macro-moth populations. *Curr. Biol.* **30**, R694–R695. (doi:10.1016/j.cub.2020.04.083)
18. van Langevelde F *et al.* 2018 Declines in moth populations stress the need for conserving dark nights. *Glob. Chang. Biol.* **24**, 925–932. (doi:doi:10.1111/gcb.14008)
19. Altermatt F, Ebert D. 2016 Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. *Biol. Lett.* **12**, 20160111. (doi:10.1098/rsbl.2016.0111)
20. Witherington BE, Bjorndal KA. 1991 Influences of artificial lighting on the seaward orientation of hatchling loggerhead turtles Caretta caretta. *Biol. Conserv.* **55**, 139–149. (doi:10.1016/0006-

3207(91)90053-C)
21. Davies TW, Duffy JP, Bennie J, Gaston KJ. 2014 The nature, extent, and ecological implications of marine light pollution. *Front. Ecol. Environ.* **12**, 347–355. (doi:10.1890/130281)
22. Fobert EK, Da Silva KB, Swearer SE. 2019 Artificial light at night causes reproductive failure in clownfish. *Biol. Lett.* **15**. (doi:10.1098/rsbl.2019.0272)
23. Garratt MJ, Jenkins SR, Davies TW. 2019 Mapping the consequences of artificial light at night for intertidal ecosystems. *Sci. Total Environ.* **691**, 760–768. (doi:10.1016/j.scitotenv.2019.07.156)
24. Bolton D, Mayer-Pinto M, Clark GF, Dafforn KA, Brassil WA, Becker A, Johnston EL. 2017 Coastal urban lighting has ecological consequences for multiple trophic levels under the sea. *Sci. Total Environ.* **576**, 1–9. (doi:10.1016/j.scitotenv.2016.10.037)
25. Berge J *et al.* 2020 Artificial light during the polar night disrupts Arctic fish and zooplankton behaviour down to 200 m depth. *Commun. Biol.* **3**, 102. (doi:10.1038/s42003-020-0807-6)
26. Hölker F, Wurzbacher C, Weißenborn C, Monaghan MT, Holzhauer SIJ, Premke K. 2015 Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. *Philos. Trans. R. Soc. B Biol. Sci.* **370**. (doi:10.1098/rstb.2014.0130)
27. Grubisic M *et al.* 2017 Artificial light at night decreases biomass and alters community composition of benthic primary producers in a sub-alpine stream. *Limnol. Oceanogr.* **62**, 2799–2810. (doi:10.1002/lno.10607)
28. Grubisic M, van Grunsven RHA, Manfrin A, Monaghan MT, Hölker F. 2018 A transition to white LED increases ecological impacts of nocturnal illumination on aquatic primary producers in a lowland agricultural drainage ditch. *Environ. Pollut.* **240**, 630–638. (doi:10.1016/j.envpol.2018.04.146)
29. Maggi E, Benedetti-Cecchi L. 2018 Trophic compensation stabilizes marine primary producers exposed to artificial light at night. *Mar. Ecol. Prog. Ser.* **606**, 1–5. (doi:10.3354/meps12769)
30. Maggi E, Bertocci I, Benedetti-Cecchi L. 2020 Light pollution enhances temporal variability of photosynthetic activity in mature and developing biofilm. *Hydrobiologia* **847**, 1793–1802. (doi:10.1007/s10750-019-04102-2)
31. Henderson CJ *et al.* 2020 Landscape transformation alters functional diversity in coastal seascapes. *Ecography (Cop.).* **43**, 138–148. (doi:10.1111/ecog.04504)
32. Yi L, Qian J, Kobuliev M, Han P, Li J. 2020 Dynamic evaluation of the impact of human interference during rapid urbanisation of coastal zones: A case study of shenzhen. *Sustain.* **12**, 2254. (doi:10.3390/su12062254)
33. Spatharis S, Danielidis DB, Tsirtsis G. 2007 Recurrent Pseudo-nitzschia calliantha (Bacillariophyceae) and Alexandrium insuetum (Dinophyceae) winter blooms induced by agricultural runoff. *Harmful Algae* **6**, 811–822. (doi:10.1016/j.hal.2007.04.006)
34. Justic D, Rabalais N, Turner RE, Dıaz RJ. 2009 Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.* **66**, 1528–1537.
