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Research

Photoperiod influences the shape and scaling of freshwater phytoplankton responses to light and temperature

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Light fluctuations are ubiquitous, exist across multiple spatial and temporal scales, and directly affect the physiology and ecology of photoautotrophs. However, the indirect effects of light fluctuations on the sensitivity of organisms to other key environmental factors are unclear. Here, we evaluate how photoperiod regime (period of time each day where organisms receive light), a dynamic element of aquatic ecosystems, can influence the interactive effects of temperature and irradiance (intensity of light) on the growth rate of phytoplankton populations. We first completed a literature review and meta-analysis that suggests photoperiod alters the individual effects of temperature – but not irradiance – on algal growth rates and that highlights how few studies experimentally manipulate photoperiod, temperature and irradiance. To address this empirical gap, we conducted a set of laboratory experiments on three freshwater phytoplankton species (*Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Cryptomonas ovata*). We measured performance surfaces relating growth rate to irradiance and temperature gradients for each species in constant (24:0 h of light:dark) environments. We then evaluated whether analogous surfaces measured under different photoperiods (6:18, 12:12 and 16:8 h of light:dark) and scaled by the duration of light availability could be inferred from results under constant light. For a majority of the combinations of species and photoperiods examined, photoperiod meaningfully altered the intercept and shape of performance surfaces. These differences were most pronounced under the shortest photoperiod (6:18 h light:dark), where populations underperformed expectations. Alterations to performance surfaces were non-linear and mostly structured by temperature with higher temperatures yielding higher than anticipated growth rates. Collectively, these experiments and synthesis reveal the potential for photoperiod regime to influence the effects of temperature, irradiance and their interaction on phytoplankton growth. Beyond the environmental variables and organisms presently considered, this research highlights the capacity for dynamic, abiotic variables to exert direct effects while also influencing relationships among other environmental factors.

Keywords: acclimation, environmental variation, light fluctuations, multiple stressors

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Introduction

Understanding the effects of abiotic conditions on the growth and distribution of organisms is fundamental to ecology and critical in anticipating the consequences of anthropogenically-induced environmental change across ecosystems. Beyond examining the main effects of single environmental factors (also called drivers or stressors), research is increasingly exploring: 1) the interactive effects of multiple abiotic drivers (Koussoroplis et al. 2017, Orr et al. 2020) and 2) the importance of both the mean and temporal variability of abiotic factors (Thompson et al. 2013, Coble et al. 2016). Across terrestrial, freshwater and marine systems, studies looking at the effects of multiple factors reveal that significant interactions commonly occur (Crain et al. 2008, Piggott et al. 2015, Orr et al. 2020), making it challenging or impossible to predict the response of individuals, populations and ecosystems to simultaneous changes of several environmental conditions (Sala et al. 2000, Boyd and Brown 2015). Temporal variation or fluctuations in even a single environmental factor can substantially affect the growth and performance of organisms (Vasseur et al. 2014, Fey et al. 2021) as well as competition and coexistence (Chesson 1994, Litchman and Klausmeier 2001, Kremer and Klausmeier 2017). In the following paper, we explore how the interactive effects of two broadly important abiotic factors – irradiance (the intensity of light; Table 1) and temperature – are influenced by the temporal patterning of light availability (photoperiod regime, i.e. the period of time each day where organisms receive light).

Light is among the most heterogeneous environmental factors on Earth. Organisms experience light fluctuations at multiple spatial and temporal scales, which are further modified by the movement, behavior and physiology of organisms (i.e. the ability and mechanisms by which organisms sequester light). In terrestrial ecosystems, light fluctuations from large-scale planetary processes, vegetation geometry, sun angle and small-scale disturbances give rise to long-term, predictable patterns punctuated by stochastic events. For example, sunflecks in understory forest environments increase irradiance by several orders of magnitude yet persist for only seconds to minutes (Chazdon 1988, Beaudet et al. 2004). In aquatic ecosystems, light fluctuations occur over timescales ranging from minutes to hours (e.g. surface waves or Langmuir cells (Thorpe 2004)), to hours to days (e.g. storms, diurnal patterns or turbulent mixing), to months or years (e.g. slow fluctuation due to seasonal variability); these can occur from either stochastic (e.g. storms) or deterministic (e.g. daylength) processes. Fluctuations can be further modified by depth, where light intensity diminishes with depth (Litchman 1998).

Many ecological consequences of light fluctuations are widely understood and appreciated (Li et al. 2017). For photoautotrophs such as phytoplankton, light can be an important resource limiting phytoplankton growth. Shorter photoperiods (i.e. short days) result in less total light over the course of a day, causing growth rates of aquatic primary producers to decline (Litchman 2000). Organismal responses to changes or fluctuations in light – in addition to the direct effects of reduced light – generate additional complexities. For example, phytoplankton exhibit reversible phenotypic responses to light fluctuations (i.e. photoacclimation) (Falkowski and LaRoche 1991). These can occur across multiple timescales as a result of alterations to photosynthetic pigment content complement (e.g. complementary chromatic adaptation in cyanobacteria on scales of ~7 days (Stomp et al. 2008)) and alterations to electron transfer chain components and Calvin cycle enzymes (which are rapidly modified across natural diel light fluctuation (Becker et al. 2020)). Such adjustments may also be influenced by the costs of photoinhibition and photooxidative stress (Raven and Samuelsson 1986, Falkowski and LaRoche 1991, MacIntyre et al. 2002). Although the timing of light fluctuations can often be anticipated (Mittag et al. 2005, Becker et al. 2020), associated phenotypic changes are not instantaneous. For example, the short-term response of algal pigment profiles can take up to 5 h to reach a stable state (Algarra and Xavier Niell 1990). Additionally, transitions from light to dark promotes carbon assimilation and yield increases in pigment stores to maximize photosynthetic rate across a day (Prézelin and Ley 1980, Becker et al. 2020). This suggests that performance may differ in environments with different photoperiods as algal pigment profiles determine the photosynthetic ability of individuals.

