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LETTER

Thermal acclimation influences the growth and toxin production of freshwater cyanobacteria

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Scientific Significance Statement

A central goal of aquatic science is to understand the ecological factors that promote harmful cyanobacterial blooms, especially in light of climate change, which is altering the temporal variability of water temperature, in addition to increasing mean temperatures. However, we have yet to consider how this variability affects cyanobacteria populations that may more gradually adjust their physiology (acclimate) relative to the pace of temperature change. Using laboratory experiments, we show that acclimation dramatically affects the growth and toxin production of cyanobacteria exposed to different temperature perturbations. This occurs in both oligotrophic (low-nutrient) and eutrophic (high-nutrient) conditions. Thus, changes in the thermal structure of aquatic habitats may interact with organismal physiology to determine the frequency, severity, and harmful effects of cyanobacterial blooms.

Abstract

Understanding how altered temperature regimes affect harmful cyanobacterial bloom formation is essential for managing aquatic ecosystems amidst ongoing climate warming. This is difficult because algal performance can depend on both current and past environments, as plastic physiological changes (acclimation) may lag behind environmental change. Here, we investigate how temperature variation on sub-weekly timescales affects population growth and toxin production given acclimation. We studied four ecologically important freshwater cyanobacterial strains under low- and high-nutrient conditions, measuring population growth rate after acclimation and new exposure to a range of temperatures. Cold-acclimated populations (15.7°C) outperformed fully acclimated populations (held in constant conditions) across 65% of thermal environments, while hot-acclimated populations (35.7–42.6°C) underperformed across 75% of thermal environments. Over a 5-day

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Data Availability Statement: Corresponding data, metadata, and code are available in a GitHub repository at https://github.com/laydent/thermal_acc_and_cyanos

Additional Supporting Information may be found in the online version of this article.

period, cold-acclimated *Microcystis aeruginosa* produced ~2.5-fold more microcystin than hot-acclimated populations experiencing the same temperature perturbation. Our results suggest that thermal variation and physiology interact in underappreciated ways to influence cyanobacterial growth, toxin production, and likely bloom formation.

Freshwater phytoplankton pose a challenging and dynamic problem in aquatic ecosystem management: they provide critical ecosystem services, yet the formation and decomposition of blooms degrades the health of ecological communities (Paerl and Huisman 2009). Cyanobacterial blooms can be especially problematic as certain species produce toxins that disrupt trophic interactions (Babica et al. 2006). Toxins may be transferred up the food chain (via biodilution or biomagnification; Pham and Utsumi 2018) and across ecosystem boundaries (Landsberg 2002). Sublethal effects can impact the immune function, behavior, development, reproduction, and physiology of co-occurring organisms, including humans (Harke et al. 2016). Despite the associated public health concern and economic costs to restore ecosystem services, cyanobacterial blooms remain difficult to manage (Landsberg 2002; Wilhelm et al. 2020).

Predicting and managing cyanobacterial blooms require understanding their environmental and ecological drivers. Nutrient supply and temperature are among the suite of factors contributing to blooms (Carey et al. 2012). Both are strongly influenced by anthropogenic activities at multiple scales and subject to targeted management (Paerl and Huisman 2009). Eutrophication, including both high total N and P or high P only (e.g., for N-fixing species), encourages cyanobacteria biomass accumulation and toxin production (Wagner et al. 2019; but see Cottingham et al. 2015). Higher temperatures also promote accelerated growth and toxin production in these species (Walls et al. 2018; but see Rose and Caron 2007). By escalating thermal stratification and increasing surface heatwave events within lakes (both of which can unfold over short, e.g., weekly, timescales), climate warming may favor the occurrence of blooms (Carey et al. 2012; Woolway et al. 2021).