35. Edwards KF, Thomas MK, Klausmeier CA, Litchman E. 2015 Light and growth in marine phytoplankton: Allometric, taxonomic, and environmental variation. *Limnol. Oceanogr.* **60**, 540–552. (doi:10.1002/lno.10033)
36. Luimstra VM, Verspagen JMH, Xu T, Schuurmans JM, Huisman J. 2020 Changes in water color shift competition between phytoplankton species with contrasting light-harvesting strategies. *Ecology* **101**, 1–16. (doi:10.1002/ecy.2951)
37. Lohrenz SE, Weidemann AD, Tuel M. 2003 Phytoplankton spectral absorption as influenced by community size structure and pigment composition. *J. Plankton Res.* **25**, 35–61. (doi:10.1093/plankt/25.1.35)
38. Serrano-bueno G, Romero-campero FJ, Lucas-reina E, Romero JM, Valverde F. 2017 Evolution of photoperiod sensing in plants and algae. *Curr. Opin. Plant Biol.* **37**, 10–17. (doi:10.1016/j.pbi.2017.03.007)
39. Duanmu D. 2017 Algal light sensing and photoacclimation in aquatic environments. , 2558–2570. (doi:10.1111/pce.12943)
40. Wobbe L, Bassi R, Kruse O. 2016 Multi-level light capture control in plants and green algae. *Trends*

Plant Sci. **21**, 55–68. (doi:10.1016/j.tplants.2015.10.004)
41. Hartmann Harz CN, Hegemann P. 1992 The photoreceptor current of the green alga Chlamydomonas. *Philos. Trans. R. Soc. B Biol. Sci.* **338**, 39–52.
42. Sineshchekov OA, Govorunova EG. 1999 Green flagellated algae. *Trends Plant Sci.* **4**, 58–63.
43. Poulin C, Bruyant F, Laprise MH, Cockshutt AM, Marie-Rose Vandenhecke J, Huot Y. 2014 The impact of light pollution on diel changes in the photophysiology of Microcystis aeruginosa. *J. Plankton Res.* **36**, 286–291. (doi:10.1093/plankt/fbt088)
44. Grubisic M. 2018 Waters under artificial lights: does light pollution matter for aquatic primary producers? *Limnol. Oceanogr. Bull.* **27**, 76–81. (doi:10.1002/lob.10254)
45. Gaston KJ, Bennie J, Davies TW, Hopkins J. 2013 The ecological impacts of nighttime light pollution: A mechanistic appraisal. *Biol. Rev.* **88**, 912–927. (doi:10.1111/brv.12036)
46. Dominoni DM *et al.* 2020 Why conservation biology can benefit from sensory ecology. *Nat. Ecol. Evol.* **4**, 502–511. (doi:10.1038/s41559-020-1135-4)
47. Ulgezen Z, Käpylä T, Meerlo P, Spoelstra K, Visser M, Dominoni D. 2019 The preference and costs of sleeping under light at night in forest and urban great tits. *Proc. R. Soc. B Biol. Sci.* **286**, 20190872. (doi:10.1098/rspb.2019.0872)
48. Donners M, van Grunsven RHA, Groenendijk D, van Langevelde F, Bikker JW, Longcore T, Veenendaal E. 2018 Colors of attraction: Modeling insect flight to light behavior. *J. Exp. Zool. Part A, Ecol. Integr. Physiol.* **329**, 434–440. (doi:10.1002/jez.2188)