Phytoplankton are an ideal study system to measure the effects of photoperiods as these individual effects can tractably scale up to population and community level processes. During periods of darkness, algae can utilize photosynthetic products to fuel maintenance and biosynthesis (Kliphuis et al. 2011) and can carry out respiration of storage lipids that accumulate during the day (Becker et al. 2020). In some cases, reductions in growth due to reduced light can be accurately predicted by the linear scaling of constant light conditions (Litchman 2000, Shatwell et al. 2012). This suggests that the population-level effects of photoacclimation are limited. In other cases, growth rates of organisms are not proportional to the length of the photoperiod, precluding

Table 1. The definitions of key light-related terms used in the manuscript.

Term	Definition
Photoperiod	The period of time each day where organisms receive light (hours light:hours dark).
Irradiance	Intensity of light in PPFD (μmol photons m^{-2} s^{-1}).
Constant light	A photoperiod of 24:0 (hours light:hours dark) at a single specified irradiance level.
Fluctuating light	Any photoperiod that involves both periods of light and darkness where all periods of light are at a single irradiance level.

accurate predictions of growth under different photoperiods (Post et al. 1986, Ibelings et al. 1994), particularly when fluctuations occur over long intervals (Litchman 2000). Fluctuating light also affects community level processes, such as coexistence via gleaner–opportunist tradeoffs (Litchman and Klausmeier 2001, Tsakalakis et al. 2018), parasitism, and the rate of infection and lysis in some viruses (Piedade et al. 2018). Due to the dependence of most primary production on light, food web structure and energy transfer within and between ecosystems ultimately depend on levels and variation in irradiance (Beardall et al. 1994).

To date, research on light fluctuations primarily addresses their direct effects on organisms (and hence, ecology), rather than examining how fluctuations change the effects of other environmental factors (Ferris and Christian 1991). For example, experiments investigating the response of phytoplankton to varying photoperiods have done so under a limited number of temperatures or irradiances, despite these being two of the most important and commonly manipulated environmental factors in experimental studies (Shatwell et al. 2012). Conversely, research on the interactive effects of irradiance and temperature on phytoplankton growth rates has historically been conducted using a single photoperiod, commonly constant light. This is potentially problematic: very few natural ecological systems experience constant or near constant light over meaningful time scales. If the interactive effects of temperature and irradiance on phytoplankton growth differ among photoperiod regimes, mismatches between the photoperiods employed in controlled laboratory studies and photoperiods experienced in natural systems could hinder efforts to predict phytoplankton dynamics.

Indeed, differences in diel light fluctuations appear, in theory, capable of modifying the light harvesting ability of phytoplankton via photoacclimation in a manner that is irradiance or temperature-sensitive (Yoder 1979). Many biological processes are temperature dependent (Dell et al. 2013). If, following a period of darkness, phytoplankton in warmer conditions alter their pigmentation faster than those experiencing colder conditions, the effects of photoacclimation may be weaker in warm environments. Empirically, complex and non-linear interactions between either photoperiod and irradiance level (Li et al. 2017) or photoperiod and temperature (McQuoid and Hobson 1995) have been previously documented. However, without incorporating interactions between temperature, irradiance and photoperiod into a single framework, current knowledge is insufficient for understand how light fluctuations influence ecological responses to multiple environmental factors.

Here, we address this gap by evaluating the ability of photoperiod to influence environmental interactions across multiple factors (temperature and irradiance) via effects on phytoplankton growth. To test the hypothesis that the interactive effects of environmental temperature and irradiance on phytoplankton growth rates are influenced by photoperiod, we present the results of a meta-analysis and quantitative review of algal monoculture growth rates as well as a series of new laboratory experiments using three phytoplankton monocultures. If our results indicate that photoperiod can influence the effects of temperature, irradiance and their interaction on growth, caution should be used in attempting to relate physiological responses measured under one set of conditions (photoperiod, irradiance level, temperature) to other conditions whether in the lab or in the field.

Methods

Literature search and meta-analysis

We reviewed the primary literature in November 2020 by searching the ISI Web of Knowledge database to evaluate the extent to which photoperiod has been included in assessing the effects of irradiance and temperature on phytoplankton growth. Combinations of the following terms resulted in 1352 papers: Phytoplankton AND growth rate* AND light* AND temperature*. We further narrowed our results by including only studies satisfying the following a priori criteria: studies must have presented original experimental data, focused on aquatic primary producers, measured growth rate or a proxy of growth rate (e.g. change in fluorescence over time), and assessed light fluctuations over a period of 24 h (e.g. only those occurring on diel timescales). For each study, we recorded the system (freshwater, marine or brackish); the number of different photoperiods, temperatures and irradiance levels; the photoperiod regimes; the maximum environmental temperature and irradiance level; the units used to measure irradiance; and the number of additional experimental factors manipulated. In studies with non-fully factorial experimental designs, we only used a subset of the data corresponding to the largest subset of observations spanning treatments that were factorial. For example, if growth was assayed at three levels of irradiance and a single temperature and seven levels of temperature and two irradiance levels, then only the latter was recorded. When experiments were conducted outside, we equated photoperiod with daylight duration on the start date of the experiment (obtained from <www.suncalc.org>). Additionally, several studies manipulated UV radiation, which we treated as an additional factor. Our final database yielded 323 experiments from 295 unique peer-reviewed manuscripts (Fig. 1).

