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# Local adaptation mediates direct and indirect effects of multiple stressors on consumer fitness

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## Abstract

Anthropogenic impacts are expected to increase the co-occurrence of stressors that can fundamentally alter ecosystem structure and function. To cope with stress, many organisms locally adapt, but how such adaptations affect the ability of an organism to manage co-occurring stressors is not well understood. In aquatic ecosystems, elevated temperatures and harmful algal blooms are common co-stressors. To better understand the role and potential trade-offs of local adaptations for mitigating the effects of stressors, *Daphnia pulicaria* genotypes that varied in their ability to consume toxic cyanobacteria prey (i.e., three tolerant and three sensitive) were exposed to five diets that included combinations of toxic cyanobacteria, *Microcystis aeruginosa*, and a green alga, *Ankistrodesmus falcatus*, under two temperatures (20 °C vs. 28 °C). A path analysis was conducted to understand how local adaptations affect energy allocation to intermediate life history traits (i.e., somatic growth, fecundity, survival) that maximize *Daphnia* fitness (i.e., population growth rate). Results from the 10-day study show that tolerant *Daphnia* genotypes had higher fitness than sensitive genotypes regardless of diet or temperature treatment, suggesting toxic cyanobacteria tolerance did not cause a decrease in fitness in the absence of cyanobacteria or under elevated temperatures. Results from the path analysis demonstrated that toxic cyanobacteria had a stronger effect on life history traits than temperature and that population growth rate was mainly constrained by reduced fecundity. These findings suggest that local adaptations to toxic cyanobacteria and elevated temperatures are synergistic, leading to higher survivorship of cyanobacteria-tolerant genotypes during summer cyanobacterial bloom events.

**Keywords** *Daphnia* · Toxic cyanobacteria · Harmful algal blooms (HABs) · Path analysis · Climate change

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Declaration of authorship: EGF and AEW conceived and designed the experiment. EGF performed the experiment, analyzed the data, and wrote the manuscript. AEW provided editorial advice.

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## Introduction

The ability of a population to manage stressors dictates its fitness, ultimately shaping population dynamics, community structure, and ecosystem function. The mechanisms through which adaptive traits improve fitness are complex given that the value of adaptive traits may vary based on the magnitude and duration of exposure as well as the presence of additional stressors that often lead to unpredictable responses. Anthropogenic activities have been shown to increase the severity, frequency, and co-occurrence of stressors, therefore, untangling the interactive effects of multiple stressors and the strategies employed by organisms to tolerate them is critical for increasing our basic understanding of ecological interactions as well as managing ecosystems (Nõges et al. 2016).

Aquatic ecosystems are particularly sensitive to anthropogenic stressors (Dudgeon et al. 2007). Factors, such as temperature increases, agricultural fertilizer run-off, and water flow disruptions, can affect the life history patterns and distribution of aquatic organisms by changing the physical (i.e., thermal stratification) and chemical (i.e., nutrient cycling) conditions of aquatic ecosystems (Hering et al. 2015). These changes are known to alter freshwater food web dynamics by expanding the spatial range and promoting the growth of undesirable or detrimental taxa (e.g., toxic or invasive species, Strayer 2010). Cyanobacteria thrive in eutrophic systems with elevated surface water temperatures. As such, anthropogenic impacts often disrupt freshwater aquatic food webs by promoting cyanobacterial growth and the competitive exclusion of ecologically important phytoplankton with lower temperature optima for growth, such as chlorophytes and diatoms (Jöhnk et al. 2008; Dupuis and Hann 2009). Cyanobacterial dominance can disrupt the trophic transfer of energy through aquatic food webs, as cyanobacteria lack essential polyunsaturated fatty acids necessary for zooplankton grazer growth, and many cyanobacterial species mechanically inhibit grazing by aggregating into colonies or producing long filaments (Müller-Navarra et al. 2000). Furthermore, some cyanobacterial species produce toxic secondary metabolites (i.e., cyanotoxins) known to elicit hepatotoxic (i.e., microcystin) or neurotoxic (i.e., anatoxin-*a* and saxitoxin) effects that may weaken or kill grazers or other phytoplankton (Lampert 1987; Wilson et al. 2006; Huisman et al. 2018). The common freshwater cyanobacterium, *Microcystis* spp., thrives in lentic systems worldwide under elevated temperature conditions and is known to reduce the fitness of some grazers by synthesizing microcystin, aggregating into colonies, and depriving grazers of unsaturated fatty acids necessary for growth (Dupuis and Hann 2009). As climate change increases global surface water temperatures, aquatic organisms will have to cope with thermal stress and cyanobacterial blooms more frequently and for longer periods (Griffith and Gobler 2020).

