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Food quality mediates responses of *Daphnia magna* life history traits and heat tolerance to elevated temperature

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Abstract

1. Surface waters are warming due to climate change, potentially pushing aquatic organisms closer to their thermal tolerance limits. However, cyanobacterial blooms are expected to occur more often with rising temperature, increasing the likelihood of poor-quality food available for herbivorous zooplankton. Zooplankton can adapt locally by genetic differentiation or via adaptive phenotypic plasticity to increasing temperatures, but there is limited knowledge on how these processes may be affected by food quality limitation imposed by cyanobacteria.

2. To test the effects of cyanobacteria-mediated food quality on local temperature adaptation, we measured juvenile somatic growth and reproduction of five *Daphnia magna* clones from different latitudinal origin grown on three food qualities at 20, 24, and 28°C. Additionally we estimated short-term heat tolerance, measured as knockout time (time to immobility) at lethally high temperature, of two clones acclimated to the three temperatures and two food quality levels to test for the effects of food quality on adaptive plastic responses.

3. As expected, clones from lower latitudes showed on average better somatic growth and reproduction than clones from higher latitudes at higher temperatures. However, the difference in somatic growth diminished with increasing cyanobacteria abundance in the diet, suggesting constraints on local genetic adaptation under predicted decreases in food quality. As expected, short-term heat tolerance of the clones generally increased with increasing acclimation temperature. However, heat tolerance of animals acclimated to the highest temperature was larger when grown at medium than at good food quality, whereas the opposite response was observed for animals acclimated to the lowest temperature. This suggests a better adaptive phenotypic response of animals to elevated temperatures under higher cyanobacteria abundance, and thus shows an opposite pattern to the results for somatic growth.

4. Overall, we demonstrate that food quality limitation can mediate responses of *D. magna* life history traits and heat tolerance to increasing temperatures, and that the effects differ depending on the time scale studied, that is, mid-term (somatic growth) versus short-term (tolerance to acute heat stress). These aspects

1 | INTRODUCTION

Climate change is increasing mean temperatures and the frequency of thermal extremes and heatwaves (Easterling et al., 2000; Meehl & Tebaldi, 2004). Therefore, the frequency and severity of summer heatwaves is also increasing in aquatic habitats (Frölicher et al., 2018; Woolway et al., 2021). Because species or populations have specific temperature preferences, increasing temperature will have far reaching consequences on local community composition and ecosystem functioning (e.g. by leading to range shifts in the occurrence of species, Easterling et al., 2000; Pinsky et al., 2020). Longer periods of high temperature may also push organisms closer to their limits of physiological tolerance, which, if crossed, may ultimately cause local extinctions (Ma et al., 2021; Stillman, 2019; Vasseur et al., 2014).

Body temperature of ectotherms is tightly linked to environmental temperature, especially for planktonic organisms due to their small body size. Ectotherms can adapt to changing temperature by a variety of regulatory mechanisms. Ectotherm species have to adapt to elevated temperatures locally by behavioural or physiological means or through phenotypic plasticity or evolutionary adaptation, if not they will be replaced by other, more warm-tolerant species (DeWitt et al., 1998; Kawecki & Ebert, 2004; Pigliucci, 2001; Stillman, 2019; Yampolsky et al., 2014).

Increasing temperature favours growth of cyanobacteria (Jöhnk et al., 2008) and therefore the frequency of harmful cyanobacterial blooms is increasing with global warming (Paerl & Huisman, 2008). Cyanobacterial blooms can have many negative effects in aquatic ecosystems, including the poor usability for secondary production by herbivorous zooplankton (Martin-Creuzburg et al., 2009; Von Elert et al., 2003; Wilson et al., 2006). Cyanobacteria can be toxic, develop colonies or filaments to hinder ingestion, and are of poor nutritional quality for zooplankton due to the lack of specific micronutrients, such as phytosterols and polyunsaturated fatty acids (PUFAs) (Martin-Creuzburg et al., 2009; Von Elert et al., 2003; Wilson et al., 2006). The lack of phytosterols and PUFAs due to high proportions of cyanobacteria in the diet can limit zooplankton growth and reproduction (Martin-Creuzburg et al., 2009; Sperfeld et al., 2012; Sperfeld & Wacker, 2015). The dietary supply of sterols seems to be especially important for physiological adaptation to higher temperatures (Hassett & Crockett, 2009; Sperfeld & Wacker, 2009), whereas PUFAs are more important at colder temperatures (Martin-Creuzburg et al., 2012; Sperfeld & Wacker, 2012).

Daphnia spp. are key freshwater zooplankton linking primary production with higher trophic level consumers such as fish (Ogorelec et al., 2021). *Daphnia magna*, the largest *Daphnia* species, is often used as model organism in ecological, evolutionary or eco-toxicological studies (Miner et al., 2012). *Daphnia* females usually reproduce asexually under favourable conditions, making it a suitable organism to study local genetic adaptation or adaptive phenotypic plasticity by investigating clonal lineages originating from different localities (Yampolsky et al., 2014). *Daphnia magna* is geographically widely distributed (Benzie, 2005) and different clonal lines can occur across seasons (e.g. Paul et al., 2012). Earlier studies found mixed evidence for genetic temperature adaptation in local *D. magna* populations (across a geographic gradient, Mitchell & Lampert, 2000; Yampolsky et al., 2014) or for seasonal temperature adaptation of clonal lines (Carvalho, 1987; Mitchell et al., 2004; Paul et al., 2012). A more recent study, using many more *D. magna* clones, shows strong evidence for local temperature adaption in summer-active populations (latitudes higher than *c.* 43°), whereas winter-active clones at latitudes lower than *c.* 43° are not locally adapted to temperature as they endure the very high summer temperatures in a dormant stage (Seefeldt & Ebert, 2019). *Daphnia magna* also shows strong adaptive phenotypic plasticity by being more heat tolerant when pre-acclimated to higher temperatures, allowing this species to better tolerate suboptimal temperatures (MacIsaac et al., 1985; Yampolsky et al., 2014).