We then conducted a meta-analysis to examine whether photoperiod alters the relationship between irradiance and temperature and the effects of irradiance and temperature on growth rate (Fig. 2, Supporting information). Because very few studies included multiple photoperiods and multiple levels of both temperature and irradiance (Fig. 1), we considered studies from our database that assessed growth rate across at least two photoperiods and a minimum of two temperatures or two levels of irradiance and that included quantitative estimates of mean growth rates, standard deviations and sample sizes (Supporting information). These criteria yielded 10 instances of temperature and photoperiod manipulations (from six studies) and six instances of irradiance and photoperiod manipulations (from five studies). For

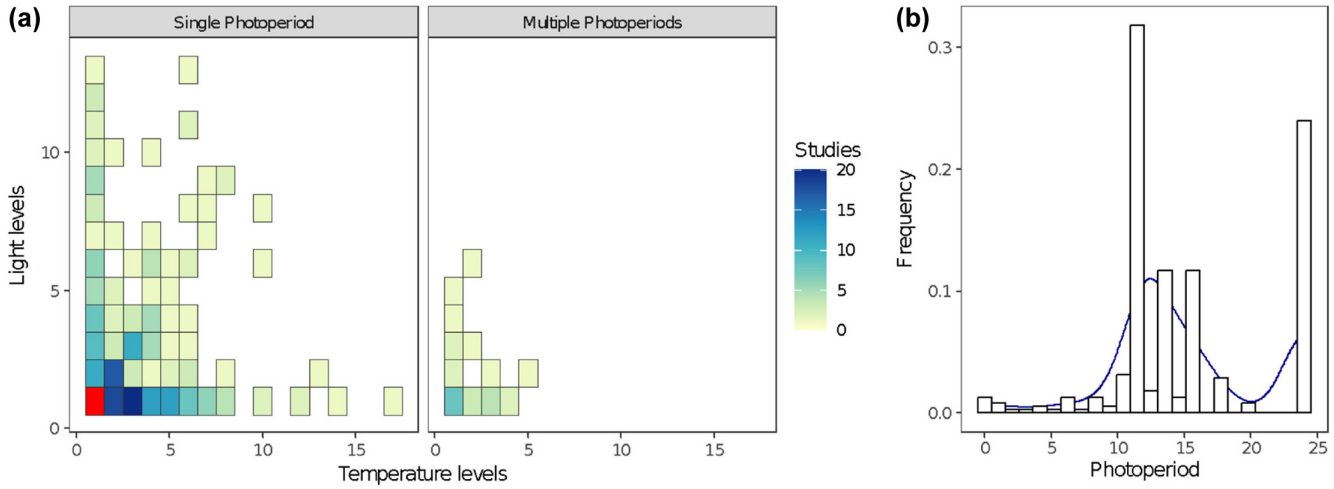

Figure 1. Experimental studies of how light and temperature influence phytoplankton growth are biased towards manipulations of small numbers of temperature or light treatments and either 12:12 or constant photoperiods. (a) The number of temperature (x-axis) and irradiance (y-axis) levels present in studies using either a single (n = 296) or multiple (n = 27) photoperiods. 64 studies used only a single light and temperature level (red). (b) The frequency distribution of photoperiod regimes used (number of hours of light across a 24-h day) across all experimental studies; the blue line indicates a smoothed trend.

each experiment, we extracted the mean growth rate, standard deviation and sample size for phytoplankton cultured in the lowest and highest photoperiod regime and under the highest and lowest environmental factor (temperature or irradiance). These data enabled us to calculate standardized mean differences in growth rates that existed between low versus high values of abiotic factors (abiotic factors were either temperature (Fig. 2A) or light (Fig. 2B)) for both low and high photoperiod regimes. Positive standardized mean differences indicate that faster growth rates occur when either temperature or irradiance increases. For each abiotic factor, we used a random effects (hierarchical) meta-analysis to estimate whether photoperiod influenced the independent effects (standardized mean differences) of temperature or irradiance, considering sampling error and variation between and within studies. We fit photoperiod as a categorical factor representing the lowest or highest photoperiod regime in a study and fit study ID as a random factor that included a random intercept to estimate the standardized mean difference and 95% CI of growth rates between treatments. Results were considered significant at $\alpha = 0.05$. Models were fit using the metafor package (Viechtbauer 2010) for R ver. 4.0.3.