Many studies have investigated the effects of temperature and nutrients on cyanobacterial growth and toxin production, but predominantly under constant conditions (Paerl and Huisman 2009; but see Peng et al. 2018). It is unclear how well such results apply to populations experiencing dynamic lake environments, especially over the short-term daily to weekly timescales (see Figure S1). Conditions in aquatic ecosystems are variable across time (e.g., substantial diel temperature fluctuations exist in small lakes; Figure S1a; Woolway et al. 2016) and space (e.g., temperature can vary 10–15°C across depths in stratified summer conditions; Figure S1b; Kremer et al. 2018). In response to environmental changes, many microbial populations exhibit phenotypic plasticity (nongenetic alterations to behavior, physiology, development, etc.; Yvon-Durocher et al. 2017; Kremer et al. 2018; Rescan et al. 2020). For example, phytoplankton can upregulate heat-shock protein production during prolonged periods of thermal stress (Magni et al. 2018), alter their stoichiometry in cold or nutrient-rich environments (Yvon-Durocher et al. 2017), and move to find suitable thermal environments (i.e., thermotaxis; Sekiguchi et al. 2018). These changes, when in response to the thermal environment, can be referred to as thermal acclimation. Thermal acclimation, like other phenotypic changes, may occur gradually relative to rates of environmental change, causing ecological outcomes in variable environments to diverge from predictions based on performance in constant conditions (Kremer et al. 2018; Rescan et al. 2020). As temperature and nutrients vary concurrently in natural systems and phenotypic (i.e., acclimation) responses to temperature changes may require specific nutrients, it is critical to examine whether acclimation effects on cyanobacterial growth persist under different nutrient regimes and if these effects extend to cyanotoxin production.

Here, we test the hypotheses that thermal acclimation alters both physiological (i.e., toxin production) and demographic responses of cyanobacteria to temperature perturbations. Previous research shows that thermal acclimation can influence growth rates of eukaryotic phytoplankton (Kremer et al. 2018). Whether these impacts extend to cyanobacteria populations and cyanotoxin production, or are mediated by nutrients regimes, remains unclear. To address this, we investigated how thermal acclimation affects (1) the growth rates of ecologically important cyanobacterial taxa, including *Anabaena* (*Dolichospermum*) *flos-aquae*, *Phormidium foveolarum*, and two strains of *Microcystis aeruginosa*, and (2) the production of microcystin (MC), one of the most potent and common cyanotoxins (Harke et al. 2016), by *M. aeruginosa*. We select these taxa because they are common across North America (National Aquatic Resource Surveys 2012) and are sufficiently tolerant of laboratory conditions. We focus our efforts on examining how thermal acclimation temperature (i.e., previously experienced temperature) affects responses to a range of acute (current) temperatures and whether nutrient limitation influences acclimation. Acknowledging and developing pathways to incorporate thermal acclimation into cyanobacterial bloom forecasting is especially pertinent amidst existing and anticipated patterns of environmental variation.

Materials and methods

Experiment 1: Thermal acclimation and cyanobacteria performance

We experimentally measured the population-level responses (i.e., exponential growth rate) of cyanobacteria to temperature variation in low- and high-nutrient environments. We studied *A. flos-aquae*, *P. foveolarum*, and a toxic and nontoxic strain of *M. aeruginosa* (from University of Texas, UTEX, Culture Collection of Algae, Austin, TX, USA; UTEX Number 2558, 427, 2385, and 2386, respectively). To manipulate temperature, we used a thermal gradient block (TGB)—a rectangular block of solid aluminum metal heated on one end and cooled on the other. Wells set into the block at nine different positions allowed us to grow cultures at distinct temperatures (15.7°C, 18.5°C, 22.1°C, 25.4°C, 28.6°C, 32.0°C, 35.7°C, 39.1°C, 42.6°C; Figures S2 and S3). Prior to our experiments, we acclimated all species to each of these temperatures for 2 weeks in both high-nutrient conditions (COMBO medium, 1000 μmol/L N and 50 μmol/L P, representative of a hypereutrophic system) and low-nutrient conditions (10 μmol/L N and 0.5 μmol/L P, analogous to an oligotrophic system; see Table S1; Abell et al. 2010). We diluted all populations periodically during the acclimation phase to maintain exponential growth and keep nutrient conditions close to their starting values, though it is likely these values decreased following the start of the experiments.

