Thermal acclimation influences the growth and toxin production of freshwater cyanobacteria

Authors

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Table 1. Description of the creation of corresponding datasets.

Title of dataset	Cyanobacteria performance measures in response to laboratory-induced temperature perturbations conducted in 2019
URL of dataset	https://github.com/laydent/thermal_acc_and_cyanos
Abstract	Understanding how altered temperature regimes affect the onset of harmful cyanobacterial blooms is essential for managing aquatic ecosystems amidst ongoing climate warming. This is difficult because algal growth and 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 time-scales) affects population growth and toxin production given acclimation. We studied four ecologically important freshwater cyanobacterial strains under nutrient-limited and -replete conditions, measuring the growth rate of populations fully acclimated to a range of temperatures. Cold-acclimated populations outperformed fully-acclimated populations across 65% of thermal environments, while hot-acclimated populations underperformed across 75% of thermal environments. Cold-acclimated *Microcystis aeruginosa* produced ~2.5-fold more microcystin per day than hot-acclimated populations following 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.
Keywords	Phenotypic plasticity, environmental variability, harmful algal blooms, temperature, acclimation, cyanobacteria
Lead author for the dataset	Tamara Layden
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Organization associated with the data	NA

Funding	Samuel Fey; funders include Reed College and the National Science Foundation (NSF DEB 1856415 and NSF DEB 1856279)
License	CCBY
Geographic location – verbal description	Reed College, Portland, Oregon, United States
Geographic coverage bounding coordinates	45.4811° N, 122.6308° W
Time frame - Begin date	6/10/2019
Time frame - End date	10/2/2019
General study design	Generating Cyanobacteria Growth Dataset ("Cyano_growth_data.csv"): After exposing four cyanobacteria taxa to three distinct thermal histories for two weeks, we measured population-level exponential growth rate (1/day) of all strains cultured across a thermal gradient (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) for 40 hours. We conducted this and subsequent experiments using nutrient replete (high N and P concentrations) and nutrient limited (low N and P concentrations) freshwater media. Generating Cyanotoxin Dataset ("Cyano_toxin_data.csv"): After exposing the toxic strain of Microcystis aeruginosa to three distinct thermal histories (15.7°C, 28.6°C, or 39.1°C) for two weeks, we measured initial and final fluorescence and microcystin concentration following a 5-day temperature perturbation of 28.6°C.
Methods description	Generating Cyanobacteria Growth Dataset: We experimentally measured the population-level responses (i.e. exponential growth rate) of cyanobacteria to temperature variation in nutrient-replete and nutrient-limiting environments. We studied *A. flos-aquae*, *P. foveolarum*, and a toxic and non-toxic strain of *M. aeruginosa* (from University of Texas, UTEX, Culture Collection of Algae, Austin, TX, USA; UTEX Number 2558, 427, 2385, and 2386, respectively). We selected these taxa because they are nationally common (based on the most recent (2012) US EPA's National Aquatic Resource Surveys data, retrieved Dec 12, 2019, from: https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys), commercially available, and tolerant of laboratory conditions. To control growth temperatures, we used a thermal gradient block (TGB). The TGB is comprised of a 0.88 m x 0.29 m aluminum block with channels on either end through which hot (left, red) and cold (right, blue) water are pumped, developing a stable, gradual gradient of hot to cold temperatures. 8 temperature sensors equally distributed along the TGB provide real-time temperature monitoring. Each block contains a total of 27 equally spaced wells: 9 across the length of the temperature gradient by 3 along the width. Each well holds up to 6 replicate 12x75mm test tubes (pictured with white opaque caps) suspended in deionized water that acts as a heat conductive medium between the blocks and the samples. Wells are covered at the bottom with clear Plexiglass and are lit from below by full spectrum, 5w LEDs (380-850nm, Shenzhen Chanzon Technology Co., China). The horizontal temperature gradient remained stable throughout the experiment

($\pm$ 0.5°C; monitored using temperature sensors and manual probe readings) and light intensities fell within the range of 66.52 (mean $\pm$ 4.32 SE) µmol m^{-2} s^{-1} with no significant difference across the TGB ($F_{1,25}$ = 1.505, p = 0.23; measured spectrophotometerly).

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). Prior to our experiments, we acclimated all species to the nine temperatures across the TGB for a period of two weeks in both nutrient-replete (COMBO medium, most representative of a hypereutrophic system) and nutrient-limited conditions (1% N and P relative to COMBO, analogous to an oligotrophic system). To create and modify media, we used Kilham et al. 1998 COMBO protocol (DOI:10.1023/A:1003231628456). We diluted all populations periodically during the acclimation phase to promote exponential growth.