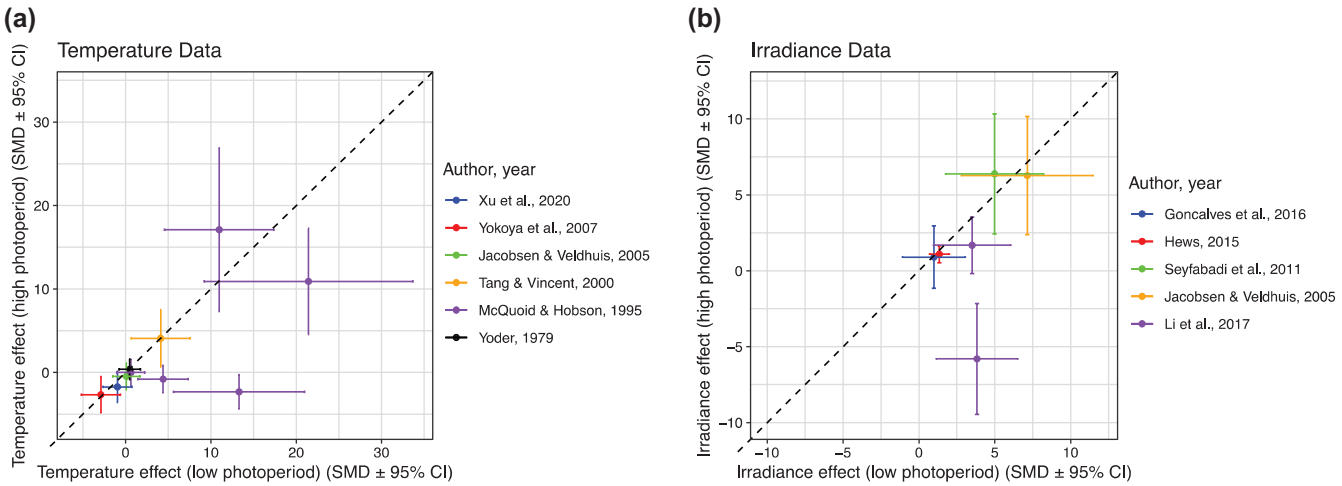

Figure 2. (a) Mean (± 95% CI) standardized mean differences between the effects of low and high temperatures on algal growth rates for low photoperiod regimes (x-axis) and high photoperiod regimes (y-axis). (b) Mean (± 95% CI) standardized mean differences between the effects of low and high irradiance levels on algal growth rates for low photoperiod regimes (x-axis) and high photoperiod regimes (y-axis). For both panels, positive values indicate that growth rates in high temperature (in a) or irradiance (in b) environments are faster than in low temperature (in a) or irradiance (in b) environments. The 1:1 dashed line indicates conditions where photoperiod is not altering the temperature-dependence (in a) or irradiance-dependence (in b) of algal growth rates.

Growth rate experiment

We experimentally measured the exponential growth rate of three species of phytoplankton – the green algae *Chlamydomonas reinhardtii* (from E. Litchman, Michigan State Univ.) and *Chlorella vulgaris* (Univ. of Texas, UTEX, Culture Collection of Algae, Austin, TX, UTEX no. 26) and the cryptomonad *Cryptomonas ovata* (UTEX, no. 2783) – under factorial combinations of temperature, irradiance and photoperiod. These species were selected for their abilities to tolerate a range of irradiance and temperature levels and because of their common usage in phytoplankton research. We used a thermal gradient block (TGB), an aluminum rectangle heated at one end and cooled at the other with wells across the surface, to impose nine temperature treatments (13.9, 17.9, 22.2, 24.6, 28.5, 32.1, 36.3, 39.5 or 42.4°C) (Fey et al. 2021, Layden et al. 2021). Each well had an individual source of light at the bottom of the well, and samples were exposed to one of 6 irradiance levels (PPFD = 0, 36.44, 95.44, 140.00, 237.44 or 451.22 µmol m^{-2} s^{-1}). These conditions resulted in 54 unique combinations of irradiance and temperature from which we were able to generate a performance (growth rate) surface (in response to this irradiance by temperature arrangement). The lights were controlled using an automatic timer allowing for four photoperiods: a short photoperiod (6:18 h of light:hours of dark), a medium photoperiod (12:12), a long photoperiod (16:8) and constant light (24:0). Importantly, natural environments will experience additional layers of fluctuations that are more complex than these simple light:dark diel patterns.

Populations were acclimated to temperature and irradiance levels over a period of two weeks under continuous irradiance, and we diluted all populations periodically during the acclimation phase to maintain exponential growth. Following acclimation, we measured the growth of replicate populations (n = 3 for each species) under 24 h of irradiance, then randomly determined the order of the three remaining photoperiod regimes to complete assays sequentially on the same block. To begin each assay, we inoculated the acclimated cultures in COMBO nutrient media (Kilham et al. 1998) at a starting density of 20 000 cells ml^{-1}. Cultures were placed on the TGB in the temperature and irradiance combination to which they were previously acclimated and then exposed to one of the four photoperiods for 48 h starting with the beginning of the light phase. This ensured growth was maintained in the exponential phase, while encompassing the timeframe of photoacclimation (Algarra and Xavier Niell 1990). Populations were not acclimated to various photoperiod regimes prior to experiments due to the logistical constraints surrounding the time required to maintain existing acclimating populations. We measured fluorescence as a proxy for cell density using a Trilogy Laboratory Fluorometer with a chlorophyll-a in-vivo module four times: at 0, 16, 24 and 48 h during each assay. Such an approach assumes that chlorophyll-a is a reliable indicator of biomass over the duration of the experiment in the context of experienced light fluctuations. Growth rates for each replicate were estimated over the 48-h assay as the slope of the linear model of the log of population density plotted through time, smoothing over potential fluctuations from light to dark and vice versa (see the Supporting information for details).

Data analysis

Our analysis of the experimental growth rate data focused on characterizing the interactive effects of temperature and irradiance level on growth rate (hereafter, a performance surface) and considered how these relationships change under different photoperiods. We generated performance surfaces

