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Seasonal variation in thermal plasticity of an alpine lake *Daphnia* population

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ABSTRACT

Background: Temperature changes dramatically throughout the growing season in temperate latitudes, and seasonal changes in temperature are especially pronounced in alpine lakes where water stratifies into distinct thermal layers during summer.

Hypothesis: Populations are expected to maintain a greater degree of plasticity in more heterogeneous environments, such as when lake stratification occurs.

Organism: We studied seasonal variation in plasticity of a population of *Daphnia*, a key grazer in alpine lakes.

Methods: We isolated maternal lines of *Daphnia pulicaria* from Blue Lake (Sierra Nevada, CA) at four different times throughout the growing season, then measured phenotypic traits and survivorship after individuals were reared at two temperatures (17°C and 24°C).

Results: We found mixed evidence for the role of thermal variation in maintaining plasticity. Thermal plasticity for offspring number and age at maturity varied seasonally; however, inconsistent with our hypothesis, neither response was related to stratification. Similarly, we observed lower plasticity for the clutch interval when the lake experienced peak thermal stratification in mid-summer compared with early-fall conditions. In contrast, plasticity for critical maximum temperature (CT_{max}) was highest during peak stratification. As CT_{max} is a direct measurement of upper thermal limits, it should be related to maximum temperature experienced in the water column. Thus, this result is consistent with a positive correlation between thermal variation and plasticity.

Conclusion: Our results suggest that the degree of plasticity in response to temperature varies throughout the season in relation to thermal stratification, with different life-history traits showing distinct seasonal patterns of plasticity.

Keywords: acclimation, alpine lakes, critical thermal maximum, plasticity, temperature variation, thermal stratification.

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INTRODUCTION

Temporal variation in environmental conditions is ubiquitous in nature. Populations living in intermediate and high latitude climates encounter tremendous seasonal variation in environmental conditions. For instance, the water temperature of temperate lakes can vary from 4°C during the winter to over 20°C during the summer months (O'Reilly *et al.*, 2015). Seasonal temperature changes impose selective pressure on organisms to maintain fitness under variable conditions. Phenotypic plasticity and genetic adaptation are two mechanisms by which organisms may respond to environmental variation. Phenotypic plasticity allows a single individual to express a phenotype with greater fitness in response to variation in conditions (Scheiner, 1993; Sultan, 2003). Genetic adaptation, on the other hand, results from environment-dependent selection among individuals based on heritable variation in traits (Debat and David, 2001). Seasonal environments in general exhibit predicable changes in conditions. By sensing cues early in the season, individuals can adjust, thereby matching their phenotypes to the expected conditions (Reed *et al.*, 2010).

Strong seasonal variability in temperature causes most lakes in temperate climates to experience thermal stratification where the water column separates into distinct thermal layers. The most extreme thermal stratification occurs during the summer, when lakes have a warm epilimnion (upper layer) and a cold hypolimnion (lower layer) separated by a steep cline in temperature (thermocline). Stratification affects physical, chemical, and biological processes in lakes, such as transport of nutrients and oxygen between the surface and deep water, the light environment of phytoplankton cells in the mixed layer, and zooplankton behaviour (Boehrer and Schultze, 2008).

Thermal stratification affects zooplankton in temperate lakes because they often exhibit a behavioural syndrome where they perform diel vertical migration throughout the water column. Diel vertical migration (DVM) represents a trade-off between the functions of food gathering and predator avoidance (Kaartvedt *et al.*, 1996). In classic DVM behaviour, individuals spend the daytime in the deeper darker layer (hypolimnion) of a lake to reduce the probability of an attack by optically orientated predators, then migrate upwards at night to warmer layers to either feed on phytoplankton or take advantage of higher temperatures to speed up metabolism in order to increase growth and reproductive rates (Lampert, 1989; Dawidowicz and Loose, 1992; Loose *et al.*, 1993). Hence, migrating zooplankton regularly experience a large daily temperature range as they travel throughout the water column (Stich and Lampert, 1981; Ringelberg, 1991). Ectotherms that occupy heterogeneous thermal environments are hypothesized to evolve physiological or behavioural capacity to optimize performance in variable thermal environments (Chown and Terblanche, 2006; Angilletta, 2009). Thus, organisms from variable environments are expected to display a high degree of phenotypic plasticity (Kingsolver *et al.*, 2016).

Daphnia is a key species in freshwater ecosystems due to its role as an effective phytoplankton grazer and preferred prey for fish. *Daphnia* also often exhibit DVM in alpine lakes. Previous studies have found genetic turnover throughout the growing season for *Daphnia*, with clones from different time periods having distinct responses (e.g. Carvalho, 1987; Paul *et al.*, 2012). For instance, winter clones have lower survivorship and thermal tolerance than summer clones (Carvalho, 1987; Paul *et al.*, 2012). In addition, *Daphnia* populations can evolve plasticity in response to temperature over short time scales. Cavalheri *et al.* (2019) found that *D. pulicaria* populations evolve higher plasticity for intrinsic growth rate after two years of selection at warm temperatures. Similarly, Van Doorslaer *et al.* (2009) found that phenotypic

plasticity increased the intrinsic growth rate for *D. magna* after only 3 months of selection at elevated temperatures. These studies show that natural populations of *Daphnia* contain standing genetic variation for plasticity. Theory predicts that higher plasticity should evolve in more heterogeneous environments (Berrigan and Scheiner, 2004). Individuals that occur during periods of thermal stratification and migrate vertically may experience the greatest thermal variability over a spatial scale of metres. Together, the results of previous work suggest that the ability of genotypes to induce phenotypic plasticity may also have a temporal signal. However, to date we lack experiments that test how seasonal variation shapes the phenotypic plasticity of *Daphnia* populations in response to temperature.