Next, we measured acute thermal performance curves (TPCs) by exposing differently acclimated taxa (see Table 1) grown in both nutrient conditions (following Kremer et al. 2018) to all nine distinct temperatures across the TGB. "Fully acclimated" populations reflect the growth rates of populations held under constant conditions (i.e., acclimated to and maintained at each experimental temperature). "Cold-acclimated" reflect populations acclimation to a temperature below their thermal optima (here, the coldest temperature achievable on the TGB design) and "hot-acclimated" reflect populations acclimated to a temperature above their thermal optima (warmest temperature permitting positive growth for each taxon). This design allows us to test how various perturbations from hot and cold initial conditions affect acute growth rates relative to fully acclimated populations. We established three replicate populations ($N = 3$) per treatment by inoculating 3 ml of sterile media in 12×75-mm test tubes with ~50,000 cells/ml (for *P. foveolarum* and *A. flos-aquae*) or ~100,000 cells/ml (for both strains of *M. aeruginosa*), chosen to keep populations growing exponentially. We measured fluorescence (a proxy for cell density) of each culture four times from 0 to 40 h (see Figure S4 for details) using a Trilogy Laboratory Fluorometer (Turner Designs, Inc, San Jose, CA, USA) with a chlorophyll a in vivo module. We confirmed a linear relationship ($R^2 > 0.99$) between fluorescence and particle (cell) counts (from a Spectrex Laser Particle Counter, Spectrex Corp., Redwood City, CA, USA) for each species and across treatments (Figure S5).

Table 1. Cold- and hot-acclimation temperatures for all species used in Experiment 1 and for *Microcystis aeruginosa* (toxic) in Experiment 2.

Species	Cold-acclimated temperature (°C)	Hot-acclimated temperature (°C)
Anabaena (*Dolichospermum*) *flos-aquae*	15.7	39.1
M. aeruginosa (nontoxic)	15.7	35.7
M. aeruginosa (toxic)	15.7	39.1
Phormidium foveolarum	15.7	42.6

We performed all analyses in R (R Core Team 2019), unless otherwise noted. We estimated growth rate by calculating the slope of the linear relationship between ln(fluorescence) and time (days) for each replicate population (Figure S4; using the growthTools package, DOI:10.5281/zenodo.3634918; Layden 2020). We then used these growth rates to generate TPCs (relating growth rate to temperature, Figure 1), which we characterized using generalized additive models (GAMs) via the mgcv package (Wood 2017). To examine which factors influence TPCs, we fit and compared three different GAMs using Akaike information criterion (AIC). These include (GAM 1) TPCs are independent of acclimation temperature, (GAM 2) acclimation temperature influences the elevation of TPCs, and (GAM 3) acclimation temperature influences the elevation and shape of TPCs (Table S2). We conducted separate model comparisons for each species and nutrient condition to facilitate interpretation of results. We used the best fit model (i.e., the model with the lowest AIC, GAM 3) for the remaining analyses. To test how often cold- or hot-acclimated populations differed in growth rates from fully acclimated populations, we considered the overlap of 95% confidence bands between respective TPCs. Lastly, we quantified the magnitude of under- or over-performance of hot- and cold-acclimated populations relative to fully acclimated populations (see Table S3 for details). We consider instances where treatments exhibited nonoverlapping confidence intervals to be statistically significantly different.

Experiment 2: Thermal acclimation and cyanobacterial toxin production

We measured MC production in the toxic strain of *M. aeruginosa* following a temperature perturbation. Using both low- and high-nutrient conditions, we acclimated populations below, near, and above their thermal optima (15.7°C, 28.6°C, or 39.1°C) for 2 weeks. Subsequently, we inoculated replicate populations ($N = 6$) at ~100,000 cell/ml