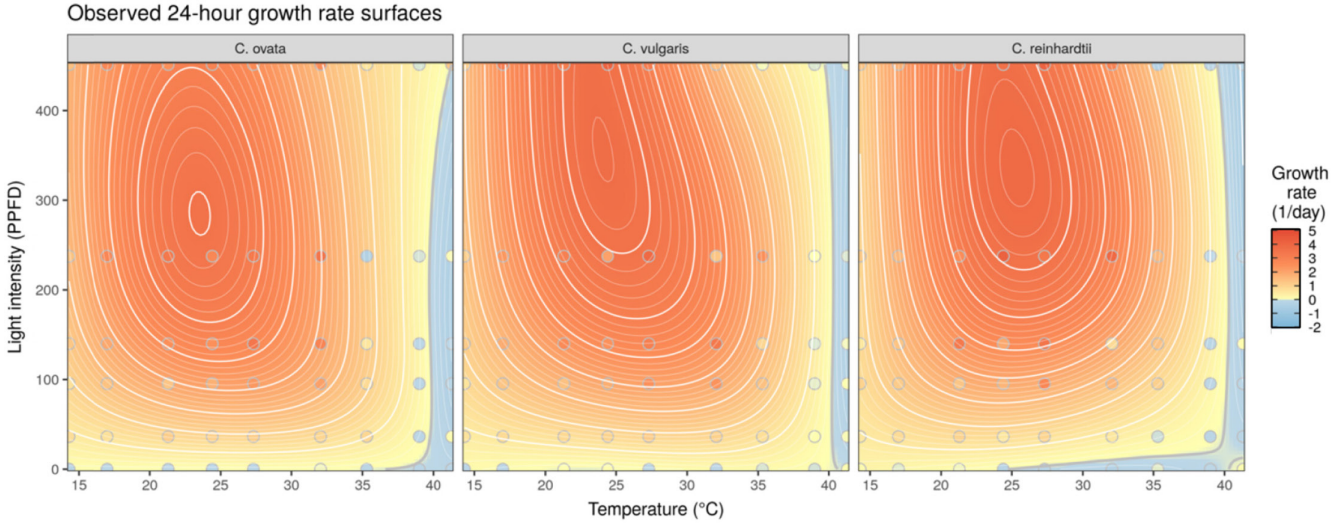

Figure 3. Observed 24 h (constant irradiance) performance surfaces depicting positive population growth rates as warm colors and negative growth as cool colors; gray line represents zero net growth and thick and thin contours indicate changes of 0.5 and 0.1 in growth rate, respectively. Colored dots indicate the mean measured growth rates for each combination of temperature and irradiance level.

for each species and photoperiod using generalized additive models (GAMs, (Wood 2017)) with a tensor product smooth allowing interactions between temperature and irradiance level and an initial basis dimension (k) of 4 (Fig. 3, Supporting information). We fit the GAMs using the mgcv package in R (Wood 2017) using the REML method, which is more robust to overfitting smaller data sets. From these GAM fits, we estimated key traits including optimal temperature (T_{opt}) and irradiance (I_{opt}) levels (the conditions maximizing the predicted growth rate) as well as the critical thermal maxima (CT_{max}, the highest temperature yielding positive growth) (Supporting information).

Having characterized the effects of temperature and irradiance on growth rate, we next considered how photoperiod affects these relationships. Specifically, we investigated whether results under 24 h of constant irradiance can be used to reliably estimate growth under shorter photoperiods. The simplest approach is to scale growth under constant irradiance (μ_1) by the fraction of a day where irradiance was provided (ϕ) to obtain new growth rate estimates (μ_ϕ). This proportional scaling implies that a plot of growth rate under a partial day of irradiance versus growth rate under a full 24 h of irradiance would follow a linear trend through the origin with a slope corresponding to ϕ. We investigated this possibility (Fig. 4a) by fitting three linear models (one per photoperiod) that allow the slope of this relationship to vary by species, while being constrained to an intercept of zero. We then considered estimates of uncertainty around these slope estimates relative to the predicted values of ϕ for each photoperiod (Supporting information).

We also considered a more nuanced approach that accounts for the fact that respiration and mortality occur for a full 24 h each day – regardless of photoperiod – while photosynthesis and growth depend on the duration of light availability (Litchman and Klausmeier 2001, Kremer and Klausmeier 2013). However, adjusting predicted growth to account for this mortality did not ultimately improve on the proportional approach, so we present only the simpler results below (see the Supporting information for additional details). We also note that neither approach accounts for photoacclimation and that because the proportional approach already underestimates growth in most cases, it is perhaps unsurprising that adding further reductions due to mortality fails to improve the situation.

Using the proportional approach, we scaled the observed growth rates under constant light and averaged the results across replicates (n = 3) for each unique combination of temperature, irradiance level and species. Next, we compared the resulting predicted growth rates to observed values, calculating the mean error (bias) and mean absolute error for each species and photoperiod (Fig. 4b). Lastly, we investigated whether these errors were systematically structured by temperature and irradiance levels which would indicate that photoperiod can affect how temperature and irradiance interact to influence growth rate (versus constant light). To accomplish this, we used GAMs to quantify the relationship (surface) between prediction errors and temperature and irradiance level for each photoperiod and species. In each case, we considered three competing GAM fits which assumed: 11) constant error unrelated to temperature or irradiance (model 1), 2) error that varies linearly with temperature, irradiance or their interaction (model 2) and 3) error that varies with temperature, irradiance and their interaction according to a tensor product smooth (model 3). After fitting each model, we used Akaike information criteria to compare models, selecting the best performing model to visualize using the method described above for generating performance surfaces (Fig. 4c, Supporting information). If error is unrelated to temperature and irradiance level, then we would expect model 1 to perform better than the alternative models. See the Supporting information for how these surfaces comparing observed versus expected outcomes relate to meta-analysis results.

Results

Literature review and meta-analysis

Of the 323 experimental papers about the impact of light and temperature on phytoplankton growth, 91.6% (n = 296) of measurements were taken from a single photoperiod (Fig. 1a). Of the remaining studies conducted across multiple photoperiods, 13 studies included more than 1 level of temperature, 10 studies included more than 1 level of irradiance and 4 studies included more than one level of both irradiance and temperature. The most common photoperiod regimes across all studies were a medium photoperiod (12:12 hours of light:hours of dark) (33.2%) and constant light (24.9%). Photoperiod regimes with less than 12 h of light (10.3% of all studies) were relatively under-represented, compared to the 56.5% of studies that exhibited photoperiods of greater than 12L:12D (Fig. 1b).