49. Brüning A, Hölker F, Franke S, Kleiner W, Kloas W. 2016 Impact of different colours of artificial light at night on melatonin rhythm and gene expression of gonadotropins in European perch. *Sci. Total Environ.* **543**, 214–222. (doi:10.1016/j.scitotenv.2015.11.023)
50. Longcore T, Aldern HL, Eggers JF, Flores S, Franco L, Hirshfield-Yamanishi E, Petrinec LN, Yan WA, Barroso AM. 2015 Tuning the white light spectrum of light emitting diode lamps to reduce attraction of nocturnal arthropods. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140125. (doi:10.1098/rstb.2014.0125)
51. Davies TW, Bennie J, Inger R, de Ibarra NH, Gaston KJ. 2013 Artificial light pollution: Are shifting spectral signatures changing the balance of species interactions? *Glob. Chang. Biol.* **19**, 1417–1423. (doi:10.1111/gcb.12166)
52. Davies TW, McKee D, Fishwick J, Tidau S, Smyth T. 2020 Biologically important artificial light at night on the seafloor. *Sci. Rep.* **10**, 1–10. (doi:10.1038/s41598-020-69461-6)
53. Marshall J. 2017 Vision and lack of vision in the ocean. *Curr. Biol.* **27**, R494–R502. (doi:10.1016/j.cub.2017.03.012)
54. Jaubert M, Bouly JP, Ribera d'Alcalà M, Falciatore A. 2017 Light sensing and responses in marine microalgae. *Curr. Opin. Plant Biol.* **37**, 70–77. (doi:10.1016/j.pbi.2017.03.005)
55. Miller DC, Bretschneider E. 2006 Method of Lighting for Protecting Sea Turtles. **2**.
56. Ayalon I, de Barros Marangoni LF, Benichou JIC, Avisar D, Levy O. 2019 Red Sea corals under Artificial Light Pollution at Night (ALAN) undergo oxidative stress and photosynthetic impairment. *Glob. Chang. Biol.* **25**, 4194–4207. (doi:10.1111/gcb.14795)
57. Poot H, Ens BJ, de Vries H, Donners MAH, Wernand MR, Marquenie JM. 2008 Green light for nocturnally migrating birds. *Ecol. Soc.* **13**, 47. (doi:10.5751/ES-02720-130247)
58. Bennie J, Davies TW, Cruse D, Gaston KJ. 2016 Ecological effects of artificial light at night on wild plants. *J. Ecol.* **104**, 611–620. (doi:10.1111/1365-2745.12551)
59. Falcón J, Torriglia A, Attia D, Viénot F, Gronfier C, Behar-Cohen F, Martinsons C, Hicks D. 2020 Exposure to Artificial Light at Night and the Consequences for Flora, Fauna, and Ecosystems. *Front. Neurosci.* **14**, 1–39. (doi:10.3389/fnins.2020.602796)
60. Levy O, Fernandes de Barros Marangoni L, Cohen JI, Rottier C, Béraud E, Grover R, Ferrier-Pagès C. 2020 Artificial light at night (ALAN) alters the physiology and biochemistry of symbiotic reef building corals. *Environ. Pollut.* , 114987. (doi:10.1016/j.envpol.2020.114987)
61. Tamir R, Lerner A, Haspel C, Dubinsky Z, Iluz D. 2017 The spectral and spatial distribution of light pollution in the waters of the northern Gulf of Aqaba (Eilat). *Sci. Rep.* **7**, 1–10.