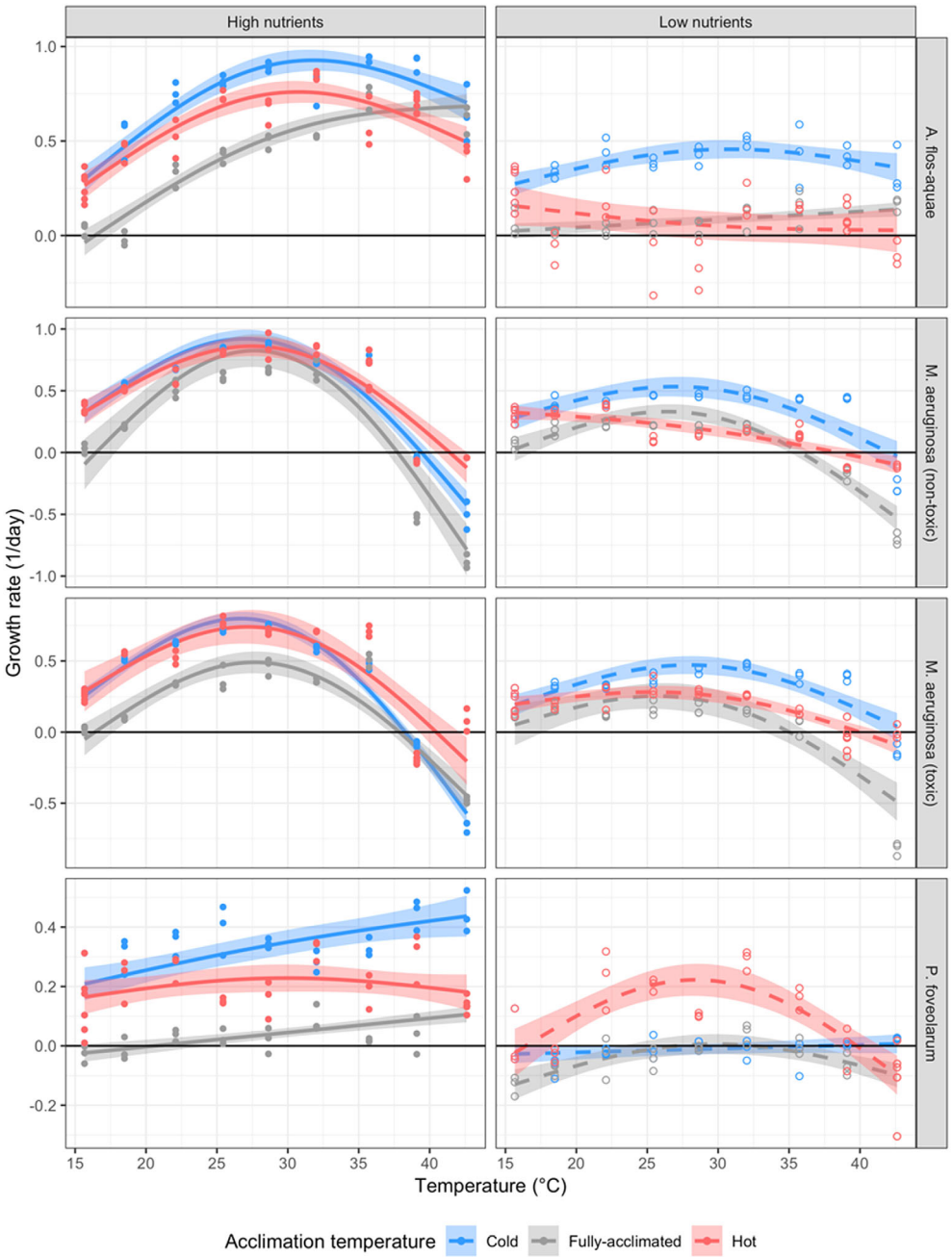

Fig. 1. Thermal performance curves for three freshwater cyanobacteria species. Growth rates vary with temperature and the acclimation temperature of populations. Specifically, populations acclimated to two different environmental conditions, cold (blue; 15.7°C) and hot (red; 39.1°C, 35.7°C, 39.1°C, and 42.6°C for *Anabaena* (*Dolichospermum*) *flos-aquae*, *Microcystis aeruginosa* (nontoxic), *M. aeruginosa* (toxic), and *Phormidium foveolarum*, respectively), perform differently from fully acclimated populations (gray) in high (left) and low (right) nutrient conditions. *Y*-axis is scaled by species. Horizontal line represents zero growth. Curves represent generalized additive model (GAM) regression fits and shading represents 95% confidence bands. Data correspond to Experiment 1 methods.