We collected *D. pulicaria* from Blue Lake (Sierra Nevada, CA) four times throughout the growing season, then measured phenotypic traits and survivorship to determine how plasticity for tolerance at their thermal maximum changes over time. We conducted an experiment using constant rearing conditions of 17°C (benign temperature) or 24°C (high temperature) to determine whether individuals collected at different times during the growing season show a distinct response to acclimation temperature. Our goal was to test whether individuals collected during periods of greater vertical temperature variation in the water column (stratification) exhibit higher levels of plasticity. The results of this work provide insights on the importance of seasonal variation in adaptive plasticity for underlying thermal adaptation across the growing season.

MATERIAL AND METHODS

Lake sampling

Blue Lake (latitude 38.051164, longitude −119.270342, elevation 3013 m), located in Inyo National Forest (CA), was sampled six times during the summer of 2017 (15 July, 1 August, 16 August, 4 September, 13 September, and 5 October). *Daphnia pulicaria* tested in the common-garden experiment were collected in the last four sample days, since *Daphnia* abundance was very low in the first two samples (Fig. 1c). At every sampling date, we recorded water temperature at 1 m intervals throughout the water column using a field probe (YSI Incorporated, Yellow Springs, OH; Fig. 1a, b) and collected zooplankton from the deepest point of the lake using a 63 μm mesh conical net with a 30 cm diameter drawn through the water column, starting 1 m above the lake bottom. Zooplankton samples were preserved in 70% ethanol and the total number of *Daphnia* was counted under a stereomicroscope (Fig. 1c).

We also collected live zooplankton samples from the deepest point of the lake (10 m) using the same approach. These samples were kept cold until we returned to the laboratory, where we searched for live *Daphnia*. When present, 30 *D. pulicaria* females carrying eggs in the brood pouch were separated in individual 50 mL Falcon tubes filled with COMBO medium (Kilham *et al.*, 1998). Each individual *Daphnia* was considered a maternal line and cultured for at least five generations in separate 50 mL Falcon tubes filled with COMBO medium under standardized conditions (17 ± 1°C and photoperiod 12/12 hours light/dark). All animals were fed the green alga *Nanochloropsis* sp. at a constant high rate of 24×10^6 cells every 2 days.

We measured the chlorophyll-*a* concentration for the first five samples by collecting lake water from 1 m below the surface. A known volume of water was filtered through a GF/F filter that was frozen until processing. We measured the concentration of chlorophyll-*a*

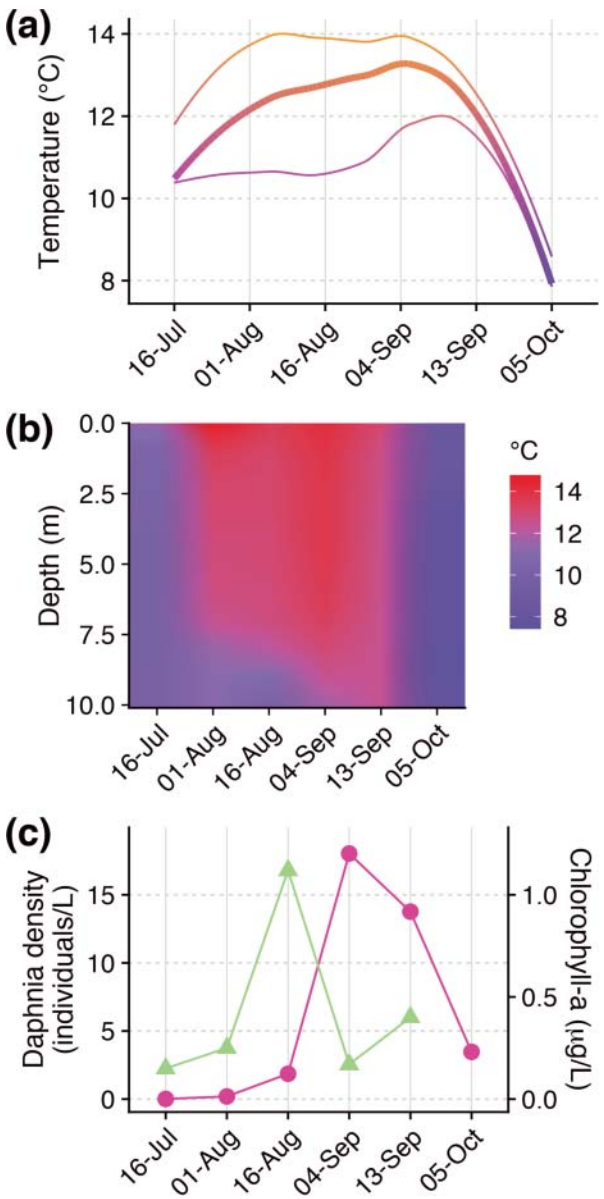

Fig. 1. (a) Maximum (top line), average (middle line), and minimum (bottom line) temperature of the water column measured throughout the summer of 2017 in Blue Lake (Sierra Nevada, CA). (b) Temperature variation of water column of Blue Lake during summer 2017. (c) Variation in *Daphnia* density (dots) and chlorophyll-*a* concentration (triangles) in Blue Lake during summer 2017.