Ectothermic aquatic organisms, such as the large-bodied cladoceran genus *Daphnia,* are particularly sensitive to environmental changes. *Daphnia* are dominant generalist grazers of phytoplankton as well as an important prey for planktivorous fish in lakes (Dodson and Hanazato 1995; Ebert 2005). The metabolic rate of ectothermic organisms increases under elevated temperature conditions, resulting in accelerated maturation, somatic growth, population growth, and higher energy demands (Orcutt and Porter 1984; Lampert and Trubetskova 1996; Hietala et al. 1997; Paul et al. 2004; Masclaux et al. 2009). Elevated temperatures can interact with diet to exacerbate detrimental effects in *Daphnia*. Cyanobacteria, like *Microcystis*, are often sterol-deficient, but daphniid sterol demand generally increases with temperature leading to reduced fitness (Sperfeld and Wacker 2009; Przytulska et al. 2015). For example, Hietala et al. (1997) showed that *Daphnia pulex* fed the nutritious green alga, *Scenedesmus obtusiusculus,* had a higher rate of instantaneous increase (*r*) when cultured at 24 °C than 19 °C. Yet, when fed toxic *Microcystis aeruginosa,* the same *Daphnia* had lower *r* when cultured at 24 °C than 19 °C. Another study focused on *Daphnia magna* fed high- vs. low-quality food at different temperatures showed that the negative effect of food quality on somatic growth and reproduction decreased at higher temperatures (Masclaux et al. 2009).

The effect of temperature on *Daphnia* sensitivity to toxic cyanobacteria (Claska and Gilbert 1998) is likely to vary between species and locally adapted populations given known large variation for other ecologically relevant traits. The effect can also depend on several additional factors, including the intensity and duration of thermal stress as well as the specific cyanobacterial species, abundance and induced toxicity (Hochmuth and Schamphelaere 2014). Claska and Gilbert (1998) found that higher temperature treatments reduced the population growth rate, survivorship, and fecundity of *Daphnia pulex* when fed two toxic cyanobacterial species, *Anabaena flos-aquae* and *A. affini.* This observation was expanded by Hochmuth and Schamphelaere (2014) who tested the effect of temperature on the reproduction of *Daphnia magna* fed six cyanobacterial species and found that higher culture temperatures further reduced the reproduction of *Daphnia* fed *Anabaena* and *Oscillatoria,* but decreased the harmful effects on reproduction of *Daphnia* fed *Microcystis*, *Nodularia,* and *Aphanizomenon*.

While these past studies have examined the interaction between temperature and cyanobacterial exposure, few studies have explored how combined temperature and cyanobacterial stressors affect multiple genotypes of the same *Daphnia* species. Hietala et al. (1997) found intraspecific variations in the effect of stressors on life-history traits, but did not consider the role of adaptations due to previous

exposure to cyanobacteria. Recent research has shown that some *Daphnia* clones from eutrophic systems with frequent cyanobacterial blooms can locally adapt to toxic cyanobacteria (i.e., cyanobacteria-tolerant genotypes) and ultimately significantly reduce toxic cyanobacteria (Blom et al. 2006; Chislock et al. 2013, 2019b; Schwarzenberger et al. 2017). In contrast, *Daphnia* clones from oligotrophic systems with limited prior exposure to cyanobacteria (i.e., cyanobacteria-sensitive genotypes) cannot control cyanobacteria in situ (Chislock et al. 2013).

*Daphnia* adaptations to tolerate toxic cyanobacteria can have significant effects on ecosystem-evolutionary dynamics (Chislock et al. 2013, 2019a), yet the mechanisms driving these effects are not well understood. Moreover, it is unclear how temperature stress will affect the ability of *Daphnia* to tolerate cyanobacteria. In this study, we explored how a range of dietary proportions of toxic cyanobacteria affected the fitness of cyanobacteria-sensitive and -tolerant *Daphnia pulicaria* genotypes at two temperatures. Cyanobacteria-tolerant *Daphnia* genotypes are expected to optimize life history traits, such as survival, fecundity, and somatic growth, to maximize fitness differently than cyanobacteria-sensitive *Daphnia* genotypes when fed diets containing increasing concentrations of cyanobacteria. To test these hypotheses, empirically generated data were used to create a path diagram to track the mechanisms by which temperature and cyanobacteria affected life history traits (i.e., survivorship, fecundity, and somatic growth) and, ultimately, fitness (i.e., population growth) of cyanobacteria-sensitive and -tolerant *Daphnia pulicaria* genotypes.

## Materials and methods

*Daphnia pulicaria* genotypes were collected as diapausing (ephippial) eggs from the surface sediment of six small glacial lakes (one genotype per lake) in southern Michigan (annual air temperature range 1–29 °C) and hatched via light-induced hatching in 2017 (Weider et al. 1997). Three cyanobacteria-sensitive *D. pulicaria* genotypes were isolated from the sediment of three oligotrophic lakes with low cyanobacterial abundance, and three cyanobacteria-tolerant *D. pulicaria* genotypes were isolated from the sediment of three eutrophic lakes known to experience cyanobacterial blooms (Supplementary table 1; Sarnelle and Wilson 2005). *Daphnia* parthenogenetic lines were maintained for a single representative isolate (i.e., genotype) from each lake at room temperature conditions (~24 °C) in filtered and autoclaved lake water and fed *Ankistrodesmus falcatus* ad libitum for at least 30 generations to minimize maternal effects. Preliminary juvenile survivorship assays were then conducted to confirm the sensitivity of the six representative genotypes to toxic cyanobacteria (Sarnelle and Wilson 2005, Supplementary table 1). *Daphnia pulicaria* neonates (< 24 h) were grouped by genotype and placed in *Daphnia* culture medium (see below) without food for 5 h to purge their guts. Seven neonates were then placed in 500-ml jars and fed either 100% *Ankistrodesmus falcatus* or 100% *Microcystis aeruginosa* for 9 days, with water changes conducted every 72 h. Four replicates per genotype and diet combination were included, for a total of 48 jars. Juvenile survivorship was determined daily.