in 12 × 75-mm test tubes in a 28.6°C Percival Incubator (Percival Scientific, Inc., Perry, IA, USA). This temperature (28.6°C) maximized growth in fully acclimated, toxic *M. aeruginosa* (Figure 1). We measured the initial and final fluorescence of populations after 5 days, at which point we also measured MC concentrations (using ELISA EP-022, QuantiPlate™ Kit, Envirologix, Inc., Portland, ME, USA; see procedures in Appendix S1; Layden 2020). Although these thermal perturbations are simple relative to what cyanobacteria will experience in aquatic ecosystems, they have plausible

magnitudes (Figure S1) and provide an initial step in assessing whether acclimation meaningfully alters rates of toxin production.

In addition to quantifying total MC content over 5 d, we also estimated the per cell MC production rate (day^{-1}). To accomplish this, we used the following model of population growth and toxin production:

$$\frac{dN}{dt} = rN,$$
$$\frac{d\tau}{dt} = xN - l\tau. \quad (1)$$

Here, the density of cyanobacteria cells, N, changes exponentially over time with growth rate r. Toxin, τ, is produced at a per cell rate of x and degrades at rate l. The analytical solution of Equation (1) provides the time dynamics of the toxin, $\tau_t = \tau_0 e^{-lt} + \frac{1}{l+r}\left[x\mathrm{N_t} - x\mathrm{N_0}e^{-lt}\right]$, where N_0 and τ_0 are the initial cell density and toxin concentration, while N_t is the final cell density. Solving for x, we get:

$$x = (l+r)\left(\frac{\tau_t}{N_t - \frac{N_0}{e^{lt}}} - \frac{\tau_0}{N_t e^{lt} - N_0}\right). \quad (2)$$

Importantly, the above expression demonstrates per cell toxin production x is not simply equivalent to the total MC content divided by the final cell density (τ_t/N_t).

Empirically, we can estimate x using Equation (2). We have direct measurements of r by taking ln(final/initial cell density)/5 d. We obtained cell density values using the established linear relationship between fluorescence and particles/ml (from a FlowCam 5000, Flow Imaging Particle Analyzer, Fluid Imaging Technologies, Inc., Scarborough, ME, USA) for each nutrient condition for toxic *M. aeruginosa* and confirmed these relationships persisted over relevant timescales (Figure S6(a),(b)). Previous research has indicated the importance of temperature for influencing cell size (Martin et al. 2020), while not considered in this study, could influence the relationship between density and biovolume. Finally, we assume negligible initial MC concentrations ($\tau_0 \approx 0$) and a loss rate of $l = 0.09$/day (given a half-life of 7 days reported by Zastepa et al. 2014).

We used two-way analysis of variances (ANOVAs) to determine whether differences in growth rate, total MC, and per cell MC production rate was explained by acclimation temperature, nutrient condition, or their interaction. These analyses revealed a dramatic effect of nutrient condition; low-nutrient treatments also displayed substantially less variation (depicted in Figure 2). Consequently, to assess pairwise differences between groups due to acclimation temperature, we performed post hoc Tukey tests on the results of two separate one-way ANOVAs (one for each nutrient condition) per response variable.

Synthesis of Experiments 1 and 2: Implications of acclimation for cyanobacterial bloom formation

To explore the sensitivity of toxic bloom formation to acclimation, we used our results from Experiment 2 to predict MC and biomass accumulation over 5 d of exponential growth for *M. aeruginosa* populations acclimated to different temperatures (cold, hot, and fully acclimated) under favorable conditions (high nutrients and approximate acute temperature that maximizes growth). We assumed initial populations had 13,500 cells/ml, half the mean level for *M. aeruginosa* across 94 sites spanning 31 states in the summer (National Aquatic Resource Surveys 2012), and no MC. We used our estimated growth and per capita toxin production rates (from Experiment 2) at 28.6°C (see Figure 2). Then, we calculated cell density and MC concentration (μg/L) over a 5-day period at 28.6°C using Equation (1) in Mathematica v. 12.0 (Wolfram Research, Inc.). This period matches the duration of the toxin assay above. We compared these values to thresholds of a MC concentration of 1.6 μg/L (above which water is unsafe for adults to drink according to the US EPA) or a density of 100,000 cells/ml (bloom threshold defined by the World Health Organization; D'Anglada and Strong, 2015). We also used this approach to explore the consequences of two assumptions used in Experiment 2: (1) the half-life of MC (Figure S7) and (2) the duration of acclimation effects persisting over time (Figure S8).