We next measured the acute and acclimated thermal performance curves (TPCs) of all species and strains under both nutrient conditions (following Kremer et al. 2018) to obtain "cold", "hot", and "fully-acclimated" curves. Cold TPCs consist of the short-term (or acute) growth rates of populations acclimated to 15.7°C and grown at all nine experimental temperatures. We obtained hot TPCs similarly, although the acclimation temperature varied by species to reflect the warmest temperature permitting positive growth (35.7°C, non-toxic *M. aeruginosa*; 39.1°C, toxic *M. aeruginosa* and *A. flos-aquae*; 42.6°C, *P. foveolarum*). Fully-acclimated TPCs reflect the growth rates of populations acclimated to and maintained at each temperature. This design allows us to test how both subtle and high magnitude thermal perturbations from hot and cold initial conditions compare to fully-acclimated populations in a given acute environment. We established three replicate populations (N=3) per treatment by inoculating in 3 mL sterile growth media in test tubes with a starting density of ~50,000 cells/mL (for *P. foveolarum* and *A. flos-aquae*) or ~100,000 cells/mL (for both strains of *M. aeruginosa*) to keep populations in exponential phase. We measured the fluorescence (proxy for cell density) of each culture four times over 40 hours using a Trilogy Laboratory Fluorometer (Turner Designs, Inc, San Jose, CA, USA) with a chlorophyll-a non-acidification module.

Generating Cyanotoxin Dataset:

We measured microcystin (MC) production in the toxic strain of *M. aeruginosa* following a temperature perturbation. Using both nutrient-limited and replete conditions, we first acclimated populations to one of three temperature conditions: 15.7°C, 28.6°C, or 39.1°C, for a period of two weeks. Subsequently, we inoculated replicate populations (N = 6) at ~100,000 cell/mL in 12x75mm test tubes in a 28.6°C Percival Incubator (Percival Scientific, Inc., Perry, IA, USA). This temperature (28.6°C) maximized growth in toxic *M. aeruginosa*. We measured the initial and final fluorescence of populations after five days, at which point we also measured MC concentrations of sample replicates (using ELISA EP-022, QuantiPlate^{TM} Kit, Envirologix, Inc., Portland, ME, USA).

To obtain MC concentration values, we first diluted samples grown in the nutrient-replete media by a factor of 10-15 and samples grown in the nutrient-limited media by a factor of 4. We then prepared duplicates of each sample for toxin analysis by splitting all replicates within each treatment in

	half (1.5 ml of cells suspended in respective liquid media) and adding them to 2 ml centrifuge tubes. To release MC content from the cells, we completed three cycles of freezing (-80°C), thawing, sonicating (1 minute in sonicating water bath), and vortexing (5 seconds), following protocols developed by Banack et al. 2015 (DOI: 10.3390/toxins7020322). We then centrifuged the tubes at 1200 rpm for 5 minutes and retrieved the supernatant for MC concentration (in ppb) analysis using an enzyme-linked immunosorbent assay (ELISA EP-022, QuantiPlateTM Kit, Envirologix, Inc.,Portland, ME, USA). This method does not distinguish between MC variants (four possible microcystin types include MC-LR, MC-LA, MC-RR, or MC-YR).
Laboratory, field, or other analytical methods	Generating Cyanobacteria Growth Dataset & Analysis: **See "Cyano_TPC_stats_code" R script for more details on the following analyses.** We performed all analyses in R (R Core Team 2019), unless otherwise noted. We used the slope of the linear relationship between log fluorescence and time in days to represent growth rate (calculated in R using the growthTools package, DOI:10.5281/zenodo.3634918). These values are provided in the "Cyano_growth_data.csv" dataset. We then used these growth rates to generate TPCs (relating growth rate to temperature) characterized using generalized additive models (GAMs) via the mgcv package (Wood 2017, DOI:10.1201/9781315370279; see "Cyano_TPC_code" R script for more details). To verify the chosen model, we tested three hypotheses using AIC for three different GAM-fits (binned by species and nutrient condition): (1) TPCs are independent of acclimation history (GAM 1, generates a single intercept and curve for compiled data), (2) acclimation history influences the elevation of TPCs (GAM 2, generates different intercepts by acclimation temperature), and (3) acclimation history influences the elevation and shape of TPCs (GAM 3, generates different intercepts and curve shapes by acclimation temperature). We used the best fit model (3) for the remaining statistical analysis, binned by species and nutrient condition. To test how often cold- or hot-acclimated populations differed in growth rates from fully-acclimated populations, we quantified the extent of overlap in CIs between respective TPCs and considered instances where treatments exhibited non-overlapping confidence intervals to be statistically significantly different. Lastly, we quantified the magnitude of under- or over-performance of hot- and cold-acclimated populations relative to fully-acclimated populations by subtracting the fully-acclimated growth rates (i.e. "expected") from respective cold- or hot- acclimated growth rates (i.e. "observed") and divided this by the absolute value of "expected," all averaged over replicates. Generating Cyanotoxin Dataset & Analysis: **See "Cyano_toxins_code" R script for more details on the following analyses.** Sample dilutions prior to analyses allowed us to obtain concentration values within the limits of detection set by the ELISA standards (0-2 ppb). We used the exponential decay curve (MC concentration by absorbance at 450nm) generated using the ELISA standards to predict concentration values (in ppb