(doi:10.1038/srep42329)
62. Davies TW, Coleman M, Griffith KM, Jenkins SR. 2015 Night-time lighting alters the composition of marine epifaunal communities. *Biol. Lett.* **11**. (doi:10.1098/rsbl.2015.0080)
63. Underwood CN, Davies TW, Queirós AM. 2017 Artificial light at night alters trophic interactions of intertidal invertebrates. *J. Anim. Ecol.* **86**, 781–789. (doi:10.1111/1365-2656.12670)
64. Abiusi F, Sampietro G, Marturano G, Biondi N, Rodolfi L, D'Ottavio M, Tredici MR. 2014 Growth, photosynthetic efficiency, and biochemical composition of Tetraselmis suecica F&M-M33 grown with LEDs of different colors. *Biotechnol. Bioeng.* **111**, 956–964. (doi:10.1002/bit.25014)
65. Aidar E *et al.* 1994 Effects of light quality on growth, biochemical composition and photo synthetic production in Cyclotella caspia Grunow and Tetraselmis gracilis (Kylin) Butcher. *J. Exp. Mar. Bio. Ecol.* **180**, 175–187. (doi:10.1016/0022-0981(94)90065-5)
66. Schulze PSC, Pereira HGC, Santos TFC, Schueler L, Guerra R, Barreira LA, Perales JA, Varela JCS. 2016 Effect of light quality supplied by light emitting diodes (LEDs) on growth and biochemical profiles of Nannochloropsis oculata and Tetraselmis chuii. *Algal Res.* **16**, 387–398. (doi:10.1016/j.algal.2016.03.034)
67. Montero MF, Aristizábal M, García Reina G. 2011 Isolation of high-lipid content strains of the marine microalga Tetraselmis suecica for biodiesel production by flow cytometry and single-cell sorting. *J. Appl. Phycol.* **23**, 1053–1057. (doi:10.1007/s10811-010-9623-6)
68. Muller-Feuga A. 2013 Microalgae for aquaculture: the current global situation and future trends. In *Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition*, (doi:10.1002/9781118567166.ch33)
69. Parsons TR, Maita Y, Lalli CM. 1984 *A manual of chemical and biological methods for seawater analysis*. Pergamon, Oxford.
70. Guillard RRL. 1975 Culture of phytoplankton for feeding marine invertebrates. In *Culture of Marine Invertebrate Animals*, pp. 29–60. New York: Plenum Press.
71. Menhinick EF. 1964 A Comparison of some species-individuals diversity indices applied to samples of field insects. *Ecology* **45**, 859–861. (doi:10.2307/1934933)
72. Pielou EC. 1966 No Title The measurement of diversity in different types of biological collections. *J. Theor. Biol.* **13**, 131–44.
73. Spatharis S, Tsirtsis G. 2010 Ecological quality scales based on phytoplankton for the implementation of Water Framework Directive in the Eastern Mediterranean. *Ecol. Indic.* **10**. (doi:10.1016/j.ecolind.2010.01.005)
74. Arhonditsis G, Karydis M, Tsirtsis G. 2003 Analysis of phytoplankton community structure using similarity indices: A new methodology for discriminating among eutrophication levels in coastal marine ecosystems. *Environ. Manage.* **31**, 619–632. (doi:10.1007/s00267-002-2903-4)
75. Wickham H, Chang W, Henry L, Pedersen TL, Takahashı K, Wilke C, Woo K, Yutani H, Dunnington D. 2020 Package 'ggplot2' : Create Elegant Data Visualisations Using the Grammar of Graphics. *R Packag. version 3.3.0*
76. Kassambara A. 2020 'ggpubr': 'ggplot2' Based Publication Ready Plots. *R Packag. version 0.2.5.*
77. de Vries A, Ripley BD. 2016 Package 'ggdendro': Create Dendrograms and Tree Diagrams Using 'ggplot2' (R package). *R Packag. version 0.1-20*
78. Galili T. 2015 dendextend: An R package for visualizing, adjusting and comparing trees of hierarchical clustering. *Bioinformatics* **31**, 3718–3720. (doi:10.1093/bioinformatics/btv428)
79. Lenth R V. 2018 emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.1. *https://CRAN.R-project.org/package=emmeans*.
80. Wickham H. 2007 Reshaping Data with the reshape Package. *J. Stat. Softw.* **21**, 1–20.
81. Wickham H. 2011 The split-apply-combine strategy for data analysis. *J. Stat. Softw.* **40**, 1–29. (doi:10.18637/jss.v040.i01)
82. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated Generalized Linear Mixed Modeling. *R J.* **9**, 378–400. (doi:10.32614/rj-2017-066)