Results

Experiment 1: Thermal acclimation and cyanobacteria performance

Acclimation temperature strongly influenced the performance (growth rate) of all species across a range of acute temperatures, under low- and high-nutrient conditions. In all cases, models allowing thermal acclimation to affect both the elevation and shape of TPCs (GAM 3) performed best (Table S2). In addition, across the majority of acute temperatures (60% for cold-acclimated populations and 64% for hot-acclimated populations), there was a significant difference in growth rates relative to fully acclimated populations (Figure 1). The growth rates of cold-acclimated populations outpaced those of fully acclimated populations across 65% of acute environments, with the most extreme differences generally arising in hot and low nutrient environments (Figure 1, gray vs. blue; Table S3). In contrast, hot-acclimated populations exhibited reduced growth and toxin production in nearly all cases. Across 75% of acute environments, growth rates were reduced (Figure 1, gray vs. red).

Experiment 2: Thermal acclimation and cyanobacterial toxin production

Acclimation temperature, nutrient availability, and their interactions also impacted growth rate and both total and per capita toxin production in *M. aeruginosa* over a 5-day incubation period (Figure 2; Table S4). In this case, cold-acclimated

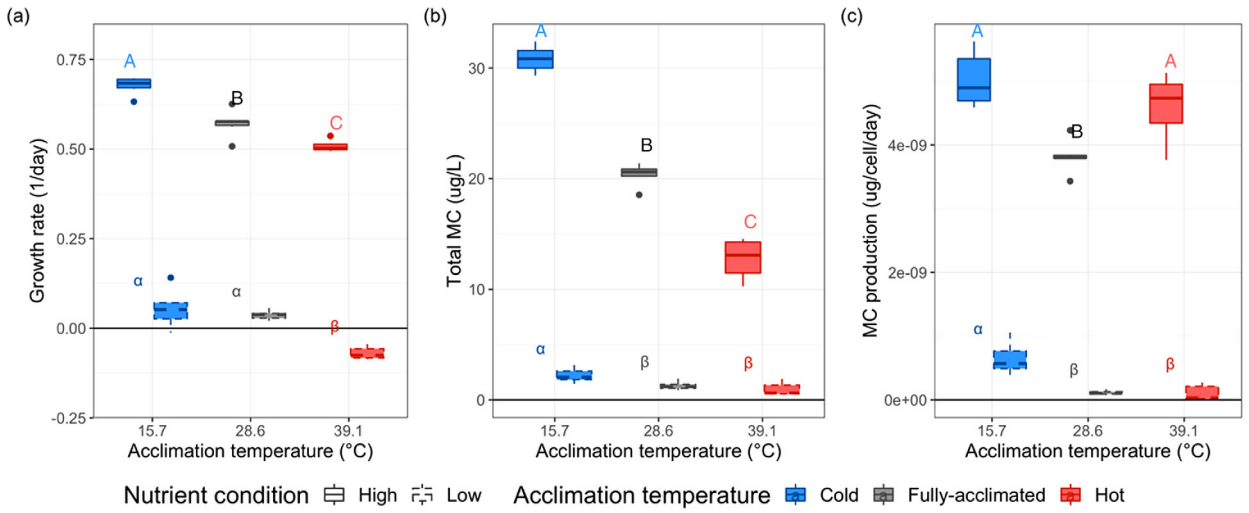

Fig. 2. *Microcystis aeruginosa* (toxic) microcystin (MC) production and growth. (a) Population growth rates (day^{-1}), (b) total concentration of MC (μg/L) accumulated over 5 days, and (c) per cell MC (μg) production rates (day^{-1}) of *M. aeruginosa* (toxic) populations acclimated to three acclimation temperatures: Cold (15.7°C), hot (39.1°C), and fully acclimated (28.6°C) after 5 days of exposure to the same environmental temperature (28.6°C) in both high-nutrient condition (solid) and low-nutrient condition (dashed). These conditions reflect Experiment 1 at the combination of acute and acclimation temperatures that achieved maximum growth. Letters above each boxplot depict significant treatment differences based on the results of Tukey tests on separate one-way analysis of variance (ANOVAs) for high-nutrient condition (alphabets A–C) and low-nutrient condition (Greek characters α, β). Data correspond to Experiment 2 methods.