*Daphnia* culture medium was prepared by filtering water from an oligotrophic lake (Lake Martin, Alabama) through a 1.2 µm glass microfiber filter followed by a 0.45 µm cellulose nitrate membrane filter and then sterilized in an autoclave. The nutritious chlorophyte, *Ankistrodesmus falcatus* (unicellular, mean cell dimensions 2.5 µm × 45 µm), and toxic cyanobacterium, *Microcystis aeruginosa* (UTEX 2667, uni- or bicellular, mean cell diameter 4 µm), were maintained in a nutrient-rich medium (modified BG-11, Vanderploeg et al., 2001) as semi-continuous cultures at 25 °C with a 8 h light: 16 h dark conditions in an incubator. Microcystin content of the diets containing *Microcystis aeruginosa* was determined via enzyme-linked immunosorbent assay (ELISA, An and Carmichael 1994) after extraction in 75% aqueous, acidified methanol followed by removing the solvent and resuspending the extract in 5 ml of phosphate buffer (Wilson et al. 2008).

Prior to the experiment, ~60 neonates from each of the three cyanobacteria-sensitive and three cyanobacteria-tolerant genotype parent cultures were placed in genotype-specific 50 ml vials filled with *Daphnia* culture medium and fed *Ankistrodesmus* ad libitum until maturity. Neonates (< 24 h) from mature females were then pooled by genotype and placed in *Daphnia* culture medium without food for 5 h to purge their guts. To determine *Daphnia* neonate lengths at the beginning of the experiment ($L_{t0}$), a random subset of two neonates from each genotype was placed onto a water droplet on a slide and measured from the top of the head over the eye to the base of the spine with a compound light microscope (40x). The six genotypes were combined into either tolerant or sensitive genotype groups by placing two neonates from each tolerant or sensitive genotype into 500 ml jars of the corresponding diet mixture equivalent to 1 mg carbon $L^{-2}$, for a total of 6 neonates total/jar (Kilham et al. 1997). Diets were prepared by centrifuging cultures of exponentially growing cells, discarding the supernatant, and then resuspending the cells in *Daphnia* culture medium. Food concentrations were determined by counting ten fields of two replicate 0.18 ml subsamples of each culture using a Palmer-Maloney chamber. Diet treatments based on algal biomass included a 100% *Ankistrodesmus* (highest quality diet), 75% *Ankistrodesmus* + 25% *Microcystis*, 50% *Ankistrodesmus* + 50% *Microcystis*, and 25% *Ankistrodesmus* + 75% *Microcystis* (lowest quality diet) treatments. A

starvation treatment of only *Daphnia* culture medium was included to determine if the effect of toxic cyanobacteria was greater than the effect of starvation. The jars were then sealed and placed in incubators set to either 20 °C or 28 °C in an 8 h light: 16 h dark cycle. In total, there were 2 genotype groups, 2 temperature treatments, and 5 diets with 4 reps per treatment combination for a total of 80 jars. Jars were inverted to resuspend algae and randomly reorganized to minimize variation in light exposure across jars daily.

*Daphnia* survivorship and number of neonates were recorded daily. *Daphnia* were transferred to new jars with fresh diet mixtures every 72 h for 10 days. After each 72 h span, the number of females carrying eggs and number of eggs per female were determined and neonates were counted, measured, and discarded. Three *Daphnia* from each jar were placed onto a water droplet on a slide and measured with a compound light microscope to determine length ($L_{tx}$) and were returned to their respective jars. *Daphnia* length measurements were used to estimate juvenile somatic growth rate (length, µm/day) based on the formula: ($lnL_{tx}$—$lnL_{t0}$)/time, where $L_{t0}$ and $L_{tx}$ are animal lengths at day 0 and day x, respectively.

*Daphnia* percent survival was calculated using the formula: ($A_{t10}$ / $A_{t0}$)*100, where $A_{t0}$ and $A_{t10}$ are the number of live *Daphnia* females at day 0 and day 10, respectively. Survivorship curves were generated based on the Kaplan–Meier estimator of survival and compared via Cox proportional hazards regression using the open-source s*urvival R* package (Kaplan and Meier 1958; Therneau 2021). *Daphnia* fecundity was estimated by calculating the total number of neonates produced per female by dividing the number of neonates produced in each jar by the number of live females and then summing these values for each day across the entire experiment.

The intrinsic rate of population increase (*r*) was calculated for each jar using the Euler equation: $1 = \sum_{x=0}^{10} e^{-rx} l(x)m(x)$, where *r* is the rate of population growth per day, x is the age class (day; 0 to 10), l(x) is the probability of surviving to age x, and m(x) is the number of neonates produced per *Daphnia* per jar on day x. For jars with no reproduction, *r* was determined from changes in *Daphnia* abundance over time based on the formula: $r = [\ln \text{density}_{t+1} — \ln \text{density}_t]/$ time, where t equals time (Allan 1976; Wilson and Hay 2007).