Meta-analysis results indicate that the impact of temperature on algal growth rates was greater in low photoperiod regimes than in high photoperiod regimes (Fig. 2; $Q = 5.26$, $df = 1$, $p = 0.0218$; Supporting information). Conversely, the impact of irradiance on growth rate did not differ between low and high photoperiod regimes (Fig. 2; $Q = 0.279$, $df = 1$, $p = 0.597$; Supporting information).

Interactions between irradiance and temperature in constant light environments

The phytoplankton growth rates we measured under constant light (Supporting information) depended on an interaction between temperature and irradiance (GAMs allowing smoothed, interactive effects of both factors performed better than simpler models, Fig. 3, Supporting information). The three species differed in their optimum irradiance and temperature conditions with *C. reinhardtii* achieving the highest T_{opt} (25.5°C) and *C. vulgaris* achieving the highest I_{opt} (351 PPFD) compared to the other species. However, global CT_{max} values were comparable across all species (Fig. 3, Supporting information).

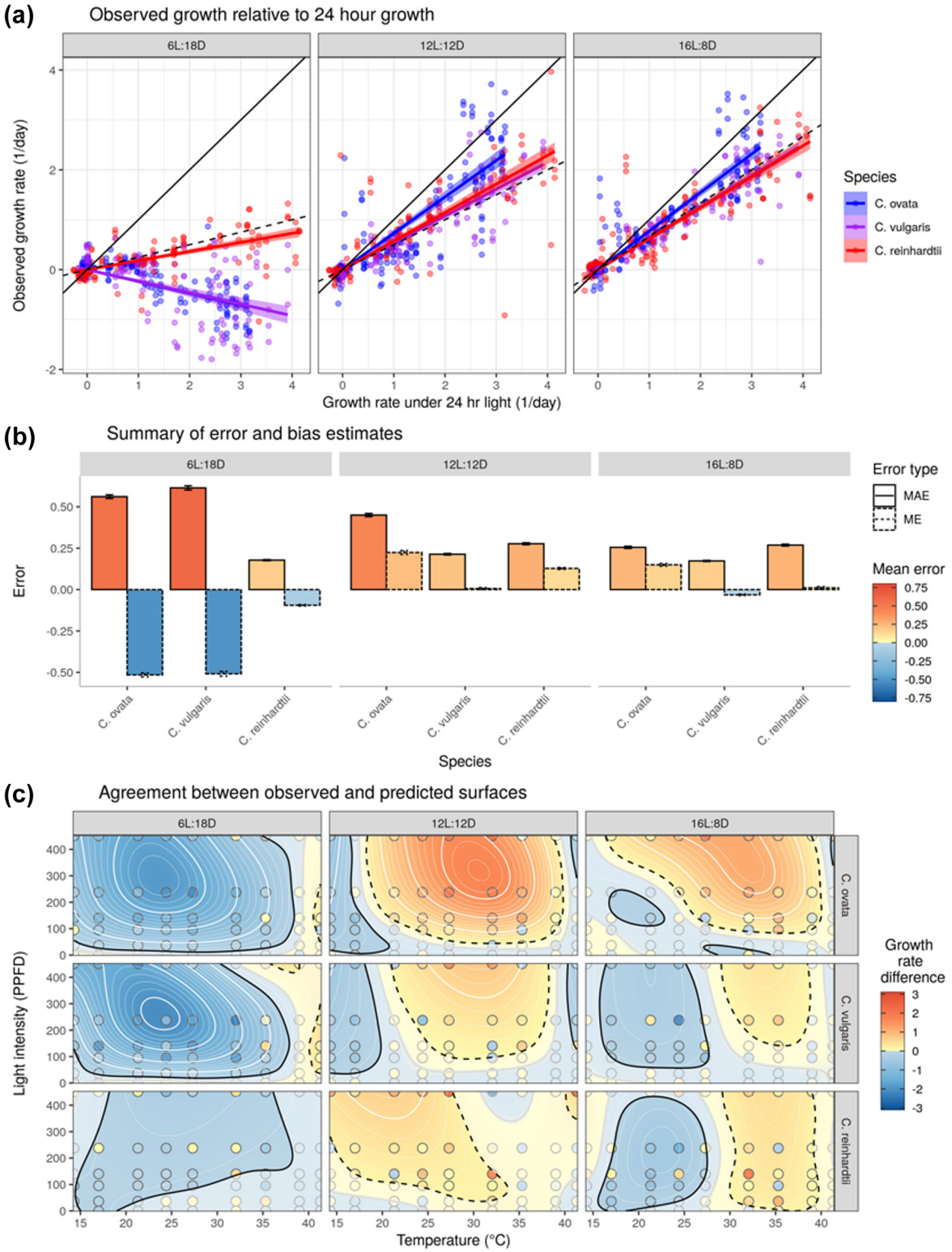

Figure 4. (a) Observed growth rates of all three focal species (by color) under shorter photoperiods relative to growth under 24 h of constant light. Solid black line indicates 1:1, dashed black line indicates the expectation if growth rates scale directly with the proportion of each day where light is supplied. Regressions (solid colored lines) and 95% confidence bands (shaded) indicate the observed linear relationship for each species, constrained to run through the origin. (b) Summary of error (MAE, mean absolute error) and bias (ME, mean error) estimates for the differences presented in (c). Error bars represent standard error based on 54 comparisons across environmental conditions. (c) Agreement between empirically resolved surfaces and predicted surfaces, shown as a difference in growth rate values (observed – predicted growth rates); gray line indicates no difference, while white shading around this indicates 95% confidence (solid black line = lower boundary, dotted black line = upper boundary); thick and thin white contours indicate 0.5 and 0.1 change in differences, respectively. Colored dots indicate the mean calculated growth rate differences (observed – predicted growth rate).