using a Turner Trilogy fluorometer (Turner, USA) following a 24 hour ~4°C methanol extraction (Fig. 1c).

Common-garden experiment

We performed a common-garden experiment with maternal lines that survived and reproduced until the end of the summer 2017. In total, we had two maternal lines from 16 August, one from 4 September, one from 13 September, and three from 5 October. We separated all neonates born within a 24 hour period and kept up to three *Daphnia* individuals from the same maternal line in 100 mL jars under standardized conditions to minimize maternal effects (17 ± 1°C and photoperiod 12/12 hours light/dark). Individuals were fed daily and transferred every 2 days to fresh media. We established three independent grandmother cultures of each maternal line. The second and third clutches of the grandmother generation were pooled together and used to establish 12 cultures of the mother generation per maternal line. This approach enabled us to establish 12 replicate cultures of each maternal line from the mother generation onwards.

Neonates of the second clutch of the daughter generation were pooled together and randomly assigned to one of the two acclimation treatments (17°C and 24°C). We chose the acclimation temperatures based on field sampling and a pilot experiment; 17°C is close to the maximum temperature *Daphnia* experience in Blue Lake (Fig. 1) and is near the optimum temperature for most populations of *Daphnia* from Sierra Nevada (CA) lakes including Blue Lake. We chose 24°C as the 'stress' acclimation temperature because it is high enough to impact life-history traits, but low enough to not induce high mortality. After culturing the *Daphnia* for another two generations at their acclimation temperatures (17°C or 24°C), all neonates born from the second and third clutches were separated in individual 50 mL Falcon tubes and used as the experimental generation. Upon reaching maturity, 30 individuals per maternal line were randomly assigned to one out of six stress temperatures and scored for survivorship: control (remain at ambient temperature for 1 hour and return to acclimation temperature), 28°C, 30°C, 32°C, 34°C, and 36°C. In addition, five individuals per maternal line were assigned to (a) a life-history assay or (b) a critical maximum temperature assay. In total, 563 individuals were tested. We analysed results for 5–6 replicates per maternal line at each acclimation treatment.

Critical maximum temperature

We defined critical maximum temperature (CT_{max}) as the temperature at which individuals lose motor control (Angilletta, 2009). To assess *Daphnia* critical maximum temperature, we placed individuals in 0.5 mL Eppendorf tubes after a resting period of 30 minutes at ambient temperature (~20°C). The measurement was done in a thermal heater (4 × 6 Corning Digital Dry Bath Heater Dual Block). The tubes were randomly divided over the thermal heater. The starting temperature was 25°C, and the temperature was gradually increased from 25°C to 40°C in 1°C steps of 45 ± 5 seconds. Once *Daphnia* lost motor control and stopped moving, they were transferred to ambient conditions to recover for 30 minutes. Because body size can also impact critical maximum temperature (Pörtner and Farrell, 2008), after recovery we measured the body size of each individual from the top of the head to the base of the tail (Gliwicz, 1990; Yurista and O'Brien, 2001).

Life-history assay

For the life-history assay, we scored age and size at maturity, and age and number of offspring from the first three clutches, and the average interval between clutches for individuals from the experimental generation. The average interval was the mean difference between the first and second and second and third clutches. Age at maturity, age at each clutch, and number of offspring in each clutch were used to calculate intrinsic population growth rate for each maternal line following the Lotka–Euler equation (Roff, 1997).

Survivorship

We examined the effect of temperature stress on survivorship by placing individuals in 0.5 mL Eppendorf tubes placed in a thermal heater (4 × 6 Corning Digital Dry Bath Heater Dual Block). First, individuals had a 1 hour rest period at ambient temperature (approximately 20°C). Hence, all individuals experienced the same rate of increase in temperature when placed in the thermal heater. Also, the resting period reduced the effect of stress that is caused by transferring individuals from the 50 mL tube to the 0.5 mL tube. We assessed survivorship at two-degree intervals for temperatures ranging from 28°C to 36°C. A pilot experiment found that individuals can reproduce at 27°C, thus we elected to begin the thermal trials at 28°C and increased temperatures up to 36°C, which was the minimum temperature at which all individuals died. Next, individuals were exposed to a stress temperature for 1 hour. Following the exposure to temperature stress, individuals were moved back to 50 mL tubes and rested for 30 minutes at ambient temperature before being transferred back to their respective acclimation temperature (17°C or 24°C). In all trials we included a control, which consisted of placing the individual in a 0.5 mL Eppendorf tube for 1 hour at ambient temperature and transferring it back to a 50 mL tube and its acclimation temperature. Survivorship was scored after 72 hours.