Statistical differences among treatment effects were analyzed using analysis of variance (ANOVA). One-way, two-way, and three-way ANOVAs were calculated to assess the importance of interactions of temperature, diet, and *Daphnia* genotypes. Extra sum of squares *F* tests were calculated to determine whether models that included interactions were a significant improvement in fit to the data. Differences among treatments were determined via Tukey's HSD post hoc test. All statistical analyses were performed with the open-source software RStudio version 4.0.2 (R Core Team 2020). A path model was used to determine how sensitive and tolerant *Daphnia* genotypes differed in how they allocated resources across three life history traits (i.e., percent survival, fecundity, and somatic growth) to maximize fitness (i.e., population growth). The path model includes causal arrows from the two experimental treatments to the three life history traits that mediate population growth. The indirect effect of the treatments on fitness through each intermediate life history trait was determined by calculating the product of path coefficients from a treatment through an intermediate life history trait to fitness. The total effect of each experimental variable was determined by adding the direct and indirect effects of the variable on fitness. A structural equation model (SEM) was generated using the *sem* R package (Fox et al. 2017) to test the causal structure of the path model. Diets were ranked for the model from least nutritious to most nutritious (starved = 0, 25% *Ankistrodesmus* and 75% *Microcystis* = 25, 50% *Ankistrodesmus* and 50% *Microcystis* = 50, 75% *Ankistrodesmus* and 25% *Microcystis* = 75, 100% *Ankistrodesmus* and 0% *Microcystis* = 100). Results were log-transformed prior to statistical analysis to meet the assumption of linearity for the path analysis. To evaluate the fit of the data for the models, Chi-square goodness of fit tests were conducted and showed that the models are a good fit to the data (sensitive $p = 0.22$ and tolerant $p = 0.59$). The two treatments (temperature and diet) and three intermediate life history traits (survival, somatic growth, and fecundity) included in the path models explained 92.3% and 93.1% of the observed patterns in population growth of sensitive and tolerant *Daphnia* genotypes, respectively. The AIC of the sensitive (41.52) and tolerant (40.3) models were relatively high, but all parameters had to be included in the model to understand the effect of all measured parameters on population growth.

## Results

The combined effects of toxic cyanobacteria and elevated temperature on cyanobacteria-tolerant and cyanobacteria-sensitive *Daphnia* genotypes were measured as *Daphnia* survival (percent survival and Kaplan–Meier), juvenile somatic growth rate, fecundity (total number of neonates produced per *Daphnia* female), and population growth (intrinsic rate of population increase (*r*)).

Both cyanobacteria-sensitive and -tolerant *Daphnia* had significantly lower ($p < 0.05$) population growth when fed diets containing no food (starvation) or ≥ 50% *Microcystis* compared to the high-quality 100% *Ankistrodesmus* diet at 28 °C (Fig. 1). Sensitive genotypes had significantly lower ($p < 0.05$) population growth when starved or fed any diet containing *Microcystis* relative to 100% *Ankistrodesmus* at 28 °C, which suggests cyanobacteria, even at low concentrations, had a detrimental impact on the population growth