populations experienced a 51% (high nutrients) and an 80% (low nutrients) increase in total MC relative to fully acclimated populations, when both were grown at 28.6°C (Figure 2(b)). Accounting for growth rate differences (Figure 2(a)) and MC decay (Equation (2)) reveals substantial impacts of acclimation history on per cell toxin production rate (Figure 2(c)). Here, cold-acclimated populations produced more MC per cell per day than fully acclimated populations (a 32% and 473% increase in high- and low-nutrient environments, respectively; Figure 2(c)). Meanwhile, hot-acclimated *M. aeruginosa* (toxic) populations produced fewer total toxins than cold-acclimated populations, though this was likely mediated by slower growth (Figure 2(a),(b)). Hot-acclimated populations also produced the least amount of toxins in low nutrients under these same experimental conditions (Figure 2(b),(c)).

Implications of acclimation for cyanobacterial bloom formation

We estimate that the combined effects of the differences in growth rates and per cell toxin production rates (from Experiment 2) can allow cold-acclimated populations to reach thresholds that constitute a harmful algal bloom roughly 1 day faster than fully acclimated populations in favorable environments (Figure 3).

Discussion

Our results indicate that acclimation temperature has substantial influences on cyanobacteria growth rates and toxin production in both high- and low-nutrient environments. Acclimation-driven effects on growth rate approached an order of magnitude higher than that of fully acclimated populations (Table S3; mean = 153%), and similarly, acclimation temperature resulted in up to a threefold change in toxin accumulation observed over a 5-day period. However, despite having similar magnitude impacts as nutrient regime alone (Jiang and Zheng 2018), thermal acclimation is yet to be included among the many factors that have been reported to influence growth and toxin production (e.g., temperature, nutrients, light, metal ions, and pollutants; Dai et al. 2016). Given recent worldwide increases in cyanobacterial, and in particular *Microcystis* blooms, and the existing uncertainly surrounding the formation of these blooms in nature (Wilhelm et al. 2020), we posit that thermal acclimation may be an underappreciated mediator of rapid growth and toxin production.

The single thermal perturbations used in our experiments are deliberately simplified relative to patterns of temporal variability that phytoplankton will likely encounter in nature. Given that thermal acclimation is a short-term, reversible process unfolding within ~2 weeks (Kremer et al. 2018), single perturbations will have lasting impacts only if events are capable of producing priority effects—a benefit obtained from reaching high abundances (e.g., altering pH or reducing herbivore grazing pressure; Gremberghe et al. 2009, Wilhelm et al. 2020). Such high-magnitude perturbations would be most likely to occur during recruitment of benthic populations migrating from the cold sediment to warm