Statistical analysis

We tested for seasonal variation in phenotypic plasticity by including sample day (time) in our analyses, and plasticity by analysing the effect of the temperature acclimation treatments. The effect of collection date might indicate either genetic adaptation through seasonal turnover in *Daphnia* genotypes, or long-term, trans-generational effects of lake temperature. Since all maternal lines were maintained in the lab for several generations and acclimated to the experimental temperatures for two generations before the experiment, it is likely that differences among sampling dates reflect genetic variation. We tested effects of time, acclimation treatment, and stress temperature (28–36°C) and their interactions on survivorship using generalized linear models. We also tested whether survivorship at each stress temperature differed with acclimation temperatures (17°C and 24°C) across all maternal lines using a non-parametric Wilcoxon test on survivorship data at each stress temperature.

We log-transformed all continuous variables after visually assessing the probability distribution that best fit the data. We analysed the effects of the acclimation treatments (17°C and 24°) and time using linear mixed-effects models for each trait using the *lmerTest* package in the R statistical software (Kuznetsova *et al.*, 2017; R Core Team, 2018). Acclimation treatment and time were modelled as fixed effects and replicate was nested within maternal line

as random effects for age and size at maturity, number of offspring, average interval between clutches, and intrinsic growth rate. Any interaction that includes acclimation treatment would indicate an effect on the level of plasticity (i.e. effect on the slope of the reaction norms).

We also used linear mixed-effects model to analyse the effects of acclimation treatment and time on critical maximum temperature with the same fixed and random parameters, but with the addition of size at maturity as a fixed effect because CT_{max} can be negatively correlated with body size. Larger individuals can have lower thermal tolerance than smaller individuals (Geerts *et al.*, 2014, 2015). In this case, we used a backward selection model where we started with the most complex model for fixed effects (the random effect was kept in all models) and dropped higher-order interactions if they did not significantly improve model fit (using log-ratio tests) until we arrived at a best-fit model.

To evaluate differences in the magnitude of plasticity through time, we calculated the pairwise slopes of each replicate tested at 17°C to all tested at 24°C within a maternal line. We tested for differences in slopes among traits and sample time using a generalized linear model with an interaction between trait and sample time as a fixed effect. We also computed pairwise differences between sample days for each trait using the least square mean values based on the generalized linear model using the *lsmeans* function in the *lmerTest* package in R (Kuznetsova *et al.*, 2017; R Core Team, 2018). In the significant difference test for multiple contrasts, alpha was set to 0.001 following Bonferroni correction. All analyses were performed using the R statistical software (R Core Team, 2018).

RESULTS

Environment

The temperature of Blue Lake varied considerably throughout the summer (Fig. 1a). Surface temperature ranged from 8.6°C to 14.6°C, while bottom temperature ranged from 7.8°C to 11.6°C. The highest temperatures were reached during late August and early September. The largest difference between minimum and maximum temperatures due to thermal stratification occurred in mid-summer, from August to September (Fig. 1a). The lake was stratified by 1 August, with the thermocline occurring at a depth of approximately 7.5 m (Fig. 1b). Following the first observation of thermal stratification, the thermocline became deeper until disappearing on 13 September. The water column was vertically mixed in the final two samples, on 13 September and 4 October, and the temperature decreased from 12°C to 8°C during that period, as fall began in the eastern Sierra mountain range (Fig. 1b). Zooplankton abundance and chlorophyll-*a* concentration changed coincidentally with temperature throughout the growing season. The highest chlorophyll-*a* concentration occurred in mid-August and was followed in early September by a peak in zooplankton density (Fig. 1c), the same period when the water reached its highest temperatures. *Daphnia* reached the highest densities in early September followed by an overall decrease in abundance as water temperature declined (Fig. 1c).

Traits

The linear mixed-effects model revealed that traits responded differently to acclimation temperature (Table 1). Age at maturity was affected by acclimation temperature

(acclimation: $P < 0.001$, Table 1) and decreased 2.33 ± 0.04 days on average when clones were grown at 24°C compared with clones tested at 17°C (Fig. 2a). There was also an interactive effect of acclimation temperature and time (acclimation × time: $P < 0.001$, Table 1). Similarly, size at maturity also showed an interactive effect of acclimation temperature and time (acclimation × time: $P = 0.021$, Table 1), and we also observed a significant effect of maternal line (random effect, $P < 0.001$, Table 1), indicating that for the sampling dates where we tested multiple maternal lines (the first and last sample) there were different responses to acclimation temperature (Fig. 2b). In contrast, the average number of offspring was unaffected by acclimation temperature or time (Table 1, Fig. 2c). The interval

Table 1. Results of the linear mixed-effects model analysis of the acclimation temperature and time on phenotypic traits of *Daphnia pulicaria* collected during the 2017 growing season in Blue Lake

Factor	*df*	Sum of squares	*F*-value	*P*-value
Age at maturity				
Acclimation	1, 563	19.141	1230.362	<**0.001**
Time	3, 563	0.048	1.039	0.374
Acclimation × Time	3, 563	0.327	7.012	<**0.001**
Size at maturity				
Acclimation	1, 133.02	0.012	2.561	0.111
Time	3, 7.001	0.032	2.208	0.174
Acclimation × Time	3, 133.03	0.048	3.325	**0.021**
Random effect:		LRT	$Pr(>\chi^2)$	
Maternal line		13.043	<**0.001**	
Average number of offspring				
Acclimation	1, 20.579	0.013	0.406	0.530
Time	3, 40.782	0.029	0.302	0.823
Acclimation × Time	3, 20.903	0.197	2.040	0.139
Interval between clutches				
Acclimation	1, 37.754	0.54234	13.925	<**0.001**
Time	3, 41.253	0.04757	0.407	0.748
Acclimation × Time	3, 38.480	0.15007	1.284	0.293
Intrinsic growth rate				
Acclimation	1, 70	0.087	77.516	<**0.001**
Time	3, 70	0.006	1.898	0.137
Acclimation × Time	3, 70	0.004	1.221	0.308
Critical maximum temperature				
Size at maturity	1, 71	0.001	0.272	0.603
Acclimation	1, 71	0.001	0.065	0.799
Time	3, 71	0.003	3.747	**0.014**
Size × Acclimation	1, 71	0.001	3.106	0.082
Size × Time	3, 71	0.002	2.971	**0.037**
Acclimation × Time	3, 71	0.003	3.410	**0.022**

Note: Likelihood ratio test statistic (LRT) and *P*-value of the random effect of replicate nested within maternal line is shown when significant.

between clutches showed a significant impact of acclimation temperature (acclimation: $P < 0.001$, Table 1), with a 0.6 day shorter interval at 24°C compared with 17°C conditions (Fig. 2d). Similarly, intrinsic growth rate was 7% higher when maternal lines were raised at 24°C compared with 17°C (acclimation: $P < 0.001$, Table 1, Fig. 2e).

Across all sample dates, acclimation at 24°C resulted in a directional shift that increased the critical maximum temperature. The 7°C difference between acclimation treatments increased critical maximum temperature by an average of 1.2°C (Fig. 2f). Although critical maximum temperature did not vary with body size, it did differ among sample dates (time: $P = 0.014$; and size × time: $P = 0.037$, Table 1), mostly because different maternal

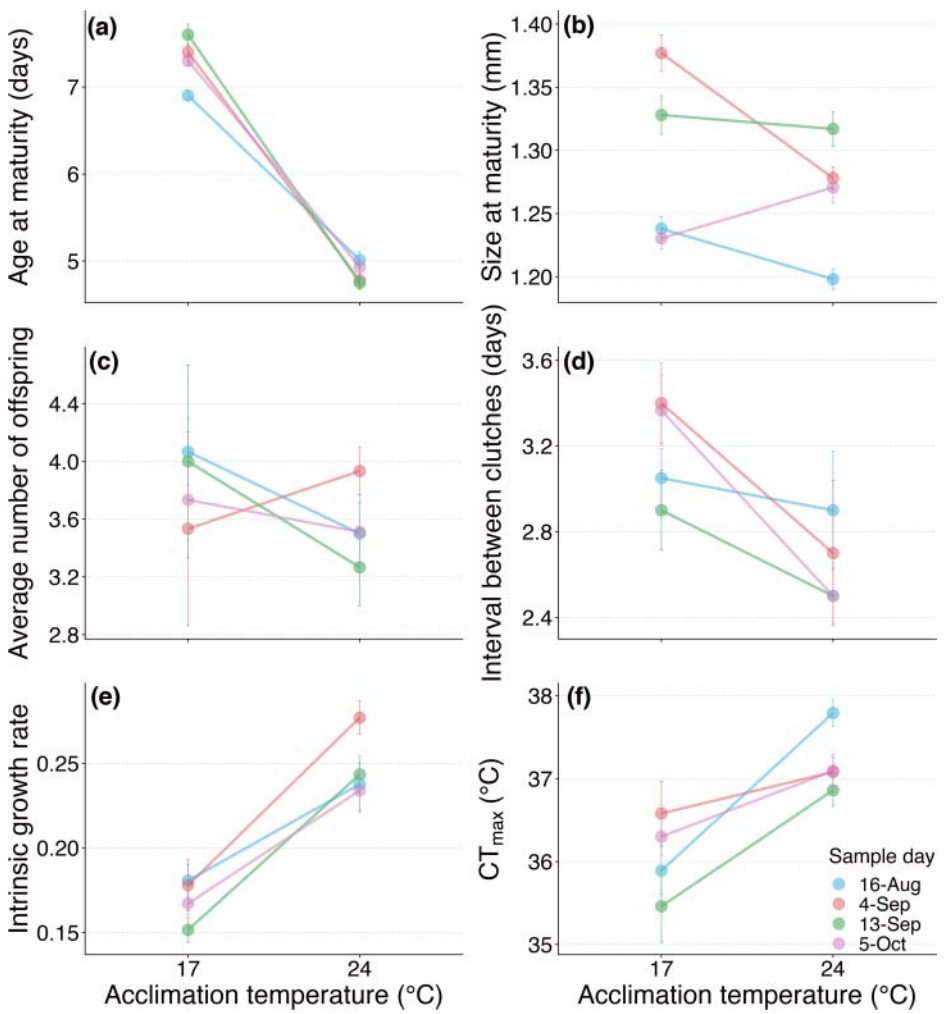

Fig. 2. Age at maturity (a), size at maturity (b), average number of offspring of the first three clutches (c), interval between clutches (d), intrinsic growth rate (e), and critical maximum temperature (f) of *Daphnia pulicaria* collected at different times during summer 2017 at Blue Lake as a function of acclimation temperature. Values are means ± 1 standard error.

lines show distinct responses to acclimation temperature for size at maturity. There was a significant interaction between acclimation temperature and time, showing differences among maternal lines in response to acclimation temperature (acclimation × time: $P = 0.022$, Table 1).

Levels of plasticity were affected by both the trait and the sample time (trait: $F_{5,1401} = 510.735$, $P < 0.001$; sample: $F_{3,1406} = 20.107$, $P < 0.001$; trait × sample: $F_{15,1386} = 13.648$, $P < 0.001$). Pairwise comparisons of least square means shows that samples have different slopes for age at maturity, number of offspring, clutch interval, and CT_{max}. The *Daphnia* sampled on 13 September, after the lake was no longer stratified, had the greatest plasticity for age at maturity (i.e. steeper slopes for the reaction norms) compared with all the other maternal lines (Fig. 3a). In contrast, the *Daphnia* sampled on 4 September, during stratification, was the only maternal line that had more offspring when reared at 24°C (Fig. 3b). For

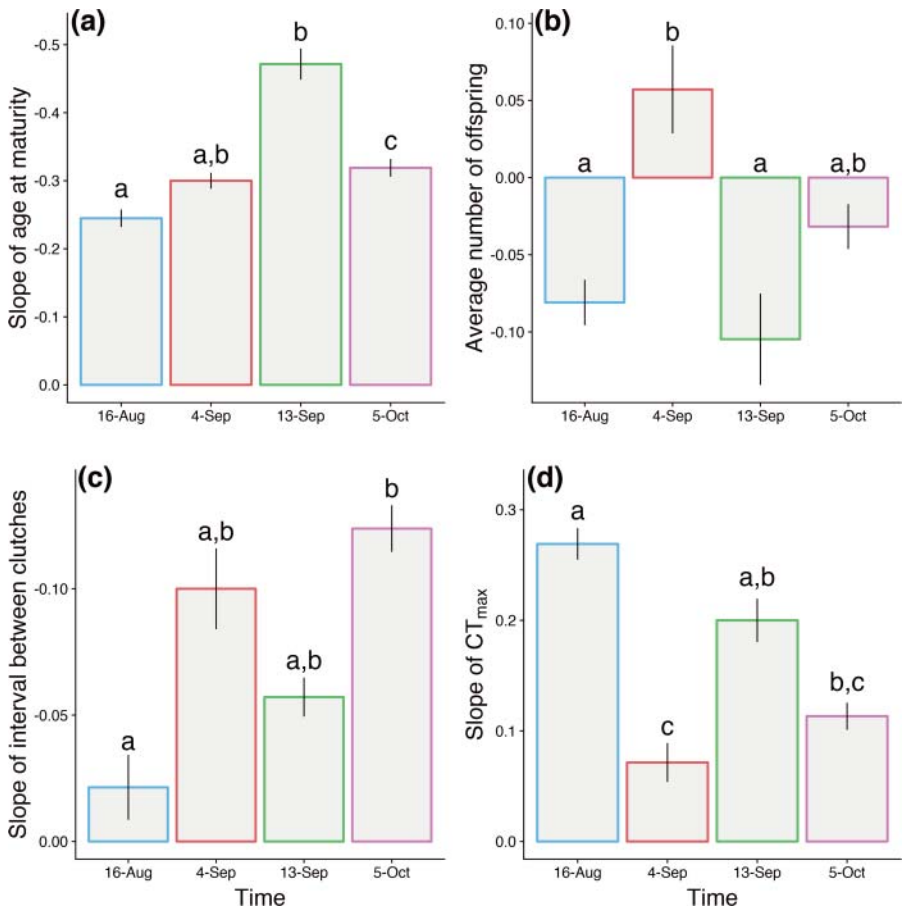

Fig. 3. Slopes for the reaction norms of age at maturity (a), average number of offspring (b), interval between clutches (c), and critical maximum temperature (d) of *Daphnia* in response to two acclimation temperatures (17°C and 24°C) collected throughout the growing season of 2017 in Blue Lake. Values are means ± 1 standard error. Letters indicate significantly different values based on least square means pairwise differences. Colours represent the sampling date as in Fig. 2.

interval between clutches, the first (16 August) and last samples (5 October) displayed the lowest and highest levels of plasticity, respectively (Fig. 3c). For CT_{max}, the earliest sample, taken on 16 August during stratification, displayed the highest level of plasticity, followed by the 13 September and 5 October samples, while that collected on 4 September had the lowest (Fig. 3d). All samples were grouped together for size at maturity and intrinsic growth rate because the slopes did not change over time.

Survivorship

The two temperature acclimation treatments affected *Daphnia* survivorship, but those effects were not influenced by time (acclimation: $P = 0.030$; time: $P = 0.118$, Table 2). Survivorship curves approximated a sigmoidal shape, starting with a plateau of high survivorship at low temperatures, followed by a steep decrease in survivorship as temperatures increased between 30°C and 32°C (Fig. 4). Stress temperature (28–36°C) also had a significant effect on thermal performance curves (stress: $P < 0.001$, Table 2). However, there was no significant effect of time in our model, indicating that *Daphnia* collected at different times throughout the growing season showed similar survivorship responses to stress temperature, and acclimatiion equally affected survivorship. Wilcoxon tests show that, across all maternal lines, acclimation only increased survivorship at 32°C, and not at any of the other stress temperatures ($W = 525$, $P = 0.039$).

DISCUSSION

In the present study, we found variation in phenotypic plasticity in response to temperature among *Daphnia* clones isolated at different times of the growing season in an alpine lake. Phenotypic traits showed distinct responses to time and acclimation. Thermal survivorship curves were similar over time, indicating that only acclimation temperature affected survivorship. Similarly, the effect of acclimation temperature on growth rate and age at maturity did not change throughout the growing season. By contrast, plasticity in size at maturity, the interval between clutches, and CT_{max} varied among maternal lines isolated on different

Table 2. Results of the generalized linear model analysis of the survivorship probability at each stress temperature (Stress) of *Daphnia pulicaria* collected throughout the 2017 growing season (Time) and reared under different acclimation treatments (17°C and 24°C)

Factor	*df*	Deviance	Residual deviance	Pr(>χ^2)
Survivorship				
Stress	1, 350	361.35	120.06	<**0.001**
Acclimation	1, 349	4.67	115.38	**0.030**
Time	3, 346	5.86	109.52	0.118
Stress × Acclimation	1, 345	1.43	108.09	0.230
Stress × Time	3, 342	5.99	102.09	0.111
Acclimation × Time	3, 339	0.19	101.90	0.978
Stress × Acclimation × Time	3, 336	7.10	94.80	0.069

Note: Error distribution: binomial; link function: logit for survivorship analysis.

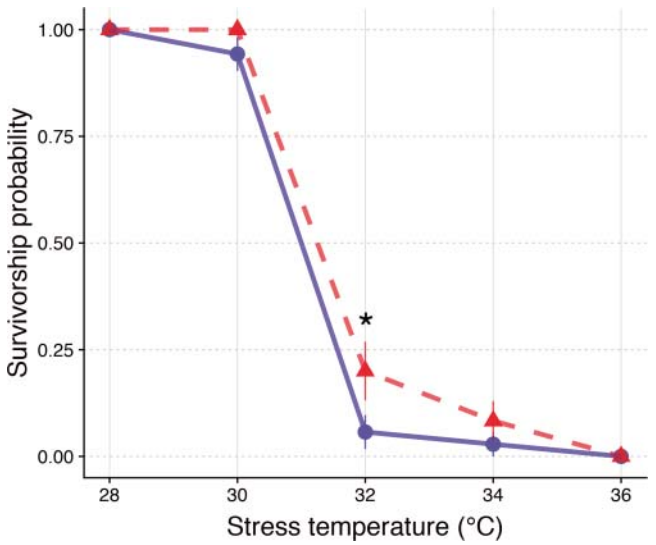

Fig. 4. Probability of survival of *Daphnia* from Blue Lake sampled during summer 2017 to exposure to stress temperatures for 1 hour across all maternal lines. Survivorship to stress temperature was tested in individuals from different samples reared at 17°C (blue) and 24°C (red) acclimation temperatures. Asterisk indicates significant differences in survivorship between acclimation treatments.

dates, indicating potential seasonal genetic variation in plasticity in response to temperature. Different traits showed distinct seasonal variation in plasticity, indicating that plasticity in general does not show clear seasonal patterns. Rather, different traits tend to be most plastic at different times of the year. Our results indicate that genetic diversity and seasonal variation in plasticity may maintain fitness and allow *Daphnia* to persist over a broad phenological temperature range encountered in alpine lakes.

The amount of plasticity in the interval between clutches changed throughout the growing season. *Daphnia* from mid-summer (16 August) had the lowest level of plasticity, while individuals from early fall (5 October) had the highest. This result contradicts our hypothesis that spatial environmental heterogeneity in the form of vertical stratification should select for greater plasticity. Plasticity for interval between clutches could be related to timing for egg production that could respond to food availability (Bradley *et al.*, 1991). Phytoplankton were most abundant in mid-summer (Fig. 1c), but declined in the early fall. When food is scarce, *Daphnia* decrease total energy investment in reproduction, but optimize their investment per offspring (Goulden *et al.*, 1987), since larger egg size is positively correlated with offspring fitness. Thus, individuals produce fewer, but larger, eggs. Even though different life-history traits showed seasonally variable plasticity, plasticity in intrinsic growth rate was consistent among sampling periods. This result supports Lynch (1989), who concluded that despite life-history changes in *D. pulex* in response to food availability, reproductive trade-offs can lead to the same population growth rate with many different life-history strategies.

Previous studies have shown that *Daphnia* reared at higher temperatures had increased thermal tolerance, indicating that this trait is plastic and the response is adaptive (Paul *et al.*,

2004; Yampolsky *et al.*, 2014). Critical maximum temperature is a measurement of the upper thermal limits (i.e. tolerance to extreme temperatures), thus adaptive changes in the mean CT_{max} response are most likely due to extreme temperatures or heat waves (Yampolsky *et al.*, 2014; Geerts *et al.*, 2015; Brans *et al.*, 2017). Therefore, we cannot infer changes in the thermal niche curve because we do not know whether or not minimum temperature also shifted, since we did not measure critical minimum temperature. Furthermore, thermal tolerance is related to aerobic capacity, which reflects the relationship between oxygen supply and demand of organisms' tissues (Pörtner and Knust, 2007). Thus, adaptive changes in slopes of the reaction norms indicate that organisms have greater aerobic capacity to cope with a wider range of temperatures. Our results show that maternal lines from the beginning of the growing season (16 August) experienced an increase of up to 1.9°C in CT_{max} after acclimation at the higher temperature (24°C), followed by increases of 1.4°C (13 September) and 0.8°C (5 October). In general, CT_{max} decreased during the fall compared with summer, except for the *Daphnia* collected on 4 September. Paul *et al.* (2012) found that thermal tolerance of summer clones was greater than that of spring or fall clones, followed by winter clones. This corroborates the general pattern found in the present study that CT_{max} seems to decrease throughout the growing season, suggesting that thermal variability might play an important role in selecting plasticity for CT_{max}.

Our data provide support for other studies of the role of genetic adaptation and plasticity in *Daphnia*'s response to temperature and seasonal cycles. Carvalho (1987) compared clones of *Daphnia magna* before and after seasonal shifts in temperature and found that the transition from spring to summer was associated with selection for clones that exhibit increased survivorship and fecundity at high temperatures. Intrinsic growth rate is often greater at higher temperatures due to an increase in metabolism (e.g. Mitchell and Lampert, 2000; Weetman and Atkinson, 2004), which permits rapid maturation and reduction in age at release of each clutch, resulting in larger populations at warmer temperatures (Stich and Lampert, 1981; Kingsolver and Huey, 2008; Henning-Lucass *et al.*, 2016). We also found that the maternal line collected on 13 September exhibited a higher level of plasticity for age at maturity compared with the other maternal lines. This result is also consistent with the response of *Daphnia* collected on 4 September, which showed an increase in offspring number at the higher acclimation temperature.

The inferences we draw from our study are constrained by a number of limitations. Most importantly, we only had one maternal line from the two intermediate samples due to high mortality, thus our maternal line might not represent the average plasticity for that time. Low survival in laboratory conditions is often observed in alpine and high latitude zooplankton. Additionally, the establishment and maintenance of maternal lines in the laboratory might cause artificial selection that may have prevented us from recording more prominent differences in life-history traits between maternal lines. A more comprehensive assessment of the genetic diversity present in Blue Lake is needed to assess the generality of our results. In addition, there is evidence that variation in individual thermal preference in *D. pulicaria* can influence diel vertical migration (DVM) (Glaholt *et al.*, 2016), but we did not determine the thermal preference of the maternal lines studied here. However, we still expect *Daphnia* to exhibit DVM behaviour because the presence of fish causes *Daphnia* to migrate (Glaholt *et al.*, 2016) and fish are present in Blue Lake. Nevertheless, we observed clear evidence for phenotypic plasticity and variation among maternal lines isolated at different times of the growing season.

Genetic variation reflects an evolutionary potential to respond to future disturbances or gradual changes, such as warming, either through adaptation (Geerts *et al.*, 2015; Brans *et al.*, 2017)

or adaptive plasticity (Cavalheri *et al.*, 2019). Previously, we showed that different populations of *D. pulicaria* from Sierra Nevada (CA) lakes evolved plasticity in response to temperature, indicating the presence of genetic variation for plasticity among populations (Cavalheri *et al.*, 2019). The level of plasticity depended on the trait and the time of year. While the interval between clutches, number of offspring, and age at maturity did not support our initial hypothesis, that *Daphnia* would exhibit higher levels of plasticity in more heterogeneous environments, thermal tolerance was consistent with our hypothesis, suggesting that *Daphnia* populations exhibit genetic variation for plasticity in response to temperature. Our results build on our prior work by documenting variation in plasticity through time, possibly because of tracking changes in water column temperature. Lakes are already affected by climate warming reflected in longer duration of ice-free periods, stratification, and reduced vertical mixing (McCormick, 1990; Schindler *et al.*, 1990; Adrian *et al.*, 1995; O'Reilly *et al.*, 2015). Thus, changes in genetic structure of *Daphnia* populations favouring individuals capable of coping with a wider range of environmental conditions (i.e. higher levels of plasticity) may be expected in the future.

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