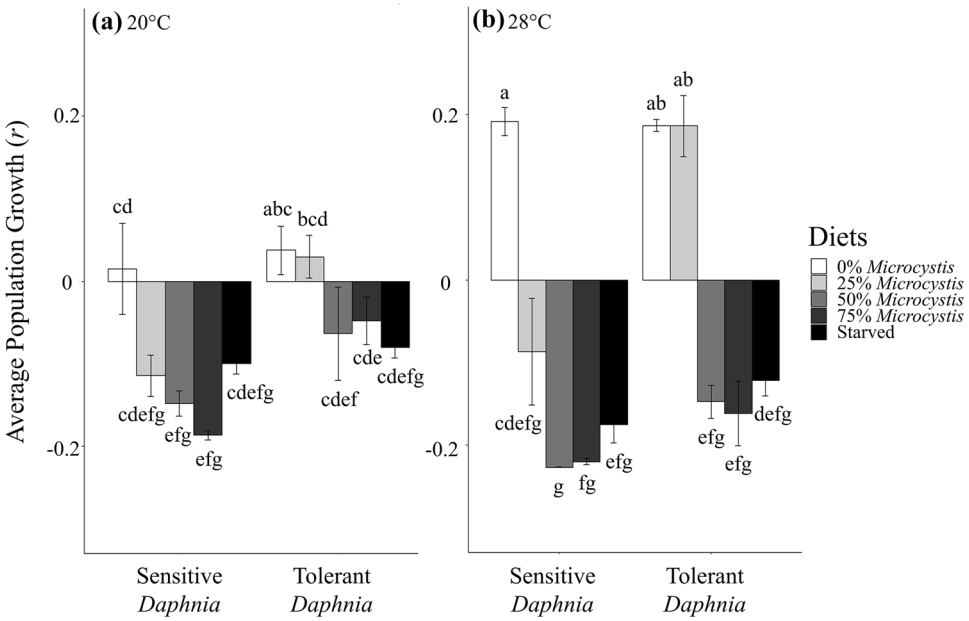


**Fig. 1** Average population growth rates ($r$, day$^{-1}$) of cyanobacteria-sensitive and cyanobacteria-tolerant *Daphnia pulicaria* genotypes cultured over 10 days at (**a**) 20 °C and (**b**) 28 °C. Diet treatments include *Ankistrodesmus* only (0% *Microcystis*), 75% *Ankistrodesmus* and 25% *Microcystis* (25% *Microcystis*), 50% *Ankistrodesmus* and 50% *Microcystis* (50% *Microcystis*), 25% *Ankistrodesmus* and 75% *Microcystis* (75% *Microcystis*), and a starvation treatment (starved). Unique letters represent statistically different observations ($p < 0.05$) across both genotypes and temperature treatments. Error bars = ± SE. Sample size per treatment = 4

**Table 1** Analysis of variance results for population growth ($r$, day$^{-1}$) over 10 days of cyanobacteria-sensitive and cyanobacteria-tolerant *Daphnia pulicaria* genotypes exposed to two temperatures (20 °C and 28 °C) and five diet treatments (*Ankistrodesmus* only (0% *Microcystis*), 75% *Ankistrodesmus* and 25% *Microcystis* (25% *Microcystis*), 50% *Ankistrodesmus* and 50% *Microcystis* (50% *Microcystis*), and 25% *Ankistrodesmus* and 75% *Microcystis* (75% *Microcystis*), and a starvation treatment (starved))

| Test | Source | *df* | MS | *F*-ratio | *p*-value |
|---|---|---|---|---|---|
| One-way ANOVA | Temperature | 1 | 0.0014 | 0.18 | 0.67 |
| | Genotype | 1 | 0.1511 | 20.36 | < 0.0001 |
| | Cyanobacteria | 4 | 0.2081 | 28.04 | < 0.0001 |
| | Error | 73 | 0.0074 | | |
| Two-way ANOVA | Temperature | 1 | 0.0014 | 0.35 | 0.55 |
| | Genotype | 1 | 0.1511 | 39.31 | < 0.0001 |
| | Cyanobacteria | 4 | 0.2081 | 54.14 | < 0.0001 |
| | Temperature x Genotype | 1 | 0.0005 | 0.13 | 0.72 |
| | Temperature x Cyanobacteria | 4 | 0.0502 | 13.07 | < 0.0001 |
| | Cyanobacteria x Genotype | 4 | 0.0236 | 6.14 | 0.0003 |
| | Error | 64 | 0.0038 | | |
| | | *F*-Test *p*-value: < 0.0001 | | | |
| Three-way ANOVA | Temperature | 1 | 0.0014 | 0.36 | 0.55 |
| | Genotype | 1 | 0.1511 | 40.93 | < 0.0001 |
| | Cyanobacteria | 4 | 0.2081 | 56.37 | < 0.0001 |
| | Temperature x Genotype | 1 | 0.0005 | 0.13 | 0.72 |
| | Temperature x Cyanobacteria | 4 | 0.0502 | 13.61 | < 0.0001 |
| | Cyanobacteria x Genotype | 4 | 0.0236 | 6.39 | 0.0002 |
| | Genotype x Temperature x Cyanobacteria | 4 | 0.0061 | 1.66 | 0.17 |
| | Error | 60 | 0.0037 | | |
| | | *F*-Test test *p*-value: 0.17 | | | |

Extra sum of squares *F*-test results determine whether two- and three-way ANOVAs are a significant improvement in fit to the data compared to one-way ANOVA. *df* degrees of freedom, *MS* means square error

of sensitive genotypes at elevated temperatures. Tolerant *Daphnia* genotypes had similar growth rates ($p > 0.05$) when exposed to 25% *Microcystis* and 100% *Ankistrodesmus* diets under both temperature treatments, which suggests tolerant genotypes can tolerate low cyanobacterial densities even under elevated temperature conditions. Cyanobacteria-sensitive genotypes exhibited significantly higher population growth at 28 °C than 20 °C ($p < 0.0001$) when fed 100%

*Ankistrodesmus* diets. Elevated temperatures also accelerated the population growth of tolerant *Daphnia* genotypes fed 100% *Ankistrodesmus* and 25% *Microcystis*, though these effects were not significant ($p > 0.05$). There was a significant interaction between temperature and diet as well as *Daphnia* genotype and diet, which suggests the effect of diet on population growth was altered by both temperature and *Daphnia* genotype ($p \leq 0.0003$, Table 1).