Photoperiod alters interactive effects of irradiance and temperature

As expected, the growth rates we empirically measured were generally reduced under short, medium or long photoperiod cycles relative to those observed under constant irradiance (Fig. 4a, b, Supporting information); however, these reductions did not consistently agree with simple expectations based on the proportional decrease in light availability (Fig. 4a, Supporting information). Of all three species, *C. reinhardtii* came the closest to matching these expectations across all three photoperiods. For *C. ovata* and *C. vulgaris*, observed growth rates under the short photoperiod were substantially lower than predicted and often negative while growth under the medium photoperiod exceeded predictions. It is also noteworthy that in some cases growth was actually lower under constant irradiance than under photoperiods in which darkness occurred (Fig. 4a). These results are summarized by considering the mean error and mean absolute error between observed and predicted growth rates (Fig. 4b).

We also found that the differences between predicted and observed growth rates varied with respect to the temperature and irradiance level experienced by populations (Fig. 4c). In support of this conclusion, GAMs that allowed interactive effects of irradiance and temperature fit the structure of these errors better than simpler models that assume no relationship between error and temperature, irradiance or their interaction (Supporting information). This indicates that the interactive relationship between temperature and irradiance, observable under constant irradiance, does not apply similarly under shorter photoperiods (Supporting information). Across species, under longer photoperiods (16L:8D), growth rates generally exceeded predictions at higher temperatures while falling short of predictions at lower temperatures (Fig. 4c). Similar patterns occurred for *C. ovata* and *C. vulgaris* under 12L:12D. Under the shortest photoperiod (6L:18D), growth rates were consistently lower than predicted across species, temperature and irradiance levels with the largest over-predictions occurring in the vicinity of the optimum temperature and irradiance levels observed for each species. The latter pattern is driven by growth under constant irradiance which yields much clearer growth optima (Fig. 3, Supporting information) than under short photoperiods, where growth rates are more uniform across conditions (Supporting information). To a lesser extent but evident in *C. ovata* in medium and long photoperiod regimes and *C. vulgaris* in medium photoperiod regimes, higher irradiance levels were associated with populations overperforming.

Discussion

Our study explores the ways in which temporal variability influences the effects of two fundamental, environmental factors on population growth. In particular, our results indicate that photoperiod regime (temporal variability in light availability, Table 1) influences the interactive effects of irradiance and temperature on phytoplankton growth. The meta-analysis results show that among suitable, published studies – which are sparse – the individual effect of temperature on growth rate was influenced by photoperiod while the effect of irradiance on growth rate was not. Our empirical results are consistent with these findings and indicate that while species- and photoperiod-specific patterns exist, different photoperiod regimes have the capacity to alter the shape and height of surfaces relating growth rate to temperature and irradiance. Below we discuss insights from our results regarding 1) how photoperiod length alters growth rates relative to constant light, 2) how both the meta-analysis and experimental results suggest temperature and irradiance interact to influence growth rates and 3) the ecological implications of these findings.

Consequences of photoperiod for phytoplankton growth rates

Our results show that the differences between phytoplankton growth rates observed in fluctuating light regimes and those observed in a constant light environment are generally consistent with established mechanisms of photoacclimation; however, they are not consistent with a simple proportional reduction in growth (Fig. 4).

The low to negative growth rates observed under short photoperiods, particularly for *C. vulgaris* and *C. ovata*, were among the most conspicuous effects of photoperiod on phytoplankton performance and were consistent with a previous study (Litchman 2000). Our simple predictions account for the short temporal duration of light, so our results are not attributable to that alone. There are at least two alternative mechanisms that may be operating: 1) naïve predictions underestimate the effects of respiration/mortality and 2) photoacclimation is not instantaneous. We considered the first possibility, motivated by previous studies (Litchman 2000, Litchman and Klausmeier 2001, Kremer and Klausmeier 2013), but found that it did not significantly improve the predictability of growth even under the shortest photoperiod examined, where the potential effects would be greatest (Supporting information). However, it is worth noting that estimates of mortality rate are difficult to directly obtain using available methods. Regarding the second possibility, it is known that algal pigment profiles (e.g. the distribution of content among existing pigments) require multiple hours to adjust following exposure to a novel irradiance level or when experiencing light after a period of darkness (Algarra and Xavier Niell 1990). Consequently, populations may not be able to immediately make use of light following the end of a dark period, and growth rates may lag behind the beginning of the light period. Populations experiencing short photoperiods would be most affected by this delay. While we did not explore this possibility, it is a fruitful avenue for future work.

In contrast, observed growth under medium and long photoperiods met or exceeded predictions based on constant light. The tendency for overperformance across various irradiances and temperatures suggests that sustained periods of

dark offer physiological benefits and that constant light may, in fact, be stressful. Such conditions can allow for beneficial light-independent photosynthetic processes (Brand and Guillard 1981), such as increasing pigment stores at night (Prézelin and Ley 1980), and other processes tuned to circadian rhythms. Indeed, many species of phytoplankton are sensitive to continuous light. For example, continuous exposure to ultraviolet radiation may damage photosynthetic machinery (i.e. photosystem II) by precluding the re-oxidation of electron transporters (Sforza et al. 2012). Additionally, variation among species suggests that species-specific traits may determine how light, temperature and photoperiod affect growth. This could reflect past adaptation to different environmental regimes (or lab conditions). For example, although each species used in this experiment is capable of mixotrophy (Tranvik et al. 1989, Heredia-Arroyo et al. 2011, Moon et al. 2013), differential rates of mixotrophy could alter the consequences of photoperiod regime by augmenting biomass production amidst periods of darkness.