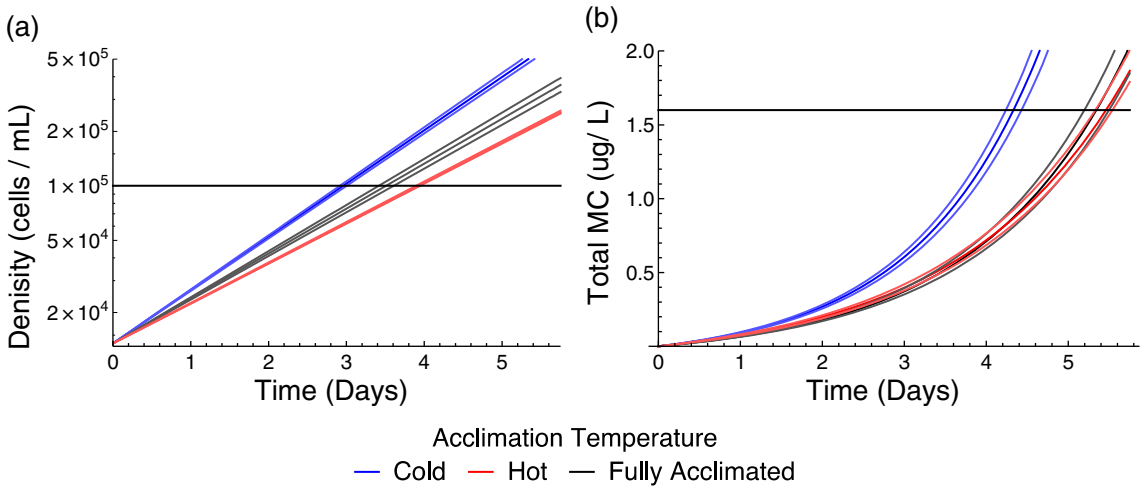

Fig. 3. *Microcystis aeruginosa* (toxic) growth and microcystin (MC) accumulation. (a) Predicted cell density (cells/ml) and (b) MC concentration (μg/L) over a 5-day period in high-nutrient conditions at 28.6°C. Growth rate (1/day) and toxin production rate (μg MC/cell/day) was derived from Experiment 2 for *M. aeruginosa*. Colors represent temperature treatments used in Experiment 2: cold (blue, 15.7°C), hot (red, 39.1°C), and fully acclimated (gray, 28.6°C). Horizontal lines depict harmful bloom thresholds based on density and toxin concentrations (see Materials and Methods section).

surface waters or during periods of rapid warming (Figure S1). However, because most freshwater environments undergo continuous shifts across space and time, it is plausible that acclimation due to less dramatic, but more frequent changes relative to our experimental conditions, could produce sustained changes in growth and toxin production. Indeed, our data indicate a large departure from fully acclimated responses for subtler, albeit more common (Figure S1), thermal transitions (e.g., growth rate increased 28.5% on average from 15.7°C to 22.1°C in high-nutrient conditions across all species; Figure 1).

The mechanisms driving thermal acclimation and the timescales over which they operate deserve further study. Freshwater phytoplankton exhibit flexible stoichiometry in response to shifting environmental conditions, such as temperature and nutrient availability. Recent research shows that nitrogen and phosphorus stores decrease with increasing temperature (Yvon-Durocher et al. 2017). This is consistent with the asymmetric patterns we observed in growth rates, where cold-acclimated populations overperform (potentially by capitalizing on stored nutrients) and hot-acclimated populations underperform, relative to acclimated expectations. Phytoplankton exposed to warm environmental temperatures for extended periods of time may also invest in energetically costly heat-shock responses that may allow cold acclimated populations to gain a short-term advantage (Magni et al. 2018), potentially explaining why the overperformance of cold-acclimated populations is temporary. Meanwhile, the cost of producing MC may contribute to the diminished performance of hot-acclimated populations, especially considering the comparable per cell toxin production at high nutrients. In addition, while these experiments focused on thermal acclimation, acclimation to short-term changes in other environmental variables likely also impacts these species (Rescan et al. 2020), representing an important area for future research.

In conclusion, our results demonstrate that past thermal environmental exposures play an important role in determining growth and toxin production in cyanobacteria, and that acclimation to novel conditions can occur gradually relative to the pace of environmental change. Our study identifies a gap in current paradigms of bloom formation, suggests that thermal acclimation is a salient ecological process, and offers a novel approach to quantifying per capita cyanotoxin production rate. Yet, future studies should investigate how more complicated characteristic features of the thermal environmental variation can mediate the effects of acclimation. As freshwater ecosystems worldwide experience environmental change—including increasing diel variation in surface temperatures, episodic events, or altering the extent of thermal stratification (Maberly et al. 2020; Woolway et al. 2021)—a concerted, physiologically grounded focus on organismal responses to temperature variability may be critical to anticipating bloom dynamics.

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