The effect of cyanobacteria on the survival of both genotype groups was affected by temperature (Supplementary Fig. 1). When cultured at 20 °C, cyanobacteria did not significantly affect the survivorship of either genotype. But when cultured at 28 °C, the presence of cyanobacteria significantly decreased the survivorship of both cyanobacteria-sensitive and -tolerant genotypes. Based on Cox proportional hazard regression, 0.44 ($p < 0.001$) times as many tolerant *Daphnia* genotypes died as sensitive at any given time. Under elevated temperature conditions, 1.06 ($p < 0.01$) times as many *Daphnia* died when compared to those cultured at 20 °C. Diet had a significant negative impact on survival, with 3.03 ($p < 0.01$), 3.86 ($p < 0.01$), and 2.31 ($p < 0.01$) times as many *Daphnia* dying when fed 50% *Microcystis,* 75% *Microcystis*, or no food (starvation) when compared to the 0% *Microcystis* treatment, respectively. The 25% *Microcystis* treatment did not significantly decrease survival probability, when compared to the 0% *Microcystis* treatment.

The juvenile somatic growth rate of both sensitive and tolerant genotypes decreased as cyanobacterial abundance in diets increased under both temperature conditions (Supplementary Fig. 2). Tolerant *Daphnia* neonates grew significantly faster than sensitive genotypes when fed 25% *Microcystis* at 28 °C ($p < 0.001$). There was a significant interaction between temperature and diet as well as *Daphnia* genotype and diet, which suggests the effect of diet on somatic growth was affected by temperature and local adaptations to cyanobacteria ($p \leq 0.05$, Supplementary Table 2). There were no data for the somatic growth of sensitive *Daphnia* genotype females fed 50% and 75% *Microcystis* at 28 °C, because all adult females died prior to day 7.

Fecundity was the main driver of population growth of both genotypes. Both *Daphnia* genotypes had higher fecundity at 28 °C than 20 °C when fed 100% *Ankistrodesmus* and 25% *Microcystis* diets, though these patterns were not statistically significant ($p > 0.05$, Supplementary Fig. 3). Neither sensitive nor tolerant *Daphnia* genotypes reproduced under starvation treatments. Tolerant *Daphnia* genotypes cultured at 20 °C were unique in their ability to produce offspring when fed high cyanobacteria diets (50–75% *Microcystis*). Tolerant *Daphnia* genotypes also produced more offspring than sensitive genotypes ($p < 0.0001$) when fed 25% *Microcystis* diets at 28 °C. There was a significant interaction between temperature and diet as well as *Daphnia* genotype

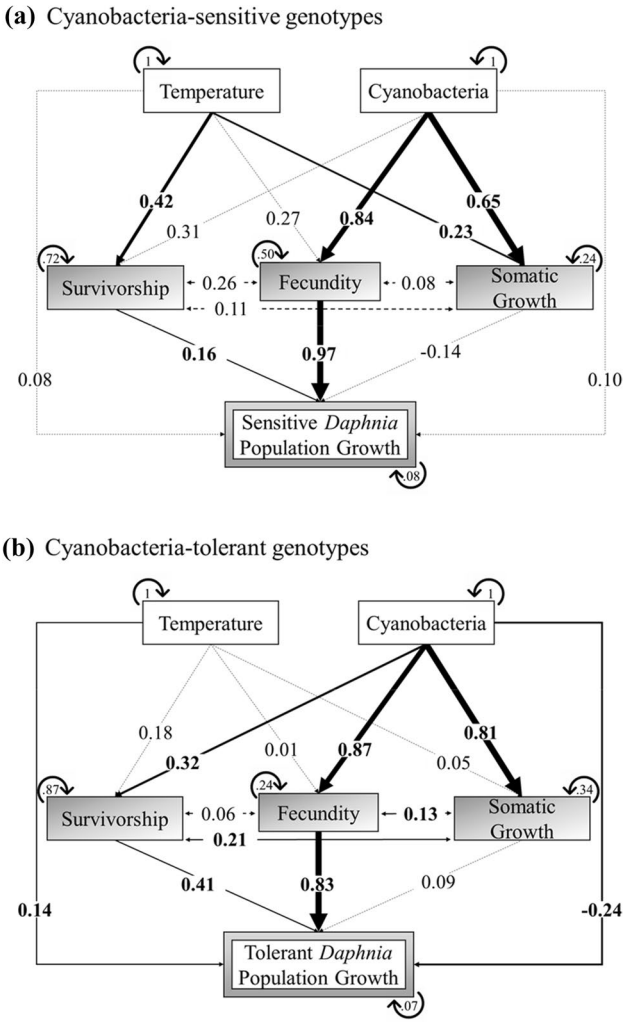


**Fig. 2** Path diagram of direct and indirect effects of temperature and cyanobacteria on the population growth (*r*) of (**a**) cyanobacteria-sensitive and (**b**) cyanobacteria-tolerant *Daphnia pulicaria* genotypes. Solid arrows represent significant paths ($p < 0.05$), and the arrow thickness represents effect strength. Double-sided arrows represent correlations between the three intermediate life history traits: survivorship, fecundity, and somatic growth. Looped arrows represent the variance terms of each variable

and diet, which suggests that the effect of diet on fecundity was affected by temperature and *Daphnia* genotype ($p \leq 0.002$, Supplementary Table 3).