Consequences of photoperiod for the interaction of irradiance and environmental temperatures

While the individual effects of temperature and irradiance on growth are well appreciated, their interactive effects are less understood, and the influence of temporal variation of irradiance (or temperature) on these interactions has not been thoroughly studied (Fig. 1). Our results suggest that these interactions are, in fact, sensitive to photoperiod as demonstrated by the influence of temperature and irradiance on the deviations between observed and predicted growth under each photoperiod (Fig. 4c). Temperature, in particular, appears to play an important role (consistent with the results of our meta-analysis, Fig. 2) in influencing growth rate. Photoacclimation is likely to be a temperature-dependent process, consistent with most biological rates (Dell et al. 2013), and as such, may occur faster in warmer environments. Assuming that photoacclimation works to increase fitness in a particular environment (Yoder 1979, Litchman 2000), this could account for both the enhanced growth rates under warmer, near-optimal temperatures (versus predictions based on constant irradiance) as well as the deficits occurring at colder, sub-optimal temperatures, at least under 12L:12D and 16L:8D.

Irradiance level had a less pronounced effect and was less likely to explain the discrepancies observed between scaled 24 h growth and observed growth under shorter photoperiods. The meta-analysis results indicated that while higher irradiance values tended to be coincident with higher growth rates, the impact of irradiance on growth rates was comparable between low and high photoperiod environments (Fig. 2). In some of our experimental results (*C. ovata* at medium and long photoperiods and *C. vulgaris* in medium photoperiods), populations overperformed expectations in high irradiance environments. Such a feature might be anticipated given that growth in fluctuating light environments can be faster than growth in constant environments when irradiance is particularly high, such that dark cycles can allow the re-oxidation of electron transporters, thus helping to avoid radiation damage (Mallin and Paerl 1992, Sforza et al. 2012).

Implications for understanding phytoplankton dynamics in nature

Our findings have a number of implications for understanding the ecology of phytoplankton in natural environments yet several limitations as well. In our empirical study, transitions between dark and light conditions occurred instantaneously; however, in natural systems these occur more gradually with patterns of variability in irradiance that unfold on multiple temporal scales (e.g. daily, yearly). The effects of these more complex patterns of variation remain an area of future research. Additionally, while the patterns we examined are strong and differences between treatments are informative, our characterizations of the interactive effects of light and temperature on growth remain largely descriptive and phenomenological. Developing a more mechanistic foundation for these patterns and building on prior work in this area (Geider et al. 1997, 1998, Li et al. 2017) would be invaluable. Finally, while our results indicate that photoperiod and photoacclimation can alter the interaction between irradiance and temperature, fluctuations in temperature are also common in nature, introducing the potential complication of thermal acclimation (Kremer et al. 2018, Fey et al. 2021).

Our results have implications for phytoplankton dynamics in nature. First, efforts to use physiological data derived from laboratory studies to predict real-world responses of algae to climate warming (Thomas et al. 2017) should be done with caution if the experimental conditions do not match the natural environment. Given that the greatest difference between predicted and observed growth rates occurred in short photoperiod regimes, researchers should be particularly cautious when making predictions for high latitude environments and/or winter months when daily irradiance is low. In fact, our review indicates that the recent emphasis on exploring the wintertime dynamics of freshwater environments (Bertilsson et al. 2013, Hrycik and Stockwell 2021) is unfolding in the backdrop of photoperiod regimes that have been typically understudied in laboratory environments (Fig. 1). Additionally, we show that measurements made under constant light, which can be preferable as constant light reduces or removes circadian rhythms that may be undesirable for certain studies, may underestimate organismal performance. Secondly, because light can act as both a source of energy to power photosynthesis and as a source of information (e.g. regulating circadian rhythms and resource allocation) (Bennie et al. 2016, O'Connor et al. 2019), our results suggest that anthropogenic sources of light may contribute to the known effects of light on aquatic primary producer community composition and biomass (Grubisic et al. 2017) in unanticipated ways that interact with temperature and daytime irradiance. These effects could occur by altering the intensity of irradiance, the length of daily light and dark

periods, or the photoacclimation capacity of phytoplankton amidst variable irradiance levels.

Beyond microbes and aquatic systems, our results speak to several broader points. First and foremost, it is clear that abiotic factors interact to affect the growth of populations in complex ways that depend, in part, on the temporal variability of these factors. Measuring growth under constant conditions may rarely be sufficient to reveal how populations will function under variable conditions, and the decay of this predictability may be steep (e.g. we observed major differences between 6 and 12 h of light per day). Yet, it is logistically impossible to perform fully factorial studies manipulating both the intensity and variability of abiotic factors, given the large diversity of species and relevant abiotic factors that exist. Instead, our results argue for increasing effort in developing mechanistic physiological models of organisms that emphasize how they are affected – and how they respond – to abiotic stressors with explicit attention paid to the time scale and consequences of plastic responses. Given ongoing and intensifying changes to environmental regimes due to climate change and other anthropogenic stressors, this work will be critical to understanding corresponding ecological changes.

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Author contributions

Meredith E. Theus: Investigation (lead); Writing – original draft (equal); Writing – review and editing (equal). **Tamara J. Layden**: Formal analysis (equal); Investigation (supporting); Writing – original draft (equal); Writing – review and editing (equal). **Nancy McWilliams**: Investigation (supporting); Methodology (supporting). **Stephen Crafton-Tempel**: Investigation (supporting); Writing – review and editing (supporting). **Colin T. Kremer**: Conceptualization (equal); Formal analysis (lead); Funding acquisition (equal); Project administration (supporting); Writing – original draft (equal); Writing – review and editing (equal). **Samuel B. Fey**: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (equal); Project administration (lead); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.5tb2rbp61> (Theus et al. 2022).

Supporting information

The supporting information associated with this article is available from the online version.

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