83. Oksanen J *et al.* 2019 Package 'vegan' : community ecology package. *Community Ecol. Packag.* **2**, 1–297.
84. Rousseeuw P *et al.* 2019 *Package 'cluster' for R: "Finding Groups in Data": Cluster Analysis Extended*.
85. Kent M, Whyte J, LaTrace C. 1995 Gill lesions and mortality in seawater pen-reared Atlantic salmon Salmo salar associated with a dense bloom of Skeletonema costatum and Thalassiosira species. *Dis. Aquat. Organ.* **22**, 77–81. (doi:10.3354/dao022077)
86. Oh SJ, Kim D Il, Sajima T, Shimasaki Y, Matsuyama Y, Oshima Y, Honjo T, Yang HS. 2008 Effects of irradiance of various wavelengths from light-emitting diodes on the growth of the harmful dinoflagellate Heterocapsa circularisquama and the diatom Skeletonema costatum. *Fish. Sci.* **74**, 137–145. (doi:10.1111/j.1444-2906.2007.01503.x)
87. Clementson LA, Wojtasiewicz B. 2019 Dataset on the in vivo absorption characteristics and pigment composition of various phytoplankton species. *Data Br.* **25**, 104020. (doi:10.1016/j.dib.2019.104020)
88. Edmunds LN. 1984 *Cell Cycle Clocks*. New York, NY: M. Dekker Inc.
89. Rockwell NC, Duanmu D, Martin SS, Bachy C, Price DC, Bhattacharya D, Worden AZ, Lagarias JC. 2014 Eukaryotic algal phytochromes span the visible spectrum. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 3871–3876. (doi:10.1073/pnas.1401871111)
90. Ridge KD. 2002 Algal rhodopsins: Phototaxis receptors found at last. *Curr. Biol.* **12**, 588–590. (doi:10.1016/S0960-9822(02)01099-0)
91. Banerjee R, Schleicher E, Meier S, Viana RM, Pokorny R, Ahmad M, Bittl R, Batschauer A. 2007 The signaling state of Arabidopsis cryptochrome 2 contains flavin semiquinone. *J. Biol. Chem.* **282**, 14916–14922. (doi:10.1074/jbc.M700616200)
92. Battle MW, Jones MA. 2020 Cryptochromes integrate green light signals into the circadian system. *Plant Cell Environ.* **43**, 16– 27. (doi:10.1111/pce.13643)
93. Battle MW, Vegliani F, Jones MA. 2020 Shades of green: untying the knots of green photoperception. *J. Exp. Bot.* **71**, 5764–5770. (doi:10.1093/jxb/eraa312)
94. Trupkin SA, Karayekov E, Buchovsky AS, Rossi C, Jose J. 2020 Cryptochrome as a sensor of the blue / green ratio of natural radiation in Arabidopsis 1 [C][W][OA]. **154**, 401–409. (doi:10.1104/pp.110.160820)
95. Spoelstra K, van Grunsven RHA, Ramakers JJC, Ferguson KB, Raap T, Donners M, Veenendaal EM, Visser ME. 2017 Response of bats to light with different spectra: Light-shy and agile bat presence is affected by white and green, but not red light. *Proc. R. Soc. B Biol. Sci.* **284**, 11–15. (doi:10.1098/rspb.2017.0075)
96. Ouyang JQ, de Jong M, van Grunsven RHA, Matson KD, Haussmann MF, Meerlo P, Visser ME, Spoelstra K. 2017 Restless roosts: Light pollution affects behavior, sleep, and physiology in a free-living songbird. *Glob. Chang. Biol.* **23**, 4987–4994. (doi:10.1111/gcb.13756)
97. Spatharis S, Tsirtsis G, Danielidis DB, Chi TD, Mouillot D. 2007 Effects of pulsed nutrient inputs on phytoplankton assemblage structure and blooms in an enclosed coastal area. *Estuar. Coast. Shelf Sci.* **73**. (doi:10.1016/j.ecss.2007.03.016)
98. Diamantopoulou C, Christoforou E, Dominoni DM, Kaiserli E, Czyzewski J, Mirzai N, Spatharis S. 2021 Wavelength-dependent effects of artificial light at night on phytoplankton growth and community structure. , 1–26.

678 Figure captions