	of MC) for the samples over the same absorbance spectrum, averaged over duplicates. We later corrected these values based on their respective dilution factors.
	In addition to total MC content over 5 days, we estimated the per cell MC production rate (day^{-1}). We estimated cell counts using an established linear relationship ($R^2 = 0.99$) between raw fluorescence units and particle (cell) counts (from a Spectrex Laser Particle Counter, Spectrex Corp., Redwood City, CA, USA). To infer the per cell toxin production rate, x, we then used the following model:
	$$\frac{dN}{dt} = rN$$ $$\frac{d\tau}{dt} = xN - l\tau$$ [E.1]
	Here, the density of cyanobacteria cells, N, changes exponentially over time with growth rate r. Toxin, τ, is produced by each cell at rate x and degrades at rate l. The analytical solution of E.1 provides the time dynamics of the toxin, $\tau_t = \tau_0 e^{-lt} + \frac{1}{l+r}[x\,N_t - x\,N_0 e^{-lt}]$, where N_0 and τ_0 are the initial population size and toxin concentration. Solving for x, we get:
	$$x = (l + r)\left(\frac{\tau_t}{N_t - N_0/e^{lt}} - \frac{\tau_0}{N_t\,e^{lt} - N_0}\right)$$ [E.2]
	Importantly, the above expression demonstrates per capita toxin production x is not simply equivalent to the total MC content divided by the final cell density (τ_t/N_t). However, knowing r, we can assume a negligible initial MC concentration ($\tau_0 \approx 0$) and a loss rate of $l = 0.09$/day (given a half-life of 7 days reported by Zastepa et al. 2014, DOI:10.1080/10807039.2013.854138) to estimate x using E.2.
	Having quantified growth rate, total MC, and MC production per cell, we used two-way ANOVAs to determine whether variation in these quantities was explained by acclimation history, nutrient condition, or their interaction. These analyses revealed a dramatic effect of nutrient condition; nutrient-limited treatments also displayed substantially less variation. Consequently, to assess pairwise differences between groups due to acclimation history, we performed post hoc Tukey tests on the results of two separate one-way ANOVAs (one for each nutrient condition) per response variable.
Taxonomic species or groups	Cyanobacteria, *including Anabaena* (*Dolichospermum*) *flos-aquae*, *Phormidium foveolarum*, and two strains of *Microcystis aeruginosa* (toxic and non-toxic)
Quality control	Each dataset was appropriately scanned and visualized using R to check for inconsistencies, outliers, and potential errors.
Additional information	NA

Table 2. Data dictionary: description of the variables in each dataset.

Dataset filename: *Cyano_growth_data.csv*

Dataset description: *Measurements of growth (1/day) for four cyanobacteria strains across a thermal gradient*