Local adaptations allow organisms to allocate resources among life history traits to maximize fitness. A path diagram was generated to better understand how local adaptations of *Daphnia pulicaria* to toxic cyanobacteria affected their allocation of resources to somatic growth, reproduction, and survival to maximize population growth when exposed to various temperatures and cyanobacterial diets. The path diagram suggests that a reduction in fecundity due to exposure to cyanobacteria was the main determinant of population growth of both cyanobacteria-sensitive and

**Table 2** Effect of temperature and cyanobacterial diets on the population growth ($r$) of cyanobacteria-sensitive and cyanobacteria-dominant *Daphnia pulicaria* genotypes

| Causal Variable | Direct Effect | Indirect Effect via Survivorship | Indirect Effect via Fecundity | Indirect Effect via Somatic Growth | Total Effect |
|---|---|---|---|---|---|
| Sensitive genotypes | | | | | |
| Temperature | 0.08 | 0.07 | 0.26 | -0.03 | 0.38 |
| Cyanobacteria | – 0.10 | 0.05 | 0.63 | – 0.12 | 0.46 |
| Survivorship | **0.16** | – | – | – | 0.16 |
| Fecundity | **0.97** | – | – | – | 0.97 |
| Somatic Growth | – 0.14 | – | – | – | – 0.14 |
| Tolerant genotypes | | | | | |
| Temperature | **0.14** | 0.07 | 0.01 | 0.00 | 0.22 |
| Cyanobacteria | **– 0.24** | 0.13 | 0.72 | 0.07 | 0.68 |
| Survivorship | **0.41** | – | – | – | 0.41 |
| Fecundity | **0.83** | – | – | – | 0.83 |
| Somatic Growth | 0.09 | – | – | – | 0.09 |

Bolded values: $p$-value < 0.05

Path coefficients are listed for the direct effects of each causal variable. Indirect effects are the products of the direct effect of the two treatments and the path coefficients for the intermediate life history trait variables, including percent survival, fecundity and somatic growth. Total effects are the sum of direct and indirect effects

-tolerant genotypes (Fig. 2). Interestingly, temperature had a significant effect on the somatic growth (0.23, $p = 0.02$) and survivorship (0.42, $p = 0.02$) of cyanobacteria-sensitive *Daphnia* genotypes, but did not significantly affect survivorship, somatic growth, or reproduction of tolerant genotypes (Table 2). The significant direct effects of temperature (0.14, $p = 0.003$) and cyanobacteria (-0.24, $p = 0.005$) on tolerant *Daphnia* population growth suggests these treatments affected population growth through a life history trait or behavioral modification not measured in this study. Although cyanobacterial diets significantly affected the somatic growth rate of cyanobacteria-sensitive (0.65, $p < 0.0001$) and -tolerant genotypes (0.81, $p < 0.0001$), there was no direct effect of somatic growth on the population growth rate of either genotype group. This suggests that although cyanobacterial diets decrease the somatic growth rate of *Daphnia*, it did not cause a significant decrease in overall fitness.

## Discussion

*Daphnia pulicaria* genotypes collected from eutrophic lakes are known to locally adapt to tolerate, and ultimately control, toxic cyanobacteria that are common in these systems (Chislock et al. 2013, 2019b). Cyanobacteria-tolerant *Daphnia* genotypes maintained positive population growth and fecundity when exposed to low concentrations of toxic cyanobacteria (25% *Microcystis*) under both temperature conditions (20° and 28 °C), whereas cyanobacteria-sensitive *Daphnia* genotypes were negatively affected regardless of cyanobacterial concentration in the diet (25–75% *Microcystis*) or temperature treatment. In fact, tolerant genotypes performed as well or better than sensitive *Daphnia* genotypes under the various temperature and diet combinations for all life history traits recorded. These results suggest tolerance to cyanobacteria did not come at a cost of lower fitness when *Daphnia* were exposed to elevated temperatures. The observed negative effects of cyanobacteria on *Daphnia* are believed to be associated with the presence of known or unknown toxins and/or lack of polyunsaturated fatty acids rather than *Daphnia* not being able to ingest the cyanobacterial cells, as *Daphnia* are considered generalist grazers and have been observed to easily ingest individual *Microcystis aeruginosa* cells (Chislock et al. 2013). Cyanobacterial blooms are well-documented climate change co-stressors, therefore cyanobacterial and thermal tolerance are likely coupled due to simultaneous exposure in nature (Griffith and Gobler 2020). However, trade-offs have been recorded by others. For example, Schaffner et al. (2019) documented the change in *Daphnia mendotae* clonal diversity throughout a growing season, culminating in a summer cyanobacterial bloom, and found a drastic decrease in clonal diversity as cyanobacteria increased in dominance. The authors then isolated cyanobacteria-sensitive and -tolerant *D. mendotae* clones and found tolerant clones had lower juvenile growth rate than sensitive clones when fed spring diets (i.e., mixture of diatoms, cryptophytes and chlorophytes) when compared to summer diets (i.e., mixture of cyanobacteria and chlorophytes), which suggests there was an unidentified cost of cyanobacterial tolerance. Although results from the present study suggest that local adaptations to toxic cyanobacteria do not lead to a decrease in fitness when exposed to elevated temperatures (i.e., energetic trade-off), findings by Schaffner

et al. (2019) suggest cyanobacterial tolerance may decrease *Daphnia* fitness when exposed to other environmental parameters not considered in this study.

Although *Daphnia* tolerance to toxic cyanobacteria has been well-documented through space and time (Hairston et al. 1999; Sarnelle and Wilson 2005), how adaptations allow *Daphnia* to prioritize life history traits to maximize fitness is not well understood. A path analysis was generated to quantify how cyanobacteria-sensitive and -tolerant *Daphnia* genotypes allocate finite resources between life history traits to maximize fitness. Path analysis results show toxic cyanobacteria had a stronger effect than temperature on the fitness of both *Daphnia* genotype groups, mainly by decreasing fecundity (Table 2). Toxic cyanobacterial abundance was also the main driver of survivorship, fecundity, and somatic growth of both sensitive and tolerant *Daphnia,* except for survivorship of sensitive *Daphnia* which was controlled by temperature (Fig. 2). Fecundity was the life history trait most sensitive to exposure to toxic cyanobacteria, likely because the sterol or fatty acid content of cyanobacteria do not meet the high energetic demands of reproduction or the presence of toxic secondary metabolites.

While cyanobacteria had a clear negative impact on *Daphnia* fitness, the effect of temperature varied based on diet. In fact, elevated temperatures (28 °C) either significantly increased or had no significant effect on the life history traits, and ultimately fitness, of cyanobacteria-sensitive and -tolerant *Daphnia* fed the nutritious green algae*, Ankistrodesmus falcatus.* Temperature increases the metabolic rate of ectothermic organisms, such as *Daphnia*, which accelerates somatic growth, maturation times and increases fecundity as long as sufficient nutritious food is available and temperatures do not exceed the thermal optimum of an organism (Orcutt and Porter 1984; Korpelainen 1986; Hietala et al. 1997). However, elevated temperatures further decreased the population growth of *Daphnia* genotypes fed diets that included the sterol-deficient and toxic cyanobacteria, *Microcystis aeruginosa*, though these differences were not statistically significant (Fig. 1). Based on the results, it appears the 28 °C treatment was not sufficient to induce lethal thermal stress, although this temperature is representative of elevated temperatures experienced in the lakes from which the *Daphnia* genotypes were isolated. Interactive effects between temperature and diet have been recorded in other *Daphnia* studies (Hietala et al. 1997; Hochmuth and Schamphelaere 2014), as well as other ectothermic organisms, such as copepods (Malzahn et al. 2016), fish (Vagner et al. 2015), mollusks (Wacker and von Elert 2003) and insects (Clissold and Simpson 2015).

Juvenile somatic growth rate based on weight or carbon content, but not necessarily length, is frequently used as a predictor of population growth ($r$) of *Daphnia* fed high-quality diets (Lampert and Trubetskova 1996), however, the strength of the relationship between these two traits can weaken when zooplankton are fed poor quality diets (Masclaux et al. 2009). While somatic growth was significantly affected by cyanobacteria in the diet for both *Daphnia* genotype groups, fecundity, and survival had larger impacts on population growth rate than somatic growth especially for tolerant *Daphnia* (Fig. 2). Fecundity is directly correlated to body size of *Daphnia* (Lampert 1993), which suggests the fecundity of tolerant *Daphnia* was controlled by higher survivorship and somatic growth rate. Fecundity and somatic growth were not closely related in sensitive genotypes, likely due to low survivorship and reproduction especially at 28 °C (Supplementary Fig. 3). The negligible direct effect of somatic growth on $r$ suggest somatic growth rate may not be a reliable predictor of population growth rate when *Daphnia* are exposed to multiple stressors, including cyanobacteria in their diet. Lampert and Trubetskova (1996) found that juvenile growth rates were a reliable index of fitness, as long as *Daphnia* had sufficient quantities of high-quality food. When *Daphnia* were exposed to temperature stress, or when comparing *Daphnia* of different strains and adaptations, somatic growth was less of a reliable estimate of fitness.

## Conclusions

Climate change is expected to negatively affect aquatic environments directly through changes in temperature and precipitation patterns, as well as indirectly by promoting the growth of sterol-deficient and toxic cyanobacteria (O'Neil et al. 2012). This study shows that under the culture conditions tested, *Daphnia pulicaria* adaptations to toxic cyanobacteria lead to higher population growth rate in the presence of toxic cyanobacteria as well as elevated temperatures. Future studies should consider climate change stressors across multiple trophic levels by taking into account effects on predator feeding rates (Beisner et al. 1996), chemical signaling (Larsson and Dodson 1993), and susceptibility to parasites (Manzi et al. 2020). Additionally, exploring additional indirect effect of elevated temperature on *Daphnia* fitness, such as hypolimnetic oxygen depletion, the effect of simultaneous exposure to multiple cyanotoxins, as well as additional stressors associated with climate change, such as acidification, will be important for understanding the value of adaptive traits (Griffith and Gobler 2020).

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**Author contribution statement** EGF and AEW conceived and designed the experiment. EGF performed the experiment, analyzed the data, and wrote the manuscript. AEW provided editorial advice.

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**Data availability** The data collected during the current study are available from the corresponding author on reasonable request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Ethics approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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