There is limited knowledge about how food quality limitation imposed by cyanobacteria affects life history traits of locally adapted *Daphnia* populations and their capacity to tolerate high temperatures. In this study, we investigated the effects of cyanobacteria-mediated food quality and warmer temperature on life history traits and heat tolerance of *D. magna* clones originating from different latitudes. For this, we measured somatic growth and reproduction of five *D. magna* clonal lineages kept at 20, 24, and 28°C on three food mixtures that differed in their relative abundance of cyanobacteria, resulting in poor, medium, and good food quality. We also measured short-term heat tolerance by knockout time, that is, time until immobilisation when exposed to lethally high temperature (37°C), for two clones differing in thermal sensitivity that have been acclimated to 20, 24, and 28°C and kept on medium and good food quality. We expected that clones from lower latitudes would grow better at higher temperatures than clones from higher latitudes due to local adaptation and that food quality limitation will weaken this effect due to smaller growth responses. We also expected the clone from lower latitude to be more heat tolerant than the clone from higher latitude (due to local temperature adaptation) and that heat tolerance increases with increasing acclimation temperature due to adaptive phenotypic plasticity.

2 | METHODS

2.1 | Organisms and general culture conditions

We used five *D. magna* clones from distinct geographical locations across Europe that can be considered as summer-active clones and thus have the potential to be locally temperature adapted (Seefeldt & Ebert, 2019). Three of the clones originated from lower latitudes (CH-H-5, lake, Switzerland, GPS lat./lon.: 47.5578/8.8626, altitude *c.* 400 m; CH-Z-4, pond, Switzerland, lat./lon.: 47.5170/8.8313, altitude *c.* 500 m; HU-HO-2, forest pond, Hungary, lat./lon.: 46.80/19.133333, altitude *c.* 100 m) and two of the clones originated from higher latitudes (RU-BOL1-1, rock pool, Russia, lat./lon.: 66.42571678/33.8368342, altitude *c.* 1 m; NO-M3-1, rock pool, Norway, lat./lon.: 59.0971769327/11.19664594, altitude *c.* 1 m). The sites from which the clones were collected show different monthly average temperatures during the daphnids' summer-active time (Figure S1), with the sites of lower-latitude clones experiencing higher maximum and mean temperatures than the sites of higher-latitude clones (maximum temperature of the warmest month, CH-H: 24.2°C, CH-Z: 23.3°C, HU-HO: 26.8°C vs. RU-BOL: 17.5°C, NO-M3: 20.4°C; mean temperature of the warmest month, CH-H: 18.8°C, CH-Z: 18.1°C, HU-HO: 21.0°C vs. RU-BOL: 13.9°C, NO-M3: 16.7°C; monthly averages retrieved from WorldClim version 2.1, see also Figure S1).

The *D. magna* clones were kept at 20°C in a temperature-controlled climate room with a 16:8 hr light: dark cycle for many generations. The daphniids were cultured in ADaM medium (Klüttgen et al., 1994), modified by using 0.05 times the recommended SeO_2 concentration, and fed daily with 2 mg C/L of an algae mixture composed of 60% *Acutodesmus obliquus* (formerly known as *Scenedesmus obliquus*, strain SAG 276-3a, culture collection Göttingen, Germany) and 40% *Nannochloropsis limnetica* (SAG 18.99). Carbon concentrations of the phytoplankton food suspensions were estimated from photometric light extinction (800 nm) using previously determined carbon-extinction equations for each phytoplankton species. The chlorophyte *A. obliquus* is rich in phytosterols and shorter-chain PUFAs, such as α-linolenic acid (18:3ω3) (Martin-Creuzburg et al., 2012; Sperfeld & Wacker, 2015), whereas the eustigmatophyte *N. limnetica* contains phytosterols and is rich in cholesterol and long-chain PUFAs, especially eicosapentaenoic acid (20:5ω3) (Martin-Creuzburg et al., 2009; Sperfeld & Wacker, 2015). For the experiments we also used the non-toxic, sterol- and PUFA-free cyanobacterium *Synechococcus elongatus* (SAG 89.79) (Martin-Creuzburg et al., 2009; Sperfeld & Wacker, 2015). *Acutodesmus obliquus*, *N. limnetica*, and *S. elongatus* (henceforth ACU, NAN, and SYN, respectively) were cultured semi-continuously (dilution rate: 0.1–0.4 day^{-1}, depending on density of cultures and species-specific growth rates) in aerated 2-L flasks containing modified WC medium with vitamins (Guillard, 1975) at 15.5°C under low-light conditions (20–40 μmol photons m^{-2} s^{-1}, 16:8 hr light: dark cycle) to ensure high nutrient concentrations in the algae. We did not grow phytoplankton at the same temperatures as used in the daphniid experiments, although an earlier study suggested growing phytoplankton and daphniids at the same temperature to more precisely predict the food quality effects of global warming (von Elert & Fink, 2018). As we did not aim to make precise predictions of global warming effects, but instead aimed understanding basic mechanisms, we grew phytoplankton at a single temperature to offer daphniids standardised food of the same biochemical composition across all treatments.

2.2 | Growth experiment

In the growth experiment, females of all five *D. magna* clones were grown from juvenile to adulthood at three different food quality treatments, each at 20, 24, and 28°C. Treatments were conducted in triplicate jars stocked with five juveniles per replicate jar filled with 200 mL ADaM medium (five clones × three temperatures × three food qualities × three replicates = 135 jars each stocked with 5 juveniles = 765 individuals in total). The three food quality treatments were: (1) good quality, 50:50% mixture of ACU and NAN; (2) medium quality, 10:10:80% mixture of ACU, NAN, and SYN; and (3) poor quality, 5:5:90% mixture of ACU, NAN, and SYN. Food ratios of different phytoplankton species in the mixtures were based on carbon concentrations (see above). Daphniids at 20 and 24°C were fed daily with 2 mg C/L, daphniids at 28°C daily with 3 mg C/L.

Prior to the experiment, *D. magna* females of all clones were first synchronised at 20°C for at least three generations and then acclimated to the higher treatment temperatures. Subsets of females were acclimated to 24 and 28°C by increasing temperature stepwise by 2°C every 2–3 days until 24°C had been reached, and every 4–5 days from 24°C until 28°C. Experiments were started with third or fourth clutch juveniles from individual mothers that had been temperature acclimated for at least one generation.

Hatching juveniles (neonates) from mothers of the five clones kept at the three temperatures were collected within 18 hr and subsequently distributed randomly to the different treatments. Subsamples of 2×10 neonates for each clone and acclimation temperature were taken for initial dry mass determination and weighed on an electronic ultra-micro balance (±1 μg, Sartorius, ME5, Göttingen, Germany) after drying in pre-weighed aluminium boats for 48 hr at 60°C. The animals were transferred every other day into newly prepared food suspensions and the experiments were terminated when the majority of females grown on good food quality had released their first clutch into the brood pouch. The eggs of each egg-carrying female were counted using a stereomicroscope before the total individual dry mass was determined to calculate mass-specific growth rates. Average individual dry mass for each replicate was determined by drying and weighing females (including eggs) as described above. The mass-specific growth rate (g) was calculated as the increase in average individual dry mass from the start (neonate) of the experiment (DM_0) to the day of experiment termination (DM_t) using the equation $g = (\ln DM_t - \ln DM_0)/t$, with t being the

duration of the growth period in days. We lost only one replicate due to handling (clone HU-HO-2, poor food quality, 24°C).

2.3 | Heat tolerance test

We tested the heat tolerance of one high-latitude clone (RU-BOL-1-1) and one lower-latitude clone (CH-Z-4), acclimated at different temperatures and food quality treatments, by measuring the time until immobilisation (= knockout time) of juveniles exposed suddenly to 37°C (Yampolsky et al., 2014). Previous studies showed that time until immobilisation was affected more strongly by acclimation temperature via phenotypic plastic responses than by local adaptation (i.e. differences between clones, e.g. Yampolsky et al., 2014). The main aim of our heat tolerance test was to investigate how food quality mediates the effects of acclimation temperature on time until immobilisation, and thus only two clones were used. Daphniids were pre-cultured for one generation on food of good quality (50:50% ACU:NAN) and medium quality (10:10:80% ACU:NAN:SYN) at 20 and 24°C (fed with 2 mg C/L), and at 28°C (fed with 3 mg C/L). Third clutch juveniles, collected within 24 hr, were raised on these treatments until they reached a similar body size, which took 2–3 days depending on acclimation temperature and food quality. For each treatment and clone, 24 of these juveniles were used in the heat tolerance test. 1.5 mL centrifuge tubes filled with 1.2 mL fresh ADaM medium were acclimated to the animals' rearing temperature (i.e. 20, 24, 28°C), before the juveniles were transferred individually into these tubes. Immediately afterwards the tubes containing the animals were placed in a heating block (Kleinfeld, MBT 250, Germany) that was set up to keep ADaM medium at 37°C. Time until immobilisation (T_{imm}) was measured from the moment the tube with the juvenile was placed into the heating block to the moment the individual showed no movement anymore, that is, became immobile. Up to 12 individuals were tested by one observer in one run. Preliminary tests showed that a maximum of 12 individuals could be monitored by one observer for sufficient accuracy in T_{imm} measurements. The individuals were observed consecutively for a few seconds each. Care has been taken to avoid measurements of apparent immobilisation due to temporarily immobilised individuals by observing each individual for an additional time period after movement had ceased.

After T_{imm} measurements were completed, individuals were photographed using a stereomicroscope (Leica, S9D) equipped with a computer-aided camera (Leica, DMC 2900) for measurements of body length, defined as the distance between the top of the head and the base of the tail spine (Sperfeld et al., 2020), using the Leica Application Suite software (LAS X, v. 3.7.1.21655). Body size of juveniles at the time of the heat tolerance test was within a very narrow range (body length: 1.6 ± 0.2, mean $\pm$ SD, $n = 278$, 10 measurements lost due to handling), thus avoiding potential size-dependent effects on T_{imm} (Burton et al., 2020).

2.4 | Data analysis

The five clones were assigned to groups of their latitudinal origin and nested within these groups in linear models. Mass-specific growth rates and clutch sizes (i.e. average number of eggs of the first clutch per female) were analysed using general linear models with factorial explanatory variables of acclimation temperature, food quality and group of latitudinal origin. Clutch size data from the poor food quality treatment were not available for all clones and treatment combinations, which did not allow meaningful comparisons, and thus were not analysed statistically.

To test whether lower-latitude clones cope better at higher temperatures than higher-latitude clones (or vice versa), we calculated the deviation of growth rates or clutch sizes measured at 24 and 28°C from mean growth rates or clutch sizes achieved at 20°C. This was necessary to account for differences in the clones' general capacity to grow or produce eggs (when considering 20°C as a baseline control). The deviation from 20°C (termed growth increment or clutch size response) was calculated at each food quality level separately to account for average differences depending on food quality level. This approach allows a straight-forward evaluation of whether growth rates or clutch sizes increase or decrease at higher temperatures compared to 20°C, similar to an effect size, and also allows meaningful comparison between higher and lower latitude clones. As for mass-specific growth rates and clutch sizes, growth increments and clutch size responses were analysed using general linear models with factors acclimation temperature, food quality and group of latitudinal origin, with clones nested within latitudinal group. Additional general linear models were conducted to investigate the effects of latitudinal clone origin and temperature for each food quality level separately. Variance explained by factors and their interactions in the models was calculated based on the sum of squares of the ANOVA table results.

Differences in T_{imm} of the two tested clones were analysed using general linear models with factors clone and food quality, and acclimation temperature as continuous explanatory variable. To keep the model parsimonious, body length was not included as it explained only very little variance in T_{imm} (3.5% out of c. 70%) and did not change the overall statistical results. To investigate effects on the individual clones, additional general linear models were conducted for each clone separately with food quality as factor and acclimation temperature as continuous explanatory variable.

All statistical analyses and tests of their assumptions were performed using the statistical software R, version 4.1.2 (R Core Team, 2021). The *tidyverse* package, which contains a collection of R packages including the *ggplot2* package, was used for data handling and preparation of figures (Wickham et al., 2019). We used the *autoplot()* function of the *ggfortify* package (Tang et al., 2016) to visually test the assumptions of the applied general linear models. We did not observe problems regarding the assumptions of linearity, normality of the residuals, and homogeneity of variance of the residuals. A few outliers led to high variance in some of the treatments, but

excluding these outliers in the analyses did not influence the general statistical outcomes.

3 | RESULTS

3.1 | Somatic growth

Somatic growth rate of all *D. magna* clones generally increased with increasing temperature as well as increasing food quality (Figure S2; Table 1). There were clone-specific differences within the higher-latitude and lower-latitude clones (Table 1: T×FQ×L×Cl interaction). For instance, the HU clone showed mostly faster growth compared to the other lower-latitude clones; for the higher-latitude clones at 20°C, the NO clone showed slower growth than the RU clone, whereas the opposite pattern was observed at 28°C (Figure S2).

We related the growth rates achieved at 24 and 28°C to mean growth rates at 20°C for each food quality level to account for differences in the clones' general growth capacity at different food quality levels (Figure 1). In this way, we could specifically test whether lower-latitude clones show higher growth increments at warmer temperatures compared to higher-latitude clones, or vice versa, and likewise for each food quality treatment. Not surprisingly, growth increments from 20°C to 28°C were higher than growth increments from 20°C to 24°C (Figure 1), resulting in temperature explaining the majority of the variance (Table 1). Notably, lower-latitude clones showed higher growth increments on average than higher-latitude clones at higher temperatures (Figure 1; Table 1: L), but only at medium and good food quality (Figure 1; Table 1: FQ×L interaction). The difference between latitudinal group was larger at good than at medium food quality and disappeared completely at poor food quality (Figure 1; Figure S3, slopes of dashed lines; Table 1: FQ×L interaction).

3.2 | Reproduction

The growth experiments were terminated when the majority of females grown on good food quality had released their first clutch into the brood pouch. Only very few females at poor food quality produced eggs at the end of the experimental period, with clutch sizes of maximum eight eggs per female (but mostly well below, Figure S4). Clutch sizes at poor food quality were not available for all clones and treatment combinations (Figure S4), which did not allow proper statistical analyses. In the two treatments of higher food quality, where a substantial part of the females produced eggs at the end of the experimental period, differences in clutch size were primarily driven by food quality (Figure S4; Table 1). Across all clones, females produced on average nine eggs at medium quality and 14.5 eggs at high quality for their first clutch. Notably, females of the lower-latitude clones showed higher clutch sizes on average than females of higher-latitude clones (12.4 and 8.8, respectively; Figure S4; Table 1). However, as this pattern was also observed at 20°C, one cannot test whether lower-latitude clones show higher clutch sizes at higher temperatures than higher-latitude clones.

TABLE 1 Results of three-way ANOVAs to test for effects of factorial temperature (T), food quality (FQ), and latitudinal clone origin (L) on somatic growth rates, somatic growth rate increment, clutch size, and clutch size response of *Daphnia magna*

	df	Sum Sq.	F	p	Var. (%)
Somatic growth rate (r²=0.97)					
T	**2**	**1.827**	**829.0**	**<0.001**	**59.0**
FQ	**2**	**0.890**	**404.1**	**<0.001**	**28.8**
L	1	0.021	19.1	<0.001	0.7
T×FQ	4	0.007	1.5	0.199	0.2
T×L.	2	0.020	9.0	<0.001	0.6
FQ×L	2	0.007	3.3	0.042	0.2
T×FQ×L	4	0.012	2.8	0.031	0.4
T×FQ×L×Cl	**27**	**0.212**	**7.1**	**<0.001**	**6.9**
Residuals	89	0.098			3.2
Somatic growth rate increment (r²=0.86)					
T	**1**	**0.447**	**354.7**	**<0.001**	**57.3**
FQ	2	0.006	2.5	0.091	0.8
L	**1**	**0.057**	**45.0**	**<0.001**	**7.3**
T×FQ	2	0.004	1.8	0.180	0.6
T×L	1	0.002	1.2	0.276	0.2
FQ×L	**2**	**0.027**	**10.8**	**<0.001**	**3.5**
T×FQ×L	2	0.003	1.1	0.330	0.4
T×FQ×L×Cl	**18**	**0.159**	**7.0**	**<0.001**	**20.5**
Residuals	59	0.074			9.5
Clutch size (r²=0.82)					
T	2	30.6	4.1	0.021	2.5
FQ	**1**	**651.7**	**176.7**	**<0.001**	**53.7**
L	**1**	**156.5**	**42.4**	**<0.001**	**12.9**
T×FQ	2	28.6	3.9	0.026	2.4
T×L	2	11.7	1.6	0.212	1.0
FQ×L	1	5.2	1.4	0.239	0.4
T×FQ×L	**2**	**5.1**	**0.7**	**0.505**	**0.4**
T×FQ×L×Cl	**18**	**109.3**	**1.6**	**0.078**	**9.0**
Residuals	58	213.9			17.6
Clutch size response (r² = 0.60)					
T	1	6.1	1.69	0.201	1.8
FQ	**1**	**18.4**	**5.15**	**0.029**	**5.5**
L	**1**	**35.4**	**9.90**	**0.003**	**10.5**
T×FQ	**1**	**21.3**	**5.94**	**0.020**	**6.3**
T×L	1	0.3	0.10	0.759	0.1
FQ×L	1	3.2	0.89	0.351	0.9
T×FQ×L	1	3.7	1.04	0.314	1.1
T×FQ×L×Cl	**12**	**113.2**	**2.63**	**0.011**	**33.5**
Residuals	38	136.1			40.3

Note: Clones (Cl) are nested within latitudinal origin levels. Factors and interactions marked in bold indicate variance explained ≥3.5%.

To account for clone-specific differences in the capacity to produce eggs (at lower temperature) we related clutch sizes produced at higher temperatures to mean clutch size at 20°C analogous as for growth rate. There was still the general tendency that lower-latitude clones showed larger clutches on average than higher-latitude clones (Figure 2; Table 1: L). However, this effect seems mainly driven by the RU clone, which
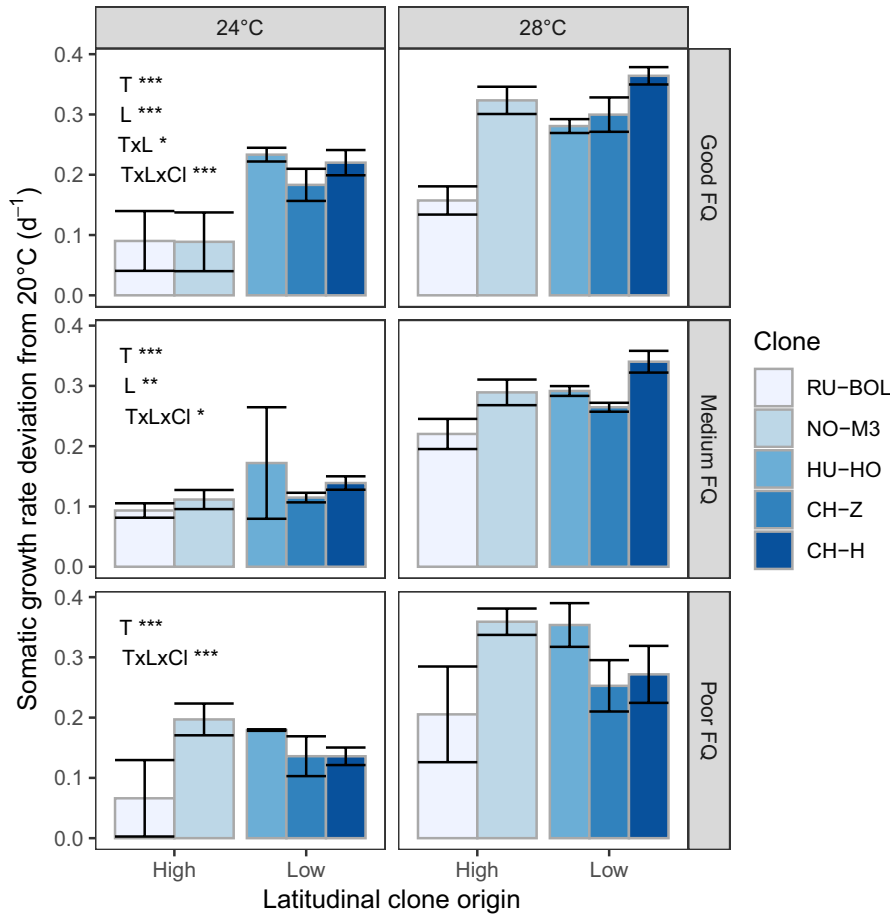
FIGURE 1 Somatic growth rate increments of *Daphnia magna* clones raised at higher temperatures (24 and 28°C) for each food quality (FQ) treatment (mean ± *SD* are presented). Growth increments were calculated by relating growth rates measured at higher temperatures to mean growth rates achieved at 20°C (= zero baseline in the plots). Asterisks indicate significance levels (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) of ANOVA results for each food quality treatment with the factors temperature (T) and latitudinal clone origin (L). Clones (cl) are nested within latitudinal origin levels. RU-BOL and NO-M3 refer to *D. magna* clones originating from higher latitudes and HU-HO, CH-Z, and CH-H refer to clones originating from lower latitudes
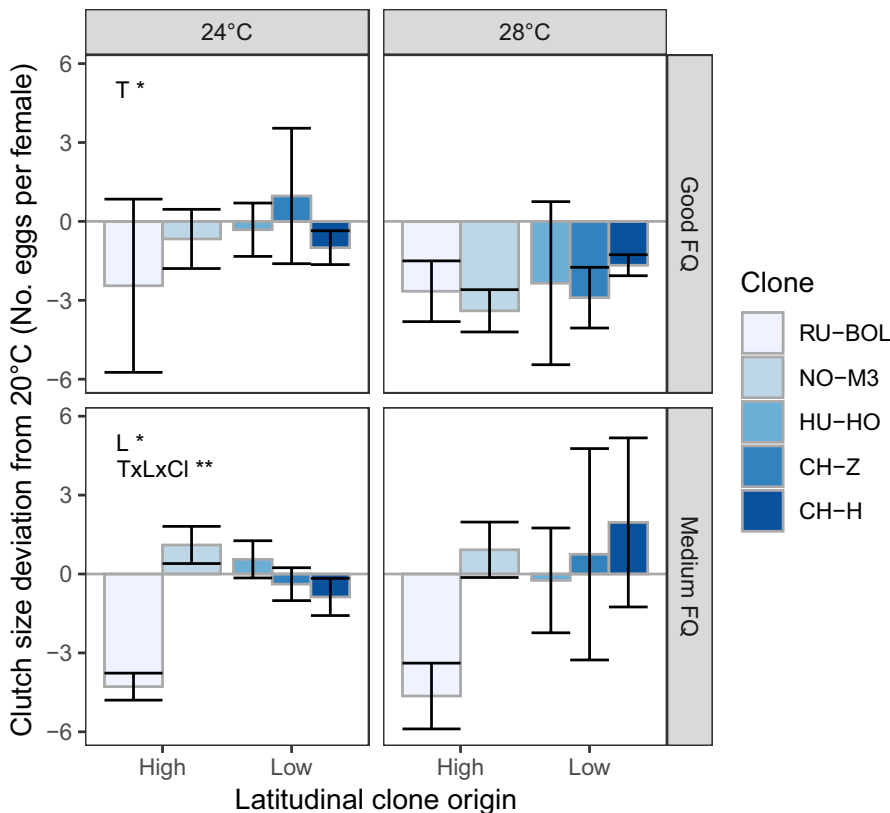
FIGURE 2 Clutch size responses of *Daphnia magna* clones raised at higher temperatures (24 and 28°C) for each food quality (FQ) treatment (mean ± *SD* are presented). Clutch size responses were calculated by relating clutch sizes measured at higher temperatures to mean clutch sizes achieved at 20°C (= zero baseline in the plots). Asterisks indicate significance levels (** $p < 0.01$, * $p < 0.05$) of ANOVA results for each food quality treatment with the factors temperature (T) and latitudinal clone origin (L). Clones (cl) are nested within latitudinal origin levels. Legend codes as for Figure 1

showed substantial negative clutch size responses especially at medium food quality, whereas the other clones did not show negative clutch size responses at medium food quality (Figure 2; Table 1: T×FQ×L×Cl interaction). At good food quality, clutch size responses across all clones were negative at 28°C, but no change in clutch sizes was observed at 24°C in relation to 20°C (Figure 2; Table 1: T×FQ interaction).

3.3 | Heat tolerance

The high and lower latitude clones showed very similar patterns in their heat tolerance across acclimation temperatures and food quality treatments (Figure 3; Table 2: clone and interactions with clone explain in sum <4% of the variance). For both clones, acclimation temperature had the largest effect, with T_{imm} increasing with increasing temperature (Figure 3; Table 2). However, the increase of T_{imm} along the temperature gradient was stronger in the medium compared to the good food quality treatment (Figure 3; Table 2: T×FQ interaction). This led to the notable pattern that T_{imm} was lower at medium than at good food quality at 20°C, whereas the opposite pattern could be observed at 28°C (Figure 3).

4 | DISCUSSION

4.1 | Temperature effects on *D. magna* life history traits and local adaptation

Temperature has strong effects on ectotherm life history traits, such as growth and reproduction (Ma et al., 2021). We observed that for all of the *D. magna* clones tested, somatic growth at all food quality levels increased with increasing temperature in the range of 20 to 28°C. This is an expected outcome as the thermal optimum

of many *D. magna* clones is above 25°C (Mitchell et al., 2004; Mitchell & Lampert, 2000), and is corroborated by other studies that investigated somatic growth rates at different food quality levels and varying high temperatures (Martin-Creuzburg et al., 2012; Masclaux et al., 2009; Sperfeld & Wacker, 2009). The size of the first clutch did not show the same pattern across temperature as growth rate, which is expected as it is a time-independent trait that depends rather on environmental factors such as food quantity or quality (Giebelhausen & Lampert, 2001; Pajk et al., 2012). The only notable response to temperature was a reduced clutch size at 28°C with a good quality diet compared to lower temperatures. Smaller clutch sizes at elevated temperature when grown on good quality food have been observed in other studies on cladocerans (Giebelhausen & Lampert, 2001; Masclaux et al., 2009). This might be explained by the common observation that the size of mature individuals decreases with increasing temperature according to the temperature-size rule (Atkinson, 1995), and that smaller *Daphnia* females produce smaller clutches (e.g. Gliwicz & Boavida, 1996; Sperfeld & Wacker, 2009).

We found that lower-latitude clones coped better at higher temperatures than higher-latitude clones, as they showed higher growth increments and better reproduction at 24 and 28°C when grown on good and medium quality food. This suggests evidence of local genetic adaptation and is supported by a recent study that characterised survival and reproductive capabilities of these clones grown on food of good quality (Seefeldt & Ebert, 2019). Seefeldt and Ebert (2019) found evidence for local temperature adaptation in *D. magna* populations that are summer-active by showing a positive relationship between temperature and heat tolerance, measured as maximal temperature for survival and reproduction in a multi-generation thermal ramp experiment. However, no such relationship was found for winter-active populations (lower latitudes), which outlive the summer in their dormant stage, supporting the hypothesis that *D. magna*
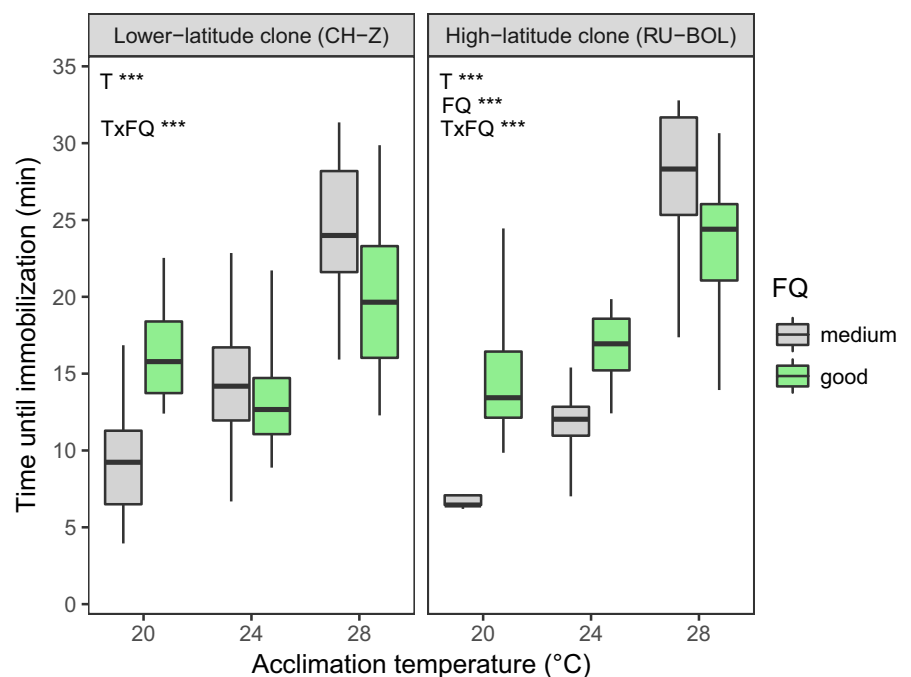
FIGURE 3 Heat tolerance (time until immobilisation) of a high-latitude and lower-latitude *Daphnia magna* clone raised at different temperatures and food qualities (FQ). Boxplots of 24 individual measurements are shown. Asterisks indicate significance levels (***$p < 0.001$) of ANOVA results for each clone with the factor food quality (FQ) and continuous temperature (T) as explanatory variables

TABLE 2 Results of three-way ANOVA to test for effects of clone (Cl), food quality (FQ), and continuous acclimation temperature (T) on heat tolerance (measured as time until immobilisation) of *Daphnia magna* ($r^2 = 0.67$)

	df	Sum Sq.	F	p	Var. (%)
Cl	1	21.6	1.3	0.253	0.2
T	**1**	**6,813.0**	**413.4**	**<0.001**	**49.0**
FQ	1	230.8	14.0	<0.001	1.7
Cl×T	1	394.3	23.9	<0.001	2.8
Cl×FQ	1	103.8	6.3	0.013	0.7
T×FQ	**1**	**1,735.9**	**105.3**	**<0.001**	**12.5**
Cl×T×FQ	1	1.1	0.1	0.792	0.01
Residuals	280	4,614.4			33.2

Note: Factors and interactions marked in bold indicate explained variance >10%.

adapts to the local temperatures experienced during the planktonic phase (Seefeldt & Ebert, 2019). Interestingly, decreasing food quality weakened the sign for local temperature adaptation as shown in our study by differences in growth increments between higher and lower latitude clones becoming increasingly smaller (see below).

4.2 | Food quality effects

Several studies have shown that pure *S. elongatus* (SYN) is of very low food quality for daphniids and that growth and reproduction can only be achieved if this cyanobacterium is supplemented directly with sterols and PUFAs (Martin-Creuzburg et al., 2009; Sperfeld et al., 2012; Von Elert et al., 2003) or if algae containing these lipids are mixed with SYN (Martin-Creuzburg et al., 2005, 2009; Sperfeld et al., 2016). Similar to the latter studies, we found that for all six *D. magna* clones somatic growth rate decreased with increasing proportion of SYN and decreasing proportion of NAN and ACU in the food, that is, with decreasing dietary sterol and PUFA supply. In our experiment, daphniids achieved surprisingly high growth rates at high proportions of cyanobacteria in the food (90% SYN in the poor food quality treatment). This is probably an effect of maternal investment as mother individuals were grown on food of very high quality (60:40% ACU:NAN) and thus, could provide offspring with high amounts of limiting sterols and PUFAs (Pajk et al., 2012; Sperfeld & Wacker, 2015). This provisioning was beneficial for the offspring used in the experiment to achieve relatively high growth rates at low dietary nutrient supply, a response also found in previous studies (Sperfeld & Wacker, 2012, 2015).

4.3 | Interactive effects of temperature and food quality

In our study, we did not find an interactive effect of temperature or food quality on somatic growth, in contrast to previous studies

showing that food quality effects in terms of essential lipids were more pronounced at colder temperatures (Martin-Creuzburg et al., 2012; Masclaux et al., 2009). It has been shown that the demand for PUFAs, especially eicosapentaenoic acid, increases at lower temperatures (Isanta et al., 2019; Martin-Creuzburg et al., 2012; Masclaux et al., 2009; Sperfeld & Wacker, 2011, 2012), whereas the demand for cholesterol, the main sterol found in animals, increases at elevated temperatures (Hassett & Crockett, 2009; Sperfeld & Wacker, 2009). Homeoviscous adaptation is likely to be the driver of these observations, which posits that ectotherms incorporate higher amounts of PUFAs and lower amounts of cholesterol into cell membranes at colder temperatures to maintain proper membrane fluidity, whereas they incorporate lower amounts of PUFAs and higher amounts of cholesterol at warmer temperatures to stabilise membranes by counteracting increasing disorder of the phospholipid bilayer (Crockett, 1998; Hochachka & Somero, 2002; Pruitt, 1990).

Interestingly, we did observe instead an interactive effect of food quality and latitudinal origin of clones on somatic growth. As hypothesised for elevated temperatures, lower-latitude clones showed better somatic growth on average than higher-latitude clones. However, this difference became smaller with increasing proportion of the cyanobacterium in the food and vanished completely at poor food quality. This may suggest constraints in adaptation to high temperatures with increasing food quality limitation imposed by cyanobacteria, a phenomenon increasingly expected in future scenarios (Jöhnk et al., 2008; Paerl & Huisman, 2008). Individuals of the lower-latitude clones may use the phytosterols supplied by food more efficiently than higher-latitude clone individuals to physiologically adapt to high temperature and achieve comparatively higher growth rates, an advantage they may lose with insufficient dietary sterol supply. Alternatively, stressful conditions, such as limiting food quality, may simply result in overall lower growth responses that make the detection of differences between growth rates more difficult.

4.4 | Heat tolerance and phenotypic plasticity

In our heat tolerance test, both clones showed very similar patterns in the time until immobilisation at lethally high temperature (37°C) across food quality and acclimation temperature treatments. This result does not support our expectation that the lower-latitude clone should be more heat tolerant than the high-latitude clone due to local adaptation, based on previous findings using *D. magna* clones of different latitudinal origin (Seefeldt & Ebert, 2019; Yampolsky et al., 2014). Statements on local adaptation using only two clones are not that meaningful, because this requires investigating many more clones as done in the previous studies (Seefeldt & Ebert, 2019; Yampolsky et al., 2014). However, adaptive responses driven by phenotypic plasticity can be more pronounced and clear as it is the case for the recurring observation that heat tolerance of daphniids increases with increasing acclimation temperature (Burton

et al., 2018; Coggins et al., 2017; Williams et al., 2012; Yampolsky et al., 2014; Zeis et al., 2004). In the present study, the same pattern was found, time until immobilisation of both clones generally increased with increasing acclimation temperature, indicating clearly a plastic phenotypic response to elevated temperature. Acclimation temperature was above all the main driving factor affecting short-term heat tolerance in our study. Interestingly, the increase in heat tolerance with increasing acclimation temperature was stronger at medium than at good food quality.

As described above, daphniids need more PUFAs at lower temperatures for physiological temperature adaptation (Schlechtriem et al., 2006; Sperfeld & Wacker, 2012). This might explain why, at lower acclimation temperature of 20°C, individuals grown on high food quality (i.e. high PUFA supply through ACU and NAN) showed longer time until immobilisation than individuals grown on medium food quality (i.e. low PUFA supply due to high percent SYN). At the highest acclimation temperature of 28°C, the opposite pattern was observed, that is, individuals grown on high food quality showed less heat tolerance than individuals grown on medium food quality. This cannot be explained by a higher requirement of sterols/cholesterol at higher temperatures (Sperfeld & Wacker, 2009), as individuals grown on high food quality had sufficient supply of dietary sterols. However, this seemingly counter-intuitive pattern could be explained by surplus supply of dietary PUFAs, which is proposed to reduce the heat tolerance of daphniids (Martin-Creuzburg et al., 2019; Werner et al., 2019; Zeis et al., 2019). Under surplus dietary PUFA supply at high temperatures, an increased incorporation of PUFAs into cell membranes may have detrimental effects on membrane functioning (Martin-Creuzburg et al., 2019; Werner et al., 2019) and could counteract or override the membrane stabilising effect of increased cholesterol supply (Chakraborty et al., 2020; Sperfeld & Wacker, 2009).

5 | CONCLUSIONS

With our experimental study we could show that food quality limitation, imposed by cyanobacterial abundance in the food, influenced life history responses of various *D. magna* clones at high temperature and mediated their heat tolerance. Lower-latitude, more warm adapted clonal lines could grow better at higher temperatures than higher-latitude clones, but lost this advantage with increasing cyanobacteria content in the diet, suggesting that adaptive constraints existed at decreasing biochemical food quality. This may suggest adverse effects on lower-latitude summer-active populations in future, as cyanobacterial blooms are expected to occur more frequently with global warming. However, we also found that short-term heat tolerance of *D. magna* acclimated to high temperature was better at poor food quality, when food contained more cyanobacteria, than at good food quality. Lower acute heat tolerance at good food quality may be caused by surplus PUFAs, which act against homeoviscous adaptation in membranes at elevated temperatures. The results

show seemingly opposite responses that act at different temporal scales for juvenile growth (mid-term) and acute heat tolerance (short-term). The net effect of the two responses on population growth is not clear and will need further investigations to better understand of how aquatic ectotherms will adapt to global warming. Future experiments should also consider simulating more natural diurnal temperature fluctuations, as peak temperatures may cross critical thermal tolerance limits more easily at elevated temperatures.

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