Column name	Description	Units	Code explanation	Data format	Missing data code
Species	Focal species/strain of cyanobacteria	NA	Abbreviated genus; toxic vs. non-toxic strain of *M. aeruginosa* indicated in parenthesis	NA	NA
Sample_ID	Well placement on TGB	NA	1=coldest, back of TGB to 27=hottest, front of TGB (read back to front across TGB from cold to hot end)	NA	NA
Treatment	Discrete acclimation temperature ID's across TGB	NA	1-9 representing the 9 distinct temperatures across the TGB from coldest to hottest.	NA	NA
Nutrient.tx	Nutrient condition	NA	LOW=Limited, NORM=Replete	NA	NA
Acute.temp.id	Discrete acute temperature ID's across TGB	NA	1-9 representing the 9 distinct temperatures across the TGB from coldest to hottest.	NA	NA
Replicate	Replicate populations (each in own test tube) and placement in individual well on TGB	NA	A-C=3 replicates for specified treatment, D-F=another 3 replicates for specified treatment; A-F all placed in a circle equidistant from one another along the inner circumference of one well in the TGB.	NA	NA
Acclim.temp	Discrete acclimation temperatures across TGB	Celsius	NA	Rounded to the nearest hundredth	NA
Acute.temp	Discrete acute temperatures across TGB	Celsius	NA	Rounded to the nearest hundredth	NA

Temp.tx	Acclimation treatment name	NA	Cold=cold-acclimated, Hot=hot-acclimated, Blank=fully-acclimated		NA
Medium	Media type	NA	B Limited=Limited, A Replete=Replete	NA	NA
mu	Best growth rate estimate (derived using growthTools package, DOI:10.5281/zenodo.3634918)	1/day	NA	NA	NA
best.model	Identity of the model that produced growth rate estimate (derived using growthTools package, DOI:10.5281/zenodo.3634918)	NA	gr=linear regression model	NA	NA
rsqr	R squared (derived using growthTools package, DOI:10.5281/zenodo.3634918)	NA	NA	NA	NA
lnf0	ln(fluorescence)	Raw Fluorescence Units (RFU)	NA	NA	NA
id	Distinct ID's for each sample	NA	Each ID corresponds to Species, Treatment, Nutrient.tx, Sample_ID, and Replicate	NA	NA

Dataset filename: *Cyano_toxin_data.csv*

Dataset description: *Measurements of fluorescence and microcystin concentration of M. aeruginosa following a temperature perturbation*

Column name	Description	Units	Code explanation	Data format	Missing data code
Species	Focal species/strain of cyanobacteria	NA	Abbreviated genus; toxic strain of *M. aeruginosa* only	NA	Not applicable to standards
Date	Date of measurement collection	NA	NA	M/DD/YY; Pacific Time Zone	NA
Treatment	Acclimation treatment (with respect to TGB	NA	1-9 representing the 9 distinct temperatures across	NA	NA

	conditions) and nutrient condition		the TGB from coldest to hottest; normal=replete nutrient condition, low=limited nutrient condition; other identifiers refer to the known concentrations of standards (controls and calibrators)		
Starting_Cond	Discrete acclimation treatment ID's (with respect to TGB conditions)	NA	1-9 representing the 9 distinct temperatures across the TGB from coldest to hottest.	NA	Not applicable to standards
Acute_temp	Acute temperature setting for duration of experiment	Celsius	NA	Rounded to nearest tenth	Not applicable to standards
Nutrients	Nutrient condition	NA	Normal=replete nutrient condition, low=limited nutrient condition, std=standard	NA	NA
Duplicate	Same sample population (i.e. replicate) split into two duplicate samples	NA	i=1 of 2 duplicates of replicate X, ii=2 of 2 duplicates of replicate X	NA	NA
Replicate	Individual replicate populations (each in own test tube)	NA	A-F=6 replicates for specified treatment	NA	Not applicable to standards
Abs_450	Absorbance at 450nm	NA	NA	NA	NA
Conc	Known microcystin concentration	ppb	NA	NA	Applicable to standards and controls only
RFU_raw_Final	Fluorescence measurements taken at the end of assay	Raw Fluorescence Units (RFU)	NA	NA	Not applicable to standards
RFU_raw_initial	Initial fluorescence measurements taken at assay	Raw Fluorescence Units (RFU)	NA	NA	Not applicable to standards

	start (5 days prior)				
Dilution_fact	Fraction to which samples were diluted	NA	NA	NA	Not applicable to standards

Table 3. Data provenance - NA

If you used data derived from other sources, provide the information here so future users know where the data came from.

Dataset title	Dataset DOI or URL	Creator (name & email)	Contact (name & email)

Scripts/code (software)

File name	Description	Scripting language
Cyano_TPC_code	Generates Thermal Performance Curves (TPCs) for each species in each nutrient condition	R
Cyano_TPC_stats_code	Statistical analyses of TPCs	R
Cyano_toxins_code	Calculates toxin production for *M. aeruginosa* (toxic), compares growth rates and toxin production for this species, and includes statistical analyses	R

Notes and Comments: